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HELENA SIRCELJ

MAJA MIKULIC PETKOVSEK

ROBERT VEBERIC

METKA HUDINA

ANA SLATNAR

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Lipophilic antioxidants in edible weeds from agricultural areas

Helena ŠIRCELJ[1], Maja MIKULIC-PETKOVSEK[2,*], Robert VEBERIČ[2], Metka HUDINA[2], Ana SLATNAR[2]

[1]Chair for Applied Botany, Ecology, Plant Physiology, and Informatics, Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

[2]Chair for Fruit, Wine, and Vegetable Growing, Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

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Abstract: The present paper reports the contents of individual lipophilic antioxidants in fourteen species of edible common agricultural weeds, typical of agricultural areas such as fields and orchards. Young edible green aboveground parts of weeds were analyzed for their chlorophyll, carotenoid, and tocopherol qualitative profiles and contents. To the best of our knowledge, this is the first report on the complete lipophilic antioxidant composition of the edible weeds examined in this study. The results revealed that all examined leafy plant species are good sources of lipophilic antioxidants, the richest source being *Urtica dioica* (255.64 mg 100 g^{-1} fwt), followed by *Cardamine hirsuta* (159.85 mg 100 g^{-1} fwt), *Cichorium intybus* (150.87 mg 100 g^{-1} fwt), *Aegopodium podagraria* (146.07 mg 100 g^{-1} fwt), *Taraxacum officinale* (123.35 mg 100 g^{-1} fwt), and *Capsella bursa-pastoris* (117.59 mg 100 g^{-1} fwt), all with higher or similar contents compared to spinach (138.72 mg 100 g^{-1} fwt), proving the value of these weeds for nutrition. The shoot vegetable *Humulus lupulus* had the lowest lipophilic antioxidant content (22.98 mg 100 g^{-1} fwt), but this was still 3.8-fold higher than that of cultivated lettuce. Although all weeds examined in our study are valuable sources of health-promoting lipophilic antioxidants, comparison with cultivated spinach revealed that the general belief that all wild edible greens are richer in lipophilic antioxidants than cultivated leafy vegetables is not valid.

Key words: Edible weeds, wild vegetables, chlorophyll, carotenoid, tocopherol, lipophilic antioxidant

1. Introduction

Renewed and increasing interest in wild food plant use in recent years is a well-known phenomenon that has drawn the attention of many scientists. Numerous studies of traditional knowledge have been conducted by anthropologists and ethnobotanists, especially in the Mediterranean region (e.g., Ertuğ, 2004; Kargıoğlu et al., 2010; Dogan et al., 2004, 2012, 2013; *Łuczaj* et al., 2012; Ranfa et al., 2013; Sánches-Mata and Tardío, 2016). Although much traditional knowledge has been lost and the traditional use of wild edible plants has been largely decreasing due to socioeconomic and ecological changes, wild plants are becoming a part of the new thinking about food, especially because they are considered to be health-promoting (*Łuczaj* et al., 2012). Agricultural weeds are one very interesting group of traditional wild food plants, which have provided farmers with a 'hidden harvest', since they have used weeds on their farms to supplement their diets (Bharucha and Pretty, 2010).

In addition to valuable traditional knowledge, knowledge about the chemical and nutritional characteristics of edible weeds and wild plants is also of great interest for understanding their potential value and stimulating their commercial exploitation. Wild plants are considered excellent sources of different health-promoting bioactive substances (Yıldırım et al., 2001; Coruh et al., 2007, 2008; Šircelj and Batič, 2007; Ercişli et al., 2008; Šircelj et al., 2010; Sanchez-Mata et al., 2012; Samancioglu et al., 2016). Defining the content of these substances is essential among other things to confirm or reject traditional beliefs of health promotion by wild plants. Knowledge of the chemical composition of wild, noncultivated edible plants is still much less than that of cultivated crop plants (Ranfa et al., 2013). In view of this, the main objective of this study was to contribute to knowledge of the chemical composition of some edible common weeds typical of agricultural areas in terms of their lipophilic antioxidant profiles.

The major plant lipophilic antioxidants in green organs are located in the plastids and include carotenoids, chlorophylls, and tocopherols. Chlorophylls and carotenoids are crucial molecules in photosynthesis, whereas tocopherols are important membrane stabilizers in plant cells (Dillard and German, 2000; Yoshida et

* Correspondence: maja.mikulic-petkovsek@bf.uni-lj.si

al., 2003). Although not synthesized by humans, these substances are also of great value for our health, being important antioxidants, and some of them also essential vitamins (Yoshida et al., 2003; Fiedor and Burda, 2014). Carotenes and tocopherols are sources of vitamins A and E, respectively. Together with chlorophylls, they are also known for their cancer-preventive properties. The xanthophylls, lutein and zeaxanthin, are important for human vision. Other beneficial effects of lipophilic antioxidants for human health have been reported, including enhancement of immune system function and prevention of cardiac and degenerative diseases (Dillard and German, 2000; Yoshida et al., 2003; Hsu et al., 2013; Fiedor and Burda, 2014).

