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THE CONTENT OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF GARLIC (*ALLIUM SATIVUM* L.)

Natália Čeryová, Iveta Čičová, Judita Lidiková, Marek Šnirc, Jarmila Horváthová, Helena Lichtnerová, Hana Franková

ABSTRACT

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Garlic (*Allium sativum* L.) is one of the most commonly grown vegetables and thanks to its sensory properties, it has an important place in numerous world cuisines. Garlic is also known for its health-promoting properties, which are attributed to its chemical composition. The health benefits of garlic depending on the content of biologically active compounds, which vary between cultivars and geographical areas. Seven cultivars of garlic, namely Sukoradsky, Zahorsky, Germidour, Mojmir, Karel IV., Arkus, Makoi, were analyzed in this study. This study aimed to determine the total polyphenol content, total flavonoid content, and antioxidant activity of garlic. Studied characteristics were analyzed by the UV-VIS spectrometry method. Total polyphenol content ranged from 430.26 to 640.04 mg GAE.kg⁻¹ FW. Total flavonoid content ranged from 10.29 to 60.49 mg CE.kg⁻¹ FW. Antioxidant activity measured by ABTS assay ranged from 1.098 to 1.955 mmol TE.kg⁻¹ FW. Antioxidant activity measured by FRAP assay ranged from 0.63 to 1.467 mmol.kg⁻¹ FW. Highest TPC, TFC, and AA were determined in the cultivar Mojmir. The lowest TPC and TFC were determined in the cultivar Zahorsky. The lowest AA was determined in the cultivar Sukoradsky. High positive correlations were determined between individual parameters.

Keywords: Allium sativum; garlic; polyphenols; flavonoids; antioxidants

INTRODUCTION

The genus Allium includes the most widespread and commonly grown vegetables such as onion (Allium cepa L.), garlic (Allium sativum L.), leek (Allium porrum L.), and many others. All these species are very important for agriculture, gastronomy, and the food industry. This genus includes plants with a very specific aroma and taste, for which sulfur-containing phytochemicals are responsible. Substances in these species have a positive effect on human health (Poojary et al., 2017). Allium vegetables have maintained a leading position in cultivation throughout the centuries. All these species are characterized by a content of valuable components with significant beneficial effects on human health. In addition to their popularity in the therapeutic area, they have an important place in almost every kitchen. From a nutritional point of view, it contains important components such as chemoprotective substances, which also include natural plant antioxidants. They also contain a wide range of vitamins and trace elements that are essential for the proper functioning of the body (Hedges and Lister, 2007; Choi et al., 2011). The genus Allium is rich in flavonoids, saponins, sapogenins, and volatile sulfur compounds. Due to the content of rare phytochemicals, Allium plants have several positive effects on human health, including antimicrobial, antidiabetic, antioxidant, antiviral,

anticarcinogenic, antimutagenic, hepatoprotective, and neuroprotective. Since ancient times, these plants have been used in the treatment of cardiovascular diseases, inflammatory diseases, vascular diseases, elevated blood cholesterol levels, and various degenerative diseases (**Putnik et al., 2019**). Plant species belonging to the genus Allium are characterized by their pungent taste and significant medicinal properties. Individual plant species differ mainly in morphological structure, taste, and color. In terms of chemical composition, plants belonging to the genus Allium are similar (**Lorigooini et al., 2014**).

Garlic (*Allium sativum* L.) is a monocotyledonous species belonging to the *Alliaceae* family and is one of the oldest traditional crops in the world (**Khan et al., 2017**). It is one of the most common types of vegetables and is an important part of many world cuisines. Garlic is used mainly for its sensory properties, but also for its health-promoting properties, which are attributed to its chemical composition. The health benefits of garlic depending on the content of biologically active compounds, which vary between cultivars and geographical areas (**Szychowski et al., 2018**). The chemical composition of garlic is significantly affected by the cultivar, growing conditions, and cultivation practices. In particular, the fertilization regime and soil properties can have a significant effect on quality properties

