

Phytochemical Profiles and Antioxidant Activities of Serbian Table and Wine Grapes

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Abstract Three table grapes and 4 wine grapes collected from a southern Serbian vineyard were evaluated and compared for their antioxidant properties and phenolic profile. Among the varieties tested, ‘Cabernet Sauvignon’ contained the highest total phenolic content with 173.6 mg/100 g of fresh weight. Also, the total flavonoid and anthocyanin content of ‘Cabernet Sauvignon’ was significantly higher from the other. ‘Cabernet Sauvignon’ and ‘Merlot’ had the strongest DPPH· radicals scavenging activity (1,318.6 and 1,282.0 μmol Trolox equivalent/100 g, respectively). ‘Cabernet Sauvignon’, ‘Merlot’, ‘Prokupac’, ‘Vranac’, ‘Muscat Hamburg’, and ‘Ribier’ grape varieties were found to be rich in malvidin-3-*O*-glucoside, while ‘Cardinal’ grape variety was found to be rich in peonidin-3-*O*-glucoside. The following compounds were also identified and quantified using HPLC-DAD: 2 flavan-3-ols and 4 hydroxycinnamic acids in all grape samples. The results suggested that phytochemicals in the selected table and wine grapes have potent antioxidant activities in correlation with phenolic content.

Keywords: phenolics, antioxidant, anthocyanin, hydroxycinnamate, flavan-3-ol

Introduction

Phenolic compounds are responsible for the antioxidant

activity of several vegetables, thus are potential inhibitors of carcinogens, acting in different stages of the pathological process of initiation of tumors and may be relevant in the prevention of cardiovascular disorders (1,2).

Among fruits and vegetables, grapes (*Vitis vinifera*) are considered as the largest source of phenolic compounds. Grape berries are consumed as table fruit, wine, juice, and raisins. Approximately 80% of all grapes are used in winemaking, and 13% are consumed as table grapes (3). Grape phenolics are classified into 2 groups: the flavonoids and nonflavonoids. The flavonoids include flavan-3-ols (catechin), flavonols, (quercetin), and anthocyanins. The nonflavonoids encompass hydroxybenzoates (gallic acid), hydroxycinnamates, and stilbenes. In the grape berry, the flavonoids are mainly localised in the skins, such as the anthocyanins and resveratrol, while the flavan-3-ols (catechins and proanthocyanidins) are present in the skins, in the seeds and in the pulp (4). However, the composition and concentration of phenolics in grapes vary with variety, species, season, and environmental and management factors such as soil conditions, climate, and crop load (3).

Anthocyanins are directly responsible for color. The grape anthocyanins are monoglucosides of 5 anthocyanidins, namely delphinidin, cyanidin, petunidin, peonidin, and malvidin. The acylated anthocyanins are esters of the glucose moiety of the free anthocyanins with acetic, *p*-coumaric, or caffeic acids. The composition of anthocyanins is primarily determined by genetic factors (5). Hydroxycinnamic acids and their derivatives are widely distributed in the plant kingdom and play a very important role in the secondary metabolism in plants. Caffeoyl, coumaroyl, and feruloyl acids are the main hydroxycinnamic acids in grape and wine. They are tartaric ester of caffeoyl, coumaroyl, and feruloyl acids.

The Serbian wine industry has a lengthy history dating back to Roman times, thus wine growing is well known in

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the region. The west Morava wine region is famous for the producers in Kruševac ('Rubin'), Prokuplje, and above all in Aleksandrovac, center of the Župa district. Župa is a term for a valley with a mild climate, and the one along the river Rasina is perfect for wine growing. The most widely grown here are the indigenous 'Prokupac', named after the town of Prokuplje, and 'Tamjanika', a 'Muscat' variety that originated in France but has been very popular in Serbia since the middle Ages. International varieties such as 'Cabernet Sauvignon', 'Gamay', 'Merlot', and other pure grape varieties are important for the future of the industry. 'Merlot' grape is a particular example of the variety that is adaptable to the climate of Serbia. Particularly for the grape varieties cultivated in Serbia and the wines product, the polyphenolic composition has not been examined in detail. The aim of the study was to determine the free radical scavenging capacity of the 3 table grapes and 4 wine grapes and to correlate these with total polyphenolic content and the amount of anthocyanins.

