# Antioxidative and Antiradical Properties of Plant Phenolics

Zbigniew Sroka

Department of Pharmacognosy, Wrocław University of Medicine, pl. Nankiera 1, 50-140 Wrocław, Poland. Fax: 48 71 784 02 18. E-mail: zbsroka@bf.uni.wroc.pl

Z. Naturforsch. **60 c**, 833–843 (2005); received July 28, 2004/April 25, 2005

The plant phenolic compounds such as flavonoids, tannins and phenolic acids appeared to be strong antiradical and antioxidant compounds. The number of hydroxy groups and the presence of a 2,3-double bond and orthodiphenolic structure enhance antiradical and antioxidative activity of flavonoids. The glycosylation, blocking the 3-OH group in C-ring, lack of a hydroxy group or the presence of only a methoxy group in B-ring have a decreasing effect on antiradical or antioxidative activity of these compounds. Tannins show strong antioxidative properties. Some tannins in red wine or gallate esters were proved to have antioxidative effect *in vivo*. The number of hydroxy groups connected with the aromatic ring, in *ortho* or *para* position relative to each other, enhance antioxidative and antiradical activity of phenolic acids. The substitution of a methoxy group in *ortho* position to the OH in monophenols seems to favour the antioxidative activity of the former.

*Key words:* Plant Phenolics, Antiradical Activity, Antioxidative Activity

## Introduction

Plant phenolics can be divided into: a) simple phenols such as phenolic acids (caffeic acid, gallic acid, rosmarinic acid); b) flavonoids with more complicated structure which are divided into flavanols (epicatechin, catechin), flavanones (naringin, taxifolin), flavonols (kaempferol, quercetin, myricetin), flavones (chrysin, apigenin); c) tannins with relatively high molecular weight which are divided into condensed and hydrolyzable tannins. Most of the above-mentioned compounds occur in free form or bound with sugar.

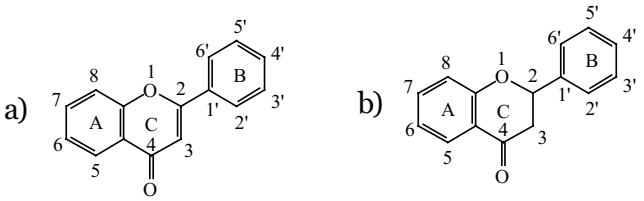
Plant phenolics are important low molecular mass antioxidants coming from the diet (Halliwell and Gutteridge, 1999; Papas, 1999). Many plant phenolics exhibited antiradical or antioxidative activity *in vitro* and *in vivo* (Prior, 2003; Pietta, 2000; Williamson *et al.*, 1998; Hassig *et al.*, 1999; Lairon and Amiot, 1999; Terao, 1999; Vinson, 1998; Lopez-Velez *et al.*, 2003; Fuhrman *et al.*, 1995). The intensity of antiradical activity of phenols depends on many factors such as number of hydroxy groups bound to the aromatic ring (Rice-Evans *et al.*, 1996; Burda and Oleszek, 2001; Sroka and Cisowski, 2003), number and places of double bonds in the molecule (Rice-Evans *et al.*, 1996; Burda and Oleszek, 2001; Croft, 1998; Arora *et al.*, 1998). There was shown that plant phenols can act *in vivo* saving blood lipoproteins from oxidation and as a result can inhibit progress of arteriosclerosis. The commonly known phenomenon so cold "French paradox" consists on the protection of coronary vase from arteriosclerosis by phenols found in red wine (Fuhrman *et al.*, 1995; de Gaetano and Cerletti, 2001; Wollin and Jones, 2001).

In this work, the antiradical and antioxidative activity of plant phenolics as well as the dependence of this activity from the molecular structure of compounds is discussed.

## Results and Discussion

### *Flavonoids*

The flavonoids belong to plant phenolic dyes with antiradical properties. The structure of these compounds is demonstrated in Fig. 1. The intensity of antiradical features of flavonoids strongly depends on its chemical structure. For example glycosylation of these compounds mostly decreases their antiradical activity (Shahidi and Wanasundara, 1992). Blocking the hydroxy group at 3 position in C-ring (Fig. 1) or removing of the 3-hydroxy group decrease antioxidative properties of flavonoids. Burda and Oleszek (2001) used heat-induced oxidation of an aqueous emulsion system of $\beta$-carotene and linoleic acid as the antioxidative activity test. The results are expressed as percentage of inhibition of oxidation process in comparison to control sample (without compound under investigation). They calculated the antioxidative




| | 3 | 5 | 7 | 2′ | 3′ | 4′ | 5′ |
|---|---|---|---|---|---|---|---|
| (a) | | | | | | | |
| Quercetin | –OH | –OH | –OH | | –OH | –OH | |
| Quercetin 3-*O*-glucoside-7-*O*-rhamnoside | –O–Glc | –OH | –O–Rha | | –OH | –OH | |
| Kaempferol | –OH | –OH | –OH | | | –OH | |
| Kaempferide | –OH | –OH | –OH | | | $-OCH_3$ | |
| Kaempferol 3,7-*O*-dirhamnoside | –O–Rha | –OH | –O–Rha | | | –OH | |
| Galangin | –OH | –OH | –OH | | | | |
| Morin | –OH | –OH | –OH | –OH | | –OH | |
| Myricetin | –OH | –OH | –OH | | –OH | –OH | –OH |
| Chrysin | | –OH | –OH | | | | |
| Apigenin | | –OH | –OH | | | –OH | |
| Rutin | –O–Glc–Rha | –OH | –OH | | –OH | –OH | |
| Laricytrin | –OH | –OH | –OH | | $-OCH_3$ | –OH | –OH |
| Laricytrin 3,7,3′-*O*-triglucoside | –O–Glc | –OH | –O–Glc | | $-OCH_3$ | –OH | –O–Glc |
| Robinetin | –OH | | –OH | | –OH | –OH | –OH |
| Fistein | –OH | | –OH | | –OH | –OH | |
| (b) | | | | | | | |
| Chrysin | | –OH | –OH | | | | |
| Naringenin | | –OH | –OH | | | –OH | |
| Hesperetin | | –OH | –OH | | –OH | $-OCH_3$ | |
| Taxifolin | –OH | –OH | –OH | | –OH | –OH | |
| Dihydrokaempferol | –OH | –OH | –OH | | | –OH | |
| Narirutin b) | | –OH | –O–Glc–Rha | | | –OH | |

