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### **Antioxidant properties of Chamisso arnica (*Arnica chamissonis* Less.) water infusions**

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Właściwości antyoksydacyjne ekstraktów wodnych arniki łąkowej  
(*Arnica chamissonis* Less.)

**Summary.** *Arnica chamissonis* (Less.) is a perennial plant of the Asteraceae family, providing flowers heads used in the production of medicines and cosmetics. Their chemical composition and properties are relatively well known, however the characteristic of the other raw materials is incomplete. The aim of the study was to assess the content of flavonoids and polyphenols as well as the antioxidant properties of Chamisso arnica infusions depending on the type of raw material (*Arnicae anthodium*, *Arnicae herba*, *Arnicae rhizoma*) and soil conditions in the cultivated area. Raw material for chemical analyses was obtained from 3-year-old plants grown on sandy and loamy soil. The obtained results indicate that the highest flavonoid content was found in flower anthodium infusions. In turn, herb infusions contained the most polyphenols and showed the greatest ability to reduce Fe<sup>3+</sup> ions and ABTS cation radical. The flavonoid content and value of the reduction strength of flower anthodium and herb infusions was significantly modified by soil conditions. Chamisso arnica obtained from loamy soil showed higher content of flavonoids and greater ability to reduce Fe<sup>3+</sup> ions than that from sandy soil.

**Key words:** *Arnica chamissonis*, water infusions, flavonoids, polyphenols, antioxidant properties

#### INTRODUCTION

Chamisso arnica (*Arnica chamissonis* Less.) belongs to the Asteraceae family, and is closely related to the extinction endangered mountain arnica (*Arnica montana* L.) valued by medicine and cosmetics. The main raw material obtained from both species are flowers heads anthodia (*Arnicae anthodium*), while folk medicine also uses rhizome with roots (*Arnicae rhizoma*), leaves (*Arnicae folium*) and herb (*Arnicae herba*) [Kaur et al. 2006].

Initially, the healing properties of Chamisso arnica inflorescences were used in North America, where it occurs in a natural state [Maguire 1943]. In Europe, at the end of the 20th century, due to the shrinking natural resources of mountain arnica, research on Chamisso arnica was undertaken. Their effect was the inclusion of inflorescences of this species in the German Pharmacopoeia as a substitute for raw mountain arnica [Passreiter 2011]. Also in Poland, arnica anthodia were considered a pharmaceutical raw material, equivalent to the mountain arnica inflorescences [FP VI 2002].

Popularity and wide use of arnica in many medicinal and cosmetic preparations is due to the presence of large group of biologically active substances in the raw material. Flowers heads contain sesquiterpene lactones, flavonoids (quercetin, kaempferol, luteolin and others) and essential oil as well as phenolic acids (including caffeic, chlorogenic, gallic), coumarins and tannins [Leven and Willuhn 1987, Gawlik-Dziki et al. 2009, Kowalski et al. 2015, Patel and Patel 2017, Sugier et al. 2017, Ivanova et al. 2019]. Herb and rhizomes with roots remain much less recognized in terms of chemical composition.

Chamisso arnica inflorescences are used for the production of medicines and cosmetics, especially for vascular skin care. The active substances contained in flowers heads are easily absorbed by the epidermis, reach the capillaries and strengthen their walls. In the case of capillary damage, they inhibit plasma penetration, have positive effect on the absorption of exudate fluid and prevent from the formation of venous clots [Lamer-Zarawska et al. 2007]. The raw material also finds application in the case of skin and furuncle irritation [Mamedov et al. 2005]. Chamisso arnica anthodia also have anti-inflammatory and antioxidant properties [Gawlik-Dziki et al. 2009, Oleszek and Kazachok 2018]. In addition, due to the ability to inhibit acetylcholinesterase and butyrylcholinesterase activity, the possibility of using Chamisso arnica infusions in the treatment of Alzheimer's disease is indicated [Wszelaki et al. 2010].

The available literature lacks studies comparing chemical composition and antioxidant properties of raw materials obtained from various parts of Chamisso arnica plant. Data on the impact of soil conditions on the quality characteristics of the raw material are also scarce. This study aims at assessing the content of flavonoids, polyphenols and antioxidant properties of meadow arnica infusions depending on the type of raw material (flower baskets, herbs and rhizome with roots) and the impact of these characteristics on different soil conditions.

