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EVALUATION AND COMPARISON OF THE CONTENT OF TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN GARLIC (*ALLIUM SATIVUM* L.)

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ABSTRACT

Garlic (*Allium sativum* L.) is one of the oldest cultivated plants in the world and highly valued throughout the ages as a culinary spice. It is a hardy perennial belonging to the *Alliaceae* family. The garlic bulb is the most commonly used portion of the plant, composed of 5 – 20 individual. It is a very good source of manganese, selenium, vitamin C and vitamin B6 (pyridoxine). In addition, garlic is a good source of other minerals, including phosphorous, calcium, potassium, iron and copper. Many of the perceived therapeutic effects of garlic are thought to be due to its active ingredient allicin. This sulphur-containing compound gives garlic its distinctive pungent smell and taste. Garlic possesses antiviral, antibacterial, anti-fungal properties allowing it to stand against all infections. This work has focused on the evaluation and comparison of total content of polyphenols and antioxidant activity in five varieties of garlic – Mojmír, Záhorský, Lukan, Havran and Makoi. Samples of plant material were collected at the stage of full maturity in the area of Nitra. The total content of polyphenols was determined using the spectrophotometric method of Folin-Ciocalteu agents. Determined the content of total polyphenols in garlic were in the range 621.13 mg.kg^{-1} (Záhorský) to 763.28 mg.kg^{-1} (Havran). Total polyphenols content in garlic declined in the following order: Havran >Mojmír >Makoi >Lukan >Záhorský. Antioxidant activity was measured by the spectrophotometric method using a compound DPPH (2.2-diphenyl-1-picryhydrazyl). Statistically significant highest value of antioxidant was recorded in 20.22% (Mojmír) and the lowest value was in 13.61% (Záhorský). The values of antioxidant activity observed in the varieties of garlic may be arranged as follows: Mojmír >Havran >Lukan >Makoi >Záhorský. In all the analysed varieties of garlic was confirmed by the strong dependence of the antioxidant activity and the total content of polyphenols.

Keywords: antioxidant activity; garlic; total polyphenols

INTRODUCTION

Fruits and vegetables have historically been considered rich sources of some essential dietary micronutrients and fibers, and more recently they have been recognized as important sources for a wide array of phytochemicals that individually, or in combination, may benefit health **(Elhamidi, 2010)**. Epidemiological studies have indicated that the consumption of vegetables is associated with a reduced risk for the development of chronic diseases, such as cardiovascular disease and cancer **(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)** and contains a number of pharmacological effects, including chemopreventive activity and tumor cell growth inhibition **(Vlase et al., 2013)**. *Allium* species, the most important genus of the *Alliaceae* family, are among the oldest cultivated vegetables. They have been used as ornamentals, spices, vegetables, or as medicines for curing various diseases **(Tepe et al., 2005)**. The *Allium* genus includes more than 800 species widely distributed all over the world **(Fritsch et al., 2010)** and appreciated due to their flavour, easy growth and long storage time. 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)**. The genus is characterized by having bulbs enclosed in membranous tunics. Most species produce remarkable amounts of cysteine sulphoxides causing the well-known characteristic aroma and taste. The genus is naturally distributed in southwest and central Asia, in Europe and North America. *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)**.

Garlic (*Allium sativum* L.) is a plant which has been grown for more than 5000 years for culinary and therapeutic purposes. Bulb is used for its medical properties, which is formed of several cloves, wrapped in individual membrane **(Trifunschi et al., 2015)**. The importance of garlic has already been recognized in early Egyptian, Chinese and Indian civilizations centuries ago as an herbal or traditional medicine. 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 like pneumonia, dysentery, typhoid fever, cholera, gastritis, tuberculosis, diabetes mellitus, heart disease and hypertension **(Srivastava and Pathak, 2012)**. Garlic is a source of various biologically active phytomolecules, including organosulfur compounds, polyphenolics (phenolic acid, flavonoids) and vitamins. The health properties of garlic depend on its bioactive compounds and particularly on phenolic compounds. Garlic is used to protect humans against oxidative stress **(Chen et al., 2013)**.

