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herba polonica
From Botanical to Medical Research
Vol. 62 No. 4 2016
DOI: 10.1515/hepo-2016-0019

EXPERIMENTAL PAPER

Yield and level of phenolic compounds in the inflorescence of yellow everlasting *Helichrysum arenarium* (L.) Moench collected from natural sites

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Summary

Introduction: The inflorescence of *Helichrysum arenarium* (L.) Moench shows anti-inflammatory, antioxidant, detoxifying properties and is traditionally used in liver and biliary tract diseases. Because of its difficult and expensive cultivation, the plant raw material is mainly harvested from natural sites. **Objective:** The research aimed to determine the local variation in yield and content of flavonoids and phenolic acids in the yellow everlasting inflorescence against the background of the layer structure of vegetation as the rate of plant succession. **Methods:** The plant raw material was collected from 30 plots of 1 m^2, established for three separate populations developing on sandy fallows near Zielona Góra (western Poland). For each study area, percentage cover of the moss-lichen and herb layers, the height, cover and yield of *H. arenarium* as well as the height and cover of other herbaceous plants were determined. Total contents of flavonoids (expressed as quercetin) and phenolic acids (calculated as caffeic acid) were measured spectrophotometrically, according to Polish Pharmacopoeia. **Results:** Everlastings reached a cover of up to 70% and the maximum air-dry matter yield of 46.42 g/m^2. The height, coverage and yield of *H. arenarium* were correlated with the parameters describing the herb layer. The content of flavonoids ranged from 0.56 to 0.99%, while that of phenolic acids from 0.82 to 1.80% *DM*. **Conclusions:** Yellow everlasting is an important species of early fallows on poor sandy soils

and these habitats constitute a rich natural source of herbal raw material. Inflorescences harvested from natural sites are distinguished by a high and similar content of polyphenols and usually meet the requirements of Polish Pharmacopoeia.

Key words: *Helichrysum arenarium, yellow everlasting, yielding, polyphenols, flavonoids, phenolic acids, fallows, psammophilous grasslands, layer structure of vegetation*

INTRODUCTION

The inflorescence of *Helichrysum arenarium* (L.) Moench is traditionally used in liver and biliary tract diseases, showing choleretic, cholagogic, and spasmolytic activity as well as stimulating digestion [1]. This plant raw material is known for its anti-inflammatory, antioxidant, and detoxifying properties. The main active compounds are flavonoids, but the above-mentioned species also contains other phenolics, such as α-pyrone derivatives, phthalides, coumarins, and phenolic acids [2-6].

Because of difficulties in cultivation of *H. arenarium* and the high cost of plantation, the plant raw material is mainly collected from natural sites [7-9]. Yellow everlasting is characteristic for psammophilous grasslands of the *Koelerio-Corynephoretea* class [10-12] and occurs on sandy fallows, dry roadsides, deforested areas, heaths, and dunes [13-15]. This heliophilous species has rather high thermal requirements, prefers warm regions and microhabitats as well as dry oligotrophic soils poor in organic matter [16]. *H. arenarium* forms large populations in areas where there is no dense vegetation cover and strong competition of other species [17-19]. General observations indicate regression of everlastings in the next stages of succession of psammophilous grasslands, while the layer structure of vegetation changes significantly, in particular by the increase in density and height of other herbaceous plants. These processes not only reduce the growth and development of *H. arenarium*, but they can also affect the accumulation of active compounds.

The aim of the present study was to determine local variation in yield and content of flavonoids and phenolic acids in the inflorescence of *H. arenarium*. The diversity was shown against the background of the layer structure of vegetation as the rate of the succession of sandy grasslands on fallows.

MATERIAL AND METHODS

Plant material

The field study was carried out at the turn of July and August 2014 in western Poland, near Zielona Góra. Inflorescences of *H. arenarium* were collected from 30 plots with an area of 1 m^2, established for three separate populations in the vicinity of Niedoradz (51°51′40″N, 15°41′10″E), Kiełpin (51°52′05″N, 15°30′45″E)

and Ochla (51°52′27″N, 15°28′56″E) villages. For each study area, percentage cover of the moss-lichen and herb layers, the height, cover and yield of yellow everlasting as well as the height and cover of other herbaceous plants were determined. The height of plants was defined as the average of four measurements in the experimental plot. Inflorescences of *H. arenarium* were dried at room temperature and then used for phytochemical analysis. Water content [%] in air-dry powdered raw material was measured after drying at 105°C in a HR73 Halogen Moisture Analyzer (Mettler Toledo).

