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EVALUATION OF THE ANTIOXIDANT ACTIONS OF FERULIC ACID AND CATECHINS

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We have evaluated the abilities of ferulic acid, (±) catechin, (+) catechin and (−) epicatechin to scavenge the reactive oxygen species hydroxyl radical ($OH^{\cdot}$), hypochlorous acid (HOCl) and peroxyl radicals ($RO_2^{\cdot}$).

Ferulic acid tested at concentrations up to 5 mM inhibited the peroxidation of phospholipid liposomes. Both (±) and (+) catechin and (−) epicatechin were much more effective. All the compounds tested reacted with trichloromethyl peroxyl radical ($CCl_3O_2^{\cdot}$) with rate constants $> 1 \times 10^6 M^{-1}s^{-1}$.

A mixture of $FeCl_3$-EDTA, hydrogen peroxide (H_2O_2) and ascorbic acid at pH 7.4, has often been used to generate hydroxyl radicals ($OH\cdot$) which are detected by their ability to cause damage to the sugar deoxyribose. Ferulic acid, (+) and (±) catechin and (−) epicatechin inhibited deoxyribose damage by reacting with $OH\cdot$ with rate constants of $4.5 \times 10^9 M^{-1}s^{-1}$, $3.65 \times 10^9 M^{-1}s^{-1}$, $2.36 \times 10^9 M^{-1}s^{-1}$ and $2.84 \times 10^9 M^{-1}s^{-1}$ respectively. (−) Epicatechin, ferulic acid and the (+) and (±) catechins exerted pro-oxidant action, accelerating damage to DNA in the presence of a bleomycin-iron complex. On a molar basis, ferulic acid was less effective in causing damage to DNA compared with the catechins.

A mixture of hypoxanthine and xanthine oxidase generates $O_2^{\cdot-}$ which reduces cytochrome c to ferrocytochrome c. (+) Catechin and (−) epicatechin inhibited the reduction of cytochrome c in a concentration dependent manner. Ferulic acid and (±) catechin had only weak effects.

All the compounds tested were able to scavenge hypochlorous acid at a rate sufficient to protect alpha-1-antiproteinase against inactivation. Our results show that catechins and ferulic acid possess antioxidant properties. This may become important given the current search for "natural" replacements for synthetic antioxidant food additives.

KEY WORDS: Catechin, ferulic acid, pro-oxidant, anti-oxidant, lipid peroxidation, hypochlorous acid.

INTRODUCTION

The rapid growth of interest in free radical research relating to human diseases in the era following the discovery of the enzyme superoxide dismutase[1] seems to parallel the interest in the role of free radical reactions in nutrition and in food deterioration.[2-5]

Generation of reactive oxygen species (ROS) beyond the antioxidant capacity of a biological system, gives rise to *oxidative stress*. There are suggestions that oxidative stress may play a role in heart diseases, cancer and in the aging pro-

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cess.[6-8] The free radical chain reaction of lipid peroxidation is responsible for the development of off-flavours in manufactured foods containing lipids: lipid peroxidation affects the shelf life and nutritional quality of foods. Use of antioxidants during the manufacturing process minimises lipid peroxidation and helps maintain food stability.[3-5,9,10]

Food antioxidants are often synthetic molecules such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate.[3,4,11] However, there is a growing interest in replacing them by "natural" antioxidants in the preservation of food material,[3,4,9-11] partly driven by safety considerations (real or imagined). Fruits and some plant materials contain components that can exhibit antioxidant actions in some test systems.[2,6,9,10] By definition,[8,12] antioxidants (at low concentrations compared with those of an oxidizable substrate) significantly delay or prevent oxidative damage to that substrate.

