

Evaluation and comparison of the content of total polyphenols and antioxidant activity of selected species of the genus *Allium*

Vyhodnotenie a porovnanie obsahu celkových polyfenolov a antioxidačnej aktivity vo vybraných druhoch rodu *Allium*

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Abstract

The species of the genus *Allium* are very important crops for human health. They contain many health beneficial substances, such as polyphenols (especially flavonoids), sulphur compounds, vitamins, mineral substances and substances with antioxidant activity. This work has focused on the comparison of total phenolic content and antioxidant activity of selected species of the genus *Allium* – garlic (*Allium sativum* L.), chives (*Allium schoenoprasum* L.), ramson (*Allium ursinum* L.) and red, yellow and white onion (*Allium cepa* L.). Samples of plant material were collected at the stage of full maturity in the area of Nitra. Total polyphenols content was determined using the spectrophotometric method of Folin-Ciocalteu agents. Determined the content of total polyphenols were in the range 444.3 - 1591 mg*kg⁻¹. Total polyphenols content in the observed crops declined in the following order: chives > red onion > garlic > yellow onion > ramson > white onion. Antioxidant activity was measured by the spectrophotometric method using a compound DPPH (2,2-diphenyl-1-picrylhydrazyl). Determined the value of antioxidant activity ranged 12.29 – 76.57%. Antioxidant activity observed in crops declined in the following order: chives > ramson > red onion > yellow onion > garlic > white onion. In all the analysed crop plants was confirmed by the strong dependence of the antioxidant activity and the total content of polyphenolic substances.

Keywords: antioxidant activity, chives, garlic, onion, polyphenols, ramson

Abstrakt

Druhy z rodu *Allium* sú veľmi dôležité plodiny pre ľudské zdravie. Obsahujú množstvo dôležitých zdraviu prospešných látok, medzi ktoré patria polyfenoly (hlavne flavonoidy), sírne zlúčeniny, vitamíny, minerálne látky a látky s antioxidantnou aktivitou. Táto práca bola zameraná na porovnanie obsahu celkových polyfenolov a antioxidantnej aktivity vo vybraných druhoch z rodu *Allium* – cesnak (*Allium sativum* L.), pažitka (*Allium schoenoprasum* L.), cesnak medvedí (*Allium ursinum* L.) a červená, žltá a biela cibuľa (*Allium cepa* L.). Vzorky rastlinného materiálu boli zhromažďované v štádiu plnej zrelosti v lokalite Nitra. Obsah celkových polyfenolov bol stanovený spektrofotometrickou metódou s použitím Folin-Ciocalteuovho činidla. Stanovené obsahy celkových polyfenolov sa pohybovali v rozmedzí 444.3 – 1591 mg*kg⁻¹. Obsah celkových polyfenolov v sledovaných plodinách klesal v nasledujúcom poradí: pažitka > červená cibuľa > cesnak > žltá cibuľa > cesnak medvedí > biela cibuľa. Antioxidantná aktivita bola meraná spektrofotometrickou metódou za použitia zlúčeniny DPPH (2,2-difenyl-1-pikrylhydrazyl). Stanovené hodnoty antioxidantnej aktivity sa pohybovali v rozmedzí 12.29 – 76.57%. Antioxidantná aktivita v sledovaných plodinách klesala v nasledujúcom poradí: pažitka > cesnak medvedí > červená cibuľa > žltá cibuľa > cesnak > biela cibuľa. Vo všetkých analyzovaných plodinách bola potvrdená silná závislosť medzi antioxidantnou aktivitou a celkovým obsahom polyfenolických látok.

Kľúčové slová: antioxidantná aktivita, cesnak, cesnak medvedí, cibuľa, pažitka, polyfenolové zlúčeniny

Introduction

Food of plant origin, such as vegetables and fruit which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the risk of chronic diseases (Liu, 2003; Yang et al., 2004). Genus *Allium* is characterized by large number of primary and secondary types of nutritional and medicinal components (Rizwani and Shareef, 2011).

