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Comparative study of eight well-known polyphenolic antioxidants

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Abstract

Eight antioxidants from five different polyphenolic classes (cinnamic acids, benzoic acids, flavonoids, proanthocyanidins and stilbenes), and the water-soluble vitamin E derivative trolox were examined for their antioxidant activity in-vitro. In addition, the compounds were tested for their cytotoxicity on growing fibroblasts and their inhibition of the classical pathway of the complement system. Procyanidin C1 was shown to be a good scavenger of both DPPH$^{\bullet}$ and HO$^{\bullet}$, and a strong inhibitor of lipid peroxidation and the classical pathway of the complement system. Consequently, procyanidin C1 was classified as the most promising antioxidant in-vitro of all compounds tested. In contrast, genistein exhibited a very low antioxidant activity in both the lipid peroxidation and the DPPH$^{\bullet}$ scavenging assay, a high cytotoxicity and a low complement-inhibiting activity.

Introduction

Reactive oxygen species (ROS) are widely believed to be involved in the aetiology of many diseases, as indicated by the signs of oxidative stress seen in those diseases. Consequently, antioxidants could play an important protective role in those diseases. This hypothesis is supported by the strong inverse relationship between the intake of fruit and vegetables and the risk of cancer (Hertog et al 1996; Steinmetz & Potter 1996; Lampe 1999) and coronary heart diseases (Diplock et al 1998; Law & Morris 1998). Food and beverages contain a wide range of antioxidants, many of which are polyphenolic compounds that have antioxidant activity in-vitro. Nevertheless, it must be emphasized that several of these antioxidants, including flavonoids, exhibit a wide range of biological activity, some of which could be beneficial or detrimental depending on specific circumstances (Ross & Kasum 2002). Consequently, further studies both in the laboratory and with populations are warranted.

Polyphenolic compounds constitute one of the most numerous and widely distributed secondary plant metabolite groups (Bravo 1998). They arise biogenetically from two main synthetic pathways (the shikimate pathway and the acetate pathway), and produce thereby a large variety of classes of polyphenolics, such as cinnamic acids (C_6-C_3), benzoic acids (C_6-C_1), flavonoids (C_6-C_3-C_6), proanthocyanidins ((C_6-C_3-C_6)$_n$), stilbenes (C_6-C_2-C_6) and coumarins (C_6-C_3). Most studies on the antioxidant activity of polyphenolics have examined only a limited number of compounds originating from one or two different classes and tested in one or two different antioxidant assays (Cos et al 1998; Choi et al 2002; Heijnen et al 2002). Therefore, in this study eight well-known antioxidants were selected from five different classes and were subsequently examined for their antioxidant activity in-vitro. Since a compound may not be classified as an antioxidant on the basis of a single antioxidant experiment, the compounds were tested in three antioxidant assays: the DPPH$^{\bullet}$ scavenging and HO$^{\bullet}$ scavenging assays and the microsomal lipid peroxidation assay. To evaluate their safety and antioxidant activity profile, the promising antioxidants were further tested for their cytotoxicity on growing fibroblasts.

Additionally, the compounds were also evaluated for their complement inhibiting properties. Complement is a defensive system consisting of serum proteins that

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participate in lysis of foreign cells, inflammation and phagocytosis. It can be activated by two major pathways – in the classical pathway by an immune reaction or in the alternative pathway by some polysaccharides, lipopolysaccharides, immune-complexes or aggregated immunoglobulins of classes (e.g. IgA) that do not usually activate the classical sequence. Complement activation promotes phagocytosis, which can lead to inflammation and an increased level of ROS. In addition, both the complement system and ROS play an important role in several diseases, such as Alzheimer's disease (Markesbery & Carney 1999) and ischaemia–reperfusion injury (Collard et al 1998, 2000). For example, Collard and coworkers demonstrated that re-oxygenation of hypoxic human umbilical vein endothelial cells (HUVECs) activated the classical complement pathway and augmented iC3b deposition on the endothelial cells (Collard et al 1998). They suggested that the iC3b deposition on the vascular endothelium may be regulated by intracellular ROS-induced activation of NF-kappa B (Collard et al 2000). Despite the strong relationship between complement-inhibiting and antioxidant activity, antioxidants are hardly evaluated for their complement-inhibiting activity. Therefore, in this study the promising antioxidants are tested for their inhibition of the classical pathway of the complement system.

