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Antioxidant properties of catechins: Comparison with other antioxidants

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ABSTRACT

Antioxidant properties of five catechins and five other flavonoids were compared with several other natural and synthetic compounds and related to glutathione and ascorbate as key endogenous antioxidants in several *in vitro* tests and assays involving erythrocytes. Catechins showed the highest ABTS$^{\cdot}$-scavenging capacity, the highest stoichiometry of Fe^{3+} reduction in the FRAP assay and belonged to the most efficient compounds in protection against SIN-1 induced oxidation of dihydrorhodamine 123, AAPH-induced fluorescein bleaching and hypochlorite-induced fluorescein bleaching. Glutathione and ascorbate were less effective. (+)-catechin and (−)-epicatechin were the most effective compounds in protection against AAPH-induced erythrocyte hemolysis while (−)-epicatechin gallate, (−)-epigallocatechin gallate and (−)-epigallocatechin protected at lowest concentrations against hypochlorite-induced hemolysis. Catechins [(−)-epigallocatechin gallate and (−)-epicatechin gallate)] were most efficient in the inhibition of AAPH-induced oxidation of 2′7′-dichlorodihydrofluorescein contained inside erythrocytes. Excellent antioxidant properties of catechins and other flavonoids make them ideal candidates for nanoformulations to be used in antioxidant therapy.

1. Introduction

Plant antioxidants are generally recognized as synonyms of nutriceuticals, at least among consumers and producers. Chemical compounds displaying reductive properties have been long used in food industry to protect foods against oxidation; however, the current interest stems from their ability to combat oxidative stress (OS) in the human organism.

Oxidative stress is an imbalance between antioxidant defence system and the production of reactive oxygen species (ROS). The collective term “reactive oxygen species” includes both free radicals [molecules having an odd electron, like superoxide radical anion ($O_2^{\cdot -}$) and hydroxyl radical ($^{\cdot}OH$)] and species that are not free radicals, like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and ozone (O_3). Reactive nitrogen species (RNS) include, i.a., nitric oxide ($^{\cdot}NO$) and peroxynitrite ($ONOO^-$) formed in a reaction of $^{\cdot}NO$ with $O_2^{\cdot -}$. Reactive halogen species (RXS) include such species as HOCl, HOBr, HOJ, chlorine, bromine, iodine etc. Hypohalogenous acids are formed in the body mainly by oxidation of halogen ions by myeloperoxidase. The imbalance between the production of ROS, RNS or RXS, and the antioxidant defense, in favor of prooxidants, is called oxidative, nitr(os) ative and halogenative stress, respectively. Although at physiological concentrations ROS, RNS and RXS can function as signaling molecules regulating cell proliferation, growth, differentiation and apoptosis (Barbieri & Sestili, 2012; Bartosz, 2009), they react with and damage all classes of endogenous macromolecules including proteins, nucleic acids, lipids and carbohydrates (Sadowska-Bartosz & Bartosz, 2015). OS has a devastating effect causing cell death and tissue damage and is commonly observed in several conditions such as cardiovascular diseases, diabetes, neuronal disorders, and in aging (Kandikattu et al., 2015; Treml & Šmejkal, 2016). Recently, there has been an increase in interest in natural substances with antioxidant properties, which reduce or prevent negative effects of OS on living tissues, and inhibit aging processes and the development of many diseases, especially polyphenols (Stolarzewicz, Ciekot, Fabiszewska, & Białecka-Florjańczyk, 2013).

Polyphenols or phenolic compounds are one of the most important groups of secondary metabolites of plants. They are widely distributed in the plant kingdom (Losada-Barreiro & Bravo-Díaz, 2017). Dietary polyphenols comprise a wide range of aromatic compounds that are responsible for organoleptic characteristics of plant-derived food and beverages. The polyphenols that are present in foods can be divided into two main groups: non-flavonoids and flavonoids. Non-flavonoids are mostly monocyclic acids and can be further divided into two main sub-classes: phenolic acids and stilbenes (e.g. resveratrol). Phenolic acids are subdivided into benzoic acids and hydroxycinnamic acids. Flavonoids share a common nucleus consisting of two phenolic rings and an oxygenated heterocycle. They form a diverse range of

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compounds and can be categorized into many classes, such as anthocyanins, flavonols (e.g. morin), flavanols (e.g. catechins), flavones, and chalcones. Catechins are present in many dietary products, plants, fruits (such as apples, blueberries, gooseberries, grape seeds, kiwi, strawberries), green and black tea, red wine, beer, cacao liquor, chocolate and cocoa. The main catechins present in the green tea include (−)-epigallocatechin gallate, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate and (+)-catechin (Zeeb, Nelson, Albert, & Dalluge, 2000). One gram of dried green tea leaves contains more than 200 mg catechins (Yen & Chen, 1996), although total catechin content varies widely depending on species, variety, growing location, season, illumination, and altitude. The best studied catechin is (−)-epigallocatechin gallate, the major polyphenol in green tea (Fujiki, 2005). The antioxidant properties of polyphenols are mostly due to their redox properties, which let them act as reducing agents, hydrogen donors and singlet oxygen quenchers (Lima, Vianello, Corrêa, da Silva Campos, & Borguini, 2014). In contrast to glutathione (GSH), polyphenols cannot be synthesized by humans, but are obtained through the diet; so, free radicals that are originated during body metabolism can be better neutralized by regular intake of foods containing a high content of fruits and vegetables (Milella et al., 2011).

Glutathione is present in the cytoplasm in millimolar concentrations. Reduced glutathione is a linear tripeptide of L-glutamic acid, L-cysteine, and glycine, which plays a key role in the cellular antioxidant system, and is the main determinant of the intracellular redox state (Schafer & Buettner, 2001).

The purpose of this study was to compare the antioxidant properties of catechins with those of other natural and synthetic antioxidants (Table 1) including main intracellular antioxidants (GSH and ascorbic acid). The antioxidant activities were estimated by commonly-used methods of scavenging of a model radical, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\cdot}$), and ferric reducing antioxidant power (FRAP) assays. We also checked the ability of the compounds studied to protect against dihydrorhodamine 123 (DHR123) oxidation by 3-morpholinosydnonimine (SIN-1) and against fluorescein bleaching by hypochlorite and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), a model source of peroxyl radicals ($ROO^{\cdot}$), in cell-free systems. Moreover, we investigated the anti-hemolytic activity of the selected antioxidant against hypochlorite or AAPH-induced OS of human erythrocytes and inhibition of oxidation of intracellular fluorogenic probe 2′,7′-dichlorofluorescein (H_2DCF-DA) by AAPH. This study is a part of a larger project aimed at comprehensive comparison of beneficial and adverse effects of natural and synthetic antioxidants.

2. Materials and methods

2.1. Materials

Dimethyl sulfoxide (DMSO; purity: ≥99.9% Sterile Filtered) produced by BioShop Canada Inc. (Burlington, Ontario, Canada) was purchased from Lab Empire (Rzeszów, Poland).

3-Morpholinosydnonimine (SIN-1) was obtained from Tocris Bioscience (Bristol, United Kingdom). 3-Morpholinosydnonimine stock solutions (1 mM) were prepared in phosphate-buffered saline (PBS: 145 mM NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4), and aliquots were frozen immediately at −80 °C until use. Under these conditions, SIN-1 was stable for several months, as assessed by HPLC analysis. 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Polysciences (Warrington, PA, USA). A stock solution of AAPH was freshly prepared in PBS before each experiment.

Dihydrorhodamine 123, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) and rutin trihydrate were purchased from Thermo Fisher Scientific (Warsaw, Poland). Fluorescein and sodium hypochlorite (NaOCl, 15% active chlorine basis) were obtained from CHEMPUR (Piekary Slaskie, Poland). A stock solution of NaOCl was diluted in 0.1 M NaOH and its concentration was determined spectrophotometrically at 290 nm using the molar absorption coefficient of $\varepsilon_{290\ nm} = 350\ M^{-1}\ cm^{-1}$ (Morris, 1966). Under such conditions NaOCl exists exclusively as OCl^-. A stock solution of NaOCl was diluted in PBS, before use. At pH 7.4 the both forms, HOCl and OCl^- are present in the solution at comparable concentrations.

Selected compounds such as (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epigallocatechin gallate were obtained from Extrasynthese (Genay, France). Curcumin and hydrocinnamic acid were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Glutathione was obtained from Fluka Chemica-Biochemika (Buchs, Switzerland). For every assay, experiments concerning the effect of solvent alone were always performed.

All other reagents, if not mentioned otherwise, were purchased from Sigma (Poznan, Poland) and were of analytical grade. Distilled water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Fluorometric and absorptiometric measurements were done in a Tecan Infinite 200 PRO multimode reader (Tecan Group Ltd., Männedorf, Switzerland). All measurements were performed in triplicate and repeated at least three times. Selected compounds were dissolved in PBS, DMSO or ethanol (in studies of the effects of NaOCl). Minimal amounts of the solvents present in the samples had a small effect on the protection (up to several %). The effect of DMSO or ethanol was subtracted from the effects of substances introduced in this solvent. In cell-free systems, GSH and ascorbate served as reference antioxidants.

2.2. Experiments in cell-free systems

2.2.1. Antiradical activity of selected exogenic and endogenic compounds

2.2.1.1. $ABTS^{\cdot}$ assay. The ability of selected compounds to scavenge the 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical ($ABTS^{\cdot}$) was measured according to a procedure previously proposed by us (Sadowska-Bartosz, Gajewska, Skolimowski, Szewczyk, & Bartosz, 2015), modified for application in a microplate. Briefly, appropriate amounts of compounds were added to a solution of $ABTS^{\cdot}$, diluted so that 200 µl of the solution had absorbance of 1.0 in a microplate well, at 734 nm. The decrease in $ABTS^{\cdot}$ absorbance was measured after 1 min ("fast" scavenging) and between 10 and 30 min ("slow" scavenging) of incubation at ambient temperature (21 ± 1 °C). From the plots of the dependence of absorbance decrease (ΔA) on the compound concentration, the value of ΔA/mM was calculated for the compounds tested.