Materials and Methods

Chemicals Standards of catechin, epicatechin, malvidin-3-*O*-glucoside, and the phenolic acid standards, such as gallic, ferulic, *p*-coumaric, and caffeic acids, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Malvidin-3-glucoside was purchased from Extrasynthèse S.A.S (Ganay, France). DPPH were purchased from Sigma-Aldrich (Steinheim, Germany). Trolox and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). Other chemicals and solvent were of analytical grade.

Plant materials Two autochthonous red wine grape varieties: 'Prokupac' and 'Vranac' and 5 varieties used in many countries: 'Cardinal', 'Ribier', 'Muscat Hamburg' (table grape), 'Merlot', and 'Cabernet Sauvignon' (wine grape) were harvested at their stage of optimum maturity in the summer of 2008. They were grown in southern Serbia vineyard. Plantations were built on sandy loam soil type in region of continental climate with much sun during the summers. Each grape variety was collected randomly from 3 vineyard block from the southern Serbia vineyard. All the analyzed table grapes are frequently consumed in Serbia.

Firstly, grape berries were removed from each bunch and homogenized in blender. For sample extraction, 10 g of blended grape was macerated in 30 mL methanol containing 0.1% HCl and set aside overnight in darkness. Then the extract was filtered over Whatman no. 1 paper under vacuum, and the residue was repeatedly extracted with some solvent until it was colorless. Extraction

continued 7 time until the extraction solvents became colorless (the total solvent volume was 100 mL). All extracts were stored at -20°C in the dark until analyzed.

Determination of total phenols The total phenolic content in the grapes was determined using the Folin-Ciocalteu colorimetric method (6). The grape extracts were oxidized by the Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance was measured at 760 nm after 120 min at room temperature by a UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA) in 10-mm cell cuvette. The absorbance values were then compared with the standards with known gallic acid concentrations. All values were expressed as the mean [mg gallic acid equivalents (GAE)/100 g of fresh sample] \pm standard deviation (SD) for 3 replications.

Determination of total flavonoids The total flavonoid content of the grape extract was determined using a modified colorimetric method (7,8). Briefly, 0.1 mL grape extracts was mixed with 2.0 mL of deionised water and subsequently with 0.15 mL of 5% sodium nitrite solution and was allowed to react for 5 min. Then, a 0.75 mL of 2% aluminium chloride was added and allowed to further react for 5 min before 1.0 mL of 1 M NaOH was added. Deionised water was added to bring the final volume of the mixture to 5 mL. The absorbance of the mixture was immediately measured at a 510 nm wavelength against a prepared blank using an Agilent UV-Vis spectrophotometer. The flavonoid content was determined by a catechin standard curve and expressed as the mean [mg catechin equivalents (CE)/100 g of fresh sample] \pm SD for 3 replications.

Determination of total anthocyanins The monomeric anthocyanin content of the grape extracts was measured using a modified pH differential method (9). The grape extracts were mixed thoroughly with 0.025 M KCl buffer pH 1.0. The grape extracts were then mixed similarly with a sodium acetate buffer pH 4.5. Agilent UV-Vis spectrophotometer was used to measure absorbance at 520 and 700 nm against a buffer blank at pH 1.0 and 4.5. Absorbance readings were converted to total mg malvidin-3-*O*-glucoside (M3G). The anthocyanin content was calculated as follows:

$$\text{TMA (mg/100 g)} = \frac{\Delta A \cdot M_w \cdot 1000}{\epsilon \cdot c}$$

$$\Delta A = (A_{520 \text{ nm, pH}=1.0} - A_{700 \text{ nm, pH}=1.0}) - (A_{520 \text{ nm, pH}=4.5} - A_{700 \text{ nm, pH}=4.5})$$

where, A is absorbance, TMA is total monomeric anthocyanins, M_w (493.5) is molecular weight for M3G, ϵ (28,000, pH 1.0 in methanol solvent) is the molar

absorptivity of M3G and c is the concentration of the grape extract in mg/mL. The anthocyanin content was expressed as mg of M3G equivalents/100 g of fresh grape for 3 replications.

Measurement of the DPPH \cdot scavenging activity The free radical scavenging activity of the grape samples was analyzed by the DPPH \cdot assay (10–12). The antioxidant assay is based on the measurement of the loss of DPPH \cdot with the tested sample. The reaction was monitored by a UV-Vis spectrophotometer. The extract diluted with methanol (1:12.5 v/v) (2.5 mL) and 2.5 mL of freshly prepared DPPH \cdot in methanol 1×10^{-4} mol/L were put into a cuvette at room temperature. After the incubation period of 30 min at room temperature, the absorbance was read against a blank (methanol-DPPH \cdot) at 517 nm. The total antioxidant activity for each grape extract sample was expressed as μ mol of Trolox equivalent (TE)/100 g fresh weight of grape.

