

## EVALUATION AND COMPARISON OF BIOACTIVE SUBSTANCES IN SELECTED SPECIES OF THE GENUS *ALLIUM*

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### ABSTRACT

*Allium* is a genus of some 650 species belonging to the family *Liliaceae*. However only a few of these are important as food plants, notably garlic (*Allium sativum* L.), onion (*Allium cepa* L.), leek (*Allium porrum* L.) and wild garlic (*Allium ursinum* L.). They contain many health beneficial substances, such as sulphur compounds, vitamins (vitamin C), mineral substances (Fe, Mg, Ca, P), polyphenols (especially quercetin) and substances antioxidant activity and fiber. In this work we evaluated the content of bioactive substances, especially the content of total polyphenols and antioxidant activity in several species (red onion, yellow onion, white onion, garlic, leek, wild garlic) of the genus *Allium*. Samples of plant material were collected at full maturity stages from Zohor (Slovak Republic). Zohor is an area without negative influences and emission sources. Samples of fresh species of the genus *Allium* were homogenized and prepared as an extract: 25 g cut plants material (red onion, yellow onion, white onion, garlic, leek, wild garlic) extracted with 50 mL of 80% ethanol for sixteen hours. These extracts were used for analyzes. The content of total polyphenols was determined using the Folin-Ciocalteu reagent (FCR). The absorbance was measured at 765 nm of wavelength versus blank. Antioxidant activity was measured using DPPH' (2,2-diferyl-1-picrylhydrazyl) at 515.6 nm in the spectrophotometer. In the present experiment it was found that total polyphenols content in samples ranged from 83.59 mg.kg<sup>-1</sup> to 758.63 mg.kg<sup>-1</sup> and values of antioxidant activity were in the range from 7.19% to 53.55%.

**Keywords:** polyphenols; antioxidant activity; *Allium*; species; variety

### INTRODUCTION

Vegetables are an important part of any dietary pattern. Because vegetables are typically high in nutrients and low in calories, they can play an essential role in health promotion and disease prevention (Manero et al., 2017). An increased intake of fruits and vegetables has been linked to lowering of important cardiovascular disease risk factors including hypertension, obesity, and type II diabetes mellitus. Furthermore, an inverse association between vegetables and fruits intake and cardiovascular disease incidents such as coronary heart disease and stroke has also been established (Bvenura and Sivakumar, 2017). Miller et al. (2017) published that the World Health Organization recommends a daily intake of five to eight portions of fruits and vegetables to reduce the risk of micronutrient deficiencies, cardiovascular disease, cancer, cognitive impairment, and other diet-related health conditions.

Currently, a major goal among researchers in food science is finding objective evidence that demonstrates food functionality. Consequently, studies regarding both the biological properties of putative phytochemicals and the chemical composition of plant species are of interest

(Ramirez et al., 2017). Onion (*Allium cepa* L.), garlic (*Allium sativum* L.), leek (*Allium porrum* L.), wild garlic (*Allium ursinum* L.) and chive (*Allium schoenoprasum* L.) are known species of the genus *Allium* which is important in the agriculture, food industry, gastronomy and modern food technologies. The genus *Allium* is composed of 600-700 species plants with specific taste and aroma. The specific aroma and taste of *Allium* plants caused by sulfur containing phytochemicals (Poojary et al., 2017; Kamenetsky and Rabinowitch, 2017). Lee et al. (2011) reported that sulfur containing phytochemicals in plants of *Allium* have anti-platelet and potential cancer-prevention activities.

Onions and garlic are universally used spice plants, and their medicinal properties are well known. Chive and the more recently introduced species are also much appreciated as spices, but leek and welsh onion are more important as vegetables with additional flavouring properties (Fritsch and Keusgen, 2006).

Onion (*Allium cepa* L.) is a plant of the *Liliaceae* family (of the genus *Allium*) and it contend phytochemicals, such as copaeenes, flavonoids, anthocyanins, minerals,

phenolics, phytoestrogens, terpenoids, vitamins, anthocyanins, and amino acids (Saxena et al., 2013).

Garlic (*Allium sativum* L.) is one of the most extensively studied species, not only among *Alliums*, but among all vegetables, and it has been considered a medicinal food for centuries, being used as a traditional remedy for common disorders (Ramirez et al., 2017). Naheed et al. (2017) reported that garlic is a rich source of health-promoting phytochemicals including antioxidants such as phenolics, flavonoids, and allicin.