Polyphenols are bioactive substances widely distributed in natural products. They have been reported to have multiple biological properties, such as antioxidant, antimutagenic, antibacterial, antiviral and anti-inflammatory activities. Medicinal plants rich in polyphenols can retard the oxidative degradation of lipids and improve the quality and nutritional value of food **(Vlase et al., 2013)**. More than 8000 phenolic structures are currently known, and among them over 4000 flavonoids have been identified. Polyphenols have been classified by their source of origin, chemical structure and biological function. 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: phenolic acids, flavonoids, polyphenolic amides, other polyphenols **(Tsao, 2010)**. Flavonoids 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. Subclasses of flavonoids can be further differentiated on the basis of the number and nature of substituent groups attached to the rings. Flavonols and anthocyanins are the main subclasses of flavonoids present in genus Allium **(Pérez-Gregorio et al., 2009)**.

An antioxidant can be defined as: "any substance that when present in low concentrations compared to that of an oxidisable substrate significantly delays or inhibits the oxidation of that substrate" **(Young and Woodside, 2001)**. There are two major groups of antioxidants: enzymatic antioxidants and non-enzymatic antioxidants. These groups are divided into several subgroups. The enzymatic antioxidants are divided into primary and secondary enzymatic defenses. The primary defense is composed of three important enzymes that prevent the formation of and neutralize free radicals: glutathione peroxidase, catalase, superoxide dismutase. The secondary enzymatic defense includes glutathione reductase and glucose-6-phosphate dehydrogenase. The group of non-enzymatic antioxidants contains several subgroups, the main ones being: vitamins (A, E, C), enzyme cofactors (Q10), minerals (zinc and selenium), peptides (glutathione), phenolic acids, and nitrogen compounds (uric acid) **(Shebis et al., 2013)**. The antioxidants protect against oxidative stress, causing serious damage to the cells, resulting in various human diseases such as Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer, arthritis, immunological disorders and neurodegenerative the failure **(Shalaby and Shanab, 2013)**. The aim was to determine and compare the content of total polyphenols and antioxidant activity in 5 varieties of garlic (*Allium sativum* L.).

MATERIAL AND METHODOLOGY

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 ºC.

Samples of plant material

The samples of plant material – garlic (Mojmír, Záhorský, Lukan, Havran and Makoi) 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 m^2) was used for the achievement of favourable soil macroelements content.

Preparations of samples

Extract was prepared from the 25 g samples garlic, 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 μL 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.

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

Vegetable	K	Ca	Mg	P	pH_{KCl}	Humus %	Cox %
garlic	392 ±4.67	3861.7 ±2.15	1312.3 ±0.98	97.4 ±3.61	6.91 ±0.073	2.9 ±0.026	1.68 ±0.011

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

Vegetable	Zn	Cu	Ni	Pb	Cd
garlic	55.7 ±2.03	27.1 ±1.43	42.3 ±1.87	40.7 ±1.69	4.04 ±0.042
Limit *	150	60	50	70	0.7

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

Determination of antioxidant activity

Antioxidant activity (AOA) was measured according to **Brand-Williams et al. (1995)**. The method is based on using DPPH˙ (2.2-diphenyl-1-picrylhydrazyl). DPPH˙ (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˙ 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˙ radical at the given period of time.

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 studied samples are presented in Table 3. The total content of polyphenols of the samples is varied from 621.13 ±4.45 to 763.28 ±3.60 $mg.kg^{-1}$ and statistically significant differences in the strength of total polyphenols content were also detected among analysed crops. The highest value of total polyphenols was observed in Havran. The lowest level of total polyphenols was measured in Záhorský. In Havran average level of total polyphenols is 1.22-times higher than in Záhorský and 1.09-times higher than in Mojmír. According to determined values of TPC the analysed samples of garlic can be arranged in the following order: Havran >Mojmír >Makoi >Lukan >Záhorský. **Charles (2013)** indicated highest total polyphenol content in garlic is 812 $mg.kg^{-1}$. In comparison with this study highest content of TPC in garlic (780 $mg.kg^{-1}$) was measured by **Batcioglu et al. (2012)** as well as by. Some authors reported even a lower value of TPC in garlic: 410 $mg.kg^{-1}$ **(Wangcharoen and Morasuk, 2007)**, 436 $mg.kg^{-1}$ **(Chekki et al., 2014)**, 493 $mg.kg^{-1}$ **(Jastrzebski et al.,**

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

Vegetable	Variety	TPC	AOA
Garlic	Mojmír	698.82 ±3.43[c]	20.22 ±0.62[d]
	Záhorský	621.13 ±4.45[a]	13.61 ±0.38[a]
	Lukan	628.76 ±8.61[a]	15.25 ±0.40[b]
	Havran	763.28 ±3.60[d]	16.52 ±0.32[c]
	Makoi	678.18 ±6.43[b]	13.71 ±0.35[a]
$HD_{0.05}$		8.52866	0.646596
$HD_{0.01}$		11.7908	0.893914

Note: Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a, b, c and d) between the factors show statistically significant differences (p <0.05) – LSD test, TPC – total polyphenols content, AOA – antioxidant activity.