HPLC-photodiode array detection (DAD) determination of polyphenolic compounds The concentration of individual anthocyanins, flavan-3-ols, and hydroxycinnamic acids was determined by HPLC, employing a direct-injection method (13,14). The equipment used was an HPLC Agilent-1200 series with UV-Vis DAD for multi wavelength detection and fluorescence detection for acquisition of the emission response. The column was thermostated at 30°C. After injecting 5 μ L of sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6-150 mm column. Two solvents were used for the gradient elution: A-(H₂O+5% HCOOH) and B-(80% ACN+5% HCOOH+H₂O). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increases 0–25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25–50% B, from 35 to 40 min gradually increases 50–80% B, and finally for the last 5 min gradually decreases 80–0% B. All quantifications were carried out with external standards. The identification of individual compounds was based on the retention times of the original standards, where available, and spectral

data. Monitoring of the eluate was performed at 520 nm for the identification of anthocyanins and 320 nm for the identification of hydroxycinnamic acids. Three flavan-3-ols including catechin, procyanidin B2 (dimer of proanthocyanidins epicatechin-epicatechin), and epicatechin were monitored at 275/322 nm ($\lambda_{Ex}/\lambda_{Em}$) with fluorescence detector. The results were expressed as mg/100 or 1,000 g fresh berry weight.

Statistical analysis The experiments were reported at least 3 times, and the data were analyzed statistically. All results are given as mean \pm SD. Statistical analysis was done by one-way analysis of variance (ANOVA), and significant differences between the results were determined by Duncan's multiple range test. The differences were considered significant at $p < 0.05$.

Results and Discussion

Total phenolic content Total phenolic contents of the 7 tested grapes are presented in Table 1. Among all the grape varieties analyzed, 'Cabernet Sauvignon' and 'Merlot' had the highest total phenolic content (173.6 and 169.2 mg of GAE/100 g of grape, respectively), followed by 'Vranac', 'Muscat Hamburg', 'Ribier', 'Prokupac', and 'Cardinal'. The total phenolic content of table grape variety 'Cardinal' was significantly lower from the other ($p < 0.05$). Significant differences were found in the total phenolic content in comparing between 'Cabernet Sauvignon' and 'Vranac' and 'Prokupac' ($p < 0.05$); however, significant differences in total phenolic content not found between 'Vranac' and 'Prokupac'. It is an ancient red wine grape indigenous to the Republic of Macedonia, Serbia, and Montenegro, and one of the most important in these areas. The 'Prokupac' grape is a red wine grape indigenous to Serbia. The skin of 'Prokupac' is of dark-blue color, with characteristic dots. Because of the strong qualities of these grapes, it are often blended with 'Cabernet Sauvignon' and 'Merlot'. 'Muscat Hamburg' grapes, a traditional table grape variety, also contained a high level of polyphenolics

Table 1. Total phenol (TP), total flavonoid (TF), and total anthocyanin (TA) contents of grapes

| Grape variety | TP (mg GAE/100 g) | TF (mg CE/100 g) | TA (mg M3GE/100 g) |
|--------------------|----------------------------------|--------------------------------|--------------------------------|
| Cardinal | 142.32 \pm 1.55 ^{a1)} | 59.46 \pm 0.81 ^a | 44.80 \pm 1.93 ^a |
| Muscat Hamburg | 157.46 \pm 4.32 ^{bc} | 101.52 \pm 1.42 ^b | 85.13 \pm 1.70 ^b |
| Ribier | 157.30 \pm 5.32 ^{bc} | 95.52 \pm 1.24 ^b | 79.10 \pm 1.64 ^c |
| Prokupac | 156.28 \pm 4.93 ^b | 105.80 \pm 1.68 ^b | 88.80 \pm 4.58 ^{bc} |
| Vranac | 158.56 \pm 1.94 ^{bc} | 122.14 \pm 1.34 ^c | 106.97 \pm 1.20 ^e |
| Merlot | 169.20 \pm 2.69 ^{cd} | 134.80 \pm 1.17 ^d | 110.13 \pm 1.64 ^e |
| Cabernet Sauvignon | 173.60 \pm 1.91 ^d | 146.20 \pm 1.42 ^e | 120.27 \pm 2.00 ^f |

¹⁾Data are reported as mean \pm SD ($n=3$); Bars with no letters in common are significantly different ($p < 0.05$) in the same column.