Leek (*Allium porrum* L.) is a member of the genus *Allium* and grown is cultivated in Asia, America, and Europe, especially in the Mediterranean region (Tighe-Neira et al., 2017). Leek is source of flavonoids, kaempferol derivatives quercetin derivatives, flavonoids, phenolic, saponins, steroidal saponin, essential oils (Mohamed et al., 2016).

Wild garlic (*Allium ursinum* L.) also known under the name "bear's garlic" is a wild plant of the genus *Allium*. Wild garlic prefer nutritive substrate in the forest near rivers and streams. The important part of plant are leaves which are used in the food industry. The flowers and bulbs also are edible. The species has antiseptic, bacteriostatic, anti-parasitic properties and it is used during hypertension, hyperlypemia, and hypercholesterolemia treatment in alternative medicine. The leaves of wild garlic are highly appreciated as a spice, salads or soups, as raw, pickled, or as a vegetable in the gastronomy (Kęsík et al., 2011).

Antioxidants are defined as compounds present at low concentration compared to the oxidizable substrate that can significantly delay or prevent oxidation of that substrate. Phytochemical components, especially polyphenols are known to reduce oxidative stress. Phenolic compounds are secondary metabolites are known to be responsible for the antioxidant activity of plants. These compounds are suggested to contribute to the health-promoting properties. In addition to nutritive dietary components plants are a good source of different classes of polyphenolic components as well as flavan-3-ols, hydroxybenzoic and hydroxycinnamic acids, anthocyanins, stilbenoids and other flavonoids (Radovanović et al., 2015).

### Scientific hypothesis

Our hypothesis is that different species of the genus *Allium* has different content of total polyphenols, values of antioxidant activity and we expects the positive correlation between antioxidant activity values and total polyphenols content in this experiment.

## MATERIAL AND METHODOLOGY

### Plant material

Samples of plant material were collected at full maturity stages from area of Zohor (Slovak Republic). The sample of plant material were analyzed individually by selected methods, and were used in fresh material on analysis. The analysed *Allium* species (leek, garlic, wild garlic, white onion, yellow onion and red onion) are the most grown in Slovakia. The analysed species of the genus *Allium* (leek, garlic, wild garlic, white onion, yellow onion and red onion) are shown in Figure 7 – Figure 12.

### The local climate conditions

This study was performed in area of Zohor, Slovak Republic. It is situated on the western Slovakia (Zahorska lowland). Zohor belongs to warmer areas in Slovakia. Zahorska lowland is characterized by the cultivation of *Allium* plants such as onion, leek and garlic. The average annual rainfall is 600 mm and the average annual temperature is 9.5 °C.

### Chemicals and extraction

High-purity chemical reagents were used for all operations. Folin-Ciocalteu assay and gallic acid were purchased from Merck, Darmstadt, Germany. Sodium carbonate, ethanol and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Ethanol extracts were prepared by adding 50 mL of 80% ethanol to 25 g milled sample and was extracted in the Twisselmann apparatus 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.

### Spectrophotometric determination of total polyphenols

Total polyphenols were determined by the method of Lachman et al. (2003) and expressed as milligrams of gallic acid equivalent per kilogram (mg GAE.kg<sup>-1</sup>) fresh mater (FW). Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteu assay. The Folin-Ciocalteu (Merck) phenol reagent was added to a volumetric flask containig 100 mL of extract of plants samples (leek, onion, garlic, wild garlic).

The content was mixed and 5 mL of a sodium carbonate solution by Merck (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After two hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm (Spektrofotometer Shimadzu UV-1800; Shimadzu, Kyoto, Japan) of wavelength against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

### Spectrophotometric determination of antioxidant activity

Antioxidant activity was measured by the (Brand-Williams et al., 1995) method-using a compound DPPH<sup>•</sup> (2,2-diphenyl-1-pikrylhydrazyl). 2,2-diphenyl-1-pikrylhydrazyl (DPPH<sup>•</sup>) by Sigma – Aldrich, USA was pipetted to cuvette (3.9 cm<sup>3</sup>) then the value of absorbance, which corresponded to the initial concentration of DPPH<sup>•</sup> solution in time A<sub>0</sub> was written. Then 0.1 cm<sup>3</sup> of the followed solution was added and then the dependence A = f (t) was immediately started to measure. The absorbance of 10 minutes at 515.6 nm in the spectrophotometer (Shimadzu UV – 1800, Shimadzu, Kyoto, Japan) was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove DPPH<sup>•</sup> radical at the given time.

