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Antioxidant effectiveness of selected wines in comparison with (+)-catechin

V. Katalinić [a], M. Milos [b,*], D. Modun [a], I. Musić [a], M. Boban [a]

[a] *Faculty of Medicine, University of Split, Šoltanska 1, 21000 Split, Croatia*
[b] *Department of Biochemistry and Food Chemistry, Faculty of Chemical Technology, University of Split, Teslina 10, Split 21000, Croatia*

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Abstract

The antioxidant effectiveness of selected wines and pure (+)-catechin were determined using three different methods. The radical scavenging potential was investigated using α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging, the reducing power was determined using ferric reducing/antioxidant power (FRAP) assay and β-carotene bleaching (BCB) method. Six red and four white wines from different grape cultivars were analyzed.

Unpaired test between FRAP, DPPH, and BCB analysis of red and white wines showed significant difference. Different reducing/antioxidant power of red and white wines was elucidated in view of their different phenolic composition. As expected, the red wines had much higher flavonoid, anthocyanin, and catechin content than white wines. There was no significant difference between nonflavonoids phenols.

All analyzed wines demonstrate significant antioxidant capacity with FRAP test. The average FRAP of red wines was almost 10-fold higher than the average FRAP of white wines. The reducing ability of red wines can be directly correlated with its flavonoid and catechin concentration. The relative antioxidant efficiency of pure (+)-catechin in FRAP assay was same as for ascorbate and Trolox.

Keywords: Antioxidant activity; Wine; (+)-Catechin; Flavonoids; Ferric reducing/antioxidant power; α,α-Diphenyl-β-picrylhydrazyl; β-Carotene bleaching

1. Introduction

Phenolic compounds are naturally occurring substances in fruit, vegetables, nuts, seeds, flowers, and some herb beverages, and are integral part of human diet. Epidemiological studies have shown that consumption of phenol-rich beverages, such as tea and wine, correlates with reduced coronary heart disease mortality (Balentine, Wiseman, & Bouwens, 1997; Cul, Juhasz, & Tosaki, 2002; Friedman & Kimball, 1986; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hertog et al., 1995; Serafini, Laranjinha, Almeida, & Mainai, 2000). The strikingly low incidence of coronary heart disease in France as compared with other western countries with comparable dietary intake has been regarded as "French paradox". Although several hypotheses have been proposed, there is strong believe that lower risk of heart disease is associated with the increased wine consumption in France (St. Leger, Cochrane, & Moore,. 1979; Xia, Allenbrand, & Sun, 1998). The protective effects of vegetable, fruit, and red wine consumption against coronary artery disease and certain types of cancer are partly attributed to the flavonoid content of these foods (Bell et al., 2000; Frankel, Kanner, German, Parks, & Kinsella, 1993). It has been demonstrated both in vitro and in vivo that these phenolic compounds can offer significant antiatherogenic protection by inhibiting the oxidation of low density lipoproteins (LDLs) (Ghiselli, Nardini, Baldi, & Scaccini, 1998; Jamroz & Beltowski, 2001; Miller & Rice-Evans, 1995; Rice-Evans, Miller, & Bolwell, 1995; Vinson, 1998;

* Corresponding author. Fax: +385-21-384-964.
E-mail address: milos@ktf-split.hr (M. Milos).

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Serafini, Laranjinha, Almeida, & Mainai, 2000). Nigdikar, Williams, Griffin, and Howard (1998) study provides additional support for protective effects of polyphenolic antioxidants on cardiovascular disease (CVD).

The phenolic compounds in wine range from relatively simple compounds to complex tannin-type substances. The composition of phenolics in wine depends on the type of fruits used (usually grapes) for vinification, their extraction, procedures employed for wine making, and the chemical reactions that occur during the aging of wine (Katalinić, 1997, 1999; Katalinić, Maleš, & Konja, 1997; Macheis, Fleureit, & Billot, 1990). The antioxidant compounds present in wine are derived almost exclusively from grapes and have been identified as phenolic acids, flavonols, monomeric catechins, and anthocyanidins. One of the most abundant of these phenolic compounds is the flavan-3-ol compound, catechin (Singleton, 1988; Singleton & Essau, 1969). Presence of catechin and its derivatives in wines has been well documented.

In the present study we have investigated the polyphenolic composition and antioxidant capacity of selected red and white wines.