The antioxidant actions of pure and crude extracts or components from plant sources are frequently tested only in lipid systems. The authors have suggested that in testing putative antioxidant activity, reactions involving biologically-relevant oxygen-derived species should be used and targets in addition to lipids must be considered.[2,5,12]

(±) Catechin, (+) catechin and (−) epicatechin (Figure 1) are natural products which occur in green tea[10] and have been claimed to possess antioxidant actions superior to BHA and *dl* α-tocopherol.[13,14] Ferulic acid (caffeic acid 3 methyl ester,

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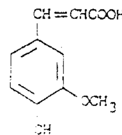
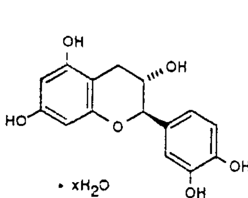
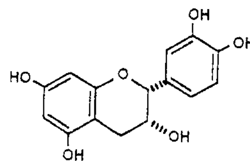


Ferulic acid



(+)-Catechin hydrate



(-)-Epicatechin

FIGURE 1 Structures of the compounds studied.

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Figure 1) is widely distributed in plants. For example, concentrations of up to 1.1×10^{-2} moles/kg have been reported in *Chia* seeds[15] (*Chia* is a desert plant found predominantly in Mexico), and up to 1.5×10^{-4} mole/kg in hydrolysed soy vegetable protein.[16] Graf[17] has discussed the potential use of ferulic acid as an antioxidant. In this paper, we have evaluated the antioxidant (protection against oxygen-derived species) and the possible pro-oxidant actions of ferulic acid, (±) catechin, (+) catechin and (−) epicatechin using established assays.[12,18]

MATERIALS AND METHODS

Chemicals and reagents were purchased either from Sigma Chemical Company (Poole, Dorset, UK) or BDH Chemical Company (Poole, Dorset, UK), and were of the highest purity available.

Assays

Reactions with OH·: Deoxyribose assay The deoxyribose assay allows the determination of the rate constants for reaction between antioxidant molecules and OH·.[19] The assay was conducted essentially as described in Halliwell *et al.*[19] with some modifications. Reaction mixtures contained in a final volume of 1.2 ml, the following reagents at the final concentrations stated: deoxyribose (2.8 mM), $FeCl_3$ (50 μM), EDTA (100 μM) (EDTA and Fe^{3+} ions are pre-mixed at the ratio given prior to the addition of deoxyribose), KH_2PO_4-KOH buffer at pH 7.4 (10 mM), H_2O_2 (2.8 mM), compounds under test and 100 μM of ascorbate where used.

(±) and (−) Catechin and (−) epicatechin were dissolved in double distilled water. Ferulic acid was used as a suspension in water. Unfortunately ferulic acid solubilized in ethanol cannot be used in the deoxyribose assay as ethanol itself is able to scavenge OH·.[19]

Tubes were incubated at 37°C for 1 hr. Products of OH· attack upon deoxyribose were measured as previously described.[19] The rate constants of reaction were calculated from competition plots obtained with varying concentrations of the antioxidant in the assay mixtures.[19,20]

Phospholipid Liposome Peroxidation

The ability of (±) and (+) catechin, (−) epicatechin and ferulic acid to inhibit lipid peroxidation at pH 7.4 was tested using bovine brain phospholipid liposomes.[18,21] The compounds were tested dissolved in ethanol, which does not affect this assay.

Reactions with Trichloromethyl Peroxyl Radicals

Reaction with trichloromethyl peroxyl radical was conducted using the Linear Accelerator facility at the Paterson Institute, Christie Hospital, Manchester. Reaction mixtures contained 1% (v/v) CCl_4, 50% (v/v) isopropyl alcohol and 49% (v/v) 10 mM KH_2PO_4-KOH buffer pH 7.4.

Bleomycin-Dependent DNA Damage

The bleomcyin assay[22] was conducted using a modified method described in.[20]

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Reaction with Hypochlorous Acid

