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ORIGINAL PAPER

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BIOLOGICALLY ACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF BORAGE (*Borago officinalis* L.) FLOWERS AND LEAVES

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ABSTRACT

Recently the interest in borage as a vegetable and medicinal plant has increased, yet the knowledge about a content of biologically active compounds in borage grown in Poland remains very scanty. In the experiment carried out in the south-eastern part of the country, fresh borage flowers and leaves contained 0.02% and 0.12% of lipids with the highest level of palmitic acid, i.e. 44.5% and 33.4%, respectively. Flower lipids contained stearic, oleic, elaidic, linoleic, linolelaidic, arachidonic, myristic and lauric acid as well. Besides, leaf lipids were rich in α-linolenic acid (17.7%), less in palmitoleic acid with absence of arachidonic acid. Leaves were found to have 0.16% of essential oil, 1.0% of flavonoids, 9.2 mg vitamin C·100 g f.w.$^{-1}$, 1.9 mg carotenoids·100 g f.w.$^{-1}$, 0.77 mg chlorophyll a·1 g f.w.$^{-1}$ and 0.22 mg chlorophyll b·1 g f.w.$^{-1}$. Flowers contained more essential oil and vitamin C while less carotenoids, chlorophyll a and b and flavonoids. 45 components were found in the flower essential oil and among them 16 were identified with cumene (62.9%) as a major one. The content of other components was below 3%. As for the leaf essential oil, 18 compounds were detected, and 5 were identified with the highest level of cumene (58.5%) and hexenyl (13.3%). Generally, flowers had more polyphenols and their ferric reducing ability was higher than that of leaves. Besides, a content of remaining unreduced DPPH radical was higher in the flowers. Notably, the time necessary for 50% reduction of the initial concentration of DPPH radical was 2.5 times longer in flowers, whereas their antiradical efficiency 3 fold lower compared to leaves.

Key words: carotenoids, chlorophyll, flavonoids, polyphenols, vitamin C, essential oil, lipids, DPPH, FRAP

INTRODUCTION

Borage is an annual plant from the Boraginaceae family native to the Mediterranean region and naturalized in some other parts of Europe, among others in Poland [Pieszak et al. 2012, Asadi-Samani et al. 2014].

At the vegetation onset, borage forms a rosette of elongated basal leaves and after entering the generative phase it grows dynamically developing the main stem and several lateral ones. The main stem is branched in the upper part and grows up to 60–80 cm height [Suchorska and Osińska 1997]. On the stem top, blue rarely white flowers arise along branched cymes [Król 2010]. In Poland, the borage flowering season is from June to July. The flowers secrete large amounts of nectar and are visited readily by honey bees and bumble-bees [Suchorska and Osińska 1997, Osborne 1999]. Stems, leaves and calyx of borage are covered with coarse and dense hairs producing essen-

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tial oils [Al-Nowaihi et al. 1987, Król 2010, Pieszak et al. 2012].

Borage with its cucumber-like smell [Król 2010, Pieszak et al. 2012] is cultivated in gardens or collected in natural stands, used in salads, soups or some vegetable and meat dishes [Simon et al. 1984, del Río-Celestino et al. 2008, Biscotti and Pieroni 2015] or for preparing beverages [Zemmouri et al. 2014]. Furthermore, its fresh or candied star-shaped blue flowers serve as an edible decoration of cocktails and confectionery [Adreon 1956, Larson et al. 1984]. In northern Spain borage rosette leaves, leaf petioles and stems are consumed in fresh or lightly fried salads [Medrano et al. 1992, del Río-Celestino et al. 2008]. In south-eastern Italy, consumption of boiled borage leaves and flowers with dressing, in omelet, in soups and stews, pickled or preserved in oil is common [Bianco et al., Biscotti and Pieroni 2015]. In Poland, however, borage is cultivated as a medicinal plant and herb harvested during flowering (*Boraginis herba*), its seeds (*Boraginis semen*) also make raw material [Król 2010, Pieszak et al. 2012]. A Polish borage ecotype was studied in Italy by Peiretti et al. [2004].