The aim of this study was to investigate the antioxidant capacity of selected wines and their correlation with wine polyphenolic composition and antioxidant capacity of equimolar concentrations of (+)-catechin. For this purpose, the DPPH (α,α-diphenyl-β-picrylhydrazyl) method, the FRAP (ferric reducing/antioxidant power) assay and the BCB (β-carotene bleaching) technique were applied.

2. Materials and methods

2.1. Materials and chemicals

All chemicals and reagents were of analytical grade and were obtained from Sigma Chemical Co. (St. Louis, MQ, USA), Aldrich Chemical Co. (Steineheim, Germany), Merck (Darmstadt, Germany), and Kemika (Zagreb, Croatia).

Ten selected wines (with controlled geographical origin) from different grape cultivars grown in Croatia were analyzed. Selected wines included six red wines: Dingač 2001; Babić 2001; Cabernet Sauvignon 1998; Faros 1998; Faros barique 1999; Merlot 1998; and four white wines: Maraština 2000; Pošip 2001; Traminac 2000 and Graševina 2000. To separate the nonalcoholic fraction rich in phenolics from the ethanol contained in the wine, wine samples were dealcoholized in vacuo in a rotary evaporator (at 35 °C for 4 h) and were then diluted to the original volume with distilled water. To avoid the mechanical stress, the vacuum was applied progressively.

2.2. Spectrophotometric measurements

Spectrophotometric measurements were performed by UV–Vis spectrophotometer (double-beam) Specord 200 Analytik Jena GmbH, Germany.

2.3. Analyses of phenolic compounds

2.3.1. Total phenol concentration

Total phenol concentration in selected wine samples was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method (Amerine & Ough, 1980; Singleton & Rossi, 1965), calibrating against gallic acid standards and expressing the results as GAE. The level of total phenol in wines determined according to the Folin–Ciocalteu method are not absolute measurements of the amounts of phenolic materials but are in fact based on their chemical reducing capacity relative to an equivalent reducing capacity of gallic acid. Data presented are average of three measurements.

2.3.2. Anthocyanins

The anthocyanin content in wines was determined using bisulfite bleaching method (Riberau-Gayon & Stonestreet, 1965). Sulfur dioxide additions cause changes in absorbance in the unpolymerized pigments but not in the condensed or polymerized pigments (Amerine & Ough, 1980). Data presented are average of three measurements.

2.3.3. Catechins

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. Catechins, as main reactants with reactive phloroglucinol moiety in wines, were reacted with vanillin and resulting colored compound was quantitatively determined. (+)-Catechin, a monomeric flavan-3-ol, was used as a standard. Data presented are average of three measurements.

2.3.4. Flavonoids

The possible use of formaldehyde to precipitate the flavonoid phenolic compounds has been proposed for wine. The method has been developed by Kramling and Singleton (1969) (Amerine & Ough, 1980). Formaldehyde react with 6- or 8-position on the 5,7-dihydroxy flavonoids forming a methylol derivative that will attach to another 6- or 8-position on another flavonoid and so on. These condensed molecules were removed by filtration. The residual nonflavonoid phenolic tannin was analyzed by Folin–Ciocalteu method. The amount of flavonoids was calculated as difference between total phenols and nonflavonoids in wine. The presented data are the average of three measurements.

2.4. Determination of antioxidant capacity

2.4.1. Determination of FRAP – ferric reducing/antioxidant power

FRAP is a simple direct test of antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but can be used for plant extracts too. The total antioxidant potential of sample was determined using a ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (1996) as a measure of "antioxidant power". FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} form by the action of electron donating antioxidants. The working FRAP reagent was prepared by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 1 volume of 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L hydrocloric acid and with 1 volume of 20 mmol/L ferric chloride. Freshly prepared FRAP reagent (1.5 ml) was warmed to 37 °C and a reagent blank reading was taken at 593 nm (M1). Subsequently, 50 μl of sample and 150 μl of deionized water was added to the FRAP reagent. Final dilution of sample in reaction mixture was 1:34. Absorbance readings were taken after 0.5 s and every 15 s thereafter during the monitoring period (8 min). Because there was little decrease in absorbency between 4 and 8 min of incubation, we used its value after 4 min for further calculation. The sample was incubated at 37 °C throughout the monitoring period. The change in absorbance between the final reading selected (4-min reading) and the M1 reading were selected for calculation of FRAP values. Standard curve was prepared using different concentrations (100–1000 μmol/L) of $FeSO_4 \cdot 7H_2O$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the antioxidant under the test was calculated with reference to the reaction signal given by an Fe^{2+} solution of known concentration, this representing a one-electron exchange reaction. The results were corrected for dilution and expressed in mmol Fe^{2+}/L. (+)-Catechin, ascorbate, quercetin, and BHT were measured within 1 h after preparation. All determinations were performed in triplicate.