Reaction with hypochlorous acid (HOCl) was studied using the assay of Wasil *et al.*[23] as modified in.[18] For the assay, 155 μM HOCl (produced immediately before use by adjusting NaOCl to pH 6.2 with dilute H_2SO_4) and the compounds were each incubated in a final volume of 1 ml in phosphate buffered saline pH 7.4[18,23] for 20 minutes. To the reaction mixture, 0.1 ml of α_1-antiproteinase (Sigma type A9024; 4 mg/ml) was added. This allows any HOCl remaining to inactivate α_1AP. After a 20 minute further incubation, 0.05 ml of 10 mg/ml elastase (Sigma type E0258) was added. This mixture was allowed to stand a further 30 min to allow any α_1AP still active to inhibit elastase. Any HOCl remaining is diluted out to the point at which it cannot affect elastase, by addition of 2 ml of phosphate buffered saline. The elastase activity remaining was measured by adding elastase substrate (5 mg/ml N-succinyl triala-p-nitroanilide), and monitoring increases in A_{410} nm.[18,23]

Reaction with Superoxide Radical

Generation of O_2^- was by the hypoxanthine-xanthine oxidase system.[1] Reaction mixtures contained, in a final volume of 3 ml, 0.1 ml of 0.3 mM EDTA, 0.1 ml of

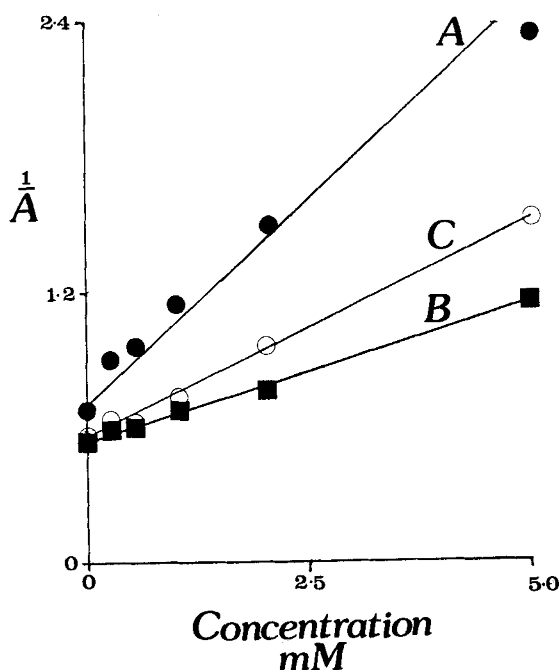

FIGURE 2 Scavenging of hydroxyl radical by (A) ferulic acid, (B) (±) catechin, and (C), (−) epicatechin in the presence of Fe^{3+}-EDTA. Experiments were conducted essentially as described in Halliwell *et al.* (1987). Ferulic acid was used as a suspension in water. Catechins and epicatechin were dissolved in double distilled water with pH adjustment close to 7.4 prior to use. Full details of the assay are given in the Materials and Methods section. The rate constant (k_2) for reaction with OH· was calculated from the equation k_2 = slope × k_{DR} × [DR] × A° where k_{DR} is 3.1 × 10^9 $M^{-1}s^{-1}$ (the rate constant of reaction between OH· and deoxyribose), A° is the absorbance in the absence of the compounds and [DR] is the concentration of deoxyribose (2.8 mM) in the respective assay mixtures.

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30 mM hypoxanthine (dissolved in minimum potassium hydroxide solution), 0.1 ml of 3 mM cytochrome c and a final concentration of 10 mM KH_2PO_4-KOH buffer (pH 7.4). In each case, 0.16 units/ml (final concentration) of xanthine oxidase (Sigma type X1875) was added to start the reaction. The rate of cytochrome c reduction was measured at 550 nm at 25°C. The effect of additives upon xanthine oxidase was measured by omitting cytochrome c from the reaction mixture and measuring uric acid formation by the rise in absorbance at 290 nm.

RESULTS

Reaction with Hydroxyl Radicals

The deoxyribose assay is a useful experimental tool for investigating the ability of molecules to react with OH·, by competing with deoxyribose for this radical and inhibiting deoxyribose degradation.[19] Hydroxyl radical is generated by a mixture of $FeCl_3$-EDTA, H_2O_2 and ascorbic acid at pH 7.4.