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

**Statistical analysis**

Results were statistically evaluated by the Analysis of Variance. All the assays were carried out in quadruplicates and results are expressed as mean ±SD. The data were subjected to the F-test in the one-way analysis of variance (ANOVA) If the *p*-value of the F-test is less than 0.05, there is a statistically significant difference between the at the 95% confidence level; the Multiple Range Tests will tell which means are significantly different from which others. The method currently being used to discriminate among the means of Fisher's least significant difference (LSD) procedure. Using statistical software Statgraphics Centurion XVI.I (Statpoint Technologies, The Plains, Virginia, USA) and a correlation analysis (Microsoft Excel, Washington, USA) was used.

**RESULTS AND DISCUSSION**

In this work the content of polyphenols and antioxidant activity (% inhibition) in leek, wild garlic, garlic, white onion, yellow onion and red onion was tested and evaluated. The results of antioxidant activity value and the content of total polyphenols in selected samples of the

genus *Allium* are summarized in Table 1.

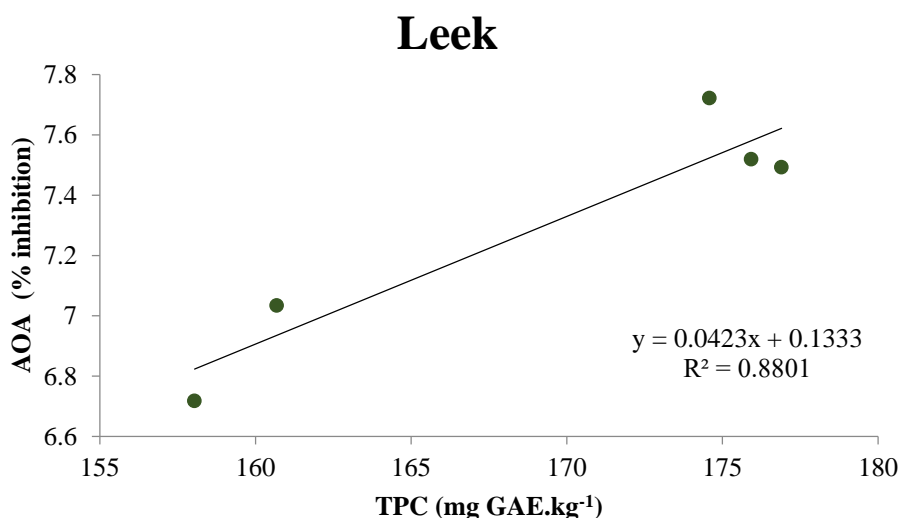
The content of total polyphenols in selected samples species of the genus *Allium* ranges from 83.59 mg GAE.kg<sup>-1</sup> (white onion) to 758.63 mg GAE.kg<sup>-1</sup> (red onion). Based on the measured content of total polyphenols in leek, wild garlic, garlic, white onion, yellow onion and red onion can be in the ensuing order: white onion <leek <yellow onion <garlic <wild garlic <red onion.

Dalaram (2016) published that the content of total polyphenols was recorded in selected species of the genus *Allium* (garlic, white onion, yellow onion and red onion) in the interval from 322.83 mg.kg<sup>-1</sup> FW to 626.61 mg.kg<sup>-1</sup> FW. In comparison to our determined values of polyphenols their results were in similar interval. Our results are higher compared to Benkeblia (2005), who has published the content of total polyphenols in garlic (490 mg.kg<sup>-1</sup> FW). Kavalcová et al. (2014) that the content of total polyphenols was recorded in selected varieties of leek is in the interval from 210.67 mg GAE.kg<sup>-1</sup> FW to 254.80 mg GAE.kg<sup>-1</sup> FW. In comparison to our determined values of polyphenols their results were in similar interval. Statistically significant highest content of total polyphenols (*p* <0.05) was recorded in red onion in variety of Karmen (758.63 ±12.30 mg GAE.kg<sup>-1</sup> FW). Statistically significant the lowest content of total

**Table 1** The averag values of antioxidant activity (% inhibition FW) and content of total polyphenols (mg GAE.kg<sup>-1</sup> FW) in selected species of the genus *Allium*.