Table 2. Comparison of our and published value of phenolic compounds content in grapes (mg GAE/100 g fresh grape)

| Country | Total phenolic content | Reference |
|---------------|------------------------|-----------|
| Serbia | 142.3-173.6 ($n=7$) | Our study |
| Croatia | 73.1-348.6 ($n=7$) | (15) |
| Spain | 176.210 ($n=2$) | (16) |
| Spain | 130-360 ($n=2$) | (17) |
| United States | 201.1-426.6 ($n=14$) | (3) |
| Andalusia | 119.1-336.1 ($n=5$) | (18) |
| China | 63-182 ($n=3$) | (19) |
| China | 250.480 ($n=2$) | (20) |
| Georgia | 140-310 ($n=10$) | (21) |
| Macedonia | 114-258 ($n=3$) | (26) |

components, thus it is recommended for regular human consumption. The mean concentration of the phenolic content of the Serbian grapes was 159.3 mg GAE/100 g fresh berry weight. Our values for table and wine grape are in the range of values determined by other authors (63-480 mg GAE/100 g f.w.) (Table 2). Katalinić *et al.* (25) measured 103.1 and 225.2 mg GAE/100 g f.w. for cultivars ‘Merlot’ and ‘Vranac’ respectively. Also, Ivanova *et al.* (26) measured 258, 165, and 114 mg GAE/100 g f.w. for cultivars ‘Vranec’, ‘Cabernet Sauvignon’, and ‘Muscat Hamburg’, respectively. In the 5 Spain red grape cultivars (‘Jean tinto’, ‘Palomino negro’, ‘Tintillade de Rota’, ‘Cabernet Sauvignon’, and ‘Tempronillo’) total phenolic content ranged from 119.1 to 336.1 mg GAE/100 g f.w. (18). There are significant differences between the investigated values. Thus difference may be a consequence of the influence of geographical location in addition to varietal differences.

Total flavonoid content Total flavonoid content of the 7 grape extracts are presented in Table 1. ‘Cabernet Sauvignon’ presented the highest flavonoid content (146.2 mg of CE/100 g of f.w.), followed by ‘Merlot’, ‘Vranac’, ‘Muscat Hamburg’, ‘Prokupac’, ‘Ribier’, and ‘Cardinal’. The flavonoid contents of ‘Cabernet Sauvignon’, ‘Merlot’, ‘Vranac’, and ‘Cardinal’ was significantly different from the others ($p<0.05$). However, significant differences in the total flavonoid content were not found in comparison among ‘Muscat Hamburg’, ‘Ribier’, and ‘Prokupac’ ($p<0.05$). ‘Prokupac’ had a significantly lower total flavonoid content compared to the 4 analysed wine grapes. An approximately 2.5-fold difference in total flavonoid content was found between the highest and lowest ranked varieties, ‘Cabernet Sauvignon’ and ‘Cardinal’ ($p<0.05$). The obtained results for the analyzed grapes were in agreement with previously published data. Katalinić *et al.* (25) measured 139.6 and 106.8 CE/100 g f.w. for cultivars ‘Vranac’ and ‘Merlot’, respectively. But, Ivanova *et al.*

(26) measured lower values 60.3 CE/100 g f.w. the average total flavonoids content of grape cultivars ‘Vranec’, ‘Cabernet Sauvignon’, and ‘Muscat Hamburg’.

Total anthocyanin content Anthocyanins are natural pigments, responsible for a wide range of colors in grapes and red wines. The anthocyanins in red grapes vary greatly with the species, maturity, production area, seasonal conditions, and yield of the fruit. Total anthocyanin content of the table and wine grapes are presented in Table 1. ‘Cabernet Sauvignon’ had the highest total anthocyanin content [120.3 mg of M3G equivalent (M3GE)/100 g of f.w.], followed by ‘Merlot’, ‘Vranac’, ‘Muscat Hamburg’, ‘Prokupac’, ‘Ribier’, and ‘Cardinal’. The total anthocyanin content of ‘Cardinal’ was significantly lower from the others ($p<0.05$). However, Serbia-grown table grape varieties had a higher anthocyanin content compared to the previously reported 7 table grapes with 11.5-36.1 mg/100 g f.w. of grapes (2). Upon M3G as a standard, the lowest anthocyanin content in ‘Primitivo’ variety was found to be 25.0 mg/100 g whereas the maximum amount was found in a ‘Teroldego’ grape with 232.3 mg/100 g (22). In this study, ‘Cabernet Sauvignon’ variety contained the highest total anthocyanin content, whereas ‘Cardinal’ had the lowest, one indicating a small a 2.7-fold difference compared to the 30-fold difference between ‘DeChaunac’ and ‘Catawba’ (3).

