

## A comparative study of the content of some antioxidants in the fruits of autochthonous pomegranate varieties

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### Abstract

The contents of total phenolics, anthocyanins, flavan-3-ols, and the fruit antioxidant activity of 9 pomegranate varieties ('Zumnarija', 'Bejnarija', 'Valandovska kisela', 'Valandovska kiselo-slatka', 'Hidjas', 'Kisela', 'Karamustafa', 'Lifanka', and 'Ropkavec') were examined. Autochthonous pomegranate varieties contained 4354.4 – 6480.9 mg/kg FW total phenols, 160 – 698.2 mg/kg FW anthocyanins, 96.9 – 157.2 mg/kg FW flavan-3-ols, and high antioxidant activity of fruits (71.2 – 92.5% inhibition). The 'Valandovska kisela' variety had the highest content of total phenolics (6480.87 mg/kg FW), flavan-3-ols (157.190 mg/kg FW) and higher fruit antioxidant activity (92.5% inhibition) compared to the standard 'Karamustafa' variety and the other autochthonous varieties. The 'Hidjas' variety had the highest content of anthocyanins (698.20 mg/kg FW). A positive correlation (0.9815) was found between the content of total phenolics and the fruit antioxidant activity. A moderate positive, insignificant correlation was also found between the content of flavan-3-ols and anthocyanins.

*Key words:* Autochthonous pomegranate varieties, Total phenolics, Anthocyanins, Flavan-3-ols, Antioxidant activity.

### Introduction

Fruit is a rich source of antioxidants that have a preventive, curative, and therapeutic effect on human health, scavenging free radicals that cause cancer

and degenerative diseases. The content of antioxidants depends on the genotype, environmental conditions, agricultural practices, fruit storage, and processing (Bassi et al., 2017).

Polyphenolics are very important secondary metabolites in fruit which have antioxidant, anti-inflammatory, and anti-cancer effects (Hertog et al., 1993; Goldner et al., 2015), anti-mutagenic, antiallergenic, antimicrobial action, provide protection against infections, reduce the risk of chronic diseases, cardiovascular, and neurodegenerative diseases (Vauzour et al., 2010). More than 40 phenolic compounds have been identified. Flavonoids are also another class of plant secondary metabolites, classified into 12 subclasses in terms of chemical structure; some of these groups are flavonols, flavanols, flavones, isoflavonoids, etc. They are most common in fruit (especially berry fruits) and grapes. Some of them contribute to the formation of fruit colour and taste, others protect plants from various biotic factors (predators and pests), and abiotic factors (frost, drought) (Panche et al., 2016). Flavonols belong to flavonoids and represent building blocks of proanthocyanidins. They are found in apples, strawberry fruits, and grapes. Flavanols or flavan-3-ols are important secondary metabolites that show high antioxidant activity. They are a large family of phenolic compounds, mainly responsible for the astringency, bitterness, and nutrient structure. Flavan-3-ols include catechins and their derivatives (catechin, epicatechin, epigallocatechin, gallic acid, gallic acid-3-O-gallate). They are mostly present in apple, blueberry, strawberry, and grape (Ullah et al., 2017).

Anthocyanins are a group of over 500 different compounds that contribute to the red, purple, and blue colour of many plants, especially fruit and vegetables, in the form of heterosides. Anthocyanin aglycones are known as anthocyanidins. The most important representatives of the group of anthocyanidins found in fruit and grapes are: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. They are most common in berry fruit species (black currant, blueberry, strawberry, raspberry), grapes, and some tropical species (Paz & Fredes, 2015; Khoo et al., 2017).

Macedonia is plentiful in autochthonous fruit varieties which represent a rich starting material in the further selection process. The aim of this study is to detect and determine the content of specific biologically active substances (antioxidants) in the fruits of each autochthonous pomegranate variety.

## Material and methods

The study was part of the scientific project *Antioxidant activity of fruits of autochthonous varieties and populations of fruits, vegetables and grapes*. The laboratory tests were performed in the laboratory at the Institute of Agriculture in Skopje.

Nine autochthonous varieties of pomegranate ('Zumnarija', 'Bejnarija', 'Valandovska kisela', 'Valandovska kiselo-slatka', 'Hidjas', 'Kisela', 'Karamustafa',

‘Lifanka’, and ‘Ropkavec’), were taken as material for analysis. Comparative studies were performed in relation to the standard ‘Karamustafa’ variety.

