Insight in the phenolic composition and antioxidative properties of *Vitis vinifera* leaves extracts

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Summary

In the present investigation, leaf ethanolic extracts of *Vitis vinifera* were assayed for their polyphenolic composition and antioxidative properties. The leaves were collected during lush vegetation period (*May leaves*) and after the harvest (*September leaves*). Air dried plant material was homogenized and the polyphenolic constituents were extracted using conventional solvent extraction procedure. Total phenolics, flavonoids, non-flavonoids, catechins and flavanols were determined using spectrophotometric methods. Both extracts were very rich in phenolic compounds. The concentration of total phenols in *September leaves* extract was about 30 % higher compared to *May leaves* extract, due to the increase of flavonoid (catechin) fraction. Non-flavonoid compound content was almost equal in both extracts. The amount of flavanols, determined with *p*-dimethylaminocinnamaldehyde method, was taken as indicator of flavan-3-ol monomers, while high catechin content determined by vanillin method, indicated the presence of polymeric fraction. The total catechin content in *September leaves* extract was more than 3 folds higher in comparison to *May leaves* extract. Principal phenolic compounds were separated by high pressure liquid chromatography on reverse phase. Antioxidant properties, determined as: 2,2-diphenyl-1-picrylhydrazyl radical and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) radical cation scavenging ability, ferric reducing/antioxidant power, Fe^{2+} chelating activity, and using β-carotene bleaching assay, were total phenol concentration dependent. *September leaves* extract had better free radical scavenging capacity, higher reducing power, and was more efficient in protecting the oxidation of emulsified linoleic acid, in comparison with *May leaves* extract which showed better chelating ability. The presence of active phenolic compounds: phenolic acids (3-hydroxybenzoic acid, caffeic acid, gallic acid, vanillin acid), flavonoids ((+)-catechin, (-)-epicatechin, apigenin, myricetin, quercetin, quercetin-4'-glucoside, rutin), and stilbenes (*trans*-resveratrol and resveratrol derivatives) was confirmed in both extracts. According to the results achieved, vine leaf extracts can be considered rich natural source of polyphenols with significant antioxidant properties.

Keywords: *Vitis vinifera*, leaves, phenolic composition, antioxidant activity

Introduction

There is considerable interest in the analysis of phenolic compounds due to their potential contribution in protecting the cells against the oxidative damage and thus preventing many human diseases including neurodegenerative disease, cardiovascular disorders and cancer (Rice-Evans and Packer, 2003; Borbalan et al., 2003; German et al., 1997; Prior and Ciao, 2000; Poudel et al. 2008). Our research up to date have proved that many medicinal plants, well known in Croatian folk medicine, are extremely rich in phenolic compounds with significant antioxidative activity, which can be linked with their therapeutic properties (Katalinić et al., 2006).

Grape vine is cultivated predominantly because of its fruits, which are used fresh, dried or processed into wine. A known herbal medicine practitioner Marušić (1990) points out the singnificance of vine in his book *Through herbal medicine to health*, noting that primary material for preparation of herbal remedies should be grapes, flowers, leafes and vine tendrils. Knowledge of the medicinal properties of grape vine (*Vitis vinifera* L.) can be traced far back in history. In Ayurvedic (Indian) system, grape leaves are used as a folk remedy for the treatment of diarrhea and vomiting (Pari and Suresh, 2009). In Europe, the leaves of *Vitis vinifera* are documented in the literature of traditional medicine for their astringent and homeostatic properties where they are utilized in the treatment of diarrhea, bleeding, haemorrhoids, varicose veins and other circulatory diseases (Bombardelli and Morazzoni, 1995; Lardos and Krauter, 2000; Orhan et al., 2009; Pari and Suresh, 2009).

Recent studies are focused on wines produced from *Vitis vinifera*. While grape skin, seed, and wine

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phenolics and their potential biological activities, including antioxidant, antiinflamatory, antimicrobial, anticarcinogenic properties were widely studied (Frankel et al., 1995; German et al., 1997; Katalinić et al., 2010; Mudnić et al., 2010; Yang et al., 2009), research on phenolic composition and biological activities of grape vine leaves are scarce (Bombardelli and Morazzoni, 1995; Balik et al., 2009; Orhan et al., 2009).