Also, the significant correlation between the anthocyanin content and antioxidant activity ($R=0.926$) of the tested grapes was confirmed.

Total antioxidant activity (TAA) of grapes using the DPPH scavenging assays The radical scavenging activity was evaluated by measuring the scavenging activity of the examined grape sample extracts on DPPH radicals. A high phenolic content in the investigated table and wine grapes contributes to their increased antioxidant activity.

The TAA of the 7 red grapes, expressed as μmol of TE/100 g of fresh weight of grapes, is shown in Fig. 1. ‘Cabernet Sauvignon’ had the highest antioxidant activity, followed by ‘Merlot’, ‘Vranac’, ‘Muscat Hamburg’, ‘Prokupac’, ‘Ribier’, and ‘Cardinal’. A significant difference was found among ‘Cabernet Sauvignon’, ‘Vranac’, and ‘Prokupac’. The TAAs of ‘Vranac’ and ‘Prokupac’ were similar ($p<0.05$), but lower ($p<0.05$) than that of ‘Merlot’. No significant difference ($p<0.05$) was found among ‘Cardinal’ and ‘Ribier’. The varieties containing high total phenolic contents had higher antioxidant activities. The present study reveals a good correlation between TAAs and total phenolics ($R^2=0.902$). Also, the significant correlation between the total flavonoid content and antioxidant activity ($R^2=0.945$) of the tested grape extracts was confirmed. TAA of 7 grape cultivars was in a range from 953.1 to

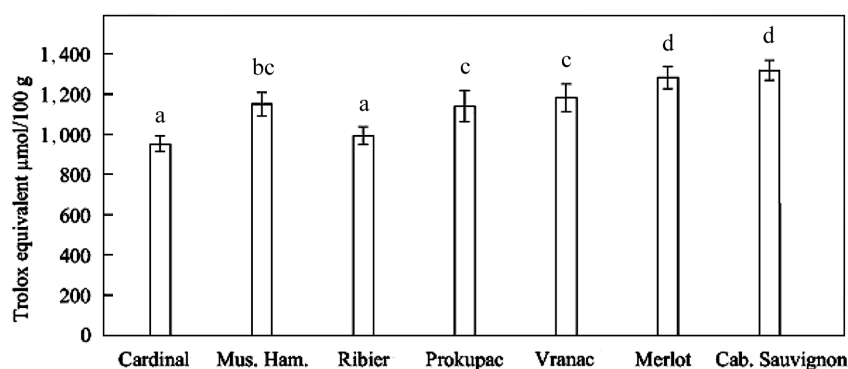


Fig. 1. DPPH free radical scavenging capacity of grape varieties. Results of the grape extract were expressed as Trolox equivalent/100 g of sample ($\mu\text{mol}/100\text{ g}$) mean \pm SD ($n=3$); Bars with different letters are statistically significant ($p<0.05$).

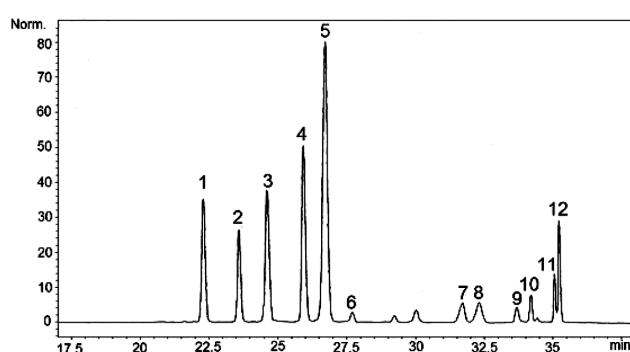


Fig. 2. Chromatogram of 'Vranac' grape extract monitored at 520 nm. Identification of compounds: (1) delphinidin-3-*O*-glucoside; (2) cyanidin-3-*O*-glucoside; (3) petunidin-3-*O*-glucoside; (4) peonidin-3-*O*-glucoside; (5) malvidin-3-*O*-glucoside; (6) delphinidin-3-acetylglucoside; (7) delphinidin-3-*p*-coumaroylglucoside; (8) malvidin-3-acetylglucoside; cyanidin-3-*p*-coumaroylglucoside (9); (10) petunidin-3-*p*-coumaroylglucoside; (11) peonidin-3-*p*-coumaroylglucoside; (12) malvidin-3-*p*-coumaroylglucoside

1,318.0 $\mu\text{mol TE}/100\text{ g f.w.}$ Our results were similar with other authors (21,27,28). Postrana-Bonilla *et al.* (21) measured 1,530 $\mu\text{mol TE}/100\text{ g f.w.}$ the average total antioxidant activity of 10 grape cultivars.