Fig. 1. The structure of some flavonoid aglycones and glycosides. Glc, glucose; Rha, rhamnose.

activity as percentage of inhibition of oxidation process in comparison to control sample (without flavonoid). They demonstrated the activity as percentage of inhibition of oxidation process in comparison to control sample (without flavonoid). They showed that flavonols with a free 3-hydroxy group such as kaempferol, galangin, quercetin, morin, robinetin, fistein, kaempferide and laricytrin (Fig. 1) were stronger antioxidants than flavonoids with blocked 3-hydroxy group by glycosylation such as laricytrin 3,7,3′-*O*-triglucoside, quercetin 3-*O*-glucoside-7-*O*-rhamnoside, rutin, kaempferol 3,7-*O*-dirhamnoside (Table I).

Rice-Evans *et al.* (1996) performed so-called Trolox equivalent antioxidative activity test (TEAC). The TEAC is defined as the concentration of Trolox (water-soluble vitamin E analogue) solution with equivalent antioxidative potential to an 1 mM concentration of investigated compound. They showed that the 2,3-double bond in the C-ring seems to be important for antiradical features of flavonoids but only for compounds with 3′,4′-orthodiphenolic structure in B-ring. The 2,3-double bond has no influence for compounds with only one hydroxy group in B-ring such as for kaempferol (Fig. 1). Antioxidative activity of quercetin (Fig. 1) was equal to (4.7 ± 0.1) mM (Table II). Removing the 2,3-double bond in the C-ring as in taxifolin (dihydroquercetin) (Fig. 1) gives a TEAC value of (1.90 ± 0.03) mM (Table II). Elimination of 2,3-unsaturated bond in the C-ring of kaempferol (Fig. 1) and converting it to dihydrokaempferol (Fig. 1) has no influence on antioxidative activity (Table II).

The orthodiphenolic structure of B-ring appeared to be also important for antiradical fea-

Table I. Antioxidative activity of flavonoids (Burda and Oleszek, 2001).

| Flavonoids with strong antioxidative activity | |
|---|---|
| Compound | $A_a$ (%) |
| Kaempferol | 65.3 |
| Galangin | 64.9 |
| Quercetin | 63.6 |
| Morin | 63.5 |
| Robinetin | 61.7 |
| Fistein | 61.6 |
| Kaempferide | 60.0 |
| Laricytrin | 28.5 |
| Myricetin | 18.4 |
| **Flavonoids with weak antioxidative activity** | |
| Compound | $A_a$ (%) |
| Laricytrin 3,7,3′-*O*-triglucoside | – 6.2 |
| Quercetin 3-*O*-glucoside-7-*O*-rhamnoside | – 6.2 |
| Rutin | – 10.2 |
| Kaempferol 3,7-*O*-dirhamnoside | – 17.5 |
| Apigenin | – 78.8 |

The model system of antioxidative activity of flavonoids was heat-induced oxidation of an aqueous emulsion system of β-carotene with linoleic acid. The activity was measured as sample absorbance (470 nm) decrease at 0 and 120 min of reaction. The percentage of oxidation inhibition was calculated according to the equation: $A_a = \left[1 - \frac{A_{S0} - A_{S120}}{A_{C0} - A_{C120}}\right] \times 100\%$, where $A_{S0}$, $A_{C0}$ are the absorbance of sample with antioxidant and control sample, respectively, at 0 min of reaction and $A_{S120}$, $A_{C120}$ are the absorbance of sample with antioxidant and control sample, respectively, at 120 min of reaction (Burda and Oleszek, 2001).

tures of flavonoids. Rice-Evans *et al.* (1996) showed that TEAC activity of quercetin with two hydroxy group in B-ring situated in 3′,4′-*ortho* position in relation to each other is much stronger than that of morin [Fig. 1, (2.55 ± 0.02) mM, Table II] with two hydroxy groups in the B-ring situated in the *meta* position (3′,5′) in relation to each other.

According to Rice-Evans *et al.* (1996) the presence of a third OH group in the B-ring situated in *ortho* position to other OH groups in the B-ring as in myricetin (Fig. 1) does not improve the effectiveness of flavonoids against aqueous phase radicals. The TEAC activity of myricetin [(3.1 ± 0.3) mM] (Table II) was lower than that of quercetin. Burda and Oleszek (2001) supported the opinion about relatively low antioxidative properties of myricetin. The activity of myricetin was equal to 18.4% in comparison to quercetin [63.6% (Table I)]. They explain the relatively low antiradical features of myricetin by high sensitivity of myricetin for oxidation which causes its decomposition during measurement.

We performed experiments according to the method of Buege and Aust (1978) modified later by Sroka *et al.* (1994). The different kinds of lipids such as flax, borage oils, oil from cod liver and hardened oils such as margarines were oxidized with UV radiation in the presence of some plant phenolic compounds. The antioxidative activities of investigated compounds were measured as percentage of inhibition of oxidation oils in the presence of phenolic compound in comparison to the control sample (without compound). These experiments showed much stronger antioxidative features of myricetin [95% (Table III)] during borage oil oxidation in comparison with quercetin (48%). When cod liver oil was used as oxidizing medium the inhibition of oxidation in the presence of myricetin was 72% and in the presence of quercetin it was 31%. Our results clearly showed strong positive dependence of number of hydroxy groups bound to the B-ring of flavonoids in *ortho* position in relation to each other and their antioxidative features in lipid system. It is difficult to explain surely the difference of our results and results described by Rice-Evans *et al.* (1996) and Burda and Oleszek (2001). Myricetin is probably more stable in the two phase lipid/water system.

