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REVIEW PAPER

A Review of the Antioxidant Mechanisms of Polyphenol Compounds Related to Iron Binding

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Abstract In this review, primary attention is given to the antioxidant (and prooxidant) activity of polyphenols arising from their interactions with iron both in vitro and in vivo. In addition, an overview of oxidative stress and the Fenton reaction is provided, as well as a discussion of the chemistry of iron binding by catecholate, gallate, and semiquinone ligands along with their stability constants, UV–vis spectra, stoichiometries in solution as a function of pH, rates of iron oxidation by O_2, upon polyphenol binding, and the published crystal structures for iron–polyphenol complexes. Radical scavenging mechanisms of polyphenols unrelated to iron binding, their interactions with copper, and the prooxidant activity of iron–polyphenol complexes are brie y discussed.

Keywords Polyphenol antioxidants · Iron binding · Catecholate · Gallate · Flavonoids · Catechins · Tannins · Proanthocyanidins · EGCG · Stability constant · Autooxidation · DNA damage · Fenton reaction · Cytotoxicity · Cytoprotection · Alzheimer's · Parkinson's · Blood and plasma · Preservatives · Radical scavenging · Reactive oxygen species

Abbreviations

Aβ	Amyloid-beta
EC	(–)-Epicatechin
ECG	(–)-Epicatechin-3-gallate
EGC	(–)-Epigallocatechin
EGCG	(–)-Epigallocatechin-3-gallate
GA	Gallic acid
Q	Quercetin
MEGA	Gallic acid methyl ester
MEPCA	Protocatechuic acid methyl ester
PCA	Protocatechuic acid
PrEGA	Gallic acid propyl ester
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
Rut	Rutin
TA	Tannic acid
VA	Vanillic acid
EPR	Electron paramagnetic resonance
TEAC	Trolox-equivalent antioxidant activity
ORAC	Oxygen radical absorbance capacity
DPPH	2,2-Diphenylpicrylhydrazyl

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Polyphenol Compounds: Sources, Structures, and General Biological Activities

Polyphenol compounds are widely studied for their antioxidant properties, although the term antioxidant has a broad range of meanings. For the purposes of this review, antioxidant activity refers to both the ability of polyphenol compounds to prevent damage from reactive oxygen species (ROS) (such as through radical scavenging) or to prevent generation of these species (by binding iron). As described in the title, the primary focus will be on polyphenol–iron interactions as a mechanism of antioxidant activity. The typical structural characteristic shared by most polyphenols is the three-membered avan ring system (Fig. 1), yet the combinatorial library of polyphenol compounds is widely diversi ed, collectively encompassing many thousands of different compounds [1], [which are divided into several sub-classes, such as the catechins,

avonols, avanols, avones, anthocyanins, proanthocyanidins, and phenolic acids, just to name a few (Fig. 1). Polyphenols are found in green [2, 3] and black teas [4, 5], coffee [6], fruits [7, 8], fruit juices [9–11], vegetables [12, 13], olive oil [14, 15], red and white wines [16, 17], and chocolate [18], and are found in medium to high milligram quantities per serving for all of these foods (Figs. 2, 3), although measurements of the precise concentrations of polyphenol compounds in each type of food often vary [7, 11, 12, 17–22]. Thus, people with diets rich in fruits and vegetables may consume one or more grams per day of these compounds, based on the recommendation of 5 servings/day of colorful fruits and vegetables by the Centers for Disease Control and Prevention [23]. Because polyphenols are such a large and integral part of the human diet, it is highly desirable to understand their biological functions and modes of activity.

Green tea leaf is particularly abundant in the group of polyphenols collectively referred to as catechins (Fig. 1), which constitute up to 30% of the plant's dry leaf weight [24]. Within just 2 h after consumption of one cup of green or black tea (350–600 ml) [25–28], catechins have been found in concentrations of 0.3–1 μM in human plasma and may even approach 10 μM with higher doses [29]. Flavonols (Fig. 1) such as quercetin are reportedly less bioavailable than catechins; however, they may reach similar plasma concentrations (high nanomolar to low micromolar) in people who eat large amounts of fruits and vegetables or intentionally supplement their diets with polyphenols [30, 31].

While polyphenols are primarily recognized for their antioxidant functions, they also have many other biological activities, such as anti-histamine [32], anti-in ammatory [33], antibacterial [34], and antiviral activities [35]. Cardiovascular effects such as vasodilation have been observed in tea drinkers [36, 37], and this property has been attributed to the ability of polyphenols to increase endothelial nitric oxide synthase (eNOS) activity by over 400% [36]. They have also been shown to bind many different proteins such as casein [36], and inhibit telomerase [38], α-amylase, pepsin, trypsin, and lipase [39], among many other enzymes. Furthermore, polyphenols are implicated in the prevention of neurodegenerative diseases [12, 40] and cancer [41]. They also induce apoptosis in cancer cells, implicating them in cancer senescence as well [42–46].