The aim of this study was to define the contents of individual chlorophylls, carotenoids, and tocopherols in edible young green aboveground parts (young and soft shoots and leaves) of fourteen species of common weeds typical of agricultural areas, in the developmental stage at which these weeds are normally consumed, and to compare these weeds with cultivated lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*), the former known as a poor and the latter as a rich source of lipophilic antioxidants. To the best of our knowledge, no studies of the complete lipophilic antioxidant profiles have been made of any of the fourteen weeds examined in this study. Studies on individual lipophilic antioxidants in these species are also relatively scarce, although partial data on chlorophylls, carotenoids, or tocopherols are available from various references for some of the examined species.

2. Materials and methods

2.1. Plant material

Plants were collected in the experimental field of the Biotechnical Faculty in Ljubljana, Slovenia. Edible aboveground organs of *Capsella bursa-pastoris*, *Galinsoga parviflora*, *Rorippa sylvestris*, *Portulaca oleracea*, and *Stellaria media* were collected in a field of cultivated crop plants; *Bellis perennis*, *Cardamine hirsuta*, *Cichorium intybus*, *Glechoma hederacea*, *Plantago lanceolata*, and *Taraxacum officinale* were collected in an orchard; and *Aegopodium podagraria*, *Humulus lupulus*, and *Urtica dioica* were collected in and under hedges. Young shoots, leafy shoots, or only leaves were collected in spring, as soon as they were available in quantities large enough for culinary use. The sampling dates for selected species were different (Table 1), depending on the development of each plant species. Three to five samples of specific

Table 1. Plant organs sampled for selected species, month of sampling, and water content of samples (%).

Species	Plant organ	Sampling	Water %
Aegopodium podagraria	Leaves	May	82
Bellis perennis	Leaves	April	84
Capsella bursa-pastoris	Leaves	April	80
Cardamine hirsuta	Leaves	April	82
Chenopodium album	Shoots with leaves	June	82
Cichorium intybus	Leaves	June	86
Galinsoga parviflora	Shoots with leaves	June	87
Glechoma hederacea	Shoots with leaves	May	83
Humulus lupulus	Shoots	June	85
Plantago lanceolata	Leaves	June	81
Portulaca oleracea	Shoots with leaves	June	91
Rorippa sylvestris	Leaves	May	81
Stellaria media	Shoots with leaves	April	83
Taraxacum officinale	Leaves	April	82
Urtica dioica	Shoots with leaves	May	84
Lactuca sativa	Leaves	June	94
Spinacia oleracea	Leaves	June	92

plant species were collected in the morning on the same day. Plant material was put in a 1-L plastic bag. One bag filled with plant material (several plants) represented a single sample for biochemical analysis. In the laboratory, samples were frozen in liquid nitrogen, lyophilized at –50 °C and 0.050 mbar (CHRIST GAMMA, 1-16 LSC), ground to a fine powder in a planetary micromill type MM 200 (Retsch, Haan, Germany) and stored at –20 °C in humidity-proof brown plastic containers until analysis. Samples were weighed before and after lyophilization in order to calculate the results on a fresh-weight basis (fwt).

For the purpose of comparison, cultivated lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) were sampled at technological maturity and samples were treated in the same way as described above for weeds.

2.2. Chemicals

Tocopherol standards (α-tocopherol, δ-tocopherol, γ-tocopherol) were purchased from Sigma-Aldrich (Steinheim, Germany) and carotenoids (neoxanthin, lutein, violaxanthin, antheraxanthin, zeaxanthin, α-carotene, β-carotene) and chlorophylls (chlorophyll a, chlorophyll b) were from DHI LAB products (Hoersholm, Denmark). All standards were at least 95% pure. The solvents acetone, ethyl acetate, methanol, and acetonitrile were from Merck, all HPLC-grade.

2.3. Extraction of lipophilic antioxidants

Chloroplast pigments and tocopherols were extracted from 100 mg of the dry plant powder with 5 mL of ice-cold acetone on an ice-bath, using a T-25 Ultra-Turrax (IKA-Labortechnik, Staufen, Germany) for 30 s. All extraction procedures were performed in dim light. The extract was filtered through a 0.2-μm Minisart SRP 15 filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany) in two brown vials for separate HPLC analysis of plastid pigments and tocopherols. Lipophilic antioxidants were determined using the methods described and cited by Šircelj et al. (2010).

2.4. Analysis of chlorophylls and carotenoids

The Thermo Finnigan HPLC system with diode array detector (San Jose, CA, USA) with a Spherisorb S5 ODS-2 250 × 4.6 mm column (Alltech Associates, Inc., Deerfield, IL, USA) was used for HPLC gradient analysis, using the following solvents: solvent A: acetonitrile:methanol:water (100:10:5, v/v/v), solvent B: acetone:ethylacetate (2:1, v/v), at a flow rate of 1 mL min^{-1}, linear gradient from 10% solvent B to 70% solvent B in 18 min, run time 30 min, photometric detection at 440 nm. Identification and quantification of compounds were performed with the corresponding external standards.

2.5. Analysis of tocopherols

The Spectra-Physics HPLC system with Spectra System FL 2000 detector (Fremont, CA, USA) and Spherisorb S5 ODS-2 250 × 4.6 mm column (Alltech Associates, Inc.) was used for an isocratic analysis using methanol as the solvent, flow rate 1 mL min^{-1}, run time 20 min. Tocopherols (α-tocopherol, δ-tocopherol, γ-tocopherol) were detected directly by fluorometry (excitation 295 nm, emission 325 nm) and identified by comparison of retention times, as well as by the addition of standards. Quantification of compounds was carried out with the corresponding external standards.

