sciendo

herba polonica
International journal edited by the Institute of Natural Fibres and Medicinal Plants
Vol. 66 No. 2 2020

Received: 2019-11-20
Accepted: 2020-05-30
Available online: 2020-06-30

DOI: 10.2478/hepo-2020-0008

EXPERIMENTAL PAPER

HPLC-DAD-ESI/MS comparison of the chemical composition of flowers from two *Arnica* species grown in Poland

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Summary

Introduction: Arnica flowers are used in pharmaceutical and cosmetic industry. According to EMA only endangered *Arnica montana* provides the medicinal plant material. However, some European countries also allow the use of *A. chamissonis flowers*, whose chemical composition is not known in detail.

Objective: The aim of the study was to recognize and compare the chemical composition of *A. montana* and *A. chamissonis* flowers collected from plants cultivated in Poland.

Methods: HPLC-DAD-ESI/MS analysis comprised phenolic acids, flavonoids and sesquiterpene lactones.

Results: Thirty eight flavonoids and phenolic acids were recognized and quantified, with patuletin, 6-methoxykaempferol and quercetin present only in *A. chamissonis* flowers. Moreover, helenalin and acetyl-dihydrohelenalin were identified.

Conclusion: *A. montana* and *A. chamissonis* flowers from plants grown in Poland possess similar composition of simple phenols and polyphenols, present in concentration slightly higher in *A. montana.* Helenalin and dihydrohelenalin esters identified in arnica flowers from various regions of Europe were not detected.

Key words: ***Arnica montana, Arnica chamissonis, simple phenols, polyphenols, sesquiterpene lactones***

Słowa kluczowe: ***Arnica montana, Arnica chamissonis, proste fenole, polifenole, laktony seskwiterpenowe***

INTRODUCTION

Arnica flowers, due to their anti-inflammatory and analgesic activities, as well as efficiency in reducing pain, swelling and discoloration from bruises, are widely used in pharmaceutical and cosmetic industry as ingredients of gels, ointments, creams and tinctures.

According to the European Pharmacopoeia and EMA herbal monograph [1], only *Arnica montana* provides medicinal plant raw material, while Polish Pharmacopoeia V and Comission E also permit the use of another species of the *Arnica* genus, namely *Arnica chamissonis* [2, 3]. *A. chamissonis* is easier to cultivate than the endangered *A. montana* [4], demonstrating similar biological and pharmacological activities.

It has been shown that *Arnica montana* and some of analyzed *Arnica chamissonis* extracts possess a similar, high anti-inflammatory activity by inhibition of NF-$\kappa\beta$ and release of human neutrophil elastase (HNE), what it is related to presence of sesquiterpene lactones of the helenanolide type in both species [5]. Another research on anti-inflammatory activity of *Arnica* flower extracts reports that both species effectively inhibit xanthine oxidase (XO) activity at similar concentrations. On the other hand, *A. chamissonis* flowers and herb exhibited higher LOX inhibitory activity than herb and flowers from *A. montana* [6]. Moreover, *A. chamissonis* seeds were proved to be superior towards *A. montana* in terms of the antioxidant activity, expressing the higher total polyphenols, tannins and flavonoids contents and stronger DPPH and FRAP activities [7].

Despite its promising biological potential and possibility to be a valuable substitute for *A. montana*, the chemical composition of *A. chamissonis* is not known in detail [8, 9]. Most data, mainly on sesquiterpene lactones and flavonoids, is found in the works of Merfort [8-12] and Willuhn [13-15] published in the late 20th century. Later, only a few papers have been published reporting the essential oil composition [16, 4] and identification of four new pseudoguaianolides and six flavonoid aglycones [17] in *A. chamissonis* flowers, as well as composition of lignans in the *A. montana* and *A. chamissonis* roots and rhizomes [18].