#### MATERIALS AND METHODS

In 2018, the raw material for the chemical analyses was taken from 3-year-old *A. chamissonis* individuals from two experimental fields at the University of Life Sciences in Lublin located in the eastern part of Poland. The first experimental field (51°31'25"N, 22°45'04"E) was located on sandy soil (sand 66.2%, silt 20.5%, clay 13.3%), and the other experimental field (51°29'28"N, 22°51'18"E) was located on loamy soil (sand 21.5%, silt 42.1%, clay 36.4%). The sandy soil was characterized by moderate content of organic matter (1.67%; PB-34 – Tiurin method), moderate phosphorus ( $P_2O_5$  – 138 mg · kg<sup>-1</sup>;

PN-R-04023:1996), low potassium ( $K_2O - 79 \text{ mg} \cdot \text{kg}^{-1}$ ; PN-R-04022:1996+Az1:2002), and very low magnesium levels ( $Mg - 13 \text{ mg} \cdot \text{kg}^{-1}$ ; PN-R-04020:1994+Az1:2004) and very acidic reaction. In turn, the loamy soil was characterized by high content of organic matter (1.88%), very high phosphorus ( $258 \text{ mg} \cdot \text{kg}^{-1}$ ), high potassium ( $39 \text{ mg} \cdot \text{kg}^{-1}$ ), and moderate magnesium levels ( $51 \text{ mg} \cdot \text{kg}^{-1}$ ), and neutral reaction. In the experiment, phosphorus-potassium fertilization was applied in the following doses per ha: 35 kg P and 100 kg K. Nitrogen fertilizers (in a total dose of  $60 \text{ kg N} \cdot \text{ha}^{-1}$ ) were applied twice: in the spring before the beginning of vegetation (30 kg) and in phase of the first flower buds (30 kg).

Total rainfall in the growing season (April–October 2018) was lower than the long-term average by over 90.0 mm (in the years 1967–2017 it was 422.6 mm). The average air temperature during vegetation reached  $16.2^\circ\text{C}$  and was  $2.8^\circ\text{C}$  higher than the long-term average. During the 2018 year inflorescence harvest for the period from the third decade of May to the second decade of June, an increase in average temperature and a decrease in precipitation compared to the long-term average were recorded. In May, the average temperature was higher by  $3.3^\circ\text{C}$ , while in June by  $2.1^\circ\text{C}$  in relation to an average of 50 years – in the years 1967–2017 it was  $13.4$  and  $16.2^\circ\text{C}$ , respectively. Compared to the 65.0 mm and 69.2 mm of long-term total precipitation for the corresponding months in May and June 2018 recorded was lower by 20.9 and 3.8 mm [<https://danepubliczne.imgw.pl/>].

### **Raw material collection**

The raw material for chemical analyses was obtained from the 3-year-old plants on two dates: flowers heads and herbs during full flowering of Chamisso arnica (in June), while the rhizome with roots at the end of its vegetation (in October). Immediately after harvesting, these raw materials and cleaning the rhizomes with roots, they were dried in a drying room at  $35^\circ\text{C}$ . The raw material was stored in paper bags in the dark until analysis. Prior to starting chemical analyses, individual raw materials were ground for their homogenization.

### **Preparation of a water infusion**

Infusions prepared by pouring 0.5 g weight of ground Chamisso arnica raw materials with hot distilled water (50 ml at  $+100^\circ\text{C}$ ) were used for the analysis. Infusion was carried out for 30 min, after which the solutions were filtered. All chemical analyses were performed in 5 replications.

### **Flavonoid content**

In infusions, flavonoid content was determined according to the method described by Lamaison and Carnet [1990]. Results obtained were presented as the amount ( $\mu\text{g}$ ) of quercetin (Q) per ml of infusion. The conversion was based on the performed calibration curve.

### Total polyphenol content

Total polyphenol concentration was determined using the Folin-Ciocalteu reagent according to the methodology proposed by Singleton and Rossi [1965]. The content of phenolics reducing reagent was expressed as caffeic acid equivalent (CAE) per ml of infusion.

### Strength of reduction

The reduction strength was measured applying the FRAP method [Benzie and Strain 1996], which is based on the reduction of iron ions by compounds having antioxidant activity. The data obtained are expressed as  $\mu\text{mol}$  of reduced  $\text{Fe}^{3+}$  · ml of infusion<sup>-1</sup>.

### Antiradical activity

Analysis of the anti-radical activity of infusions was performed based on the ability to neutralize the ABTS radical [Re et al. 1999]. As a result of the interaction of the antioxidant with radical, the solution is discolored in proportion to the content of antioxidants. Affinity of tested material for quenching the free radical ABTS was expressed in mg Trolox (TE) · ml of infusion<sup>-1</sup>.