Phytochemical analysis

Total contents of flavonoids (expressed as quercetin) and phenolic acids (calculated as caffeic acid) in the inflorescence of yellow everlasting were measured spectrophotometrically, according to Polish Pharmacopoeia. The flavonoid level was quantified for 0.5 g of air-dried and powdered plant material, using Christ-Müller's method by acetone and ethyl acetate as extraction solvents [20]. The amount of phenolic acids (0.4 g sample) was determined with Arnov's reagent after methanolic extraction [20, 21]. The absorbance was measured on a Cintra 20 UV-VIS spectrometer (GBC) at λ=425 nm (flavonoids) and 490 nm (phenolic acids). The content of these active compounds was given as the percentage of dry matter (*DM*) of raw material. The applied analytic methods allowed the obtained results to be compared with previous works from Poland [6, 22, 23].

Statistical analysis

Yield of *H. arenarium*, content of polyphenols, and layer structure of vegetation were characterized using the basic statistics: mean, standard deviation, minimum, maximum, and coefficient of variation. The significance of differences between populations was analyzed using the Kruskal-Wallis and post-hoc tests. To check the normality of variable distribution, we applied the Shapiro-Wilk test. Square root, inverse proportion, arcsine and Bliss' ($y=\arcsin (x/100)^{0.5}$) transformations of data were performed for the skewed distribution of variables. Pearson's and Spearman's rank correlations were used to evaluate the relationships between features.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

The investigated populations of *H. arenarium* occupied sandy post-cultivated lands in contact with pine forests (monocultures). Besides yellow everlasting,

other psammophytes characteristic for the *Koelerio-Corynephoretea* class were noted. These were as follows: *Rumex acetosella*, *Jasione montana*, *Corynephorus canescens*, *Trifolium arvense*, *Armeria maritima* ssp. *elongata*, *Chondrilla juncea*, *Brachythecium albicans*, *Ceratodon purpureus*, *Polytrichum piliferum*, *Cladonia arbuscula* ssp. *mitis*, and *C. subulata*. The share of segetal species (*Viola arvensis*, *Vicia angustifolia*, *Setaria viridis*, and *Anthoxanthum aristatum*) was not large. On the other hand, we observed plants of later stages of succession: xerothermic grasslands and thermophilous forest edge communities (*Euphorbia cyparissias*, *Artemisia campestris* ssp. *campestris*, *Centaurea stoebe* and *Hypericum perforatum*) as well as ruderal associations (*Convolvulus arvensis*, *Tanacetum vulgare*, *Linaria vulgaris*, *Melandrium album*, *Artemisia absinthium*). There were also pine seedlings (Niedoradz) and about 3–5-year-old (Kiełpin) or 8-year-old (Ochla) individuals of *Pinus sylvestris*.

The vegetation structure of the study plots was strongly differentiated (tab. 1). The cover of the moss-lichen layer varied from 5 to 95%, while that of the herb layer – from 10 to 100%. Large variability was also noted in the case of the height of *H. arenarium* layer (21–47 cm) and other herbaceous plants in the patches (26–57 cm). Both parameters were positively correlated (tab. 2). A significant relationship existed between the height of everlastings and the cover of the herb layer. The average height of *H. arenarium* in the experimental plots was usually smaller by several or a dozen centimeters than that of other herbaceous plants in total (fig. 1). Yellow everlasting reached a cover of up to 70% (31.6%, on average) and was an important component of the vegetation (tab. 1, fig. 2). The cover of *H. arenarium* was negatively correlated with the coverage of other herbaceous species and their share in patches also had a limiting effect on the inflorescence yield (tab. 2). On the other hand, the yield of raw material grew exponentially with increasing yellow everlasting coverage (fig. 3). The air-dry weight of *H. arenarium* inflorescences on the 1 m^2 plots ranged from 5.36 to 46.42 g (tab. 3). However, this large variation in raw material yield had no effect on the amounts of flavonoids and phenolic acids. No correlations were detected between these active compounds and other investigated parameters of the vegetation structure, either.