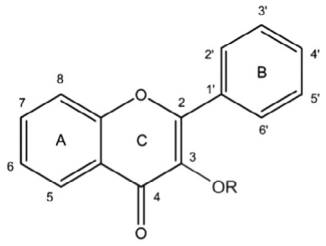
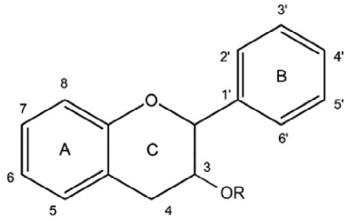
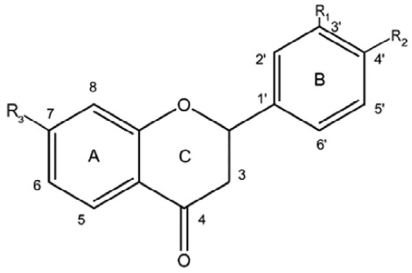
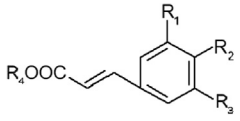
2.2.1.2. FRAP assay. The Ferric Reducing Antioxidant Potential (FRAP) was determined with 0.3 M acetate buffer (pH = 3.6), 0.01 M TPTZ (2,4,6-tripyridyl-*s*-triazine) in 0.04 M HCl and 0.02 M $FeCl_3 * 6H_2O$ mixed in a 10:1:1 vol ratio (Benzie & Strain, 1996). Absorbance was measured at the wavelength of 593 nm after 20-min incubation at room temperature.

In each case, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; CID: 40634) was used as control to obtain the standard curve and value was calculated with respect to the activity of Trolox and expressed as Trolox equivalents.

2.2.2. Protection against oxidation of dihydrorhodamine 123

Dihydrorhodamine 123 (DHR123) is a model substance that is easily oxidized by various oxidants, among them SIN-1 [a compound generating $ONOO^-$-anion of peroxynitrous acid (HONOO)], to the fluorescent rhodamine 123. SIN-1 is an active metabolite of the vasodilator drug 3-morpholinosydnonimine, which decomposes spontaneously in solution. In the presence of O_2, $^{\cdot}NO$ and $O_2^{\cdot -}$ are released, generating $ONOO^-$. Prevention of DHR123 oxidation by a compound is a measure of its property to react with a given oxidant. The lower is the concentration of a compound inhibiting DHR123 oxidation by 50%, the higher is the antioxidant potency of this compound with respect to a

Table 1
Classification and structure of flavonoids and model polyphenols used in this study.

Class of compounds	General structure	Compounds studied
Flavonols		Morin (3,5,7,2′,4′-OH) Rutin (5,7,3′,4′-OH, R = rutinose)
Flavanols (catechins)		(+)-Catechin (5,7,3′,4′-OH, R = H) (−)-Epicatechin (5,7,3′,4′-OH, R = H) (−)-Epicatechin gallate (5,7,3′,4′-OH, R = gallate) (−)-Epigallocatechin (5,7,3′,4′,5′-OH, R = H) (−)-Epigallocatechin gallate (5,7,3′,4′,5′-OH, R = gallate)
Flavanones		Hesperidin (5-OH, R_1 = OH, R_2 = OCH_3, R_3 = *O*-rutinoside) Naringenin (5-OH, R_1 = H, R_2 = R_3 = OH) Naringin (5-OH, R_1 = H, R_2 = OH, R_3 = *O*-neohesperidoside)
Hydroxycinnamic acids		Caffeic acid (R_1 = R_4 = H, R_2 = R_3 = OH) Chlorogenic acid (R_1 = H, R_2 = R_3 = OH, R_4 = quinic acid) *p*-Coumaric acid (R_1 = R_3 = R_4 = H, R_2 = OH) Ferulic acid (R_1 = R_4 = , R_2 = OH, R_3 = OCH_3) Hydrocinnamic acid (R_1,R_2,R_3, R_4 = H) Sinapic acid (R_1 = R3 = $OCH3$, R_2 = OH, R_4 = H)
Others		Ascorbic acid Butylated hydroxyanisole *tert*-Butylhydroquinone 2,6-di-*tert*-Butyl-1-4-methylphenol Carnosine Curcumin Deferoxamine Glutathione Mangiferin Propyl gallate Pyruvic acid Resveratrol Spermine Uric acid

given oxidant.

Dihydrorhodamine 123 (190 μl of 1 μM solution in 0.1 M phosphate buffer, pH 7.4) was added to each well of a 96-well plate containing the compounds studied in a range of concentrations (0.005–500 μM). The final volume of a sample was 200 μl. SIN-1 chloride (1 μl of 1 mM solution) was added to each well and kinetic measurement of fluorescence increase was carried using the excitation/emission wavelengths of 460/528 nm at 37 °C for 2 h. From the area under curve values of fluorescence, IC_{50} values were determined.

2.2.3. Protection of fluorescein against bleaching induced by NaOCl or AAPH

The fluorescence of fluorescein can be bleached by various oxidants, including hypochlorite or AAPH due to oxidation. Inhibition of fluorescein bleaching is thus a measure of a given compound to prevent reactions of oxidation by selected oxidants. AAPH is a water-soluble azo compound which is used extensively as a free radical generator. Decomposition of AAPH produces molecular nitrogen and two carbon radicals. The carbon radicals may combine to produce stable products or react with molecular oxygen to give $ROO^{\cdot}$. The half-life of AAPH is about 175 h (37 °C at neutral pH), making the rate of free radical generation essentially constant during the first several hours in solution.

Various amounts of hypochlorite were added to a well containing 0.2 μM fluorescein dissolved in PBS and the solution was mixed immediately. The amount of hypochlorite required to decrease fluorescence down to ca 5–10% of the initial value was determined (17.5 μM hypochlorite). These conditions were used for subsequent measurements, in which compounds dissolved in PBS or DMSO in a range of concentrations (usually 0.125–5 μM), were present in the fluorescein solution before addition of hypochlorite, keeping the volume of the sample constant (100 μl). Fluorescence was measured after 15 min incubation at room temperature at the excitation/emission wavelengths of 485 and 538 nm, respectively.

Table 2
Reducing activity of the antioxidants studied. Comparison with glutathione scavenging activity: [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$ (Dunnett test).

Compound	PubChem CID	$ABTS^{\cdot}$ scavenging activity [mol TE/mol]	FRAP [mol TE/mol]
Standard antioxidants			
Glutathione	124886	1.027 ± 0.004	0.010 ± 0.000
Synthetic antioxidants			
Ascorbic acid	54670067	1.114 ± 0.017	1.982 ± 0.016[a]
Butylated hydroxyanisole	8456	1.080 ± 0.006	0.756 ± 0.003[a]
tert-Butylhydroquinone	16043	0.951 ± 0.003	0.933 ± 0.005[a]
2,6-di-*tert*-Butyl-1-4-methylphenol	31404	1.016 ± 0.004	0.010 ± 0.001
Propyl gallate	4947	1.881 ± 0.028[b]	1.122 ± 0.004[a]
Catechins			
(+)-Catechin	9064	3.965 ± 0.067[a]	0.793 ± 0.004[a]
(−)-Epicatechin	72276	2.800 ± 0.051[a]	0.917 ± 0.004[a]
(−)-Epigallocatechin	72277	2.939 ± 0.037[a]	1.032 ± 0.007[a]
(−)-Epicatechin gallate	107905	7.800 ± 0.037[a]	2.335 ± 0.006[a]
(−)-Epigallocatechin gallate	65064	5.632 ± 0.027[a]	2.211 ± 0.006[a]
Other flavonoids and derivatives			
Morin	16219651	2.497 ± 0.027[a]	1.169 ± 0.003[a]
Naringenin	932	1.507 ± 0.007	0.011 ± 0.002
Naringin	442428	1.086 ± 0.018	0.030 ± 0.009[c]
Rutin	16218542	2.074 ± 0.006[a]	1.156 ± 0.011[a]
Hesperidin	10621	0.849 ± 0.034	0.101 ± 0.001[a]
Hydroxycinnamic acids and derivatives			
p-Coumaric acid	637542	2.123 ± 0.006[a]	0.008 ± 0.002
Caffeic acid	689043	0.965 ± 0.015	1.018 ± 0.004[a]
Ferulic acid	445858	1.560 ± 0.041	0.687 ± 0.002[a]
Sinapic acid	637775	1.618 ± 0.004	1.230 ± 0.008[a]
Chlorogenic acid	1794427	0.926 ± 0.056	1.061 ± 0.012[a]
Hydrocinnamic acid	107	0.264 ± 0.016[b]	0.011 ± 0.004
Other natural antioxidants			
Curcumin	969516	1.685 ± 0.019[c]	0.709 ± 0.002[a]
Resveratrol	445154	2.738 ± 0.023[a]	0.619 ± 0.002[a]
Mangiferin	5281647	2.376 ± 0.030[a]	1.719 ± 0.022[a]

TE, trolox equivalents.

Different concentrations of AAPH were added to a well containing 0.2 μM fluorescein dissolved in PBS and the solution was mixed immediately. The concentration of AAPH required to decrease fluorescence down to ca 5–10% of the initial value after 60 min was determined (10 mM AAPH). These conditions were used for subsequent measurements, in which compounds dissolved in PBS or DMSO in a range of concentrations (usually 0.25–100 μM), were present in the fluorescein solution before addition of AAPH, keeping the volume of the sample constant (100 μl). Fluorescence was measured after 1 h incubation at 37 °C temperature at the excitation/emission wavelengths of 485 and 538 nm, respectively.

