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TOTAL POLYPHENOL CONTENT, TOTAL FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITY OF GARLIC (*ALLIUM SATIVUM* L.) CULTIVARS

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ABSTRACT

Garlic (*Allium sativum* L.), is used as both a food and traditional medicine for several illnesses throughout the world. 7 garlic cultivars (Novozamocky, Lumir, Mojmir, Havran, Garpel, Eden Rose, and Bjetin) were analyzed in this study. Since modern consumer demands encourage the consumption of foods with positive effects on human health, research into the nutritional properties and chemical composition of traditional crops, like garlic, is important. Knowledge of these properties could support the cultivation of cultivars with the highest content of bioactive compounds. Total polyphenol content (TPC) determined by Folin – Ciocalteau method ranged from 797.11 to 1183.98 mg GAE.kg^{-1} DM. Total flavonoid content (TFC) determined by the aluminum chloride method ranged from 15.96 to 28.18 mg CE.kg^{-1} DM.
Antioxidant activity (AA) determined by the ABTS method ranged from 1.97 to 4.42 mmol TE.kg^{-1} DM. Antioxidant activity (AA) determined by the FRAP method ranged from 1.12 to 2.64 mmol TE.kg^{-1} DM. Statistical evaluation of results showed a difference between garlic cultivars. The highest antioxidant activity and the highest content of bioactive substances were determined in the cultivar Garpel. High positive correlations ($p < 0.001$) were determined between TPC and TFC ($r = 0.746$), TPC and AA ABTS ($r = 0.862$), TPC and AA FRAP ($r = 0.922$), TFC and AA ABTS ($r = 0.792$), TFC and AA FRAP ($r = 0.808$), and AA ABTS and AA FRAP ($r = 0.896$). Garlic is confirmed to be a natural source of antioxidants, polyphenols, and flavonoids.

Keywords: *Allium sativum* L.; garlic; polyphenols; flavonoids; antioxidants

INTRODUCTION

Garlic (*Allium sativum* L.) is an aromatic herbaceous plant, used as food and as a traditional medicine for several ailments. The use of garlic as a food and as a medicine has its earliest origins in Asia. Garlic was a common treatment in traditional Chinese medicine for breathing problems, diarrhea, and worm infestation. Many ancient Indians, Egyptians, Greeks, and Romans consumed garlic as part of their daily diets. Antioxidant, antibacterial, anticarcinogenic, antihypertensive, antidiabetic, anti-atherosclerotic, and renoprotective capabilities are only a few of the biological effects that have been linked to it **(Rivlin, 2001; El-Saber Batiha et al., 2020)**.
Garlic products showed to be effective in reducing central and peripheral blood pressure and modification of endothelial biomarkers associated with cardiovascular risk. It can also help prevent chronic inflammation and the development of chronic diseases associated with low-grade inflammation in adults with obesity, reduce the severity of cold and flu illness, reduce oxidative stress, and prevent diseases such as osteoporosis **(Tavares et al., 2021).**
According to recent studies, garlic can lower the incidence of colon, esophageal, larynx, oral, ovary, prostate, and renal cell cancers. Additionally, garlic may reduce the symptoms of several kinds of cancer conditions, including lung, breast, gastric, pancreatic, colon, and colorectal cancer **(Ansary et al., 2020).** Convincing evidence has been reported for a correlation between garlic consumption and a significant reduction of the risk of diabetes, hyperlipidemia, atherosclerosis, hypertension, ischemic stroke, and myocardial infarction **(Zhu et al., 2018)**.
The therapeutic effects of garlic are mainly due to its bioactive compounds, such as organosulphur compounds, phenolic compounds, saponins, and polysaccharides **(Ansary et al., 2020)**. Bioactive compounds of garlic show many biological functions, such as antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, cardiovascular protective, hepatoprotective, neuroprotective, renal protective, digestive system protective, immunomodulatory, anti-diabetic, and anti-obesity effects. Due to these effects and low to no toxicity, garlic is a promising ingredient in developing nutraceuticals or functional foods for the treatment and prevention of various ailments **(Shang et al., 2019).** In addition to being the main plant compounds with antioxidant activity, several phenolic compounds also exhibit antifungal and antibacterial properties and have a significant impact on the textures and flavors of food products **(Lourenço et al., 2019).**
Genetic factors, growing conditions, such as the environment, cultivation techniques, and fertilizer, as well as storage conditions and technical processing, all have an impact on the amount of phenolic compounds in garlic. Cultivar selection may be a good strategy to raise the quality of garlic, thus it's critical to discover garlic cultivars with high phenolic content and antioxidant activity as well as growing conditions that may promote these qualities **(Petropoulos et al., 2018).** The present study aimed to determine and compare the polyphenol and flavonoid content, and antioxidant activity of different garlic cultivars grown under the same conditions.