Plant	Variety	AOA (% ±SD)	TPC (mg GAE.kg <sup>-1</sup> ±SD)
Leek	Starozagorski kamuš	7.19 ±0.39 <sup>a</sup>	167.71 ±10.09 <sup>b</sup>
Wild garlic	-	26.66 ±0.74 <sup>d</sup>	687.36 ±17.85 <sup>e</sup>
Garlic	Dukat	24.25 ±0.52 <sup>c</sup>	600.30 ±7.89 <sup>d</sup>
Onion (white)	Ala	12.71 ±0.67 <sup>b</sup>	83.59 ±10.62 <sup>a</sup>
Onion (yellow)	Bamberger	22.79 ±1.36 <sup>c</sup>	466.87 ±14.39 <sup>e</sup>
Onion (red)	Karmen	53.55 ±1.84 <sup>e</sup>	758.63 ±12.30 <sup>f</sup>
	HD <sub>0.05</sub>	1.56322	18.7296
	HD <sub>0.01</sub>	2.1474	25.6612

Note: <sup>a-f</sup> values with different letters mean significant differences (*p* <0.05) among selected species of the genus *Allium*, values AOA and TPC are expressed as arithmetic mean.



**Figure 1** Correlation between antioxidant activity and total polyphenols content of leek (Starozagorski kamuš).

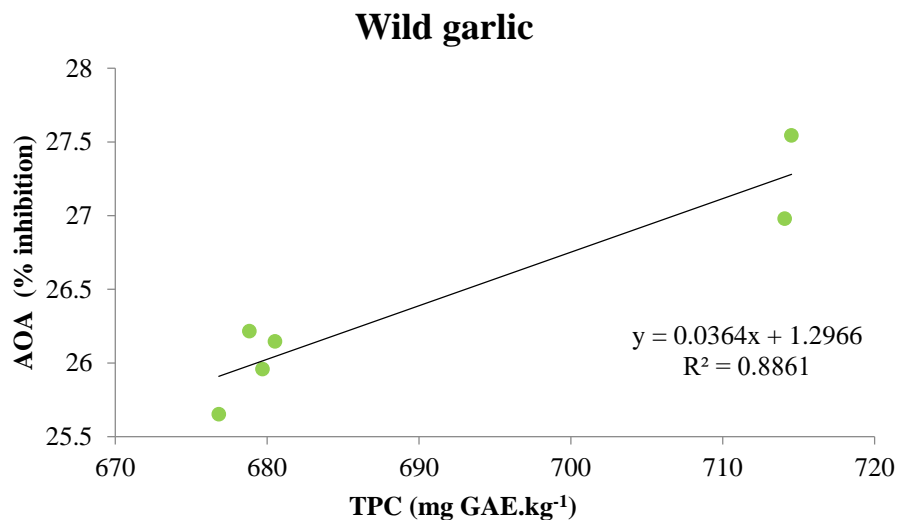


Figure 2 Correlation between antioxidant activity and total polyphenols content of wild garlic.

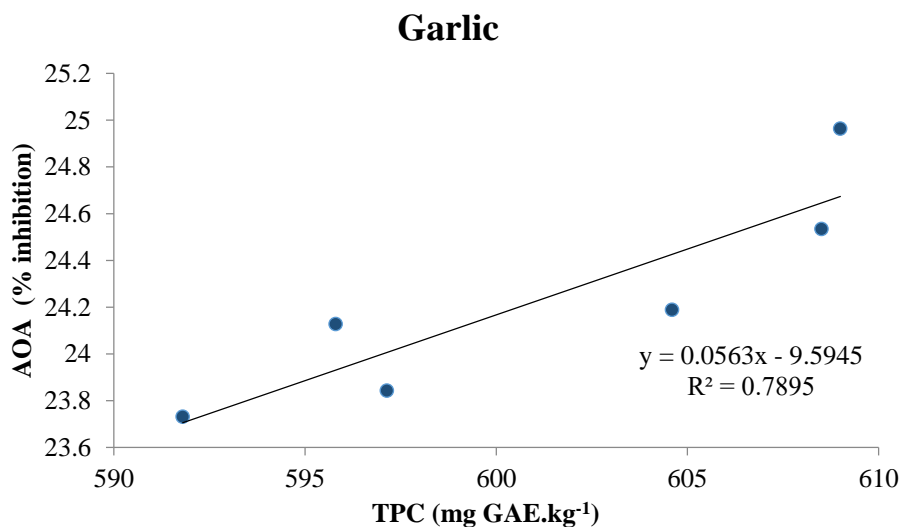


Figure 3 Correlation between antioxidant activity and total polyphenols content of garlic (Dukat).