Because a full discussion of the biological activity of polyphenols would be prohibitively long, this review will focus on the antioxidant mechanisms of polyphenols specifically related to iron-binding, with brief mention made of other mechanisms such as radical scavenging, prooxidant activity, and interactions between polyphenols and copper.

Oxidative Stress, DNA Damage, and the Fenton Reaction

Reactive oxygen species and reactive nitrogen species (RNS), such as hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^-$), and others, are major sources of oxidative stress in cells, damaging proteins, lipids, and DNA [47]. Oxidative DNA damage has been implicated as a cause of cancer [48, 49], aging and neurodegenerative diseases such as Alzheimer's and Parkinson's [50–54], cardiovascular diseases such as arteriosclerosis [55, 56], and is the primary cause of cell death and tissue damage resulting from heart attack and stroke [57, 58]. Therefore, prevention of oxidative stress caused by ROS and RNS has important implications for the prevention and treatment of disease.

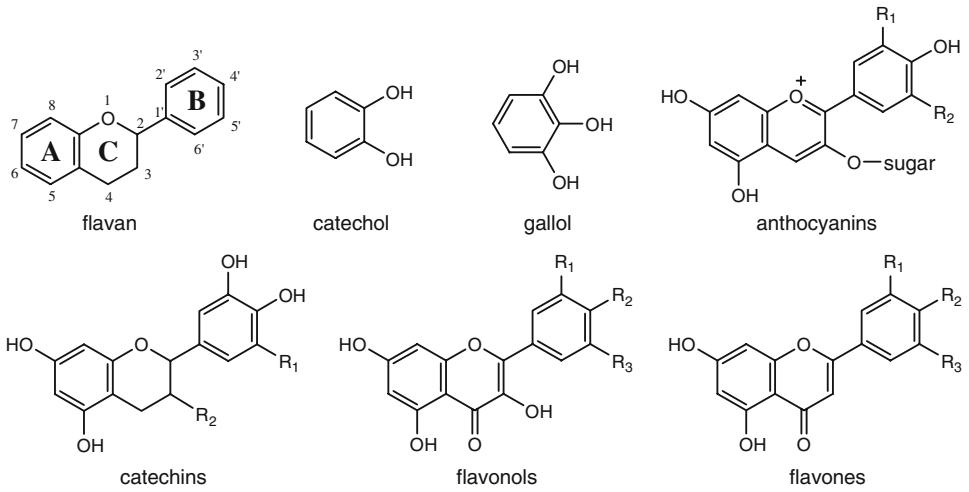

Fig. 1 Flavan general structure, showing the ring labeling and numbering system. Structures of catechol, gallol, and general structures of catechins, avonols, avones, and anthocyanins. R groups are typically H, OH, OCH_3, galloyl esters, or carbohydrate groups, depending on the compound

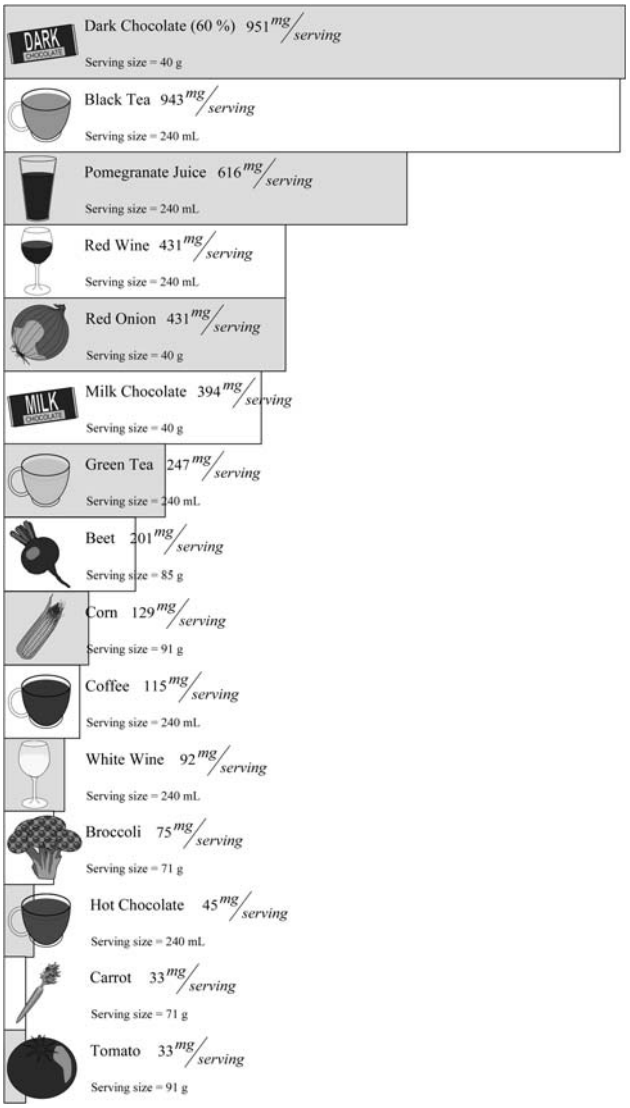

Fig. 2 A chart showing the phenolic content of selected beverages, vegetables, and chocolate in milligrams per serving. Serving size is based on a typical beverage size (240 ml), piece of chocolate (40 g), or serving of vegetables. Values are taken or calculated from data in the references [5, 6, 11, 12, 17, 18]. Reported polyphenol content varies