Table 1.

Layer structure of vegetation with *Helichrysum arenarium* on the study plots

Variables	Mean ±*SD*	Min	Max	*V* [%]
Cover of moss-lichen layer [%]	58.7 ±33.8	5	95	58
Cover of herb layer [%]	74.5 ±19.5	10	100	26
Cover of *Helichrysum arenarium* [%]	31.6 ±15.5	7	70	49
Cover of other herbaceous plants [%]	42.9 ±23.4	3	80	55
Height of *Helichrysum arenarium* [cm]	32.2 ±6.3	21	47	20
Height of other herbaceous plants [cm]	41.4 ±8.3	26	57	20

SD – standard deviation, *V* – coefficient of variation, $n=30$

Table 2.

Correlations between the height, cover and yield of *Helichrysum arenarium* and the parameters describing the herb layer

Herb layer*H. arenarium*	Height	Cover	air-*DM* yield	*DM* yield
Height of other herbaceous plants	0.64***			
Cover of herb layer	0.67***			
Cover of other herbaceous plants	0.46*	-0.50**	-0.47**	-0.46**

Pearson's correlation: *** $p<0.001$, ** $p\leq0.01$, * $p<0.05$, $n=30$. Other herbaceous plants – without *H. arenarium*. *DM* – dry matter of raw material (*H. arenarium* inflorescences).

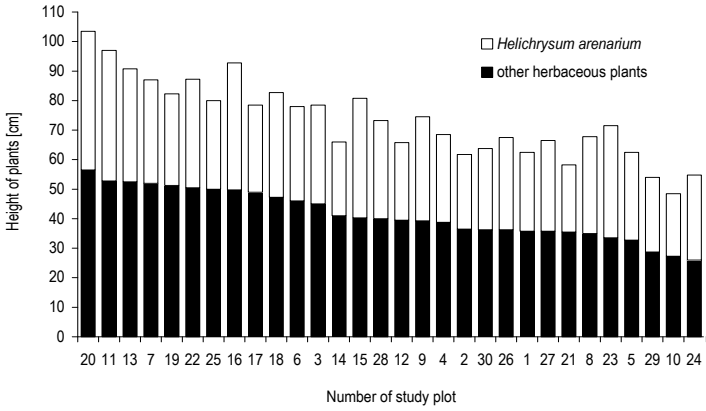

Figure 1.

Height of *Helichrysum arenarium* and other herbaceous plants on the study plots

Numbers of study plots: 1–10: Niedoradz, 11–20: Kiełpin, 21–30: Ochla

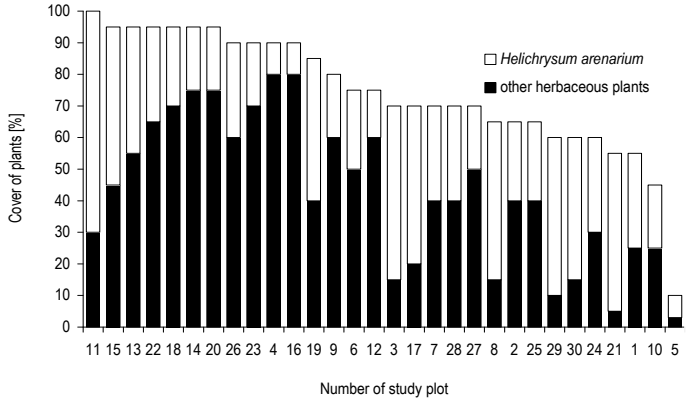

Figure 2.