Allium species, the most important genus of the *Alliaceae* family, are among the oldest cultivated vegetables (Fritsch et al., 2010). The species may differ in form and taste, but they are close in biochemical and phytochemical contents (Lanzotti, 2006). Garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) are the most important *Allium* species consumed all over the world (Kim et al., 2004). *Allium* includes some economically important species like common onion, garlic, chives, leek and also species used as herbal crops, as traditional medicines and as ornamental plants (Fritsch and Friesen, 2002; Tepe et al., 2005).

Onion (*Allium cepa* L.) is probably native of south west Asia. It has a globose bulb that is an underground part of the stem (Ozougwu, 2011). Onions contain generous amounts of the flavonoid (mainly quercetin). Studies have shown that quercetin protects against cardiovascular disease, and cancer. Onions contain organosulfur compounds that have been linked to lowering blood pressure and cholesterol levels

(Kumar et al., 2010), anthelmintic, antiseptic and hypoglycaemic (Ozougwu, 2011). Onions contain high levels of antioxidant compounds (polyphenolics) which have protective effects against different degenerative pathologies (Griffiths et al., 2002).

Garlic (*Allium sativum* L.) is a plant which has been grown for more than 5000 years for culinary and therapeutic purposes. Its best known chemical compound is allicin, which has extensive pharmacological effects (Trifunski et al., 2015). Today, in many parts of the world garlic is being used as prophylaxis and for the cure of numbers of diseases including acute and chronic infections (Srivastava and Pathak, 2012). Garlic is a source of various biologically active phytochemicals, including organosulfur compounds, polyphenolics (phenolic acid, flavonoids) and vitamins (Chen et al., 2013).

Chives (*Allium schoenoprasum* L.) come from cold regions of Europe and Asia. Chives have slim, dark green leaves and pale purple flowers. The plant was used in traditional folk medicine to stimulate digestion, treat anemia and to cleanse the blood (Grzeszczuk et al., 2011). The pharmacological effects are caused by diallyl sulphides (diallyl monosulphide, diallyl disulphide, diallyl trisulphide, diallyl tetrasulphide), polyphenolic compounds, vitamin C and carotenoids (Vlase et al., 2013). In opinion of Parvu et al. (2010) the main polyphenolic compounds of chives are p-coumaric acid, ferulic acid, isoquercitrin and rutin.

Ramson (*Allium ursinum* L.) - the species name "ursinum" is of Latin origin, being derived from "ursus" (bear), and is related to folk tales, according to which bears after awakening from winter hibernation consume this plant to remove toxins from the body and to regain strength. The plant is a bulbiferous. Its bulb is narrow, elongated, about 1.5 - 6 cm long. Ramson grows up to the height of 50 cm (Sobolewska et al., 2015). *Allium ursinum* L. is spread through the entirety of Europe, Asia Minor, Caucasus and Siberia up to the Kamchatka Peninsula (Djurdjevic et al., 2004). In recent years, the potential health benefits of ramson have been attributed mainly to the sulfur-containing compounds (Parvu et al., 2011).

The health properties of these natural plants depend on the contents of bioactive compounds, mainly polyphenolics and substances with antioxidant effects (Chun et al., 2005; Beato et al., 2011).

Polyphenols have been classified by their source of origin, biological function, and chemical structure. The majority of polyphenols in plants exist as glycosides with different sugar units and acylated sugars at different positions of the polyphenol skeletons. Distribution of polyphenols according to the chemical structures of the aglycones: phenolic acids, flavonoids, polyphenolic amides, other polyphenols (Tsao, 2010). The phenolic compounds have antiproliferative and tumour arresting effects (Kuceková et al., 2011). Plant phenolics are generally involved in protection against ultraviolet radiation or aggression by pathogens, parasites and predators (Priečina and Karlina, 2013). Flavonoids are major phenolics in genus *Allium* and can be classified in different subclasses (flavones, flavanones, flavonols, isoflavones, flavanonols, flavanols, chalcones and anthocyanins) according to the degree of unsaturation and degree of oxidation of the 3-carbon skeleton. Flavonols and anthocyanins are the main subclasses of flavonoids present in genus *Allium* (Pérez-Gregorio et al., 2009).