In Rice-Evans *et al.* (1996) research, apigenin, chrysin and naringenin (Fig. 1) with different model of hydroxylation of B- and C-rings have small antiradical activity (Table II). These compounds have the same 5,7-metadihydroxy arrangement of the A-ring. Thus weak antiradical activity of these compounds can be attributed to the 5,7-metadihydroxy substitution of A-ring. Substitution of 4′-methoxy group in the B-ring as in hesperetin (Fig. 1) slightly decreased the antioxidative activity [(1.37 ± 0.08) mM] in comparison to a little more active naringenin [(1.53 ± 0.05) mM (Table II)]. Our experiments exhibited no antioxidative activity of naringenin and hesperetin during oxidation of flax, borage oils and oil from cod liver.

Rice-Evans *et al.*, (1996) by comparing the antioxidative activity of narirutin (Fig. 1) [(0.76 ± 0.05) mM) (Table II)] and naringenin showed that

Table II. Antioxidative activity of some plant phenolic compounds (Rice-Evans *et al.*, 1996).

| Compounds with strong antioxidative activity | | Compounds with weak antioxidative activity | |
|---|---|---|---|
| Compound | TEAC [mM] | Compound | TEAC [mM] |
| Epicatechin gallate | 4.93 ± 0.02 | Naryngenin | 1.53 ± 0.05 |
| Epigallocatechin gallate | 4.75 ± 0.06 | *o*-Pyrocatechuic acid | 1.46 ± 0.01 |
| Quercetin | 4.7 ± 0.1 | Apigenin | 1.45 ± 0.08 |
| Epigallocatechin | 3.82 ± 0.06 | Chrysin | 1.43 ± 0.07 |
| Myricetin | 3.1 ± 0.3 | Dihydrokaempferol | 1.39 ± 0.02 |
| Gallic acid | 3.01 ± 0.05 | Hesperetin | 1.37 ± 0.08 |
| Morin | 2.55 ± 0.02 | Syringic acid | 1.36 ± 0.01 |
| Epicatechin | 2.5 ± 0.02 | *m*-Coumaric acid | 1.21 ± 0.02 |
| Catechin | 2.4 ± 0.05 | Protocatechuic acid | 1.19 ± 0.03 |
| Rutin | 2.4 ± 0.06 | *o*-Coumaric acid | 0.99 ± 0.15 |
| *p*-Coumaric acid | 2.22 ± 0.06 | 2,5-Dihydroxyphenylacetic acid | 0.91 ± 0.05 |
| 3,4-Dihydroxyphenylacetic acid | 2.19 ± 0.08 | Narirutin | 0.76 ± 0.05 |
| $\alpha$-Resorcylic acid | 2.15 ± 0.05 | 4-Hydroxyphenylacetic acid | 0.34 ± 0.10 |
| Luteolin | 2.1 ± 0.05 | 4-Hydroxybenzoic acid | 0.08 ± 0.01 |
| Taxifolin | 1.90 ± 0.03 | 2-Hydroxybenzoic acid | 0.04 ± 0.01 |

Trolox equivalent antioxidant activity (TEAC) measures the concentration of Trolox (water-soluble vitamin E analogue) with an equivalent antioxidant potential to a standard concentration of the compound under investigation. TEAC reflects the ability of antioxidants to the elimination of $ABTS^{\bullet+}$ radical. The TEAC is defined as the concentration of Trolox solution with equivalent antioxidative activity to an 1 mM concentration of the compound under investigation (Rice-Evans *et al.*, 1996)

glycosylation of the 7-OH group in the A-ring leads to suppression of antioxidative activity.

Comparing the activity of kaempferol (65.3%) with apigenin (– 78.8%) (Table I) Burda and Oleszek (2001) showed that existing free hydroxy group in C3 position has primary effect on antioxidative features of flavonoids.

Croft considered antioxidative features of flavonoids in relation to the structure. He ascertained that the main structural features of flavonoids necessary for efficient radical scavenging are: a) *o*-dihydroxy structure in the B-ring because it makes possible electron delocalization; b) 2,3-double bond in the C-ring in conjugation with a 4-keto function provides electron delocalization from the B-ring; c) free hydroxy groups at 3 (ring C) and 5 (ring A) positions provide hydrogen bonding to the 4-keto group (Croft, 1998).

Flavonoids can act also as antioxidants in oxidation systems with transition metal ions such as Cu(I) and Fe(II) by chelation of these metals. In this way flavonoids may prevent their involvement in Fenton reaction leading to dangerous hydroxyl radical formation. We oxidized lipids in the two phase lipid/water system using Fe(II) ions as oxidizing factor. Myricetin inhibited lipid oxidation by 86% at the concentration of 0.17 mg/ml in the test sample, kaempferol inhibited 67% of lipid oxidation at the same concentration but the antioxidative efficiency of quercetin-3-rhamnoside (Fig. 1) and quercetin-3-rutinoside (rutin) (Fig. 1) was, respectively, 10 and 12% (Table III). These results clearly show that blocking the 3-hydroxy group in flavonols by sugars leads to decreasing of antioxidative features of flavonols when oxidation is stimulated by metal ions. This could prove the importance of the free 3-hydroxy group for elimination of oxidizing features of metal ions during lipid oxidation.

Plant polyphenols reacting with metal ions such as Fe(III) or Cu(II) may also lead to a prooxidant effect. Reducing of these ions by phenolic compounds leads to the formation of hydroxyl radicals according to the following reactions: 1.) Fe(III) + phenolic compound $\rightarrow$ Fe(II) + phenolic compound$^{\bullet}$ (radical); 2.) Fe(II) + $H_2O_2 \rightarrow$ Fe(III) + $OH^{\bullet} + OH^{-}$. Yamanaka *et al.* (1997) demonstrated prooxidant activity of catechins isolated from tea during lipid oxidation.