Material and Methods

Materials

RPMI 1640 with L-glutamine, fetal bovine serum and trypsin-EDTA solution were obtained from Life Technologies. Caffeic acid, (+)-catechin, diethylenetriaminepentaacetic acid (DETAPAC), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH$^{\bullet}$), ferrous sulfate, gallic acid, genistein, hydrogen peroxide, kaempferol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), quercetin, resveratrol, sodium dodecyl sulfate, 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid and trolox were purchased from Sigma (USA). Ascorbic acid, Coomassie blue G250, and trichloroacetic acid were obtained from Merck (Germany). Procyanidin C1 was isolated and identified as described previously (Shahat et al 1996).

The purity of kaempferol was 94.3%, while the purity of the other commercial test compounds was at least 98% (as reported by Sigma-Aldrich). The purity of procyanidin C1 was evaluated by HPLC and was minimum 96%.

Cell cultures

Human skin fibroblasts from normal donors were obtained from the Laboratory of Medical Genetics, University of Antwerp, Antwerp, Belgium. Fibroblasts were seeded into culture flasks with growth medium (RPMI1640 medium supplemented with 15% fetal bovine serum). Every ten days, cell lines were passaged using trypsin-EDTA to detach the cells. The fourth or fifth passage was used for the experiments.

Preparation of test solutions

Test solutions were freshly prepared by dissolving test compounds in DMSO, followed by dilution with growth medium (i.e. RPMI1640 medium supplemented with 15% fetal bovine serum). For each compound tested, at least six different test solutions were used from the following dilution series: 200, 180, 160, 140, 120, 100, 80, 60, 40, 30, 20, 15, 10, 5 and 1 μg mL^{-1}.

Cytotoxicity test

Fibroblasts were seeded into 96-well plates at a density of 10^4 cells per well in growth medium. The plates were incubated during 24 h at 37 °C under a humidified atmosphere containing 5% CO_2. Then the medium was discarded and test solutions were added. Four wells were used for each concentration and the last two rows of each plate for appropriate cell controls. After 72 h incubation at 37 °C the medium was removed and 20 μL of MTT solution (5 mg MTT dissolved in 1 mL phosphate-buffered saline (PBS)) was added to each well. Four hours at 37 °C later the formazan product was solubilized by the addition of 200 μL 10% (w/v) SDS in acidified DMSO (600 μL acetic acid per 100 mL solvent). The optical density of each well was measured using an automatic plate reader (Multiscan MCC/340) with a test wavelength of 570 nm and a reference wavelength of 690 nm. The absorbance is directly proportional to the number of living cells. The cytotoxicity of each test compound was expressed as an ID50 value (the concentration in μM that inhibits cell growth by 50% compared with cell controls, calculated by linear regression analysis). The maximal non-toxic dose (MNTD) was defined as the highest concentration of the test compound that did not affect cell growth comparable with control cells.

Microsomal lipid peroxidation

The lipid peroxidation inhibiting activity was determined as described previously (Cos et al 2001). Briefly, reaction mixtures contained 80 μM potassium phosphate buffer (pH = 7.4), microsomes (350–500 μg protein mL^{-1}) and test products in various concentrations. Peroxidation was started by adding vitamin C at a final concentration of 200 μM. The reaction mixture was incubated in an open Eppendorf tube for 90 min at 37 °C. The reactions were terminated by addition of 250 μL of a 20% trichloroacetic acid solution followed by centrifugation at 10 000 *g* for 3 min. The supernatant (600 μL) was boiled with 250 μL of a 0.67% thiobarbituric acid solution for 20 min. At room temperature, the amount of lipid peroxidation was determined by measuring the absorbance of the pink chromogen at 535 nm.

The antioxidant activity of each compound was expressed as IC50 value (the concentration in μM able to inhibit 50% of the thiobarbituric acid reactive substances

(TBARS), calculated from the corresponding log-dose inhibition curve). Appropriate sample blanks were taken into account to calculate the IC50 value. The test was conducted in triplicate and the data were collected as mean ± s.d. Different concentrations of quercetin were measured in multiplicate on separate days and the reproducibility was found to be 7%. The water-soluble vitamin E derivative trolox was used as a positive control in the microsomal lipid peroxidation assay. The IC50 value of trolox was found to be 2.8 ± 0.14 μM.

