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Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia)

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ABSTRACT

The aim of the present study was to determine polyphenolic composition, related antioxidative and antimicrobial properties of grape skin extracts from 14 grape varieties (seven white and seven red grape) grown in Dalmatia (Croatia). The content of total phenols, flavonoids, catechins, flavanols and individual polyphenols ((+)-catechin, (−)-epicatechin, epicatechin gallate, procyanidin B1 and procyanidin B2, quercetin glucoside, resveratrol monomers, piceid and astringin) was variety dependent. Antioxidant properties were determined as DPPH radical-scavenging ability (IC_{50}), ferric reducing/antioxidant power (FRAP), Fe^{2+}-chelating activity (IC_{50}), and using β-carotene bleaching assay. The high antioxidant capacity of all extracts, both red and white, has been observed and related to the relative amounts of polyphenolic compounds with good antioxidant properties. The antimicrobial activity was screened by broth microdilution test using Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli* O157:H7, *Salmonella* Infantis, *Campylobacter coli*). It was confirmed against all tested organisms. Minimum inhibitory concentrations (*MIC*s), were found in the range 0.014–0.59 mg of gallic acid equivalents (GAE)/ml, with lower *MIC*s of white cultivars, especially against *Campylobacter* and *Salmonella*.

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1. Introduction

Over the past years great attention has been paid to plant polyphenols. These secondary plant metabolites, naturally present in fruit and vegetables, are part of our everyday diet. As efficient free radical scavengers they can potentially interact with biological systems and play a role in preventing human neurodegenerative diseases, cardiovascular disorders and cancer (German, Frankel, Waterhouse, Hansen, & Walmez, 1997; Poudel, Tamura, Kataoka, & Mochioka, 2008). Phenolics display a wide variety of structures, ranging from simple moieties containing a single hydroxylated aromatic ring to highly complex polymeric substances. These compounds arise biogenetically from two main primary synthetic pathways: the shikimate pathway and the acetate pathway. Based on their carbon skeleton polyphenols can be classified into non-flavonoid compounds (stilbenes) and flavonoid compounds (Hakkinen, 2000). Positive correlation between total phenolic content of plant extracts and related antioxidant capacity has been reported (Katalinic, Milos, Kulisic, & Jukic, 2006).

The skins and seeds of grapes are known to be rich sources of phenolic compounds, both flavonoids and non-flavonoids (Arnous & Meyer, 2008; Negro, Tommasi, & Micelli, 2003; Pinelo, Arnous, & Meyer, 2006; Poudel et al., 2008). The concentration of phenolic compounds in grapes depends on the variety of grapevine and is influenced by viticultural and environmental factors. The synthesis of flavonoid and non-flavonoid plant polyphenols such as stilbenes is increased in plant tissues following wounding or infection by pathogenic organisms (Hakkinen, 2000; Montealegre, Peces, Vozmediano, Gascuena, & Romero, 2006). Amongst plant polyphenols with good antioxidant and other properties, phytoalexin resveratrol (*trans*-3,4′,5-trihydroxystilbene) has aroused great interest in scientists after being identified in wine and linked to the "French paradox". It is found principally in the skins of grape berries, but is also present in other parts of the grape cluster, both grape seeds and stems. The useful effects of resveratrol are well documented (Aggarwal & Shishodia, 2006). Although the relative concentration of stilbenes in the overall phenolic ripe grape skin content is low, it is interesting to know the potential of individual

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varieties with regard to the concentration of each of these biologically-active compounds.

Knowledge of polyphenolic content of grape skins is relevant for their future use. Of special interest are white grape skins which are not subjected to lasting maceration in the process of vinification, and can be a cheap and valuable raw material for the production of biologically interesting polyphenolic compounds (or products based on them). Whilst grape skin flavonoids, especially anthocyanins, flavonols and flavanols have been widely studied (Kammerer, Claus, Carle, & Scheiber, 2004; Montealegre et al., 2006; Pinelo et al., 2006; Yang, Liu, & Martinson, 2009), there is a lack of a more systematic investigation on stilbenes in grape skin extracts of different grape varieties.

Although grape skins are confirmed as rich sources of phenolic compounds and recent results indicate higher antimicrobial potential of natural extracts than shown by selected antioxidants alone against several microorganisms (Serra et al., 2008) reports for antimicrobial activity of grape skin extracts are scarce.

The aim of this study was to determine polyphenolic composition, related antioxidative and antimicrobial properties of grape skin extracts from 14 grape varieties grown in Dalmatia (Croatia). The research includes seven white and seven red grape varieties, eleven native and three introduced ones. The compounds of interest in the present study were (+)-catechin, (−)-epicatechin, epicatechin gallate, procyanidin B1 and procyanidin B2 (flavanols), quercetin glucoside (flavonol), *cis*- and *trans*-resveratrol monomers, piceid and astringin (stilbenes). These compounds were selected because of their proposed health-beneficial properties (Aggarwal & Shishodia, 2006; Hakkinen, 2000). Separation and quantitative determination of individual polyphenolic compounds was done using reverse-phase HPLC with diode array detection (RP–HPLC–DAD). Antioxidant properties of extracts were determined as free radical-scavenging ability (DPPH method), ferric reducing/antioxidant power (FRAP method), Fe^{2+}-chelating ability, and using β-carotene bleaching assay. Additionally, all extracts have been screened by broth microdilution test for antimicrobial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative (*Escherichia coli* O157:H7, *Salmonella* Infantis, *Campylobacter coli*) food-borne pathogenic bacteria.

2. Materials and methods

2.1. Reagents, solvents and standards

The following reagents were obtained from Sigma (Sigma–Aldrich GmbH, Steinheim, Germany): (+)-catechin hydrate, β-carotene (>97%), *p*-dimethylaminocinnamaldehyde (DMAC), ferric chloride ($FeCl_3$), ferrozine, Folin–Ciocalteau reagent, formaldehyde, gallic acid, linoleic acid (>95%), Tween 20, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) and Trolox. Chloroform, ethanol and methanol (all analytical grade), acetic acid and acetonitrile (HPLC grade), and $FeCl_2 \times 4H_2O$ were purchased from Merck (Darmstadt, Germany). Procyanidin dimers B1 and B2, epicatechin gallate, (+)-catechin and (−)-epicatechin were obtained from Extrasynthèse (Genay, France). Gallic acid, vanillin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Fluka (Buchs, Switzerland). The *trans*-resveratrol was acquired from Sigma (St Louis, MO). Piceid (*trans*-3,5,4′-trihydroxystilbene-3-*O*-β-D-glucopyranoside), astringin (*trans*-3,4,3′,5′-tetrahydroxystilbene-3′-*O*-β-D-glucopyranoside), and quercetin 4′-*O*-β-glucoside were obtained from Polyphenols Laboratories (Sandnes, Norway). The stock solution of resveratrol *cis*-isomer was prepared by UV irradiation at 254 nm of alcoholic solution of *trans*-resveratrol according to Romero-Perez, Lamuela-Raventos, Waterhausee, and De la Torre-Boronat (1996). Water was prepared by purification with a Milli-Q water purification system (Millipore, Bedford, MA).