Anthocyanins, hydroxycinnamic acids, and flavan-3-ols composition The total of 12 phenolics compounds was identified as anthocyanins due to the information provided by their UV-Vis spectra (Fig. 2). This primary information differentiated 5 glucosylated anthocyanins (λ maximum at 520 nm): delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside; and 7 acylglucosylated anthocyanins: delphinidin-3-acetylglucoside, malvidin-3-acetylglucoside, delphinidin-3-*p*-coumaroylglucoside, cyanidin-3-*p*-coumaroylglucoside, petunidin-3-*p*-coumaroylglucoside, peonidin-3-*p*-coumaroylglucoside, and malvidin-3-*p*-coumaroylglucoside.

Certain quantitative differences in anthocyanins between table grape and wine grape varieties were presented in

Table 3. Total anthocyanin content ranged from 32.50 mg/100 g f.w. for 'Cardinal' to 96.60 mg/100 g f.w. for 'Cabernet Sauvignon'. M3G was the main anthocyanin in all the varieties (from 38% of total anthocyanin in 'Vranac' to 56% in 'Cabernet Sauvignon'), except for 'Cardinal' and 'Muscat Hamburg' because these are the rosé grape varieties where dominated peonidin-3-*O*-glucoside (17). This was the first time that these data were reported for autochthonous Serbian varieties ('Vranac' and 'Prokupac').

Among the wine grape varieties the main anthocyanin is the studied malvidin acetylated derivative. However, not all acetylated derivatives were detected in all varieties. Malvidin-3-acetylglucoside was detected in all wine grape varieties, and accounted for 3-6% of total anthocyanin. In one of the 2 autochthonous varieties studied ('Prokupac') delphinidin-3-acetylglucoside was not detected.

Malvidin-3-*p*-coumaroylglucoside was the most abundant derivative in all the wine grape varieties (accounting for around 13.7% of total anthocyanin) (Table 3). In contrast with the other varieties, 'Cardinal' and 'Muscat Hamburg' showed a higher content of peonidin-3-*p*-coumaroylglucoside ('Cardinal': malvidin-3-*p*-coumaroylglucoside 8.2% and peonidin-3-*p*-coumaroylglucoside 5.0%; 'Muscat Hamburg': malvidin-3-*p*-coumaroylglucoside 3.0% and peonidin-3-*p*-coumaroylglucoside 2.0%).

Other minor *p*-coumaroyl derivatives were also detected in wine grape varieties, however, some of them only in trace amounts. Cyanidin-3-*p*-coumaroylglucoside was not detected in 'Prokupac'.

Although anthocyanin profile may be complex and quite different for each variety studied, it is notable that the 'Vranac' variety showed the considerable concentration of anthocyanins in grapes, largely due to the small berry size, among other factors. On the other hand, 'Prokupac' displayed the narrowest variety of anthocyanin types. In the nonautochthonous varieties, the total anthocyanin content of 'Cabernet Sauvignon' was triplicated in comparison to the one of 'Cardinal'.