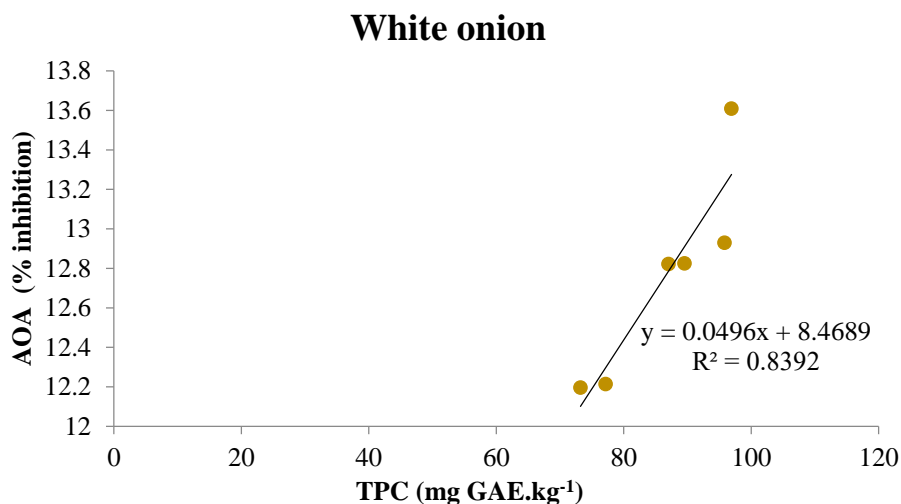


Figure 4 Correlation between antioxidant activity and total polyphenols content of white onion (Ala).

polyphenols ( $p < 0.05$ ) was recorded in white onion in variety of Ala ( $83.59 \pm 10.62$  mg GAE.kg<sup>-1</sup> FW).

The last indicator that has been evaluated and compared was the antioxidant activity of selected species of the genus *Allium* (wild garlic, leek, garlic, white onion, yellow

onion red onion). The values of antioxidant activity were in interval from  $7.19 \pm 0.39\%$  (leek) to  $53.55 \pm 1.84\%$  inhibition FW (red onion). Based on the measured values of antioxidant activity in leek, wild garlic, garlic, white onion, yellow onion and red onion can be in the ensuing

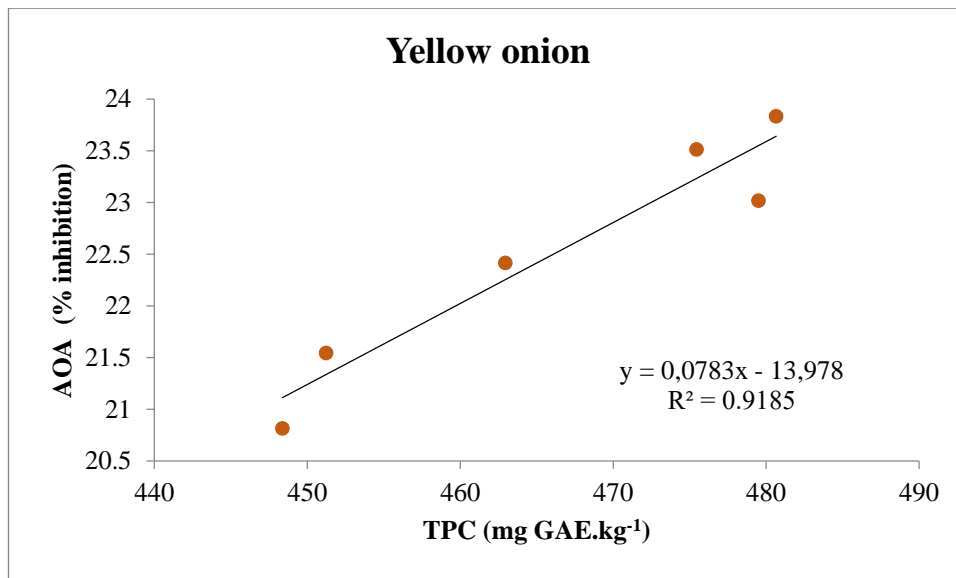


Figure 5 Correlation between antioxidant activity and total polyphenols content of yellow onion (Bamberger).