Arora *et al.* (1998) used the fluorescent method with 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl] phenylpropionic acid to measure the antioxidative activity of flavonoids and demonstrated strong activity of quercetin as well as its sugar derivatives such as rutin. They also observed higher antioxidative features of naringenin than hesperetin. Hesperetin

Table III. Antioxidative ($I_p$), anti-$H_2O_2$ ($S_h$) and antiradical ($S_r$) activity of phenolic compounds (Sroka and Cisowski, 2003).

| Phenolic compounds with strong antioxidative, anti-$H_2O_2$ and antiradical activity | | | | Phenolic compounds with weak antioxidative, anti-$H_2O_2$ and antiradical activity | | | |
|---|---|---|---|---|---|---|---|
| Compound | [a]$I_p$ (%) | [b]$S_h$ (%) | [c]$S_r$ (%) | Compound | [a]$I_p$ (%) | [b]$S_h$ (%) | [c]$S_r$ (%) |
| Tannins | 100 ± 3[d] | – | – | 3,4-Dihydroxyphenylacetic acid | 56 ± 6 | 52 ± 2 | 70.8 ± 0.3 |
| Gallic acid | 96 ± 2 | 90.0 ± 0.1 | 75 ± 2 | $\alpha$-Resorcylic acid | 64 ± 6 | 18 ± 2 | 0.60 ± 0.08 |
| Myricetin | 95 ± 2[d] | – | – | *m*-Coumaric acid | – | – | 0.38 ± 0.01 |
| Pyrogallol | 95 ± 3 | 74 ± 2 | 79.5 ± 0.6 | *o*-Coumaric acid | – | – | 0.25 ± 0.01 |
| Chlorogenic acid | 93 ± 4 | 20.2 ± 0.6 | 93 ± 4 | *p*-Coumaric acid | – | – | 0.25 ± 0.01 |
| Protocatechuic acid | 89 ± 4 | 55 ± 2 | 41.2 ± 0.6 | $\beta$-Resorcylic acid | 60 ± 10 | 0.3 ± 1.43 | 0.11 ± 0.07 |
| Myricetin | 86 ± 3[e] | – | – | 3-Hydroxybenzoic acid | 48 ± 13 | 0.3 ± 1.00 | 0.07 ± 0.15 |
| Isochlorogenic acid | 86 ± 3 | 19.8 ± 0.7 | 86 ± 3 | Quercetin | 48 ± 5[d] | – | – |
| Caffeic acid | 81 ± 5 | 59 ± 3 | 44.0 ± 0.5 | 4-Hydroxyphenylacetic acid | 20 ± 7 | 18 ± 5 | 0.11 ± 0.07 |
| Kaempferol | 67 ± 2[e] | – | – | Rutin | 12 ± 5[e] | – | – |
| Gentisic acid | 67 ± 9 | 59 ± 3 | 30.5 ± 0.07 | Quercetin-3-rhamnoside | 10 ± 5[e] | – | – |
| *o*-Pyrocatechuic acid | 65 ± 7 | 47 ± 3 | 46 ± 3 | Salicylic acid | 0 ± 13 | 0 ± 2 | 0.11 ± 0.07 |

[a] Antioxidative activity ($I_p$) was measured during rapeseed oil oxidation by the determination of thiobarbituric acid reactive substances formed during the peroxidation process by the method of Buege and Aust (1978). Inhibition of peroxidation was measured according to the equation:

$I_p = \frac{A_0 - A_{0.1}}{A_0} \times 100\%$, where $A_0$, $A_{0.1}$ are the absorbance of sample without phenolic acid and with phenolic acid at 0.1 mg/ml. Lipid oxidation was initaiated by UV light at 254 nm.

[b] Scavenging of hydrogen peroxide ($S_h$) was measured by the method of Pick and Keisari (1980) as inhibition of peroxidase reaction with $H_2O_2$. The hydrogen peroxide scavenging activity was measured according to the following equation:

$S_h = \frac{B_0 - B_{0.05}}{B_0} \times 100\%$, where $B_0$, $B_{0.05}$ are the absorbance of sample without phenolic acid with phenolic acid at 0.05 mg/ml.

[c] Antiradical activity of phenolic acids ($S_r$) was measured as decrease of absorbance of sample containing DPPH$^\bullet$ radicals in comparison with control sample (without phenolic acid) according to Brand-Williams *et al.* (1995). The antiradical activity was measured according to the equation:

$S_r = \frac{C_0 - C_1}{C_0} \times 100\%$, where $C_0$ and $C_1$ are the absorbance of sample at the beginning and after 1 min of the reaction of DPPH$^\bullet$ elimination. Concentration of compounds in the sample was 0.0028 mg/ml.

[d] Antioxidative activity ($I_p$) was measured as described above but the oxidized medium was Borage oil and the concentration of compound under investigation was 0.17 mg/ml. The oxidation process was initiated with UV light at 254 nm.

[e] Antioxidative activity ($I_p$) was measured as described above but the concentration of compound under investigation was equal to 0.17 mg/ml and the oxidized medium was rapeseed oil. Lipid oxidation was initiated with Fe(II).

with a methoxy group in the B-ring (Fig. 1) was less antioxidant than naringenin which lacks the methoxy substitution. They also showed that flavones with no hydroxy group in the B-ring such as chrysin (Fig. 1) or compounds with only a methoxy group bound to the B-ring were quite ineffective as antioxidants. Of interest is the strong antiradical activity of compounds with no functional group bound to the B- and C-rings but with two hydroxy groups bound to the A-ring in 7 and 8 position.

### *Tannins, catechins, catechin gallate*

Polyphenolic compounds such as the condensed and hydrolysable tannins have shown strong antioxidative potential in various antioxidant tests

(Bors *et al.*, 2001; Buege and Aust, 1978). There is a little literature information demonstrating the tannins antiradical activity in relation to the structure.

Our experiments showed that tannins have strong antioxidative properties during lipids oxidation. Tests performed with the two phase lipid/water system showed that tannin (Serva, Feinbiochemica, Heidelberg, Germany) inhibited malonaldehyde formation (product of lipid oxidation) with efficiency 100% at 0.17 mg/ml (Table III). Probably the large number of free hydroxy groups bound to the aromatic ring in the tannin molecule is the main factor causing the strong antiradical features of these compounds.

