

## Preliminary communication

# THE CONTENT OF POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN LEAVES AND FLOWERS OF WILD GARLIC (*ALLIUM URSINUM* L.)

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Wild garlic (*Allium ursinum* L.) is a wildy growing plant that grows in forests and next to the streams. It has a specific aroma and taste resembling garlic. Wild garlic is increasingly favoured in gastronomy, food industry, and modern food technologies. The aim of this study was to analyse the dynamics of changes in total polyphenols content (TPC) and antioxidant activity (AOA) in wild garlic leaves and flowers during the vegetation period (from April to June). The samples of plant material were collected from the area of Bratislava – Železná studienka. The samples of fresh leaves and flowers were homogenized and a methanolic extract was prepared. These extracts were used for analyses. Total polyphenol content in the samples ranged from  $726 \pm 10$  mg GAE/kg FW to  $14.1 \times 10^2 \pm 13$  mg GAE/kg FW. The values of antioxidant activity were from  $10.7 \pm 1.57$  to  $25.9 \pm 1.06\%$  inhibition FW.

**Keywords:** wild garlic (*Allium ursinum* L.), total polyphenols, antioxidant activity, leaves, flowers

*Allium* is one of the largest plant genera in the world, including important and economically valuable agricultural crops such as garlic (*Allium sativum* L.), onion (*Allium cepa* L.), leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum* Hort.), elephant garlic (*Allium ampeloprasum* L. var. *ampeloprasum*), chive (*Allium schoenoprasum* L.), Chinese chive (*Allium tuberosum* L.), and less known wild garlic (*Allium ursinum* L.). The species of the genus *Allium* are characteristic of aroma and taste (HIRSCHEGGER et al., 2010).

Wild garlic (*Allium ursinum* L.), also known as ‘bear garlic’, is a wild growing plant. Wild garlic grows by streams and in the forests of Europe and Asia (GOĐEVAC et al., 2008). It prefers humid soils and humus and areas with full shade and semi-shade. The vegetation period of wild garlic is from early spring to the beginning of summer (DJURDJEVIC et al., 2004; SOBOLEWSKA et al., 2015).

Except for sulphur compounds such as cysteine-sulfoxides (methiin, alliin, isoalliin, propiin, ethiin) and thiosulfonates (allicin, methyl-allyl thiosulfinate, allyl-methyl thiosulfinate, dimethyl thiosulfinate) ajoenes and dithiins, the wild garlic contains phenolic compounds (flavonoids, mainly kaempferol derivatives), saponins and vitamins (C, E, A) (RADULOVIČ et al., 2015; SOBOLEWSKA et al., 2015).

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It is also important to note, that the main usable parts of wild garlic are the smooth, flat, fine, and brittle leaves. Flowers and bulbs are also consumable (BLAZEWICZ-WOZNAK & MICHOWSKA, 2011).

Wild garlic has become very popular in gastronomy in the last years, and it is used as salad, spice, boiled as a vegetable, or as an ingredient for pesto in lieu of basil (WU et al., 2009). Wild garlic has been known in folk medicine for ages, it has antibacterial, antiviral, and antifungal activities, which are associated with the presence of sulphur components (LACHOWICZ et al., 2017).

To date, only a few studies of wild garlic (*Allium ursinum* L.) has been published. Therefore, the aim of the study was to evaluate the content of polyphenols and the antioxidant activity in flowers and leaves of wild garlic (*Allium ursinum* L.) often used in gastronomy and food technologies.

## 1. Materials and methods

### 1.1. Plant material

The leaves and the flowers of wild garlic (*Allium ursinum* L.) were collected in Bratislava – Železná studienka (48°10'43.6"N 17°04'22.0"E) (Slovakia) in spring 2017 (leaves from April to June and flowers only in May) at regular monthly intervals. Wild garlic flowers (*Allium ursinum* L.) were collected only in May, because wild garlic flowers blossom only for a limited time. In each growing period 100 plants were collected, and morphological parts of plants were separated (leaves and flowers). The samples were collected from five different places.