Figure 2 shows a typical competition plot obtained when we assessed the ability of (±) and (+) catechin, (−) epicatechin or ferulic acid to inhibit deoxyribose degradation. From the slopes of the plots, rate constants were calculated (Table 1). Ferulic acid, (±) and (+) catechins and (−) epicatechin all reacted with OH· with second order rate constants close to the diffusion-controlled limit, as expected for phenolic compounds.[12,18]

Inhibition of Phospholipid Liposome Peroxidation and Scavenging of Trichloromethyl Peroxyl Radical

Bovine brain phospholipid liposomes undergo rapid non-enzymic peroxidation when incubated in the presence of $FeCl_3$ and ascorbic acid. The effects of the plant phenolics are shown in Table 2. Graf[17] quoted a 70.9% inhibition of *in vitro* lipid peroxidation in rat brain homogenates by 5 mM ferulic acid. Ferulic acid provided an 80% inhibition at this concentration in the liposome system (Table 2). (±) and (+) catechins and (−) epicatechin were much more effective at lower concen-

TABLE 1
Rate constants of reaction with OH· obtained by use of the deoxyribose assay.

Compounds	Calculated Rate Constants ($M^{-1}s^{-1}$)
Ferulic acid	4.55×10^9
(+) Catechin	3.65×10^9
(±) Catechin	2.36×10^9
(−) Epicatechin	2.84×10^9
Vanillic acid	1.6×10^{10} (ref 25)
Inosinic acid	3.9×10^9 (ref 25)
Carnosol	8.7×10^{10} (ref 18)
Carnosic acid	5.9×10^{10} (ref 18)

Rate constants were calculated from data in Figure 2 using the method described in Halliwell *et al.* (1987).[19] Representative values for vanillic acid, inosinic acid, carnosol and carnosic acid are given for comparison. Results are the means of 3 determinations that differed by less than 10%.

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TABLE 2
Phospholipid liposome peroxidation by an $FeCl_3$-ascorbate system: effect of catechin, epicatechin and ferulic acid.

Compound tested	Concentration (mM)	A_{532}* Extent of Peroxidation	% Inhibition
Control, no compound plus ethanol	–	2.76	–
(+) Catechin	0.25	0.417	85
(±) Catechin	0.25	0.536	81
(−) Epicatechin	0.25	0.394	86
Ferulic acid	0.25	2.45	8
	1.25	2.22	20
	2.5	0.648	77
	5.0	0.540	80
Propyl gallate	0.10	0.324	88

*Values are the means from duplicate measurements that varied by less than 10%. Concentrations quoted are the final concentrations of the respective compounds in the reaction mixtures. Experiments were conducted essentially as described in Aruoma *et al.* 1992.[18] Ascorbate and $FeCl_3$ were at a final concentration of 100 μM respectively in a 1 ml final volume.

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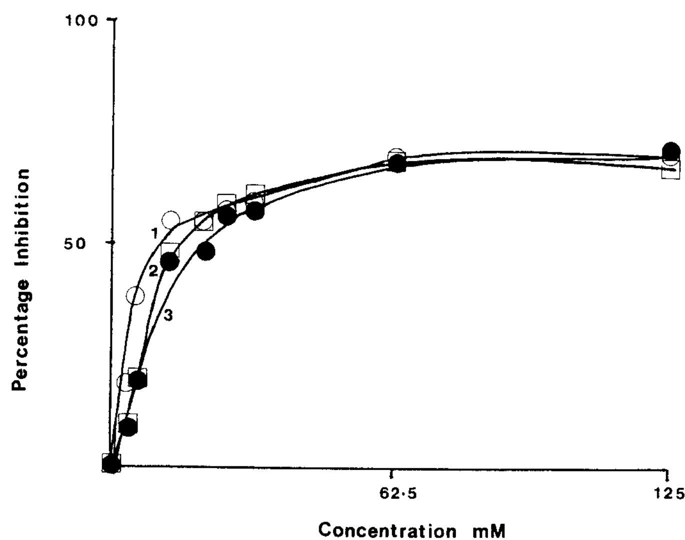

FIGURE 3 Inhibition of lipid peroxidation by (±) and (+) catechins and (−) epicatechin. Experiments were performed essentially as described in Aruoma *et al.*[18] and in the legend to Table 2. All concentrations were the final concentrations in the reaction mixtures. The IC_{50} for (±) catechin, (+) catechin and (−) epicatechin are approximately (20 ± 5) μM, (22 ± 5) μM and (12.5 ± 2) μM respectively.
Line (1), (−) epicatechin
Line (2), (±) catechin
Line (3), (+) catechin

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TABLE 3
Rate constants for reaction of compounds with trichloromethyl peroxyl radicals.