Borage as a valuable medicinal plant has enjoyed more popularity last years [Mhamdi et al. 2007, Gupta and Singh 2010, Asadi-Samani et al. 2014]. Feeding the diet with entire borage plant could also make an alternative to pharmacological treatment of animals [Peiretti et al. 2004]. The herb contains mucous compounds (up to 12%), tannins (about 4%), saponins, flavonoids (among others quercetin, isorhamnetin and kaempferol), phenolic acids (vanilic, p-coumaric, p-hydroxybenzoic, gentisic, caffeic, rosmarinic, chlorogenic), organic acids (apple and citric), scopoletin, soluble silica (2.5%) and mineral salts [Gudej and Tomczyk 1996, Król 2010, Zemmouri et al. 2014]. The studies by Zemmouri et al. [2014] showed that water extract from borage leaves contained 35.48 mg of polyphenols·g of extract^{-1} (gallic acid equivalent), 20.79 mg of flavonoids·g of extract^{-1} (quercetin equivalent), 10.88 mg of flavonols expressed as dry extract mg·g rutin equivalents^{-1}, 3.14 mg of tannin expressed as mg catechin equivalent·g of dry extract^{-1} and 0.09 mg of anthocyanins·g of extract^{-1}. Total phenolic content determined by Segovia et al. [2014] in ethanol extract from borage leaves obtained in the local market in Spain and expressed as mg gallic acid equivalent·g dry weight^{-1} ranged from 19.16 up to 27.49 subject to ethanol concentration as well as temperature and extraction time. Borage petioles are abundant in protein, fiber, potassium, iron and contain about 94% of water [Medrano et al. 1992]. Edible parts are also rich in calcium but with low levels magnesium and natrium and medium amount of nitrate [Bianco et al. 1998]. The studies conducted under partially controlled greenhouse conditions in southern Spain by del Río-Celestino et al. [2008] demonstrated that rosette leaves of several white and blue flowering borage accessions contained 2.7% of oil, while petioles and stems 1.1% and 1.4% of oil on average in relation to dry mass basis, respectively. In the leaves, depending on flower color, the highest levels were recorded for α-linolenic (26.2–32.2%), palmitic (19.8–24.4%), stearidonic (13.8–15.2%), linoleic + linolelaidic (9.8–12.3%) and myristic (5.5–11.8%) fatty acids. The share of γ-linolenic (3.3–5.0%), stearic (3%), oleic + elaidic (1.9–3.0%) and erucic (0.4–0.6%) acids was much smaller. Moreover, the leaf lipids contained very low quantities of lauric, palmitoleic, eicosenoic, behenic, lignoceric and nervonic acids. As for the petioles and stems, myristic and linoleic fatty acids prevailed and made about 60% of total leaf lipids. The experiment carried out by Griffiths et al. [1966] highlighted that lipids obtained from young borage leaves had 43% of α-linolenic acid, 23% of stearidonic acid, 12% of palmitic acid, 9% of linoleic + linolelaidic acids, 6% of γ-linolenic acid, 3% of oleic + elaidic acids, and 2% of stearic acid. The results obtained by Sayanova et al. [1999] show that the fatty acid composition of borage leaves depends on their stage of development with the highest levels of palmitic (18.6–24.8%), linoleic (13.3–20.9%), α-linolenic (17.3–38.4%) and γ-linolenic (8.0–22.0%) acids. Peiretti et al. [2004] also reported that the chemical composition of borage was closely associated with the developmental stages of the plant, with a progressive increase in a crude protein content and moderate lignin growth as a result of plant ageing. Besides, the fatty acid profile of the plant also varied depending on the stage of devel-

opment. At the late vegetative stage, α-linolenic and stearidonic acids were the main components which decreased afterwards. At the early seed stage, linolenic acid was found most abundant and then γ-linolenic and oleic acid levels elevated. Bandoniene and Murkovic [2002] showed the presence of rosmarinic acid in borage leaves.