2.6. Statistical analysis

The data were analyzed using one-way ANOVA followed by Fischer's least significant difference (LSD), with α = 0.05. The StatGraphics Centurion XVI program (StatPoint Technologies, Inc., Warrenton, VA, USA) was used for analysis.

3. Results and discussion

The contents of important plant lipophilic antioxidants in edible young green organs of fourteen common species of weeds were assessed. All selected weeds are known for their use as food (Grlić, 1990; Dogan et al., 2004, 2012, 2013; Ertuğ, 2004; Kargıoğlu et al., 2010; *Łuczaj* et al., 2012; Ranfa et al., 2013; Sánches-Mata and Tardío, 2016). Twelve individual lipophilic antioxidants were detected in the examined weeds. The results of analysis of chlorophylls a and b; carotenoids neoxanthin, lutein, violaxanthin, antheraxanthin, zeaxanthin, α-carotene, and β-carotene; and tocopherols α, δ, and γ are presented in Tables 2 and 3. For the purpose of comparison, cultivated spinach, already known as a rich source of these substances, and lettuce, known as a poor source of these substances, were also analyzed.

The qualitative HPLC profiles of the edible weeds analyzed in this study were typical of green plant organs and were similar (Figure 1). On the other hand, the concentration of each single compound and the concentration of total lipophilic antioxidants varied among species. The highest concentration of total lipophilic compounds was found in *Urtica dioica* leafy shoots (255.64 mg 100 g^{-1} fwt), and the lowest was found in *Humulus lupulus* shoots (22.98 mg 100 g^{-1} fwt). As expected, all examined weeds had much higher total lipophilic antioxidant contents than cultivated lettuce (6.09 mg 100 g^{-1} fwt). Compared to spinach, with 138.72 mg of total lipophilic antioxidants per 100 g fwt, *Urtica dioica*, and also *Cardamine hirsuta* (159.85 mg 100 g^{-1} fwt), had significantly higher total lipophilic antioxidant contents; *Cichorium intybus* (150.87 mg 100 g^{-1} fwt), *Aegopodium podagraria* (146.07 mg 100 g^{-1} fwt), *Taraxacum officinale* (123.35 mg 100 g^{-1} fwt), and *Capsella bursa-pastoris* (117.59 mg 100 g^{-1} fwt) had similar; and all other examined species had significantly lower contents of total lipophilic antioxidants. The contents of total lipophilic antioxidants

Table 2. Contents (mean ± SE in mg 100 g^{-1} fwt) of chlorophylls and tocopherols in edible aboveground organs of selected weeds, lettuce, and spinach. Values are the mean ± SD of 3–5 samples, each measured in two replicates.

Species	Chlorophyll a	Chlorophyll b	δ-Tocopherol	γ-Tocopherol	α-Tocopherol
Aegopodium podagraria	75.88 ± 19.34gh*	31.77 ± 2.32hi	0.04 ± 0.01b	0.11 ± 0.02b	3.44 ± 0.11a
Bellis perennis	50.45 ± 14.68def	23.87 ± 5.79efgh	nd	nd	1.63 ± 0.45a
Capsella bursa-pastoris	61.77 ± 20.60fg	29.16 ± 8.80fghi	nd	0.05 ± 0.00b	2.88 ± 0.93a
Cardamine hirsuta	61.73 ± 14.61fg	31.50 ± 6.73ghi	nd	3.85 ± 0.84a	47.05 ± 13.55b
Cichorium intybus	91.04 ± 22.92h	43.11 ± 9.92j	nd	nd	3.28 ± 0.71a
Galinsoga parviflora	23.96 ± 3.45bc	10.62 ± 1.96bc	nd	nd	0.51 ± 0.11a
Glechoma hederacea	40.43 ± 7.69cde	20.55 ± 3.99def	0.05 ± 0.01c	0.28 ± 0.06b	2.88 ± 0.89a
Humulus lupulus	11.27 ± 1.08b	3.59 ± 0.43ab	0.02 ± 0.01a	0.25 ± 0.04b	1.32 ± 0.12a
Plantago lanceolata	46.34 ± 4.41cdef	21.43 ± 1.20defg	nd	nd	2.84 ± 2.10a
Portulaca oleracea	37.96 ± 12.69cd	19.22 ± 5.72cde	nd	0.07 ± 0.03b	3.22 ± 0.66a
Rorippa sylvestris	60.43 ± 9.46efg	27.46 ± 4.18efghi	nd	nd	2.84 ± 0.51a
Stellaria media	36.79 ± 8.21cd	14.69 ± 3.85cd	nd	nd	1.52 ± 0.32a
Taraxacum officinale	64.27 ± 6.29fg	32.83 ± 7.53hi	nd	nd	2.85 ± 0.48a
Urtica dioica	158.51 ± 24.43i	51.77 ± 10.66j	0.06 ± 0.01c	0.18 ± 0.09b	3.67 ± 0.68a
Lactuca sativa	3.18 ± 0.39a	1.00 ± 0.21a	nd	0.02 ± 0.00b	0.21 ± 0.04a
Spinacia oleracea	78.40 ± 6.11gh	32.09 ± 2.52i	0.03 ± 0.01b	0.03 ± 0.01b	1.61 ± 0.19a

*In the same column means marked with the same letter do not differ significantly according to Fischer's LSD test ($P < 0.05$).
nd - Not detected.