The aim of this study was to determine and compare phenolic composition and antioxidative profile of *Vitis vinifera* leaves collected in two different phenophases. Phenolic composition of leaf extract was analysed using spectrophotometric and chromatographic methods. The antioxidant potential was tested using five distinct assays: ferric reducing ability (FRAP), iron chelating (CA), scavenging of DPPH, and ABTS radicals, and by β-carotene bleaching assay (BCB).

Materials and methods

Reagents, solvents and standards

All reagents and solvents used in the experiments were of adequate analytical grade and were obtained from Fluka (Buch, Switzerland), Kemika (Zagreb, Croatia), Merck (Darmstadt, Germany) and Sigma (Sigma–Aldrich GmbH, Steinheim, Germany). HPLC standards were obatined from Extrasynthese (Genay, France), Sigma (Milwaukee, USA) and Polyphenols Laboratories (Sandnes, Norway). Acetic acid and acetonitril (HPLC grade) were purchased from Merck (Darmstadt, Germany). Water was prepared by purification with a Millli-Q-water purification system (Millipore, Bedford, Massachusetts).

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, Gremany). The HPLC system used for separation and quantification of individual polyphenolic compounds was composed by 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.

Plant material

The present research includes two samples of *Vitis vinifera* L. leaves. Fully expanded, green, healthy leaves were collected in May and in September (after vintage) in Teskera vineyards, Kijevo, Dalmatia, Croatia). The plant material was washed with cold water and air dried in shade at room temperature. The leaf petioles were carefully manually separated. After that dry leaves were pulverized (1 min in high speed grinder) into powder. Two samples: *May leaves* and *September leaves*, were prepared by mixing equal mass amounts of powdered plant material obtained from different grape varieties. The voucher specimens of *Vitis vinifera L.* plant material are deposited in the Department of Food technology, Faculty of Chemistry and Technology, University of Split, Croatia.

Extractions of polyphenols

The polyphenolic constituents were extracted using conventional solvent extraction procedure. Five grams of dry, homogenized *Vitis vinifera* leaves (1 min in high speed grinder) was subjected to solvent extraction with 250 mL of alcoholic solvent (ethanol/water 80/20, v/v; 60 °C; 60 min). After the cooling at room temperature (30 min), the extract was filtered with Whatman No. 1 filter paper to remove residual particles, and the residual tissue was washed with 3x10 mL of solvent. Extractions were done in triplicate replica for each plant material. Extracts obtained for the same plant material were combined together in total extract. The total extract was concentrated *in vacuo* (< 40 °C) to 150 mL. Thus obtained extracts (MLE: *May leaf extract*; SLE: *September leaf* extract) were centrifuged and used for spectrophotometric and HPLC measurements.

Determination of total phenols, flavonoids, non-flavonoids, total catechins and flavanols

Total phenolic concentration (TP) in *Vitis vinifera* leaf extract was determined spectrophotometrically according to the Folin-Ciocalteu (FC) colorimetric method (Amerine and Ough, 1980), calibrating against gallic acid standards and expressing the results in gallic acid equivalents (GAE)/L of leaf extract. Data presented are average of three measurements.

The amount of total non-flavonoids was determined by method developed by Kramling and Singleton (1969). The content of total flavonoids (TFLO) was calculated as difference between total phenols and non-flavonoids. The results are reported in mg GAE/ L of extracts.

Catechins (CAT) were determined using vanillin assay (Amerine and Ough, 1980). The results are expressed in catechin equivalents (CE) per liter of extract.

The amount of total flavanol content was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method (Arnous, Makris and Kefalas, 2001). The concentration of total flavanols was calculated from a calibration curve, using (-)-epicatechin as a standard. The results are reported in epicatechin equivalents (ECE)/ L of leaf extract.
Each determination was performed in triplicate. Results are expressed as means ± SD.

HPLC separation of phenolic compounds

The polyphenolic compounds were separated on an octadecyl column (Zorbax Eclipse XDB-C18; 4.6x250, 5μ, Agilent) maintained at 25 ^{o}C. Plant extracts were filtered through 0.45-μm syringe filters and directly injected through a 20 μL fixed loop into a guard C_{18} column.

Separation of polyphenols: 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^{-1} as follows: 0 min 92 % A and 8 % B; 18 min 80 % A and 20 % B; 25 min 60 % A and 40 % B; 30 min 55 % A and 45 % B; 40 min 35 % A and 65 % B; 50 min 20 % A and 80 % B; 54 min 20 % A and 80 % B; 57 min 90 % A and 10 % B; 60 min 90 % A and 10 % B.