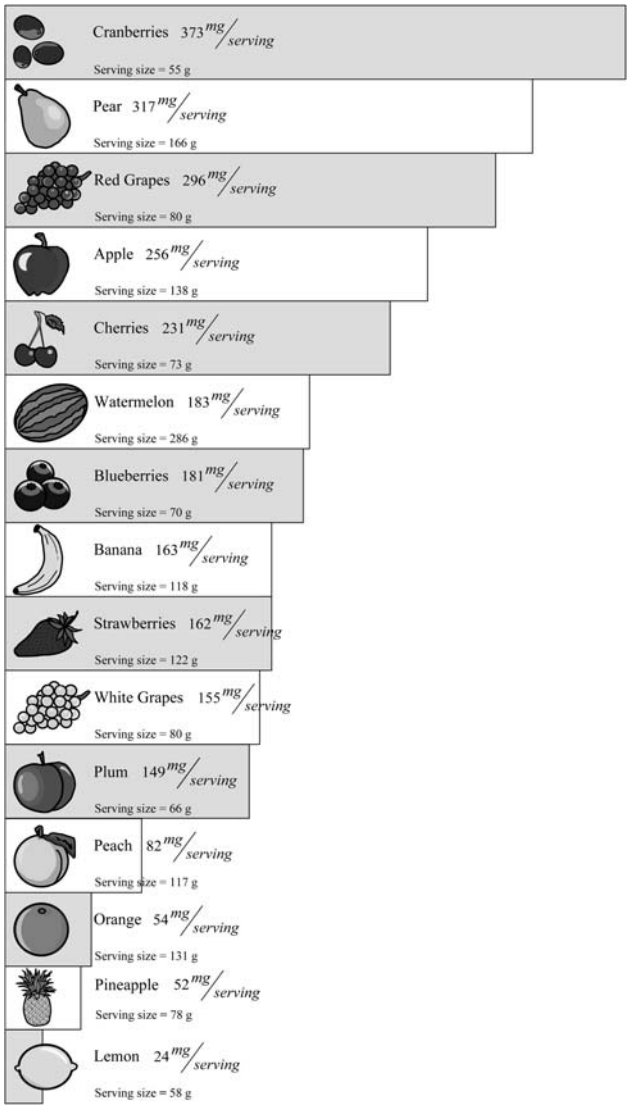

Fig. 3 A chart showing the phenolic content of selected fruits in milligrams per serving. Serving size is based on a typical serving of fruit. Data from [7]. Reported polyphenol content varies

Radical Scavenging Pathways of Polyphenol Antioxidant Activity

Many mechanisms have been proposed for polyphenol prevention of oxidative stress and ROS/RNS generation both in vitro and in vivo. Radical scavenging by polyphenols is the most widely published mechanism for their antioxidant activity, with over 700 papers since 1995 alone [59]. In this radical scavenging mechanism, polyphenols sacri cially reduce ROS/RNS, such as $^{\bullet}OH$, $O_2^{\bullet-}$, $NO^{\bullet}$, or $OONO^{-}$ after generation, preventing damage to biomolecules or formation of more reactive ROS [15, 60–68]. Several assays, such as the trolox-equivalent antioxidant activity (TEAC) and oxygen radical absorbance capacity (ORAC) assays as well as 2,2-diphenylpicrylhydrazyl (DPPH) scavenging, are commonly used to study the radical-scavenging ability of polyphenols [69–72]. These assays provide a relative measure of antioxidant activity, but often the radicals scavenged have little relevance to those present in biological systems. In addition, radical scavenging assays do not account for the iron-binding properties of polyphenol antioxidants.

It is clear that polyphenols have many different biological activities; among them are enzyme regulation and antioxidant behavior. Radical scavenging is a probable mechanism for reduction of oxidative stress by these compounds, but as it does not involve iron binding, it is therefore outside the scope of this review.