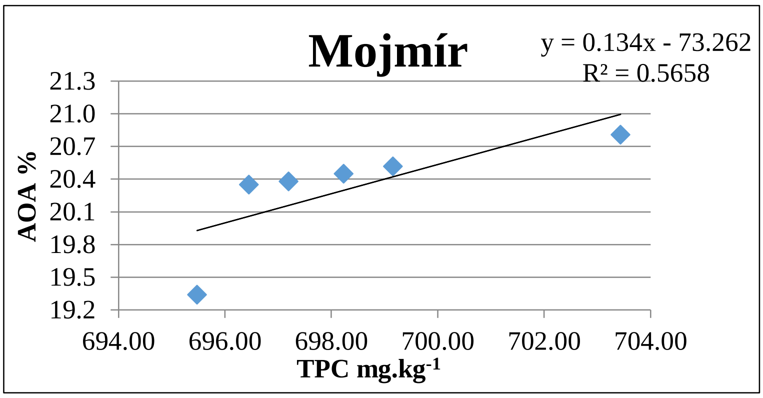

Figure 1 Relationship between TPC and AOA in Mojmír.

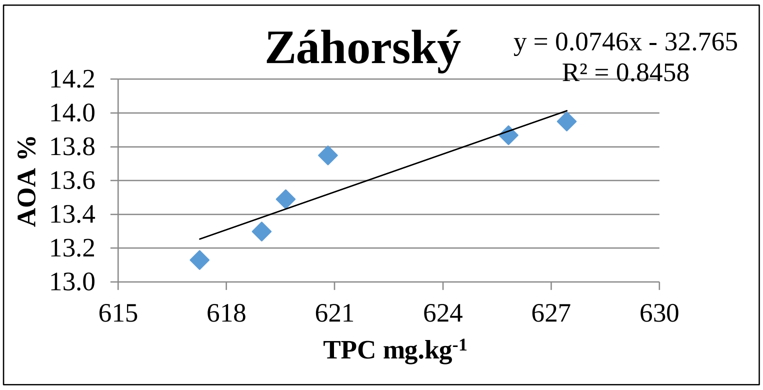

Figure 2 Relationship between TPC and AOA in Záhorský.

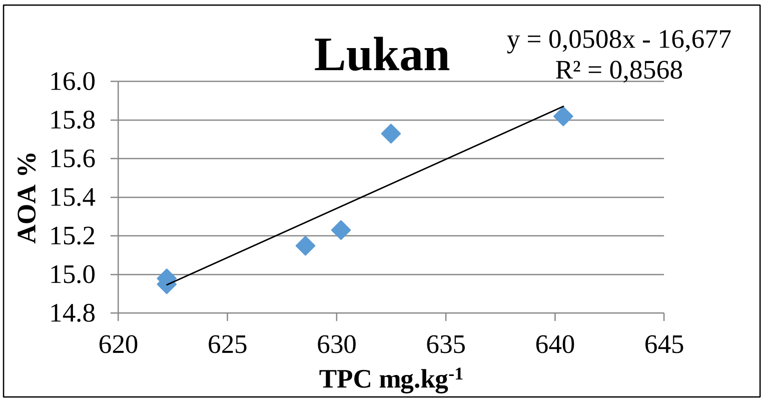

Figure 3 Relationship between TPC and AOA in Lukan.