Compound	Rate Constant $M^{-1}s^{-1}$ (Compound + $CCl_3O_2^{\cdot}$)
Ferulic acid	5.6×10^6
(+) Catechin	7.5×10^6
(±) Catechin	6.1×10^6
(−) Epicatechin	7.3×10^6
Lovastatin	5.2×10^8 (ref 2)
Quercetin	3.9×10^7 (ref 2)
Trolox c	2.23×10^8 (ref 2)
Gallic acid lauryl ester	2.33×10^7 (ref 2)
Propyl gallate	1.67×10^7 (ref 2)
Carnosic acid	2.7×10^7 (ref 18)
Phenol	$< 1 \times 10^5$ (ref 18)

Analysis using pulse radiolysis was carried out essentially as described in the Materials and Methods section. Values quoted are averages of two independent measurements which varied by less than 5%. Rate constants for reaction with quercetin, Trolox C, gallic acid lauryl ester, propyl gallate, carnosic acid, phenol and lovastatin are given for comparison.

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trations compared with ferulic acid (Table 2 and Figure 3). Control experiments showed that none of the compounds tested interfered with the TBA assay.

Inhibition of lipid peroxidation may be due to scavenging of intermediate peroxyl radicals. This was investigated directly using trichloromethyl peroxyl radical ($CCl_3O_2^{\cdot}$), a reactive organic radical,[26,27] frequently used in assessment of the ability of compounds to react with peroxyl radicals. The abilities of (±) and (+) catechins, (−) epicatechin, and ferulic acid to interact with $CCl_3O_2^{\cdot}$ generated by radiolysis of a mixture of propan-2-ol and CCl_4 are summarized in Table 3. Quercetin and carnosic acid are natural products of plant origin. They react rapidly with $CCl_3O_2^{\cdot}$ with rate constants comparable to the widely used antioxidant propyl gallate and the lauryl ester of gallic acid. By comparison, ferulic acid, (±) and (−) catechin, and (−) epicatechin reacted more slowly with the trichloromethyl peroxyl radical, although still at significant rates.

Scavenging of Hypochlorous Acid

Hypochlorous acid, HOCl, is produced by the neutrophil-derived enzyme myeloperoxidase at sites of inflammation and when activated neutrophils infiltrate reoxygenated tissue.[28] In the context of food handling, HOCl is a major active constituent of chlorine-based bleaches often used to disinfect equipment with which foods will come into contact.

An assay involving the enzyme elastase and its protein inhibitor α_1-antiproteinase (α_1AP) has been adopted as a method for assessing the antioxidant action of compounds that could react with HOCl.[23] Table 4 shows representative results. Incubation of HOCl (155 μM) with varying concentrations of ferulic acid prior to addition of α_1AP resulted in the protection of the elastase inhibitory activity of α_1AP. Ferulic acid was dissolved in ethanol in this set of experiments, but controls for the effect of ethanol were included and show that ethanol alone had much less effect.[18] (Ethanol was included in equal amounts in all reaction mixtures). Control

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TABLE 4
Reactions with hypochlorous acid and protection of alpha-1-antiproteinase. Effects of ferulic acid.

Additions to reaction mixture		ΔA_{410} per min Elastase Activity	Activity of α_1AP in inhibiting elastase (%)
Elastase only		0.600	–
+ α_1AP		0.066	100
+ α_1AP + HOCl		0.400	33
+ α_1AP + HOCl + ferulic acid	0.1 mM	0.273	55
	0.5 mM	0.081	87
	2.5 mM	0.027	96
	5.0 mM	0.029	95

Experiments were conducted essentially as described in the Materials and Methods section. The reaction mixtures contained equal amounts of ethanol. Ferulic acid is only soluble in organic solvents.