The Role of Iron in ROS Generation

Hydroxyl radical, the most reactive ROS known, abstracts a hydrogen atom from biological substrates at diffusion-limited rates [67]. Multiple pathways generate $^{\bullet}OH$, including the decomposition of peroxynitrous acid [73], or the metal-mediated reduction of peroxides. Formation of biological peroxides, such as H_2O_2, is a process that occurs naturally during cellular respiration [74], and cell signaling mechanisms often involve ROS or RNS such as H_2O_2, $O_2^{\bullet-}$, and $NO^{\bullet}$. These species can also form more potent oxidants if not closely regulated, leading to cellular damage and oxidative stress [75–80]. For example, $O_2^{\bullet-}$ and $NO^{\bullet}$ can react to form $OONO^-$ [81, 82], which can then decompose into $^{\bullet}OH$ [73]. H_2O_2 is commonly reduced in vivo by either Fe^{2+} or Cu^{+}, resulting in the formation of $^{\bullet}OH$ via Fenton-type reactions (reaction 1). Superoxide forms H_2O_2 upon protonation in aqueous solution (reaction 2) [83]. DNA damage is observed directly from $^{\bullet}OH$ [84–86], and indirectly from $O_2^{\bullet-}$ oxidation of [4Fe–4S] iron–sulfur clusters (reaction 3) to form H_2O_2 [87]. In addition to H_2O_2 formation, $O_2^{\bullet-}$ also releases Fe^{2+} from enzymes, such as ferritin [88] and the [4Fe–4S]-containing dehydratases by reducing Fe^{3+}, generating an unstable iron–sulfur cluster, and releasing Fe^{2+} (reaction 4) [83, 87, 89]. Superoxide can also reduce aqueous Fe^{3+} or Cu^{2+} (reaction 5), making these metal ions available to react with H_2O_2, although the rate of iron reduction is slow (10 h is the proposed half-time for this reaction in vivo), and it is generally assumed that more abundant cellular reductants such as NADH commonly reduce cellular Fe^{3+} (Fig. 4) [67]. The Haber–Weiss reaction was once thought to be a source of cellular $^{\bullet}OH$ (reaction 6) [90, 91], but it has since been determined that this reaction does not occur in vivo [91, 92].

$$Fe^{2+}\text{or } Cu^{+} + H_2O_2 + H^{+} \rightarrow Fe^{3+}\text{or } Cu^{2+} + {}^{\bullet}OH + H_2O \quad (1)$$

$$2O_2^{\bullet-} + 2H^{+} \rightarrow H_2O_2 + O_2 \quad (2)$$

$$\left[2Fe^{2+}2Fe^{3+} - 4S\right] + O_2^{\bullet-} + 2H^{+} \rightarrow \left[Fe^{2+}3Fe^{3+} - 4S\right] + H_2O_2 \quad (3)$$

$$\left[Fe^{2+}3Fe^{3+} - 4S\right] \rightarrow \left[3Fe^{3+} - 4S\right] + Fe^{2+} \quad (4)$$

$$O_2^{\bullet-} + Fe^{3+}\text{or } Cu^{2+} \rightarrow O_2 + Fe^{2+}\text{or } Cu^{+} \quad (5)$$

$$O_2^{\bullet-} + H_2O_2 + H^{+} \rightarrow O_2 + {}^{\bullet}OH + H_2O \quad (6)$$

Radical-induced damage to DNA occurs at both the phosphate backbone (strand breakage) and nucleotide bases, and both of these sites of damage are widely used to quantitatively determine the extent of oxidative DNA damage [93–101]. Because the DNA backbone is composed of negatively charged phosphate groups, as well as electron-rich nucleotide bases, metal ions such as Na^{+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, Cu^{+}, Cu^{2+}, etc. localize via electrostatic interaction near the phosphate backbone and transition metal ions such as iron and copper can covalently bind to the nucleotide bases of DNA. Metal ion localization stabilizes DNA by balancing the charge of the oxygen atoms of the phosphate backbone [94] [or coordination to electron pairs donated by nitrogen atoms of the bases, particularly at guanine-rich sequences [102–104].

When H_2O_2 is also present as a result of oxidative stress, redox active metal ions such as Fe^{2+} or Cu^{+} that are localized or bound to the DNA react with H_2O_2 to form highly reactive $^{\bullet}OH$ in immediate proximity to DNA. Hydroxyl radical then abstracts the 4′ hydrogen atom from the deoxyribose sugar backbone, leaving a DNA radical adduct that rearranges, ultimately cleaving the phosphodiester backbone and resulting in strand scission [84, 85, 102, 104]. Alternatively, $^{\bullet}OH$ may damage the nucleotide bases themselves, resulting in oxidized base products such as 8-oxo-guanine and fragmented or ring-opened derivatives [105–107]. DNA damage of both types (strand breakage or base damage) can ultimately result in genetic mutations, cancer, or cell death [105].

Both Imlay et al. and Mello-Filho et al. have determined that iron-mediated oxidative DNA damage by $^{\bullet}OH$ is the primary cause of cell death under oxidative stress conditions for both prokaryotes [108], and eukaryotes (including humans) [109, 110]. Iron-mediated DNA damage is primarily thought to originate from solvated iron that is not bound to proteins (such as hemoglobin, transferrin, or ferritin in eukaryotes [111], or the ferritin-like *dpr* protein and ferric uptake regulatory (*fur*) protein of prokaryotes) [112], which would otherwise prevent the iron from participating in the Fenton reaction. In *Escherichia coli*, the concentration of non-protein-bound iron is 10–30 μM [113], and it is believed to be coordinated to low molecular weight intracellular ligands such as ascorbate or citrate [114, 115]. However, if iron homeostasis is not maintained, the intracellular concentration of non-protein-bound iron may increase to between 80 and 320 μM [88, 113], causing a much greater susceptibility to oxidative DNA damage [88, 116]. Whole-cell electron paramagnetic resonance (EPR) indicates that most of this non-protein-bound iron in *E. coli* exists as Fenton-active Fe^{2+} [113]. Oxidative stress also causes release of iron from proteins (reactions 3 and 4), resulting in increased non-protein-bound iron concentrations [67, 88, 89, 117]. An increase in intracellular non-protein-bound iron concentration is associated with oxidative stress in humans and is also implicated in both Alzheimer's and Parkinson's diseases [118, 119], and cardiovascular disease [120]. In addition, even slightly elevated iron levels have been linked to increased cancer incidence in humans [48].