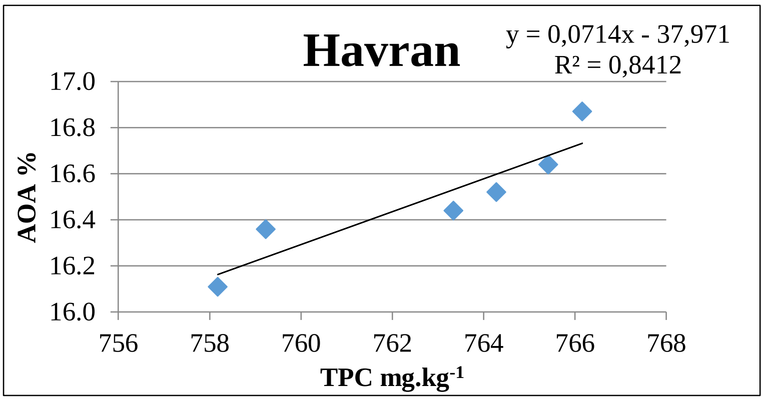

Figure 4 Relationship between TPC and AOA in Havran.

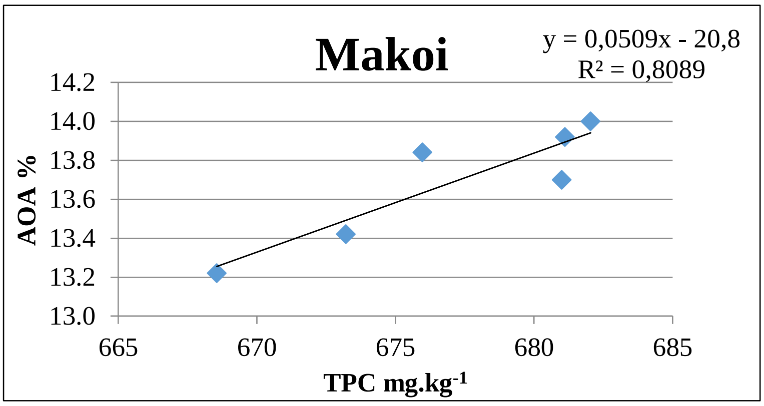

Figure 5 Relationship between TPC and AOA in Makoi.

2007). By way of contrast **Wangcharoen and Morasuk (2009)** measured in another study higher level of TPC (1290 mg.kg^{-1}).

The values antioxidant activity in the samples ranges from 13.61 ±0.38% to 20.22 ±0.62% (Table 3). Statistically significant differences in the antioxidant activity were detected among all analysed varieties of garlic. The highest value of antioxidant activity was measured in Mojmír. The lowest antioxidant activity was observed in Záhorský. In Mojmír average value of antioxidant activity is 1.49-times higher than in Záhorský and 1.22-times higher than in Havran. The determined quantity of antioxidant activity in the analysed samples can be arranged in the following order: Mojmír >Havran >Lukan >Makoi >Záhorský. **Elhamidi and Elshami (2015)** indicate that the values of the antioxidant activity of garlic move in wide range from 16.39% to 27.25%. **Chen et al. (2013)** provides a greater range of antioxidant activity of garlic, which in the wide range from 3.60% to 45.63%. **Charles (2013)** indicates that the value of the antioxidant activity of garlic is 15.5%, which good correlate with the results of this work.

Relations among the content of polyphenols and the antioxidant activity in analysed varieties of garlic (Mojmír, Záhorský, Lukan, Havran and Makoi) were evaluated (Figure 1 – 5). The coefficient of correlation (r = 0.7522 - 0.9195) confirmed strong dependency between the content of polyphenols and the antioxidant activity and the results are in good agreement with the findings of **Hu (2012)**, **Chekki et al. (2014)**, **Ramkissoon et al. (2012)** and **Chen et al. (2013)**, who also indicated correlations between antioxidant activity and content of polyphenols in garlic, onion and other vegetable.

CONCLUSION

The total content of polyphenols and antioxidant activity of different varieties of garlic (Mojmír, Záhorský, Lukan, Havran and Makoi), grow in locality Nitra were comparable with literature. The statistically significant differences in both the antioxidant activity and the total content of polyphenols were detected among all varieties of garlic (Mojmír, Záhorský, Lukan, Havran and Makoi). The highest value of total polyphenols was determined in Havran (763.28 $mg.kg^{-1}$) and the highest level of antioxidant activity was found in Mojmír (20.22%). The lowest value of both followed indicators as well as determined in Záhorský (AOA -13.61%, TPC –621.12 $mg.kg^{-1}$). The coefficient of correlation (r = 0.7522 - 0.9195) confirmed strong dependency between the total content of polyphenols and the antioxidant activity. Garlic should we include into our diet, it is an important raw material for our health, because they provide culinary, nutritional and health benefits.

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