The antioxidant activity of *Allium* species is due to several of sulphur-containing compounds and their precursors, but it is also related to other bioactive compounds such as polyphenols, dietary fiber and microelements (Vlase et al., 2013).

The physiological role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals (Young and Woodside, 2001). Antioxidants may be divided into enzymatic (superoxide dismutase - SOD, catalase, glutathione peroxidase - GSHP) and non-enzymatic (vitamins E, C, A) groups (Capasso, 2013).

Polyphenolic compounds are effective antioxidants due to their capability to scavenge free radicals of fatty acids and oxygen (Lachman et al., 2003). Genus *Allium* has been widely used to scavenge reactive oxygen species (ROS) and treat a variety of diseases including heart disease and cancer (Oak et al., 2005; Chen et al., 2013). These antioxidant properties were highly correlated with the presence of the primary phenolic compounds (Chen et al., 2013). Polyphenols behave as antioxidants by a variety of way including trapping of reactive oxygen species, inhibition of enzymes responsible for superoxide anion production, chelation of transition metals involved in process forming radicals and prevention of the peroxidation process by reducing alcoxyl and peroxy radicals (Priecina and Karlina, 2013). It is known that the contents of bioactive compounds and related antioxidant activity in vegetables are influenced by geographical region, climatic and storing conditions, and degree of ripeness (Saxena et al., 2007).

Materials and methods

The local climate conditions

This study was performed in area of Nitra, Slovak Republic. She is situated on the southern Slovakia. Nitra belongs to warmer areas in Slovakia. Nitra has very good natural and climatic conditions for crop growth, without any adverse effects. The average annual rainfall is 550 – 600 mm and the average annual temperature is 9.9 °C.

Samples of plant material

The samples of plant material (yellow, red and white onion, garlic, chives and ramson) were collected in the phase of full ripeness from area of Nitra. For analysis was used fresh material soil samples and plant, samples were analysed by selected methodologies (determination of total polyphenols and antioxidant activity). All samples of plant material were grown under the same conditions. The soil samples from the area, where was grown plant material, was analysed (Table 1 and Table 2). The analysis of soil samples was carried out four times in four sampling sites. Only NPK fertilization (200 g per m²) was used for the achievement of favourable soil macroelements content.

Table 1. Agrochemical characteristic of soil substrate in $\text{mg}\cdot\text{kg}^{-1}$, content of nutrients from locality Nitra

Tabuľka 1. Agrochemická charakteristika pôdneho substrátu v $\text{mg}\cdot\text{kg}^{-1}$, obsah živín z lokality Nitra

Locality	Cultivar	K	Ca	Mg	P	pH _{KCl}	Humus %	Cox %
1	garlic, chives, red, yellow, white onion	392	$38.6\cdot 10^2$	$13.1\cdot 10^2$	97.4	6.91	2.9	1.68
		±	±	±	±	±	±	±
		4.67	2.15	0.98	3.61	0.073	0.026	0.011
2	ramson	412	$61.9\cdot 10^2$	362	46.9	7.2	3.47	2.01
		±	±	±	±	±	±	±
		5.19	2.33	4.29	1.72	0.094	0.035	0.017

Table 2. Content of heavy metals ($\text{mg}\cdot\text{kg}^{-1}$) in soil substrate (extraction by aqua regia)

Tabuľka 2. Obsah ťažkých kovov ($\text{mg}\cdot\text{kg}^{-1}$) v pôdnom substráte (extrakcia lúčavkou kráľovskou)