2.2. Instruments

Spectrophotometric measurements were performed on a UV–Vis double beam Specord 200 spectrometer (Analytik Jena GmbH, Germany) and model UV–Vis 8453 Hewlett Packard spectrophotometer (Hewlett Packard, Waldbronn, Germany). The HPLC system used for separation and quantification of individual polyphenolic compounds was composed of a Varian UV–Vis PDA 330 detector, a ternary gradient liquid Pro Star 230 pump, column, heater Model 500 and Star chromatography workstation Version 6.0 (Varian, Palo Alto, CA).

2.3. Plant material

The present research includes eleven native and three introduced* grape varieties (white: *Debit*, *Kuč*, *Kujundžuša*, *Maraština*, *Medna*, *Rkaciteli**, *Zlatarica*; red: *Babić*, *Lasin*, *Merlot**, *Plavina*, *Rudežuša*, *Trnjak*, *Vranac**). Grapes were harvested at their technological maturity, from Vrgorac and Imotski vineyards, region Dalmatia (Croatia), 2006 vintage. Approximately 300 undamaged and disease-free berries were randomly snipped from clusters. The berries were dried with filter paper and weighed (Table 1). The skins were carefully manually separated from pulp, dried with filter paper, weighed and frozen at −20 °C until analysed.

2.4. Bacterial strains, culture media and growth conditions

Antimicrobial activity was screened against *B. cereus* WSBC 10530 (clinical isolate), *S. aureus* ATCC 25923 (clinical isolate), *C. coli* ATCC 33559 (pig faeces isolate), *E. coli* O157:H7 ZM370 (clinical isolate) and *Salmonella* Infantis ZM9 (poultry meat isolate). *B. cereus*, *S. aureus* and *S.* Infantis were incubated aerobically at 37 °C in Müller-Hinton broth or agar (MHB, MHA) (Oxoid, Basingstoke, UK), whilst *E. coli* was incubated in Tryptone soya broth or agar (TSB, TSA) (Oxoid, Hampshire, UK). *C. jejuni* was incubated microaerobically at 42 °C in MHB with defibrinated horse blood (Oxoid) added. For inocula preparation all bacteria were incubated for 20 h in MHB and for antibacterial activity assays, 1 ml of each, appropriately diluted (in MHB) to *ca.* 10^6 CFU/ml, was used.

2.5. Extractions of polyphenols

The polyphenolic constituents from grape skins were extracted using conventional solvent extraction procedure. Ten grams of homogenised grape skins (1 min in high speed grinder) were extracted with 100 ml of alcoholic solvent (ethanol/water 80/20, v/v) at 60 °C. Contact time was 60 min. After the extraction, samples were filtered with Whatman No. 1 filter paper and the residual tissue was washed with 2 × 25 ml of solvent. The filtrates were combined in a total extract, which was dried by vacuum rotary evaporator at 50 °C. The dry residues were weighed and redissolved with 50% methanol reaching a volume of 25 ml and centrifuged at 5000 rpm for 10 min. The obtained extracts were used for spectrophotometric and HPLC measurements. Extraction was done in duplicate for each grape cultivar. Average content of dry matter in skins subjected to extraction ranged from 19.68% to 25.05%.

2.6. Determination of total phenols (TPC), total flavonoids (TFLO), total flavanols (TFA) and total anthocyanins (TA) of the grape skin extracts

The total phenols, flavonoids, catechins, flavanols and anthocyanins were determined spectrophotometrically.

Table 1
Total phenolics (TPC), flavonoids (TFLO), catechins, flavanols (TFA) and anthocyanins (TA) in the grape berry skin extracts of seven white and seven red *Vitis vinifera* L. varieties (11 native and three introduced). The results are expressed in milligrams per kilogram of grape berries fresh weight.

Locality	Grape varieties		Weight (g) of 100 grape berries	TPC mg GAE/kg	TFLO mg GAE/kg	Catechins mg CE/kg	TFA mg ECE/kg	TA mg MglcE/kg
Imotski	Kujundžuša	W	242.9	1121 ± 23.5	872 ± 23.9	943 ± 15.3	77.9 ± 0.29	–
Vrgorac	Rkaciteli	W	184.5	539 ± 12.8	478 ± 27.3	121 ± 0.49	27.8 ± 0.98	–
Vrgorac	Zlatarica	W	267.2	1232 ± 3.52	1182 ± 3.78	896 ± 1.37	88.6 ± 0.48	–
Vrgorac	Medna	W	273.9	435 ± 1.69	389 ± 2.23	202 ± 1.47	30.3 ± 0.69	–
Vrgorac	Kuć	W	295.4	545 ± 3.66	456 ± 4.00	160 ± 5.00	42.2 ± 3.24	–
Drniš	Maraština	W	153.5	956 ± 7.05	768 ± 8.77	191 ± 4.40	25.5 ± 1.07	–
Drniš	Debit	W	237.8	1294 ± 21.0	654 ± 10.9	808 ± 0.70	93.2 ± 2.34	–
Average for white cultivars				**875 ± 362**	**686 ± 281**	**474 ± 385**	**55.1 ± 30.2**	–
Imotski	Vranac	R	227.3	2252 ± 10.9	1396 ± 21.1	707 ± 22.5	104 ± 1.61	739 ± 39.0
Imotski	Trnjak	R	340.8	3486 ± 54.7	2556 ± 44.0	1406 ± 42.4	158 ± 1.35	1286 ± 32.8
Imotski	Rudežuša	R	244.1	2972 ± 18.2	2594 ± 21.7	873 ± 33.0	75.9 ± 1.57	1848 ± 60.0
Vrgorac	Merlot	R	127.0	1666 ± 21.1	1068 ± 22.4	798 ± 15.5	100 ± 1.33	739 ± 18.8
Vrgorac	Babić	R	279.0	1031 ± 16.0	756 ± 17.6	830 ± 35.9	49.9 ± 1.53	336 ± 12.4
Drniš	Lasin	R	219.5	731 ± 9.06	400 ± 9.60	326 ± 22.7	91.8 ± 3.47	158 ± 4.53
Vrgorac	Plavina	R	258.8	820 ± 8.61	554 ± 4.23	486 ± 36.6	42.9 ± 1.35	238 ± 6.25
Average for red cultivars				**1851 ± 1089**	**1332 ± 910**	**775 ± 342**	**88.9 ± 38.6**	**763 ± 616**

Total phenols and flavonoids are expressed as gallic acid equivalents (GAE), catechins as catechin equivalents (CE), total flavanols as epicatechin equivalents (ECE), and total anthocyanins as malvidin-3-glucoside equivalents (MglcE).