Percent of protection was calculated according to the formula and the concentration of a compound providing 50% protection (IC_{50}) against the fluorescein bleaching was calculated.

$$\%Protection = (F_n - F_0)/(F_c - F_0) \times 100\%$$

F_n – fluorescence of sample containing fluorescein, hypochlorite/AAPH and a compound studied; F_0 – fluorescence of fluorescein treated with hypochlorite/AAPH; F_c – fluorescence of non-treated fluorescein.

2.2.4. Iron chelating assay

To examine the strong iron chelating properties of the compounds studied, the deoxyribose test was performed as described previously by Sadowska-Bartosz, Galiniak, and Bartosz (2017).

2.2.5. Comparison with standard antioxidants

In order to evaluate the antioxidant power of compounds studied, standard antioxidants, glutathione and ascorbic acid, were assayed in parallel.

2.3. Erythrocytes hemolysis assays

2.3.1. Ethical approval

The study was approved by the Research Bioethics Committee of the University of Łódź (Poland).

2.3.2. Preparation of erythrocytes

Eight ml of peripheral blood from a healthy donor (lab volunteer, a 39-year-old woman) was collected in EDTA tubes and used within the day of its collection. Erythrocytes were isolated by centrifugation for 10 min at 3000 rpm, at 4 °C. The plasma and buffy coat were removed by aspiration. The red blood cells (RBCs) were washed four times with ice-cold PBS. Washed RBCs were suspended to a final hematocrit of 10% in PBS.

2.3.3. The assay of AAPH-induced hemolysis

The inhibition of free radical-induced RBCs hemolysis was performed by a modification of a previously published method (Wang, Sun, Cao, & Tian, 2009), in which hemolysis was monitored turbidimetrically. The erythrocyte hemolysis was induced by thermal decomposition of AAPH as an alkyl radical initiator. The protective effect of exogenic/endogenic compound against AAPH-induced hemolysis was measured only for compounds dissolved in PBS. The RBCs suspension was mixed with a selected endogenic or exogenic compound solution at the final concentration range 0.05–1 mM or 0.025–0.5 mM, respectively and incubated with shaking in the presence/absence of 75 mM AAPH, as optimal concentration to induce hemolysis at 37 °C. The turbidance (600 nm) was measured every 15 min for 10 h using an automated Bioscreen C turbidity reader [Oy Growth Curves Ab Ltd. (Helsinki, Finland)]. For all determinations, hemolysis time and percentage of hemolysis time with respect control erythrocytes were calculated 100% * [time (seconds) for test compound/mean time (seconds) for control sample containing RBCs and AAPH only].

2.3.4. The assay of hypochlorite-induced hemolysis

Aliquots of erythrocyte suspensions in PBS were mixed with a selected exogenic/endogenic compound solution at the final concentration range 5–25 μM (a final volume of 200 μl) and incubated 15 min with shaking at 37 °C. Then 0.15 mM hypochlorite (final), as optimal concentration to induce hemolysis, was added and turbidance (700 nm) was measured every 2 min for 240 min using a Tecan Infinite 200 PRO multimode reader. We prefer monitoring hemolysis at 700 nm whenever possible, as hemoglobin has some absorbance at 600 nm which may interfere with turbidance measurements especially when hemoglobin is oxidized. For all determinations, hemolysis time (seconds) and percentage of hemolysis time with respect control RBCs were calculated [100% * time (seconds) for test compound/mean time (seconds) for control sample containing RBCs and NaOCl only].

2.3.5. Determination of the intracellular ROS generation

2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) (also known as 2′,7′-dichlorofluorescin diacetate) is a chemically reduced form of 2′7′-dichlorofluorescein diacetate used as an indicator for ROS in cells. Upon cleavage of the acetate groups by intracellular esterases and oxidation, the nonfluorescent H_2DCF-DA is converted to the highly fluorescent 2′,7′-dichlorofluorescein (DCF) (Wang et al., 2017). The indicator H_2DCF-DA [dissolved in ethanol (0.4 mM), 10 μM final concentration] was added to the erythrocyte suspension (10% final hematocrit) with subsequent incubation at 37 °C for 30 min. The samples were centrifuged, and supernatant was discarded to remove excess H_2DCF-DA. Selected compounds were added in a range of

Table 3
Protection against dihydrorhodamine 123 oxidation by SIN-1 and against fluorescein bleaching by hypochlorite and AAPH (IC_{50} values). IC_{50} values compared to glutathione: [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$ (Dunnett test).

Compound	Protection against DHR123 oxidation (IC_{50}, μM)	Protection against fluorescein bleaching by NaOCl (IC_{50}, μM)	Protection against fluorescein bleaching by AAPH (IC_{50}, μM)
Standard antioxidants			
Ascorbic acid	13.900 ± 1.012	> 5[a]	11.850 ± 0.001[a]
Glutathione	14.070 ± 0.359	3.519 ± 0.048	15.440 ± 0.009
Synthetic antioxidants			
Butylated hydroxyanisole	0.933 ± 0.055[a]	0.594 ± 0.080[a]	1.503 ± 0.090[a]
tert-Butylhydroquinone	0.456 ± 0.028[a]	0.486 ± 0.037[a]	2.093 ± 0.081[a]
2.6-di-*tert*-Butyl-1-4-methylphenol	105.924 ± 8.844[a]	1.743 ± 0.109[a]	83.350 ± 0.854[a]
Propyl gallate	0.435 ± 0.012[a]	0.503 ± 0.022[a]	2.923 ± 0.233[a]
Catechins			
(+)-Catechin	0.805 ± 0.072[a]	0.341 ± 0.002[a]	0.671 ± 0.037[a]
(−)-Epicatechin	1.359 ± 0.044[a]	0.511 ± 0.026[a]	1.076 ± 0.060[a]
(−)-Epicatechin gallate	1.207 ± 0.049[a]	0.246 ± 0.034[a]	0.658 ± 0.025[a]
(−)-Epigallocatechin	1.076 ± 0.039[a]	0.307 ± 0.011[a]	1.872 ± 0.074[a]
(−)-Epigallocatechin gallate	1.147 ± 0.002[a]	0.355 ± 0.007[a]	2.156 ± 0.170[a]
Other flavonoids and derivatives			
Hesperidin	4.988 ± 0.748[a]	0.332 ± 0.027[a]	0.918 ± 0.033[a]
Morin	0.183 ± 0.014[a]	0.280 ± 0.007[a]	0.598 ± 0.010[a]
Naringenin	4.910 ± 0.374[a]	0.693 ± 0.048[a]	0.483 ± 0.011[a]
Naringin	40.507 ± 22.813[a]	0.458 ± 0.037[a]	1.478 ± 0.120[a]
Rutin	0.324 ± 0.009[a]	0.338 ± 0.009[a]	0.966 ± 0.014[a]
Hydroxycinnamic acids and derivatives			
Caffeic acid	0.390 ± 0.020[a]	0.625 ± 0.123[a]	1.662 ± 0.021[a]
Chlorogenic acid	0.718 ± 0.166[a]	0.506 ± 0.017[a]	1.138 ± 0.024[a]
p-Coumaric acid	4.252 ± 0.199[a]	0.627 ± 0.014[a]	0.976 ± 0.014[a]
Ferulic acid	4.694 ± 0.294[a]	0.595 ± 0.020[a]	1.132 ± 0.067[a]
Hydrocinnamic acid	> 500[a]	2.781 ± 0.160[a]	282.703 ± 0.009[a]
Sinapic acid	0.359 ± 0.018[a]	0.614 ± 0.041[a]	1.254 ± 0.033[a]
Other natural antioxidants			
Curcumin	0.903 ± 0.086[a]	0.328 ± 0.045[a]	0.793 ± 0.038[a]
Deferoxamine**	5.403 ± 3.301[b]	2.462 ± 0.055[a]	2.183 ± 0.032[a]
Mangiferin	0.412 ± 0.017[a]	0.339 ± 0.022[a]	0.879 ± 0.036[a]
Resveratrol	0.295 ± 0.025[a]	0.346 ± 0.007[a]	0.353 ± 0.009[a]
Uric acid*	0.880 ± 0.100[a]	5.243 ± 0.155[a]	6.469 ± 0.194[a]

* CID:1175;
** CID:62881.

concentrations (25–200 μM for uric acid, hydroxycinnamic acid, spermine, deferoxamine and pyruvic acid, 0.10–25 μM for other selected substances). The samples were maintained at 37 °C in a dark environment in a shaking incubator for 20 min. AAPH (25 mM final concentration) was then added with good mixing. The fluorescence (485 nm/535 nm) was measured every 1 min for 30 min. The reaction percent and IC_{50} were calculated.

2.4. Statistical analysis

Statistical significance of differences was evaluated using the Dunnett's test or paired Student's "t" test. Statistical analysis of the data was performed using STATISTICA software package (version 12, StatSoft Inc. 2014, Tulsa, OK, USA, www.statsoft.com).