Wine FRAP was total phenol concentration-dependent. The wine to be analyzed was first adequately diluted to fit within the linearity range (Fig. 1).

2.4.2. Free radical scavenging capacity

The free radical scavenging capacity of the wine samples and pure compound was analyzed by using 1,1-diphenyl-2-picrilhydrazyl (DPPH) assay (Fuhrman, Volkova, Suraski, & Aviram, 2001; Vongadow, Joubert, & Hansmann, 1997). Aliquots (50 μl) of the tested samples were mixed with 2 ml of 6×10^{-5} methanolic solution of DPPH radical. Wine samples were tested

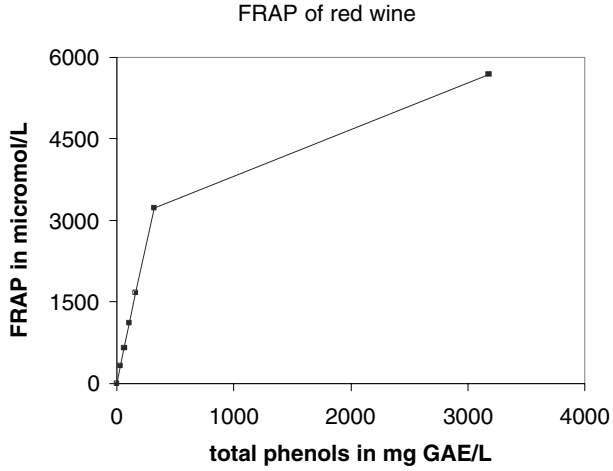

Fig. 1. Typical profile of ferric reducing/antioxidant power (FRAP) in correlation with decreasing amount of total phenols in wine (for selected red wine Dingač).

before and after dilution 10-fold with water. A methanolic solution of pure compounds were tested too (at 0.5 mol of antioxidant/mol of DPPH).

All determinations were performed in triplicate. The percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

$$\%\ \text{inhibition} = \left[\left(A_{C(0)} - A_{A(t)}\right)/A_{C(0)}\right] \times 100,$$

where $A_{C(0)}$ is the absorbance of the control at $t = 0$ min and $A_{A(t)}$ is the absorbance of the antioxidant at $t = 16$ min.

2.4.3. Determination of antioxidant activity with β-carotene bleaching method

Antioxidant activity with BCB method was measured using the procedure of Vongadow et al. (1997). A stock solution of 1.3×10^{-3}M (+)-catechin in methanol was prepared, of which 200 μl was added in reaction mixture, giving a final concentration of 5×10^{-5}M of (+)-catechin. Red wines were diluted with water 1:5 (v:v), and 200 μl was added to a reaction mixture. White wines were added without dilution. The antioxidant activity coefficient (AAC) was calculated from data with the formula (Mallet, Cerrati, Ucciani, Gamisiana, & Gruber, 1994):

$$\text{AAC} = \left[\left(A_{A(120)} - A_{C(120)}\right)/\left(A_{C(0)} - A_{C(120)}\right)\right] \times 1000D,$$

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, $A_{C(0)}$ is the absorbance of the control at $t = 0$ min, and D is dilution. Dilution factor for red wines was 5. All determinations were performed in duplicate. Using the concentration of catechins, the theoretical AAC of tested red wines for phenol concentration of 1.3×10^{-3}M (+)-catechin was calculated. All data in Tables 1 and 3 are average of determinations performed in duplicate or triplicate.