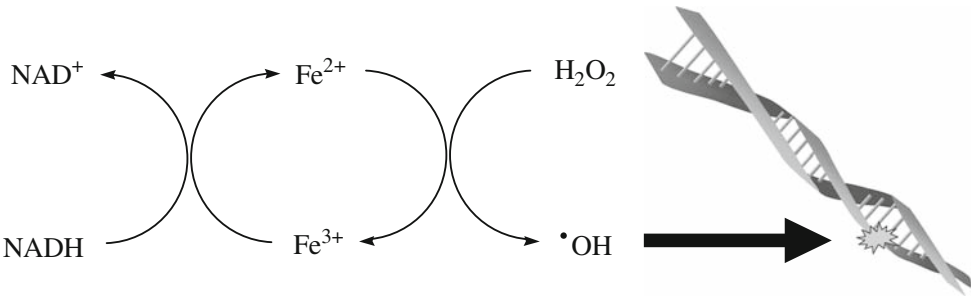

Fig. 4 Reduction of Fe^{3+} by cellular reductants (NADH shown) recycles iron for reaction with H_2O_2 in the Fenton reaction, leading to DNA damage

Damage to both nuclear and mitochondrial DNA occurs in cancer and other diseases linked to iron mis-regulation [121, 122]. Although nuclear DNA is packaged with histone proteins in chromatin, several studies have shown that oxidative damage to nuclear DNA occurs even in the presence of histone proteins; in fact, histone proteins can increase metal-mediated oxidative DNA damage because redox-active metal ions are associated with these proteins [123–126]. Mitochondrial DNA is particularly at risk for oxidative damage due to its proximity to respiratory processes that produce $O_2^{\bullet-}$, H_2O_2, and other ROS [127–129]. In fact, oxidative damage to mitochondrial DNA may actually be a more relevant cause of cell death than nuclear DNA damage because of this higher risk of damage [130, 131].

Because iron is a primary cause of ROS generation in vivo and because it plays such a pivotal role in contributing to oxidative stress, DNA damage, and cell death, iron has been the target of many antioxidant therapies. Due to their ability to coordinate iron, polyphenols are one large class of antioxidants that has been extensively examined for treatment and prevention of conditions associated with iron-generated ROS and oxidative stress.

Iron Binding by Catecholate, Gallate, and Semiquinone Ligands

Stability Constants for Iron–Polyphenol Complexes

It is well known that catechol and gallol (Fig. 1) and the many functionalized derivatives thereof (including most polyphenol compounds) are effective metal chelators When deprotonated, as is required for metal binding, catechol and gallol functionalities are referred to as catecholate and gallate groups, respectively. Metal ions that prefer octahedral geometry, such as Fe^{2+} and Fe^{3+}, can coordinate up to three catecholate or gallate groups (Fig. 5). Because of this, it might be expected that polyphenols with catechol or gallol groups would always bind iron in a 3:1 fashion (Fig. 5). However, since polyphenol compounds are so structurally varied and the complexes formed are pH dependent, they often exhibit variable coordination modes. Despite K_a values in the range of 7–9 for the most acidic phenolic hydrogen, polyphenols are easily deprotonated at or below physiological pH in the presence of iron and form very stable complexes [132]. Because iron binding has been proposed as a mechanism for polyphenol antioxidant activity, stability constants for polyphenol–iron interactions provide insight into their antioxidant behavior. For the purposes of this review, "stability constant" is defined as the equilibrium constant, K, of one or more polyphenols binding to an iron ion in aqueous solution. Multiple equilibrium constants (K_1, K_2, and K_3) arise when several polyphenol ligands are bound to one iron center. The product of the individual equilibrium constants is referred to as the overall stability constant of the complex, β.