### Statistical analysis

The two-way analysis of variance (ANOVA) and subsequent Tukey's tests were used. The differences were considered significant at  $p < 0.05$ . The statistical analyses were carried out using the Statistica 6.0 software.

## RESULTS AND DISCUSSION

In the tested Chamisso arnica infusions, the flavonoid content was significantly modified by the factors used in the experiment, i.e. assessed plant materials and soil conditions (Fig. 1). The highest content of flavonoids was found in infusions of flowers heads of Chamisso arnica obtained from plants derived from loamy soil ( $64.64 \mu\text{g Q} \cdot \text{ml}$  of infusion<sup>-1</sup>). Also, water infusions from the herb obtained from this soil contained significantly more flavonoid compounds compared to the raw material derived from sandy soil. Regardless of soil conditions, the flavonoid content was five times higher in anthodia infusions compared to rhizomes with roots (Fig. 1). Stefanache et al. [2016] report that the content of phenolic compounds, including flavonoids, depends on the part of the plant and the plant's response to environmental factors. They showed that the mountain arnica leaf blades contained less flavonoids compared to the inflorescences. Also other authors [Karimi et al. 2011, Jamuna et al. 2012, Stojković et al. 2014, Šibul et al. 2016] show significant differences in chemical composition of the raw materials from the same plant from different herbal plant species. Jamuna et al. [2012] report that infusions of *Hypochaeris radicata* L. leaves contained almost twice as many flavonoids as compared to the roots (leaf –  $105.76$ , root –  $55.16 \mu\text{g}$  quercetin ·  $10 \text{mg}^{-1}$ ). Differentiation in the content of flavonoid compounds was also found in the case of water infusions of *Tanacetum vulgare* L. – higher content was found in leaf

infusions compared to infusions made from inflorescences (18.2 and 10.5 mg catechin · g<sup>-1</sup> DM, respectively) [Stojković et al. 2014]. Karimi et al. [2011] and Šibul et al. [2016] also noted differences in the content of flavonoids in infusions prepared from different parts of the plant.

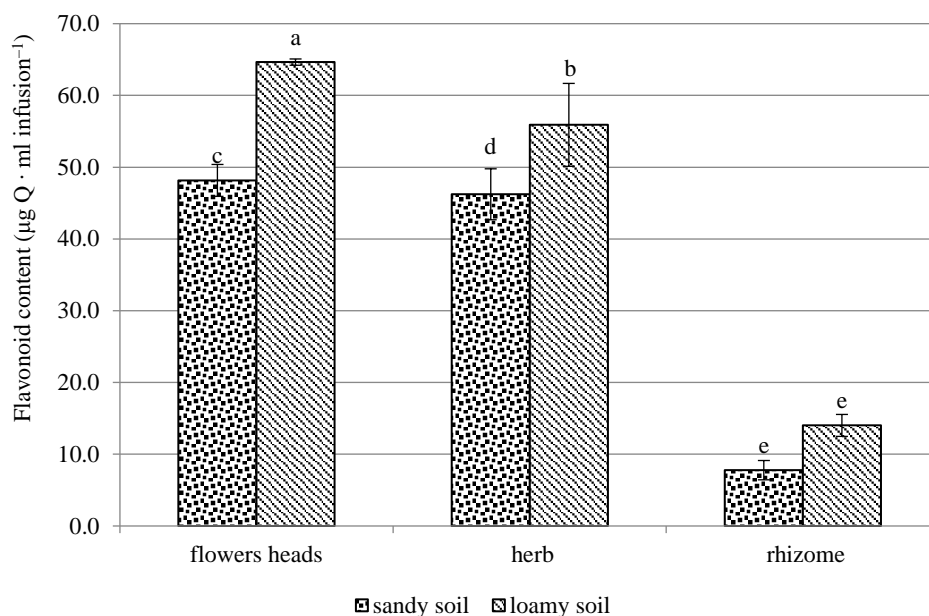


Fig. 1. Flavonoid content in *A. chamissonis* water infusions depending on experimental factors. Q – quercetin. Values marked with different letters are significantly different ( $p = 0.05$ ) (Tukey test,  $p < 0.05$ )