### 1.2. Chemicals and extraction

Folin–Ciocalteu reagent and gallic acid were purchased from Merck (Darmstadt, Germany). Sodium carbonate, methanol, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The fresh leaves and flowers of wild garlic were homogenised in a mixer (Kinematica AG, Luzern, Switzerland). Methanol extracts were prepared by adding 50 ml of 80% methanol to 25 g milled sample and extracting in a shaker (Shaker GFL 3006, 125 r.p.m.) for 12 h. Samples were then filtered through filter paper (130 g m<sup>-2</sup>, Filtrak, Thermalbad Wiesenbad, Germany) and kept at 8 °C for further analysis. Each determination was carried out in six replications.

### 1.3. Total polyphenolic content (TPC)

The total polyphenolic content was assessed by the method used by SINGLETON and ROSSI (1965) employing the reduction of a phosphowolframate–phosphomolybdate complex to blue products by phenolic compounds. Briefly, an aliquot of the extract, blank, or standard was placed in a 50 ml flask, where the Folin–Ciocalteu reagent (2.5 ml) was added and the mixture was allowed to react for 3 min under continuous stirring, then a solution of sodium carbonate (5 ml) was added and mixed thoroughly. The volume was then made up to 50 ml with distilled water and left standing at room temperature in darkness for 2 h. The absorbance was measured at 765 nm using Shimadzu UV-1800 spectrophotometer (Japan). Results were expressed as mg gallic acid equivalents (GAE) per kg fresh weight (FW).

#### 1.4. Antioxidant activity (AOA)

Antioxidant activity was measured by the method BRAND-WILLIAMS and co-workers (1995) using a compound DPPH<sup>·</sup> (2,2-diphenyl-1-picrylhydrazyl). To obtain a stock solution, 0.025 g of DPPH<sup>·</sup> was diluted to 100 ml with methanol and kept in a cool and dark place. For the analysis, 3.9 ml of the DPPH<sup>·</sup> working solution (10× dilution of stock) was added to a cuvette, and the value of absorbance, which corresponded to the initial concentration of DPPH<sup>·</sup> solution ( $A_0$ ), was registered. Absorbance was read at 515.6 nm with Shimadzu UV-1800 spectrophotometer. Subsequently, 0.1 ml of the extract was added to the cuvette with DPPH<sup>·</sup>, and the absorbance was measured after 10 min ( $A_t$ ). The percentage of inhibition reflects the ability of antioxidant compounds to remove DPPH<sup>·</sup> radical at the given time.

Computation:

$$\% \text{ inhibition DPPH}^{\cdot} = \frac{A_0 - A_t}{A_0} \times 100 (\%)$$

#### 1.5. Statistical analysis

Results were statistically evaluated by Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD) using statistical software STATGRAPHICS (Centurion XVI.I, USA), also correlation analysis (Microsoft Excel) was used. The data are presented as mean ± standard deviation (SD) of six measurements.

## 2. Results and discussion

The total polyphenol contents and antioxidant activities of the studied wild garlic (*Allium ursinum* L.) samples from Bratislava surroundings – Železná studienka are presented in Table 1 and Table 2. The total polyphenols contents of the studied samples varied from 726±10 mg GAE/kg FW to 14.1×10<sup>2</sup>±13 mg GAE/kg FW. The highest levels of total polyphenols contents were detected in the flowers. The lowest polyphenol content in leaves was measured in April. In the flowers, the average content of total polyphenols was 1.69-times higher than those measured in the leaves obtained in June. LENKOVÁ and co-workers (2016) measured the total polyphenols content as 871±8 mg GAE/kg FW, which is consistent with our results. Lower total polyphenol content in wild garlic was found by MIHAYLOVA and co-workers (2014) – 609 mg GAE/kg FW and by ALEXIEVA and co-workers (2014) – 400 mg GAE/kg FW.