MATERIAL AND METHODS

Plant material

7 cultivars of garlic, namely Novozamocky, Lumir, Mojmir, Havran, Garpel, Eden Rose, and Bjetin were collected in the state of full ripeness. All samples were grown under the same agroenvironmental conditions, in the Experimental Garden of the Slovak University of Agriculture in Nitra, Slovakia. Nitra lies in a warm and dry climate region, with average annual precipitation of 550-600 mm. Soil from the growing area is medium heavy, sandy-loamy with neutral soil reaction.

Extract preparation

100 g of peeled garlic cloves were homogenized using a Microtron MB 550 laboratory mixer at 11,000 rpm for 2 minutes. Extracts were prepared by shaking 25 g of homogenized sample in 50 g of 80% methanol on Heidolph Promax 1020 horizontal shaker (Heidolph Instruments GmbH, Schwabach, Germany) for 16 hours at room temperature. The extracts were filtered through Munktell No. 392 filtrating paper (Munktell & Filtrac GmbH, Bärenstein, Germany) and stored at 4 °C before analysis.

Dry matter analysis

Samples were subjected to dry matter analysis on a Kern DLB moisture analyzer (Kern & Sohn GmbH, Albstadt, Germany).

Total polyphenol content

For the total polyphenol content determination, Folin – Ciocalteau phenol reagent, 20% Na_2CO_3, and distilled water were used (**Lachman et al., 2003)**. Into a 50 mL volumetric flask, 0.1 mL of extract was pipetted, diluted with distilled water, and 0.85 mL of Folin – Ciocalteau reagent was added. After 3 minutes, 5 mL of 20% Na_2CO_3 was added. Then, the volume was made up to 50 mL with distilled water. A blank solution was prepared at the same time. Flasks were then left for 2 hours at laboratory temperature. Absorbance was measured using Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan) against a blank solution at 765 nm.
Based on the calibration curve (R^2 = 0.995), total polyphenol content was expressed as mg of gallic acid equivalent (GAE) in 1 kg of fresh garlic.

Total flavonoid content

For the total flavonoid content, 5% $NaNO_2$, 10% $AlCl_3$, 1M NaOH, and distilled water were used **(Chang et al., 2002)**. Into a 10 mL volumetric flask, 1 mL of extract was pipetted, diluted with 5 mL of distilled water, and 0.3 mL of 5% $NaNO_2$ was added. After 6 minutes, 0.6 mL of 10 % $AlCl_3$ was added. After 5 minutes, 2 mL of 1M NaOH was added. Then, the volume was made up to 10 mL with distilled water, and the solution was mixed on a vortex. A blank solution was prepared at the same time. Flasks were then left for 15 minutes at laboratory temperature. Absorbance was measured using Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan) against a blank solution at 510 nm.
Based on the calibration curve (R^2 = 0.998), total flavonoid content was expressed as mg of catechin equivalent (CE) in 1 kg of fresh garlic.