The contents of total phenolics, anthocyanins, and flavan-3-ols were determined by the spectrophotometric method, expressed in mg/kg FW. Their content was determined with the Agilent 8453 UV-VIS spectrophotometer. Total phenolics in the samples were determined by the Folin-Ciocalteu method (Slinkard and Singelton, 1977). The content of total anthocyanins was determined according to the acid ethanol method (Somers and Evans, 1977). *p*-dimethylaminocinnamaldehyde (*p*-DMACA) was used to quantify total flavan-3-ols in the tested samples (Di Stefano et al., 1989). The absorbance was measured at 640 nm. Defining of the antioxidant activity was performed as an anti-radical activity against stable product DPPH (2,2-diphenyl-1-picrylhydrazil). It was determined spectrophotometrically, at 517 nm.

A correlation analysis was carried out between the examined parameters using the XLStat software 2014 5.03. The coefficient of determination  $R^2$  and certain general standard parameters, such as maximum and minimum values, average value, and standard deviation for each of the parameters were calculated.

## Results and discussion

In Table 1, results from the chemical analysis performed on the fruits of the autochthonous varieties of pomegranate are given.

Tab. 1. Chemical analysis of fruits of some indigenous pomegranate varieties

Variety	Total phenolics (mg/kg FW)	Anthocyanins (mg/kg FW)	Flavan-3-ols (mg/kg FW)	Antioxidant activity (% inhibition)
‘Zumnarija’	4354.4	169.3	139.2	71.2
‘Bejnarija’	5562.3	216.4	108.4	82.9
‘Valandovska kisela’	6480.9	275	157.2	92.5
‘Valandovska kiselo-slatka’	5775.5	308.6	106.6	85.5
‘Hidjas’	5132.7	698.2	150.2	79.8
‘Kisela’	5064.5	358.2	108.9	80.9
‘Lifanka’	4505.5	160	112.8	72.8
‘Ropkavec’	5500.2	238.5	96.9	82.3
‘Karamustafa’-standard	5859	490	122.4	86.3
Average	5359.4	323.8	122.5	81.6
Standard deviation	672.6	173.6	21.3	6.6
Standard error	224.1934	57.8681	7.1076	2.1973

The content of anthocyanins in the fruits of the autochthonous pomegranate varieties varied from 160 to 698.2, average 323.78 mg/kg FW. The ‘Hidjas’ variety had the highest content of anthocyanins (698.2 mg/kg FW). Due

to its high content of anthocyanins, it was necessary to add a larger amount of activated carbon to the tested solution for decolourization.

The accumulation of anthocyanins is primarily influenced by genetic and external factors (light, temperature, etc.). The high amount of anthocyanins was also found in other fruits: chokeberry (300-2000 mg/100g), blueberry (300-698 mg/100g), blackberry (82.5-325.9 mg/100g), and pomegranate (15-252 mg/100g) (Ćujić et al., 2013; Selamovska & Miskoska-Milevska, 2021). According to Dumlu and Gürkan (2007), the average content of anthocyanins in 20 tested varieties of pomegranate was found to be between 2100-4400 mg/L. Kaur and Kapoor (2005) found a positive correlation between the content of anthocyanins and phenolics in pomegranate.

As expected, the autochthonous varieties of pomegranate had higher content of total phenolics (4354.4 – 6480.9 mg/kg FW). The ‘Valandovska kisela’ variety had the highest content of total phenolics (6480.9 mg/kg FW). A positive correlation (0.9907) was found between the content of total phenolics and the antioxidant activity of the fruits.

Kaur and Kapoor (2005) found a positive correlation between the content of phenolics and anthocyanins in some fruit species, while Murillo et al. (2012) found a positive correlation between polyphenol content and the antioxidant activity.

The content of flavan-3-ols in the fruits of the autochthonous varieties was 96.9 – 157.2 mg/kg FW. The highest content of flavan-3-ols compared to the standard variety and the other autochthonous varieties was measured in ‘Valandovska kisela’ (157.2 mg/kg FW), ‘Hidjas’ (150.2 mg/kg FW), and ‘Zumnarija’ (139.2 mg/kg FW). The accumulation of flavan-3-ols is primarily influenced by genetic and external factors. According to Mikulic-Petkovsek et al. (2016), bird cherry had higher content of total phenolics (11053 mg GAE/kg) than wild cherry (2373 mg GAE/kg).

According to the results of the study, the pomegranate fruits showed very high antioxidant activity (81.6% inhibition). The ‘Valandovska kisela’ variety had the highest antioxidant activity (92.5% inhibition). A positive correlation was found between the content of total phenolics and the antioxidant activity of the fruits.

The antioxidant activity of the fruits depended on the type and age of the plant material. The greatest antioxidant activity was found in strawberries, black raspberry (*Rubus occidentalis* L. – jewel variety), blackberry, and red raspberry. In some cherry varieties of the species *Elaeagnus umbellata*, the antioxidant activity was 4.32-9.49 mM TE/100g (Lachowicz et al., 2019), by other authors for bird cherry (*Prunus padus*) it was 7.26-31.54 mM/kg (Mikulic-Petkovsek et al., 2016), and in apple and strawberry it was 12- 64 mM FRAP (Kaur & Kapoor, 2005).