Deprotonated polyphenol ligands behave as hard Lewis bases, and give rise to particularly large metal-binding stability constants with hard Lewis acids, such as Fe^{3+} [133]. In particular, catecholate complexes of Fe^{3+} often have extremely large stability constants ($\log \beta \sim$ 40–49) when three catecholate groups are bound to one iron center [134, 135]. Despite the importance of iron binding by polyphenols, only a handful of stability constants are reported for catecholate and gallate complexes of Fe^{3+}, other than siderophore complexes (both naturally occurring and synthetic). Fe^{2+}, in contrast to Fe^{3+}, is a borderline Lewis acid, and does not bind as strongly to the hard oxygen atoms of polyphenol ligands. The stability constant for Fe^{2+} monocatecholate is 7.95, much lower than the

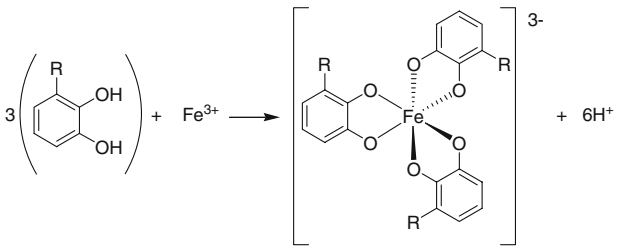

Fig. 5 Expected octahedral coordination geometry of general iron–polyphenol complexes. Gallols, R=OH; catechols, R=H. Coordination requires deprotonation of the polyphenol ligands

Fe^{3+} monocatecholate complex (20.01) [136]. In addition to catechol [136, 137], stability constants for the Fe^{2+} complexes of quercetin and 1,2-dihydroxynaphthalene-4-sulfonate have been reported [138, 139], as well as Fe^{2+} complexes of gallic acid and *n*-propyl gallate (Fig. 6) [137, 140], with limited data for the latter two compounds (Table 1). The scarcity of Fe^{2+}-polyphenol stability constants is most likely due to the fact that performing these measurements requires oxygen-free conditions to prevent oxidation of Fe^{2+} to Fe^{3+}. Log K and β values for catecholate and gallate complexes with iron are given in Table 1, and structures for selected ligands are shown in Fig. 6.

Because polyphenol ligands strongly stabilize Fe^{3+} over Fe^{2+}, catecholate and gallate complexes of Fe^{2+} rapidly oxidize in the presence of O_2 to give Fe^{3+}-polyphenol complexes, a process commonly referred to as autooxidation (Fig. 7a) [141–145]. Typically, Fe^{2+} oxidation occurs slowly in the presence of O_2, but binding of polyphenol ligands to Fe^{2+} lowers the reduction potential of iron [146] and enhances the rate of iron oxidation [137, 143, 147]. This iron oxidation rate varies for polyphenol complexes, with gallate complexes having faster oxidation rates than catecholate complexes [148]. This oxidation of Fe^{2+} to Fe^{3+} upon binding to polyphenol ligands is facilitated by the greater stability of the harder Fe^{3+} metal ion interactions with the hard oxygen ligands of the polyphenol moieties as well as the strongly electron-donating properties of the oxygen ligands that stabilize the higher iron oxidation state. Fe^{2+} autooxidation is not unique to polyphenol ligands; however, this phenomenon has been noted in the presence of various anions such as hydroxide [149], pyrophosphate and phosphate [150], chloride [151], sulfate [152], and perchlorate [153], with the rate of Fe^{2+} autooxidation dependent on the counterion of the Fe^{2+} salt.

Reduction of Fe^{3+} by Polyphenol Ligands

Upon binding of a catecholate or gallate ligand to Fe^{3+}, the polyphenol can reduce the iron to Fe^{2+}. The polyphenol is oxidized to a semiquinone during this process (Fig. 7b) [132, 154–158]. At low pH, the semiquinone ligand is

