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Evaluation of selected nutritional factors in *Aposeris foetida* (L.) Less. during the harvesting period

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Summary

Aposeris foetida (L.) Less. is a plant native to central European forests. In Slovenia it is known as an early spring wild food. Considering the total absence of studies of the leaves of *Aposeris* regarding its use as food, the present study was made to investigate its nutritional value concerning important vitamins and antioxidants. Foliar contents of ascorbic acid, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, and chlorophyll in young edible plants during the harvesting period are reported. Comparison of *Aposeris* with supermarket lettuce analysed in our study and with numerous cultivated and wild edible plants from other studies showed that *Aposeris* is one of the best sources of ascorbic acid, tocopherol and carotenoids. The results of our study justify its use in the early spring wild food cuisine and may stimulate consumption of this nutritious plant.

Introduction

Aposeris foetida (L.) Less., (*Asteraceae*) is a perennial herbaceous plant native to central European forests. It reminds a little of the dandelion (*Taraxacum officinale* Weber). It has about 10 cm long, lance-shaped, deeply toothed, usually glabrous leaves, always growing in a basal rosette. It flowers between May and August. The composite flowers grow individually on 10 cm to 20 cm high flower stalks. The bright yellow flower heads are 2.5 cm to 4 cm broad. The whole plant (especially the roots and to smaller extent also the mature leaves) contains white milky sap. It smells like cooked potatoes. This is why *Aposeris* is called forest dandelion, stinking dandelion or odorous pig-salad.

Forest dandelion is known as an early spring wild food in Slovenia. The young, soft leaves are at their best when they have just emerged and they are collected before flowering. The leaves usually emerge as early as the beginning of April. This makes the forest dandelion one of the first wild-growing plants available for the table. Usually the leaves are eaten raw in salads or cooked in different dishes. After flowering the leaves become harder and the quantity of white milky sap increases.

To our knowledge no studies on the nutritional value of *Aposeris* have been made. Since this plant is quite popular in the wild food cuisine in Slovenia and abundant in the alpine and prealpine central European region, we conducted a study on the nutritional value of *Aposeris*. The aim was to evaluate the contents of selected bioactive compounds in young leaves of *Aposeris* during the harvesting period which in Slovenia is in April and May. We analysed ascorbic acid, tocopherols, carotenoids and chlorophylls in leaves collected weekly from forest dandelion rosettes growing at several locations in central Slovenia. The most important criterion for the selection of nutritional factors for analysis was the actual purpose of collecting wild-growing plants for culinary use; that is an intake of vitamins and antioxidants from fresh natural sources. All the selected factors represent important vitamins and antioxidants which are indispensable for normal functioning of the human body. Ascorbic acid, carotenes and tocopherols are the sources of vitamins C, A and E respectively. Xanthophylls lutein and zeaxanthin are crucial factors for human vision. Neoxanthin and chlorophyll were found to reduce the risk of certain types of cancer (DASWOOD, 1997; DILLARD and GERMAN, 2000).

The present study reports foliar contents of ascorbic acid, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, and chlorophyll in young edible *Aposeris foetida* plants at the stage normally consumed in Slovenia, and compares the nutritional value of *Aposeris* with other cultivated and wild plants.

Material and methods

Plant material

Leaves of forest dandelion were collected in the way consumers do when this plant is picked for home cuisine. Entire leaf-rosettes were separated with a knife from the roots and put in a plastic bag (volume 2L) until the bag was full (at least 6 plants). One bag filled with leaves represented one sample for biochemical analyses. Two samples were collected from each of four selected locations in central Slovenia.

Leaves were collected weekly (7 weeks) from the beginning of April (when the first leaves emerged) until the end of May (one week after the onset of flowering) in the morning (6:00 to 8:00 solar time). They were transported in a portable refrigerator to the laboratory (in less than 1.5 hours) where leaves were frozen in liquid nitrogen, lyophilized, ground to a fine powder and stored at -20°C in humidity-proof plastic containers until analysis. Before and after lyophilization samples were weighed in order to recalculate data obtained from biochemical analyses from mg per dry weight (dwt) to mg per 100g fresh weight (fwt). The foliar moisture content was 88.1 ± 2.1 g/100g fwt.