Locality	Cultivar	Zn	Cu	Ni	Pb	Cd
1	garlic, chives, red, yellow, white onion	55.7	27.1	42.3	40.7	4.04
		±	±	±	±	±
		2.03	1.43	1.87	1.69	0.042
2	ramson	190	24.8	39.1	40.8	3.88
		±	±	±	±	±
		3.15	1.02	1.62	1.73	0.047
Limit ¹		150	60	50	70	0.7

¹ Limitná hodnota pre lúčavku kráľovskú – zákon č. 220/2004

¹ Limit value for aqua regia – Law No. 220/2004

Preparations of samples

Extract was prepared from the 25 g samples yellow, red and white onion, garlic, ramson and chives, which were shaken (shaker GFL 3006, 125 rpm) in 50 ml of 80% ethanol for sixteen hours. Samples were kept at laboratory room temperature in dark conditions until the analysis. Each determination was carried out in six replications.

Determination of total polyphenols

Total polyphenols content (TPC) was determined by the method according to Lachman et al. (2003). It is expressed as mg of gallic acid equivalent per kg of fresh matter. Total polyphenols content was determined using the Folin-Ciocalteu reagent. 2.5 ml of Folin-Ciocalteu reagent was added to 100 μ l extract to volumetric flask. The content was mixed. After 3 minutes, 5 ml 20% solution of sodium carbonate was added. Then the volume was adjusted to 50 ml with distilled water. After 2 hours, the samples were centrifuged (centrifuges UNIVERSAL 320, 15000 rpm) for 10 minutes. The absorbance was measured of the spectrophotometer Shimadzu UV/VIS – 1240 at 765 nm. The concentration of polyphenols was calculated from a standard curve with known concentration of gallic acid.

Determination of antioxidant activity

Antioxidant activity (AOA) was measured according to Brand-Williams et al. (1995). The method is based on using DPPH \cdot (2,2-diphenyl-1-picrylhydrazyl). DPPH \cdot (3.9 ml) was pipetted into the cuvette and the absorbance was measured using the spectrophotometer Shimadzu UV/VIS – 1240 at 515.6 nm. The measured value corresponds to the initial concentration of DPPH \cdot solution at the time A_0 . Then 0.1 cm 3 extract was added to start measuring dependence $A=f(t)$. The content of cuvette was mixed and the absorbance was measured at 1, 5 and 10 minutes in the same way as DPPH solution. The percentage of inhibition expresses how antioxidant compounds are able to remove DPPH \cdot radical at the given period of time.

$$\text{Inhibition (\%)} = (A_0 - A_t / A_0) \times 100$$

Statistical analysis

Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD). It was used by the statistical software STATGRAPHICS (Centurion XVI.I, USA).

Results and discussion

The total content of polyphenols in the samples ranges from 444.3 ± 6.93 to 1591 ± 10.89 mg*kg $^{-1}$ (Table 3). Statistically significant differences in the content of polyphenols were detected among all analysed crop. The highest value of total polyphenols was measured in chives. The lowest total polyphenol content was observed in the white onion. The determined quantity of TPC in the analysed samples can be arranged in the following order: chives > red onion > garlic > yellow onion > ramson > white onion. In comparison with this study Batcioglu et al. (2012)

indicated lower total polyphenol content in garlic is $780 \text{ mg} \cdot \text{kg}^{-1}$, similarly Charles (2013) recorded that TPC in garlic is $812 \text{ mg} \cdot \text{kg}^{-1}$. Some authors reported even a lower value of TPC in garlic: $436 \text{ mg} \cdot \text{kg}^{-1}$ (Chekki et al., 2014), $410 \text{ mg} \cdot \text{kg}^{-1}$ (Wangcharoen and Morasuk, 2007), $493 \text{ mg} \cdot \text{kg}^{-1}$ (Jastrzebski et al., 2007). By way of contrast Wangcharoen and Morasuk (2009) recorded in another study higher values of TPC in garlic ($1290 \text{ mg} \cdot \text{kg}^{-1}$). A wide range of TPC in ramson from 190 to $910 \text{ mg} \cdot \text{kg}^{-1}$ was indicated by Sapunjieva et al. (2012). Even higher value of TPC in ramson ($1280 \text{ mg} \cdot \text{kg}^{-1}$) was recorded by Mahmutovic et al. (2014). In comparison with this study lower content of TPC in chives ($844 \text{ mg} \cdot \text{kg}^{-1}$) was measured by Greszczuk et al. (2011) as well as by Charles (2013) found $749 \text{ mg} \cdot \text{kg}^{-1}$. Nuutila et al. (2003) indicated that total polyphenol content in onion is like in the ramson in the wide range from 845 to $2075 \text{ mg} \cdot \text{kg}^{-1}$. In agreement with Lachman et al. (2003) the highest content of TPC was determined in red onion, and followed the yellow onion. The lowest content of total polyphenols contained white onion. Pérez-Gregorio et al. (2009) also noted that white onion had the lowest content of total polyphenols.