DPPH

The stable free radical DPPH• was dissolved in ethanol to give a 100 μM solution; 0.5 mL of a test compound in ethanol (or ethanol itself as control) was added to 3.0 mL of the ethanolic DPPH• solution. For each test compound, different concentrations were tested. The mixtures were shaken vigorously and left to stand in the dark for 20 min. The decrease in DPPH• absorption was measured at 517 nm and the actual decrease in absorption induced by the test compound was calculated by subtracting that of the control.

The test was conducted in triplicate and the antioxidant activity of each test compound was expressed as an IC50 value ± s.d. (i.e. the concentration in μM that inhibits DPPH• absorption by 50%, calculated by linear regression analysis). Different concentrations of quercetin were measured in multiplicate on separate days and the reproducibility was found to be 8%. Trolox was used as a positive control and its IC50 value was found to be 24.0 ± 1.5 μM.

Hydroxyl radical scavenging activity

The HO• scavenging activity was measured according to the method described by Taira et al (1992) and further adapted in our laboratory (Cos et al 2002). Hydroxyl radicals were generated in a Fenton-type reaction and were visualized by DMPO in an electron paramagnetic resonance (EPR) instrument. The spin trap DMPO was checked for radical and coloured impurities. No EPR signal was detected and a single absorption peak of DMPO at 227 nm was measured with a UV spectrophotometer. The final reaction mixture contained the following reagents (mM): 10 DMPO, 0.1 ferrous sulfate, 0.1 DETAPAC, 50 phosphate buffer pH = 7.4 and test compounds in various concentrations. The reaction mixture was transferred to a quartz flat cell with a volume of 200 μL. After 2 min the EPR spectrum was recorded at room temperature using a Magnettech miniscope MS100 instrument (Magnettech, Berlin, Germany). Scan conditions were as follows: microwave frequency 9.4 GHz; microwave power 10 mW; modulation frequency 100 kHz; modulation amplitude 0.1 mT; scan time 42 s; center field 336.6 mT and sweep width 6 mT.

Different concentrations of a test compound were analysed and then the half-maximal inhibitory concentration (IC50) was calculated by linear regression analysis. The test was conducted in quadruplicate and the data were collected as mean ± s.d.

Anticomplement activity

The anticomplement activity on the classical pathway was measured in a microassay according to the procedure developed by Klerx et al (1983) and adapted in our laboratory (Huang et al 1998). Human pooled serum (HPS) from healthy subjects was used as source of complement.

The assay was performed in U-well microtiter plates (Falcon 3077). All samples were diluted in the microtiter plate (7 consecutive logarithmic dilutions) with the appropriate buffer, resulting in a final volume in each well of 50 μL. Subsequently, 50 μL of a CP-HPS (classical pathway, CP) was added to each well. After incubating for 30 min at 37 °C, 50 μL of a suspension of sensitized sheep erythrocytes was added and the plates were then incubated for 60 min at 37 °C. Subsequently, the plates were centrifuged at 800 *g* for 6 min. To quantify haemolysis, 50 μL of the supernatant was mixed with 200 μL water in a flat-bottom microtiter plate (Falcon 3072) and the absorbance at 414 nm was measured in an automatic plate reader (Multiscan MCC/340). Controls in this assay consisted of erythrocytes incubated in demineralized water (100% haemolysis), erythrocytes incubated in buffer (0% haemolysis), erythrocytes incubated with sample (colour and toxicity control), erythrocytes incubated in buffer and HPS (50% haemolysis control) and the colour of HPS-dilution (complement blank). The HPS-dilution used in the classical standard assay gave rise to approximately 50% hemolysis.

Inhibitory activity was evaluated by comparing the concentrations (w/v) inducing 50% inhibition of hemolysis (IC50 value). The test was conducted in quadruplicate and the data were collected as mean ± s.d. Dextran sulfate was used as a positive control and its IC50 value was found to be 19.2 ± 5.4 nM.

Statistical analysis

The number of replicates was increased to six for those compounds of which the statistical difference between their IC50 values had to be tested. A one-way analysis of variance with a Tukey's post-hoc test was used.

Results and Discussion

A number of well-known antioxidants were selected from five different polyphenolic classes – hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, proanthocyanidins and stilbenes – and were evaluated for their antioxidant and complement-inhibiting properties and their cytotoxicity. The water-soluble vitamin E derivative trolox was included as standard antioxidant.