The conducted experiment showed significant differences in the content of polyphenolic compounds in the assessed parts of arnica plant. The highest concentration of polyphenols was found in the herb (irrespective of the soil – 0.61 mg CAE · ml of infusion<sup>-1</sup>), lower in flowers heads, and definitely the lowest in rhizomes with roots (on average 0.17 mg CAE · ml of infusion<sup>-1</sup>) (Fig. 2). This indicates a large variation in the content of polyphenolic compounds depending on the analyzed part of the plant. Also other studies [Jamuna et al. 2012, Stojkovic et al. 2014] conducted within the Asteraceae family indicate significant variation in the polyphenol content in infusions prepared from different parts of plants. Infusions from underground parts of *H. radicata* and *Anthemis cretica* L. were characterized by higher content of polyphenolic compounds – water infusions from chamomile roots (34.72 mg GAE · g DM<sup>-1</sup>) were particularly abundant in the analyzed group of compounds. They were characterized by more than 30% higher concentration of polyphenols compared to leaf infusions and nearly 20% in relation to flowers heads [Jamuna et al. 2012, Stojkovic et al. 2014].

Based on the results obtained, it was found that the value of the reduction power of the analyzed infusions was differentiated by the soil type, on which the plants were grown.

Significantly more iron ions were reduced by flower and herb infusions that were obtained from loamy soil (Fig. 3). In addition, infusions obtained from root rhizomes have been shown to have significantly lower ability to reduce  $\text{Fe}^{3+}$  ions ( $2.39 \mu\text{mol Fe}^{3+} \cdot \text{ml of infusion}^{-1}$ ) compared to herb and inflorescences ( $7.68$  and  $6.33 \mu\text{mol Fe}^{3+} \cdot \text{ml of infusion}^{-1}$ , respectively). Other authors also point to significant differences in the content of antioxidants in individual parts of plants: *Hypochaeris radicata* [Jamuna et al. 2012], *Taraxacum officinale* Web. [García-Carrasco et al. 2015] and *Anthemis cretica* [Stojkovic et al. 2014]. Jamuna et al. [2012] examining the reducing power of methanol infusions from various parts of *Hypochaeris radicata* showed higher absorbance of leaf infusions compared to roots. Leaf infusions contain more antioxidants than those obtained from the roots. Similar data were obtained after analyzing methanol infusions from *Taraxacum officinale* – infusions from *Taraxacum officinale* leaves ( $302 \mu\text{mol TE} \cdot \text{g}^{-1}$ ) were particularly capable of reducing  $\text{Fe}^{3+}$  ions. They were characterized by more than twice the reduction power compared to the root infusions [García-Carrasco et al. 2015]. Less varied results were obtained by analyzing the water infusions of the roots, leaves and flowers of *Anthemis cretica* – in this case the reduction power also depended on parts of plants, but the differences were not so significant. The ability to reduce iron ions by water infusions from roots and flowers was over  $27 \text{ mg GAE} \cdot \text{g DM}^{-1}$ . However, in leaf infusions, the parameter was determined at a level of less than  $25 \text{ mg GAE} \cdot \text{g DM}^{-1}$  [Stojkovic et al. 2014].