The total phenolic concentration in grape skin extract (TPC) was determined by the Folin–Ciocalteau colorimetric method (Singleton & Rossi, 1965) calibrated against gallic acid standards and expressing the results as mg of gallic acid equivalents (GAE) per kg of grape berries fresh weight (FW).

The amount of total flavonoids (TFLO) was determined using a method developed by Kramling and Singleton (1969). Grape skin extracts were treated with formaldehyde to precipitate the flavonoid phenolic compounds. Condensed molecules were removed after 24 h by filtration. The remaining phenolics (non-flavonoids) were analysed by the Folin–Ciocalteau procedure. The content of TFLO was calculated as difference between total phenols and non-flavonoids (also reported as mg GAE per 1 kg of grape berries FW).

Catechins were determined using vanillin assay (Amerine & Ough, 1980). The vanillin test is specific for flavan-3-ols, proanthocyanins, and dihydrochalcones which have a single bond at the 2,3-position and possess free metahydroxy groups on the B-ring. The results are expressed in catechin equivalents (CE) per kg of grape berry FW.

The total flavanol (TFA) content was estimated using the *p*-dimethylaminocinnamaldehyde (DMAC) method (Arnous, Makris, & Kefalas, 2001). The concentration of total flavanols was calculated from a calibration curve, using (−)-epicatechin as a standard. The results are expressed in epicatechin equivalents (ECE) per kg of grape berries FW.

The amount of total anthocyanins (TA) in skin extracts from selected grape cultivars was determined using bisulphite bleaching method (Amerine & Ough, 1980). Absorbances were measured at 520 nm. Monomeric anthocyanins in grape skin extracts were calculated using the molar absorption coefficient for malvidin 3-glucoside. Results were expressed as mg of malvidin 3-glucoside equivalents (MglcE) per kg of grape berries FW.

Each determination was performed in triplicate. Results are expressed as means ± SD.

2.7. HPLC analysis of phenolic compounds

The polyphenolic compounds were separated on an octadecyl column (Zorbax Eclipse XDB-C18; 4.6 × 250 mm, 5 μm, Agilent) maintained at 25 °C. Grape skin extracts were filtered through 0.45-μm syringe filters and directly injected through a 20 μl fixed loop into a C_{18} guard column. Two gradient elution methods were used.

Method 1: A gradient consisting of solvent **A** (water/acetic acid, 98:2, v/v) and solvent **B** (acetonitrile/acetic acid, 98:2, v/v) was applied at a flow rate of 1.0 ml/min as follows: 0 min, 95% **A** and 5% **B**; 18 min, 75% **A** and 25% **B**; 25 min, 50% **A** and 50% **B**; 30 min, 20% **A** and 80% **B**; 35 min, 20% **A** and 80% **B**; 36 min, 20% **A** and 80% **B**; 38 min, 95% **A** and 5% **B**; 40 min, 95% **A** and 5% **B**.

Method 2: A gradient consisting of solvent **A** (water/acetic acid, 98:2, v/v), solvent **B** (water/acetonitrile/acetic acid, 78:20:2, v/v) and solvent **C** (acetonitrile/acetic acid, 98:2, v/v) was applied at a flow rate of 1.0 ml/min as follows: 0 min, 95% **A** and 5% **B**; 30 min, 50% **A**, 48% **B** and 2% **C**; 55 min, 5% **A**, 70% **B** and 25% **C**; 60 min, 0% **A**, 50% **B** and 50% **C**, 62 min, 0% **A**, 25% **B**, and 75% **C**; 64 min, 0% **A**, 25% **B**, 75% **C**; 66 min, 90% **A** and 10% **B**.

The signal was monitored at 280 nm. Each sample was injected two times. The stilbenes (*trans*- and *cis*-resveratrol monomers, piceid and astringin), flavan-3-ols ((+)-catechin, (−)-epicatechin, epicatechin gallate), procyanidin dimers (B1 and B2), and flavonol (quercetin 4-glucoside), were quantified from the areas of their peaks at 280 nm using external standard calibration curves.

2.8. Free radical-scavenging ability (DPPH)

Free radical-scavenging ability of grape skin extracts was determined by use of a stable 2,2-diphenyl-2-picrylhydrazyl radical ($DPPH^*$) (Von Gadow, Joubert, & Hansmann, 1997). Free radical working solution was prepared dissolving stock solution of $DPPH^*$ (4 mg DPPH in 100 ml EtOH) with EtOH to get an initial absorbance of 1.2 ± 0.02. An aliquot of 100 μl of the sample, adequately diluted with EtOH, was placed in a cuvette and reacted with 3 ml of $DPPH^*$ working solution. The mixture was shaken vigorously and left to stand for 60 min at room temperature in the dark. The decrease in absorbance was measured at 517 nm after 60 min, *versus* ethanol as blank. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. All determinations were performed in triplicate. The affinity of the test material to quench DPPH radicals (% Inh DPPH) was calculated using the following equation:

$$\%\,\mathrm{Inh\,DPPH} = \{(A_{control} - A_{sample})/A_{control}\} \times 100,$$

where A_{control} was calculated as a sum of initial absorbance of $DPPH^*$ plus sample (100 μl) in ethanol (3 ml). Sample concentration

providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Using the obtained curve, final results were expressed as IC_{50} values, i.e., mg GAE/l of grape skin extract needed to reduce DPPH radical by 50%.

2.9. Reducing power (FRAP)

The reducing capability of ethanolic extracts was measured as ferric reducing antioxidant power (FRAP). In this assay, antioxidants are evaluated as reductants of Fe^{3+} to Fe^{2+}, which is chelated by TPTZ to form a Fe^{2+}–TPTZ complex absorbing at 593 nm (Benzie & Strain, 1996). Absorbance was monitored by UV–Vis spectrophotometer, equipped with a six-cell holder and a thermostatically controlled bath. Standard curve was prepared using different concentrations (100–2000 μM) of Trolox. All solutions were used on the day of preparation. The antioxidant efficiency of grape skin extract was calculated with reference to the reaction signal given by the Trolox solution of known concentration. The plant extract to be analysed was first adequately diluted to fit within the linearity range. The results were corrected for dilution and expressed in mmol Trolox equivalents (TE). All determinations were performed in triplicate.