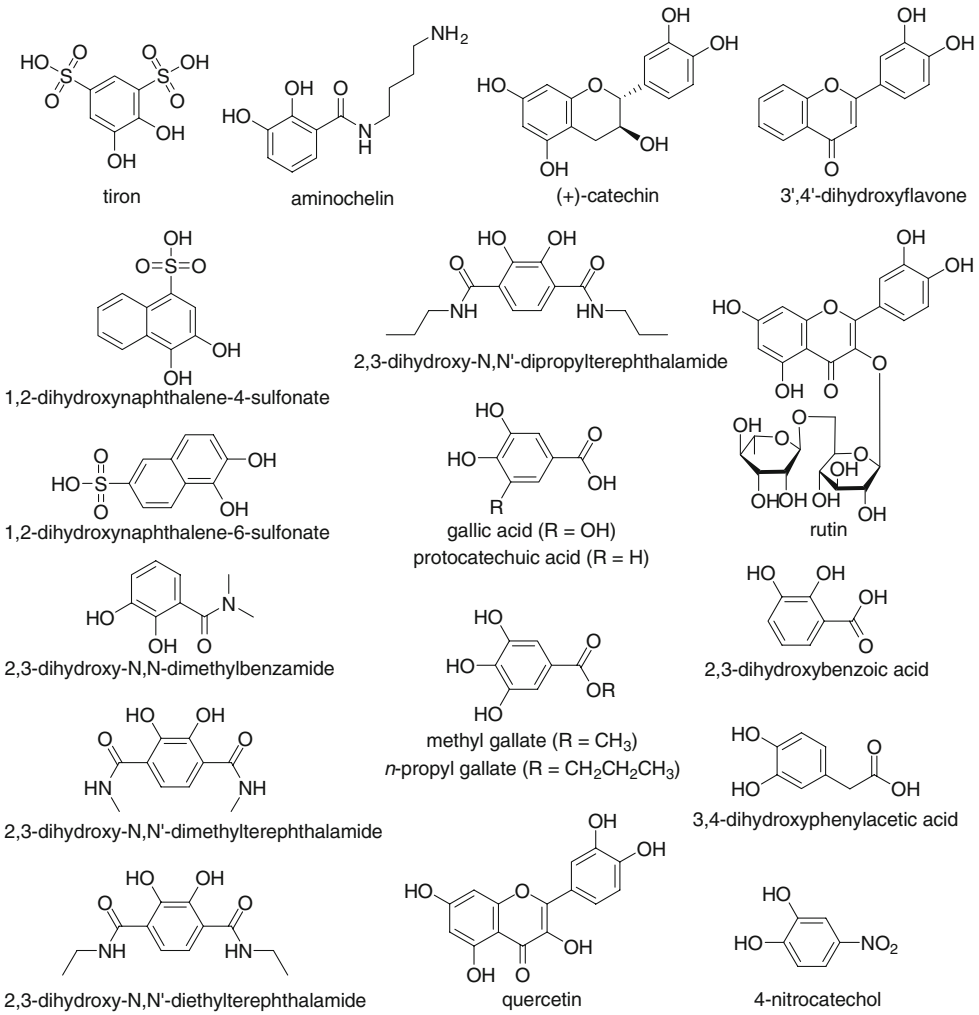

Fig. 6 Structures of polyphenol compounds for which iron-binding stability constants have been measured

Table 1 Reported stability constants for catechol and gallol polyphenol ligands with both and Fe^{2+} Fe^{3+}

Compound	Fe^{2+}, n =	Log K_1	Log K_2	Log K_3	Log β	Reference
1,2-Dihydroxynaphthalene-4-sulfonate	2	8.99	7.5		16.49	[139]
	3	19.84	15.11		34.95	[139]
1,2-Dihydroxynaphthalene-6-sulfonate	3	19.88	14.47	9.87	44.22	[297]
2,3-Dihydroxybenzoic acid	3	7.7[a]	4.0[a]	24.0[a]	35.7[a]	[135]
2,3-Dihydroxy-*N*,*N*′-diethylterephthalamide	3	16.22	14.32	11.48	42.02	[298]
2,3-Dihydroxy-*N*,*N*-dimethylbenzamide	3	17.8	13.9	8.5	40.2	[298]
	3				39.8	[299]
2,3-Dihydroxy-*N*,*N*′-dimethylterephthalamide	3	16.37	14.40	10.91	41.68	[298]
2,3-Dihydroxy-*N*,*N*′-dipropylterephthalamide	3	16.0	15.17	11.97	43.14	[298]
3,4-Dihydroxyphenylacetic acid	3	14.85[a]	8.9[a]		23.75[a]	[135]
	3				43.9	[299]
3′,4′-Dihydroxy avone	3	20.87				[300]
4-Nitrocatechol	3	18.29[a]	14.17[a]	9.2[a]	41.66[a]	[135]
Aminochelin	3	19.06	11.71	10.54	41.31	[132]
(+)-Catechin	3	21.8	15.7	9.9	47.4	[301]
Catechol	2	7.95	5.55		13.5	[136]
	2	8.4				[137]
	3	20.01	14.69	9.06	43.76	[135]
3,3,4-CYCAM	3				40	[299]
Enterobactin	3				49	[134]
Gallic acid	2	7.0				[137]
	3	14	8.5	5	27.5	[180]
	3	11.15	8.53	4.42	24.09	[302]
LYSCAM	3				46	[303]
MECAM	3				46	[299]
Methyl gallate	3	12.4	11.7	9.93	34.03	[302]
n-Propyl gallate	2	9.1				[140]
Protocatechuic acid	3	19.50	14.32	8.70	42.52	[185]
Protochelin	3				44.6	[132]
Quercetin	2	9.44	3.86		13.3	[138]
	3				44.2	[187]
Rutin	3				44.1	[187]
Tiron	3	20.07[a]	15.01[a]	9.8[a]	44.88[a]	[135]
	3	20.7	15.2	11	46.9	[297]
	3	20.4	15	10.4	45.8	[136]
TRENCAM	3				43.6	[304]
TRENCAMS	3				43.6	[305]

[a] Listed values here are the averages of either two or three trials reported separately in the referenced publication

protonated and is therefore a neutral ligand [132]. According to Basolo and Pearson [159], Fe^{2+} is stabilized relative to Fe^{3+} by neutral unsaturated ligands due to the greater crystal eld stabilization of a d^6 electronic con g-uration (Fe^{2+}) than that of a d^5 system (Fe^{3+}). These Fe^{2+}-semiquinone complexes are green in color, likely due to stabilization of the semiquinone radical by the aromatic ring, and may often be mistaken for mono(polyphenol)-Fe^{3+} complexes at low pH. However, the presence of Fe^{2+} in these semiquinone complexes has been con rmed by both Mössbauer spectroscopy [160] and magnetic moment measurements [161].