Moreover, it was described in several papers [19-22] that differences observed in the chemical composition of arnica flowers may depend not only on the species, but also the growth conditions. It was reported that the concentration of phenolic acids and the ratio of *o*-dihydroxylated flavonoids versus others belonging to this group in *A. montana* flowers increases with elevated altitude [19]. While Spitaler *et al.* found no significant relationship between helenalin levels and growing site altitude, the research on *A. montana* collected from the wild sites in Spain showed that the helenalins content was the highest at the highest altitude sites (1330–1460 m a.s.l.) [20]. Similar observations were noted in the arnica flowers collected in North East Romania, where those collected at higher altitudes (1000–1700 m above sea level, as compared to 800–1000 m above sea level) were richer in phenolic acids and sesquiterpene lactones [21]. On the other hand, studies of *A. montana* flowers from various wild populations from northern Italy did not show such correlation and among samples taken at altitudes from 1227 to 2060 m above sea level the highest concentrations of flavonoids, sesquiterpene lactones and phenolic acids were observed in those growing at medium altitudes (1608–1817 m above sea level) [22].

The aim of the study was to compare by use of chromatographic methods the chemical composition of flowers from *Arnica montana* L. and *Arnica chamissonis* L. (*Compositae*) cultivated in Poland.

MATERIALS AND METHODS

Chemicals and reagents

Standard compounds of apigenin, kaempferol, quercetin, isoquercetin, hyperoside, chlorogenic acid, caffeic acid, ferulic acid were obtained from Fluka (St. Gallen, Switzerland). Luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, luteolin, astragalin, galangin, tectochrysin, genkwanin, isorhamnetin, quercetin 3-glucuronide, kaempferol 3-*O*-glucuronide from Extrasynthèse (Lyon, France). Hispidulin, pectolinaringenin were obtained from PhytoLab (Vestenbergsgreuth, Germany). Naringenin, isochlorogenic acid and *p*-coumaric acid were obtained from Koch-Light (Colnbrook, Great Britain). Chrysin and vanillic acid were obtained from Sigma-Aldrich (Saint Louis, USA). Helenalin was purchased from Abcam (Cambridge, Great Britain). Cynarin originated from the standard collection of the Department of Pharmacognosy, Medical University of Gdańsk (Poland). Analytical-grade chloroform and methanol were obtained from POCH S.A. (Gliwice, Poland). Analytical-grade formic acid (89–91% purity) was purchased from Merck (Dramstadt,

Germany). HPLC-grade acetonitrile (LC/MS) Lichrosolv was purchased from Sigma-Aldrich (Steinheim, Germany). Demineralised water was prepared by using Millipore Water Purification System (Molsheim, France).

Plant material and sample preparation

Dried flowers of *Arnica montana* L. cultivated in Greater Poland and *Arnica chamissonis* L. cultivated in Lublin region were obtained from "Runo" company (Poland) and from "Kawon" company (Poland), respectively. Dried flowers of arnica were extracted according to the method described by Zheleva-Dimitrova *et al.* [23]. Plant material (1 g) was extracted with 80% methanol (2x5 ml each) on the ultrasonic bath (temp. 35°C, 30 min). The obtained extracts were centrifuged (15 min) and next transferred to a flask and supplemented with 80% methanol to a volume of 10 ml. The samples was filtered through a 0.22 μm membrane syringe filter (ChemLand, Stargard Szczeciński, Poland).

HPLC-DAD-ESI/MS analysis of biologically active compounds

HPLC analysis was performed using an LC system by Shimadzu (Kyoto, Japan) consisting of two pumps LC-20AD, semi-micro mixer, CBM-20A system controller, CT0-20AC column thermostat, SIL $20AC_{XR}$ autosampler, UV–vis detector (Diode Array Detector) SPD-M20A, LCMS-2020 mass spectrometer with ESI ionization. Data was acquired and processed by LabSolution software.

The HPLC separation and further analysis were conducted according to the method described in the literature [24]. Injection volume: for phenolic compounds - 1 μl (qualitative analysis) and 1 μl of extract diluted with methanol in proportion 1:1 (quantitative analysis), for sesquiterpene lactones – 3 μl.

The method was validated according to Food and Drug Administration, Center for Drug Evaluation and Research Guidelines [24, 25].

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

RESULTS AND DISCUSSION

The results of qualitative and quantitative analysis carried out under the conditions of HPLC-DAD-ESI/MS methods are presented in tables 1 and 2 and in figure 1. According to the obtained DAD and ESI spectral data, 38 simple phenols and polyphenols next to 2 sesquiterpene lactones were identified by comparison with authentic standards (t_R value) and literature data [7-9, 21, 23, 26].