Separation of phenolic acids: A gradient consisting of solvent A (acetonitrile), solvent B (water/acetic acid, 99:1, v/v) and solvent C (methanol) was applied at a flow rate of 1.0 mL min^{-1} as follows: 0 min 1 % A, 95 % B and 4 % C; 15 min 5 % A, 85 % B and 10 % C; 45 min 15 % A, 35 % B and 50 % C; 60 min 20 % A, 5 % B and 75 % C; 72 min 1 % A, 95 % B and 4 % C; 75 min 1 % A, 95 % B and 4 % C.
The signal was monitored at 280 nm wavelength. Each sample was injected two times in the chromatographic system. The phenolics were quantified from the areas of their peaks at 280 nm using external standard calibration curves.

Antioxidative capacity of leaf extracts

Free radical scavenging ability

Free radical scavenging ability of *Vitis vinifera* LE was determined by use of two stable free radicals: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Von Gadow, Joubert and Hansmann, 1997), and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) radical cation (Re et al., 1999; Katalinić et al., 2006).

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical (% Inh DPPH), or ABTS radical (% Inh ABTS), by the samples was calculated according to the formula of Yen and Duh (1994).
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}, inhibitory concentration, mg GAE per L of leaves extract needed to reduce DPPH (or ABTS) radical by 50 %.

Reducing power (FRAP)

The reducing capability of ethanolic extracts was measured as ferric reducing antioxidant power (FRAP) as described by Benzie and Strain (1996). Standard curve was prepared using different concentrations (100–2000 μmol/L) of Trolox. 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.

Measurement of chelating activity on metal ions

The chelating of ferrous ions by the sample was estimated by method described by Dinis et al. (1994). The adequately diluted *Vitis vinifera* leaves extract (1 mL) was mixed with methanol (3.7 mL) and 2 mM $FeCl_2$ (0.1 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left standing at room temperature in dark for 10 min. Absorbance of the resulting solution was measured spectrophotometrically at 562 nm. A low absorbance of the resulting solution indicates 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:

$$\text{Metal chelating activity (CA \%)} = [1-(A_{\text{sample}}/A_{\text{control}})] \times 100 \quad (1)$$

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

Determination of antioxidant activity with the β-carotene bleaching (BCB) test

Antioxidant activity of *Vitis vinifera* leaf extract in an aqueous emulsion system of linoleic acid and β-carotene was determined according to a slightly modified method of Pratt (1980) as described by Kulišić et al. (2004). The total phenol concentration in plant extract to be analysed was 1000 mg GAE/L. All determinations were performed in duplicate. The efficiency of investigated sample in protecting the oxidation of emulsified linoleic acid was calculated according to the formula (Mallet et al., 1994):

$$\text{BCB (\% Inhibition)} = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)})] \times 100 \qquad (2)$$

where $A_{A(120)}$ is the absorbance of the antioxidant at t = 120 min, $A_{C(120)}$ is the absorbance of the control at t = 120 min, and $A_{C(0)}$ is the absorbance of the control at t = 0 min.

Statistical analysis

All values are expressed as the mean ± SD. Statistical analysis was performed with the GraphPad InStat3 statistical program.

Results and discussion

A range of different spectrophotometric and chromatographic methods were used to analyze the phenolic content and antioxidative profile of *Vitis vinifera* leaf extracts. Extraction of phenolics was done using conventional extraction method taking care of solubility of polyphenolic compounds and aggressiveness of extraction procedure. Results of *Vitis vinifera* leaf extracts phenolic content determination point to differences in content of total phenols, phenolic subgroups and indvidiual phenolic compounds between MLE and SLE (Table 1, Table 3, Fig. 1).

Table 1. Phenolic composition of *Vitis vinifera* leaf extracts determined using different spectrophotometric methods

Selected plant material	Total phenols (mg GAE/L)	Flavonoids (mg GAE/L)	Non-flavonoids (mg GAE/L)	Catechins (mg CE/L)	Flavanols (mg ECE/L)
May leaves	2910.5 ± 16.5	1165.3 ± 4.8	1745.1 ± 21.2	223.4 ± 1.2	11.7 ± 10.0
September leaves	3338.7 ± 29.5	1579.9 ± 17.4	1758.8 ± 12.6	717.9 ± 36.8	87.3 ± 7.1

Total phenols, flavonoids and non-flavonoids are expressed as gallic acid equivalents (GAE); Catechins as catechin equivalents (CE); Flavanol monomers as epicatechin equivalents (ECE).