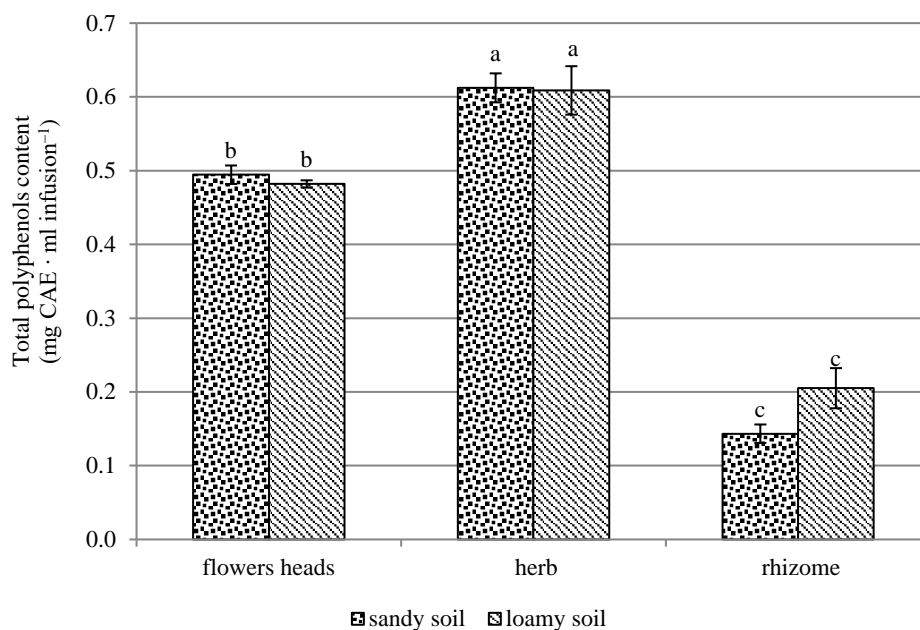


Fig. 2. Polyphenols content in *A. chamissonis* water infusions depending on experimental factors. CAE – caffeic acid. Values marked with different letters are significantly different ( $p = 0.05$ ) (Tukey test,  $p < 0.05$ )

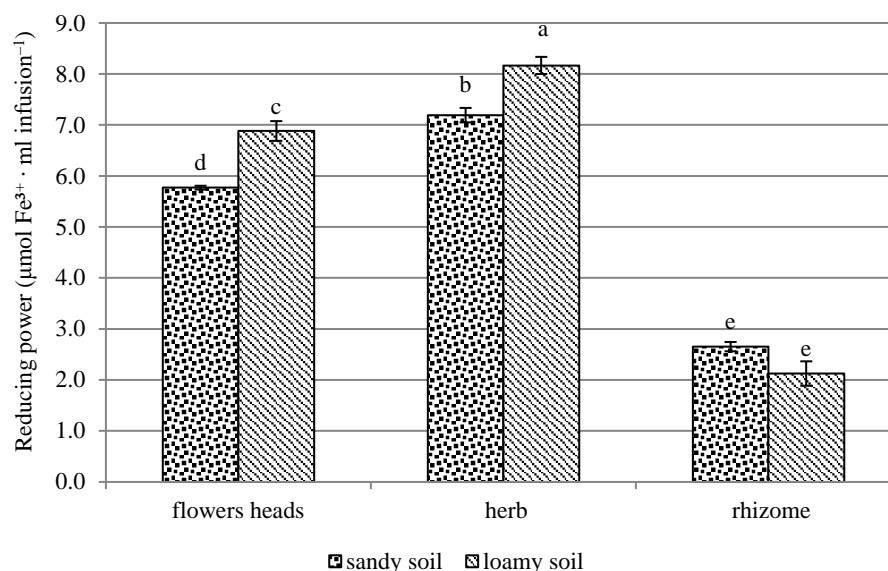


Fig. 3. Reducing power of *A. chamissonis* water infusions depending on experimental factors. Values marked with different letters are significantly different ( $p = 0.05$ ) (Tukey test,  $p < 0.05$ )

In the conducted experiment, the greatest antioxidant abilities (quenching the ABTS radical) were found in herbal infusions (mean 63 mg TE), while the smallest – for rhizomes with roots ( $0.22 \text{ mg TE} \cdot \text{ml of infusion}^{-1}$ ). From the tested parts of arnica plants, only in the case of herb, soil conditions had significant impact on the ability to quench the ABTS radical. Better antioxidant properties were found in infusions obtained from herbs obtained from plants grown on loamy soil than on lighter sandy soil (Fig. 4). Gawlik-Dziki et al. [2010] indicate that both *A. chamissonis* herb and flowers heads reduce about 90% of the cation radical and have better antioxidant properties compared to analogous *A. montana* raw materials. In turn, infusions from rhizomes and roots of both species of arnica showed low activity, and the results obtained were similar (about 35% of the reduced radical). Other authors also point to the diverse antioxidant capacity of various parts of herbal plants (*Tanacetum vulgare*, *Hypochaeris radicata*, *Anthemis cretica* and *Vernonia cinerea* L.) [Jamuna et al. 2012, Stojkovic et al. 2014, Goggi and Malpathak 2017]. Stojković et al. [2014] indicate that water infusions of *Tanacetum vulgare* leaves and flowers differ in their antioxidant properties. Their ability to reduce the ABTS radical was 22.2 and 19.2 mol TE · g DM<sup>-1</sup>. However, according to data published by Jamuna et al. [2012], methanol infusion obtained from the leaves of *Hypochaeris radicata* has higher antioxidant activity ( $2706.73 \text{ μmol TE} \cdot \text{g infusion}^{-1}$ ) than root infusion ( $2028.37 \text{ μmol TE} \cdot \text{g infusion}^{-1}$ ). In turn, in the case of *Anthemis cretica* and *Vernonia cinerea*, root infusions were characterized by similar antioxidant properties compared to flowers [Stojkovic et al. 2014, Goggi and Malpathak 2017].