Table 3. Contents (mean ± SE in mg 100 g^{-1} fwt) of carotenoids in edible aboveground organs of selected weeds, lettuce, and spinach. Values are the mean ± SD of 3–5 samples, each measured in two replicates.

Species	Lutein	Neoxanthin	Violaxanthin	Antheraxanthin	Zeaxanthin	β-Carotene	α-Carotene
Aegopodium podagraria	11.94 ± 0.47h*	4.59 ± 0.27gh	6.39 ± 2.41g	0.73 ± 0.10e	nd	11.12 ± 0.93h	0.05 ± 0.01b
Bellis perennis	7.53 ± 1.38ef	3.37 ± 0.41de	3.34 ± 0.43cde	0.57 ± 0.08de	nd	4.81 ± 0.73efg	nd
Capsella bursa-pastoris	9.51 ± 1.09g	2.48 ± 0.37ef	5.38 ± 0.48fg	0.48 ± 0.25cd	nd	5.88 ± 0.88fg	nd
Cardamine hirsuta	6.80 ± 1.04def	2.41 ± 0.33ef	3.39 ± 1.17cde	0.29 ± 0.08abc	nd	2.84 ± 1.00bcd	nd
Cichorium intybus	5.09 ± 0.97bcd	1.17 ± 0.27bc	1.61 ± 0.27ab	0.46 ± 0.15bcd	nd	5.12 ± 0.90efg	nd
Galinsoga parviflora	3.61 ± 0.76b	1.11 ± 0.11bc	1.99 ± 0.32abc	nd	nd	2.33 ± 0.54bc	nd
Glechoma hederacea	8.14 ± 1.15fg	2.15 ± 0.42e	3.69 ± 0.70de	0.77 ± 0.15e	nd	4.55 ± 0.52ef	nd
Humulus lupulus	1.88 ± 0.27a	0.61 ± 0.06ab	2.15 ± 0.13bc	0.19 ± 0.06a	nd	1.70 ± 0.09b	nd
Plantago lanceolata	6.73 ± 3.03cdef	1.40 ± 0.92cd	2.20 ± 0.53bcd	0.24 ± 0.13ab	nd	4.15 ± 2.50de	nd
Portulaca oleracea	4.94 ± 1.27bc	2.03 ± 0.37de	4.49 ± 0.83ef	1.11 ± 0.13f	nd	5.70 ± 0.51efg	0.06 ± 0.02b
Rorippa sylvestris	6.24 ± 1.22cde	1.40 ± 0.23cd	2.17 ± 0.41bcd	0.22 ± 0.07a	nd	3.13 ± 0.32cd	nd
Stellaria media	6.52 ± 0.91cdef	2.16 ± 0.47e	4.70 ± 0.40ef	1.03 ± 0.03f	nd	5.41 ± 0.42efg	nd
Taraxacum officinale	7.15 ± 0.58ef	3.04 ± 0.25f	5.47 ± 1.23fg	1.35 ± 0.29g	0.09 ± 0.01a	6.16 ± 0.81g	0.14 ± 0.05c
Urtica dioica	15.58 ± 1.92i	4.95 ± 0,92h	8.65 ± 1.89h	0.57 ± 0.17de	0.05 ± 0.05b	11.63 ± 1.84h	nd
Lactuca sativa	0.59 ± 0.15a	0.15 ± 0.04a	0.49 ± 0.15a	nd	nd	0.36 ± 0.05a	nd
Spinacia oleracea	7.99 ± 0.78fg	4.32 ± 0.44g	8.34 ± 0.75h	0.23 ± 0.03a	nd	5.64 ± 0.48fg	0.03 ± 0.01a

* In the same column means marked with the same letter do not differ significantly according to Fischer's LSD test ($P < 0.05$).
nd - Not detected.