In the middle of the forest dandelion harvesting period we collected also three samples (one sample comprised one plant) of soft lettuce (*Lactuca sativa* 'Majniška Kraljica') from a supermarket. The lettuce samples were treated exactly like forest dandelion samples.

Analysis of chloroplast pigments

Chloroplast pigments (neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, α-carotene, β-carotene, chlorophyll a, chlorophyll b) were determined using the methods described by PFEIFHOFER (1989). Pigments were extracted from the dry leaf powder with ice-cold acetone. All extraction procedures were performed in dim light. Acetone extracts were subjected to an HPLC gradient analysis (Spherisorb S5 ODS-2 250 x 4.6 mm column with an S5 ODS-2 50 x 4.6 mm precolumn), 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 $mLmin^{-1}$, linear gradient from 10% solvent B to 70% solvent B in 18 min was applied, run time 30 min, photometric detection at 440 nm.

Analysis of tocopherols

Concentrations of tocopherols (α-tocopherol, γ-tocopherol, δ-tocopherol) were measured following the method reported by TAUSZ

et al. (2003). Tocopherols were extracted from the dry leaf powder with ice-cold acetone. The acetone extracts of lyophilized leaf powder were subjected to isocratic HPLC analysis (Spherisorb S5 ODS-2 250 x 4.6 mm column with a S5 ODS-250 x 4.6 mm precolumn) using methanol as solvent. Tocopherols were detected directly by fluorometry (excitation 295, emission 325).

Analysis of ascorbic acid

The concentration of ascorbic acid was measured as reported by TAUSZ et al. (2003). Ascorbic acid was extracted from the lyophilized leaf powder with 1.5 % (w/v) metaphosphoric acid containing 1mM EDTA. Extracts were subjected to isocratic HPLC analysis (Lichrosorb RP-8 250 x 4.6 mm column with a Lichrosorb RP-8 50 x 4.6 mm precolumn) using methanol/water (1/3, v/v) containing 1 mM hexadecylammoniumbromide and 0.05% (w/v) sodium dihydrogen phosphate monohydrate (pH 3.6) as solvent, at a flow rate of 1 $mLmin^{-1}$, run time 20 min, photometric detection at 248 nm.

Statistical analysis

Data obtained from biochemical analyses were statistically evaluated with the Statgraphics programme, version 4.0. Differences between sampling dates were evaluated by one-way ANOVA. Fischer's least significant difference (LSD) was used for comparisons between means for the different sampling dates of each factor analysed. Significance was accepted at $p<0.05$.

Results and discussion

In this work the contents of important nutritional factors in the leaves of *Aposeris* during the harvesting period were assessed. The analysis results of ascorbic acid, α-tocopherol, β-carotene, lutein, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin and chlorophyll are presented in Table 1. The foliar contents of α-carotene and γ-tocopherol were too low to contribute significantly to the nutritional value of forest dandelion (12 -112 µg/100g of fresh weight (fwt) for α-carotene and 14 - 70 µg/100g fwt for γ-tocopherol respectively) and are consequently not presented in detail in the table.

One of the most important factors influencing the contents of above mentioned nutritional factors in plant leaves is the degree of maturity at harvest (KIMURA and RODRIGUEZ-AMAYA, 2003; FOYER, 1993; MUNNE-BOSCH and ALEGRE, 2002). For this reason we sampled the leaves weekly during the entire harvesting season. Statistically significant differences ($p<0.05$) between different dates for each factor analysed are denoted by different letters in Tab. 1.

Ascorbic acid: The most important vitamin in vegetables for human nutrition is vitamin C. Plant derived ascorbate is the major source of vitamin C in the human diet (WHEELER et al., 1998). In green tissues of plants ascorbic acid is the major water soluble antioxidant. The contents of ascorbic acid change seasonally and increase with leaf age; over short time intervals the foliar ascorbate contents remain at a relatively constant level, except under stress which can affect the ascorbate pool significantly (FOYER, 1993). The mean concentrations of ascorbic acid measured in *Aposeris* leaves were between 57 and 101 mg/100g fwt (Tab. 1). In the first week after leaf emergence the concentration of ascorbic acid was significantly lower compared to the last three weeks of the harvesting period (Fig. 1). The highest concentration of ascorbic acid was measured in leaves collected one week before flowering (May-7). Lettuce bought in a supermarket during the *Aposeris* sampling period had only 1.1 ± 0.7 mg ascorbic acid per 100g fresh weight. SHEELA et al. (2004), GUPTA et al. (2005) and KRUMBEIN et al. (2005) reported foliar ascorbic acid contents for 57 leafy vegetables. Only *Brassica oleracea* had a higher ascorbic acid content compared to *Aposeris* in our study. GUIL et al. (1997) analysed 16 wild edible plants and compared to *Aposeris,* found similar vitamin C contents in 11 and higher content in 5 plants species.