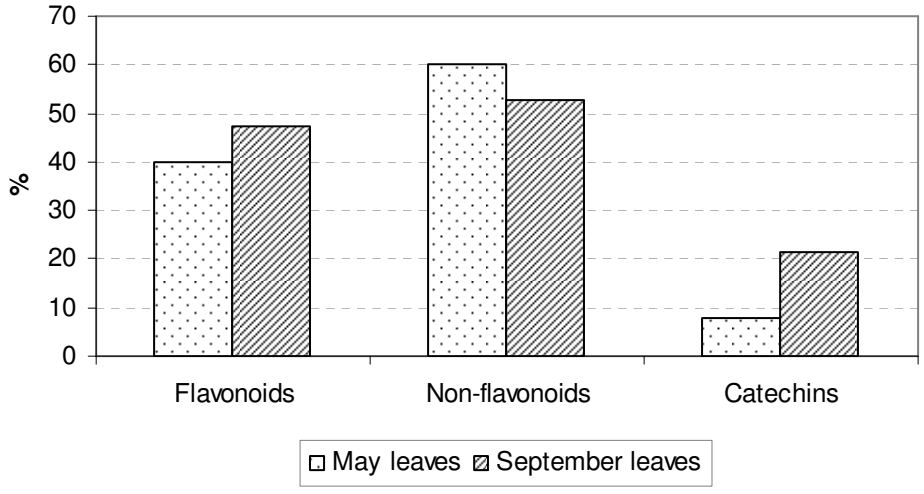

Fig. 1. The relative content of main phenolic groups in the *Vitis vinifera* leaf extracts

Grape leaf extracts from both phenophases, MLE and SLE, were rich in total phenolics. The average concentration of total phenols in SLE was 3338.7±29.54 mg GAE/L, and was about 30 % higher compared to MLE, due to the significant increase of total flavonoid, especially total catechin, fraction. The content of non-flavonoid phenolics was almost equal in both extracts (1745.1±21.2 mg GAE/L of MLE and 1758.8±12.6 mg GAE/L of SLE). The average concentration of total catechins in MLE (223.4 mg CE/L) was more than 3 folds lower compared to average catechin concentration in SLE (717.9±36.8 mg CE/L). The amount of flavanols, determined with DMACA protocol which provides higher specificity than vanillin protocol, was taken as indicator of flavanol monomer content (Li et al., 1996; Arnous et al., 2001). Thies and Fischer (1971) first reported the deep blue colouration of 4-dimethylaminocinnamaldehyde (DMACA), aromatic aldehyde, after reaction with catechins. The average content of flavanols was 11.7±10.0 mg ECE/L and 87.3±7.1 mg ECE/L, successively given for MLE and SLE. These results are coherent with HPLC analysis of extracts (Table 3). Flavan-3-ol monomers: (+)-catechin and (-)-epicatechin, were identified in both extracts. Average amount of these two catechin monomers was 6.6 mg/L of MLE, and 38.46 mg/L of SLE, what indicates that increase in total flavanols was mostly influenced by increase of (+)-catechin content. The total catechin content in leaves extracts (vanillin method) was taken as indicator of the proanthocyanidins, polymeric flavanols formed by condensation of monomeric flavanols (as initiating unit) and flavan-3,4-diols (leukoanthocyanidins, as extension units). From the results presented in Table 1 and Table 3, it can be concluded that during the period between flourishing till the end of ripening a significant accumulation occurred not only of catechin monomers but also of condensed catechin forms in grape vine leaves. The total catechin content in SLE was more than 3 folds higher in comparison to MLE. Although absolute concentration of total flavans in SLE was almost 8 times higher than in MLE, relative flavanol monomer content was smaller in SLE compared to MLE (Fig. 2).