Identification of flavonoids

The analysis carried out with use of standard compounds revealed the presence of seven flavonoids, namely: isoquercetin (**9**), apigenin (**36**), kaempferol (**37**), apigenin 7-*O*-glucoside (**21**), luteolin (**33**), luteolin 7-*O*-glucoside (**11**) present in both analysed plant materials and quercetin (**32**) which was recognized only in flowers of *A. chamissonis* (tab. 2). All these compounds have been previously described in flowers of *A. montana* and *A. chamissonis* [15-20, 31].

Based on the obtained UV spectra and *m/z* values of molecular $[M + H]^+$ and fragmentation ions $[Ag+H]^+$ in ESI spectra and comparing them with literature data [8, 26], the presence of three more flavonoids, compounds **13**, **30** and **31** was confirmed in the plant material analysed.

The ESI/MS spectra of compounds **13** and **31** showed similar UV spectra and presence of molecular ion $[M+H]^+$ (**13**) or fragmentation ion $[Ag+H]^+$ (**31**) at *m/z* 317 corresponding to the molecular weight of 6-methoxykaempferol (tab. 1). The molecular ion $[M+H]^+$ at *m/z* 479 visible in the ESI mass spectrum of compound **13** indicated that its structure contains a glucose unit. As a result, compound **13** was identified as 6-methoxykaempferol-3-*O*-glucoside [8] and compound **31** as a free aglycone: 6-methoxykaempferol. Another free aglycone present in the studied plant material, compound **30** exhibiting the molecular ion $[M+H]^+$ at *m/z* 333 in the ESI spectrum, was identified as patuletin (tab. 2). Both compounds (**30** and **31**) were recognized only in *A. chamissonis* flowers.

Identification of phenolic acids

A number of phenolic acids were identified in the tested plant material (tab. 1, fig. 1). Analysis using standard compounds confirmed the presence of a benzoic acid derivative: protocatechic acid (**2**) and three derivatives of cinnamic acid, namely: chlorogenic acid (**4**), caffeic acid (**5**) and cynarin (1,3-di-*O*-caffeoylquinic acid) (**6**).

Table 1

Polyphenols and simple phenols identified by HPLC-DAD-ESI/MS in the flowers of *A. montana* and *A. chamissonis*

Peak number	Compound	t_R (min)	UV λ_{max} (nm)	ESI-MS (m/z) [M+H]+, [Ag+H]+	Correlation with standard
1	Isomer of caffeoylquinic acid	4.57	234, 243sh, 296sh, 326	355	
2	Protocatechic acid	5.97	259, 292	155	0.8591
3	Unidentified compound	8.71	230, 293sh, 314		
4	Chlorogenic acid	10.46	234, 243sh, 296sh, 324	355	0.943
5	Caffeic acid	12.23	232, 241sh, 294sh, 322	181	0.9981
6	Cynarin	16.61	230, 243sh, 299sh, 325	516	0.9813
7	Patuletin 3-*O*-glucoside	24.51	256, 269sh, 348	495, 333	
8	Patuletin 3-*O*-glucuronide	24.66	256, 267sh, 347	509, 333	
9	Isoquercetin	25.21	255, 265sh, 310sh, 352	465	0.9813
10	Quercetin 3-*O*-glucuronide	25.49	255, 264sh, 310sh, 353	479	
11	Luteolin 7-*O*-glucoside	27.10	254, 264sh, 347	449	0.9787
12	Eupafolin 7-*O*-glucoside	27.76	269, 296sh, 330	479, 317	
13	6-methoxykaempferol-3-*O*-glucoside	28.10	249sh, 270, 344	479, 317	
14	Isomer of dicaffeoylquinic acid	28.14	234, 246sh, 298sh, 325	517	
15	Eupafolin 7-*O*-glucuronide	28.35	269, 334	493, 317	
16	Kaempferol 3-*O*-glucuronide	29.07	260, 300sh, 347	463	
17	Derivative of caffeic acid	29.21	233, 240sh, 300sh, 324		
18	3,5-dicaffeoylquinic acid	29.49	234, 246sh, 296sh, 328	517	
19	1,5-dicaffeoylquinic acid	29.89	235, 246sh, 296sh, 329	517	
20	4,5- dicaffeoylquinicacid	30.74	235, 265sh, 300sh, 352	517	
21	Apigenin 7-*O*-glucoside	31.23	267, 334	433	0.9377
22	1-methoxyoxaloyl-3,5-dicaffeoylquinic acid	31.62	235, 241sh, 298sh, 329	603	
23	Isomer of dicaffeoylquinic acid	33.74	235, 242sh, 297sh, 328	517	
24	Luteolin 3'-*O*-glucoside	33.99	267, 301sh, 331	449, 317	
25	Isomer of dicaffeoylquinic acid	34.37	230, 240sh, 297sh, 330		
26	Feruloyl-caffeoylquinic acid	34.82	235, 242sh, 300, 329	531	
27	Kaempferol 3-*O*–acetylglucoside	34.98	264, 300sh, 345	491	
28	Derivative of caffeic acid	35.50	232, 245sh, 300sh, 330		
29	Derivative of caffeic acid	36.08	232, 240sh, 300sh, 327		
30	Patuletin	40.20	256, 271sh, 366	333	
31	6-Methoxykaempferol	40.59	252sh, 270, 344	317	
32	Quercetin	41.21	254, 265, 310sh, 367	303	0.984
33	Luteolin	41.75	253, 265sh, 298sh, 347	287	0.9928
34	Derivative of caffeic acid	42.08	232, 243sh, 300sh, 329		
35	Derivative of caffeic acid	42.79	233, 245sh, 300sh, 329		
36	Apigenin	45.28	267, 304sh, 329	271	0.9231
37	Kaempferol	45.94	253sh, 267sh, 299sh, 341	287	0.9231
38	Derivative of caffeic acid	46.97	235, 241sh, 307sh, 330		