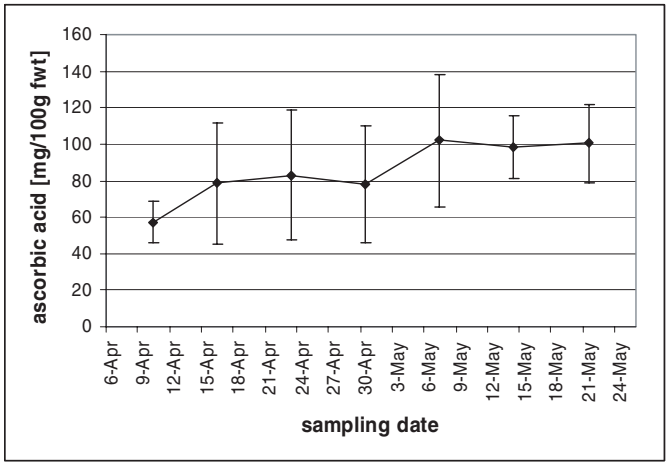

Fig. 1: Ascorbic acid (mg/100g fwt) in leaves of *Aposeris foetida* L. during harvesting period. Symbols represent mean ± S.D. of eight plant samples.

Tocopherols: In the human body tocopherols are recognized as the most important lipid soluble antioxidants, together with the tocotrienols known as vitamin E. Tocopherols are only synthesized by plants and other oxygenic, photosynthetic organisms (DELLAPENNA, 2005). The

Tab. 1: Ascorbic acid, tocopherol and chloroplast pigments (mg/100g fwt) in leaves of *Aposeris foetida* L. during harvesting period. Values are means of eight plant samples. Statistically significant differences ($p < 0.05$) between different dates are denoted by different letters. VAZ - violaxanthin + antheraxanthin + zeaxanthin, E-emergence of first leaves, F-flowering.

	E-April-10	April-16	April-23	April-30	May-7	F-May-14	May-21
ascorbic acid	57.3 ± 11.2**a**	78.6 ± 33.1**ab**	83.0 ± 35.4**ab**	78.2 ± 32.0**ab**	101.9 ± 36.2**b**	98.3 ± 17.1**b**	100.3 ± 21.7**b**
α-tocopherol	0.41 ± 0.10**a**	1.87 ± 0.14**b**	2.22 ± 0.38**bc**	2.29 ± 0.32**bc**	2.69 ± 0.33**cd**	3.09 ± 0.61**de**	3.17 ± 0.35**e**
β-carotene	7.88 ± 0.81**a**	9.42 ± 1.46**ab**	10.29 ± 1.32**bc**	10.70 ± 2.14**bc**	11.30 ± 1.32**bc**	12.28 ± 2.91**bc**	11.64 ± 1.11**c**
lutein	9.43 ± 0.77**a**	10.07 ± 0.99**ab**	10.35 ± 0.97**abc**	10.36 ± 0.69**abc**	11.21 ± 1.16**bc**	11.61 ± 2.45**bc**	10.82 ± 1.02**c**
violaxanthin	4.77 ± 0.84**a**	6.06 ± 1.53a**b**	6.67 ± 1.18**b**	7.40 ± 1.03**bc**	8.18 ± 1.07b**cd**	8.86 ± 1.47**cd**	7.33 ± 0.76**d**
antheraxanthin	0.385 ± 0.033**a**	0.501 ± 0.114**ab**	0.744 ± 0.143**ab**	0.583 ± 0.093**bc**	0.577 ± 0.123**bc**	0.552 ± 0.189**bc**	0.682 ± 0.170**c**
zeaxanthin	0.047 ± 0.095**a**	0.245 ± 0.284**a**	0.317 ± 0.311**a**	0.070 ± 0.142**a**	0.136 ± 0.184**a**	0.055 ± 0.100**a**	0.459 ± 0.255**a**
VAZ	5.20 ± 0.82**a**	6.81 ± 1.72**ab**	7.74 ± 1.27**bc**	8.06 ± 0.92**bcd**	8.90 ± 1.22**bcd**	9.47 ± 1.59**cd**	8.47 ± 0.89**d**
neoxanthin	3.49 ± 0.32**a**	4.12 ± 0.65**ab**	4.55 ± 0.28**bc**	4.27 ± 0.26**bc**	4.39 ± 0.32**bc**	4.48 ± 0.90**bc**	4.86 ± 0.54**c**
chlorophyll	129.4 ± 13.6**a**	136.6 ± 32.8**ab**	144.2 ± 16.3**abc**	146.2 ± 9.4**abc**	159.2 ± 8.4**bc**	166.8 ± 24.4**c**	166.1 ± 16.7**c**