such as mineral composition, dry matter, protein content, and total soluble solids content. Garlic is considered a rich source of volatile compounds, which are responsible for the typical taste and bioactive properties of garlic. There is also a high content of non-volatile compounds with well-known medicinal and therapeutic properties, such as amides, nitrogen oxides, proteins, saponins as well as antioxidants, minerals (especially P, K, and Se), vitamins (vitamin C and vitamins), and phenolic compounds, especially В flavonoids and phenolic acids (Petropoulos et al., 2018). Garlic is characterized by a high content of polyphenolic compounds that have a positive effect on the human body. Phenolic acids and flavonoids account for the largest proportion of polyphenolic compounds in garlic. The total content of polyphenols in garlic can be influenced by variety, environmental influences (sun exposure, precipitation, different types of cultivation, fruit yield), but also by the storage and technological processing of garlic (Srivastava et al., 2013; Rasouli et al., 2017).

Polyphenol compounds have been identified as nutrients, plant secondary metabolites, phytonutrients, antioxidants, dietary bioactive agents, and protective factors (Cory et al., 2018). Polyphenols form one of the most important groups of secondary metabolites of plants. They are widespread in the plant kingdom and can be obtained directly from plants and other foods rich in antioxidants, gaining worldwide attention as nutraceuticals in the prevention of multiple diseases. The health and antitumor effects associated with regular consumption of foods rich in polyphenols are an effective therapy for maintaining health against reactive oxygen species (Losada-Barreiro and Bravo-Díaz, 2017). Polyphenol compounds are partly responsible for determining the sensory and nutritional characteristics of foods. They represent a wide variety of different pigments, are involved in attracting pollinators, performing structural functions, protecting against ultraviolet radiation, and protecting plants from microbial invasion and herbivores (Cutrim and Cortez, 2018). The availability of polyphenols depends primarily on their bioavailability. The rate of absorption in the intestines depends on their chemical structure (Abbas et al., 2017).

Polyphenol compounds are potent antioxidants. Unlike several sulfur compounds in garlic, polyphenols are more stable and can be extracted from fresh, frozen, or dried plant samples. The content of biologically active compounds in garlic varies between cultivars grown in different geographical areas (Szychowski et al., 2018). Given the bioactive unique combination of compounds, representatives of the genus Allium should be a regular part of our diet. Garlic has a wide range of applications as an antioxidant, antifungal, antithrombotic and hypoglycemic agent. It reduces glucose metabolism in diabetics, slows the development of arteriosclerosis, and reduces the risk of the development of various types of cancer. In addition, it reduces the possibility of myocardial infarct in patients and could also improve the function of the immune system (Suleria et al., 2015).

Scientific hypothesis

Garlic is a natural source of polyphenols and antioxidants. The concentration of polyphenols in garlic is cultivar dependant.

MATERIAL AND METHODOLOGY Samples

Seven cultivars of garlic, namely Sukoradsky, Zahorsky, Germidour, Mojmir, Karel IV., Arkus, and Makoi, were conventionally cultivated and harvested in full ripeness. **Chemicals**

Folin-Ciocalteau reagent (Merck, Germany), anhydrous p.a. (Sigma-Aldrich, natrium carbonate USA), monohydrate of gallic acid p.a., (Sigma-Aldrich, USA), aluminium chloride p.a., (Sigma-Aldrich, USA), sodium nitrite p.a., (Sigma-Aldrich, USA), sodium hydroxide p.a., (Sigma-Aldrich, USA), catechin hydrate 98%, (Sigma-Aldrich, USA), methanol p.a., 80% (Sigma-Aldrich, USA), Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, USA), ABTS (2,2'-azino-bis (3 ethylbenzthiazoline-6-sulfonic) acid, Sigma-Aldrich, USA), potassium persulfate p.a. (Sigma Aldrich, USA), acetic acid p.a. (CH3COOH, Sigma Aldrich, USA), sodium acetate p.a. (COONa, Sigma Aldrich, USA), TPTZ (2,4,6--s-triazine, Sigma-Aldrich, USA), tri (2-pyridyl) hydrochloric acid (HCl, Sigma Aldrich, USA), ferric chloride p.a. (FeCl₃), distilled water

Biological material

Garlic (*Allium sativum* L.)