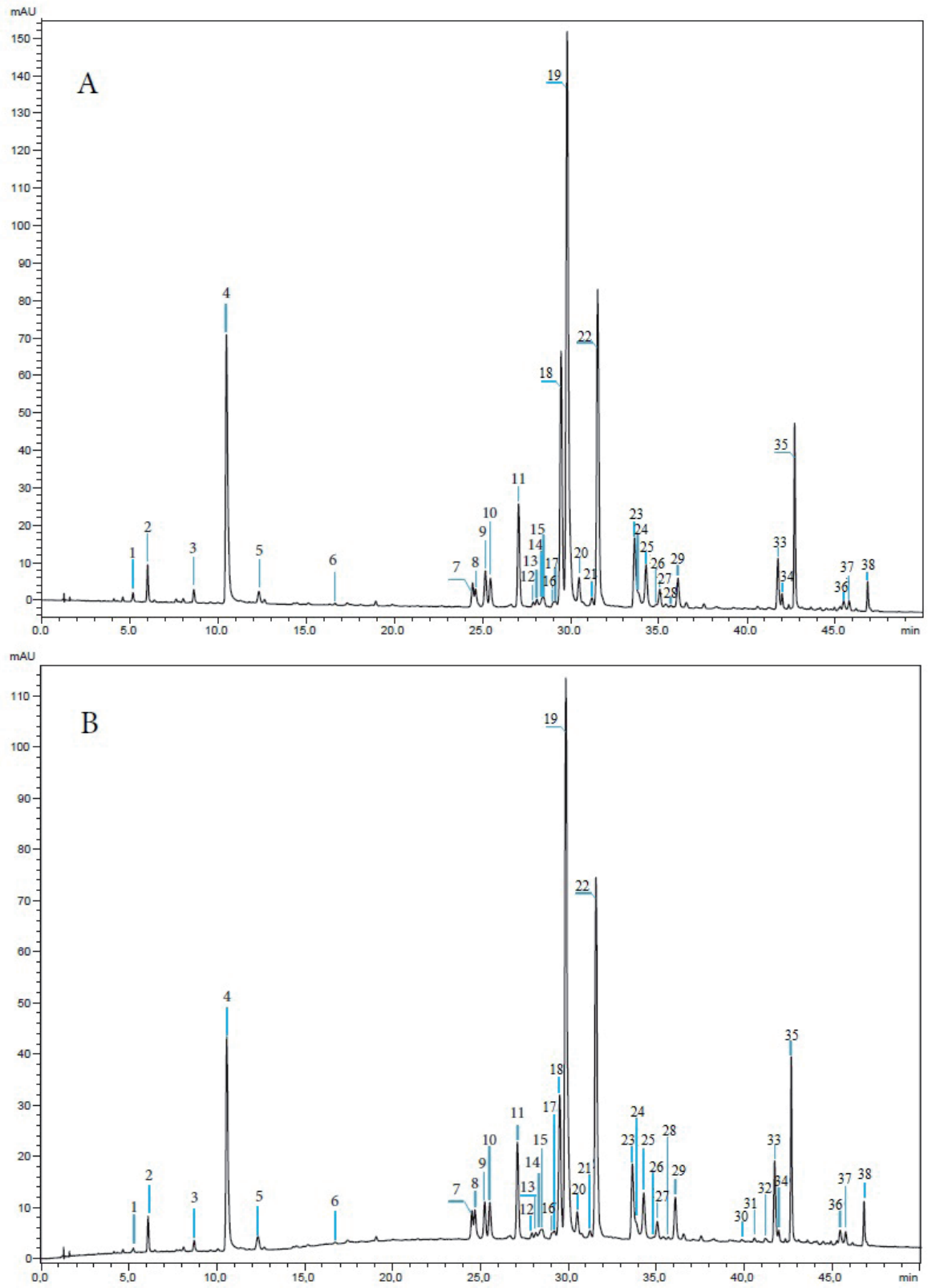

Figure 1

HPLC chromatograms of the extracts from the flowers of *Arnica montana* (**A**) and *Arnica chamissonis* (**B**). Kinetex PFP column (2.6 μm, 4.6 x 100 mm), gradient program I, UV λ-330 nm

Table 2

Sesquiterpene lactones determined by HPLC-DAD-ESI/MS in the flowers of *A. montana* and *A. chamissonis*

Peak number	Compound	t_R [min]	$[M+H]^+/[M+Na]^+$	Amount [mg/100g d.w.]	
				Arnica montana	*Arnica chamissonis*
39	*Helenalin*	17.3	263	1.50 ± 0.40	0.764 ±0.026
40	*Acetyl-dihydrohelenalin*	20.4	307	*	*

*not quantitatively determined

Moreover, comparing the obtained DAD and MS spectra, a number of caffeic acid derivatives has been showed. These were: 3,5-di-*O*-caffeoylquinic acid (**18**), 1,5-di-*O*-caffeoylquinic acid (**19**), 4,5-di-*O*-caffeoylquinic acid (**20**), 1-methoxyoxaloyl-3,5-di-*O*-caffeoylquinic acid (**22**), two unidentified isomers of dicaffeoylquinic acid (**1** and **14**), six caffeic acid derivatives (**17**, **28**, **29**, **34**, **35** and **38**) and compound **26.** The latter compound, based on the presence of the molecular ion $[M + H]^+$ at *m/z* 531 in the ESI spectrum, may be feruloylquinic acid, previously described in both arnica species [27]. Identification of other di-*O*-caffeoylquinic acid derivatives (**18**, **19** and **20**) was based on Lin *et al.* work [28], in which, using isolated compounds with the structure confirmed by H^1 NMR, it was proved that the dominant compound in arnica flowers is 1,5-di-*O*-caffeoylquinic acid, and not 3,5-di-*O*-caffeoylquinic acid as described in previous works [19, 29].