contents of tocopherols change diurnally and seasonally and differ between plant organs (MUNNE-BOSCH and ALEGRE, 2002). The main tocopherol in leaves of *Aposeris* was α-tocopherol which represented approximately 98% of total tocopherols. The remainder was in the form of γ-tocopherol. The contents of α-tocopherol in *Aposeris* leaves were between 0.32 and 3.65 mg/100g fwt (Tab. 1). Foliar concentrations of α-tocopherol increased significantly during the harvesting period, by approximately 670% from the first to the last sampling date (Fig. 2). Lettuce bought in a supermarket during the *Aposeris* sampling period had only 0.186 ± 0.042 mg α-tocopherol per 100g fresh weight. In comparison with other studies on leafy vegetables *Aposeris* leaves in the harvesting period contain more α-tocopherol than lettuce (BURNS et al., 2003; CHUN et al., 2006), cabbage and Chinese cabbage (SINGH et al., 2007) and the wild leafy vegetable hummayd (*Rumex vesicarius*) (ALFAWAZ, 2006), but spinach leaves had approximately the same content of α-tocopherol (CHUN et al., 2006). CHING and MOHAMED (2001) analysed 28 tropical leafy edible plants and 16 of them had higher α-tocopherol contents than *Aposeris* from our study. But because foliar tocopherol content depends strongly on leaf age, a better comparison of our tocopherol results with the results from the above mentioned studies on wild plants would be possible if the leaf age or plant developmental stage at the time of plant collection were also reported in the papers.

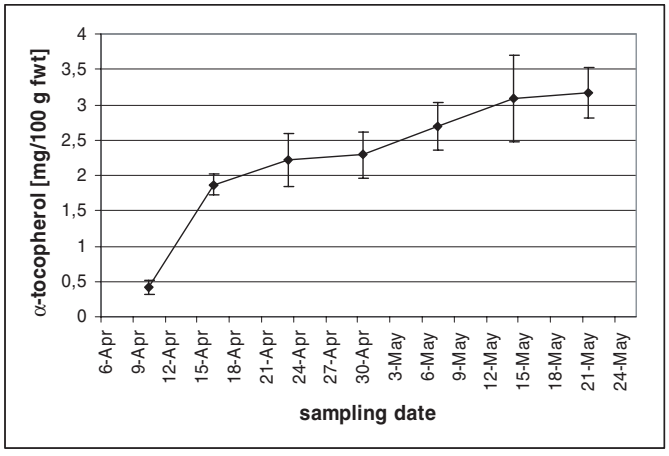

Fig. 2: α tocopherol (mg/100g fwt) in leaves of *Aposeris foetida* L. during harvesting period. Symbols represent mean ± S.D. of eight plant samples.

Carotenoids: Carotenoids are the main lipid-soluble antioxidants of plant cells (MUNNE-BOSCH and ALEGRE, 2000). Plant leaves usually contain β-carotene, lutein, violaxanthin and neoxanthin, and small amounts of zeaxanthin, antheraxanthin and α-carotene. Foliar carotenoid content depends on plant species, leaf age and environmental conditions (GOODWIN, 1980).