Instruments

Unimax 2010 Horizontal Shaker (Heidolph Instrument, GmbH, Germany).

Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer (Shimadzu, Japan).

Laboratory Methods

Folin – Ciocalteau method was modified by Lachman et al. (2003).

Aluminium chloride method was modified by Chang et al. (2002).

ABTS radical scavenging assay (Re et al., 1999).

FRAP assay by (Pedersen et al., 2000).

Description of the Experiment

Sample preparation: To prepare extracts, 25 g of homogenized peeled garlic bulbs were shaken in 50 mL of 80% methanol for 16 hours on the Unimax 2010 horizontal shaker (Heidolph Instrument, GmbH, Germany), and filtered through Munktell No. 390 filtrating paper (Munktell and Filtrac, Germany).

Number of samples analyzed: 7 Number of repeated analyses: 4 Number of experiment replication: 1

Total polyphenol content

Total polyphenol content was determined by the method of **Lachman et al**. (2003), using Folin – Ciocalteau phenol reagent, 20% Na₂CO₃, and distilled water. 0.1 mL of extract was pipetted into a 50 mL volumetric flask and diluted with distilled water. Then, 0.85 mL of Folin – Ciocalteau reagent was added, and after 3 minutes, 5 mL of 20% Na₂CO₃ was added. Volume was made up to 50 mL with distilled water. and left at laboratory temperature for 2 hours. Absorbance was measured against blank solution at 765 nm, using Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer, Shimadzu, Japan.

Total polyphenol content was expressed as mg of gallic acid equivalent in 1 kg of fresh garlic, based on the calibration curve ($R^2 = 0.9948$).

Total flavonoid content

Total flavonoid content was determined by the aluminium chloride method (**Chang et al., 2002**), using 5% NaNO₂, 10% AlCl₃, 1M NaOH, and distilled water. 1 mL of extract was pipetted into a 10 mL volumetric flask and diluted with 5 mL of distilled water. Then, 0.3 mL of 5% NaNO₂ was added. After 6 minutes, 0.6 mL of 10% AlCl₃ was added. After 5 minutes, 2 mL of 1M NaOH was added. After mixing on vortex, volume was made up to 10 mL with distilled water and left at laboratory temperature for 15 minutes. Absorbance was measured against blank solution at 510 nm, using Shimadzu UV-1800 UV/ Visible Scanning Spectrophotometer, Shimadzu, Japan.

Total flavonoid content was expressed as mg of catechin equivalent in 1 kg of fresh garlic, based on the calibration curve ($R^2 = 0.9981$).

Antioxidant activity using ABTS assay

Antioxidant activity was determined by the ABTS assay (**Re et al., 1999**), using ABTS⁺⁺ radical cation - (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate (K₂S₂O₈), and acetate buffer (pH = 4.3) Working ABTS solution was produced by the reaction of ABTS⁺⁺ solution, K₂S₂O₈ solution, and acetate buffer. 0.05 mL of extract was pipetted into 3 mL of working ABTS solution, stirred, and left at laboratory temperature for 20 minutes. Absorbance was measured against blank solution at 734 nm, using Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer, Shimadzu, Japan.

Antioxidant activity was expressed as mmol of Trolox equivalent in 1 kg of fresh garlic, based on the calibration curve ($R^2 = 0.9931$).

Antioxidant activity using FRAP assay

Antioxidant activity was determined by the FRAP assay (**Pedersen et al., 2000**), using TPTZ - (2,4,6-tris(2-pyridyl)-S-triazine), ferric chloride (FeCl₃), and acetate buffer (pH = 3.5) Working FRAP solution was produced by the reaction of TPTZ solution, FeCl₃ solution, and acetate buffer. 0.05 mL of extract was pipetted into 3 mL of working FRAP solution, stirred, and left at laboratory temperature for 20 minutes. Absorbance was measured against blank solution at 593 nm, using Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer, Shimadzu, Japan.