According to the research made by Sayanova et al. [1999], fatty acid composition of borage flowers was characterized by the highest content of linoleic (41.4%), palmitic (31.9%) and γ-linolenic (10.6%) acids. Total phenolic content in three extracts of borage flowers determined by Aliakbarlu and Tajik [2012] ranged between 50.4 $mg \cdot g^{-1}$ and 64.1 $mg \cdot g^{-1}$ (gallic acid equivalent). So far, no information has been available on the content of anthocyanins, carotenoids and chlorophyll in borage plants.

Today borage is cultivated mainly for seed oil [El Hafid et a. 2002, Asadi-Samani et al. 2014]. Its beneficial effects are attributed to a high content of γ-linolenic acid [Karłowicz-Bodalska and Bodalski 2007, Tasset-Cuevas et al. 2013]. Borage seeds contain from 27% to 38% of oil subject to their maturity, ecotype and environmental conditions [Muuse et al. 1988, Gunstone 1992, del Río et al. 1993, de Haro-Bailón and del Río 1998, Zadernowski et al. 1999, de Haro et al. 2004, Borowy et al. 2016]. Unsaturated fatty acids made 60% [Król 2010] while γ-linolenic acid 8.7–30% of this oil, respectively [del Río et al. 1993, Berti et al. 1998, Zadernowski et al. 1999, Berti et al. 2002, El Hafid et al. 2002, Mhamdi et al. 2009, Borowy et al. 2016]. Borage seed oil is known to be the richest plant source of γ-linoleic acid [Beaubaire and Simon 1987, El Hafid et al. 2002, Asadi-Samani et al. 2014], however it also contains small quantities of erucic acid (0.6–3.3%) [del Río et al. 1993, Berti et al. 1998, Borowy et al. 2016].

Mhamdi et al. [2009] pointed out the presence of 16 volatile components in the essential oil produced from borage seeds with major β-caryophyllene (26%) and p-cymene-8-ol (19.7%) while minor – nonadecane (0.7%) and hexanol (0.7%). This essential oil composition was characterized by great profusion of oxygenated monoterpenes (27.7%) followed by sesquiterpenes (26%). Mhamdi et al. [2009] also reported the presence of six phenolic acids in borage seed extract with rosmarinic and p-coumaric acids as the major ones.

Several aforementioned chemical compounds produced by borage have antioxidant properties and therefore, the increasing interest in this borage capacity has been observed [Wettasinghe and Shahidi 1999, 2000, Bandoniene and Murkovic 2002, Mhamdi et al. 2007, Krishnaiah et al. 2011, Segovia et al. 2014]. Phenolic compounds are the major contributor to the antioxidant activity of borage plants [Aliakbarlu and Tajik 2012, Segovia et al. 2014, Zemmouri et al. 2014]. Borage seed extracts have been used as antioxidants in the preparation of gelatin films [Gómez-Estaca et al. 2009]. Bandoniene et al. [2002] showed that borage leaf extract stabilized rapeseed oil against its autoxidation more efficiently than synthetic antioxidant. Garciá-Iñiquez de Ciriano et al. [2009] proved that lyophilized water extract of borage leaves is a valuable alternative for synthetic antioxidants added to meat products. Similarly, Sánchez-Escalante et al. [2003] pointed out that borage seed meal can be used as a highly effective natural antioxidant for prolongation of beef patties shelf life. According to Aliakbarlu and Tajik [2012] water extract of borage flower can be employed as an effective antioxidant in food system.

A content of biologically active compounds relies on the environmental conditions to a great extent [Kołodziej and Berbeć 2010, Zemmouri et al. 2014] and majority of the mentioned above studies were carried out in the Mediterranean countries. The purpose of this research was to determine the content of several biologically active compounds and the antioxidant activity of borage flowers and leaves obtained from plants cultivated in south-eastern Poland.