The polyphenolic antioxidants were selected because of their reported antioxidant or health-promoting properties. Flavonoids are the most abundant polyphenolics in our diet and can be classified into several groups according to the oxidation level of their central C-ring. Since most studies conclude that both a catechol or pyrogallol moiety in the B-ring, a free 3-OH group, and a C2-C3 double bond are essential for a high radical scavenging activity, the flavonol

quercetin was added to our test compounds (Rice-Evans et al 1996; Cos et al 2000). Two other interesting flavonoids are the flavonol kaempferol and the flavanol (+)-catechin that are abundant in *Ginkgo biloba* L. and *Camellia sinensis* L. (tea), respectively. Both plants are well-known for their antioxidant and health-promoting properties (Rice-Evans 2001). In addition, a high consumption of soy, which contains a high amount of the isoflavonic phytoestrogens genistein and daidzein, may protect against breast cancer and osteoporosis (Adlercreutz & Mazur 1997). Similarly, several scientists are claiming that proanthocyanidins or the stilbene derivative resveratrol are the wine components responsible for the French Paradox (i.e., the low incidence of heart diseases in some parts of France, in spite of a relative high-fat diet) (Renaud & De Lorgeril 1992; Soleas et al 1997). Proanthocyanidins are oligo- or polymeric flavanols with a molecular weight between 500 and 20 000, while resveratrol, or 3,5,4′-trihydroxystilbene, is a naturally occurring phytoalexin which has been reported to exert cancer chemoprotective (Jang et al 1997) and antioxidant properties (Tadolini et al 2000).

From the classes of hydroxybenzoic and hydroxycinnamic acids, gallic acid and caffeic acid, respectively, were tested. Esterified gallic acid is found in many plants in the form of gallotannins and ellagitannins, while caffeic acid is present at high levels in coffee as an ester of quinic acid (chlorogenic acid). These compounds were evaluated for their antioxidant activity in three different assays — the DPPH$^{\bullet}$ scavenging, HO$^{\bullet}$ scavenging and microsomal lipid peroxidation assays. In all three antioxidant assays, the results were expressed as half-maximal inhibitory concentrations (IC50 values) and are listed in Tables 1 and 2. DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical that has a strong absorption band in ethanol at 517 nm, which disappears upon reduction by an antioxidant. The DPPH$^{\bullet}$ assay is an excellent in-vitro method to investigate the free radical scavenging activity of an antioxidant (i.e., the donation of a hydrogen atom to a free radical species). In contrast to DPPH$^{\bullet}$, HO$^{\bullet}$ are highly reactive radicals that react at the site of formation. HO$^{\bullet}$ were generated in a Fenton-type reaction and were visualized by the spin-trap DMPO in an EPR instrument. The EPR signal of the DMPO-OH$^{\bullet}$ adduct is inhibited by the presence of HO$^{\bullet}$ scavengers, which compete with DMPO for HO$^{\bullet}$. Since DMPO is present in the reaction mixture at a final concentration of 10 mM, the IC50 values of the HO$^{\bullet}$ scavenging assay are much higher than those of the DPPH assay.

Of all the compounds tested, procyanidin C1 showed the highest DPPH$^{\bullet}$ scavenging activity, while procyanidin C1 and gallic acid were the most active HO$^{\bullet}$ scavengers. The DPPH$^{\bullet}$ scavenging activity of the flavonoids tested decreased in the following order: quercetin > (+)-catechin > kaempferol. The IC50 values of these compounds were significantly different ($P < 0.0001$). The isoflavone genistein showed no DPPH$^{\bullet}$ activity even at concentrations up to 100 μM. These results are in agreement with the generally accepted structure–radical scavenging activity relationship of flavonoids (Rice-Evans et al 1996; Cos et al 2000). Interestingly, the IC50 value of trolox for DPPH$^{\bullet}$ scavenging activity was about four times lower than that of resveratrol, while the IC50 value of resveratrol for HO$^{\bullet}$ scavenging activity was about two times lower than that of trolox. These results indicate once again the importance of testing antioxidants in different assays.

The compounds were also tested in the microsomal lipid peroxidation assay, which is one of the most biomimetic antioxidant tests. All compounds tested showed a

Table 1 Lipid peroxidation and cytotoxic effects of some selected phenolic compounds.