Bors *et al.* (2001) investigated the antiradical activity of compounds such as catechin, epicatechin, pycnogenol, epigallocatechin, proanthocyanidin A2, epicatechin gallate, epigallocatechin gallate, pentagalloyl glucose and others. They clearly showed the positive dependence of antiradical activity of proanthocyanidins from the number of hydroxy groups in a molecule or so-called reactive centres which they defined as catechol or pyrogallol groups in molecules. Plumb *et al.* (1998) showed that this correlation is true up to trimers in aqueous environment with the TEAC assay. He showed that the number of adjacent aromatic hydroxy groups positively correlated with antiradical activity of polyphenolic compounds.

The "French paradox" phenomenon refers to relatively low incidence of coronary heart diseases in some regions of France despite the fact that the diet in these regions is similar to diets in other industrial countries. The explanation of this is high consumption of red wine which is stored in oak barrels. The tannins (mainly condensed) from the oak are leaching into the wine and might have an effect on the flavour and also on the antioxidative properties of wines. This may show an antioxidative efficiency of tannins *in vivo* (Bors *et al.*, 2001).

An interesting problem is the way of biosynthesis of tannins. There is still not explained the detailed mechanism of forming the high-molecular mass hydrolysable and condensed tannins. They may be formed by coupling of simple phenols such as proanthocyanidins during oxidation (Fig. 2). Some pathways of phenolic coupling reactions were observed during oxidation and free radical reactions of proanthocyanidins (Bors *et al.*, 2001).

Rice-Evans *et al.* (1996) demonstrated antiradical activity of catechins and catechin gallate esters. The antiradical activity of these compounds expressed as the Trolox equivalent antioxidant activity is as follows in decreasing order: epicatechin gallate (Fig. 2) > epigallocatechin gallate > epigallocatechin > gallic acid > catechin (Table II). The comparison of chemical structures of these compounds is demonstrated in Fig. 2.

The same authors described an interesting data on green tea extract (Rice-Evans *et al.*, 1996). They determined the amounts of total phenols as 26.7% per dry weight of the extract. Epigallocatechin gallate was measured as 11.16%, epicatechin gallate 2.25%, epigallocatechin 10.32%, epicatechin 2.45% and catechin 0.53%. Taking into consideration the antiradical features of these compounds they calculated its contribution to the antioxidative effectiveness in green tea: epigallocatechin 34%, epigallocatechin gallate 32%, epicatechin gallate 7%, epicatechin 6%, catechin 1% (Rice-Evans *et al.*, 1996). The catechin and catechin gallate esters appeared to be the most important antioxidants in green tea extracts. Its contribution in general antioxidative activity of extracts was 78%.

The antioxidative features of black tea extract are similar to the antioxidative activity of that of green tea. However the content of catechin gallate in general phenolic components is only 6.9%. The rest are theaflavins, thearubigens, and other polymeric polyphenols which are formed during the fermentation process.

The green and black tea exhibit *in vivo* some anticarcinogenic and antiatherosclerotic activity probably by protecting the low density lipoproteins from oxidation processes (Kono *et al.*, 1988; Leung *et al.*, 2001).

### *Phenolic acids*

The detailed analysis of antiradical and antioxidative features of phenolic acids as well as its ability for scavenging of hydrogen peroxide was made in our laboratory (Sroka and Cisowski, 2003) (Table III). The antioxidative activity of phenolic acids was measured with the thiobarbituric acid test by the method of Buege and Aust (1978). We also measured the ability of phenolic acids to eliminate hydrogen peroxide by the method of Pick and Keisari (1980). The antiradical test of phenolic acids was performed with free radical DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams *et al.*, 1995).

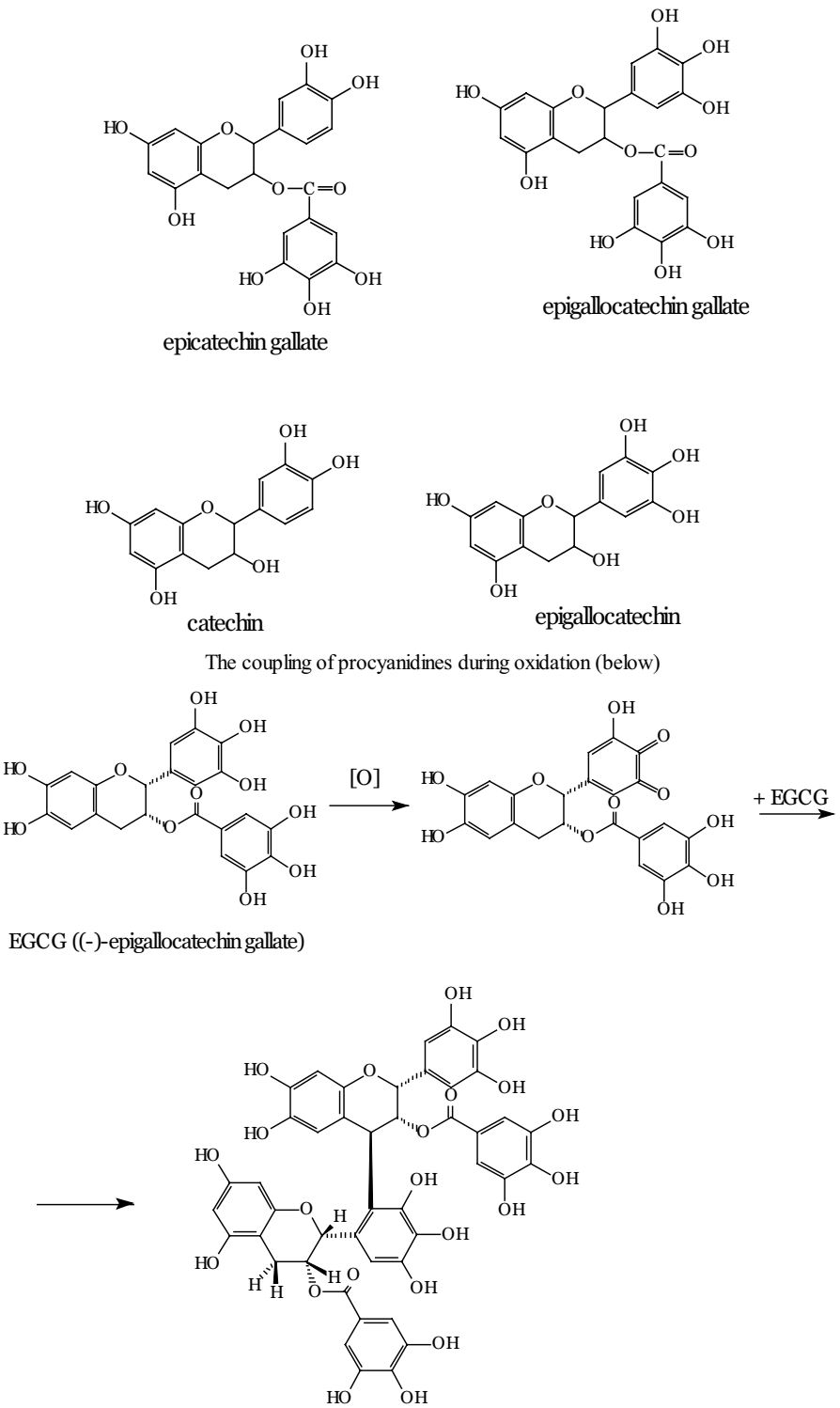
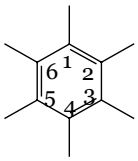
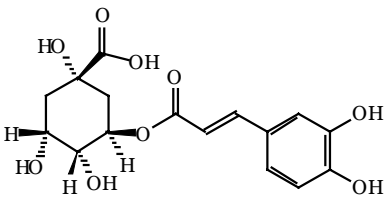