3. Results

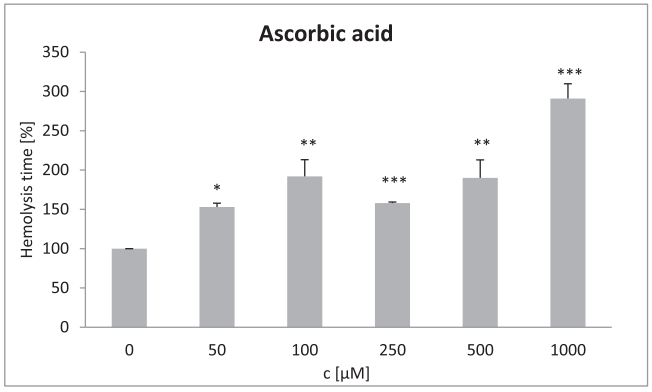
Free radical reducing capacity estimated by reduction of $ABTS^{\cdot}$ and Fe^{3+}-reducing capacity estimated by the FRAP assay of the compounds studies are compared in Table 2. From among the compounds studied, catechins showed the highest stoichiometry of $ABTS^{\cdot}$ reduction, (−)-epicatechin gallate being 7.8 times as efficient as Trolox, followed by (−)-epigallocatechin gallate (5.6 times), (+)-catechin (4 times), (−)-epigallocatechin (2.9 times) and epicatechin (2.8 times). Other antioxidants studied, including flavonoids and standard antioxidants, GSH and ascorbic acid, showed lower $ABTS^{\cdot}$-scavenging activity than catechins on the molar basis. The reactivity of both GSH and ascorbic acid was close to that of Trolox (about 1 mol Trolox equivalents/mol).

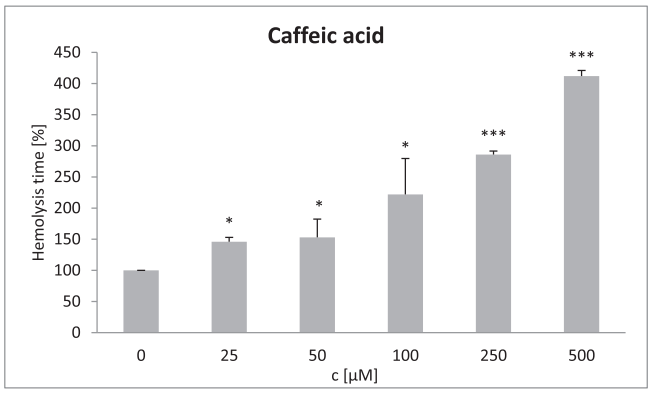
The reactivity of the antioxidants studied was generally lower in the FRAP assay than in the $ABTS^{\cdot}$ decolorization assay. However, some hydrocinnamic acids (caffeic acid and chlorogenic acid) showed comparable activity in both assays. The reactivity of catechins did not exceed 2.3 Trolox equivalents/mol and was much higher than that of GSH and comparable with that of ascorbic acid. In general, the correlation between the results of $ABTS^{\cdot}$ decolorization assay and FRAP assay within the groups of compounds studied was modest (0.615).

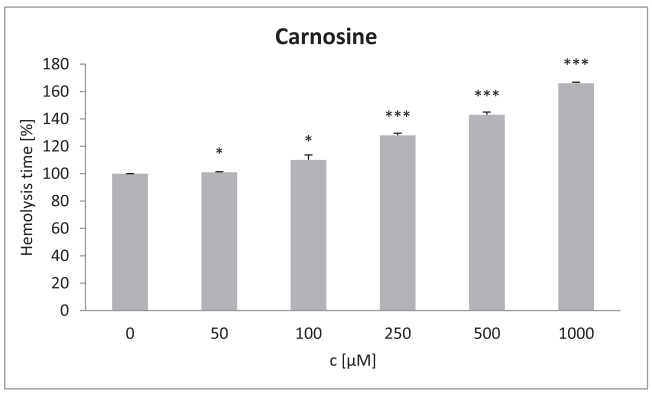
In view of many studies on the metal ion-binding properties of flavonoids, we checked catechins and other polyphenols studied for strong binding of ferrous ions using a recently developed assay (Sadowska-Bartosz et al., 2017). No compound studied showed the ability for strong ferrous iron binding except for deferoxamine (not shown).

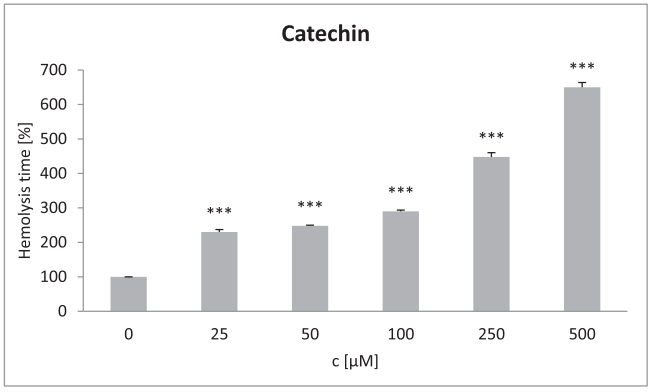
We compared the efficiency of selected antioxidants to react with other physiologically relevant oxidants such as $ONOO^-$, hypochlorite and $ROO^{\cdot}$. SIN-1 was used as a source of $ONOO^-$ and AAPH as a source of $ROO^{\cdot}$. Peroxyl radical formed via the reaction of carbon-centered radical with oxygen is a biologically relevant active species, because of its likelihood to damage cellular constituents. Furthermore, the pathological effects of $ROO^{\cdot}$ have received much attention in connection with the chain-propagation mechanism of lipid peroxidation.

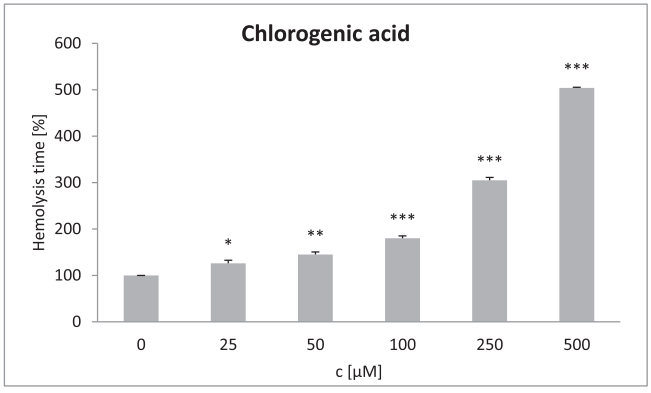
From plots of the dependence of DHR123 oxidation rate on the antioxidant concentration, IC_{50} values were derived. Morin was the most effective compound in protection against SIN-1 induced oxidation of DHR123, followed by rutin and hydroxycinnamic acids: sinapic acid











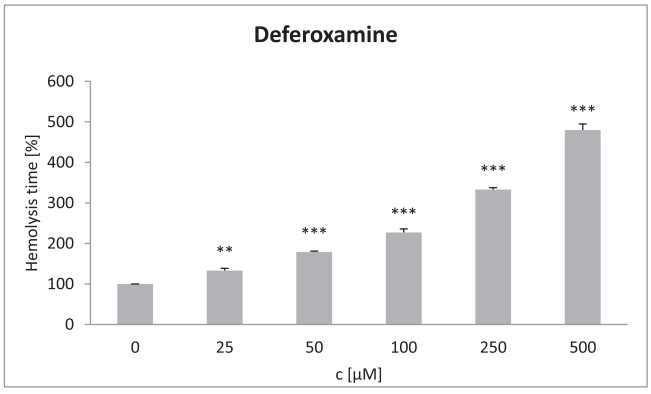


Fig. 1. Protection against AAPH-induced hemolysis by selected antioxidants.

Fig. 1. (*continued*)

and caffeic acid. Catechins showed reactivity much higher in comparison with GSH and ascorbic acid (IC_{50} values of about 1 μM, as compared with 14.1 and 13.9 μM, respectively). Interestingly, the reactivity of naringin was much lower in comparison with its aglycone naringenin (about 10-fold difference in IC_{50} values). This results point out to the important potential role of hydrolysis of flavonoid glycosides in the biological efficiency of these food components (Table 3).

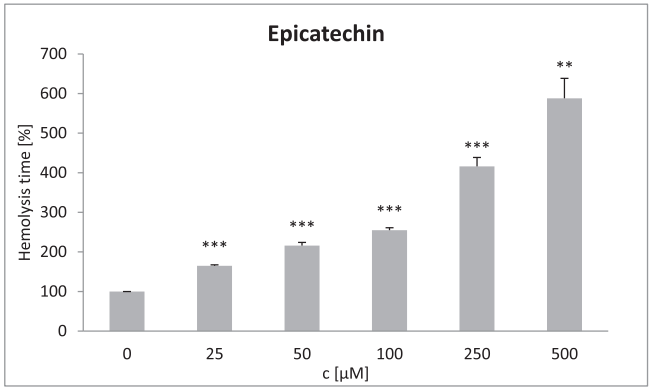
Resveratrol was the most effective in protection against AAPH-induced fluorescein bleaching, followed by naringenin and morin. Catechins, especially (+)-catechin and (−)-epicatechin gallate, showed also high reactivity, much higher than GSH and ascorbic acid. Also in this case, naringenin was more effective than naringin.

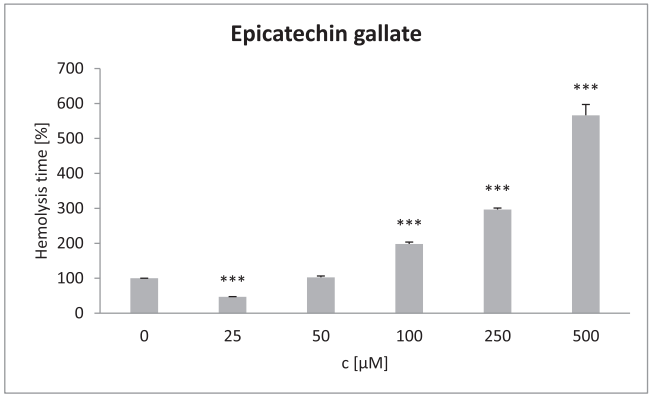
(−)-Epicatechin gallate was the most effective in protection against hypochlorite-induced fluorescein bleaching, followed by morin and (−)-epigallocatechin. Catechins, especially (+)-catechin and (−)-epicatechin gallate, showed also high reactivity, much higher than GSH and ascorbic acid; the list of other most effective compounds included flavonoids studied, especially morin, curcumin, resveratrol, as well as hydroxycinnamic acids, BHT and *tert*-butylhydroquinone. Glutathione and ascorbic acid were less effective (Table 3).