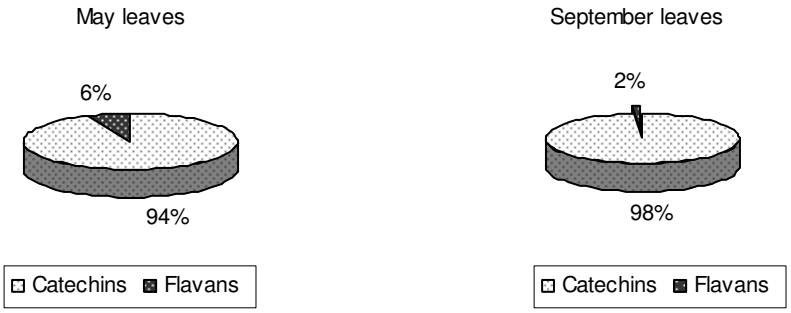

Fig. 2. The relative amount of flavan-3-ol monomers (flavans) in total catechins content. Catechins in vine leaves extracts were determined as catechin equivalents (vanillin method), and flavanols were determined as epicatechin equivalents using DMACA method

In continuation, five distinct methods were used to get insight in the antioxidant properties of leaf extracts. As previously described (Frankel et al., 1994; Koleva et al., 2002), the use of different methods is necessary to get information about antioxidant profile of an plant extract/fraction or single compound.

Antioxidant properties of *Vitis vinifera* leaf extracts, determined as free radical scavenging ability (DPPH and ABTS methods), reducing power (FRAP method), Fe^{2+} chelating ability of plant extracts, and ability to prevent oxidation of linoleic acid (BCB method), were good and total phenol concentration dependent (Table 2, Fig. 3).

Table 2. Antioxidant properties of *Vitis vinifera* leaf extracts determined as free radical scavenging activity (DPPH and ABTS), ferric reducing ability/antioxidant power (FRAP), Fe^{2+} chelating activity (CA), and using β-carotene bleaching (BCB) method

Selected plant material	DPPH[a] (% Inh)	ABTS [a] (% Inh)	CA[a] (%)	FRAP[b] (mmol TE/L)	BCB[c] (% Inh)
May leaves	61.69 ± 1.15	59.36 ± 1.19	69.35 ± 0.54	7.14 ± 0.05	81.35 ± 8.45
September leaves	70.32 ± 0.73	71.38 ± 1.15	61.40 ± 0.14	10.05 ± 0.03	92.22 ± 4.31

[a] Activity provided by leaf extract previously diluted 10 times with 80 % ethanol
[b] TE -Trolox-equivalents
[c] The total phenol concentration in vine leaf extract to be analysed was 1000 mg GAE/L.

Table 3. Concentration of individual phenolic compounds in *Vitis vinifera* leaves extracts determined by HPLC-RP–DAD.

Phenolic compound	Concentration (mg /L of plant extract)	
	May leaves extract *(MLE)*	*September leaves* extract (SLE)
Phenolic acids		
3-Hydroxybenzoic acid	34.14±2.64	8.90±0.92
Gallic acid	1.20±0.11	3.43±0.23
Caffeic acid	2.51±0.36	2.40±0.39
Vanillin acid	12.75±1.31	12.52±0.47
Flavonoids		
(+)-Catechin	5.16±1.32	33.52±2.12
(-)-Epicatechin	1.44±0.07	4.94±0.09
Apigenin	1.24±0.10	1.14±0.06
Quercetin	3.61±0.08	3.28±0.89
Myricetin	2.26±0.11	2.10±0.19
Quercetin-4'-glucoside	13.49±0.87	56.68±3.12
Rutin	57.38±2.11	100.08±2.98
Stilbenes		
trans-Resveratrol	0.33±0.01	1.11±0.02
cis-Resveratrol	n.d.	n.d.
Piceid /Isorhapontin	+++	+++
Astringin	1.21±0.10	1.31±0.07

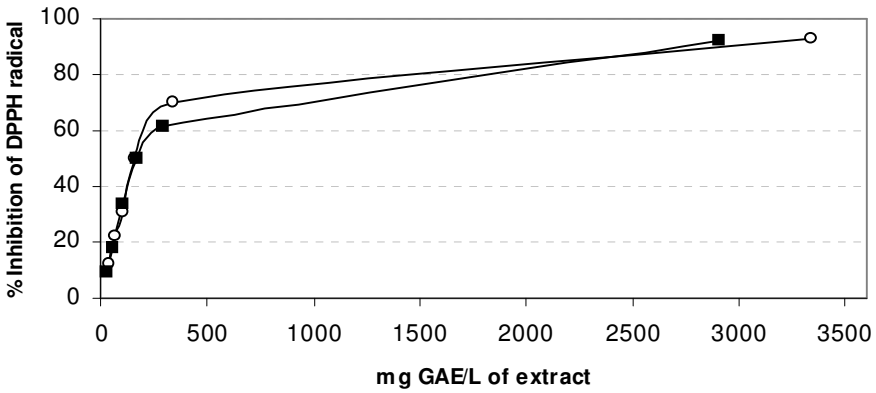

Fig. 3. The percentage inhibition of free DPPH radical in the presence of different concentrations of antioxidants: *May leaves* extract (■); *September leaves* extract (○)