Table 1
The polyphenol content and antioxidant activity of selected wine

Selected wines	Total phenols (mg GAE/L)	Anthocyanins (mg/L)	Flavonoids (mg GAE/L)	Non-flavonoids (mg GAE/L)	Catechins (mM CE/L)	FRAP (mM/L)	DPPH (% I)	BCB (AAC)
Dingač	3183	398.0	2893.1	286.9	12.69	32.280	82.6	2783
Babić	2809	222.0	2331.0	479.0	8.36	29.590	72.1	2478
Cabernet Sauvignon	3087	305.0	2813.0	276.0	12.53	27.410	82.2	2823
Faros	2193	70.0	1941.0	252.0	5.05	20.598	54.6	2520
Faros barique	2372	69.7	2124.7	247.3	6.80	24.464	65.4	2515
Merlot	2402	146.2	2105.2	297.8	7.29	22.195	68.86	2660
Maraština	402	n.d.	125.7	276.3	0.57	3.856	16.16	63
Pošip	292	n.d.	41.7	250.3	0.27	2.213	10.66	72
Traminac	338	n.d.	47.5	290.5	0.35	2.563	10.30	16
Graševina	301	n.d.	15.5	285.5	0.25	2.794	13.55	46

n.d., not detected.
FRAP, relative activities of the individual antioxidants to the reaction of Fe^{2+}.
I%, the inhibition determined by DPPH radical scavenging method (wines were diluted with water 1:10, v/v).
AAC, antioxidant activity coefficient determined with BCB method.

2.5. Statistical analysis

The *t*-test was used to compare whether the mean of variable differs between red wines and white wines. The direction and magnitude of correlation between variables was done using analysis of variance (ANOVA) and quantified by the correlation factor "*r*". The *P*-value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Concentration of phenolic compounds in selected wines

There was a wide range of phenol concentrations in selected wines. As expected, the red wines had significantly higher amounts of total phenols, flavonoids, anthocyanins, and catechins compared to white wines (Table 1). This is due to a greater grape skin and seed contact time and temperature for the fermentation process for red wines.

The phenolic component in red wine was substantially higher compared with white wines: mean 2674.5 (SD 410.6) mg of gallic acid equivalents (GAE)/L of red wine versus 333 (SD 50) mg of GAE/L of white wine. According to the Singleton and Rossi (1965), various phenolic compounds have different responses in this assay. The molar response of this method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate, but the reducing capacity is enhanced when two phenolic hydroxyl groups are oriented *ortho* or *para* (Frankel, Waterhouse, & Teissedre, 1995). Since these structural features of phenolic compounds are reportedly also responsible for antioxidant activity, measurements of total phenols in wines may be related to their antioxidant activities.

The mean amount of nonflavonoid phenols in wines was 306 (SD 86.8) mg GAE/L of red wines and 277.8 (18.7) mg GAE/L of white wines. There was no significant difference between nonflavonoids phenols in red and white wines. The mean amount of flavonoid phenolic compounds was 2368 (SD 396.56) mg GAE/L of red wines and 58.0 (SD 47.4) mg GAE/L of white wines. The average percent amount of of flavonoids in total phenols was 89% for red wines and 16% for white wines. The high flavonoid content in red wine contributes to its increased antioxidant potential in comparison to white wine.

The amount of catechins in red wine was, as expected, substantially higher compared with white wines: mean 8.80 (SD 3.147) mmol of (+)-catechin equivalents(CE)/L of red wine versus 0.36 (SD 0.147) mmol CE/L of white wine.

The grape pigments or anthocyanins are present in red grapes only. The anthocyanin content in selected red wines, determined using bisulfite bleaching method, vary from 70 to 398 mg/L (mean 202.0 mg/L; SD 132.13).

As expected significant linear correlation was confirmed between total phenols and flavonoids ($r = 0.9986$; $P < 0.0001$, $n = 10$) and total phenols and catechins ($r = 0.9663$; $P < 0.0001$; $n = 10$) in selected wines. Correlation between total phenols and nonflavonoid phenols in selected wines was not significant.

The significant linear correlations in red wines, between catechins and flavonoids ($r = 0.9940$; $P < 0.0001$; $n = 6$) and between catechins and anthocyanins ($r = 0.9940$, $P < 0.0001$, $n = 6$) were confirmed.

3.2. Antioxidant capacity of selected wine

Phenol-rich beverages like wines contain variety of low-molecular mass molecules and many of these have been ascribed primary antioxidant role. Unpaired test

with two tailed *P*-values showed significant difference between FRAP, DPPH, and AAC of red and white wines ($P < 0.0001$). The correlation (coefficient "*r*" and two-tailed "*P*"-value) between wine phenols and antioxidant capacity is shown in Table 2.