MATERIAL AND METHODS

Plant material used in the study was sampled from blue flowering borage (*Borago officinalis* L.) plants grown in the Felin Experimental Farm, University of Life Sciences in Lublin (215 m a.s.l., 51°23'N latitude, 22°56'E longitude). Borage was cultivated on podzolic soil developed from dusty medium loam containing 2.2% of organic matter and with pH (in H_2O) of 7.0. 100 g of soil containing 53.2 mg of

P_2O_5, 29.1 mg of K_2O and 5.4 mg of Mg. The borage was seeded at the 50 cm row distance at the rate of 6 kg·ha^{-1} on April 25th, 2016. The samples of fully developed flowers and leaves were collected after several warm and sunny days on 30 June 2016 when the 70–80 cm high plants started the flowering stage. Many honey bees and bumble-bees visited borage flowers during the sampling process. The samples were delivered directly to the Central Agroecological Laboratory of the University of Life Sciences in Lublin where a content of carotenoids, chlorophyll a and b, flavonoids, vitamin C, essential oil and lipids in the fresh material of borage flowers and leaves was examined. Then the composition of essential oil and lipids was analyzed. Antioxidant activity of borage flowers and leaves was determined in the Laboratory of Herb Raw Material Quality Evaluation, University of Life Sciences in Lublin.

Determination of a content of the mentioned above bioactive components was performed using the following methods: carotenoids and chlorophyll a and b – Mac Kinney's method [Charłampowicz 1966], flavonoids (recalculated onto quercetin) – Christ-Müller's method [Polish Pharmacopoeia IX 2011], vitamin C – HPLC method [PN-EN 14130:2004], lipids – Soxhlet method (hot extraction with hexane) [PN-EN ISO 734:2016-03]. Fatty acid composition was established with a gas chromatograph after the initial saponification of lipids and esterification of acids according to AOAC 969.33 and 963.22 norms [2000] and using heptadecanoic acid as an internal pattern. 1 µl of test sample (fatty acid methyl esters in hexane) was injected into the gas chromatograph Varian 450-GC equipped with a flame ionization detector FID and split injector. The injector and detector parts were set at 200°C and 240°C, respectively. The oven temperature program was initially set at 200°C for the first 10 min. and then increased up to 240°C at the rate of 3°C·min^{-1} up where it remained for the last 4.67 min. The separation of the fatty acid methyl esters was performed with a Select™ Biodisel for FAME fused silica capillary column (30 m × 0.32 mm, i.d. 0.25 µm). Helium (He) with 2.5 ml·min^{-1} flow was used as a carrier gas. Galaxie™ Chromatography Data System was used for chromatograph controlling as well as for data collecting, counting and integrating.

A content of the essential oil was determined by direct steam distillation, according to the Polish Pharmacopoeia VI [2002]. The qualitative composition of the essential oil was performed with a gas chromatograph Varian Chrompack CP-3800 coupled with mass detector Varian 4000 GC/MS/MS and the flame ionization detector (FID) using VF column – 5 ms (DB-5 equivalent). The temperature was raised up to 50°C during 1 minute followed by its increase from 50°C to 250°C (4°C per 1 min); 250°C was maintained for 10 min. Helium (He) was used as carrier gas, at a constant flow of 0.5 ml·min^{-1}. The injector temperature was 250°C and the split was 1 : 100. 1 µl of the solution was injected (1 µl of sample per 1000 µl hexane). Varian 4000 GC/MS/MS detector was employed at the registration range of 40–1000 m/z and scan rate of 0.8 seconds per scan. The Kovats retention indices were determined based on a range of *n* – alkanes from C_{10} to C_{40} [Van Den Dool and Kratz 1963]. The qualitative analysis was made on the basis of MS spectra which were compared with the obtained data and the identity of the compounds confirmed by their retention indices available in the literature [Adams 2004].