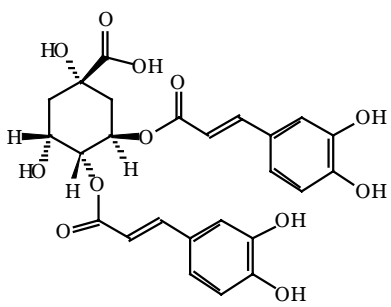

Fig. 2. The structure of catechins and catechin gallate esters. The phenolic coupling during oxidation process with an example of (–)-epigallocatechin gallate (Bors *et al.*, 2001).




| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Caffeic acid | –CH = CH–COOH | | –OH | –OH | |
| Protocatechuic acid | –COOH | | –OH | –OH | |
| Pyrogallol | –OH | –OH | –OH | | |
| Gallic acid | –COOH | | –OH | –OH | –OH |
| 3,5-Dihydroxybenzoic acid | –COOH | | –OH | | –OH |
| 2,4-Dihydroxybenzoic acid | –COOH | –OH | | –OH | |
| *p*-Coumaric acid | –CH = CH–COOH | | | –OH | |
| *m*-Coumaric acid | –CH = CH–COOH | | –OH | | |
| *o*-Coumaric acid | –CH = CH–COOH | –OH | | | |
| *o*-Pyrocatechuic acid | –COOH | –OH | –OH | | |
| Ferulic acid | –CH = CH–COOH | | $-CH_3O$ | –OH | |
| Salicylic acid | –COOH | –OH | | | |
| Resorcinol | –OH | | –OH | | |
| 3,4-Dihydroxyphenylacetic acid | $-CH_2-COOH$ | | –OH | –OH | |
| 4-Hydroxyphenylacetic acid | $-CH_2-COOH$ | | | –OH | |
| 3-Hydroxybenzoic acid | –COOH | | –OH | | |
| Gentisic acid | –COOH | –OH | | | –OH |
| Syringic acid | –COOH | | $-CH_3O$ | –OH | $-CH_3O$ |




Chlorogenic acid




Isochlorogenic acid

Fig. 3. The structure of some simple phenolic compounds.

The strongest antioxidative, antiradical and anti-$H_2O_2$ activity was observed for gallic acid (Fig. 3) with (96 ± 2)% of inhibition of lipid oxidation, (75 ± 2)% of scavenging of DPPH$^\bullet$ radical, (90 ± 0.1)% of scavenging of hydrogen peroxide (Table III). Pyrogallol (Fig. 3), also exhibited strong antioxidative, antiradical and anti-$H_2O_2$ activities [(95 ± 3)%, (79.5 ± 0.6)% and (74 ± 2)%]. Caffeic, protocatechuic, chlorogenic and isochlorogenic acids (Fig. 3) exhibited strong antioxidative properties [(81 ± 5)%, (89 ± 4)%, (93 ± 4)% and (86 ± 3)% inhibition of lipid oxidation].

The 3,5-dihydroxybenzoic, *o*-pyrocatechuic, 2,4-dihydroxybenzoic, gentisic and 3,4-dihydroxyphenylacetic acids (Fig. 3) exhibited little weaker antioxidative activities than gallic acid and pyrogallol [(64 ± 6)%, (65 ± 7)%, (60 ± 10)%, (67 ± 9)%, (56 ± 6)% (Table III)]. The antiradical activities of these compounds were (0.60 ± 0.08)%, (46 ± 3)%, (0.11 ± 0.07)%, (30.50 ± 0.07)%, (70.8 ± 0.3)%]. The lowest antioxidative activity was observed for 3-hydroxybenzoic, 4-hydroxyphenylacetic and salicylic acids (Fig. 3) [(48 ± 13)%, (20 ± 7)%, (0 ± 13)% (Table III)]. The antiradical activities of these compounds were [(0.07 ± 0.15)%, (0.11 ± 0.07)%, (0.11 ± 0.07)% (Table III)]. The anti-$H_2O_2$ activity of phenolic acids are in order of decreasing effectiveness: gallic

acid (Fig. 3) (Table III) > pyrogallol > caffeic acid = gentisic acid > protocatechuic acid > 3,4-dihydroxyphenylacetic acid > *o*-pyrocatechuic acid > chlorogenic acid > isochlorogenic acid > 3,5-dihydroxybenzoic acid = 4-hydroxyphenylacetic acid > 2,4-dihydroxybenzoic acid = 3-hydroxybenzoic acid > salicylic acid. The correlation coefficient between the antiradical activity and hydrogen peroxide scavenging activity of phenolic acids is strong ($r^2$ = 0.83), while the correlation ($r^2$) between the hydrogen peroxide scavenging activity and antioxidative activity of these compounds is 0.68. The lowest correlation ($r^2$ = 0.60) was between antiradical and antioxidative activity of phenolic acids.