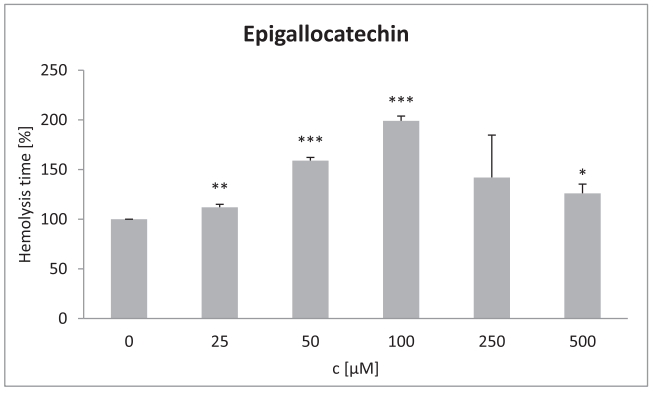
The cell-free assays are valuable for comparison of antioxidant reactivities with various oxidants, but they may not reflect the behavior of antioxidants in cellular systems where other factors such as membrane permeability for antioxidants and their location within the cell may considerably affect their effects. Using RBCs as model cells, we studied protection by antioxidants against hemolysis induced by hypochlorite and AAPH, and against intracellular ROS formation induced by AAPH. In these experiments, only water soluble antioxidants were used in order to avoid the cellular, mainly membrane effects of DMSO.

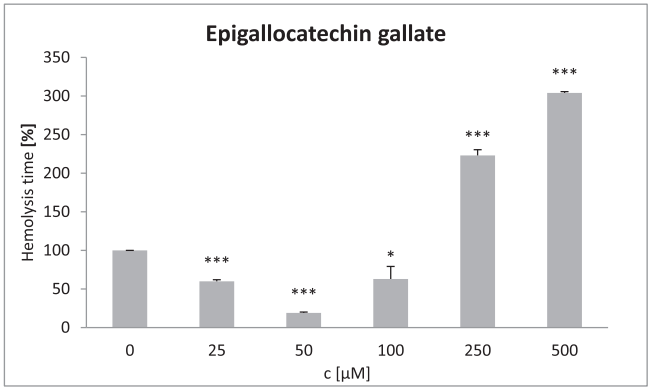
Protection by various antioxidants against hemolysis induced by AAPH is shown in Fig. 1. Catechins showed good protection. The lowest concentration of some catechins used [25 μM (+)-catechin, 50 μM (−)-epicatechin] prolonged the time of hemolysis by more than 100%. The same effect was achieved by 100 μM caffeic acid and deferoxamine, 250 μM (−)-epicatechin gallate, (−)-epigallocatechin gallate and chlorogenic acid, 1000 μM ascorbic acid and uric acid. Other antioxidants tested, including GSH, did not reach this level of protection.

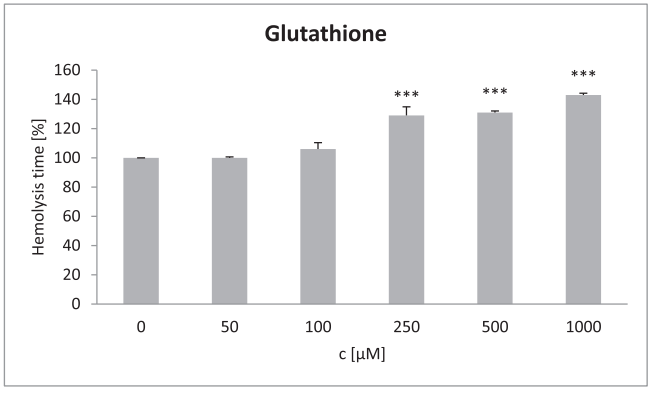
Protection against hemolysis induced by hypochlorite is presented in Fig. 2. Prolongation of the hemolysis time by more than 100% was achieved by 0.5 mM (−)-epicatechin, 1 μM (+)-catechin, 5 μM (−)-epigallocatechin and (−)-epicatechin gallate, 10 μM glutathione and epigallocatechin gallate, 15 μM spermine and 20 μM deferoxamine,











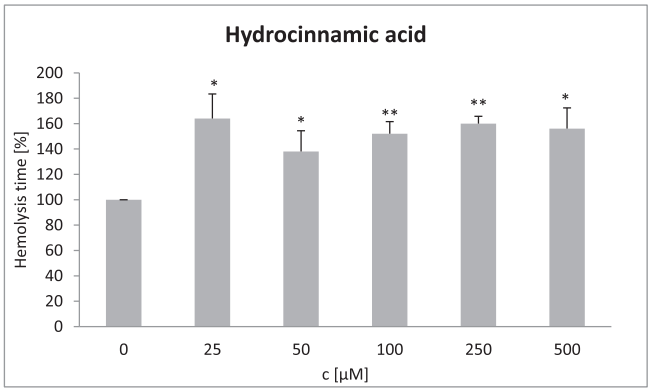


Fig. 1. (*continued*)

Fig. 1. (*continued*)

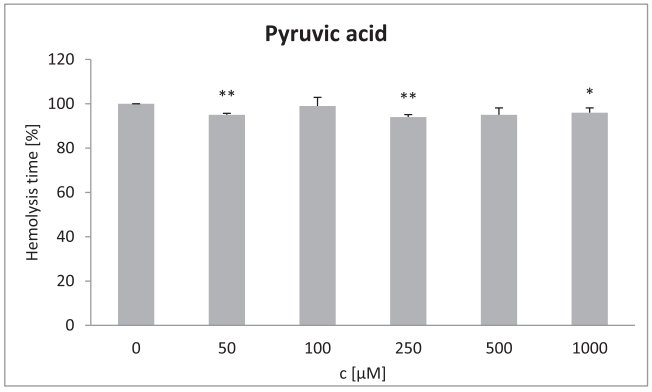
carnosine and uric acid.

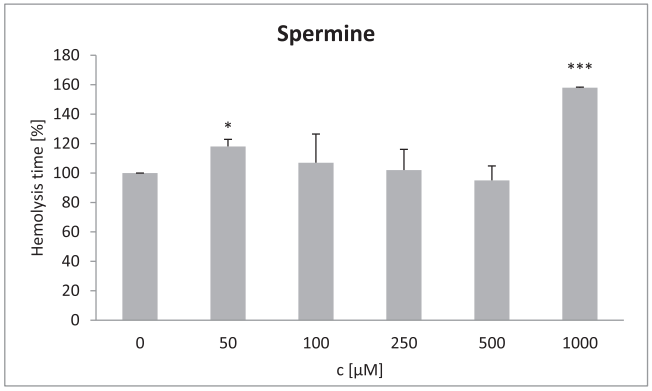
We tested the protective intracellular activity of antioxidants using a modification of the method proposed by Wang et al. (2017) in which RBCs are preloaded with H_2DCF-DA and challenged with AAPH in the absence and in the presence of the antioxidants tested. From the dependence of on the concentration of antioxidants, the concentrations providing 50% protection against intracellular H_2DCF-DA oxidation were estimated. In this assay, catechins, hydroxycinnamic acids and ascorbic acid showed the highest effectiveness [(−)-epigallocatechin gallate > (−)-epicatechin gallate > chlorogenic acid > ascorbic acid > (−)-epigallocatechin > caffeic acid] having IC_{50} values below or equal to 500 nM. The IC_{50} values of other antioxidants tested were in the range of about 5–100 μM (Table 4).

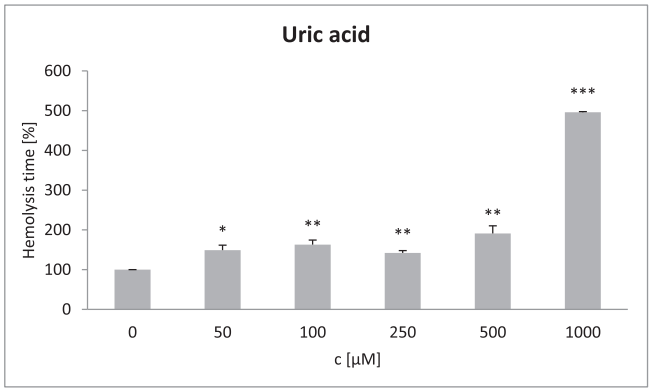
4. Discussion

There is increasing interest in the beneficial health effects of compounds present in food and beverages. Numerous studies have been devoted to the antioxidant properties of flavonoids, including catechins (Higdon & Frei, 2003; Lambert & Elias, 2010; Prior & Cao, 1999; Re et al., 1999). In particular, catechins have been found to be good chain-breaking antioxidants, inhibiting lipid peroxidation in low-density lipoprotein (LDL) induced by metmyoglobin at submicromolar concentrations, (−)-epicatechin gallate being the most effective (Rice-Evans, 1995), and bind metal ions catalyzing oxidation reactions (Lambert & Elias, 2010). The antioxidant action of catechins is well-established in various *in vitro* and *in vivo* systems. Many studies have reported that the scavenging effects of galloylated catechins were stronger than those of nongalloylated catechins and the scavenging effects of [(−)-epigallocatechin] was stronger than those of [(−)-epicatechin] and [(+)-catechin] (Jovanovic, Hara, Steenken, & Simic, 1995; Nanjo et al., 1996; Yoshida et al., 1989). Lee, Kim, Kim, and Kim (2014) have also found that (−)-epigallocatechin gallate has the highest antiradical capacity.