Antioxidant activity was expressed as mmol of Trolox equivalent in 1 kg of fresh garlic, based on the calibration curve ($R^2 = 0.9956$).

Statistical Analysis

The **RStudio** (2020) software package was used to perform statistical analysis. A nonparametric Kruskal-Wallis test was performed to obtain statistically significant information about the differences among the tested samples (p < 0.05). Each variety was compared with the median value using the Wilcoxon test. To determine the relationship between individual parameters, linear regression and Spearman's correlation test ($\alpha = 0.05$) were performed.

RESULTS AND DISCUSSION

Total polyphenol content, total flavonoid content, and antioxidant activity of garlic cultivars are given in Table 1 and Table 2.

Total polyphenol content

The total polyphenol content in studied garlic cultivars ranged from 430.26 to 640.04 mg GAE.kg⁻¹ FW (966.01 to 1365.56 mg GAE.kg⁻¹ DM), with a mean value of 505.32 mg GAE.kg⁻¹ FW (1099.83 mg GAE.kg⁻¹ DM). Highest TPC was determined in the cultivar Mojmir, while the lowest TPC was determined in the cultivar Zahorsky.

Based on their TPC, an order for garlic cultivars could be as following: Zahorsky< Sukoradsky< Germidour< Arkus< Karel IV.< Makoi< Mojmir.

Similar values were determined by other authors. Lenková et al. (2016) reported TPC in garlic cultivar Mojmir 1051 mg GAE.kg⁻¹ FW. Lenková et al. (2017) reported TPC in garlic cultivars in the range from 621.13 to 763.28 mg GAE.kg⁻¹ FW. (In Zahorsky – 621.13, in Makoi – 678.18, in Mojmir – 698.82 mg GAE.kg⁻¹ FW). Lenková et al. (2018) reported TPC in garlic cultivars in the range from 566.01 to 612.23 mg GAE.kg⁻¹ FW. (In Mojmir -612.23, in Makoi - 612.21, in Zahorsky - 577.68, in Lukan - 566.01 mg GAE.kg⁻¹ FW). Kavalcová et al. (2014) reported TPC in garlic cultivars in the range from 260.62 to 279.74 mg GAE.kg⁻¹ FW. (In Mojmir - 260.62 mg GAE.kg⁻¹FW). Kovarovič et al. (2017) determined TPC in garlic cultivar Dukat 600.30 mg GAE.kg⁻¹ FW. Micová et al. (2019) reported TPC in garlic cultivars in the range from 635.1 to 742.0 mg GAE.kg⁻¹ FW. (In Zahorsky – 665.2, in Makoi - 635.1, in Mojmir - 742.0 mg GAE.kg⁻¹ FW). Bystrická et al. (2018) determined TPC in garlic cultivars in the range from 401.25 to 595.00 mg GAE.kg⁻¹ FW. Najman et al. (2020) reported TPC in garlic in the range from 562 to 728 mg GAE.kg⁻¹ DM. Khalid et al. (2014) reported 390 mg GAE.kg⁻¹ FW. Khan et al. (2016) reported 408 mg GAE.kg⁻¹ FW.

Higher TPC was determined by **Beato et al.** (2011) – 6500 mg GAE.kg⁻¹ DM, and **Zhou et al.** (2017) – 2719.3 mg GAE.kg⁻¹ FW. Škrovánková et al. (2018) determined TPC in garlic cultivars in the range from 922 to 1196 mg GAE.kg⁻¹ FW. **Kim et al.** (2013) determined lower TPC in garlic – 105.73 mg GAE.kg⁻¹ DM.

Total flavonoid content

The total flavonoid content in studied garlic cultivars ranged from 10.3 to 60.49 mg CE.kg⁻¹FW (23.12 to 129.05 mg CE.kg⁻¹DW), with a mean value of 24.75 mg CE.kg⁻¹ FW (53.65 mg CE.kg⁻¹DW).