The conclusion is that antiradical, antioxidative and anti-$H_2O_2$ activity of phenolic acids strongly depends on the number of hydroxy groups and position of bonding of these groups in the aromatic ring: The greater the number of hydroxy groups the stronger the antioxidative activity. The *ortho* position of hydroxy groups in relation to each other was proved to be advantageous for antiradical features of phenolic acids similar to the B-ring of flavonoids (Burda and Oleszek, 2001). The *para* position of hydroxy group substitution in relation to each other seems to be also advantageous for antiradical features of phenolic acids.

The analysis of antiradical activity of phenolic acids with the Trolox equivalent antioxidative activity test was carried out by Rice-Evans *et al.* (1996). They proved the advantageous effect of the methylene group between the phenolic ring and the carboxylate group as in phenylacetic acid. The 3,4-dihydroxy-substituted compound (protocatechuic acid) (Fig. 3) with activity (1.19 ± 0.03) mM appeared to be a less effective antioxidant than 3,4-dihydroxyphenylacetic acid (Fig. 3) with activity (2.19 ± 0.08) mM (Table II). According to these authors 2,3-dihydroxysubstituted phenolic acid (*o*-pyrocatechuic acid) (Fig. 3) appeared to be a more effective antioxidant with activity (1.46 ± 0.01) mM than 3,4-substituted protocatechuic acid [(1.19 ± 0.03) mM] (Table II). Our experiments confirmed a little higher antiradical effectiveness of *o*-pyrocatechuic acid [(46 ± 3)%] than protocatechuic acids with activity (41.2 ± 0.6)% (Table III) but the antioxidative and anti-$H_2O_2$ activity of *o*-pyrocatechuic acid [(65 ± 7)%] and [(47 ± 3)%] was lower than of 3,4-dihydroxy-substituted protocatechuic acid [(89 ± 4)%] and [(55 ± 2)%] (Table III) (Sroka and Cisowski, 2003).

Rice-Evans *et al.* (1996) described 3,5-dihydroxybenzoic ($\alpha$-resorcylic) acid as strong antioxidant compound with antioxidative activity (2.15 ± 0.05) mM (Table II). Our study showed that $\alpha$-resorcylic acid is an effective antioxidant (Table III) during lipid oxidation [(64 ± 6)%] similar to other strong active dihydroxy-substituted phenolic acids but its antiradical and anti-hydrogen peroxide activity as for $\beta$-resorcylic acid (Fig. 3) was much lower [(0.60 ± 0.08)% and (18 ± 2)%] (Table III) (Sroka and Cisowski, 2003).

There was shown that substitution of the 3 and 5 hydroxy groups in gallic acid [(3.01 ± 0.05) mM] with methoxy groups as in syringic acid (Fig. 3) causes the decreasing effect of antioxidative activity [(1.36 ± 0.01) mM] (Table II) (Rice-Evans *et al.*, 1996).

The surprisingly high antioxidative features for *p*-coumaric acid (Fig. 3) were described [(2.22 ± 0.06) mM] (Table II). Lower activity exhibited *meta*- [(1.21 ± 0.02) mM] but the lowest *ortho*-coumaric acid [(0.99 ± 0.15) mM] (Rice-Evans *et al.*, 1996). The antioxidative activity of *p*-coumaric acid was even stronger than surprisingly low antioxidative activity of caffeic acid [(1.26 ± 0.01) mM] (Table II). Our experiments showed small ability of *p*-coumaric [(0.25 ± 0.01)%] as well as *m*-coumaric [(0.38 ± 0.01)%] and *o*-coumaric [(0.25 ± 0.01)%] acids for scavenging of DPPH$^\bullet$ free radical (Table III) while antiradical activity of caffeic acid in the same test was strong [(44.0 ± 0.5)%] (Table III). Castelluccio *et al.* (1995) showed during investigation of antioxidative features of cinnamates in lipid phase (LDL) that caffeic and chlorogenic acids were more effective antioxidants than ferulic or *p*-coumaric acids.

The results from the lipids studies showed that antioxidative activity of monophenols was increased substantially by methoxy substitutions in *ortho* position to the OH group (Couvelier *et al.*, 1992; Chimi *et al.*, 1991). The higher activity of methoxy-substituted compounds (ferulic acid) than *para*-monohydroxy benzoate (*p*-coumaric) could be connected with the increase of stabilisation of the aryloxyl radical by the electron-donating methoxy group.

## Conclusions

a) Antioxidative and antiradical activity of flavonoids strongly depend on the number of hydroxy groups bound to the aromatic B-ring.

b) The presence of a 2,3-double bond in the C-ring of flavonoids seems to have positive influence on antiradical features of flavonoids.
c) The glycosylation of flavonoids and blocking of the hydroxy group connected with the C-ring at position 3 decrease antiradical features.
d) An orthodiphenolic structure of the B-ring of flavonoids enhances antiradical features of these compounds.
e) Flavonoids with no group bound to the B-ring or with only a methoxy group connected to this ring seem to be ineffective as antioxidants.
f) Tannins possess strong antiradical and antioxidative properties especially due to the high number of hydroxy groups connected to the aromatic ring.
g) Tannins and gallate esters of catechins seem to be effective antioxidants *in vivo*.
h) The antiradical potency of phenolic acids strongly, positively depends on the number of hydroxy groups bound to the aromatic ring.
i) The *ortho* and *para* position in relation to each other of hydroxy groups bound to the aromatic ring favours the antioxidative and antiradical activity of phenolic acids.
j) The substitution of methoxy group in *ortho* position to the OH in monophenols causes the increased antioxidative activity of monophenols.