Relatively stable organic DPPH and ABTS radical have been widely used in the determination of the antioxidant properties of single phenolic compound, as well as of different plant extracts. The leaf extracts were able to interract with the stable free DPPH, or ABTS, radicals efficiently and quickly. SLE showed better free radical scavenging capacity, what was expected because of higher total phenol content. DPPH IC_{50} and ABTS IC_{50} were slightly lower for SLE than for MLE, indicating higher free radical scavenging efficiency of SLE phenolics (Fig. 4). Differences for DPPH, or ABTS, IC_{50} between MLE and SLE can be related to differences in polyphenolic content of analyzed extracts.

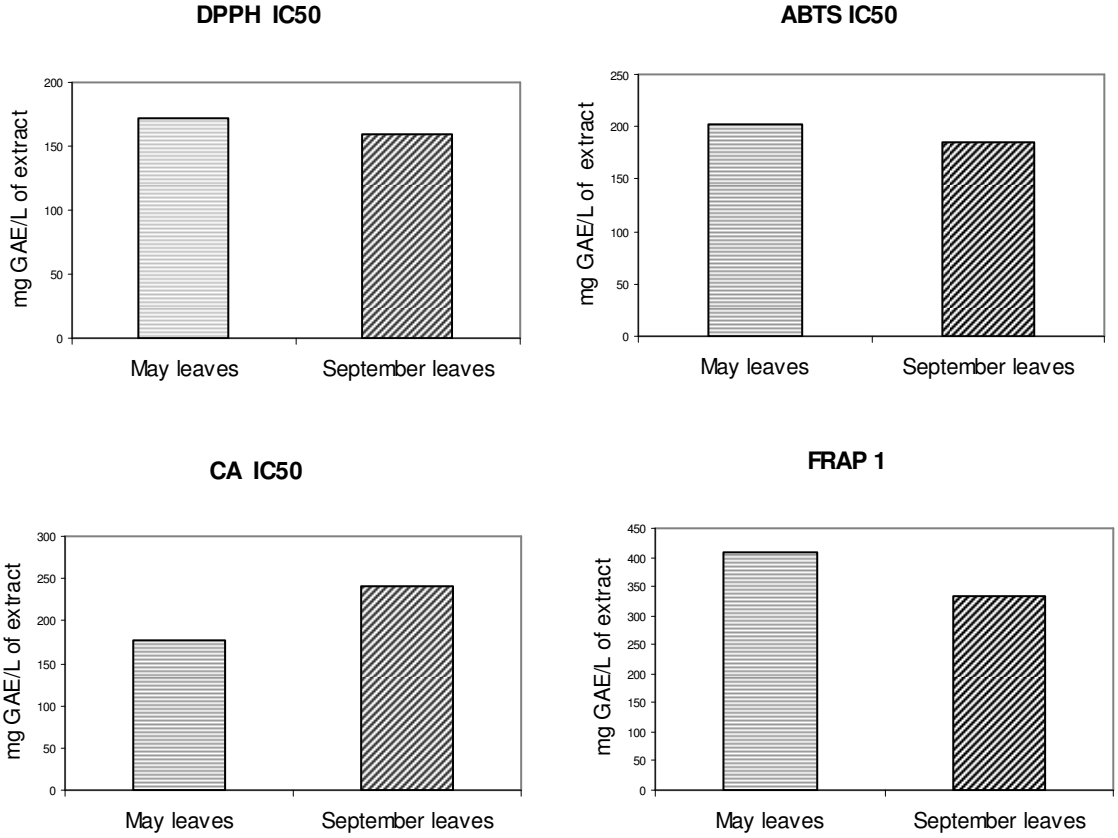

Fig. 4. Comparison of antioxidant properties of *Vitis vinifera* leaf extracts determined as DPPH radical scavenging ability (DDPH IC_{50}), ABTS radical cation scavenging ability (ABTS IC_{50}), Fe^{2+} chelating ability (CA IC_{50}), and antioxidant power (expressed as quantity of phenolic compounds with a reduction power of 1 mM of Trolox: FRAP1). IC_{50} is the sample concentration in mg GAE per L of plants extracts providing 50 % inhibition.