The present study demonstrates that catechins have remarkable antioxidant properties with respect to model free radical $ABTS^{\cdot}$, $ROO^{\cdot}$, ferric ions and other physiologically relevant oxidants such as $ONOO^-$ and hypochlorite, both in pure chemical systems and in protection of RBCs against hemolytic membrane damage and intracellular generation of free radicals. Catechins proved to be among the best antioxidants studied. This property of catechins may be of importance since after ingestion they are transported by blood, are in contact with RBCs and







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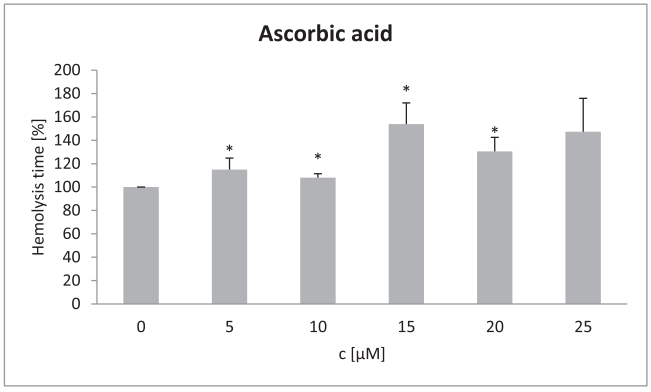
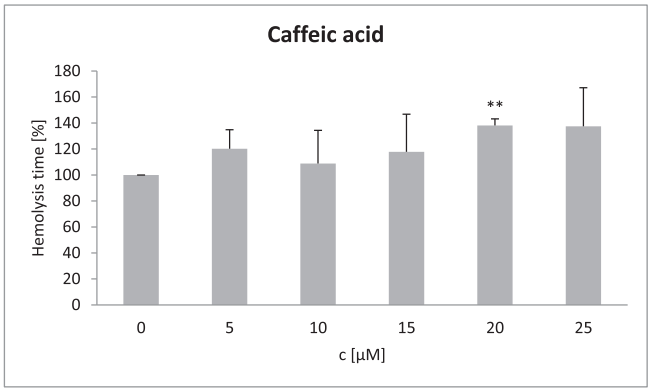




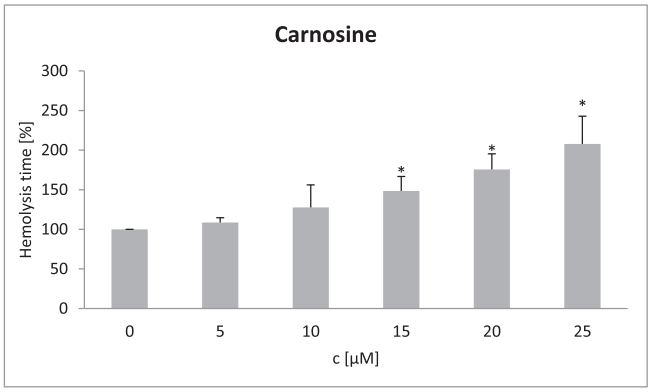


Fig. 2. Protection against hypochlorite-induced hemolysis by selected antioxidants.

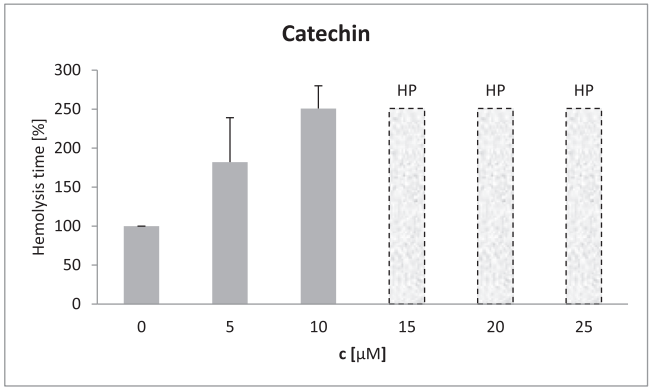
are bound by these cells.

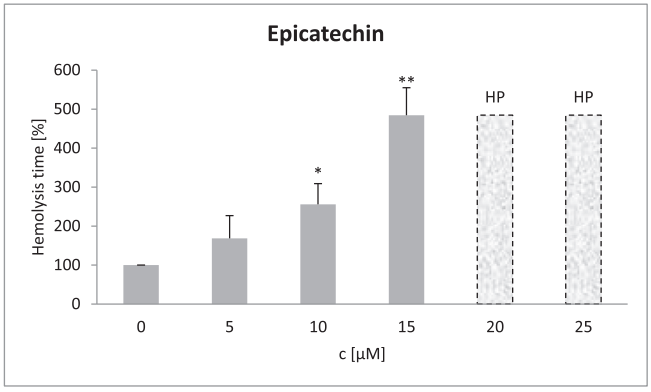
Selected compounds were assayed for antioxidant and free radical scavenging activities using the FRAP and ABTS˙ assays respectively. Although both the FRAP and ABTS˙ assays are based on electron transfer mechanisms, FRAP method measures the direct capacity of selected compounds to reduce ferric iron to ferrous iron as a measure of the antioxidant power (Benzie & Strain, 1996) and the ABTS˙ assay is based on the capacity of compounds to reduce the stable ABTS˙ radical in aqueous solutions (Re et al., 1999). Both activities were compared with that of Trolox, a water soluble derivative of vitamin E. FRAP and also ABTS˙ assays results indicate the highest antioxidant activity for the flavan-3-ols (catechins) as (−)-epicatechin gallate and (−)-epigallocatechin gallate, which have the galloyl moiety at the C-3 position.

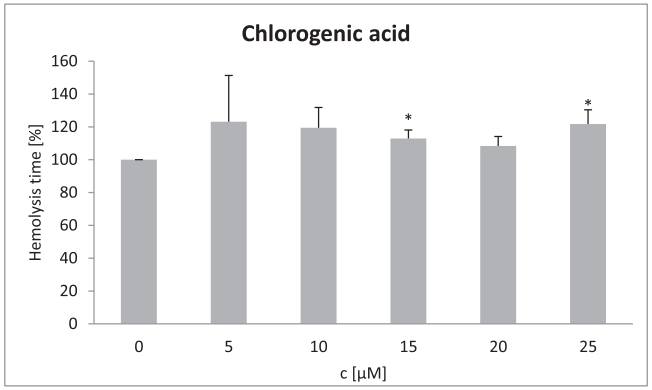
Generally, the stoichiometry of reaction of the antioxidants studied was lower in the FRAP assay than in the ABTS˙ decolorization assay. This is obvious for thiol-containing compounds such as GSH, but true also for many other antioxidants, including flavonoids. Similar differences in the reactivity of catechins with ABTS˙ and FRAP assays were found by Lee et al. (2014).

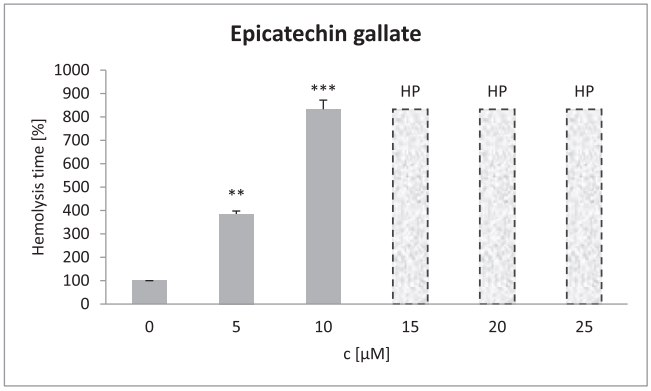
Comparison of structures and reactivity of hydroxycinnamic acids suggest that the presence of the second hydroxyl group in the phenolic ring is the main determinant of reactivity in the FRAP assay. *p*-Coumaric acid which has only one hydroxyl group shows very low reactivity, similar to that of hydroxycinnamic acid, lacking hydroxyl group. Caffeic acid (3,4-dihydroxy-cinnamic acid) having two hydroxyl groups and its ester, chlorogenic acid, show a FRAP reactivity of about 1 mol Trolox equivalents/mol. Methylation of the second hydroxyl group lowers the reactivity: ferulic acid (3-methoxy-4-hydroxycinnamic acid) has a reactivity of 0.6–0.7 mol Trolox equivalents/mol while sinapic acid, having one hydroxyl and two methoxy groups has a reactivity of about 1.2 mol Trolox equivalents/mol. Reactivity of hydroxycinnamic acids with ABTS˙ is more difficult to explain since even *p*-coumaric acid, having one hydroxyl group in the phenyl ring, shows a reactivity of about 2 mol Trolox equivalents/mol, suggesting a more complex reaction mechanism.