There are some differences in antioxidative and antiradical activities measured for the same compound in different tests. The conclusions should be drawn carefully while antioxidative activities of compared substances were measured in different manner. The comparative conclusions should be drawn when experiments are made in the same or similar model test.

Arora A., Nair M. G., and Strasburg G. M. (1998), Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radic. Biol. Med. **24**, 1355–1363.
Bors W., Foo Y. L., Hertkorn N., Michel Ch., and Stettmaier K. (2001), Chemical studies of proanthocyanidins and hydrolysable tannins. Antioxid. Redox. Sign. **3**, 995–1008.
Brand-Williams W., Cuvelier M. E., and Berset C. (1995), Use of free radical method to evaluate antioxidant activity. Lebensm.-Wiss. Technol. **28**, 25–30.
Buege J. A. and Aust S. D. (1978), Microsomal lipid peroxidation. Methods Enzymol. **52C**, 302–310.
Burda S. and Oleszek W. (2001), Antioxidant and antiradical activities of flavonoids. J. Agric. Food Chem. **49**, 2774–2779.
Castelluccio C., Pagana G., Melikian N., Bolwell G. P., Pridham J., Sampson J., and Rice-Evans C. (1995), Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. FEBS Lett. **368**, 188–192.
Chimi H., Cillard J., Cillard P., and Rahmani M. (1991), Peroxyl radical scavenging activity of some natural phenolic antioxidants. J. Am. Oil Chem. Soc. **68**, 307–312.
Couvelier M. E., Richard H., and Berset C. (1992), Comparison of the antioxidative activity of some acid-phenols: Structure activity relationship. Biosci. Biotechnol. Biochem. **56**, 324–325.
Croft K. D. (1998), The chemistry and biological effects of flavonoids and phenolic acids. Ann. N. Y. Acad. Sci. **854**, 435–442.
Fuhrman B., Lavy A., and Aviram M. (1995), Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. Am. J. Clin. Nutr. **61**, 549–554.
de Gaetano G. and Cerletti C. (2001), Wine and cardiovascular disease. Nutr. Metab. Cardiovasc. Dis. **11**, 47–50.
Halliwell B. and Gutteridge J. M. C. (1999), Plant phenols. In: Free Radicals in Biology and Medicine (Halliwell B. and Gutteridge J. M. C., eds.). Oxford University Press, Oxford, New York, pp. 225–231.
Hassig A., Liang W. X., Schwabl H., and Stampfli K. (1999), Flavonoids and tannins: plant-based antioxidants with vitamin character. Med. Hypotheses **52**, 479–481.
Kono S., Ikeda M., Tokudome S., and Kuratsune M. (1988), A case-control study of gastric cancer and diet in northern Kyushu, Japan. Jpn. J. Cancer Res. **79**, 1067–1074.
Lairon D. and Amiot M. J. (1999), Flavonoids in food and natural antioxidants in wine. Curr. Opin. Lipidol. **10**, 23–28.
Leung L. K., Su Y., Chen R., Zhang Z., Huang Y., and Chen Z. Y. (2001), Theaflavins in black tea and catechins in green tea are equally effective antioxidants. J. Nutr. **131**, 2248–2251.
Lopez-Velez M., Martinez-Martinez F., and Del Valle-Ribes C. (2003), The study of phenolic compounds as natural antioxidants in wine. Crit. Rev. Food Sci. Nutr. **43**, 233–244.
Papas A. M. (1999), Isoflavone metabolism and bioavailability. In: Antioxidant Status, Diet, Nutrition, and Health (Papas A. M., ed.). CRC Press, London, New York, Washington, pp. 211–231.
Pick E. and Keisari Y. (1980), A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J. Immunol. Methods **38**, 161–170.
Pietta P. G. (2000), Flavonoids as antioxidants. J. Nat. Prod. **63**, 1035–1042.

Plumb G. W., De Pascual-Teresa S., Santos-Buelga C., Cheynier V., and Williamson G. (1998), Antioxidant properties of catechins and proanthocyanidins: effect of polymerisation, galloylation and glycosylation. Free Radic. Res. **29**, 351–358.

Prior R. L. (2003), Fruits and vegetables in the prevention of cellular oxidative damage. Am. J. Clin. Nutr. **78** (Suppl.), 570S–578S.

Rice-Evans C. A., Miller J. M., and Paganga G. (1996), Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radic. Biol. Med. **20**, 933–956.

Shahidi F. and Wanasundara P. K. J. (1992), Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. **32**, 67–103.

Sroka Z. and Cisowski W. (2003), Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem. Toxicol. **41**, 753–758.

Sroka Z., Rządkowska-Bodalska H., and Mażol I. (1994), Antioxidative effect of extracts from *Erodium cicutarium* L. Z. Naturforsch. **49c**, 881–884.

Terao J. (1999), Dietary flavonoids as antioxidants *in vivo*: conjugated metabolites of (–)-epicatechin and quercetin participate in antioxidative defence in blood plasma. J. Med. Invest. **46**, 159–168.

Vinson J. A. (1998), Flavonoids in foods as *in vitro* and *in vivo* antioxidants. Adv. Exp. Med. Biol. **439**, 151–164.

Williamson G., Faulkner K., and Plumb G. W. (1998), Glucosinolates and phenolics as antioxidants from plant foods. Eur. J. Cancer Prev. **7**, 17–21.

Wollin S. D. and Jones P. J. (2001), Alcohol, red wine and cardiovascular disease. J. Nutr. **131**, 1401–1404.

Yamanaka N., Oda O., and Nagao S. (1997), Green tea catechins such as (–)-epicatechin and (–)-epigallocatechin accelerate $Cu^{1+}$-induced low density lipoprotein oxidation in propagation phase. FEBS Lett. **401**, 230–234.