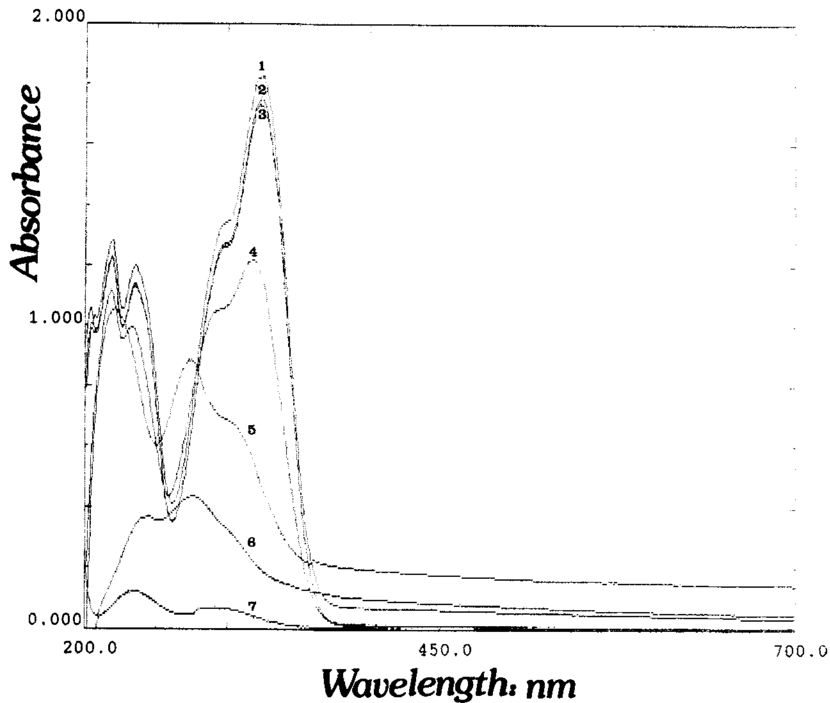

FIGURE 4 Effect of increasing concentrations of HOCl on the spectrum of ferulic acid. Ferulic acid (dissolved in ethanol) was at a final concentration of 0.1 mM in a final volume of 3 ml containing phosphate buffered saline pH 7.4 (140 mM NaCl, 2.7 mM KCl, 16 mM Na_2HPO_4 and 2.9 mM KH_2PO_4).
Line I contained no HOCl;
line 2, 0.109 mM HOCl;
line 3, 0.063 mM HOCl;
line 4, 0.52 mM HOCl;
line 5, 1.30 mM HOCl,
line 6 contained 1.73 mM HOCl and
line 7 contained only 1.93 mM HOCl (without ferulic acid).

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experiments also showed that 2.5 mM ferulic acid (dissolved in ethanol) produced a 37% inhibition of elastase activity. This compares with the 96% elastase inhibitory activity of α_1AP resulting from the scavenging of HOCl by 2.5 mM ferulic acid (Table 4). Figure 4 shows the spectral changes produced by reaction of ferulic acid with HOCl. (±) and (+) Catechins and (−) epicatechin were also powerful scavengers of HOCl (Table 5), when pre-incubated with this molecule. Experiments were also performed in which HOCl was added to mixtures of α_1AP and the phenols: 0.25 mM each of (±) and (+) catechins, (−) epicatechin and 2.5 mM of ferulic acid; α_1AP and HOCl were at the final concentrations of 0.91 mg/ml and 160 μM respectively. The compounds protected α_1AP against inactivation by HOCl, thus confirming the data in Tables 4 and 5 and in Figure 4. Active components of rosemary extract have also been reported to scavenge HOCl,[18] as do gallic acid and its derivatives[31]. This suggests that food antioxidants containing phenolic functional groups might provide protection against damage by HOCl.