Table 1. The content of total polyphenols in leaves and flowers of wild garlic in the monitored months (mg GAE/kg FW)

Part of plants	Leaves			Flowers
	April	May	June	May
mg GAE/kg FW	726±10.1 <sup>a</sup>	782±11.2 <sup>b,c</sup>	827±9.82 <sup>c</sup>	14.1×10 <sup>2</sup> ±12.5 <sup>d</sup>

The results are expressed as mean value ± standard deviation; <sup>a,b,c,d</sup>: different letters in superscript in column mean significant difference (P<0.05).

Table 2. Antioxidant activity values of wild garlic leaves and flowers in the monitored months (% inhibition FW)

Part of plants	Leaves			Flowers
Month	April	May	June	May
% inhibition	10.7±1.57 <sup>a</sup>	15.05±1.21 <sup>b</sup>	17.3±0.85 <sup>c</sup>	25.9±1.06 <sup>d</sup>

The results are expressed as mean value±standard deviation; <sup>a,b,c,d</sup>: different letters in superscript in column mean significant difference ( $P<0.05$ ).

The statistically significant highest value of antioxidant activity was detected in flowers. The lowest level of antioxidant activity was measured in April leaves. The average value of antioxidant activity in flowers was 1.48-times higher than in June leaves. SAPUNJIEVA and co-workers (2012) indicated that the value of antioxidant activity of wild garlic was 22.49% FW, which correlates with the results of this work. The highest content of antioxidant activity in wild garlic (41.81% inhibition) was measured by SAHNOUN and co-workers (2017). The process of polyphenol content formation as well as antioxidant activity were observed in the leaves of wild garlic (*Allium ursinum* L.) during the vegetation period (from April to June). Generally, from April (the beginning of vegetation) to June (the end of the vegetation), the dynamics of total polyphenols formation and antioxidant activity value formation in leaves showed a slightly increasing tendency. Our results are similar to those published by LACHOWICZ and co-workers (2017). Authors found the highest content of total polyphenols in the leaves of wild garlic in the last vegetation phase – specifically in June. The authors also published that total phenolic concentrations in all anatomical parts of the wild garlic increased with maturity.

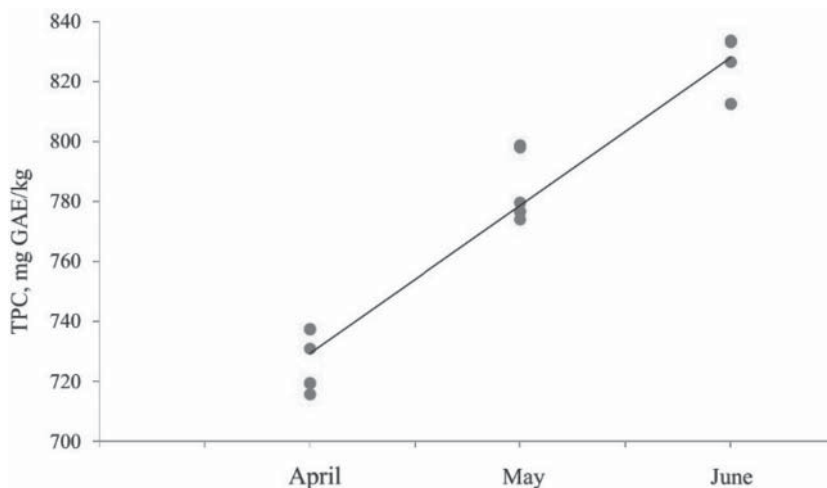


Fig. 1. Dynamics of total polyphenols content formation in wild garlic leaves ( $y=50.3x+677.68$ ;  $R^2=0.9497$ )