Reducing power of *Vitis vinifera* leaf extracts, determined as FRAP was 7.14±0.05 mmol/L of SLE and 10.05±0.03 mmol/L of MLE. As both extracts were rich in phenolics, good results for FRAP were expected. As significant correlation between total phenol content and FRAP was previously reported (Katalinić et al., 2006; 2009), good results for FRAP, especially for SLE, were expected. The quantity of phenolic compounds with a reduction power of 1 mM of Trolox (FRAP1) was calculated for each extract. FRAP1 of SLE (332.21 mg GAE/L) was lower compared to MLE (407.63 mg GAE/L). It can be concluded that phenolic mixtures of SLE have better reducing power due to the presence of flavonoids (Fig. 4).

In cells, free Fe^{2+} is toxic because it is able to catalyze the decomposition of H_2O_2 into the extremely reactive hydroxyl (OH) radical. It is therefore important to determine the iron binding activity of the natural antioxidant (Halliwell, 1991; Ebrahimzadeh et al., 2008; Orhan et al., 2009). Both extracts showed a significant capability of chelating Fe^{2+} ion (Table 2). Chelating effect of leaf extracts expressed as IC_{50} (Fig. 4) indicates that polyphenolic

compounds in SLE, and especially in MLE, can capture ferrous ions more efficiently compared to some other herb extracts (Ebrahimzadeh et al., 2008). SLE extracts with BCB inhibition percentage (BCB IP) 92.22±4.31 % exhibited, contrary to results obtained chelating activity test, higher antioxidant activity than MLE extract with BCB IP 81.35±8.45 % (Table 2, Fig. 5). The BCB method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Kulišić et al., 2004). The higher efficiency of SLE extract in protecting the oxidation of emulsified linoleic acid in comparison to MLE, can be linked to differences in polyphenolic composition of these two extracts (Table 1, Table 3).

The results of identification of individual phenolic acids and polyphenols (flavonoids and stilbenes) in *Vitis vinifera* leaf extracts are presented in Table 3. Principal phenolic compounds separated by high pressure liquid chromatography on reverse phase (HPLC-RP) were phenolic acids: 3-hydroxybenzoic acid, caffeic acid, gallic acid, vanillin acid, flavonoids: (+)-catechin, (-)-epicatechin (flavan-3-ols), apigenin, myricetin, quercetin, quercetin glucoside and rutin (flavonols), and stilbenes: *trans*-resveratrol, piceid/isorhapontin and astringin.

Presence of biologically interesting polyphenols is confirmed in both extracts, MLE and SLE. MLE extract showed twofold higher amount (50.6 mg/L) of identified phenolic acids than in SLE (27.25 mg/L), due to greater content of 3-hydroxybenzoic acid. On the contrary, the total content of identified flavonoids in SLE was 201.74 mg/L which is more than twofold higher compared to MLE (84.58 mg/L). Highest quantities of flavonoids in leaf extracts were found for rutin and quercetin-4'-glucoside, which were found in especially high concentrations in SLE. Relative content of these quercetin derivatives in SLE or MLE extract makes over 70 % from total identified flavonoids concentration. Concentrations of certain flavonols: apigenin, quercetin, and myricetin, were almost the same in both extracts, and range from 1 to 5 mg/L. The significantly higher (+)-catechin and (-)-epicatechin content was observed in SLE, compared to MLE. Because of the great interest that has recently been devoted to resveratrol and its derivatives, special attention was given to stilbene compounds. The low concentrations of *trans*-resveratrol and astringin were found in both extracts. Quantification of the piceid and isorhapontin, was not successful because the applied HPLC-RP method could not separate these two resveratrol derivatives. It is clear that better antioxidative properties of SLE, expected because of greater total phenols content, can be related to the presence of biologically interesting phenolic compounds, especially flavanoids which were found in much higher concentrations in SLE than in MLE extracts. Nevertheless, significance of other phenolic compounds may not be neglected, especially the ones from stilbene group which can add to positive biological impact of the phenolic extract.

Conclusion

According to the obtained results, *Vitis vinifera* leaves extracts, can be considered rich natural source of polyphenolic compounds with good antioxidative properties. Further research should focus on leaves remaining on the plant after harvest which can be a cheap and easily accessible material for extraction of biogically interesting phenolic compounds.

Acknowledgments

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