Bleomycin-Induced DNA Damage

The anti-tumour antibiotic bleomycin binds iron ions and the bleomycin-iron complex will degrade DNA in the presence of O_2 and a reducing agent. The bleomycin assay[22] has been adapted for assessing the pro-oxidant effects of proposed lipid antioxidants for food use.[5,18,20,25]

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TABLE 5
Reactions with hypochlorous acid and protection of alpha-1-antiproteinase. Effects of (+) and (±) catechin and (−) epicatechin.

Addition to reaction mixture		Elastase Activity ΔA_{410} per min	Activity of α_1AP in inhibiting elastase (%)
Elastase only		0.827	–
+ α_1AP		0.0	100
+ [α_1AP, HOCl]		0.807	2
α_1AP and HOCl plus (+) catechin			
	0.025 mM	0.755	9
	0.05	0.973	0
	0.125	0.464	44
	0.25	0.008	99
	0.50	0.002	100
α_1AP and HOCl plus (±) catechin			
	0.025	0.824	0.4
	0.05	0.957	0
	0.125	0.913	0
	0.25	0.316	62
	0.50	0.126	85
α_1AP and HOCl plus (−) epicatechin			
	0.025	0.872	0
	0.05	0.891	0
	0.125	1.01	0
	0.25	0.281	66
	0.50	0.021	97

Experiments were conducted as described in the Materials and Methods section. In Tables 4 and 5 values quoted are the means from duplicate independent experiments with variations of 5%.

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Table 6 shows that (±) and (+) catechin and (−) epicatechin promoted DNA damage in the system although they were less effective than ascorbate. Ferulic acid at concentrations greater than 1 mM became pro-oxidant in the bleomycin system. These results are not unexpected given that (±), (+) catechins and (−) epicatechin are phenolic compounds and can probably reduce ferric iron to ferrous. However, it is noteworthy that the catechins were antioxidant in the deoxyribose assay.

Reactions with Superoxide Radical

A mixture of hypoxanthine and xanthine oxidase at pH 7.4 generates $O_2^{\cdot-}$ which can be measured as a rise in absorbance at 550 nm.[1] We have used the assay to assess the ability of (+) and (±) catechin to react with $O_2^{\cdot-}$.[18,24] The compounds tested were dissolved in either buffer or ethanol.[1] Table 7 shows that (+) catechin and (−) epicatechin decreased the rate of reduction of cytochrome c by $O_2^{\cdot-}$. Superoxide dismutase inhibited the reduction of cytochrome c as expected. Ferulic acid and (±) catechin had only small inhibitory effects: $O_2^{\cdot-}$-dependent cytochrome c reduction was decreased by 10% and 18% respectively (Table 7). In control experiments, the formation of uric acid at 290 nm was measured using the same assay mixtures described in the Materials and Methods section but omitting cytochrome c. Ferulic acid has high absorption in the UV region (see Figure 4). However, none of the compounds changed the rate of uric acid formation. (−)

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TABLE 6
DNA damage by ferric-bleomycin: effect of (+) and (±) catechin, (−) epicatechin and ferulic acid.

Compounds added to reaction mixture	Concentrations	Extent of DNA damage A_{532} nm
None, control		0.02
Ascorbate	0.2 mM	1.22
(+) Catechin	0.05	0.03
	0.1	0.09
	0.25	0.29
	0.5	0.46
(±) Catechin	0.05	0.02
	0.1	0.07
	0.25	0.25
	0.5	0.39
(−) Epicatechin	0.05	0.03
	0.1	0.09
	0.25	0.39
	0.5	0.54
Ferulic acid	0.05	0
	0.1	0
	0.25	0.04
	0.5	0.06
	1.0	0.16
	2.5	0.39
	5.0	0.55

Experiments were conducted essentially as described in Gutteridge *et al.*[22] with modifications described by Aruoma.[20] The table shows representative data in which the mean values quoted varied by less than 10% in two experiments. All compounds tested were dissolved in ethanol: ethanol does not affect the ferric-bleomycin assay. The concentrations in the Table are final concentrations in the respective assay mixtures.