The antioxidant activity of borage flowers and leaves was evaluated using the FRAP assay (measurement of iron ion reduction ability) [Benzie and Strain 1996], using Folin's method (measurement of polyphenolics content) according to Singelton and Rossi [1965] and to Prior [2005] and the free radical method with DPPH reagent [Brand-Williams et al. 1995] As for the first method, water infusion was prepared from 1 g of plant material and 100 ml water. Then FRAP reagent was added to 0.2 ml of infusion and light absorption at 593 nm wave length was measured using Hitachi U-2900 spectrophotometer. The results were expressed in FRAP units (µmol·g^{-1} of plant material) and the standard curve was made for iron sulphate II. Concerning the second method, 2 ml of Folin reagent was added to 0.5 ml of borage water infusion and then mixed up with 10 ml of 10% solution of sodium carbonate. Light absorption was measured at 765 nm wave length using the same spectrophotometer and the results expressed in mg of

polyphenols·1 g of plant material^{-1} after recalculation onto caffeic acid. The standard curve was made for caffeic acid. As regards the third method, DPPH methanolic solution in the concentration of $6 \cdot 10^{-5}$ mol·dm^3 was added to 0.1 ml of borage water infusion and then kinetics of DPPH radical reduction was established at 515 nm during 15 min. using the same spectrophotometer. The following parameters were measured: a content of remaining unreduced DPPH radical (DPPH rem) expressed as % of total DPPH content, time (s) necessary for 50% diminution of initial radical concentration and antiradical efficiency (AE) expressed in dm^3·(μmol·s)$^{-1}$. All reagents were produced by the Sigma Aldrich and Chempur companies. The measurements were carried out in triplicate and the results reported as means with standard deviations. A content of essential oil, lipids, carotenoids, chlorophyll, flavonoids and vitamin C in the flowers and leaves was studied by analysis of variance, while significance of differences determined by the Tukey's test at 0.01 probability level.

RESULTS

Fresh borage flowers and leaves contained 0.20% and 0.12% of lipids, respectively (tab. 1). Seven fatty acids were determined in the flower lipids, while eight in the leaf lipids (tab. 2). Saturated fatty acids constituted 75.05% of the flower lipids and 53.51% of the leaf lipids. Mono- and polyunsaturated fatty acids made 17.04% and 7.91% in the flower lipids and 19.14% and 27.36% in the leaf lipids, respectively. Omega-3 fatty acids (α-linolenoic + eicosapentaenoic) were found only in the leaf lipids (17.66%) and omega-6 fatty acids (linoleic + γ-linolenic + arachidonic) in both lipids: flowers – 7.91%, leaves – 9.70%. In both lipids, the percentage of hexadecanoic (palmitic) acid was the highest, i.e. 44.51% and 33.42%, respectively. In the flower lipid, it was followed by octadecanoic (stearic) acid (18.85%), oleic + elaidic acids (17.04%), linoleic + linolelaidic acids (7.91%), arachidonic acid (5.27%), myristic acid (4.27%) and lauric acid (2.16%). Besides, a high level of α-linolenic acid (17.66%), low level of palmitoleic acid (4.59%) and absence of arachidonic acid were reported in the leaf lipids. In both lipids, the content of erucic acid and of γ-linolenic acid was below the detection limit.

Borage leaves contained 0.163% of essential oil, 1.03% of flavonoids, 9.21 mg of vitamin C in 100 g fresh weight, 1.91 mg of carotenoids in 1 g fresh weight, 0.77 mg of chlorophyll a and 0.22 mg of chlorophyll b in 1 g fresh weight. The flowers had more essential oil and vitamin C, whereas less carotenoids, chlorophyll a and b and flavonoids than the leaves (tab. 2). Content of anthocyanins in flowers was 0.68 mg·100 g f.w.$^{-1}$ and this compound was not determined in the leaves (tab. 1).