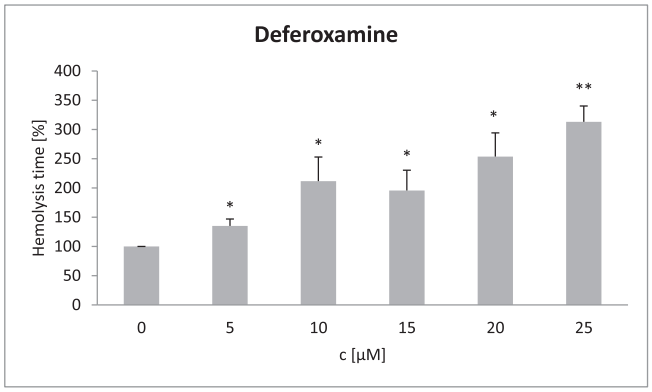
In flavonoids, the number of hydroxyl group in the A ring seems to determine the reactivity in the FRAP assay, the presence of the second hydroxyl group being again a prerequisite for reactivity. Naringin and naringenin, having one hydroxyl group in the A ring, show negligible reactivity, like hesperidin, which has one hydroxyl group and one glycosylated hydroxyl group. (+)-Catechin, (−)-epicatechin, (−)-epigallocatechin, morin and rutin, having two hydroxyl groups in the A











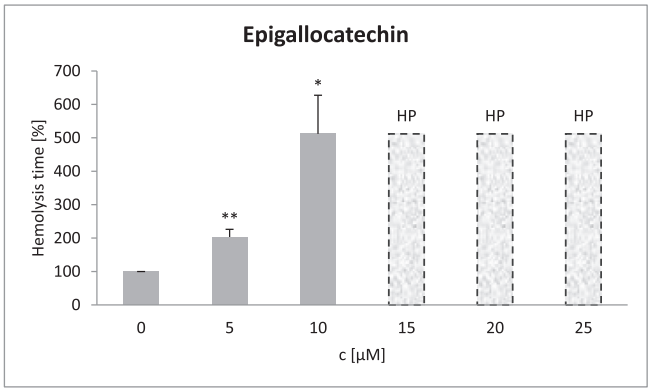


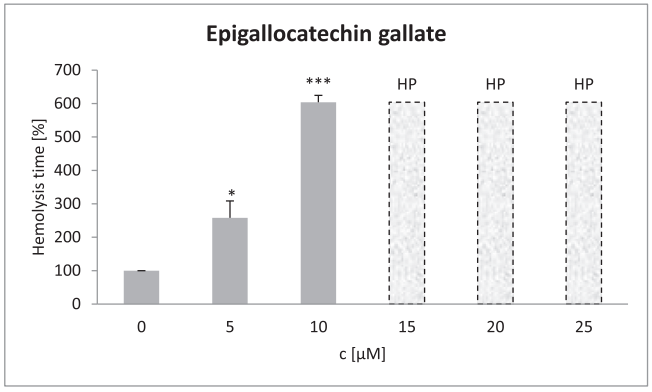
Fig. 2. (*continued*)

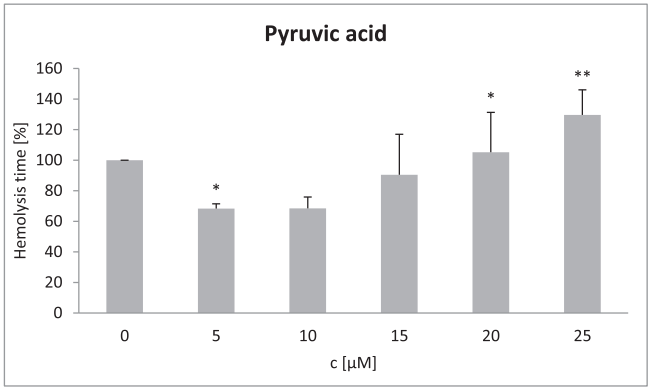
Fig. 2. (*continued*)

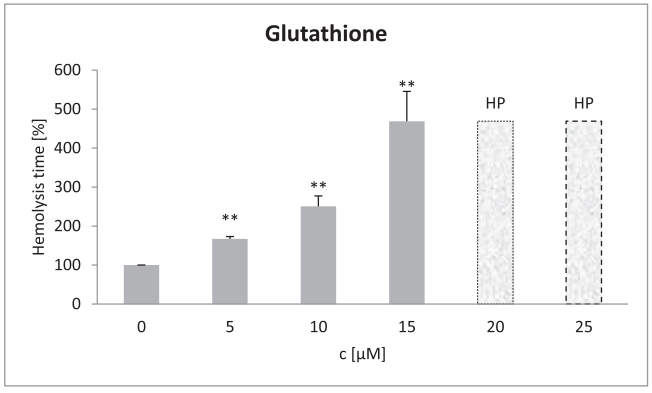
ring, have a reactivity of about 1 mol Trolox equivalents/mol. These data suggest that Fe^{3+} binding by *ortho*-hydroxyl groups in the A ring is necessary for iron reduction. The number of hydroxyl groups in the B ring does not seem to affect the flavonoid reactivity as it can be inferred, i.a., from the lack of significant difference between the reactivity of (−)-epigallocatechin (three hydroxyl groups in the B ring) and other flavonoids mentioned (two hydroxyl groups in the B ring). In flavonoid esters, hydroxyl groups of the gallic acid seem to account for the increased FRAP reactivity. Again, the $ABTS^{\cdot}$ reactivity of flavonoids is more difficult to explain. $ABTS^{\cdot}$ is a promiscuous radical in its reactivity and it could be expected that each hydroxyl group of flavonoids is able to scavenge $ABTS^{\cdot}$. However, (−)-epicatechin gallate, which has a total of 7 hydroxyl groups shows $ABTS^{\cdot}$ reactivity of about 8 mol Trolox equivalents/mol, higher than (−)- epigallocatechin gallate, which has a total of 8 hydroxyl groups. (+)-Catechin, having two hydroxyl groups in the A ring and two hydroxyl groups in the B ring shows higher reactivity, (ca 4 mol Trolox equivalents/mol) than (−)-epicatechin having the same number of hydroxyl groups and (−)-epigallocatechin having two hydroxyl groups in the A ring and three hydroxyl groups in the B ring (ca 3 mol Trolox equivalents/mol). An explanation may lie in the complex reaction of catechins with $ABTS^{\cdot}$. It has been demonstrated that during this reaction covalent adducts are formed, which further scavenge $ABTS^{\cdot}$ (Osman, Wong, Hill, & Fernyhough, 2006). These reactions are not completed within one minute (the time period of our measurement), what further complicates the comparisons. In line with these findings, Sang et al. (2003) characterized the reaction products of (−)-epicatechin with $ROO^{\cdot}$ generated by thermolysis of the azo initiator azo-*bis*-isobutyronitrile. Eight reaction products were isolated and identified using high-field 1D and 2D NMR spectral analysis. This study demonstrated that the B-ring is the initial site for formation of reaction products in the peroxyl radical oxidant system.

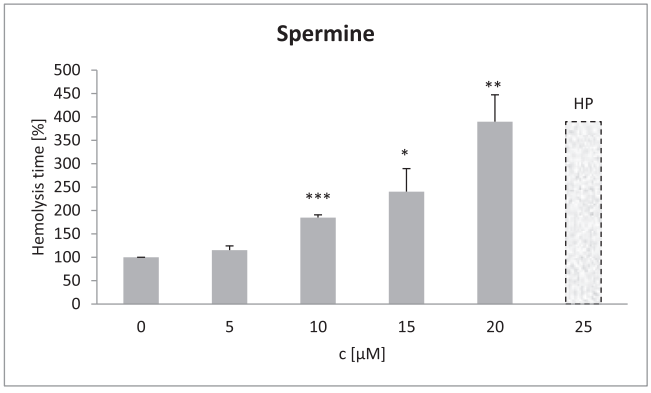
Reactivity of catechins in the FRAP assay confirms their ability to reduce metal ions reported previously (Lambert & Elias, 2010; Nagle, Ferreira, & Zhou, 2006). However, our study demonstrates that these compounds (like other natural compounds studied) are unable to bind ferrous ions strongly enough to prevent their participation in the Fenton reaction. It explains the prooxidant behavior of catechins mediated by metal ions (Lambert & Elias, 2010).

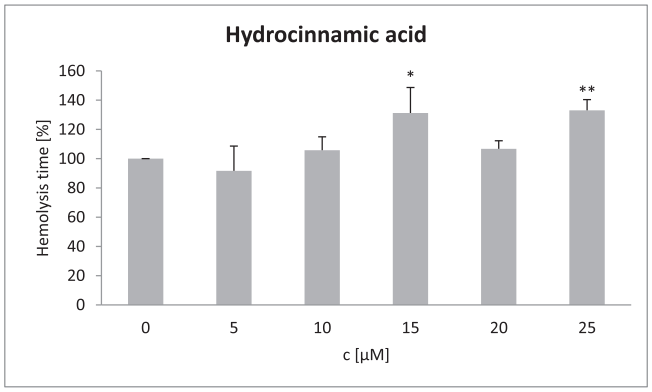
In most cases, catechins were the most effective among the compounds studies, as well as more effective than the standard antioxidant GSH, ascorbate and synthetic antioxidants tested. They inhibited the SIN-1 induced oxidation of DHR123, and fluorescein bleaching induced











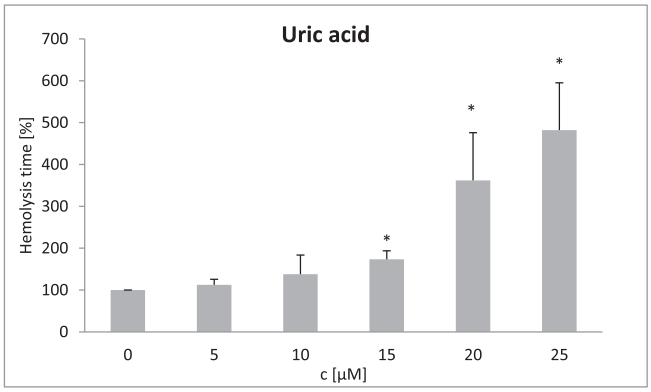


Fig. 2. (*continued*)

Fig. 2. (*continued*)

by AAPH and hypochlorite at concentrations of about 1 μM, or lower, i.e. at concentrations attainable *in vivo*. The same concentrations of some catechins were able to ameliorate hemolysis induced by AAPH and hypochlorite and diminish intracellular free radical reactions. Thus, catechins are versatile antioxidants able to protect against various oxidants formed *in vivo*, which may underlie their health-promoting effects. Martínez, Ugartondo, Vinardell, Torres, and Mitjans (2012) reported that (−)-epicatechin and its conjugates spread out in the core of the bilayer, but might also interact with its external part. They stabilize the membrane through a decrease in lipid fluidity, blocking the access of the $ROO^{\cdot}$ to the interior of RBCs membranes, which may contribute to their ability to inhibit oxidative hemolysis.