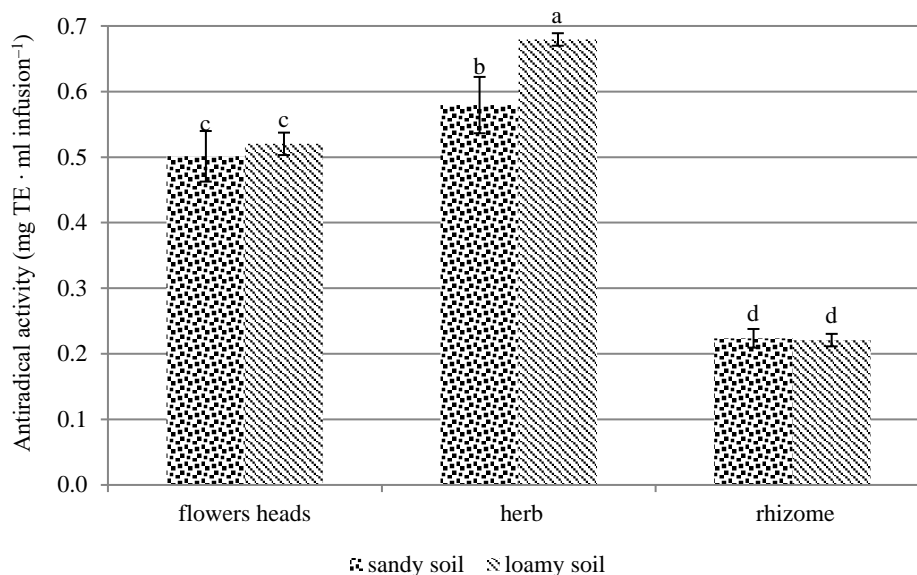


Fig. 4. Antiradical activity (ABTS) of *A. chamissonis* water infusions depending on experimental factors. TE – Trolox. Values marked with different letters are significantly different ( $p = 0.05$ ) (Tukey test,  $p < 0.05$ )

#### CONCLUSIONS

1. The analyzed parts of *Chamisso arnica* plants (flowers heads, herbs and rhizomes with roots) generally showed significantly differentiated content of flavonoids and polyphenols as well as antioxidant properties of infusions.

2. The highest amount of flavonoids was found in flowers heads extracts, while the infusions obtained from the herb had the highest content of polyphenols and showed the best antioxidant properties.

3. The flavonoid content and value of the reducing power of flowers heads and herbs infusions was significantly modified by soil conditions. *Chamisso arnica* grown on loamy soil showed higher flavonoid content and greater ability to reduce  $Fe^{3+}$  ions than on sandy soil.

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**Streszczenie.** *Arnica chamissonis* (Less.) to bylina z rodziny Asteraceae, dostarczająca surowca zielarskiego głównie w postaci koszyczków kwiatowych wykorzystywanych do produkcji leków i kosmetyków. Ich skład chemiczny i właściwości zostały stosunkowo dobrze poznane, brakuje natomiast informacji o właściwościach ziela i części podziemnej. Celem badań była ocena zawartości flawonoidów i polifenoli oraz właściwości przeciwutleniających naparów arniki łąkowej w zależności od typu surowca (*Arnicae anthodium*, *Arnicae herba*, *Arnicae rhizoma*) i warunków glebowych w miejscu ich pozyskania. Surowiec do analiz chemicznych pozyskiwano z roślin 3-letnich uprawianych na glebie piaszczystej oraz pylastej. Uzyskane wyniki wskazują, że największą zawartość flawonoidów stwierdzono w wyciągach z koszyczków kwiatowych. Z kolei wyciągi z ziela zawierały najwięcej polifenoli oraz wykazywały największą zdolność do redukcji jonów  $Fe^{3+}$  oraz kationorodnika ABTS. Zawartość flawonoidów oraz wartość siły redukcji naparów z koszyczków kwiatowych i ziela była istotnie modyfikowana przez warunki glebowe. Arnika łąkowa pozyskana z gleby pylastej wykazywała większą zawartość flawonoidów i większą zdolność redukcji jonów  $Fe^{3+}$  niż ta pozyskana z gleby piaszczystej.

**Słowa kluczowe:** *Arnica chamissonis*, ekstrakty wodne, flawonoidy, polifenole, właściwości antyoksydacyjne

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