Identification of sesquitriterpene lactones

Among sesquitriterpene lactones, only the presence of helenalin (fig. 2) and acetyl-dihydrohelenalin in *A. montana* and *A. chamissonis* flower extracts was indicated (tab. 2). The latest compound was identified by comparison of retention time value (t_R 20.4 min) with peak of acetyl-dihydrohelenalin from Arnica TM analysed in our previous research [24].

Quantification of active biologically compounds from *Arnica* species

The contents of compounds are given in mg of the compound per 100 g of analyzed dried plant material (tab. 2, 3). The content of individual flavonols was calculated on quercetin, flavones expressed as luteolin and caffeoylquinic acids/phenolic acids calculated as chlorogenic acid (tab. 3). The content of free helenalin was determined using external standard – helenalin (tab. 2).

Flavonoids

A. chamissonis flowers contained higher concentration of flavonoid compounds, including 92.2±5.7 mg/100 g d.w. of flavonols and 343±52 mg/100 g d.w. of flavones, in comparison to *A. montana* flowers where the amounts of both flavonoid groups were: 76.8±5.4 mg/100 g d.w. and 283±49 mg/100 g d.w. (tab. 3), respectively. Among two analysed species, only flavonoid concentration in *A. chamissonis* corresponds to the Polish Pharmacopoeia V [2] requirements and was higher than 0.4%.