Cover of *Helichrysum arenarium* and other herbaceous plants on the study plots

Numbers of study plots: 1–10: Niedoradz, 11–20: Kiełpin, 21–30: Ochla

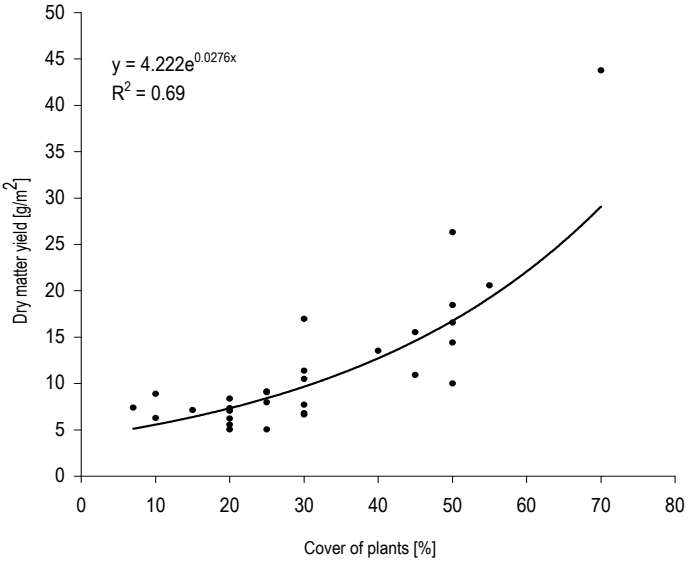

Figure 3.

Correlation between the cover and dry matter yield of *Helichrysum arenarium* on the study plots

Pearson's correlation after transformation of data: r=0.74, $p<0.001$, $n=30$

Table 3.

Phytochemical characteristic of the raw material (inflorescences) and yield of *Helichrysum arenarium* on the study plots

Variables	Mean ±*SD*	Min	Max	*V* [%]
Flavonoids [%]	0.75 ±0.11	0.56	0.99	15
Phenolic acids [%]	1.18 ±0.22	0.82	1.80	19
Water content in air-dry raw material [%]	7.06 ±1.21	5.66	9.27	17
Air-dry weight of raw material per 1 m^2 [g]	12.58 ±8.47	5.36	46.42	67
Dry weight of raw material per 1 m^2 [g]	11.69 ±7.94	5.05	43.79	68

Flavonoids – expressed as quercetin equivalent, phenolic acids – expressed as caffeic acid equivalent. *SD* – standard deviation, *V* – coefficient of variation, $n = 30$. The content of active compounds – in dry matter (*DM*) of raw material

The collected samples of *H. arenarium* were characterized by relatively low phytochemical differentiation. The level of the investigated compounds ranged from 0.56 to 0.99% and from 0.82 to 1.80% *DM* for the total flavonoids and phenolic acids, respectively. The calculated coefficient of variation was only 15 and 19% (tab. 3). The contents of the above-mentioned groups of active components in the inflorescence of everlastings were positively correlated (r=0.50, $p=0.005$).

The character of the vegetation indicates that the Kiełpin population was the most advanced in succession. It is distinguished by a significantly greater cover and height of the herb layer and a smaller cover of the lichen-moss layer (tab. 4). The relationship between the degree of development of these two layers was confirmed by the correlation analysis carried out for all the experimental plots. Coverage of the lichen-moss layer was negatively correlated with coverage of the herb layer (r_s=-0.51, p=0.004). In the case of the Kiełpin population, *H. arenarium* reached a greater height, coverage and raw material yield, but these differences were not statistically significant (tab. 4, 5). There were also no statistically significant differences between yellow everlasting populations in terms of the total content of flavonoids and phenolic acids (tab. 5).

Table 4.

Inter-population diversity of the layer structure of vegetation with *Helichrysum arenarium* (mean ±*SD*)

Variables	Niedoradz	Kiełpin	Ochla	*p*-value
Cover of moss-lichen layer [%]	68.5 ±30.0 [a]	30.0 ±30.3 [b]	77.5 ±21.0 [a]	**
Cover of herb layer [%]	62.5 ±22.3 [a]	89.5 ±9.8 [b]	71.5 ±14.7 [a]	**
Cover of *Helichrysum arenarium* [%]	27.2 ±15.4	34.5 ±19.4	33.0 ±11.4	n.s.
Cover of other herbaceous plants [%]	35.3 ±23.4	55.0 ±20.7	38.5 ±23.2	n.s.
Height of *Helichrysum arenarium* [cm]	30.1 ±4.5	36.0 ±7.8	30.4 ±4.8	n.s.
Height of other herbaceous plants [cm]	38.8 ±7.2 [a]	48.0 ±5.9 [b]	37.2 ±7.9 [a]	**

Kruskal-Wallis test: ** $p<0.01$, n.s. – not significant. Values with the same letter are not significantly different (post-hoc test, $p>0.05$). *SD* – standard deviation, n=30 (10 samples of each population)

Table 5.