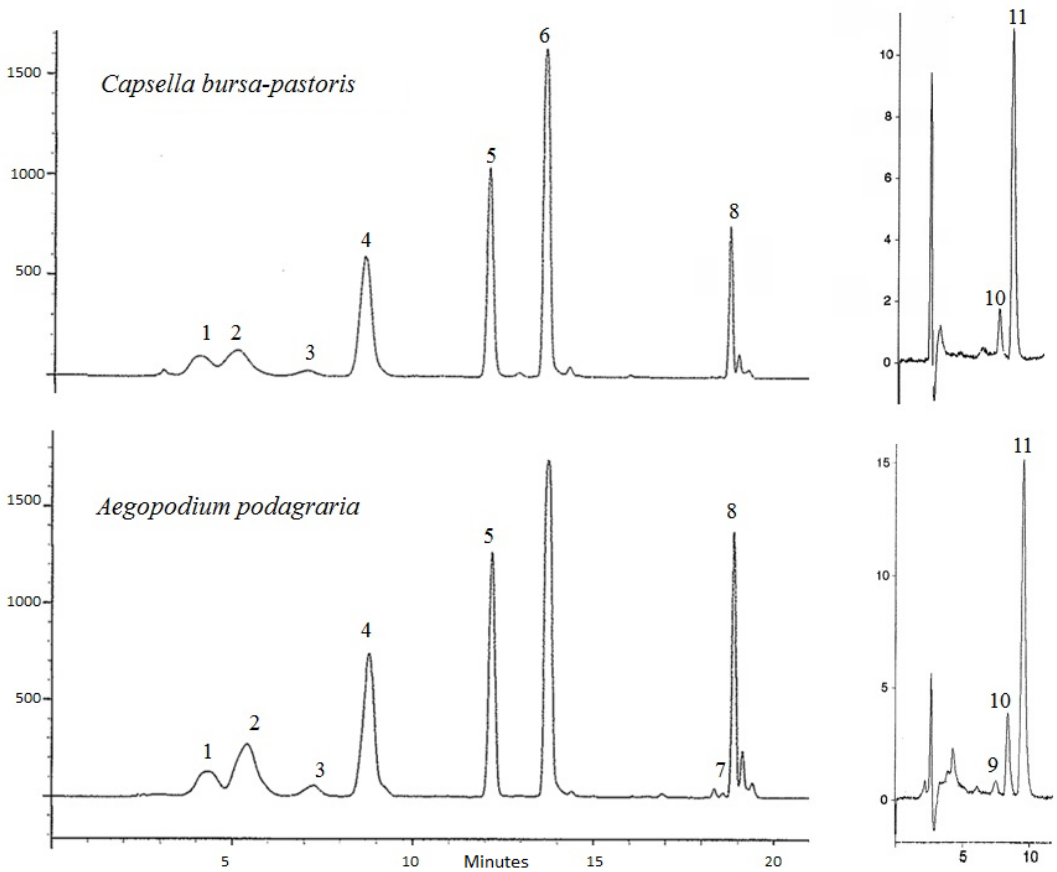

Figure 1. Samples of chromatograms for lipophilic antioxidants: 1- neoxanthin, 2- violaxanthin, 3- antheraxanthin, 4- lutein, 5- chlorophyll b, 6- chlorophyll a, 7- α-carotene, 8- β-carotene, 9- δ-tocopherol, 10- γ-tocopherol, 11- α-tocopherol.

and the contribution of chlorophylls, carotenoids, and tocopherols to the total lipophilic antioxidants content for each examined species are presented in Figure 2.

3.1. Chlorophylls

Chlorophylls were the major lipophilic antioxidants in all sampled weeds, with chlorophyll a being more abundant than chlorophyll b. The ratios of chlorophyll a/b for all examined weeds were in agreement with the well-established fact that chlorophyll a is usually 2–3 times more abundant than chlorophyll b. The ratio of chlorophyll a to chlorophyll b is known to be lower in plants in shade (Boardman, 1977). In our study, the highest chlorophyll a to b ratio was found in *Urtica dioica* (3.1) and the lowest in *Cardamine hirsuta* (1.9) The contents of total chlorophyll varied from 210.28 mg 100 g^{-1} fwt in leafy shoots of *Urtica dioica* to 14.86 mg 100 g^{-1} fwt in shoots of *Humulus lupulus*. The weeds had at least 3.5-fold (*Humulus lupulus*) and a maximum of 50-fold (*Urtica dioica*) higher chlorophyll content than cultivated lettuce with only 4.22 mg 100 g^{-1} fwt of total chlorophyll. Compared to spinach (110.49 mg 100 g^{-1} fwt), the majority of the examined weeds had lower chlorophyll contents, but *Aegopodium podagraria* (107.65 mg 100 g^{-1} fwt) had similar and *Urtica dioica* and *Cichorium intybus* (134.15 mg 100 g^{-1} fwt) had higher chlorophyll contents. Only a few other reports on the chlorophyll composition of individual plant species in this study are available for comparison with our results, mostly for *Urtica dioica*. Kukrić et al. (2012) reported lower and several authors have reported similar contents and ratios of chlorophylls in *Urtica dioica* (e.g., Hojnik et al., 2007; Duma et al., 2014; Zeipiņa et al., 2014). Žnidarčič et al. (2011) reported higher chlorophyll contents for cultivated *Cychorium intybus* and *Taraxacum officinale*. Kopsell et al. (2016), who studied two cultivars of *Portulaca oleracea* grown in nutrient solution, found significantly higher contents of chlorophylls compared to the wild *Portulaca oleracea* in our study. They showed that increased N concentrations in the growing medium resulted in higher chlorophyll contents in the same plant species.