Antioxidant activity using ABTS assay

For the antioxidant activity determination, ABTS•+ radical cation - (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate ($K_2S_2O_8$), and acetate buffer (pH= 4.3) were used **(Re et al., 1999)**. By the reaction of ABTS•+ solution, $K_2S_2O_8$ solution, and acetate buffer, a working ABTS solution was produced. Into 3 mL of working ABTS solution, 0.05 mL of extract was pipetted and stirred. A blank solution was prepared at the same time. The solution was then left for 20 minutes at laboratory temperature. Absorbance was measured using Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan) against a blank solution at 734 nm.
Based on the calibration curve (R^2 = 0.995), antioxidant activity was expressed as mmol of Trolox equivalent (TE) in 1 kg of fresh garlic.

Antioxidant activity using FRAP assay

For the antioxidant activity determination, TPTZ - (2,4,6-tris(2-pyridyl)-S-triazine), ferric chloride ($FeCl_3$), and acetate buffer (pH= 3.5) were used **(Pedersen et al., 2000)**. By the reaction of TPTZ solution, $FeCl_3$ solution, and acetate buffer, a working FRAP solution was produced. Into 3 mL of working FRAP solution, 0.05 mL of extract was pipetted and stirred. A blank solution was prepared at the same time. The solution was then left for 20 minutes at laboratory temperature. Absorbance was measured using Shimadzu UV- 1800 UV/ Visible Scanning Spectrophotometer (Shimadzu, Japan) against a blank solution at 593 nm.
Based on the calibration curve (R^2 = 0.997), antioxidant activity was expressed as mmol of Trolox equivalent in 1 kg of fresh garlic.

Statistical Analysis

Statistical analysis was performed using Jamovi software version 2.3.9. For the comparison between the tested variables, the non-parametric ANOVA test (Kruskal-Wallis) and Dunn pairwise test with Holm correction was used. To determine the relationships between individual parameters, the Spearman correlation was used **(The jamovi project, 2021; R Core Team, 2021; Brunson, 2019; Koneswarakantha, 2019; Patil, 2018; Wickham et al., 2018).**

RESULTS AND DISCUSSION

The total polyphenol content, total flavonoid content, and antioxidant activity of analyzed garlic cultivars in fresh weight and dry matter are presented in Table 1 and Table 2.

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

Cultivar	TPC (mg GAE.kg^{-1})	TFC (mg CE.kg^{-1})	AA ABTS (mmol TE.kg^{-1})	AA FRAP (mmol TE.kg^{-1})
Bjetin	380.05±4.80	7.49±0.61	1.01±0.07	0.58±0.02
Eden Rose	434.02±5.17	8.99±0.61	1.20±0.06	0.74±0.02
Garpel	472.11±7.36	11.24±1.06	1.76±0.07	1.05±0.09
Havran	430.93±5.18	9.93±0.72	1.59±0.05	0.87±0.02
Mojmir	367.11±4.55	8.05±0.72	0.91±0.02	0.51±0.01
Novozamocky	443.12±4.62	9.55±0.72	1.46±0.05	1.00±0.09
Lumir	379.73±3.39	8.61±0.75	0.94±0.07	0.62±0.04
Mean	415.29±37.64	9.12±1.35	1.27±0.33	0.77±0.21

Legend: 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 analyzed garlic cultivars (in dry matter)

Cultivar	TPC (mg GAE.kg^{-1})	TFC (mg CE.kg^{-1})	AA ABTS (mmol TE.kg^{-1})	AA FRAP (mmol TE.kg^{-1})
Bjetin	809.90±10.12	15.96±1.31	2.15±0.14	1.23±0.03
Eden Rose	994.20±12.11	20.59±1.40	2.75±0.14	1.69±0.04
Garpel	1183.98±18.75	28.18±2.66	4.42±0.18	2.64±0.22
Havran	1004.14±12.16	23.13±1.67	3.71±0.11	2.03±0.04
Mojmir	922.89±8.20	20.94±1.83	2.29±0.16	1.50±0.10
Novozamocky	797.11±9.92	17.48±1.56	1.97±0.04	1.12±0.03
Lumir	966.99±10.04	20.84±1.57	3.19±0.11	2.18±0.21
Mean	954.17±124.30	21.02±4.04	2.93±0.86	1.77±0.53