The studies presented herein demonstrated also that (−)-epicatechin gallate and (−)-epigallocatechin gallate exerted the most significant cellular protective activity against intracellular H_2DCF-DA oxidation as well as hemolysis induced by hypochlorite.

(−)-Epicatechin gallate and (−)-epigallocatechin gallate have high octanol/water partition coefficients, i.e., 23.9 and 16.0, respectively, as compared to 0.5 for (−)-epigallocatechin, suggesting mainly membrane localization of the esters in erythrocytes. The pK_a value of (−)-epigallocatechin gallate is estimated to be at 7.59 and that of (−)-epicatechin gallate at 7.74 (Kumamoto, Sonda, Nagayama, & Tabata, 2001), which suggests that their cellular absorption is, most probably, in the undissociated, nonionized form by diffusion through the phospholipid bilayer of the plasma membrane. Yet, surface receptors have been identified for (−)-epigallocatechin gallate and (−)-epicatechin gallate (Babich, Krupka, Nissim, & Zuckerbraun, 2005).

Red blood cells are considered a prime target for free radical attack due to their oxygen transport, which is a potent promoter of ROS and the presence of high contents of polyunsaturated fatty acid in their membrane. Exposure of RBCs to oxidative conditions results in successive free radical-mediated reactions that ultimately lead to cell lysis. Among the studied antioxidants dissolved in PBS, (+)-catechin was the most potent protector against AAPH ($ROO^{\cdot}$) damage, followed by (−)-epicatechin, caffeic acid, deferoxamine, (−)-epicatechin gallate, (−)-epigallocatechin gallate, chlorogenic acid, ascorbic acid and uric acid.

Several facets of interaction of antioxidants with erythrocytes, and most probably their resultant action, seem to be critical for their antioxidant effects in these cells: penetration into erythrocytes, partition into the erythrocyte membrane and interaction with the membrane

Table 4
The inhibitory effect of selected antioxidants on AAPH-induced ROS production in erythrocytes. IC_{50} values compared to glutathione: [a] $P < 0.001$; [b] $P < 0.01$ (Dunnett test).

Compound	IC_{50} [μM]
Standard antioxidants	
Ascorbic acid	0.40 ± 0.00[a]
Glutathione	19.15 ± 1.28
Catechins	
(+)-Catechin	4.95 ± 0.25[b]
(−)- Epicatechin	12.02 ± 1.29
(−)-Epicatechin gallate	0.36 ± 0.06[a]
(−)-Epigallocatechin	0.41 ± 0.11[a]
(−)-Epigallocatechin gallate	0.19 ± 0.07[a]
Hydroxycinnamic acids and derivatives	
Caffeic acid	0.50 ± 0.08[a]
Chlorogenic acid	0.37 ± 0.10[a]
Hydrocinnamic acid	45.00 ± 1.48[a]
Other natural antioxidants	
Carnosine*	19.47 ± 1.80
Deferoxamine	80.53 ± 8.13[a]
Pyruvic acid***	74.16 ± 3.16[a]
Spermine**	106.39 ± 4.85[a]
Uric acid	52.92 ± 2.11[a]

* CID: 62881;
** CID: 1103;
*** CID: 1060.

surface.

Most antioxidants, including flavonoids (Fiorani & Accorsi, 2005), are able to diffuse into erythrocytes or be transported by appropriate transport systems.

In a comparative study concerning catechins, (−)-epicatechin gallate showed the highest interaction with the lipid bilayer, followed by (−)-epigallocatechin gallate, (−)-epicatechin and (−)-epigallocatechin as reflected by their hydrophobicity (partition coefficient in the system of *n*-octanol/PBS), incorporation into lipid bilayer of liposomes and quenching of 2-anthroyloxystearic acid (Nakayama, Hashimoto, Kajiya, & Kumazawa, 2000). However, the sequence of protective efficiency against hemolysis induced by AAPH and hypochlorite (Figs. 1 and 2) does not correlate with the above sequence.

Another factor may consists in the localization of antioxidants in the membrane. It has been proposed that the high antioxidant capacity of some galloylated catechins such as (−)-epicatechin gallate could be partially due to the formation of membrane structures showing resistance to detergent solubilization and in which the phospholipids have tightly packed acyl chains and highly hydrated phosphate groups (Caturla, Vera-Samper, Villalaín, Mateo, & Micol, 2003). Interaction of antioxidants with the membrane surface protects the membrane from attack by oxidants present in the aqueous phase while interaction with both the membrane surface and hydrophobic interior protects the membrane from hydrophilic and hydrophobic oxidants. (−)-Epigallocatechin gallate, e. g., was demonstrated to interact both with the hydrophobic and hydrophilic regions of lipid bilayers (Oteiza, Erlejman, Verstraeten, Keen, & Fraga, 2005).

(−)-Epicatechin gallate was found to be the most effective compound in protection against fluorescein bleaching induced by NaOCl and in inhibiting AAPH-induced ROS formation in RBCs. In other cell-free systems (protection against DHR123 oxidation and protection against fluorescein bleaching by AAPH), another compound, the stilbene resveratrol (*trans*-3,5,4′-trihydroxystilbene), present e.g. in red wine showed the highest protection. Moderate consumption of red wine reduces the risk of heart disease and extends lifespan, but the relative contribution of wine polyphenols to these effects is still unclear.

More recent studies suggested to use of peanut sprouts as a functional food (Wang et al., 2017). However, our results showed that catechins are more potent against oxidative stress-induced RBCs hemolysis to compare with caffeic acid, which is greatly enriched in peanut sprout. In our opinion only comparative analysis of antioxidants and also, employment of more than one test method specific to a radical species, gives a better estimate of antioxidant potential of a tested compound.

Apart from the excellent antioxidant properties of catechins and other flavonoids, they may show synergistic interaction with endogenous antioxidants and act as indirect antioxidants. Pereira, Sousa, Costa, Andrade, and Valentão (2013) reported that flavonoid showed synergistic interaction in the $DPPH^{\cdot}$ scavenging assay; the presence of a catechol group in the B ring was demonstrated to be essential for synergisms with GSH, except when an OH group at C6 is also present. Moreover, adducts formed at C2′ and C5′ of the B ring seemed to be more important for the antioxidant capacity than adducts formed at C6 and C8 of the A ring.

In addition to their direct antioxidant activity, polyphenols exhibit indirect antioxidant action, consisting in induction of synthesis of endogenous antioxidants and antioxidant enzymes, and inhibition of biosynthesis of prooxidant proteins. Basu et al. (2013) reported that green tea beverage and green tea extract significantly increased plasma antioxidant capacity (from 1.5 mmol/l to 2.3 mmol/l and from 1.2 mmol/l to 2.5 mmol/l respectively) and whole blood glutathione (from 1783 to 2395 μg/g hemoglobin and from1905 to 2751 μg/g hemoglobin, respectively) versus controls at 8 weeks. Catechin intake has been also reported to increase the activity of glutathione *S*-transferase and decrease the activities of prooxidant enzymes such as xanthine oxidase or nitric oxide synthase (Butt et al., 2014). More recently, Yokotani and Umegaki (2017) reported that the administration of (−)-epigallocatechin gallate (500 mg/kg) to rats increased plasma (−)-epigallocatechin gallate (4 μmol/l as free form) and ascorbic acid levels (1.7-fold), as well as oxygen radical absorbance capacity (1.2-fold) and FRAP (3-fold) values.

It should be noted that (+)-catechin, (−)-epicatechin and (−)-epigallocatechin were classified as the least toxic for normal cells (HGF-2 fibroblasts cells from the human oral cavity; IC_{50} values > 500 μM)) to compare with (−)-epigallocatechin gallate (moderately toxic, IC ≈ 250 μM) or (−)-epicatechin gallate and catechin gallate (highly toxic, IC ≥ 100 μM) (Babich et al., 2005). However, such high concentrations of catechins are not attainable *in vivo* and significant antioxidant effects were observed at much lower concentrations in this study. (−)-Epigallocatechin gallate has been reported to exert selective toxicity to tumor cells but not to normal epithelial cells (Hsu & Liou, 2011). It was found that normal but not malignant cells expresses large amount to (−)-epigallocatechin gallate-binding protein called “Fas-like decoy proteins” which decreases the concentration of free (−)-epigallocatechin gallate, resulting in resistance to apoptosis (Suzuki, Miyoshi, & Isemura, 2012). By this mechanism, green tea catechins block carcinoma and help in modulating signal transduction pathways pertaining to cell proliferation, transformation, inflammation, and metastasis (Butt, Ahmad, Sultan, Qayyum, & Naz, 2015).

Rosenkranz et al. (2002) suggested catechin-mediated inhibition of the platelet-derived growth factor beta receptor signaling, which plays a critical role in the pathogenesis of atherosclerosis; it offers another molecular explanation for the “French paradox”.

Intestinal absorption of catechins in humans depends on their properties and is higher for catechins of lower molecular weight (Kanwar et al., 2012). The ability of catechins to cross the blood-brain barrier rose interest in using their antioxidant properties of polyphenols to prevent and treat neurodegenerative diseases (Mandel, Amit, Reznichenko, Weinreb, & Youdim, 2006). Recent evidence reveals that catechins may be a key mediator in cardiovascular health via mechanisms of blood pressure reduction, flow-mediated vasodilation and atherosclerosis attenuation (Mangels & Mohler, 2017). Japanese and Chinese people who often have the habit of drinking tea have a very low incidence of coronary heart disease.

The low bioavailability of flavonoids is the main problem in their prophylactic and pharmaceutical use. However, contemporary nanotechnology may overcome this restriction, by production of flavonoid-containing nanoparticles (Maity, Mukhopadhyay, Kundu, & Chakraborti, 2017). The versatile antioxidant properties of catechins make them ideal candidates for formulation of nanoparticles devoted to efficient antioxidant supplementation, especially in cases requiring antioxidant participation in therapy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.08.117.

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