Table 3. Average content of total polyphenols ($\text{mg} \cdot \text{kg}^{-1}$) and antioxidant activity (% inhibition)

Tabuľka 3. Priemerný obsah celkových polyfenolov ($\text{mg} \cdot \text{kg}^{-1}$) a antioxidačnej aktivity (% inhibície)

Vegetable	Variety	TPC	AOA (%)
Garlic	Mojmír	1051 ± 18.09^d	17.17 ± 0.634^b
Ramson	Wild garlic	871 ± 8.16^b	45.34 ± 0.622^e
Chives	Pražská	1591 ± 10.89^f	76.57 ± 0.67^f
Red onion	Red mate	1313 ± 29.74^e	40.58 ± 1.157^d
Yellow onion	Sherpa	935.2 ± 9.23^c	21.09 ± 2.418^c
White onion	White solid	444.3 ± 6.93^a	12.29 ± 1.592^a
	HD _{0.05} ¹	23.43	2.008
	HD _{0.01}	32.10	2.752

¹ Viac rozsahový test, Metóda 95.0 percent LSD, Písmena (a, b, c, d, e, f) medzi faktormi ukazujú štatisticky významné rozdiely ($P < 0.05$) – LSD test

¹ Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a, b, c, d, e and f) between the factors show statistically significant differences ($P < 0.05$) – LSD test

The values of antioxidant activity in the studied samples are presented in Table 3. The antioxidant activity of the sample is varied from $12.29 \pm 1.592\%$ to $76.57 \pm 0.67\%$ and statistically significant differences in the strength of antioxidant effect were also detected among all analysed crops. The highest value of antioxidant

activity was measured in the chives. The lowest value of antioxidant activity was measured in the white onion. In chives average value of antioxidant activity is 6.23-times higher than in the white onion and 4.45-times higher than in garlic. According to determined values of AOA the analysed samples of the genus *Allium* can be arranged in the following order: chives > ramson > red onion > yellow onion > garlic > white onion. Chen et al. (2013) indicate that the values of the antioxidant activity of garlic move in wide range from 3.60% to 45.63%. El-Hamidi and El-Shami (2015) recorded the values of antioxidant activity of 16.39% to 27.25%. Charles (2013) mentioned value 15.5%, which good correlate with the results of this work. But Choi et al. (2014) report a lower value of antioxidant activity in garlic (4.65%). In comparison with this study slightly lower strength of antioxidant effect in ramson (37%) was found by Gitin et al. (2014). Sapundjieva et al. (2012) measured also lower values of antioxidant activity in ramson (22.49%). Unlike above mentioned authors Mnayer et al. (2014) indicate that the antioxidant activity in ramson moves from 39.08% to 81.73% and AOA of ramson determined in this study belong to this range. Ashwini et al. (2013) noted that the scope of the antioxidant activity of onion from 13.6% to 84.1%. According to Cheng et al. (2013) the antioxidant activity in red onion is 41.22%.