2.10. Chelating of metal ions

The chelating of ferrous ions by the sample was estimated using the method described by Yen, Duh, and Chuange (2000). The adequately diluted grape skin extract (1 ml) was mixed with methanol (3.7 ml) and 2 mM $FeCl_2$ (0.1 ml) and then 5 mM ferrozine (0.2 ml). The mixture was shaken vigorously and left standing at room temperature in the dark for 10 min. Absorbance of the resulting solution was measured spectrophotometrically at 562 nm. A low absorbance of the resulting solution indicated a strong Fe^{2+}-chelating ability. The ability to chelate ferrous ion and prevent formation of ferrous ion-ferrozine complex, was calculated using the following equation:

$$\text{Chelating effect}\,(\%) = \{1 - (A_{sample}/A_{control})\} \times 100,$$

where $A_{control}$ was the absorbance of a mixture of methanol (4.7 ml), 2 mM $FeCl_2$ (0.1 ml) and 5 mM ferrozine (0.2 ml). All analyses were run in triplicate and averaged. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

2.11. Antioxidant activity in the β-carotene–linoleic acid emulsion system

The antioxidant activity of grape skin extracts in an aqueous emulsion system of linoleic acid and β-carotene was determined according to a slightly modified method of Moure et al. (2000). A solution of β-carotene in chloroform was mixed with linoleic acid and Tween 20. After evaporation of chloroform, oxygenated distilled water was added. Solution of grape skin extract was added to this emulsion. The final concentration of grape skin extract in the emulsion was 0.040 mg/ml. As a control mixture, 50% methanol added to the above emulsion was used. The tubes were maintained at 50 °C in a water bath. The absorbance was measured at 470 nm immediately after their preparation ($t = 0$ min) and at incubation times $t = 60$, 80, 100 and 120 min, against the blank. The blank was prepared by adding 50% methanol to the above emulsion without β-carotene. The efficiency of investigated grape skin extract in protecting the oxidation of emulsified linoleic acid was expressed by means of antioxidant activity coefficient, C_{AA} that was calculated according to following relation:

$$C_{AA} = 1 - \left\{ \left(A_{s\,470\,nm\,(t=0)} - A_{s\,470\,nm\,(t=x)}\right) / \left(A_{c\,470\,nm\,(t=0)} - A_{c\,470\,nm\,(t=x)}\right) \right\},$$

where $A_{s\ 470\ nm\ (t=0)}$ is the initial absorbance of the sample containing antioxidant, $A_{c\ 470\ nm\ (t=0)}$ is the initial absorbance of the control, $A_{s\ 470\ nm\ (t=x)}$ and $A_{c\ 470\ nm\ (t=x)}$ are the absorbances of the sample and of the control, respectively, at incubation times. Each absorbance was measured twice. Duplicate runs were performed for each extract.

2.12. Determination of the minimum inhibitory concentration (MIC)

The extracts were prepared as described in Section 2.5 and diluted to 10% and 15% (v/v) stock solutions in MHB (or TSB when *E. coli* was tested). For the broth microdilution test bacterial culture (50 μl) in the early stationary phase (*ca.* 10^6 CFU/ml) was added to the wells of a sterile 96-well microtitre plate containing 50 μl of 2-fold serially diluted plant extracts stock solutions. The concentrations ranged from 0.002 to 1.0 mg GAE/ml growth medium. The *MIC* was calculated from the lowest concentration where no metabolic activity was observed after 24 h on the basis of the absence of a bioluminescence signal measured by a microplate reader (Tecan, Mannedorf/Zurich, Switzerland) after adding 100 μl/well of BacTiter-Glo™ reagent and 5 min incubation in the dark (Klančnik, Guzej, Hadolin-Kolar, Abramovič, & Smole Možina, in press). Minimum inhibitory concentrations (*MIC*s) are expressed in mg of gallic acid equivalents (GAE) per ml of growth medium. All measurements of *MIC* values were performed in triplicate and the most representative values were used.

2.13. Statistical analysis

Statistical analysis was performed with the GraphPad InStat3. The *t*-test was used to compare whether the means of variables differed between the samples. The relationship amongst all parameters in grape skin extracts was described by the Pearson product-moment correlation coefficient, *r*. Differences at $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

In this investigation a conventional solvent extraction with aqueous ethanol (80%) was used as it has been demonstrated to provide efficient extraction of phenolic compounds from different plant material (Peschel et al., 2006). Possible use of ethanol as an efficient solvent for extraction of phytochemicals should be of interest to the food industry.

3.1. Polyphenolic composition of grape skin extracts

Spectrophotometric and HPLC analyses were carried out to get insight into the polyphenolic profile of grape skin extracts of 14 different *Vitis vinifera* red and white grape varieties. The results for total phenols and main phenolic groups are presented in Table 1. The research showed differences in the mass amounts of the total phenolic compounds (TPC), flavonoids (TFLO), catechins, flavanols (TFA) and anthocyanins (TA) amongst selected grape cultivars, as expected. Although the extracts of certain red grape cultivars were extremely rich in phenolic compounds, the means of white and red cultivars for TPC, TFLO or TFA did not differ significantly ($p > 0.05$). In general, highly pigmented red cultivars *Rudežuša* i *Trnjak* can be emphasised as cultivars with grape skins extremely rich in phenolic compounds. Skins of these cultivars have a significant content of TPC (>70 mg/g of grape skin dry matter; about 3000 mg GAE/kg of grape berry FW), TFLO and TA. Amongst white cultivars, the highest amounts of total phenolics

were found in grape skin extracts of cultivars *Debit*, *Zlatarica*, and *Kujundžuša* (>1000 mg GAE/kg of grape berry FW). These cultivars were also rich in total flavonoids, catechins and flavanols. The smallest amounts of phenolic compounds were found in weakly pigmented *Lasin* and *Plavina* red grape skin extracts, and skin extracts of white cultivars *Kuč*, *Medna* and *Rkaciteli* (<1000 mg GAE/kg grape berry FW). This is in agreement with the finding of Yang, Liu, and Martinson (2009) that the phenolic content of different grapes depends mainly on the varietal differences, not on grape skin colour. In our case three red cultivars had lower TPC yield than three white cultivars (*Debit*, *Zlatarica*, and *Kujundžuša*). Regarding the catechin content, significant were white cultivars *Kujundžuša*, *Zlatarica* and *Debit*, and red cultivars *Trnjak*, *Rudežuša*, *Babić* and *Merlot*. Extremely high content of catechins in the extracts of certain white and almost all red cultivars implies the presence of condensed tannins (Amerine & Ough, 1980). Very significant correlation was confirmed between catechins and TPC, or TFLO ($p < 0.01$), and extremely significant correlation ($p < 0.001$) between catechins and flavanols (TFA) in grape skin extracts. The results for anthocyanin content were variety dependent and ranged from 3.33 ± 0.10 to 49.3 ± 1.60 mg MglcE/g of grape skin dry matter (DM). (158 to 1848 mg/kg grape berry FW; Table 1). In this study *Rudežuša* cv. had the highest total anthocyanin content whereas *Lasin* cv. had the lowest, showing an almost 12-fold difference. Significant correlation between total phenols and anthocyanins ($p < 0.05$) and extremely significant correlation between flavonoid and anthocyanin content ($p > 0.001$) was confirmed, as expected. The results for total anthocyanin contents are in agreement with data published by Yang et al. (2009).