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

Total Polyphenol Content

Total polyphenol content in analyzed garlic cultivars varied from 367.11 to 472.11 mg GAE.kg^{-1} FW (797.11 – 1183.98 mg GAE.kg^{-1} DM), with a mean value of 415.29 mg GAE.kg^{-1} FW (954.17 mg GAE.kg^{-1} DM). The lowest TPC was determined in the cultivar Mojmir, and the highest TPC was determined in the cultivar Garpel. Similar values were reported by **Khalid et al. (2014)** – 390 mg GAE.kg^{-1} FW, and **Khan et al. (2016)** – 408 mg GAE.kg^{-1} FW. In our previous study **(Čeryová et al., 2021)**, we determined 430.26 – 640.04 mg GAE.kg^{-1} FW (966.01 – 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), 640.04 mg GAE.kg^{-1} (1365.56 mg GAE.kg^{-1} DM) in Mojmir.) Higher values were reported by **Denre et al. (2013)** – from 2710 to 5190 mg GAE.kg^{-1} FW, **Lenková et al. (2017)** – from 621.13 to 763.28 mg GAE.kg^{-1} FW (698.82 mg GAE.kg^{-1} FW in Mojmir), **Kovarovič et al. (2017)** – 600.30 mg GAE.kg^{-1} FW, **Lenková et al. (2018)** – from 566.01 to 612.23 mg GAE.kg^{-1} FW (612.23 mg GAE.kg^{-1} FW in Mojmir, 763.28 mg GAE.kg^{-1} FW in Havran), **Bystrická et al. (2018)** – from 401.25 to 595.00 mg GAE.kg^{-1} F, and **Micová et al. (2019)** – from 635.1 to 742.0 mg GAE.kg^{-1} FW in garlic cultivars (742.0 mg GAE.kg^{-1} FW in Mojmir). Even higher values were reported by **Lenková et al. (2016)** – 1051 mg GAE.kg^{-1} FW in cultivar Mojmir, **Zhou et al. (2017)** – 2719.3 mg GAE.kg^{-1} FW, and **Škrovánková et al. (2018)** – from 922 to 1196 mg GAE.kg^{-1}. Lower values were reported by **Kavalcová et al. (2014)** – from 260.62 to 279.74 mg GAE.kg^{-1} FW (260.62 mg GAE.kg^{-1} FW in Mojmir.)