Highest TFC was determined in the cultivar Mojmir, while the lowest TFC was determined in the cultivar Zahorsky.

Based on their TFC, an order for garlic cultivars could be as following: Zahorsky< Germidour< Sukoradsky< Arkus< Karel IV.< Makoi< Mojmir.

Similar values were determined by other authors. **Chun et al**. (**2005**) reported 54.3 mg CE.kg⁻¹ FW in garlic. **Priecina and Karlina** (**2013**) determined 89 mg CE.kg⁻¹ DM in garlic. **Bhandari et al**. (**2014**) determined TFC in garlic in the range from 100 to 219 mg CE.kg⁻¹ DM, with a mean value of 161 mg CE.kg⁻¹ DM.

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Cultivar	TPC (mg GAE.kg ⁻¹ ±SD)	TFC (mg CE.kg ⁻¹ ±SD)	AA ABTS (mmol TE.kg ⁻¹ ±SD)	AA FRAP (mmol TE.kg ⁻¹ ±SD)
Sukoradsky	456.15 ±5.93	12.73 ±0.61	1.098 ±0.021	0.63 ±0.017
Zahorsky	430.26 ± 3.65	10.29 ±0.37	1.23 ± 0.037	0.737 ± 0.018
Germidour	461.22 ± 5.91	10.86 ± 0.43	1.148 ± 0.011	0.72±0.025
Mojmir	640.04 ± 5.75	60.49 ± 0.72	1.955 ± 0.015	1.467 ± 0.018
Karel IV.	506.40 ± 4.90	26.78 ± 0.72	1.36 ± 0.013	0.888 ±0.21
Arkus	503.76 ± 4.32	20.60 ± 0.43	1.203 ± 0.018	0.791 ±0.016
Makoi	539.41 ±5.36	31.46 ±0.61	1.463 ± 0.022	1.001 ± 0.025
Mean	505.32 ± 66.06	24.75 ± 16.74	1.351 ±0.278	0.891 ± 0.266

Table 1 Total polyphenol content, total flavonoid content, and antioxidant activity of garlic (in fresh weight).

Note: TPC – total polyphenol content, TFC – total flavonoid content, AA ABTS – antioxidant activity measured by ABTS assay, AA FRAP – antioxidant activity measured by FRAP assay.

Table 2 Total polyphenol content, total flavonoid content, and antioxidant activity of garlic (in dry matter).

Cultivar	TPC (mg GAE.kg ⁻¹ ±SD)	TFC (mg KE.kg ⁻¹ ±SD)	AA ABTS (mmol TE.kg ⁻¹ ±SD)	AA FRAP (mmol TE.kg ⁻¹ ± SD)
Sukoradsky	1002.08 ±13.03	27.97 ±1.34	2.412 +0.046	1.383 ±0.039
Zahorsky	966.01 ± 8.20	23.12 ± 0.84	2.762 ± 0.083	1.655 ± 0.040
Germidour	1010.55 ±12.96	23.80 ±0.95	2.516 ±0.024	1.578 ±0.055
Mojmir	1365.56 ±12.26	129.05 ±1.53	4.17 ±0.031	3.13 ±0.039
Karel IV.	1132.63 ±10.96	59.89 ±1.6	3.043 ±0.028	1.986 ±0.047
Arkus	1026.20 ± 8.80	41.96 ±0.88	2.45 ± 0.037	1.611 ±0.034
Makoi	1195.77 ± 11.87	69.74 ±1.36	3.244 ±0.048	2.219 ± 0.054
Mean	1099.83 ±134.67	53.65 ± 35.72	2.942 ±0.591	1.937 ±0.563

Note: TPC – total polyphenol content, TFC – total flavonoid content, AA ABTS – antioxidant activity measured by ABTS assay, AA FRAP – antioxidant activity measured by FRAP assay.