Relations among content of polyphenols and antioxidant activity in garlic, ramson, chives and red, yellow and white onion were evaluated (Figure 1 – 6). The coefficient of correlation ($r = 0.827 - 0.937$) confirmed strong dependency between the AOA and the TPC and the results are in good agreement with the findings of Chen et al. (2013), Ramkissoon et al. (2012), Hu (2012) and Chekki et al. (2014) who also indicated correlations between content of polyphenols and antioxidant activity in the garlic, onion, carrot and other vegetable.

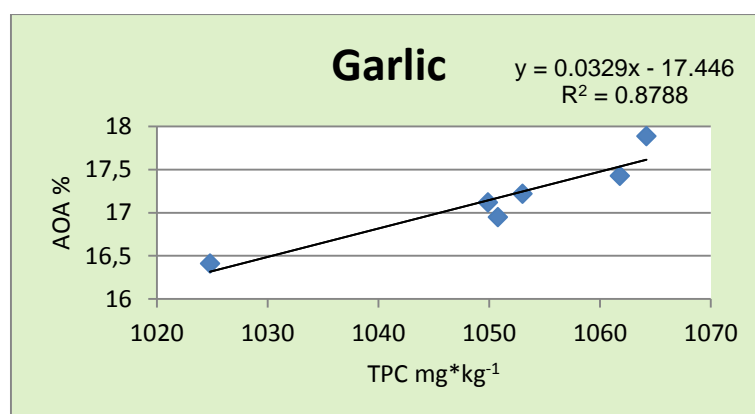


Figure 1. Relationship between TPC and AOA in garlic

Obrázok 1. Vzťah medzi TPC a AOA v cesnaku

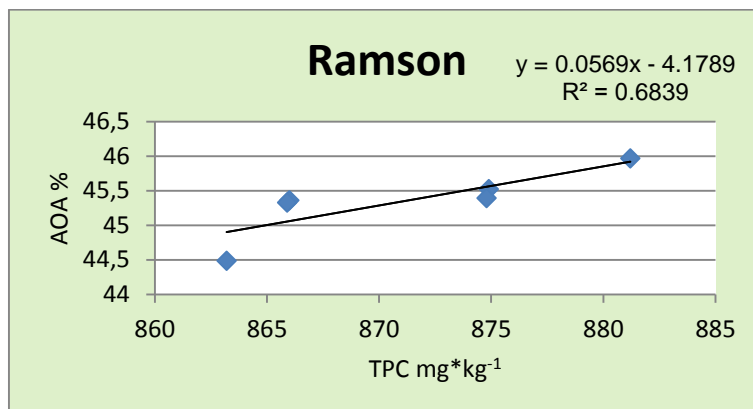


Figure 2. Relationship between TPC and AOA in ramson
Obrázok 2. Vzťah medzi TPC a AOA v cesnaku medveďom

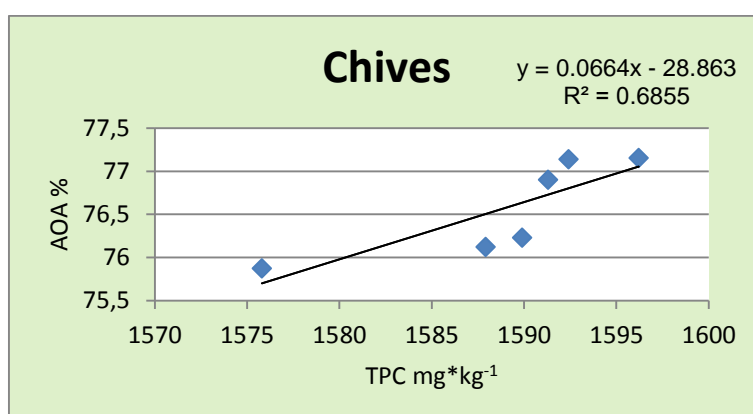


Figure 3. Relationship between TPC and AOA in chives
Obrázok 3. Vzťah medzi TPC a AOA v pažitke