Aposeris leaves contained two provitamin A carotenoids, β-carotene and α-carotene, the last representing less than 1% of total carotenes. The mean concentrations of β-carotene measured in *Aposeris* leaves were between 7.88 and 12.28 mg/100g fwt (Tab. 1). In the first week after leaf emergence the concentration of β-carotene was significantly lower than on other sampling dates (Fig. 3). The highest concentration of β-carotene was measured in leaves collected at the beginning of flowering (May-14). Lettuce bought in a supermarket during the *Aposeris* harvesting period had only 0.27 ± 0.116 mg β-carotene per 100g fresh weight. In comparison with other studies on leafy vegetables, *Aposeris* leaves contain significantly more β-carotene than lettuce (KIMURA and RODRIGUEZ-AMAYA, 2003; BURNS et al., 2003; MANGELS et al., 1993), cabbage, Chinese cabbage (SINGH et al., 2007), rocket, cress and chicory (KIMURA and RODRIGUEZ-AMAYA, 2003) and more than spinach (BURNS et al., 2003; MANGELS et al., 1993). *Aposeris* in our study had β-carotene contents higher than 56, similar to 7 and lower than 3 leafy vegetables from different parts of the world analysed in 4 different studies (GUPTA et al., 2005; KRUMBEIN et al., 2005; RAJU et al., 2007; BHASKARACHARY et al. 1995). MERCADANTE and RODRIGUEZ-AMAYA (1990) and BHASKARACHARY et al. (1995) reported foliar β-carotene for 26 edible wild plants. Eleven of these plants had similar and 15 lower β-carotene content than *Aposeris*.

We detected 5 xanthophylls in *Aposeris* leaves (Tab. 1). Lutein was the predominant xanthophyll. The mean concentrations of lutein during the harvesting period were between 9.43 and 11.61 mg/100g fwt. The mean contents of total xanthophyll cycle pigments (VAZ; the sum of violaxanthin, antheraxanthin and zeaxanthin) and neoxanthin were between 5.20 and 9.47 mg/100g fwt, and 3.49 and 4.86 mg/100g fwt, respectively. Since the leaves were sampled early in the morning the xanthophyll cycle was in the epoxidised state and consequently the major cycle pigment was violaxanthin, antheraxanthin represented 8-10% and zeaxanthin represented only 0.6-6% of the VAZ pool. The contents of xanthophylls were the lowest (significantly) in the first week after leaf emergence (Fig. 3). The highest concentrations of lutein and VAZ were measured in leaves collected at the beginning of flowering (May-14). The highest concentrations of neoxanthin were measured one week after the beginning of flowering (May-21). Lettuce from a supermarket compared to *Aposeris* had significantly lower contents of xanthophylls: 0.32 ± 0.09 mg/100g fwt lutein, 0.12 ± 0.04 mg mg/100g fwt neoxanthin, 0.26 ± 0.09 mg/100g fwt violaxanthin, 0.016 ± 0.006 mg/100g fwt antheraxanthin and no zeaxanthin.

In published studies available on carotenoids in edible leafy vegetables data for all individual xanthophylls are rarely presented. In comparison with those few studies on common leafy vegetables in which single xanthophylls were analysed, *Aposeris* had higher individual xanthophyll contents than lettuce, rocket, cress, chicory, collards, cabbage and Chinese cabbage and similar contents as kale and spinach (KIMURA and RODRIGUEZ-AMAYA, 2003; BURNS et al., 2003; SINGH et al., 2007; HUMPHRIES and KHACHIK, 2003). Comparison of our study with studies (KRUMBEIN et al., 2005; RAJU et al., 2007; MERCADANTE and RODRIGUEZ-AMAYA, 1990; KIDMOSE et al., 2006; WILLS and RANGGA, 1996) performed on 58 mostly less commonly cultivated and wild leafy plants showed that only 3 species had higher contents of one or more individual foliar xanthophyll namely *Rumex acetosella* L., *Chenopodium album* L. and *Commelina benghalensis* L. had higher lutein and violaxanthin than *Aposeris* from our study.