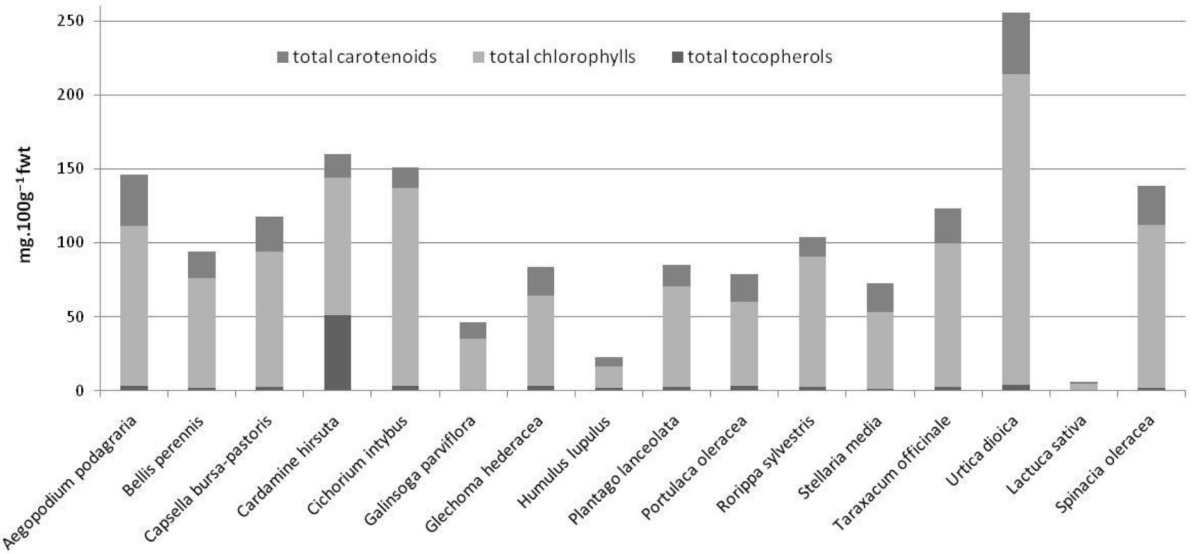

Figure 2. Contents of total lipophilic antioxidants (mg 100 g^{-1} fwt) in weeds, lettuce, and spinach and the contribution of chlorophylls, carotenoids, and tocopherols to total lipophilic antioxidants content for each examined species.

3.2. Tocopherols

Three tocopherols (α, δ, γ) were identified in the edible weeds in this study (Table 2). α-Tocopherol, which is known to have the highest vitamin E activity of all tocols (Yoshida et al., 2003), was the major tocopherol in all examined samples. The predominance of α-tocopherol in green plant organs has already been reported by several authors (Vardavas et al., 2006; Šircelj and Batič, 2007; Barros et al., 2010; Šircelj et al., 2010; Morales et al., 2013, 2014). In the present study, α-tocopherol represented 81% of the total tocopherols in *Humulus lupulus,* 89% in *Glechoma hederacea*, and 92% or more in all other species. γ-Tocopherol was found in seven weed species, ranging from 2% of the total tocopherol in *Capsela bursa-pastoris* to 16% in *Humulus lupulus*. δ-Tocopherol was found in only 4 weed species (*Aegopodium podagraria*, *Glechoma hederacea*, *Humulus lupulus*, and *Urtica dioica*) and it always represented less than 2% of the total tocopherol. The total tocopherol content in weeds in the present study ranged from 0.51 mg 100 g^{-1} fwt in *Galinsoga parviflora* to 3.92 mg 100 g^{-1} fwt in *Urtica dioica*, with one exception. Namely, *Cardamine hirsuta* had as much as 50.90 mg 100 g^{-1} fwt of tocopherol. This is an annual wintergreen plant, which germinates in the fall and remains green throughout the winter before flowering in the spring. At the time that they were collected for analysis, the leaves of *Cardamine* were older than the leaves of other sampled plants. Since the foliar content of tocopherol increases with leaf age (Šircelj et al., 2010), the highest content of tocopherols in *Cardamine* meets expectations. For comparison, cultivated spinach had only 1.67 mg 100 g^{-1} fwt and lettuce 0.22 mg 100 g^{-1} fwt of total tocopherol.

There have been reports from other studies on the tocopherol composition of five of the plant species included in our experiment. Significantly higher contents of tocopherols were reported for *Humulus lupulus* (Morales et al., 2012) and *Glechoma hederacea* (Barros et al., 2010). Reported values for *Urtica dioica* (Tardio et al., 2016) and *Taraxacum officinale* (Vardavas et al., 2006) are similar to those from our study. For *Cichorium intybus,* Morales et al. (2014) reported similar and Vardavas et al. (2006) lower contents of total tocopherols than we found in our study, both with different individual tocopherol ratios, with prevailing or at least higher γ-tocopherol compared to the *Cichorium intybus* in our study. The individual tocopherol ratios for the other four above-mentioned species were also more or less different than those in our study. Morales et al. (2012) attributed the variability of contents and ratios of individual tocopherols in the same plant species reported from different countries to different environmental conditions, postharvest conditions, and organs, but the age of the analyzed plant organ and time of sampling (diurnal changes) can also contribute to differences (Munné-Bosch and Alegre, 2002; Šircelj et al., 2010). Our results are not in agreement with the suggestion of Morales et al. (2012) that a lower content of tocopherols should be expected in stem (shoot) edible greens such as *Humulus lupulus* than in leafy edible greens, since we found lower or similar contents of total tocopherols compared to *Humulus lupulus* in leafy shoots of *Galinsoga parviflora*, in leaves of *Bellis perennis*, leafy shoots of *Stellaria media*, and even in leaves of cultivated spinach.