Comparison of results with literature data is difficult due to different authors presenting results in different ways: per extract volume, per mass of grape berry fresh weight (FW) or per mass of berry skin dry matter (DM). However, the average content of extracted phenolic compounds was 33.6 ± 22.5 mg/g of berry skin DM (45.0 ± 26.3 mg GAE/g; and 22.2 ± 9.95 mg GAE/g; respectively for red and white cultivars), which is in accordance with results published by Negro et al. (2003) for red marc peel (33.3 ± 0.3 g/kg DM). Higher content of TPC in red grape skin extracts (≈100 μg/mg lyophilised grape skin) according to Arnous and Meyer (2008) can be explained by more intensive exhaustion of plant material, seven successive extractions in comparison to our one-step extraction procedure. Our results (Table 1) are further confirmation of the varietal dependence of content of not just total polyphenols but individual phenolic subgroups as well, as was already pointed out by other authors (Montealegre et al., 2006). The obtained results can also serve as an indicator of extractability of TPC, or phenolic subgroups, from skins of different grape varieties. As can be observed, approximately 74% of TPC are due to contribution of flavonoid compounds. The average ratios TFLO/TPC (0.72 for red; 0.79 for white varieties), catechins/TPC (0.45 for red; 0.54 for white varieties) and TFA/TPC (0.048 for red; 0.063 for white varieties) show that the contribution of total flavonoids, catechins and flavanols in the total phenolic content of grape skin extracts is higher for white varieties. It is certain that differences between total phenols or individual phenolic subgroups are influenced by the natural potential of an individual cultivar, but cell-wall structural components can also be an important factor in extractability of phenolic compounds (Pinelo et al., 2006). The high anthocyanin content in skin extracts of red grape varieties (41% of TPC; 57% of TFLO) agreed well with data published by Arnous and Meyer (2008), and is further confirmation of the fact that anthocyanins are more easily extracted from grapes skins compared to other phenolic groups.

The results of identification of individual stilbenes, and flavonoids in grape skin extracts are presented in Table 2. Because of the great interest that has recently been devoted to resveratrol and its derivatives a special attention was given to stilbene compounds. Free resveratrol monomers (*cis*- and *trans*-resveratrol) were found in all analysed, white and red, grape skin extracts. The average content of resveratrol monomers in the red varieties extracts was almost three times as high, in comparison to the white varieties. The obtained results are higher in comparison to the results of Bavaresco, Pezzuto, Gatti, and Mattivi (2007). The presence of piceid and astringin has been confirmed in most skin extracts. The presence of these phytoalexins, especially astringin which is usually found in grape leaves and stems, can be of importance for antimicrobial activity of extracts. Procyanidin B1, (+)-catechin, (−)-epicatechin gallate, and flavonol quercetin 4-glucoside have been determined in all selected grape skin extracts, whilst the presence of epicatechin was confirmed only for white cultivars (Table 2). The results show that polyphenolic composition of grape skin extracts was variety dependent.

Table 2
The amounts of stilbenes and flavonoids in grape skin extracts of 14 *Vitis vinifera* L. varieties obtained by HPLC–RP–PAD.

Grape variety	Stilbenes mg/kg of grape berry fresh weight					Flavonoids mg/kg of grape berry fresh weight					
	trans-R	*cis*-R	RM	E-Piceid	Astringin	C	EC	ECG	PC B1	PC B2	Qglu
Kujundžuša	0.27 ± 0.10	0.84 ± 0.06	1.11	1.11 ± 0.05	0.25 ± 0.08	2.93 ± 0.23	2.08 ± 0.36	1.66 ± 0.35	6.75 ± 0.67	1.95 ± 0.34	2.55 ± 0.05
Rkaciteli	0.26 ± 0.05	0.20 ± 0.04	0.46	0.26 ± 0.10	0.72 ± 0.39	1.25 ± 0.26	1.25 ± 0.07	2.64 ± 0.17	3.49 ± 0.12	2.20 ± 0.36	1.31 ± 0.60
Zlatarica	0.10 ± 0.04	0.26 ± 0.07	0.36	0.10 ± 0.04	0.21 ± 0.07	4.04 ± 0.21	0.81 ± 0.04	0.28 ± 0.07	10.65 ± 1.99	2.84 ± 0.04	0.58 ± 0.31
Medna	0.07 ± 0.05	0.13 ± 0.08	0.20	0.46 ± 0.05	0.64 ± 0.01	2.19 ± 0.15	0.85 ± 0.07	0.32 ± 0.08	2.74 ± 0.41	7.13 ± 0.38	0.35 ± 0.02
Kuč	0.09 ± 0.05	0.14 ± 0.08	0.23	n.d.	0.43 ± 0.03	1.25 ± 0.16	0.76 ± 0.22	0.24 ± 0.09	1.70 ± 0.40	0.44 ± 0.15	0.53 ± 0.27
Maraština	0.17 ± 0.02	0.29 ± 0.02	0.46	0.65 ± 0.02	0.75 ± 0.02	3.39 ± 0.09	1.42 ± 0.03	0.64 ± 0.06	6.36 ± 0.06	2.80 ± 0.02	0.21 ± 0.02
Debit	0.72 ± 0.05	0.26 ± 0.01	0.98	0.29 ± 0.01	1.18 ± 0.44	2.10 ± 0.09	2.22 ± 0.44	0.63 ± 0.01	4.89 ± 0.01	4.27 ± 0.05	0.49 ± 0.07
Average (White)	**0.24 ± 0.22**	**0.30 ± 0.25**	**0.54 ± 0.36**	**0.48 ± 0.36**	**0.60 ± 0.34**	**2.45 ± 1.06**	**1.34 ± 0.61**	**0.92 ± 0.90**	**5.23 ± 3.0**	**3.09 ± 2.12**	**0.86 ± 0.82**
Vranac	0.78 ± 0.09	0.93 ± 0.04	1.71	n.d.	0.75 ± 0.05	2.05 ± 0.16	n.d.	2.83 ± 0.27	2.51 ± 0.18	2.74 ± 0.05	1.73 ± 0.10
Trnjak	0.41 ± 0.02	1.74 ± 0.25	2.15	0.11 ± 0.04	0.55 ± 0.03	2.05 ± 0.05	n.d.	1.47 ± 0.09	18.13 ± 0.16	2.54 ± 0.03	1.16 ± 0.18
Rudežuša	0.61 ± 0.03	1.62 ± 0.21	2.23	1.09 ± 0.05	0.51 ± 0.04	0.73 ± 0.04	n.d.	7.81 ± 0.17	7.34 ± 0.10	n.d.	1.62 ± 0.11
Merlot	1.02 ± 0.10	0.36 ± 0.05	1.38	0.31 ± 0.03	0.56 ± 0.03	3.12 ± 0.24	n.d.	6.54 ± 1.89	2.74 ± 0.05	n.d.	1.65 ± 0.04
Babić	0.44 ± 0.02	0.42 ± 0.06	0.86	0.18 ± 0.01	0.56 ± 0.04	2.60 ± 0.03	n.d.	0.45 ± 0.07	1.53 ± 0.10	1.19 ± 0.12	1.18 ± 0.03
Lasin	0.59 ± 0.02	0.46 ± 0.07	1.05	n.d.	n.d.	4.57 ± 0.18	n.d.	1.13 ± 0.27	1.30 ± 0.27	n.d.	1.75 ± 0.95
Plavina	0.30 ± 0.08	0.07 ± 0.02	0.37	0.17 ± 0.03	0.26 ± 0.02	2.48 ± 0.12	n.d.	0.70 ± 0.02	1.81 ± 0.14	n.d.	0.79 ± 0.25
Average (Red)	**0.59 ± 0.25**	**0.80 ± 0.65**	**1.39 ± 0.69**	**0.37 ± 0.41**	**0.53 ± 0.16**	**2.51 ± 1.17**	**n.d.**	**2.99 ± 2.98**	**5.05 ± 6.13**	**–**	**1.41 ± 0.37**