From the group of flavonoids, the dominating compounds in both analyzed samples of *Arnica* flowers were flavones: luteolin 7-*O*-glucoside (**11**) (127±22 mg/100 g d.w. in *A. chamissonis,* 140±23 mg/100 g d.w. in *A. montana*) and luteolin (**33**), present in almost twice higher quantity in *A. chamissonis* (121.1±8.7 mg/100 g d.w. in *A. chamissonis vs.* 71.7±1.3 mg/100 g d.w. in *A. montana*). Other flavones, were present at slightly higher level in *A. chamissonis* flowers: apigenin (**36**) (25.7±4.6 mg/100 g d.w. in *A. chamissonis vs.* 19.05±0.62 mg/100 g d.w. in *A. montana*), luteolin 3'-*O*-glucoside (**24**) (28.3±6.1 mg/100 g d.w. in *A. chamissonis vs.* 17.9±1.4 mg/100 g d.w. in *A. montana*) and eupafolin-7-*O*-glucuronide (**15**) (18.2±5.5 mg/100 g d.w. in *A. chamissonis vs.* 14.01±0.54 mg/100 g d.w. in *A. montana*).

In contrary to flavones, flavonols were present in smaller quantities, with the dominating compounds being: quercetin 3-*O*-glucuronide (**10**) (16.8±2.5 mg/100 g d.w. in *A. chamissonis vs.* 15.1±1.6 mg/100 g d.w. in *A. montana*), patuletin 3-*O*-glucoside (**7**) (14.7±2.8 mg/100 g d.w. in *A. chamissoni vs.* 10.9±1.5 mg/100 g d.w. in *A. montana*) and patuletin 3-*O*-glucuronide (**8**) (11.9±1.7 mg/100 g d.w. in *A. chamissonis vs.* 10.29±0.66 mg/100 g d.w. in *A. montana*).

Phenolic acids

Among the phenolic acids identified in the flowers, the dominating compounds are 1,5-di-*O*-caffeoylquinic

Table 3

Determined concentrations of identified compounds in the analyzed plant material (mg/g d.w.) – flowers of *A. montana* and *A. chamissonis*.

L.p.	Compound	Amount [mg/g d.w.]	
		A. chamissonis	*A. montana*
1	Isomer of caffeoylquinic acid	1.82 ± 0.33	2.43 ± 0.37
2	Protocatechic acid	26.0 ± 2.1	25.8 ± 3.5
3	Unidentified compound	*	*
4	Chlorogenic acid	328 ± 29	416.1 ± 6.1
5	Caffeic acid	14.4 ± 1.0	11.9 ± 2.5
6	Cynarin	1.14 ± 0.15	1.82 ± 0.20
7	Patuletin 3-*O*-glucoside	14.7± 2.8	10.9 ± 1.5
8	Patuletin 3-*O*-glucuronide	11.9 ± 1.7	10.29 ± 0.66
9	Isoquercetin	16.5 ± 2.6	18.74 ± 0.57
10	Quercetin 3-*O*-glucuronide	16.8 ± 2.5	15.1 ± 1.6
11	Luteolin 7-*O*-glucoside	127 ± 22	140 ± 23
12	Eupafolin 7-*O*-glucoside	9.7 ± 1.1	8.6 ± 1.3
13	6-Methoxykaempferol-3-*O*-glucoside	3.99 ± 0.45	4.53 ± 0.42
14	Isomer of dicaffeoylquinic acid	8.2 ± 1.3	4.86 ± 1.5
15	Eupafolin 7-*O*-glucuronide	18.2 ± 5.5	14.01 ± 0.54
16	Kaempferol 3-*O*-glucuronide	2.56 ± 0.51	2.77 ± 0.24
17	Derivative of caffeic acid	3.50 ± 0.78	3.27 ± 0.34
18	3,5-Dicaffeoylquinic acid	165 ± 23	350 ± 48
19	1,5-Dicaffeoylquinic acid	624 ± 92	700.8 ± 6.7
20	4,5- Dicaffeoylquinic acid	22.1 ± 1.1	26.0 ± 2.3
21	Apigenin 7-*O*-glucoside	12.2 ± 1.9	11.8 ± 1.0
22	1-Methoxyoxaloyl-3,5-dicaffeoylquinic acid	438 ± 28	425 ± 12
23	Isomer of dicaffeoylquinic acid	104 ± 34	82.3 ± 9.9
24	Luteolin 3'-*O*-glucoside	28.3 ±6.1	17.9 ± 1.4
25	Isomer of dicaffeoylquinic acid	57 ± 12	48.07 ± 4.14
26	Feruloyl-caffeoylquinic acid	0.59 ± 0.03	1.84 ± 0.14
27	Kaempferol 3-*O*–acetylglucoside	9.18 ± 0.38	8.9 ± 1.4
28	Derivative of caffeic acid	2.24 ± 0.37	3.14 ± 0.34
29	Derivative of caffeic acid	54.5 ± 5.5	32.5 ± 1.8
30	Patuletin	1.66 ± 0.04	-
31	6-Methoxykaempferol	2.84 ± 0.33	-
32	Quercetin	3.94 ± 0.09	-
33	Luteolin	121.1 ± 8.7	71.7 ± 1.3
34	Derivative of caffeic acid	10.0 ± 1.3	11.8 ± 1.8
35	Derivative of caffeic acid	182 ± 17	179.4 ± 9.1
36	Apigenin	25.7 ± 4.6	19.05 ± 0.62
37	Kaempferol	7.15± 0.34	5.17 ± 0.74
38	Derivative of caffeic acid	*	*
	Total flavones	343 ± 52	283 ± 49
	Total flavonols	92.2 ± 5.7	76.8 ± 5.4
	Total phenolic acids	2040 ± 170	2330 ± 200