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TABLE 7
The ability of ferulic acid, (±) catechin, (+) catechin and (−) epicatechin to scavenge superoxide.

Addition to reaction mixture		ΔA_{550} per sec Rate of cytochrome c reduction
Control, HX, XO (RM1)		8.5×10^{-3}
RM1 + 133 U/ml SOD		0
RM1 plus (±) catechin	0.16 mM	7×10^{-3}
RM1 plus (−) epicatechin	0.16 mM	5×10^{-3}
RM1 plus (+) catechin	0.16 mM	6×10^{-3}
	0.46 mM	5×10^{-3}
RM1 plus ethanol* (RM2)		10×10^{-3}
RM2 plus ferulic acid	1.64 mM	9×10^{-3}

Assays were conducted as described in the Materials and Methods section.
*Ethanol (0.1 ml absolute ethanol) was added to test the net effect of the solvent used as a vehicle for ferulic acid in this assay. Concentrations quoted are the final concentrations and values are the means from triplicate measurements which varied by no more than 5%. Units of superoxide dismutase (SOD) and xanthine oxidase (XO) were as described in the Sigma catalogue.

Epicatechin and (+) catechin decreased the rates of cytochrome c reduction somewhat, but only at concentrations greater than those of cytochrome c. None of the compounds reduced cytochrome c at a significant rate in the absence of xanthine oxidase.

DISCUSSION

The use of antioxidants to minimise the oxidation of lipid in food materials is widely practised by food manufacturers.[2-5] The increasing interest in the search for natural replacements for synthetic antioxidants, has led to the evaluation of a number of plant products for their antioxidant actions (eg refs 29–35). In this study, we have applied established *in vitro* assays to screen the antioxidant/pro-oxidant actions of (±) and (+) catechins, (−) epicatechins and ferulic acid. (±) and (+) Catechins, (−) epicatechin and ferulic acid scavenged $OH\cdot$ with rate constants $> 1 \times 10^9$ $M^{-1}s^{-1}$. The compounds reacted with $CCl_3O_2^{\cdot}$ with reasonably high rate constants (Table 2). Ferulic acid inhibited the peroxidation of bovine brain phospholipid liposomes but only at very high concentrations. The catechins were much more effective anti-oxidants (Table 3).

However, the catechins were able to cause some DNA damage in the bleomycin assay, whereas ferulic acid was not able to stimulate this reaction except at much higher concentrations (Table 6). The pro-oxidant action seen with the catechins is a feature of many phenolic compounds. The compounds were not pro-oxidant in the deoxyribose assay. The reaction mechanisms in the two assays are somewhat different, as are the redox potentials of the iron complexes. Whereas a mixture of $FeCl_3$-EDTA, H_2O_2 and ascorbate generates $OH\cdot$, a bleomycin-Fe^{3+}-ascorbate system causes damage via an oxo-iron complex in which the involvement of $OH\cdot$ is a minor side reaction.[36]

In the cytochrome c assay, $O_2^{\cdot-}$ generated by a mixture of hypoxanthine and xanthine oxidase, reduces ferricytochrome c to ferrocytochrome. (+) Catechin and (−) epicatechin inhibited the reduction of cytochrome c in a concentration dependent manner. Ferulic acid and (±) catechin had only weak effects on the reduction of

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cytochrome c, indicating that their rate constants for reaction with $O_2^{\cdot-}$ are $< 10^5$ $M^{-1}s^{-1}$.

Data in Tables 1, 5 and 6 highlight the variability in results of assays for pro-oxidant and antioxidant properties and underline the need to use several different assay systems. It is now clear that some antioxidants showing pro-oxidant actions in the bleomycin assay do not necessarily show pro-oxidant action in the deoxyribose system[33]. Ferulic acid and the catechins also react with hypochlorous acid at a rate fast enough to protect α_1-antiproteinase.

Our data are consistent with the view that ferulic acid and catechins possess useful antioxidant properties and may become good candidates in the search for "natural" replacements for synthetic antioxidant food additives.

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