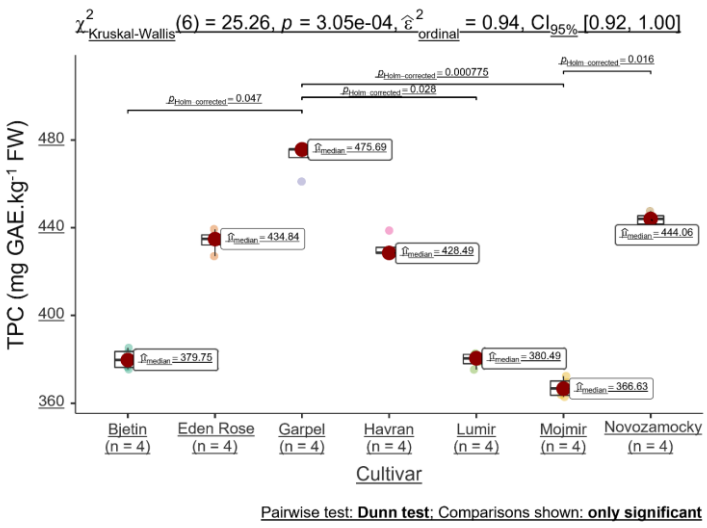

Figure 1 Differences in the content of TPC in selected garlic cultivars

As shown in Figure 1, statistical differences in TPC were observed between cultivars Bjetin and Garpel ($p_{Holm\text{-}corrected}$ = 0.047), Garpel and Lumir ($p_{Holm\text{-}corrected}$ = 0.028), Garpel and Mojmir ($p_{Holm\text{-}corrected}$ = 0.001), and Mojmir and Novozamocky ($p_{Holm\text{-}corrected}$ = 0.016).

Total Flavonoid Content

Total flavonoid content (TFC) in analyzed garlic cultivars varied from 7.49 to 11.24 mg CE.kg^{-1} FW (15.96 – 28.18 mg CE.kg^{-1} DM), with a mean value of 9.12 mg CE.kg^{-1} FW (21.02 mg CE.kg^{-1} DM). The lowest TFC was determined in the cultivar Bjetin, and the highest TFC was determined in the cultivar Garpel. In our previous study **(Čeryová et al., 2021)**, we determined 10.3 – 60.49 mg CE.kg^{-1} FW (23.12 – 129.05 mg CE.kg^{-1} DM), with a mean value of 24.75 mg CE.kg^{-1} FW (53.65 mg CE.kg^{-1} DM), 60.49 mg GAE.kg^{-1} (129.05 mg CE.kg^{-1} DM) in Mojmir. Higher values were determined by **Chun et al. (2005)** – 54.3 mg CE.kg^{-1} FW, **Priecina and Karlina (2013)** – 89 mg CE.kg^{-1} DM, **Bhandari et al. (2014)** – from 100 to 219 mg CE.kg^{-1} DM, **Soto et al. (2016)** – from 70 to 110 mg CE.kg^{-1} DM, and **Park and Kim (2015)** – 334.27 mg CE.kg^{-1} DM.

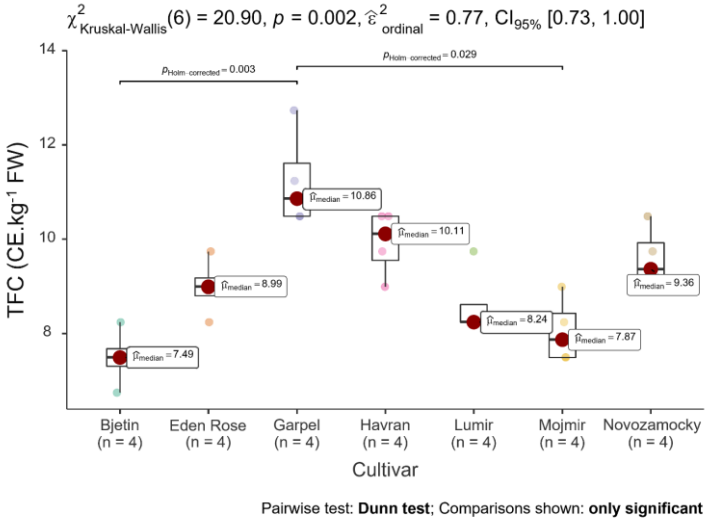

Figure 2 Differences in the content of TFC in selected garlic cultivars
As shown in Figure 2, statistical differences in TFC were observed between cultivars Bjetin and Garpel ($p_{Holm\text{-}corrected}$ = 0.003), and Garpel and Mojmir ($p_{Holm\text{-}corrected}$ = 0.029).