trans-R (*trans*-resveratrol); *cis*-R (*cis*-resveratrol); RM (resveratrol monomeres: *trans*-R + *cis*-R).
(+)-Catechin (C); (−)-epicatechin (EC); epicatechin gallate (ECG); procyanidin dimer B1 (PC B1); procyanidin dimer B2 (PC B2); quercetin 4-glucoside (Qglu).
The results are average of two injections, and expressed in mg of polyphenolic compound per kg of grape berry fresh weight.

Table 3
Antioxidant properties for grape skin extracts of 14 *Vitis vinifera* L. varieties determined as DPPH radical-scavenging capacity, Fe^{2+}-chelating ability, ferric reducing/antioxidant power (FRAP) and efficiency of investigated grape skin extract in protecting the oxidation of emulsified linoleic acid (C_{AA}). Results are mean ± SD.

Grape variety	DPPH radical-scavenging activity IC_{50} (mg GAE/l)*	Fe^{2+}-chelating ability IC_{50} (mg GAE/l)*	FRAP (mM TE)**	C_{AA} (%)
White varieties				
Kujundžuša	79.3 ± 4.04	151 ± 8.32	5.67 ± 0.10	62.4 ± 9
Rkaciteli	52.8 ± 3.33	21.5 ± 3.11	1.52 ± 0.03	72.9 ± 0.2
Zlatarica	133 ± 5.17	75.5 ± 3.31	5.79 ± 0.05	69.7 ± 7
Medna	236 ± 8.08	21.0 ± 3.61	2.46 ± 0.04	n.d.
Kuč	192 ± 3.00	21.7 ± 2.89	3.41 ± 0.04	81.2 ± 2
Maraština	291 ± 4.51	70. 7 ± 3.78	1.48 ± 0.03	69.4 ± 6
Debit	159 ± 6.11	129 ± 4.68	4.19 ± 0.11	50.2 ± 13
Red varieties				
Vranac	156 ± 6.76	475 ± 5.51	15.3 ± 0.52	87.4 ± 4
Trnjak	209 ± 6.54	482 ± 15.3	15.5 ± 0.15	87.8 ± 7
Rudežuša	239 ± 4.16	655 ± 7.07	16.4 ± 0.11	88.0 ± 4
Merlot	153 ± 3.79	300 ± 9.41	9.88 ± 0.28	89.7 ± 4
Babić	58.0 ± 3.00	235 ± 10.0	8.24 ± 0.09	85.9 ± 0.2
Lasin	64.2 ± 4.25	52.0 ± 2.65	2.68 ± 0.04	75.5 ± 3
Plavina	158 ± 3.18	102 ± 3.21	5.49 ± 0.12	87.8 ± 0.5

* IC_{50} – sample concentration in mg GAE per l of grape skin extracts providing 50% inhibition.
** TE – Trolox equivalents.

3.2. Antioxidant properties of grape skin extracts

Antioxidant properties of grape skin extracts were determined as free radical-scavenging ability (DPPH method), reducing power (FRAP method), Fe^{2+}-chelating ability of plant extracts, and ability to prevent oxidation of linoleic acid. The results are presented in Table 3, Figs. 1 and 2. The high antioxidant capacity of all extracts has been observed and related to the presence of a mixture of polyphenolic compounds with good antioxidant properties.

3.2.1. Free radical-scavenging ability

Some methods such as free radical-scavenging assays might provide information on how capable an antioxidant is in preventing reactive radical species from reaching lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars in biological and food systems. The relatively stable organic radical, DPPH, has been widely used in the determination of antioxidant activity of single compounds, as well as of different plant extracts. Antioxidants can deactivate (scavenge) stable free DPPH radical by two major mechanisms: by reduction *via* electron transfer or by hydrogen atom transfer that may occur also in parallel, and steric accessibility is one of the major determinants of the reaction. The ethanolic grape skin extracts of selected, both red and white, grape varieties were able to interact with the stable free DPPH radicals efficiently and quickly, with an IC_{50} of 148 ± 70.1 mg GAE/l. The difference between mean values for red and white cultivars was not statistically significant (Fig. 1). Differences for DPPH IC_{50} between certain grape cultivars can be related to differences in polyphenolic

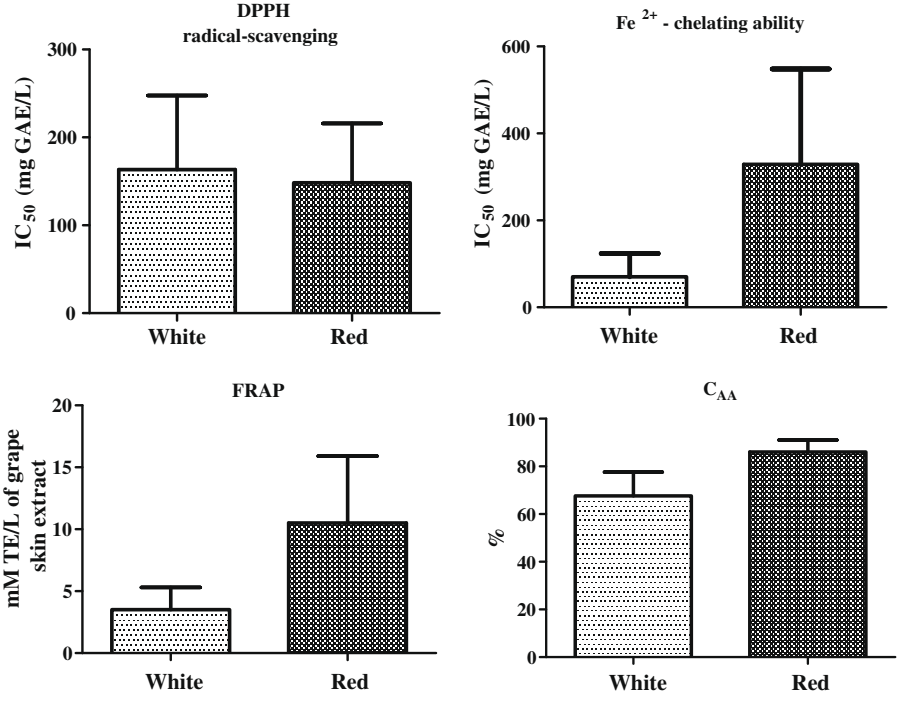

Fig. 1. Comparison of antioxidant properties of grape skin extracts from 14 grape varieties (seven red and seven white) determined as DPPH radical-scavenging ability, Fe^{2+}-chelating ability, ferric reducing/antioxidant power (FRAP method), and using β-carotene bleaching assay (C_{AA}).