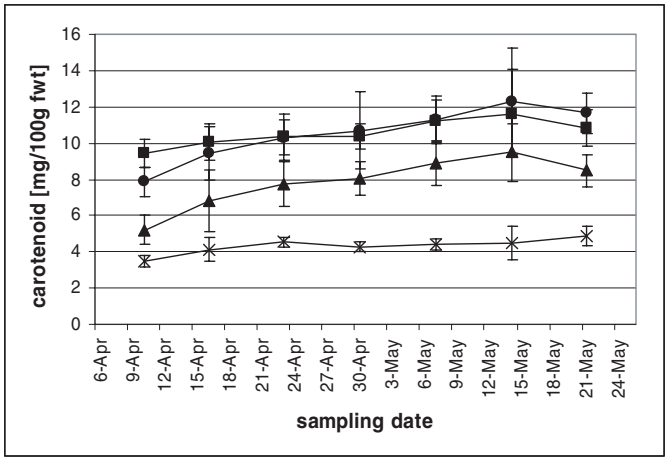

Fig. 3: Lutein, β-carotene, xantophyll cycle pigments (VAZ = violaxanthin + antheraxanthin + zeaxanthin) and neoxanthin (mg/100g fwt) in leaves of *Aposeris foetida* L. during harvesting period. Symbols represent mean ± S.D. of eight plant samples; ■ = lutein; ● = β-carotene; ▲ = VAZ; X = neoxanthin.

Chlorophyll: Chlorophylls a and b were measured in leaves of *Aposeris*. Foliar contents of total chlorophyll are presented in Tab. 1. The mean concentrations of total chlorophyll were between 129 and 166 mg/100g fwt. Contents of total chlorophyll were higher at the end of the harvesting period compared to earlier sampling dates (Fig. 4). Lettuce bought in a supermarket during the *Aposeris* harvesting period had only 2.98 ± 1.22 mg total chlorophyll per 100g fresh weight. In comparison with other studies on leafy vegetables *Aposeris* leaves contain significantly more chlorophyll than lettuce (BURNS et al., 2003) and more chlorophyll than cabbage, Chinese cabbage, savoy beet and spinach (KRUMBEIN et al., 2005; NEGI and ROY, 2000). NEGI and ROY (2000) reported similar chlorophyll contents for *Amaranthus tricolor* and higher for *Trigonella foenum graecum* compared to *Aposeris*. ŠTAJNER et al. (2006) reported similar or higher chlorophyll contents compared to *Aposeris* for 10 of 12 analysed cultivated and wild *Allium* species.

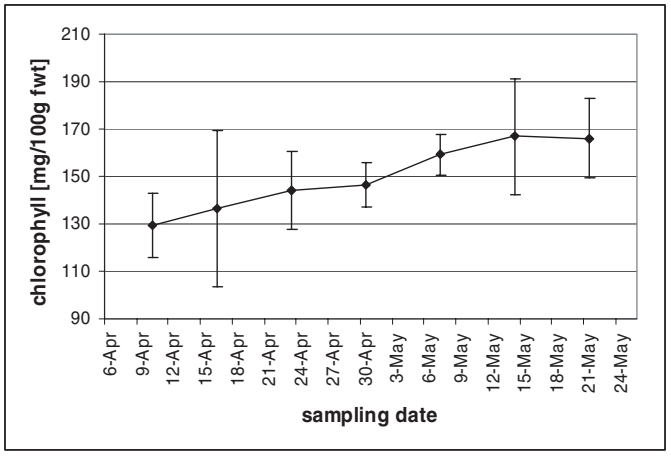

Fig. 4: Chlorophyll (mg/100g fwt) in leaves of *Aposeris foetida* L. during harvesting period. Symbols represent mean ± S.D. of eight plant samples.

Conclusion

Our study showed that *Aposeris* is an excellent source of important vitamins and antioxidants. Compared to *Aposeris* at least 20-times larger portion of supermarket lettuce is needed for the same intake of the nutrients analysed in the present study. The lowest foliar contents of vitamins and antioxidants were measured in just emerged *Aposeris* leaves and the highest contents were measured at the beginning of flowering ± one week. Because at the time of flowering the leaves became harder and the quantity of white milky sap increased, we can say that *Aposeris* leaves were at their best one week before flowering. Comparison of *Aposeris* with cultivated and wild edible plants from different studies showed that *Aposeris* is one of the best sources of ascorbic acid, tocopherol and carotenoids. The high foliar vitamin and antioxidant contents justify its use in the early spring wild food cuisine. The results may stimulate consumption of this nutritious plant, especially in home cuisine and in organic farms with an orientation towards tourism.

Acknowledgements

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