Inter-population diversity of the active compound level and the raw material yield of *Helichrysum arenarium* (mean ±*SD*)

Variables	Niedoradz	Kiełpin	Ochla	*p*-value
Flavonoids [%]	0.71 ±0.09	0.75 ±0.13	0.79 ±0.11	n.s.
Phenolic acids [%]	1.10 ±0.15	1.25 ±0.23	1.18 ±0.27	n.s.
Air-dry weight of raw material per 1 m^2 [g]	12.22 ±5.87	15.76 ±12.74	9.75 ±3.78	n.s.
Dry weight of raw material per 1 m^2 [g]	11.15 ±5.34	14.79 ±11.99	9.13 ±3.52	n.s.

Kruskal-Wallis test: n.s. – not significant ($p > 0.05$). Flavonoids – expressed as quercetin equivalent, phenolic acids – expressed as caffeic acid equivalent. *SD* – standard deviation, $n = 30$ (10 samples of each population). The content of active compounds – in dry matter (*DM*) of raw material

DISCUSSION

H. arenarium is an important species of early stages of secondary succession on sandy uncultivated fields and can grow on fallows for at least over a dozen

years [24]. Everlastings occur in the pioneer phytocoenoses of *Corniculario-Corynephoretum* (=*Spergulo vernalis-Corynephoretum*) and in initial psammophilous grasslands with the dominance of *Anthoxanthum aristatum*, *Rumex acetosella*, *Scleranthus perennis* or *Hieracium pilosella*. On post-cultivated lands, *H. arenarium* can form large populations and it sometimes codominates with *Jasione montana* [18, 25-27]. Yellow everlasting on fallows was also documented in more advanced in succession communities of *Armerio elongatae-Festucetum ovinae* (=*Diantho-Armerietum elongatae*) [27] and even in the initial wildwoods with *Padus serotina* or *Betula pendula* [25, 28]. In our investigations, *H. arenarium* constituted a significant element of vegetation (tab. 1, fig. 2). We noted a strong positive correlation between the yellow everlasting coverage in the study plots and the yield of raw material (fig. 3). However, the coverage of this species and the inflorescence yield were negatively correlated with the coverage of other herbaceous plants (tab. 2). According to Sawilska and Dąbrowska [26], the share of yellow everlasting in plant communities is reduced in the succession process in favor of competitively stronger perennials. The field study showed a negative correlation between the fresh shoot yield of *H. arenarium* and the species number which grows in plant succession. This biomass of everlastings in the species-poor phytocoenoses of *Corniculario-Corynephoretum* was much greater than in the species-richer communities of *Armerio-Festucetum* and the differences increased in the following years of observations [27]. Similarly, a higher number of specimens and higher inflorescence weight of *H. arenarium* per $1m^2$ were recorded in the *Corniculario-Corynephoretum* community than in patches of *Armerio-Festucetum* [29]. In two successive years, the air-dry matter yield in the first plant association reached 6.75 and 10.84 g, respectively, and it was close to the mean value obtained in our research (tab. 3). Moreover, the cited literature data show the influence of weather conditions on the growth and development of yellow everlasting, including its blooming and herbal raw material yield. It seems that water deficiency in the spring and high air temperature belong to the main adverse factors [27, 29].

According to the Polish Pharmacopoeia [21], the inflorescence of yellow everlasting should contain in dry matter at least 0.5% of flavonoids expressed as quercetin equivalent. In our research, the average content of this group of active compounds was 0.75% and it ranged from 0.56 to 0.99% *DM* (tab. 3). Similar results (0.49–0.84%) were reported by Sawilska and Mielcarek [23] from two populations of *H. arenarium* growing in north Poland. A slightly smaller level of flavonoids (0.5–0.6%) was determined in the plant raw material harvested from four natural sites in the middle part of the valley of the Bug River, Poland [22]. More detailed studies of 22 populations from this region [6] showed a relatively large variation range of the amount of total flavonoids (0.15–0.78%) and phenolic acids (0.45–1.52%). However, it should be noted that in most cases the average content of the above-mentioned active components varied respectively from about 0.4 to 0.6% and from about 0.5 to 0.8% *DM*. In the present work (tab. 3), phenolic acids reached the mean value of 1.18% *DM* (0.82–1.80%) and it was higher than in the cited article of Bryksa-Godzisz *et al.* [6].