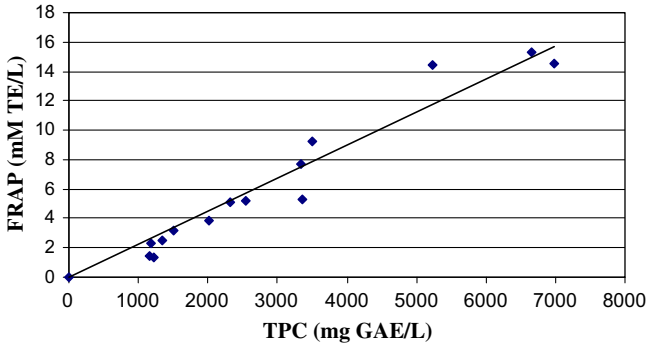

Fig. 2. Correlation between total phenolic content (TPC) and related FRAP (ferric reducing ability/antioxidant power) of phenolic extracts from a grape skins of 14 *Vitis vinifera* L. varieties ($y = 0.0022\ x$; $r^2 = 0.9456$).

content of analysed extracts. A significant positive correlation between DPPH IC_{50} and the content of total anthocyanins in red grape skin extracts point to a weaker contribution of this flavonoid group to free radical-scavenging activity.

3.2.2. Reducing power (FRAP)

Reducing power of grape skin extracts, determined as FRAP, ranged from 1.48 to 16.4 mM TE. FRAP for red cultivars was 10.5 ± 5.41 mM TE and significantly higher than FRAP for white cultivars (3.50 ± 1.80 mM TE). Extremely significant correlations ($p < 0.0001$) between FRAP and TPC, or TFLO, or catechins, and significant correlation ($p < 0.05$) between FRAP and TFA, or TA in grape skin extracts was observed (Fig. 2). The strong correlation between FRAP and TPC was expected because the Folin-Cioacalteau (FC) method is not an absolute measurement of phenolics content. The FC method is based on the reaction of reducing groups, and in fact measures the reducing capacity of any phenolic compound relative to the reducing capacity of gallic acid as standard (Frankel, Waterhouse, & Teissedre, 1995). Significant correlation ($p < 0.05$) between DPPH radical-scavenging ability and reducing power (FRAP) of extracts was observed. The quantity of phenolic compounds with a reduction power of 1 mM of Trolox (FRAP2) was calculated for each extract. The means of FRAP2 for white and FRAP2 for red grape skin extracts differed significantly. Mean ± SD FRAP2 for skin extracts of red cultivars (412 ± 54.7 mg/l) was low compared to white cultivars (574 ± 157 mg/l of extract). It can be concluded that polyphenolic mixtures of red skin extracts generally have better reducing power, due to the presence of anthocyanins.

3.2.3. Metal chelating effect

Chelating effect is one indicator of antioxidant properties in a pure compound or plant extract. Chelating of transition metal ion Fe^{2+} is an important part of antioxidative strategy because Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions even starting with relatively non-reactive radicals (Ebrahimzadeh, Poumorad, & Bekhradnia, 2008). The method employing ferrozine turned out to be very adequate for the comparison of chelating ability of berry skin extracts amongst different grape varieties. Ferrozine can quantitatively form complexes with Fe^{2+} but in the presence of chelating agents, the complex formation is disrupted with a resulting decrease the red colour of the complex. All extracts showed a significant capability of chelating Fe^{2+} ion. Chelating effect of grape skin extracts expressed as IC_{50} (Table 3) indicate that polyphenolic compounds in extracts, both red or white, can capture ferrous ions more efficiently compared to some herb extracts (Ebrahimzadeh et al., 2008). The means of IC_{50} for red and white grape skin extracts differed significantly and was more than 4-folds lower for white in comparison to red grape varieties. Amongst red cultivars lower chelating activity was observed for cultivars with higher content of anthocyanins.

3.2.4. Antioxidant activity in the β-carotene–linoleic acid emulsion system

Generally, real food is a heterogenous system that consists of multiple phases in which lipid and water coexist with some emulsifier. Therefore an aqueous emulsion system of linoleic acid and β-carotene incubated at elevated temperature was used to evaluate the antioxidant activity of investigated extracts. The free radicals (peroxyl radicals) formed when linoleic acid is oxidised attack β-carotene molecules that consequently undergo rapid decolorisation. As can be seen in Table 3, where the values of C_{AA} for grape skin extracts in β-carotene–linoleic acid emulsion system at an antioxidant concentration of 0.040 mg/ml are presented, all investigated skin extracts efficiently inhibited the oxidation of emulsified linoleic acid (inhibited β-carotene bleaching). Skin extracts of red varieties with C_{AA} averaging 86 ± 5% exhibited, contrary to results obtained in the chelating activity test, higher antioxidant activity than skin extracts of white varieties with C_{AA} that amounted on average to 68 ± 10%. The highest efficiency in protecting emulsified linoleic acid was exhibited by the extract of variety *Rudežuša*, whilst in the homogenous system (DPPH test) this extract exhibited the weakest ability in scavenging free radicals. It seems therefore that distinct compounds present in the extract may define the antioxidant efficiency, depending on the assay used, which has previously been confirmed (Klančnik et al., in press). In emulsion, antioxidative activity of a compound amongst others depends on its polarity and consequently on its partition between aqueous and lipid phase. Further, DPPH IC_{50} and C_{AA} determinations are based on different reaction mechanisms and the reaction kinetics in these assays may depend on the relative proportions of particular compounds in an extract that act additively or synergistically. In comparison to pure phenolic compound, gallic acid, grape skin extracts retarded the oxidation of emulsified linoleic acid more effectively and retained their antioxidant activity through the whole incubation time. At $t = 80$ min for red skin extracts C_{AA} was 90 ± 4%, C_{AA} of white skin extracts was 77 ± 10%, whilst for gallic acid C_{AA} amounted to 2.3 ± 0.6%. At $t = 100$ min gallic acid was no longer antioxidatively active. Many publications (Roussis et al., 2008) reported limited or even negligible antioxidant activity of extracts from white wines in emulsified linoleic acid, which can be explained by the absence of anthocyanins and other flavonoids in white wines *versus* red wines and by poorer distribution of phenolic acids between the two phases of the emulsion. Phenolic acids in grapes are located mainly in the pulp and are the most abundant phenolic fraction of white wine extracts. Stilbenes are synthesised in the leaf epidermis and the skin of grapes and only tracers are present in the pulp. Flavonoid compounds are located in skins, seeds and stems and due to lack of maceration in white wines did not reach the same amount as in red wines. In white grape skins the most abundant phenolic compounds are flavanols, proanthocyanidins and hydroxycinnamic acids (Montealegre et al., 2006), whilst in red grape skins anthocyanins, flavanols, proanthocyanidins, flavonols and hydroxycinnamic acids were determined (Kammerer et al., 2004). Anthocyanins and flavonols followed by flavanols have been reported (Fukumoto & Mazza, 2000) very active in the β-carotene bleaching test, whilst phenolic acids were less active. In this study extracts from white grape skins efficiently protected the oxidation of emulsified linoleic acid, which was confirmed by the presence of active phenolic compounds in these extracts (Tables 1 and 2).