3.2.1. Antioxidant capacity determined by FRAP

All analyzed wines demonstrate significant antioxidant capacity with FRAP test (Table 1). Red wines had stronger reducing power (20.598–32.280 mmol/L) than white wines (2.213–3.856 mmol/L). The mean (SD) FRAP of red wines was 26.091 (4.479) mmol/L and mean (SD) FRAP of white wines was 2.856 (0.708) mmol/L. The average ratio of wine FRAP to the total phenol level was about 9 (7.6–10.5) g GAE/L. Difference between ratio of wine FRAP to the total phenol level of red and white wines was not significant ($P > 0.05$).

3.2.2. Antioxidant capacity determined by DPPH

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging ability (Baumann, Wurn, & Bruchlausen, 1979). The addition of selected wines (50 μl/2 ml) to the DPPH solution induced a rapid decrease in the optical density at 517 nm which reached plateau within 5 min. Both red and white wines had significant DPPH scavenging ability. The percentage inhibition was 82.4–85.0% for red wines and 39.0–60.2% for white wines. The percentage inhibition for wines diluted with water (1:10, v/v) was 54.6–82.2% for red and 10.7–16.2% for white wines (Table 1).

3.2.3. Antioxidant capacity determined by BCB

In all cases it was demonstrated that addition of red wines inhibit oxidation of linoleic acid. Antioxidant activity coefficient (AAC) of selected wines according to the BCB method was 2478–2823 (mean 2630, SD 148.3) for red wines and 16–72 (mean 49) for white wines. Average ratio of the AAC for red wines to the total phenols in wine (in gram GAE per liter) was 998 (882–1107) for red wines and 151 (47–246) for white wines. Thus, polyphenols of the red wines were more efficient antioxidants compared with the analyzed white wines.

3.2.4. Comparison of antioxidant capacity of pure (+)-catechin with other pure antioxidants

Antioxidant activity of pure (+)-catechin is presented in Table 3. The reducing power of (+)-catechin was

Table 2
Correlation between wine phenolics and antioxidant capacity of wine determined with three different methods (FRAP, DPPH, and BCB)

	r		
	FRAP	DPPH	BCB
Total phenols			
SW	**0.9923**	**0.9963**	**0.9802**
RW	**0.9075**	**0.9535**	0.7106
Flavonoids			
SW	**0.9892**	**0.9958**	**0.9832**
RW	**0.8418**	**0.9464**	**0.8126**
Catechins			
SW	**0.9510**	**0.9675**	**0.9102**
RW	**0.8165**	**0.9690**	**0.8417**
Anthocyanins			
RW	**0.8695**	**0.9205**	0.7641

The direction and magnitude of correlation between variables was quantified by the correlation coefficient *r*. A two-tailed *P*-value less than 0.05 was considered statistically significant.

SW, selected red and white wines together ($n = 10$); RW, red wines ($n = 6$); significant results in bold; (wines were diluted with water 1:10, v/v).

Table 3
Antioxidant activity of pure(+)-catechin in comparison with other pure antioxidants determined by three methods (FRAP, DPPH, and BCB)

Individual antioxidants	FRAP[a] (relative activity)	DPPH[b] (% Inhibition)	BCB[c] (AAC[c])
(+)-Catechin	2.0 (1.9–2.1)	94.4	109
Quercetin	4.8 (4.7–4.9)	95.3	653
Vitamin C	2.0 (1.9–2.1)*	91.5	n.d.
Trolox	2.0 (2.0–2.1)	92.63	n.d.
BHT	0.2 (0.19–0.2)	49.2	706

[a] Relative activities (measured ranges) of the individual antioxidants. Relative to the reaction of Fe^{2+} (representing one-electron exchange reaction and taken as unity).

[b] Determined by DPPH radical scavenging method; mole ratio of antioxidant/DPPH = 0.5.

[c] AAC, antioxidant activity coefficient as determined with BCB method. Concentration of antioxidant in emulsion = 5×10^{-5}. n.d., not detected.

* Results for vitamin C according to Benzie and Strain (1996).