CONCLUSIONS

Our investigations and literature data show that inflorescences of *H. arenarium* harvested from natural sites are characterized by a high and similar level of the main active compounds: total flavonoids and phenolic acids. The plant raw material originating from different populations in our country usually meets the requirements of Polish Pharmacopoeia in terms of the flavonoid content (at least 0.5% *DM*, expressed as quercetin equivalent). In addition, drying at room temperature well stabilizes the high level of the above-mentioned groups of active compounds.

Yellow everlasting is a significant component of vegetation on sandy fallows, but it gradually declines under the influence of the competition of other perennials. However, poor post-cultivated lands could be a rich natural source of *H. arenarium* raw material. It would require the permanent maintenance of fallows in the early stages of succession, for example by periodic plowing (about once every ten years). The observed exponential relationship between the inflorescence yield and the percentage coverage of everlastings in patches can be used to estimate the abundance of *H. arenarium* populations.

ACKNOWLEDGEMENTS

The authors would like to thank Ms. Sława Kitkowska for help in field works and harvesting of plant raw material. The work was supported by the programme "Creation of scientific basis for biological improvement and plant genetic resources protection as a source of innovation and support of sustainable agriculture and national food security".

Conflict of interest: Authors declare no conflict of interest.

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PLON I ZAWARTOŚĆ ZWIĄZKÓW FENOLOWYCH W KWIATOSTANACH KOCANEK PIASKOWYCH *HELICHRYSUM ARENARIUM* (L.) MOENCH ZBIERANYCH ZE STANU NATURALNEGO

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

Wstęp: Kwiatostany *Helichrysum arenarium* (L.) Moench są tradycyjnie stosowane w chorobach wątroby i dróg żółciowych, wykazując właściwości przeciwzapalne, antyoksydacyjne oraz odtruwające. Ze względu na trudności w uprawie tego gatunku i wysokie koszty prowadzenia plantacji, surowiec zielarski wciąż jest pozyskiwany głównie ze stanu naturalnego. **Cel:** W prezentowanych badaniach określono lokalną zmienność plonowania oraz zawartości flawonoidów i fenolokwasów ogółem w kwiatostanach kocanek piaskowych na tle struktury warstwowej roślinności jako wskaźnika zaawansowania procesu sukcesji. **Metody:** Surowiec zielarski zebrano z 30 poletek (1 m^2), wyznaczonych w obrębie trzech populacji kocanek występujących na piaszczystych odłogach w okolicach Zielonej Góry. Na każdej powierzchni badawczej opisano strukturę warstwową roślinności: procentowe pokrycie warstwy porostowo-mszystej i zielnej, wysokość, pokrycie i plon *H. arenarium* oraz przeciętną wysokość i pokrycie pozostałych gatunków zielnych. Całkowitą zawartość flawonoidów (w przeliczeniu na kwercetynę) i fenolokwasów (w przeliczeniu na kwas kawowy) oznaczono metodą spektrofotometryczną według Farmakopei Polskiej. **Wyniki:** Kocanki uzyskiwały pokrycie do 70%, a maksymalny plon powietrznie suchej masy kwiatostanów wynosił 46,42 g/m^2. Wysokość, pokrycie i plon *H. arenarium* były skorelowane z parametrami opisującymi warstwę zielną. Poziom flawonoidów w surowcu mieścił się w granicach od 0,56 do 0,99% suchej masy (s.m.), natomiast kwasów fenolowych: od 0,82 do 1,80% s.m. **Wnioski:** Kocanki stanowią ważny element roślinności wczesnych odłogów powstałych na ubogich, piaszczystych glebach. Dlatego też, wspomniane siedliska mogą być zasobnym źródłem surowca zielarskiego. Kwiatostany zbierane ze stanu naturalnego charakteryzują się wysoką i wyrównaną zawartością związków polifenolowych i zwykle spełniają wymagania Farmakopei Polskiej.

Słowa kluczowe: *Helichrysum arenarium, kocanki piaskowe, plonowanie, polifenole, flawonoidy, kwasy fenolowe, odłogi, murawy napiaskowe, struktura warstwowa roślinności*