The concentrations of hydroxycinnamic acids determined

Table 3. Anthocyanin composition of table and winegrape varieties

| Compound ¹⁾ | Cardinal | Muscat Hamburg | Ribier | Prokupac | Vranac | Merlot | Cabernet Sauvignon |
|------------------------|-------------------------|----------------|------------|------------|------------|------------|--------------------|
| 1 | 0.53±0.08 ²⁾ | 4.11±0.35 | 1.84±0.09 | 2.47±0.18 | 10.19±0.72 | 5.36±0.12 | 8.29±0.09 |
| 2 | 1.19±0.10 | 3.54±0.28 | 0.72±0.06 | 1.25±0.09 | 6.70±0.25 | 1.62±0.06 | 0.40±0.01 |
| 3 | 0.74±0.09 | 4.23±0.18 | 2.46±0.09 | 2.93±0.11 | 11.71±0.38 | 5.97±0.18 | 4.54±0.08 |
| 4 | 14.57±0.82 | 15.72±0.83 | 5.19±0.13 | 7.92±0.29 | 14.33±0.85 | 9.35±0.27 | 7.04±0.11 |
| 5 | 11.17±0.94 | 22.23±0.92 | 17.67±0.52 | 27.64±0.98 | 29.14±0.92 | 43.97±1.18 | 54.47±1.17 |
| 6 | ND | ND | ND | ND | 0.71±0.06 | 0.89±0.05 | 0.92±0.05 |
| 7 | ND | ND | ND | 1.74±0.07 | 2.37±0.07 | 1.10±0.08 | 0.95±0.09 |
| 8 | ND | ND | 0.98±0.07 | 3.77±0.05 | 2.74±0.07 | 3.02±0.05 | 3.52±0.09 |
| 9 | ND | ND | 1.19±0.09 | ND | 1.30±0.02 | 0.40±0.01 | 0.80±0.04 |
| 10 | ND | ND | ND | 0.83±0.06 | 1.57±0.03 | 2.30±0.06 | 2.85±0.10 |
| 11 | 2.66±0.32 | 1.59±0.06 | 3.11±0.22 | 2.86±0.10 | 2.29±0.07 | 2.78±0.07 | 2.92±0.08 |
| 12 | 1.64±0.12 | 1.08±0.05 | 11.51±0.63 | 7.27±0.08 | 5.17±0.09 | 12.94±0.32 | 9.90±0.42 |
| TA | 32.50 | 52.50 | 44.67 | 58.68 | 88.22 | 89.70 | 96.60 |

¹⁾(1) delphinidin-3-*O*-glucoside; (2) cyanidin-3-*O*-glucoside; (3) petunidin-3-*O*-glucoside; (4) peonidin-3-*O*-glucoside; (5) malvidin-3-*O*-glucoside; (6) delphinidin-3-acetylglucoside; (7) delphinidin-3-*p*-coumaroylglucoside; (8) malvidin-3-acetylglucoside; (9) cyanidin-3-*p*-coumaroylglucoside; (10) petunidin-3-*p*-coumaroylglucoside; (11) peonidin-3-*p*-coumaroylglucoside; (12) malvidin-3-*p*-coumaroylglucoside

²⁾Results expressed as mg/100 g fresh sample; ND, not detected; TA, total anthocyanins

Table 4. Amount of hydroxycinnamic acids in grape extracts of 7 grape varieties obtained by HPLC

| Grape varieties | <i>t</i> -Caftaric acid | <i>c</i> -Coutaric acid | <i>t</i> -Coutaric acid | <i>t</i> -Fertaric acid | Total acids |
|--------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------|
| Cardinal | 0.68±0.05 ¹⁾ | 0.11±0.03 | 0.32±0.04 | 0.08±0.01 | 1.20±0.03 |
| Muscat Hamburg | 0.98±0.09 | 0.17±0.02 | 0.38±0.07 | 0.10±0.01 | 1.63±0.05 |
| Ribier | 0.85±0.06 | 0.19±0.05 | 0.29±0.03 | 0.19±0.02 | 1.42±0.04 |
| Prokupac | 0.47±0.02 | 0.22±0.02 | 0.21±0.01 | 0.11±0.02 | 1.01±0.02 |
| Vranac | 0.70±0.05 | 0.11±0.04 | 0.18±0.02 | 0.30±0.05 | 1.29±0.04 |
| Merlot | 1.05±0.11 | 0.25±0.02 | 0.67±0.08 | 0.12±0.04 | 2.09±0.06 |
| Cabernet Sauvignon | 0.82±0.09 | 0.09±0.01 | 0.58±0.02 | 0.25±0.02 | 1.74±0.04 |

¹⁾Results are presented with mean±SD (*n*=3) and expressed in mg of hydroxycinnamic acid/kg of grape berry fresh weight.

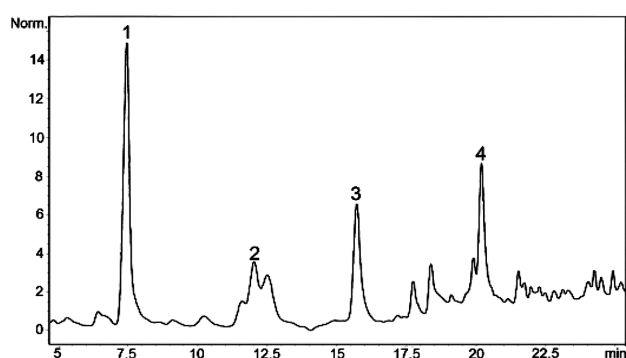


Fig. 3. Chromatogram of grape extract monitored at 320 nm. Identification of compounds: (1) *t*-caftaric acid; (2) *c*-coutaric acid; (3) *t*-coutaric acid; (4) *t*-fertaric acid