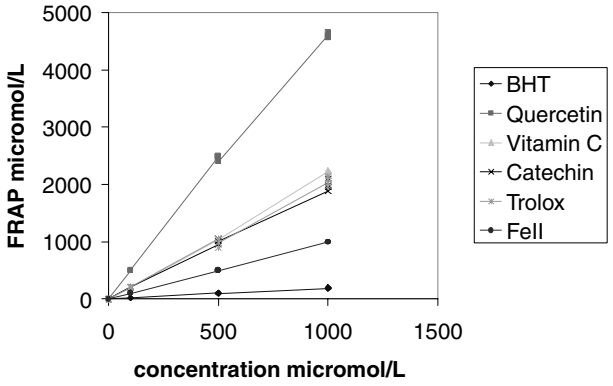

Fig. 2. Dose–response line of (+)-catechin, over the concentration range of 50–1.000 μM (the highest concentration tested), in the ferric reducing/antioxidant power test (FRAP) assay for reducing (antioxidant) activity. The relative antioxidant efficiency of (+)-catechin was 2.0 as for Trolox and vitamin C; that is, on 1 mol per mole basis (+)-catechin gave twice the signal induced by Fe^{II}, Fe^{II} representing a one-electron exchange reaction in the FRAP assay. Each point represents the mean of the three readings.

independent of concentrations, over the concentration range tested, in the FRAP assay system. The FRAP dose–response of (+)-catechin in aqueous solution was linear up at least 1000 μM catechin (the highest concentration tested). Fig. 2 shows the (+)-catechin dose–response line over the concentration range of 50–1000 μM. Slopes of the dose–response line of catechin containing solutions were twice that of the aqueous Fe^{II} dose–response line. According to the obtained results, the relative antioxidant activity in this test system was constant at 2.0 (on mole for mole basis (+)-catechin gave twice the signal induced by Fe^{II}, Fe^{II} representing a one-electron exchange reaction). The same relative antioxidant activity (2.0) was obtained for ascorbate and Trolox. The results for ascorbate are in correlation with the results of Benzie, Wai, and Strain (1999). Quercetin had stronger FRAP (4.6) compared to (+)-catechin. It is accepted that diverse polyphenols possess different antioxidant activity. Quercetin and (+)-catechin have $3,5,7,3',4'$- pentahydroxy polyphenolic structures. The results demonstrate the importance of the unsaturation in the C ring.

The antioxidant activity of tested molecular antioxidants, as determined by the DPPH radical scavenging method, decrease in the order quercetin, (+)-catechin, Trolox, ascorbic acid, and BHT (Table 3). Absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidant through donation of hydrogen to form stable DPPH-H. A rapid decrease in absorbance occurred with flavonoids (+)-catechin and quercetin when where present at 0.5 mol/mol DPPH radical. The established poor radical scavenging abilities of BHT agree with the results of other researchers (Brand-Williams, Cuvelier, & Berset, 1995; Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002). They explained that the compounds with rapid or intermediate kinetics have shown a reaction stoichiometry corresponding approximately to the number of electrons available for donation. For slow-reacting compounds (such as BHT) such correlations do not exist and this was attributed to the complex reaction mechanism, involving one or more secondary reactions. The same explanation can be related to FRAP assays.

AAC, antioxidant activity coefficient of (+)-catechin according to the BCB method was lower compared with AAC of quercetin and BHT. AAC of quercetin was 6-fold higher than for (+)-catechin. Quercetin was more potent antioxidant than the flavan-3-ol (+)-catechin in this model system (decrease in absorbance of β-carotene in the presence of different antioxidant with the coupled oxidation of β-carotene and linoleic acid). The presence of C2–C3 double bond and C4 keto group seems to be essential for high antioxidant activity (quercetin > (+)-catechin).

3.2.5. Relation among antioxidant activity and concentration of phenolic compounds

The results of this study show that red wine has potent antioxidant properties in vitro as estimated by FRAP, DPPH, and BCB methods (Table 1). FRAP, DPPH, and AAC results of selected white wines were far from those obtained for red wines. The correlations were very good between antioxidant properties and phenolic content of tested wines (Table 2). The significant correlation between catechins and antioxidant capacity of wine was confirmed with all three methods. Because of the significant correlation between $DPPH^*$ scavenging ability of tested wines and their ferric reducing ability ($r = 0.9881$ for selected wines and 0.8308 for red wines), either of these methods can be used for the quick evaluation of antioxidant capacity of wines. FRAP as a very rapid and simple method is very convenient for the screening of large number of samples.