- not detected, *not quantitatively determined

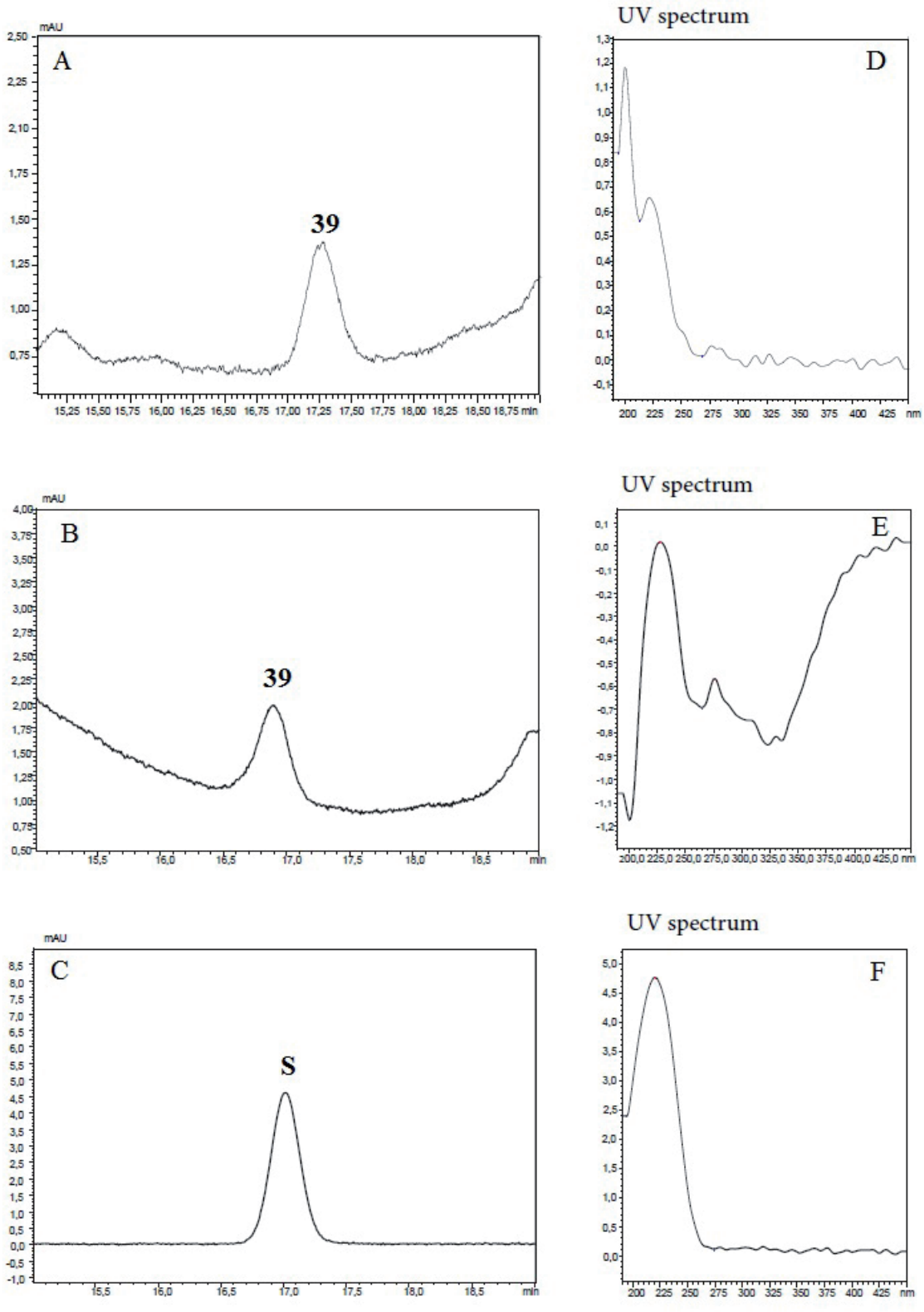

Figure 2

HPLC chromatograms of helenalin standard and the extracts from the flowers of *Arnica montana* (**A**) and *Arnica chamissonis* (**B**) helenalin standard (**C**) and UV spectra of helenalin in *A. montana* (**D**), *A. chamissonis* (**E*)* – 39 and helenalin standard (**F**) – S. Kinetex C-18 column (2.6 µm, 4.6 x 100 mm), gradient program II, UV λ-225 nm

acid (**19**) (624±92 mg/100 g d.w. in *A. chamissonis vs.* 700.8±6.7 mg/100 g d.w. in *A. montana*) (tab. 3) and 1-methoxyoxaloyl-3,5-di-*O*-caffeoylquinic acid (**22**) (438±28mg/100gd.w.in*A.chamissonisvs.*425±12mg/100g d.w. in *A. montana*) present in both species at similar levels. Chlorogenic acid (**4**) (328±29 mg/100 g d.w. in *A. chamissonis vs.* 416.1±6.1 mg/100 g d.w. in *A. montana*), as well as 3,5-di-*O*-caffeoylquinic acid (**18**) (165±23 mg/100 g d.w. in *A. chamissonis vs.* 350±48 mg/100 g d.w. in *A. montana*) were present in significantly higher quantities in *A. montana* flowers.

Both analysed species show a high total content of phenolic acids, *Arnica montana* flowers 2330±200 mg/100 g d.w. and *A. chamissonis* flowers 2040±170 mg/100 d.w (tab. 3). Unlike arnica flowers originating from different European regions, arnica plants cultivated in Poland accumulate phenolic acids in significantly higher concentrations in comparison to flavonoids and