Antioxidant Activity using ABTS assay

Antioxidant activity measured by ABTS assay (AA ABTS) varied from 0.91 to 1.76 mmol TE.kg^{-1} FW (1.97 – 4.42 mmol TE.kg^{-1} DM), with a mean value of 1.27 mmol TE.kg^{-1} FW (2.93 mmol TE.kg^{-1} DM). The lowest AA ABTS was determined in the cultivar Mojmir, and the highest AA ABTS was determined in the cultivar Garpel. In our previous study **(Čeryová et al., 2021),** we determined 1.098 – 1.955 mmol TE.kg^{-1} FW (2.41 – 4.17 mmol TE.kg^{-1} DM), with a mean value of 1.35 mmol TE.kg^{-1} FW (2.94 mmol TE.kg^{-1} DM), 1.955 mmol TE.kg^{-1} FW (4.17 mmol TE.kg^{-1} DM) in Mojmir. Slightly higher values were reported by **Azzini et al. (2014)** – from 4.42 to 6.27 mmol TE.kg^{-1} DM. Higher values were reported by **Gorinstein et al. (2006)** – 21.4 mmol TE.kg^{-1} FW, **Gorinstein et al. (2009)** – from 23.71 to 37.02 mmol TE.kg^{-1} DM, **Lu et al. (2011)** – from 57.86 to 65.22 mmol TE.kg^{-1} FW, and **Boonpeng et al. (2014)** – 7.62 mmol TE.kg^{-1} FW. Lower values were reported by **Zhou et al. (2017)** – 0.49 mmol TE.kg^{-1} DM.

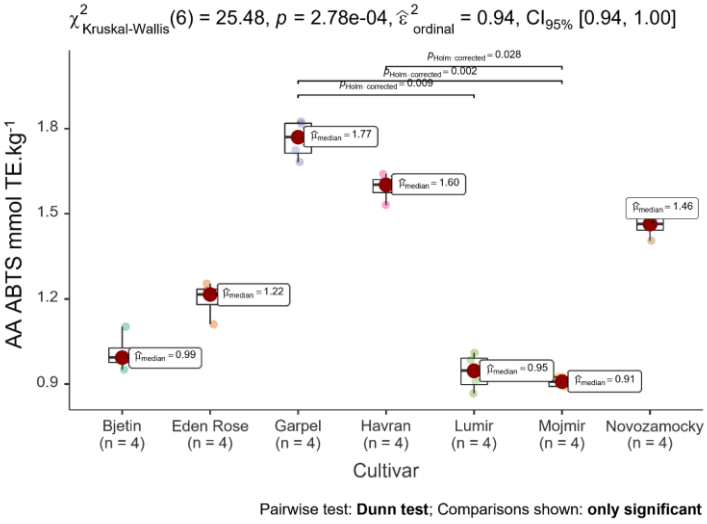

Figure 3 Differences in the content of AA ABTS in selected garlic cultivars
As shown in Figure 3, statistical differences in AA ABTS were observed between cultivars Garpel ($p_{Holm\text{-}corrected}$ = 0.04), Garpel and Mojmir ($p_{Holm\text{-}corrected}$ = 0.002), and Havran and Mojmir ($p_{Holm\text{-}corrected}$ = 0.028).

Antioxidant activity using FRAP assay

Antioxidant activity measured by FRAP assay (AA FRAP) varied from 0.51 to 1.05 mmol TE.kg^{-1} FW (1.12 – 2.64 mmol TE.kg^{-1} DM). The lowest AA FRAP was determined in the cultivar Mojmir, and the highest AA FRAP was determined in the cultivar Garpel. In our previous study **(Čeryová et al., 2021)** we reported 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). Higher values were reported by **Gorinstein et al. (2009)** – from 6.9 to 10.8 mmol TE.kg^{-1} DM, **Lu et al. (2011)** – from 7.62 to 11.45 mmol TE.kg^{-1} FW, and **Bhatt and Patel (2013)** – 2.3 mmol TE.kg^{-1} FW. Lower values were reported by **Boonpeng et al. (2014)** – 0.01 mmol TE.kg^{-1} FW.