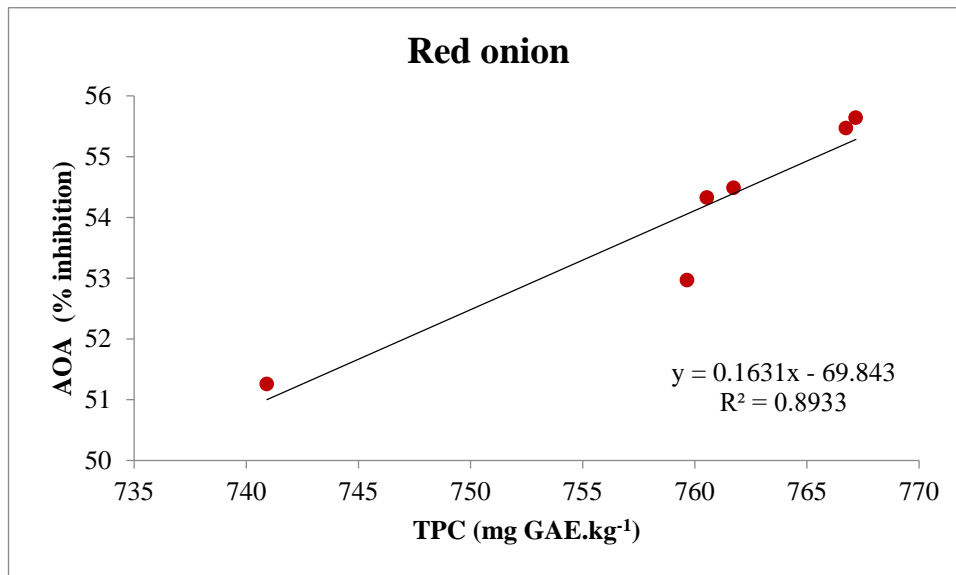


Figure 6 Correlation between antioxidant activity and total polyphenols content of red onion (Karmen).

order: leek <white onion <yellow onion <garlic <wild garlic <red onion. Our results are lower to **Ashwini (2013)** who has published that the values of antioxidant activity in onion were 57.11% inhibition.

Our results are in a similar range to **Shon et al. (2004)** and **Prakash et al. (2007)**. **Kavalcová et al. (2014)** reported that the value of antioxidant activity was recorded in leek (*Allium porrum* L.) in the interval from 8.55 to

12.92% inhibition FW. Statistically significant highest values of antioxidant activity ( $p < 0.05$ ) was recorded in red onion in variety of Karmen ( $53.55 \pm 1.84\%$  FW). Statistically significant the lowest content of total polyphenols ( $p < 0.05$ ) was recorded in leek in variety of Starozagorski kamuš ( $7.19 \pm 0.39\%$  FW). **Benkeblia (2005)** stresses that antioxidant activity depended on both phenolics and sulfur compounds of *Alliums*.



Figure 7 Red onion (Karmen).



Figure 8 White onion (Ala).



Figure 9 Yellow onion (Bamberger).





**Figure 10** Leek (Starozagorski kamuš). **Figure 11** Garlic (Dukat).

**Figure 12** Wild garlic (leaves).

In this study we have found positive correlation between the content of total polyphenols and antioxidant activity ( $r = 0.938$ ,  $r = 0.941$ ,  $r = 0.889$ ,  $r = 0.916$ ,  $r = 0.958$ ,  $r = 0.945$ ). Results are shown in Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6. These results are in good accordance with Cheng et al. (2013), who reported a positive correlation between total antioxidant activity and total phenolic content in onion ( $r = 0.793$  -  $r = 0.912$ ). Lenková et al. (2016) also observed a positive relationship between the content of polyphenolic compounds and antioxidant activity in selected species of the genus of *Allium*.

## CONCLUSION

The present paper was focused on the content of total polyphenols and antioxidant activity in selected *Allium* species. The results suggest that red onion and wild garlic contains higher amount of polyphenolic substances. The six *Allium* species for this study represent majority *Allium* species currently grown in Slovakia. The coefficient of correlation confirmed strong dependency between the antioxidant activity and the total content of polyphenols. Content of polyphenolic compounds contained in *Allium* species are quite variable. It is also important to note that the main factor affecting the content of total polyphenols are species and variety. The content of chemoprotective compounds may be affected also by agrochemical composition of the soil for example content of humus, climatic condition and nutrients. The results obtained in this work provide further information about of the content of total polyphenols and antioxidant activity in *Allium* species.

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