Soto et al. (2015) determined TFC in garlic cultivars in the range from 70 to 110 mg $CE.kg^{-1}$ DM. **Park and Kim** (2015) determined higher TFC in garlic – 334.27 mg $CE.kg^{-1}$ DM.

Antioxidant activity using ABTS assay

Antioxidant activity in analyzed garlic cultivars measured by ABTS assay ranged from 1.098 to 1.955 mmol TE.kg⁻¹ FW (2.41 to 4.17 mmol TE.kg⁻¹DM), with a mean value of 1.35 mmol TE.kg⁻¹ FW, (2.94 mmol TE.kg⁻¹DM). The highest AA was determined in the cultivar Mojmir, while the lowest AA was determined in the cultivar Sukoradsky. Based on their TPC, an order for garlic cultivars could be as following: Sukoradsky< Germidour< Zahorsky< Arkus< Karel IV. < Makoi< Mojmir.

Azzini et al. (2014) reported AA of garlic in the range from 4.42 to 6.27 mmol TE.kg⁻¹ DM. Boonpeng et al. (2014) determined 7.62 mmol TE.kg⁻¹ FW. Other authors reported higher AA in garlic. Lu et al. (2011) reported 57.86 to 65.22 mmol TE.kg⁻¹ FW. Gorinstein et al. (2006) determined 21.4 mmol TE.kg⁻¹ FW. Gorinstein et al. (2009) 23.71 to 37.02 mmol TE.kg⁻¹ DM. Zhou et al. (2017) determined lower antioxidant activity in garlic – 0.49 mmol TE.kg⁻¹ DM.

Antioxidant activity using FRAP assay

Antioxidant activity in studied garlic cultivars measured by FRAP assay ranged from 0.63 mmol TE.kg⁻¹ FW to 1.467 mmol TE.kg⁻¹ FW (1.383 to 3.13 mmol TE.kg⁻¹ DM), with a mean value of 0.89 mmol TE.kg⁻¹ FW (1.94 mmol TE.kg⁻¹ DM). The highest AA was determined in the cultivar Mojmir, while the lowest AA was determined in the cultivar Sukoradsky. Based on their AA, an order for garlic cultivars could be as following: Sukoradsky < Germidour< Zahorsky<Arkus< Karel IV. < Makoi< Mojmir.

Nencini et al. (2011) determined 0.38 mmol TE.kg⁻¹ in aged garlic extracts. **Bhatt and Patel (2013)** determined 2.3 mmol TE.kg⁻¹ FW. **Lu et al. (2011)** reported 7.62 to 11.45 mmol TE.kg⁻¹ FW in garlic. **Gorinstein et al. (2009)** reported 6.9 to 10.8 mmol TE.kg⁻¹ DM in garlic. **Boonpeng et al. (2014)** reported lower AA of garlic – 0.01 mmol TE.kg⁻¹ FW.

As shown in Figure 1., a significantly higher content of total polyphenols and total flavonoids was observed in the cultivar Mojmir. In this cultivar, significantly higher antioxidant activity was also observed. Significantly lower total polyphenol content was observed in cultivar Zahorsky. Significantly lower total flavonoid content was also observed in cultivar Zahorsky, and cultivar Germidour. Significantly lower antioxidant activity was observed in cultivar Sukoradsky.

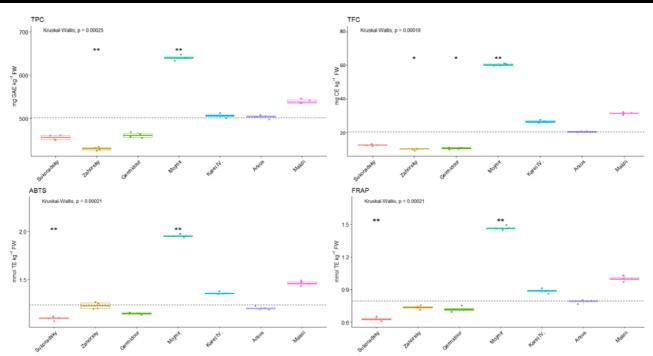


Figure 1 Differences in the content of bioactive compounds in selected garlic cultivars.