Table 1. Content of several bioactive components in borage flowers and leaves

Component		Flower		Leaf		$LSD_{0.01}$
		min.–max.	mean ±SD	min.–max.	mean ±SD	
Essential oil (% f.w.)		0.167–0.221	0.195 ±0.022	0.146–0.193	0.163 ±0.018	0.026
Lipids (% f.w.)		0.173–0.230	0.202 ±0.031	0.094–0.130	0.121 ±0.014	0.004
Carotenoids (mg · g f.w.$^{-1}$)		0.67–0.69	0.68 ±0.01	1.66–2.23	1.91 ±0.29	0.043
Chlorophyll (mg · g f.w.$^{-1}$)	a	0.22–0.25	0.23 ±0.01	0.66–0.89	0.77 ±0.12	0.009
	b	0.11–0.12	0.11 ±0.01	0.21–0.24	0.22 ±0.02	0.008
Total flavonoids (% f.w., quercetin equivalent)		0.201–0.293	0.250 ±0.038	0.869–1.194	1.030 ±0.113	0.021
Vitamin C (mg · 100 g f.w.$^{-1}$)		9.71–13.20	11.30 ±0.93	7.86–10.84	9.21 ±1.15	0.137

Table 2. Fatty acid composition of borage flower and leaf lipids

Fatty acid	Flowers		Leaves	
	%	g·100 g^{-1} f.w.	%	g·100 g^{-1} f.w.
1. N-dodecanoic (lauric)	2.16 ±-	0.004	0.66 ±-	0.001
2. Tetradecanoic (myristic)	4.27 ±0.81	0.009	5.36 ±1.02	0.006
3. Hexadecanoic (palmitic)	44.51 ±7.20	0.090	33.42 ±5.41	0.041
4. cis-9-Hexadecenoic (palmitoleic)	<LOQ = 0.05*		4.59 ±0.34	0.006
5. Octadecanoic (stearic)	18.85 ±1.11	0.038	14.08 ±0.83	0.017
6. cis-9-Octadecenoic + trans-9-Octadecenoic (oleic + elaidic)	17.04 ±1.01	0.034	14.55 ±0.87	0.018
7. (Z.Z)-9.12-Octadecadienoic + (E.E)-9.12 Octadecadienoic (linoleic + linolelaidic)	7.91 ±1.01	0.016	9.70 ±1.24	0.012
8. (Z.Z.Z)-6.9.12-Octadecatrienoic (γ-linolenic)	<LOQ = 0.05		<LOQ = 0.05	
9. (Z.Z.Z)-9.12.15-Octadecatrienoic (α-linolenic)	<LOQ = 0.05		17.66 ±4.27	0.021
10. Eicosanoic (arachidonic)	5.27 ±0.7	0.011	<LOQ = 0.05	
11. cis-13-Docosenoic (erucic)	<LOQ = 0.05		<LOQ = 0.05	
Saturated fatty acids	75.05	0.151	53.51	0.065
Monounsaturated fatty acid	17.04	0.034	19.14	0.023
Polyunsaturated	7.91	0.016	27.36	0.033
Omega–3 (α-Linolenic + Eicosapentaenoic)	0.00	0.000	17.66	0.021
Omega–6 (Linoleic + γ-Linolenic + Arachidonic)	7.91	0.016	9.70	0.012

* limit of detection

Table 3. Composition of the essential oil obtained from borage flowers and leaves (%)

No	Compound	IR	Flowers	Leaves
1.	Cumene	923	62.94	58.47
2.	n.i.	933	0.21	–
3.	n.i.	952	2.84	2.59
4.	n.i.	960	1.95	1.65
5.	n.i.	963	1.58	0.77
6.	n.i.	974	–	0.40
7.	n.i.	979	4.28	0.64
8.	n.i.	990	0.28	–
9.	n.i.	1000	0.10	–
10.	Octanal <N->	1004	2.76	–
11.	Hexenyl	1006	–	13.32
12.	Cymene <orto>	1022	0.54	–
13.	Limonene	1026	0.11	–
14.	Benzene acetaldehyde	1040	2.17	1.13