Compound	Cytotoxicity		Lipid peroxidation IC50 (μM)	ASI[a]
	ID50 (μM)	MNTD (μM)		
Hydroxybenzoic acid				
Gallic acid	31	6	1.51 ± 0.06	4
Hydroxycinnamic acid				
Caffeic acid	268	56	3.29 ± 0.08	17
Flavanol				
(+)-Catechin	> 344	> 344	3.55 ± 0.02	> 96
Flavonols				
Kaempferol	260	140	0.40 ± 0.02	350
Quercetin	90	30	0.95 ± 0.04	32
Isoflavonoid				
Genistein	160	37	12.36 ± 0.04	3
Proanthocyanidin				
Procyanidin C1	> 231	58	0.80 ± 0.03	73
Stilbene				
Resveratrol	109	44	0.86 ± 0.13	51
Vitamin E derivative				
Trolox	> 400	> 400	2.80 ± 0.14	> 143

Lipid peroxidation data are means ± s.d. [a]ASI, antioxidant selectivity index = MNTD/IC50.

Table 2 Complement inhibitory, DPPH$^{\bullet}$ and HO$^{\bullet}$ scavenging activity of some selected phenolic compounds.

Compound	Complement (CP) IC50 (μM)	DPPH$^{\bullet}$ scavenging activity IC50 (μM)	HO$^{\bullet}$ scavenging activity IC50 (μM)
Hydroxybenzoic acid			
Gallic acid	2000[a]	9.4 ± 0.4	191 ± 22
Hydroxycinnamic acid			
Caffeic acid	> 1000	19.8 ± 0.5	572 ± 19
Flavanol			
(+)-Catechin	684.1 ± 46.2	14.3 ± 0.1	693 ± 27
Flavonols			
Kaempferol	557.1 ± 105.9	27.1 ± 0.2	—
Quercetin	52.6 ± 13.2	9.7 ± 0.8	391 ± 11
Isoflavonoid			
Genistein	226.6 ± 3.4	> 100	—
Proanthocyanidin			
Procyanidin C1	6.0 ± 0.2	5.6 ± 0.2	< 250
Stilbene			
Resveratrol	229.8 ± 14.9	93.1 ± 2.9	584 ± 47
Vitamin E derivative			
Trolox	> 1000	24.0 ± 1.5	1243 ± 59

Data are means ± s.d. [a]From Kroes et al (1992).

lipid peroxidation-inhibiting activity, but the IC50 value of genistein (12.4 μM) was about 30 times that of kaempferol (0.4 μM). However, since the plasma concentration of a polyphenolic compound rarely exceeds 1 μM after the consumption of 10–100 mg of a single polyphenolic compound (Scalbert & Williamson 2000), genistein should not be classified as a lipid peroxidation-inhibiting compound. The most interesting antioxidants, with IC50 values lower than 1 μM, are kaempferol, procyanidin C1, quercetin and resveratrol. These results clearly indicate that the microsomal lipid peroxidation-inhibiting activity of polyphenolic compounds is not only related to their radical scavenging activity, but also to other parameters, such as iron-chelating ability and lipophilicity. For example, resveratrol was not a good DPPH$^{\bullet}$ and HO$^{\bullet}$ scavenger, but was a strong inhibitor of lipid peroxidation. According to Tadolini and coworkers, resveratrol inhibits lipid peroxidation mainly by scavenging LOO$^{\bullet}$ within the membrane and its capacity of spontaneously entering the lipid environment may allow resveratrol to exert a significant antioxidant activity in-vivo (Tadolini et al 2000). It is also important to consider that microsomes contain several antioxidants, including the chain-breaking antioxidant α-tocopherol. It has been demonstrated that flavonoids, such as quercetin and tea catechins, can regenerate α-tocopherol from the α-tocopherol radical (Pietta 2000).