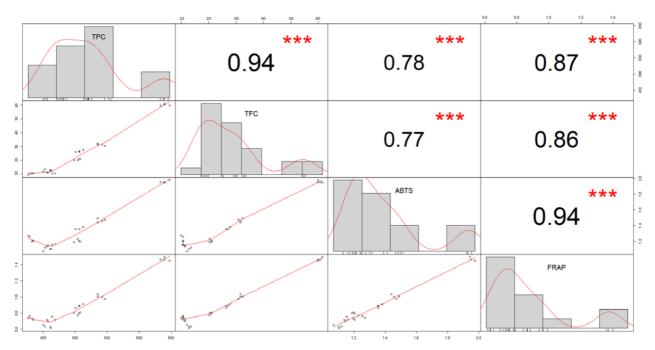


Figure 2 The relationships between the analysed parameters.

The relationships between the parameters are shown as the Spearman correlation matrix. Statistical evaluation of results confirmed strong positive linear correlation between TPC and TFC (r=0.94), TPC and ABTS (r=0.78), TPC and FRAP (r=0.87), TFC and ABTS (r=0.77), TFC and FRAP (r=0.86), and ABTS and FRAP (r=0.94). **Bhandari et al.** (2014) also determined a positive correlation between TPC and TFC of garlic. Škrovánková et al. (2018), and Locatelli et al. (2017) also determined a positive correlation between TPC and ABTS of garlic. Locatelli et al. (2017) and Chen et al. (2013) also determined a positive correlation between TPC and FRAP of garlic. Soto et al. (2015) also determined a positive correlation between ABTS and FRAP of garlic.

CONCLUSION

Total polyphenol content, total flavonoid content, and antioxidant activity of 7 garlic cultivars were determined in this study. Based on the results of the study, we can state that garlic is a natural source of polyphenols and antioxidants. Significantly higher TPC, TFC, and AA were determined in cultivar Mojmir. Significantly lower TPC and TFC were determined in cultivar Zahorsky. Significantly lower AA was determined in cultivar Sukoradsky. Based on statistical evaluation strong positive correlations

Based on statistical evaluation strong positive correlations were determined between individual studied parameters.

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Contact Address:

*Natália Čeryová, Slovak University of Agriculture, Institute of Food Science, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421376414378, E-mail: <u>xceryova@uniag.sk</u>

ORCID: https://orcid.org/0000-0002-1865-5131

Iveta Čičová, The Research Institute of Plant Production, Gene Bank of the Slovak Republic, Bratislavská cesta 122, 921 68, Piešťany, Tel.: +421337947345, E-mail: <u>iveta.cicova@nppc.sk</u>

ORCID: https://orcid.org/0000-0002-4167-3733

Judita Lidiková, Slovak University of Agriculture, Institute of Food Science, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421376414353,

E-mail: judita.lidikova@uniag.sk

ORCID: https://orcid.org/0000-0001-9922-4300

Marek Šnirc, Slovak University of Agriculture, Institute of Food Science, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421376414345,

E-mail: <u>marek.snirc@uniag.sk</u>

ORCID: <u>https://orcid.org/0000-0003-1732-0417</u>

Jarmila Horváthová, Slovak University of Agriculture, Centre of Languages, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421376414353,

E-mail: jarmila.horvathova@uniag.sk

Helena Lichtnerová, Slovak University of Agriculture, Institute of Landscape Architecture, Tulipánová 7, 949 76 Nitra, Slovakia, Tel.: +421376415443,

E-mail: Helena.lichtnerova@uniag.sk

Hana Franková, Slovak University of Agriculture, Institute of Food Science, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421376414375, E-mail: <u>xchrkavah@uniag.sk</u> ORCID: <u>https://orcid.org/0000-0003-1833-1732</u>

Corresponding author: *