STLs. Their concentration was 5-fold higher as compared to flavonoid content. In previous research, the plants cultivated in Austria [19] and harvested in Italy [22] contained phenolic acids at similar level as flavonoids.

Sesquiterpene lactones

In *A. montana* and *A. chamissonis* flowers, the determined content of helenalin was 1.50±0.40 mg/ 100 g d.w. and 0.764±0.026 mg/100 g d.w., respectively (tab. 2). Despite 2-fold difference, both analysed plant materials did not meet standard required by European Pharmacopea 9 [30], which set at least 0.4% of sesquiterpene lactones calculated as dihydrohelenalin tiglate. The low content of sesquiterpene lactones occurs probably due to the fact that apart from acetyldihydrohelenalin, no other helenalin or dihydrohelenalin derivatives have been detected in both species.

CONCLUSIONS

Both *Arnica montana* and *Arnica chamissonis* flowers originating from plants cultivated in Poland have a similar chemical composition in terms of phenolic acids, flavonoids and sesquiterpene lactones, with slight differences in their content. The performed analysis suggests that arnica plants cultivated in Poland do not accumulate helenalin and dihydrohelenalin esters and are significantly richer in phenolic acids in comparison to arnica plants collected from different European sites.

Conflict of interest: The authors declare no conflict of interest.

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