Table 4
Antimicrobial activity for grape skin extracts of 14 *Vitis vinifera* L. varieties (expressed as *MIC*s of total phenols, e.g., GAE* per ml of growth medium in broth microdilution test).

Testing organisms	*S. aureus*	*B. cereus*	*E. coli*	*S.* Infantis	*C. coli*
White varieties					
Kujundžuša	0.15 ± 0.02	0.08 ± 0.02	0.15 ± 0.02	0.11 ± 0.02	0.032 ± 0.005
Rkaciteli	0.20 ± 0.03	0.20 ± 0.03	0.15 ± 0.03	0.11 ± 0.01	0.014 ± 0.002
Zlatarica	0.21 ± 0.03	0.21 ± 0.03	0.59 ± 0.08	0.29 ± 0.04	0.042 ± 0.005
Medna	0.21 ± 0.03	0.15 ± 0.03	0.15 ± 0.03	0.10 ± 0.01	0.014 ± 0.002
Kuč	0.26 ± 0.04	0.19 ± 0.04	0.19 ± 0.04	0.13 ± 0.02	0.019 ± 0.002
Maraština	0.21 ± 0.03	0.21 ± 0.03	0.15 ± 0.03	0.11 ± 0.02	0.015 ± 0.002
Debit	0.25 ± 0.05	0.25 ± 0.05	0.25 ± 0.05	0.18 ± 0.03	0.025 ± 0.005
Average	0.22 ± 0.04	0.18 ± 0.06	0.23 ± 0.06	0.15 ± 0.07	0.023 ± 0.01
Red varieties					
Vranac	0.23 ± 0.03	0.16 ± 0.03	0.23 ± 0.03	0.16 ± 0.03	0.20 ± 0.03
Trnjak	0.22 ± 0.04	0.12 ± 0.02	0.31 ± 0.04	0.31 ± 0.04	0.14 ± 0.03
Rudežuša	0.29 ± 0.04	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.25 ± 0.06
Merlot	0.44 ± 0.08	0.22 ± 0.04	0.44 ± 0.08	0.44 ± 0.08	0.13 ± 0.03
Babić	0.42 ± 0.08	0.21 ± 0.04	0.21 ± 0.04	0.21 ± 0.04	0.08 ± 0.01
Lasin	0.34 ± 0.07	0.34 ± 0.07	0.17 ± 0.03	0.17 ± 0.03	0.04 ± 0.01
Plavina	0.29 ± 0.06	0.29 ± 0.06	0.41 ± 0.06	0.29 ± 0.06	0.09 ± 0.02
Average	0.32 ± 0.09	0.21 ± 0.08	0.25 ± 0.16	0.25 ± 0.11	0.13 ± 0.07

* Total phenols are expressed as gallic acid equivalents (GAE) in grape skin extract.

3.3. Antimicrobial effect of grape skin extracts

Although grape skins are confirmed as rich sources of phenolic compounds, both flavonoids and non-flavonoids, and recent results indicate higher antimicrobial potential of natural extracts than shown by selected components alone against several microorganisms (Serra et al., 2008), still low numbers of reports are available about antimicrobial activity of grape skin extracts or their comparison with efficiency of extracts from other by-products of grape processing (Baydar, Özkan, & Sagdiç, 2004; Luther et al., 2007).

The antimicrobial activity of all 14 phenolic extracts was screened by broth microdilution test using Gram-positive (*S. aureus*, *B. cereus*) and Gram-negative bacteria (*E. coli* O157:H7, *Salmonella* Infantis, *C. coli*). The activity was confirmed against all tested organisms. Minimum inhibitory concentrations (*MIC*s) were expressed in mg gallic acid equivalents (GAE)/ml of phenolic compounds per ml microbiological growth medium and ranged between 0.014 and 0.59 mg GAE/ml for different organisms and extracts (Table 4). The differences in efficiency of phenolics from white and red grape cultivars are seen against different testing organisms. Interestingly, on average lower *MIC*s were confirmed for phenolic mixtures from white grape cultivars for all tested species, although the differences were not statistically significant. Furthermore, no significant differences were found in susceptibility of Gram-positive and Gram-negative bacteria, although it has often been reported that plant phenolic extracts are more efficient against Gram-positive bacteria (Klančnik et al., in press). In this study, the most susceptible organism was Gram-negative *Campylobacter*, followed by Gram-negative *Salmonella* in extracts of white cultivars (and by Gram-positive *Bacillus* for red cultivars). Directly comparable results in the literature are missing due to differences in preparation of grape extract and performance of microbiological assays and due to different expression of antimicrobial activity.

4. Conclusion

Skins of pigmented red cultivars *Rudežuša* and *Trnjak* have a significant content of TPC (>70 mg/g of grape skin dry matter), TFLO and TA, and can be emphasised as the cultivars with grape skins extremely rich in phenolic compounds. Amongst white cultivars the highest amounts of total phenolics were found in grape skin extracts of cultivars *Debit*, *Zlatarica*, and *Kujundžuša* (>20 mg GAE/g berry skin DM; about 1000 mg GAE/kg of grape berry FW). These cultivars were also rich in total flavonoids, catechins and flavanols. These results are confirmation of the varietal dependence on the content of not just total polyphenols but individual phenolic subgroups as well. The contents of identified individual polyphenols, stilbenes or flavonoids, were small compared to total phenolic content in grape skin extracts. However, as these polyphenols, especially resveratrol, are the compounds postulated for health benefits of wine and grapes, the knowledge of natural potential of grape skins regarding content of these biologically-active polyphenols is important for further exploiting this plant material. Further, the results of antioxidant activity tests show that the grape skin extracts should be treated as efficient reductants, chelators and free radical scavengers in homogenous systems and in emulsion. As white grape skins leave the wine processing still rich in biologically valuable components, they could be interesting and inexpensive material for the production of new food products or food additives. In this respect our results are very promising with regard to the antimicrobial activity of grape skin extracts of white grape cultivars, especially against Gram-negative bacteria like *Campylobacter* and *Salmonella*, most frequent causes of bacterial food-borne infections in developed countries. Further tests are needed to confirm these screening results in other *in vitro* and *in vivo* assays, e.g., in selected food model systems.

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