In Tables 2, 3, and 4, the correlations between the examined parameters in the autochthonous pomegranate varieties are given.

A statistically significant positive correlation (0.9907) was found between the content of total phenolics and the antioxidant activity in pomegranate fruits.

A moderate positive, insignificant correlation was also found between the content of flavan-3-ols and anthocyanins (0.3760) in pomegranate fruits. Among the other parameters there is little or no correlation ( $>0.2557$ ) (Table 2).

Tab. 2. Correlations between the tested parameters

Variables	Phenolics	Anthocyanins	Flavan-3-ols	Antiox.activity
Phenolics	1			
Anthocyanins	0.2016	1		
Flavan-3-ols	0.1305	0.3760	1	
Antiox.activity	<b>0.9907</b>	0.2557	0.1337	1

*Values in bold are different from 0 with a significance level  $\alpha = 0.05$*

The p-value that reflected the dependence between the total phenolics and the antioxidant activity was significantly lower (0.0001) than the given  $\alpha = 0.05$  which showed high statistical significance (Table 3).

Tab. 3. p values between the tested parameters

Variables	Phenolics	Anthocyanins	Flavan- 3-ols	Antiox. Activity
Phenolics	0			
Anthocyanins	0.6030	0		
Flavan-3-ols	0.7378	0.3186	0	
Antiox.activity	<b>&lt;0.0001</b>	0.5066	0.7317	0

*Values in bold are different from 0 with a significance level  $\alpha = 0.05$*

The highest determination coefficient of almost 98% was observed between total phenolics and antioxidant activity, i.e., about 98% of the variations in the value of the antioxidant activity were due to the variations in the value of phenolics. Considering other parameters, the coefficient of determination was approximately equal to zero (Table 4).

Tab. 4. Coefficient of determination  $R^2$  between the tested parameters

Variables	Phenolics	Anthocyanins	Flavan-3-ols	Antiox. Activity
Phenolics	1			
Anthocyanins	0.0406	1		
Flavan-3-ols	0.0170	0.1414	1	
Antiox. Activity	0.9815	0.0654	0.0179	1

## Conclusion

The autochthonous Macedonian pomegranate varieties proved to have high contents of specific biologically active substances (antioxidants) and high fruit antioxidant activity. The 'Valandovska kisela' variety had the highest content of total phenolics, flavan-3-ols, and the highest fruit antioxidant activity, compared to the standard 'Karamustafa' variety and the other autochthonous varieties. The 'Hidjas' variety had the highest content of anthocyanins.

A statistically significant positive correlation (0.9815) was found between the content of total phenolics and antioxidant activity of fruit extract. A moderate positive, insignificant correlation (0.3760) was also found between the content of flavan-3-ols and anthocyanins.

After the performed analysis, the autochthonous pomegranate varieties showed high quality characteristics and can be considered as a good starting material in the further selection process.

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## Упоредни приказ садржаја неких антиоксидативних супстанци у плодовима аутохтоних сорти нара

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### Сажетак

Циљ истраживања био је да се испита садржај укупних фенола, антоцијана, флаван-3-ола и антиоксидативно дјеловање плодова девет сорти нара ('Зумнарија', 'Бејнарија', 'Валандовска кисела', 'Валандовска кисело-слатка', 'Хидјас', 'Кисела', 'Карамустафа', 'Лифанка' и 'Ропкавец'). Аутохтоне сорте нара садржале су 4354,4 – 6480,9 mg/kg FW укупних фенола, 160 – 698,2 mg/kg FW антоцијанина, 96,9 – 157,2 mg/kg FW флаван-3-ола, те високу антиоксидативну активност плодова (71,2 – 92,5% инхибиције). Сорта 'Валандовска кисела' имала је највећи удио укупних фенола (6480,87 mg/kg ТМ), флаван-3-ола (157,190 mg/kg ТМ) и већу антиоксидативну активност плодова (92,5% инхибиције) у односу на стандардну сорту 'Карамустафа' и остале аутохтоне сорте. Највиши садржај антоцијана имала је сорта 'Хидјас' (698,20 mg/kg ТМ). Утврђена је позитивна корелација (0,9815) између садржаја укупних фенола и антиоксидативне активности плодова. Умјерена позитивна, али не и значајна корелација такође је утврђена између садржаја флаван-3-ола и антоцијана.

*Кључне ријечи:* аутохтоне сорте нара, укупни феноли, антицијанини,  
флаван-3-оли, антиоксидативна активност

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