It can be concluded that inhibition of lipid peroxidation should be one of the first tests to determine the antioxidant activity of a potential antioxidant compound and should, in our opinion, always be tested before classifying a compound as an antioxidant. However, other properties, such as absorption, metabolism and cytotoxicity, must also be studied before antioxidants can be applied to man. The cytotoxicity of a compound can be determined by measuring cytotoxic parameters, such as the integrity of the cell and the effect on cell growth. Since the latter is a more sensitive cytotoxic parameter and more appropriate for pharmacological work, the cytotoxicity of the phenolic compounds was investigated on growing fibroblasts using an MTT assay. The cytotoxicity was expressed as an ID50 value (i.e., the concentration of the compound in μM that inhibits cell growth by 50% compared with cell controls). The maximal non-toxic dose (MNTD) was defined as the highest concentration of the compound that did not affect cell growth compared with cell controls. A promising antioxidant should show a lipid peroxidation-inhibiting effect at micromolar level and a low cytotoxicity. Therefore, we have introduced previously an antioxidant selectivity index, ASI (the maximal non-toxic dose divided by the IC50 value for lipid peroxidation) (Cos et al 2001). Compounds with an ASI higher than 100 would have a good safety/antioxidant activity profile.

At the highest concentration tested (200 μg mL^{-1}), the standard antioxidant trolox and the flavanol (+)-catechin did not show any cytotoxic effect on growing fibroblasts and exhibited an ASI of higher than 143 and higher than 96, respectively. However, the most interesting compound was kaempferol with an ASI of 341; this is about ten times that of quercetin and about 100 times that of gallic acid and genistein. The latter compound, which exhibited a very low antioxidant activity in both the lipid peroxidation and the DPPH$^{\bullet}$ scavenging assay, showed a high cytotoxicity and a low ASI. Genistein can therefore not be classified as an interesting antioxidant in-vitro, but it should also be emphasized that genistein exhibits an interesting phytoestrogenic activity (Wiseman 2000). In contrast, procyanidin C1 showed a very high activity in all three antioxidant assays and exhibited an ASI of 73, which prompted us to classify procyanidin C1 as a promising antioxidant. Kaempferol showed a high lipid peroxidation-inhibiti ng and a low scavenging activity, but regarding its ASI of 350, kaempferol can be a very useful antioxidant.

Besides the three antioxidant assays, the compounds were also tested for their inhibition of the classical pathway of the complement system. Quercetin exhibited an IC50 value of 52.6 μM, while procyanidin C1 was the most active inhibitor of the complement system with an IC50 value of 6 μM. None of the other compounds tested were active at concentrations lower than 100 μM. Although complement is certainly beneficial, if not essential, for the host's defence against foreign invading organisms, its activation may evoke pathological reactions in a variety of diseases, such as autoimmune diseases, rheumatoid arthritis and gout. In these diseases, there is also an overproduction of ROS that contribute to the disease pathology. Therefore, compounds that exhibit both complement inhibiting and antioxidant activity could be valuable therapeutics. In our study, it was demonstrated that procyanidin C1 is an interesting candidate for this complementary therapeutical strategy.

In conclusion, the most promising antioxidant is procyanidin C1, which shows a high scavenging, lipid peroxidation-inhibiting and complement-inhibiting activity. However, its cytotoxicity must be kept in mind. Until now, data on the bioavailability of proanthocyanidins are still scarce. Nevertheless, in-vitro absorption of proanthocyanidin dimers and trimers was demonstrated in a Caco-2 cell line model, while polymers with an average degree of polymerization of 7 were not absorbed (Déprez 1999). In addition, Rein and coworkers demonstrated the increase in plasma antioxidant status after intake of chocolate, which is a rich source of procyanidins (Rein et al 2000). Kaempferol and (+)-catechin exhibit a low cytotoxicity and a high lipid peroxidation-inhibiting activity, as shown by their high ASI values. Both compounds are rather selective antioxidants, since they do not exhibit an interesting free radical scavenging activity and do not inhibit the classical pathway of the complement system at low concentrations. Similarly, resveratrol is a relatively strong inhibitor of lipid peroxidation, but has a higher cytotoxicity and subsequently a lower ASI compared with kaempferol and (+)-catechin. Finally, genistein exhibits a very low antioxidant activity in both the lipid peroxidation and the DPPH$^{\bullet}$ scavenging assay, a high cytotoxicity and a low complement-inhibiting activity, and can therefore not be classified as an interesting antioxidant in-vitro. Some of these promising antioxidants are now being further evaluated in our laboratory for their in-vivo antioxidant activity.

Conclusions

A detailed comparative study on the in-vitro antioxidant profile of eight polyphenolic antioxidants demonstrated that genistein had a low antioxidant activity. In contrast, procyanidin C1 exhibited an interesting antioxidant activity in all the assays performed and can therefore be classified as a promising antioxidant in-vitro. Further studies are underway to evaluate its antioxidant activity in vivo.

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