15.	Terpinene <gamma->	1052	0.40	–
16.	n.i.	1061	0.37	–
17.	Totoualdehyde <para->	1064	0.35	–
18.	n.i.	1085	0.61	–
19.	n.i.	1090	0.33	–
20.	Nonanal	1095	3.00	2.42
21.	n.i.	1126	1.07	–
22.	n.i.	1132	0.36	–
23.	n.i.	1142	0.25	–
24.	n.i.	1144	0.12	–
25.	n.i.	1149	0.17	–
26.	n.i.	1152	0.39	–
27.	Nonen-1-al.<2E->	1157	0.84	–
28.	n.i.	1162	0.09	–
29.	Menthol	1176	0.30	–
30.	n.i.	1180	0.10	–
31.	n.i.	1196	0.13	–
32.	n.i.	1200	0.20	–
33.	Decanal	1208	0.92	–
34.	n.i.	1222	0.09	–
35.	n.i.	1304	1.53	–
36.	n.i.	1312	0.25	–
37.	n.i.	1393	0.25	–
38.	n.i.	1406	0.21	–
39.	Dodecanal	1413	0.66	–
40.	n.i.	1418	0.17	–
41.	Nopyl acetate	1427	0.21	–
42.	n.i.	1476	0.22	–
43.	Ionone <(E)-beta->	1493	0.26	0.89
44.	n.i.	1566	–	0.69
45.	n.i.	1582	–	4.87
46.	n.i.	1614	–	0.79
47.	Tetradecanal	1618	1.16	–
48.	n.i.	1619	–	0.67
49.	n.i.	1630	–	6.37
50.	n.i.	1712	0.48	0.36
51.	n.i.	1841	–	0.37
52.	Hexadecanal <n->	1883	1.94	–
53.	n.i.	1910	2.79	–
54.	n.i.	2108	–	3.59

n.i. – compound not identified

Table 4. Antioxidant activity of borage flowers and leaves

Method and unit of measurement		Leaves		Flowers	
		min.–max.	average	min.–max.	average
FRAP (μmol·g^{-1})		439–510.6	477.4 ±36.04	957.03–1010.20	992.01 ±30.30
Folin (mg·g^{-1})		17.44–14.89	16.26 ±1.29	25.76–26.26	25.96 ±0.26
DPPH	T(s) 50% (%)	131–237	187 ±53.25	368–571	471.33 ±101.55
	DPPH rem (%)	65.69–77.66	73.58 ±6.83	80.05319–84.04	81.74 ±2.07
	AE (dm^3·μmol s^{-1})	0.0046–0.009	0.0064 ±0.0023	0.0018–0.0026	0.0021 ±0.0004

Generally, 45 compounds were found in the flower essential oil, however only 16 of them were identified. These compounds made 78.56% of the oil (tab. 3) with cumene as the key one constituting 62.94% of the oil. A share of other identified compounds was substantially lower: nonanal – 3.0%, octanal – 2.26%, benzene acetaldehyde – 2.17%, hexadecanol – 1.94% or less. There were found 18 compounds in the leaf essential oil and 5 of them identified. The percentage of cumene was also the highest in this oil (58.47%) and hexenyl (13.32%) was the second highest which, notably, was not present in the flower essential oil. The other identified components included nonanal (2.42%), benzene acetaldehyde (1.13%) and ionone (0.89%). The identified compounds made 76.23% of the leaf essential oil.

The measurements of iron ion reduction ability, polyphenols content and DPPH radical reduction showed the good antioxidant activity of borage flowers and leaves with the indication of higher flower activity (tab. 4). An average ferric reducing ability of flowers was 992.01 μmol·g^{-1} in comparison to that of 477.40 μmol·g^{-1} determined for the leaves. The flowers also had far more polyphenols (25.96 mg·g^{-1}) as against the leaves (16.26 mg·g^{-1}). Besides, the content of remaining unreduced DPPH radical was higher in the flower. However, the time necessary for 50% reduction of initial concentration of DPPH radical was reported to be 2.5 times longer in the flowers and their antiradical efficiency 3 fold lower than that in the leaves. This finding is inconsistent with the data mentioned above.

DISCUSSION

The results obtained in this experiment have confirmed the conclusion drawn by other authors that borage contains many chemical compounds of nutritional, medicinal and antioxidant value [Gupta and Singh 2010, Pieszak et al. 2012, Asadi-Samani et al. 2014].