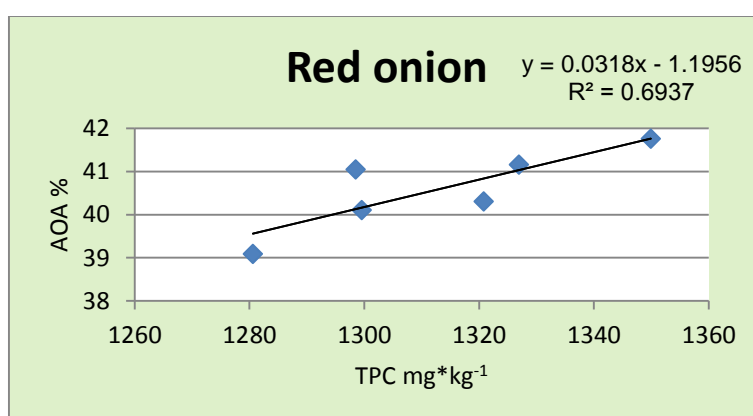


Figure 4. Relationship between TPC and AOA in red onion
Obrázok 4. Vzťah medzi TPC a AOA v červenej cibuli

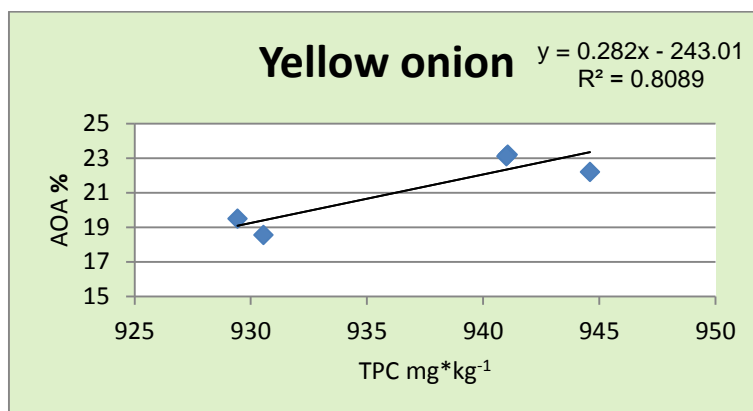


Figure 5. Relationship between TPC and AOA in yellow onion

Obrázok 5. Vzťah medzi TPC a AOA v žltej cibuli

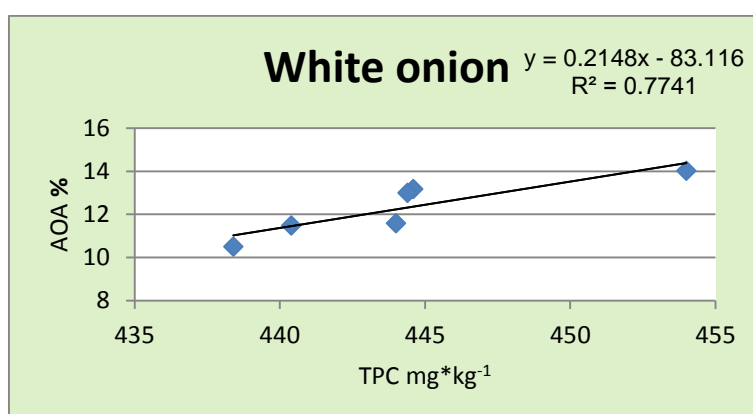


Figure 6. Relationship between TPC and AOA in white onion

Obrázok 6. Vzťah medzi TPC a AOA v bielej cibuli

Conclusions

The total content of polyphenols and antioxidant activity of selected crops of the genus *Allium* (garlic, ramson, chives and red, yellow and white onion) grown in the locality Nitra were comparable to or higher than those described in the literature but statistically significant differences in both the total content of polyphenols and the antioxidant activity were detected among all analysed crops. The highest value of total polyphenols and antioxidant activity as well as was determined in chives. The lowest level of both followed indicators was found in white onion. The coefficient of correlation confirmed strong dependency between the antioxidant activity and the total content of polyphenols. Vegetables should be an important part of your daily diet due to high content of biologically active substances that can help to protect you from some diseases.

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