by HPLC in the investigated grape samples are shown in Table 4. The HPLC chromatogram of 4 acids, i.e., *trans*-caftaric, *cis*-coutaric, *trans*-coutaric, and *trans*-fertaric acids recorded at 320 nm with a diode array detector is shown in Fig. 3. These compounds have been previously reported in grapes (18,23). The total amounts of hydroxycinnamic acids ranged from 1.01 mg/kg for ‘Prokupac’ up to 2.09

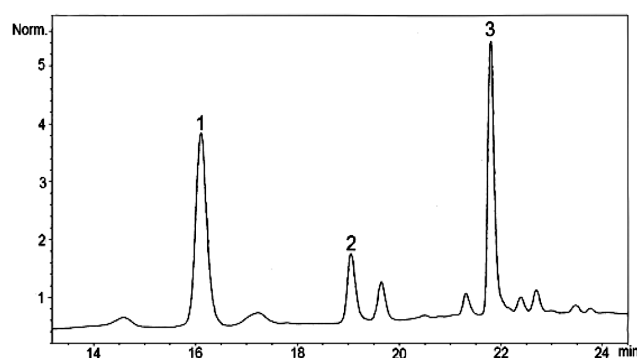


Fig. 4. Chromatogram of ‘Prokupac’ grape extract monitored at 275 nm/322 nm ($\lambda_{EX}/\lambda_{EM}$) with fluorescence detector. Identification of compounds: (1) catechin; (2) procyanidin dimer B2; (3) epicatechin

mg/kg for ‘Merlot’. The *trans* isomers presented higher concentrations than the *cis* isomers in all cases.

The use of fluorescence detector has allowed increasing selectivity and sensitivity for the determination of the concentration of catechin and epicatechin, in addition to other phenolic compounds (24). Catechin, epicatechin, and

Table 5. Amount of flavan-3-ols in grape extracts of 7 varieties obtained by HPLC

| Grape varieties | Catechin | Procyanidin dimer (B2) | Epicatechin | Total flavan-3-ol |
|--------------------|-------------------------|------------------------|-------------|-------------------|
| Cardinal | 0.63±0.05 ¹⁾ | 0.22±0.04 | 0.32±0.03 | 1.17 |
| Muscat Hamburg | 1.39±0.08 | 0.25±0.03 | 0.72±0.07 | 2.36 |
| Ribier | 0.46±0.06 | 0.27±0.05 | 0.38±0.04 | 1.11 |
| Prokupac | 1.17±0.06 | 0.30±0.05 | 0.92±0.05 | 2.39 |
| Vranac | 3.09±0.12 | 0.36±0.04 | 2.16±0.10 | 5.61 |
| Merlot | 3.62±0.10 | 0.26±0.02 | 1.69±0.09 | 5.57 |
| Cabernet Sauvignon | 4.08±0.11 | 0.21±0.03 | 2.03±0.08 | 6.32 |

¹⁾Results are presented with mean±SD ($n=3$) and expressed in mg of flavan-3-ols compound/kg of grape berry fresh weight.

procyanidin dimer (B2) were identified by fluorescence detector in grape extracts (Fig. 4). The amount of catechin, epicatechin, and B2 in the red grape of 7 different varieties are presented in Table 5. Catechin was more abundant than epicatechin. The range of catechin, epicatechin, and B2 concentration were 0.46–4.08, 0.32–2.16, and 0.21–0.36 (mg/kg of grape berry f.w.). The content of catechin found in the presented study was similar to the amount (0.73–4.57 mg/kg of red grape berry f.w.) reported by findings of Katalinic *et al.* (25), while the amount of B2 was lower than the one found by some researcher (0.0–2.74 mg/kg). In contrary to our research, Katalinic *et al.* (25) are not determined epicatechin in red grape varieties.

In recent years, phytochemicals, especially phenolics, have attracted increasing attention for their antioxidant activities. Grape provide phenolic antioxidants, which contribute to their potential health benefits. This work has shown that the phytochemicals present in grapes grown in southern Serbia have potent antioxidant activities and that the antioxidant activity in grapes is positively correlated with total phenolic content. Our results have also found that significant differences in phytochemical content can exist among grape varieties.

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