A content of lipids established in fresh borage leaves in this study is in line with that determined on a dry mass basis by del Río-Celestino et al. [2008]. The composition of the leaf lipids with palmitic, α-linolenic and linoleic + linolelaidic acids as the major components and with approximate content of myristic and lauric acids was similar to those stated by Griffiths et al. [1996], Sayanova et al. [1999] and del Río-Celestino et al. [2008]. A level of stearic, oleic + elaidic and palmitoleic acids was much higher in the present research. Griffiths et al. [1996] and del Río-Celestino et al. [2008] reported a substantial content of stearidonic acid but this component was not determined in this experiment. Griffiths et al. [1996], Sayanova et al. [1999] and del Río-Celestino et al. [2008] also found a few percentage content of γ-linolenic acid, however this component was not detected in this study. Alike, erucic acid established in very low quantities in the borage leaf lipids by del Río-Celestino et al. [2008]

was not detected in the present research. Borowy et al. [2016] obtained inconsiderable amount of erucic acid in the oil produced from borage seeds harvested at the same experimental conditions in south-eastern Poland. The research results from this experiment have confirmed a high content of palmitic acid, yet they differ with regard to a content of other five fatty acids determined in borage flower lipids by Sayanova et al. [1999].

Borage flowers contained more essential oil and this oil was composed of many more constituents than that obtained from the leaves (tabs 1 and 3) and seeds by Mhamdi et al. [2009]. This is in line with their high attractiveness to pollinators [Suchorska and Osińska 1997, Osborne 1999]. However, the composition of flower and leaf essential oil in this experiment differed significantly compared to that of seed essential oil determined by Mhmadi et al. [2009].

A flavonoid level established in the present research in borage flowers and leaves was markedly lower than that in borage leaves reported by Zemmouri et al. [2014] in northern Algeria. This can be due to the differences in both, the analytical procedure performed and environmental conditions [Kołodziej and Berbeć 2010, Zemmouri et al. 2014]. Moreover, a content of biologically active compounds depends on the growth stage of borage plants [Peiretti et al. 2004, Asadi-Samani et al. 2014] and this information is missing from many publications.

A content of polyphenolics in borage flowers and leaves obtained in this experiment was far higher than total phenolic content in various extracts of many culinary and medicinal herbs grown in Maryland, USA [Zheng and Wang 2001] and these compounds have powerful antioxidant properties [Shahidi 2000, Zheng and Wang 2001]. It was slightly lower than that determined by Segovia et al. [2014]. Importantly, the borage flowers and leaves under study contained carotenoids, chlorophyll and vitamin C (tab. 1) which are also mighty antioxidants [Zheng and Wang 2001]. However, there is no detailed information available in literature treating the subject of borage.

According to the classification proposed by Sánchez-Moreno et al. [1998], antiradical efficiency of borage flowers and leaves can be defined as medium and high respectively. The results obtained in this experiment agree with those reported by Bandoniene et al. [2002] showing the strong antioxidant activity of borage leaf extracts. The antioxidant properties of borage flowers and leaves measured as the time necessary for 50% diminution of initial DPPH concentration (T(s) 50%) and as antiradical efficiency (AE) are not consistent with those evaluated by FRAP and Folin methods (tab. 4). According to Gramza-Michałowska and Człapka-Matyasik [2011], a polyphenol content is not the only reason for antioxidant potential but, what also matters is its quality and interactions between the components. In this research, the total phenolic content of borage flower water infusion was about a half of that found in the water extract by Aliakbarlu and Tajik [2012] in Iran. This can be partially attributed to the differences in the environmental conditions during the borage vegetation season.

To acquire a more complete knowledge on borage nutritional and medicinal properties, further studies need to be conducted over a wide geographical range and in multi-year trials [Peiretti et al. 2004].

CONCLUSION

The present research has demonstrated that flowers and leaves of borage grown in the south-eastern region of Poland contain considerable amounts of essential oil, carotenoids, chlorophyll, polyphenols, flavonoids, ascorbic acid and lipids with unsaturated fatty acids. Generally, their content is similar to that established in the Mediterranean region. Notably, the study shows the strong antioxidant activity of borage flowers and leaves.

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