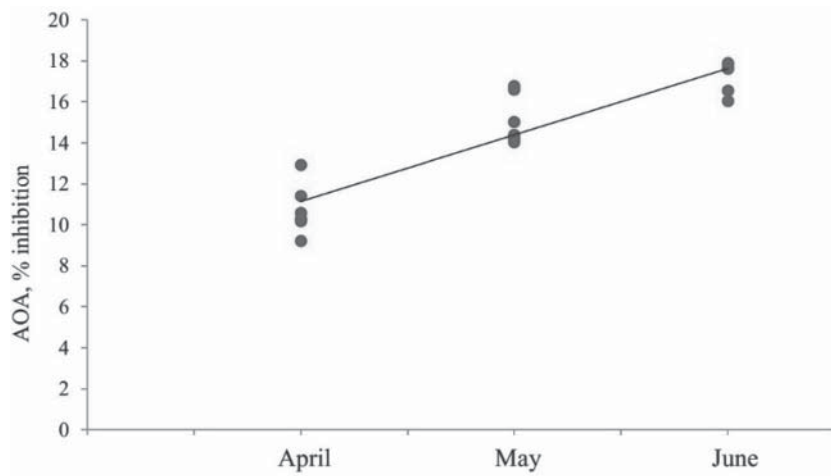


Fig. 2. Dynamics of antioxidant activity value changes in wild garlic leaves  
( $y = 3.2859x + 7.7875$ ;  $R^2 = 0.8365$ )

The last parameter evaluated in this experiment was the relation between the content of total polyphenols and the antioxidant activity value in the leaves and flowers of wild garlic (Fig. 3). The coefficient of correlation ( $r=0.917$ ) confirmed strong correlation between the content of total polyphenols and antioxidant activity value in leaves and flowers of wild garlic. Probably, the antioxidant capacity of the plant extracts is due, to a great extent, to the polyphenol content. According to BALASUNDRAM and co-workers (2006), the antioxidant activity of phenolic compounds was due to their ability to scavenge free radicals, donate

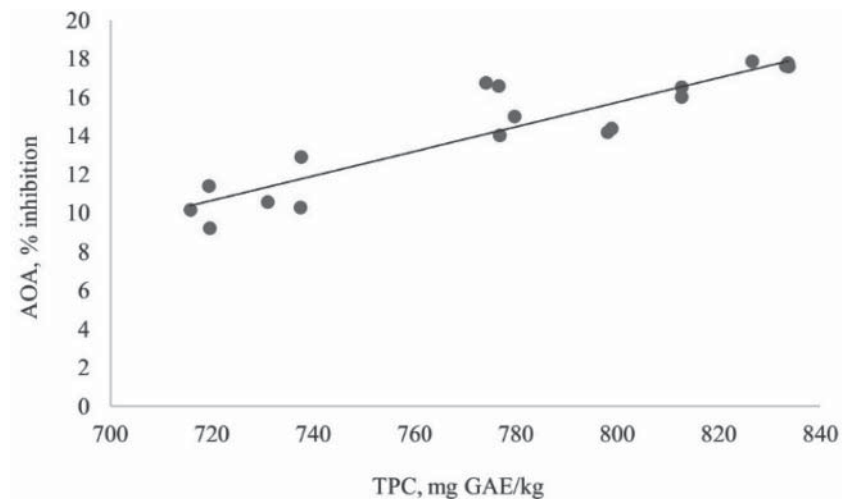


Fig. 3. Correlation between antioxidant activity and total polyphenols content in wild garlic leaves and flowers  
( $y = 0.0637x - 5.2$ ;  $R^2 = 0.8404$ )

hydrogen atoms or electron, or chelate metal cations. SANTAS and co-workers (2008) published that several studies have reported a good correlation between the phenol content of plant extracts and antioxidant activity. According to LACHOWICZ and co-workers (2017), it is also important to note that the amount of bioactive compounds in the plant is also influenced by the growing season of plants, the degree of maturity, i.e. physiological changes in the period of plant development, as well as weather conditions prevailing in the initial and final stages of plant growth.

### 3. Conclusions

The present experiment indicates that wild garlic (*Allium ursinum* L.) is an important source of polyphenolic substances. The highest content of total polyphenols and antioxidant activity values were measured at the end of vegetation period in the wild garlic leaves. We conclude that the content of total polyphenols and antioxidant activity value in the leaves of the wild garlic increases with maturity. It is also important to note that the results of the present study require further experiments regarding, for example, heat treatment and its impact on bioactive compounds in parts of plants of wild garlic, to be utilised in food technologies and gastronomy.

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