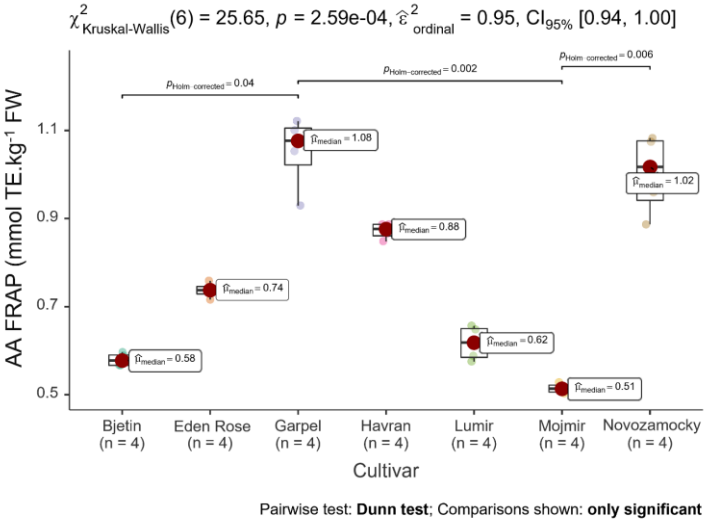

Figure 4 Differences in the content of AA FRAP in selected garlic cultivars
As shown in Figure 4, statistical differences in AA FRAP were observed between cultivars Bjetin and Garpel ($p_{Holm\text{-}corrected}$ = 0.009), Garpel and Mojmir ($p_{Holm\text{-}corrected}$ = 0.002), and Mojmir and Novozamocky ($p_{Holm\text{-}corrected}$ = 0.006).

Based on the results, and their comparison with the literature, it can be indicated that the content of bioactive compounds, as well as the antioxidant activity of garlic, can vary widely depending on a combination of factors such as cultivar and growing conditions, harvest time, processing and storage conditions, and other factors, which can all contribute to the differences in these parameters.

Relationships between analyzed parameters

In general, it has been demonstrated that both polyphenols and flavonoids have strong antioxidant properties. As a result, a plant with a high amount of either polyphenols or flavonoids may have high antioxidant potential. However, the exact relationship between the total polyphenol and flavonoid content and the antioxidant activity depends significantly on the plant species, the specific polyphenols and flavonoids present, and the conditions in which the plant was grown and processed. High positive correlations ($p < 0.001$) were determined between TPC and TFC (r = 0.746), TPC and AA ABTS (r = 0.862), TPC and AA FRAP (r = 0.922), TFC and AA ABTS (r = 0.792), TFC and AA FRAP (r = 0.808), and AA ABTS and AA FRAP (r = 0.896). These results are in agreement with our previous study **(Čeryová et al., 2021)**, and with other authors who also reported a positive relationship between total polyphenol and total flavonoid content of garlic **(Bhandari et al., 2014)**, between total polyphenol content and antioxidant activity of garlic **(Leelarungrayub et al., 2009; Chen et al., 2013; Locatelli et al., 2017; Škrovánková et al., 2018),** and between antioxidant activity of garlic determined by ABTS and FRAP assay **(Soto et al., 2016).**

Table 3 Correlation matrix

	TPC		TFC		AA ABTS		AA FRAP
TPC	—						
TFC	0.746	***	—				
AA ABTS	0.862	***	0.792	***	—		
AA FRAP	0.922	***	0.808	***	0.896	***	—

Note. *** $p < .001$

CONCLUSION

Garlic is confirmed to be a natural source of antioxidants, polyphenols, and flavonoids. The highest antioxidant activity and the highest content of bioactive compounds were determined in the cultivar Garpel. Statistical evaluation of results showed a difference between garlic cultivars, from which we can conclude that cultivar has an impact on the antioxidant activity, total polyphenol content, and total flavonoid content of garlic, however, there are probably other influences, such as growing conditions, storage, and technology processing, that must be taken into account. Therefore, further research on these influences is important to recognize conditions that may increase the content of bioactive compounds in garlic.

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