3.3. Carotenoids

In the edible weeds in the present study, xanthophylls (lutein, neoxanthin, violaxanthin, and zeaxanthin) and two carotenes (α and β) were identified and quantified. The mean concentrations of individual carotenoids are shown in Table 3. The qualitative HPLC profiles of carotenoids and chlorophylls in the extracts of wild greens examined in this study were almost identical. However, the concentration of single compounds and their ratios to total carotenoids varied among plants (Table 3; Figure 3). The highest total carotenoid concentrations were found in *Urtica dioica* and *Aegopodium podagraria* (41.45 and 34.82 mg 100 g^{-1} fwt, respectively), while the lowest was found in *Humulus lupulus* (6.53 mg 100 g^{-1} fwt). Compared to cultivated lettuce, with only 1.59 mg 100 g^{-1} fwt of total carotenoids, all weed species showed excellent (statistically significantly higher) carotenoid content. Mean concentrations of total carotenoids in *Urtica dioica* and *Aegopodium podagraria* also exceeded that in spinach (26.55 mg 100 g^{-1} fwt). *Taraxacum officinale* (23.39 mg 100 g^{-1} fwt) and *Capsella bursa-pastoris* (23.74 mg 100 g^{-1} fwt) had total carotenoid contents similar to that of spinach. Xanthophylls were more abundant than carotenes in all examined plants (Figure 3).

The xanthophyll lutein was the predominant carotenoid in all edible weeds in this study, with the exception of *Humulus lupulus,* with violaxanthin as the major carotenoid. The contents of lutein ranged from 1.88 mg 100 g^{-1} fwt in *Humulus lupulus* to 15.58 mg 100 g^{-1} fwt in *Urtica dioica.* Lutein in the examined weeds represented between 27% and 47% of total carotenoids. The content of another xanthophyll, neoxanthin, was between 0.61 mg 100 g^{-1} fwt in *Humulus lupulus* and 4.95 mg 100 g^{-1} fwt in *Urtica dioica. Aegopodium podagraria* also had a high neoxanthin content (4.59 mg 100 g^{-1} fwt). The ratios of neoxanthin to total carotenoids were between 9% and 16%.

The second most abundant carotenoid in all examined weeds was the provitamin A carotenoid β-carotene, with mean concentrations ranging from 1.70 mg 100 g^{-1} fwt in *Humulus lupulus* to 11.63 mg 100 g^{-1} fwt in *Urtica dioica*, the richest source of carotene in the present study. In eleven plant species, β-carotene was the only carotene. In *Aegopodium podagraria*, *Portulaca oleracea*, and *Taraxacum officinale,* another provitamin A carotenoid, α-carotene, was also found, but it represented only 1% to 2% of total carotenes. The ratio of total carotenes to total carotenoids was highest in *Galinsoga parviflora* (39%) and lowest in *Cardamine hirsuta* (18%) (Figure 3).

The mean concentrations of total xanthophyll cycle pigments (VAZ: the sum of violaxanthin, antheraxanthin, and zeaxanthin) were highest in *Urtica dioica* and *Aegopodium podagraria* at 9.28 and 7.11 mg 100 g^{-1} fwt, respectively. The lowest mean concentrations of VAZ were measured in *Galinsoga parviflora* and *Cichorium intybus* (1.99 mg 100 g^{-1} fwt and 2.06 mg 100 g^{-1} fwt, respectively). The VAZ pool of examined weeds was composed of 78%–100% of violaxanthin, up to 22% of antheraxanthin, and up to 1% of zeaxanthin. Zeaxanthin was found in only two plant species (*Taraxacum officinale* and *Urtica dioica*), in concentrations probably too low to contribute significantly to the nutritional value of these plants. Low zeaxanthin and high violaxanthin ratios in plant samples were expected, because plant sampling was conducted early in the morning, when there is no need for heat dissipation of excess excitation energy from the photosystem,