The higher reducing power (FRAP) and antioxidant effectiveness of red wines compared with white wines is in agreement with the results of previous studies (Jamroz & Beltowski, 2001; Nigdikar et al., 1998; Vinson & Hontz, 1995). The average FRAP of red wines was almost 10-fold higher than the average FRAP of white wines. Even the lowest observed wine FRAP value exceeded the antioxidant potential of normal human plasma (0.612–1.634 mmol/L of plasma; according to the Benzie & Strain, 1996). The reducing power of red wines highly exceeded the reducing power of black tea, and some fruits and vegetables juices such as lemon, orange, cherry, strawberry, red beet, carrot (our unpublished data, 2004).

As wine is complex mixture of phenolic compounds, the antioxidant activity of selected wines is not a property of a single phenolic compound. It is important to determine which group of phenolic compounds is most

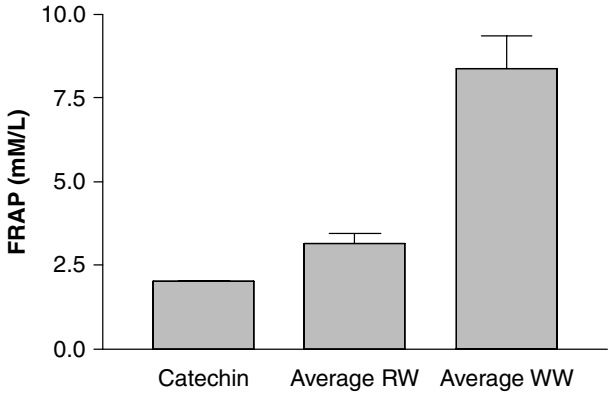

Fig. 3. FRAP of equimolar concentrations of (+)-catechin (1.0 mM) in comparison with average FRAP of red (RW) and white (WW) wine; FRAP in wines were calculated for concentrations of 1.0 mM of catechins in wine.

significant in determining antioxidant potency of wine. Grape seeds are natural reservoir of low molecular weight catechins (monomers, dimers, trimers, and oligomers), flavonoids with orthodiphenolic structure in the B ring. During maceration, the phenolic compounds from solid parts of grapes are extracted into the wine.

The study of the catechins is important for better understanding of antioxidant properties of red wines. As mentioned previously, significant correlation was confirmed between the amounts of catechins in selected wines and its antioxidant efficiencies determined as FRAP. Using the results for catechins in selected wines, the FRAP of selected wines was calculated for equimolar concentrations of catechins (1000 μM/L of wine) and average values for red and white wines were compared with FRAP of pure (+)-catechin (Fig. 3). Calculated FRAP values for equimolar concentrations of catechins were 3164 (SD 708) μM/L for average red wine and 8366 (SD 1963) μM/L for average white wine. These results suggest that both red and white wines contains polyphenols with higher reducing ability than (+)-catechin. More than 60% of the reducing ability/antioxidant power of red wines can be connected with catechins, but for white wines only 24%.

4. Conclusions

The constituents of red wine are factors of particular interest due to the intrigue created by the French paradox (Renaud & De Lorgeril, 1992). Qualitatively there is little difference between white and red wines except that red wines contain anthocyanins, the pigment molecules and large amount of catechins (low molecular weight flavans as well as polymerized tannins). Catechin polymers would not be expected to be significant antioxidants, but low molecular weight catechins, especially monomers and dimers, could significantly participate in antioxidant power of red wine. According to the obtained results free radical scavenging ability and reducing ability of red wines is strongly correlated with polyphenolic content. The significant correlation between catechins and antioxidant capacity of wines was confirmed with FRAP, DPPH, and BCB methods. High amount of catechins in selected red wines and significant reducing power of pure (+)-catechin are the confirmation of the importance of catechin flavonoids in the antioxidant capacity of red wines. Results of this study show that great percent of the reducing ability/antioxidant power of red wine can be directly correlated with its catechins concentration. The FRAP assay gives fast and reproducible results with tested wine samples and pure molecular antioxidants too. It can be useful tool in further in vitro and in vivo investigation of antioxidant activity of plant phenolics.

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