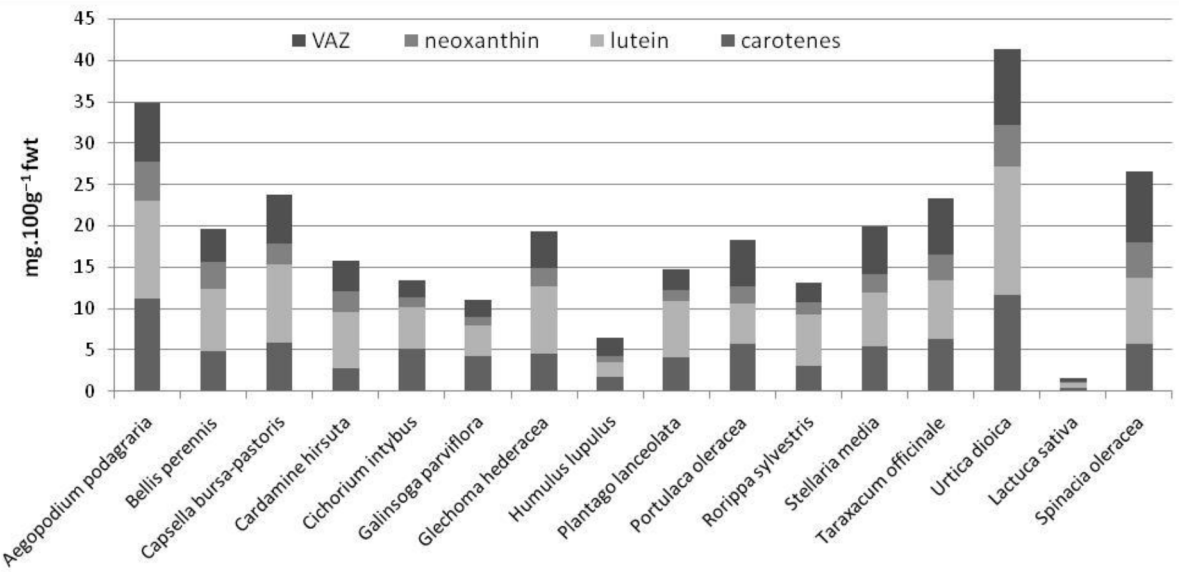

Figure 3. Contents of the xanthophylls lutein, neoxanthin and VAZ (the sum of violaxanthin, anteraxanthin and zeaxanthin) and total carotenes (mg 100g^{-1} fwt) in weeds, lettuce and spinach.

and the xanthophyll cycle may be in an epoxidized state, mainly in the form of violaxanthin. The xanthophyll cycle is the reversible deepoxidation of violaxanthin to zeaxanthin, with intermediate antheraxanthin (Demmig-Adams and Adams, 1994).

Reports on individual and even total carotenoids for the edible weeds examined in the present study are scarce. We found reports on total carotenoids for *Portulaca oleracea* and *Taraxacum officinale* with similar contents to those in our study, and for *Capsella bursa-pastoris*, *Cichorium intybus*, *Humulus lupulus*, *Plantago lanceolata*, and *Urtica dioica* with lower contents of total carotenoids compared to our study (Dias et al., 2009; Žnidarčič et al., 2011; Kukrić et al., 2012; García-Herrera et al., 2014; Kopsell et al., 2016; Tardio et al., 2016). Individual carotenoids have been reported only for wild *Humulus lupulus* (García-Herrera et al., 2014) and *Urtica dioica* (Tardio et al., 2016), and for cultivated *Portulaca oleracea* (Dias et al., 2009; Kopsell et al., 2016), *Taraxacum officinale*, and *Cichorium intybus* (Žnidarčič et al., 2011). *Humulus lupulus* in a study performed by García-Herrera et al. (2014) had a different carotenoid profile (similar % of beta carotene, lower VAZ, and higher lutein and neoxanthin) than in our study. The ratios of individual carotenoids to total carotenoids reported for plants in the other four mentioned studies, which dealt with individual carotenoids, were similar to those found in the weeds in our study.

Only a few published studies have focused on elucidation of the complete lipophilic antioxidant profile of edible weeds. To the best of our knowledge, this is the first report on the complete lipophilic antioxidant composition of the edible weeds examined in this study and the results can be very useful in completing food composition databases. To date, complete profiles of lipophilic antioxidants could only be made from various partial reports in the available literature for carotenoids, chlorophyll, or tocopherols for three of the fourteen weed species examined in the present study: *Urtica dioica*, *Taraxacum officinale*, and *Cichorium intybus*. Furthermore, the published data for the latter two species were obtained mainly from cultivated varieties. Complete profiles of lipophilic antioxidants have already been previously reported by several authors for the two cultivated plants, lettuce and spinach, also included in this study just for the purpose of comparison with weeds from the same location, because it is known that the content of bioactive compounds in the same species depends on geographical site and environmental conditions (Dias et al. 2009; Morales et al., 2012).

The present study verifies the justification for the use of the examined edible common weeds from fields and orchards as health-promoting foods due to their high contents of lipophilic antioxidants. Among the fourteen weeds examined in the present study, the highest level of total lipophilic antioxidants was detected in *Urtica dioica*, followed by *Cardamine hirsuta*, *Cichorium intybus*, *Aegopodium podagraria*, *Taraxacum officinale*, and *Capsella bursa-pastoris*, all with contents higher than or similar to those of spinach. The other examined weeds had lower total lipophilic antioxidant contents than spinach, but still significantly higher than lettuce. The qualitative HPLC profiles of the weeds from the present study were more or less similar, although the concentrations of each individual compound varied significantly among species. Comparison of our results with those from other studies once again revealed the variability of contents and ratios of individual lipophilic antioxidants in the same plant species, probably due to different environmental conditions, postharvest conditions, type of organ, and the degree of organ maturity at harvest or the time of sampling. We believe that despite this (expected) variability, all edible weeds examined in our study are valuable, rich sources of health-promoting lipophilic antioxidants, although comparison with cultivated spinach revealed that the general belief that all wild edible greens are richer in lipophilic antioxidants than cultivated leafy vegetables is not entirely correct. Since edible weeds are an inexpensive, sustainable, and nowadays increasingly popular source of foods, their use as food should be encouraged also due to their valuable nutritional characteristics, especially in traditional smallholder farms and organic farms with an orientation towards tourism. However, in the first place more research is needed to investigate other aspects of the chemical composition of these weeds, especially antinutritive substances.

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