Once the semiquinone form of the polyphenol is generated, it is capable of reducing another equivalent of Fe^{3+}, simultaneously oxidizing the semiquinone to the quinone (Fig. 7b) [132, 154–158]. The studies investigating this Fe^{3+} reduction behavior are performed at very low pH [1–3], which may be relevant for processes occurring during digestion in the stomach. At higher pH, the formation of bis- and tris-polyphenol complexes with iron

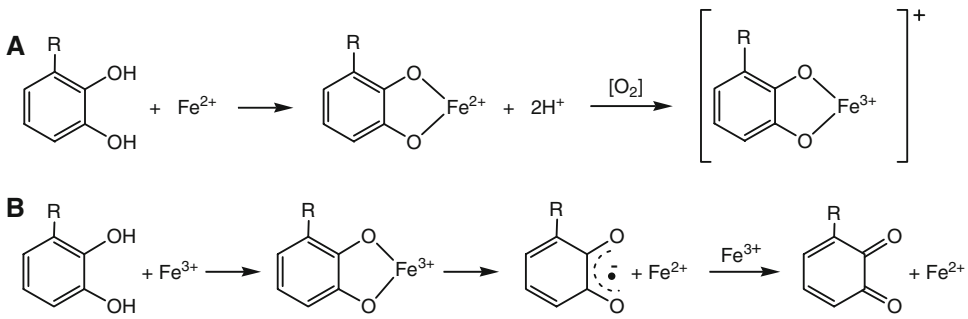

Fig. 7 a Coordination of Fe^{2+} by polyphenols and subsequent electron transfer reaction in the presence of oxygen generating the Fe^{3+}-polyphenol complex; b Coordination of Fe^{3+} by polyphenols, subsequent iron reduction and semiquinone formation, and reduction of Fe^{3+} to form a quinone species and Fe^{2+}. R=H, OH

(two and three polyphenol ligands coordinated to a single iron, respectively) inhibit these Fe reduction processes [155], so such reactions may occur much more slowly around pH 7. Nonetheless, this process of iron reduction is often attributed to both antioxidant and prooxidant activity of these compounds [154, 162]. Reduction of Fe^{3+} generates Fe^{2+} that can participate in the Fenton reaction and cause ROS generation [163–166]. This topic as it relates to DNA or cellular damage is discussed more in depth in the section on prooxidant activity of polyphenols.

Crystal Structures of Iron–Polyphenol Complexes

A number of X-ray structures of Fe-polyphenol complexes have been published, with the majority of these synthesized as structural and functional models of catechol dehydrogenase enzymes (summarized by Yamahara et al. in an excellent review) [167]. Because of their similarity to the enzyme active site, most of these synthetic structures contain catecholate ligands. Very few structures of gallate complexes with iron have been reported [168, 169], possibly due to their ability to form complexes with varying stoichiometry. Nuclearity of iron–polyphenol complexes with catecholate or gallate ligands ranges from mononuclear [170–174], to dinuclear [175] and supramolecular clusters [176], to extended polymeric structures [168, 169].

Stoichiometry and UV–Vis Spectroscopy of Iron–Polyphenol Complexes

Determining the binding interactions between polyphenol compounds and iron is vital to understanding their behavior. Generally, around physiological pH (7.2), a mixture of both the 2:1 and 3:1 ligand to metal species exist in solution, depending on the polyphenol compound and its stability constant with the metal [177]. It should be noted that changes in the ratio of metal to polyphenol as well as the pH can significantly change the coordinated species in solution. At slightly acidic pH (5–6.5), iron is bound by two catecholate or two–three gallate ligands per metal ion [138, 178–180], giving blue-purple Fe^{3+} complexes with molar extinction coefficients on the order of 10^3 for their ligand-to-metal charge transfer (LMCT) bands [137, 179, 181, 182], and λ_{max} values ranging from 542 to 561 nm for gallates, or 561 to 586 nm for catecholates (Fig. 8) [181]. At more acidic pH (<4), polyphenols bind iron in a 1:1 ratio [140, 183, 184]. Fe^{3+} monocatecholate complexes are often blue-green, with λ_{max} values ~ 670 nm [147, 185]. At alkaline pH, the octahedral tris(polyphenol) complexes of Fe^{3+} predominate (Fig. 5), which are red in color [147]. Fe^{2+} complexes of polyphenol ligands are colorless in the absence of oxygen [137, 177, 181].

Flavonols, such as quercetin (Q) and myricetin (Myr), display unique absorbances in the UV–vis spectra because these compounds are colored due to their extended conjugation. In addition, flavonols possess a second iron-binding site between the carbonyl oxygen at the 4-position and either the 3-OH or 5-OH groups as well as the catechol or gallol moiety. At pH 6.0, iron complexes of Q have absorbance maxima at 407 and 548 nm, and iron complexes of Myr have absorbances at 443 and 589 nm [181].

Stoichiometry of polyphenol ligands to iron often varies in solution, as measured by Job's method. For example, quercetin (Q) has been shown by de Souza et al. [186] to

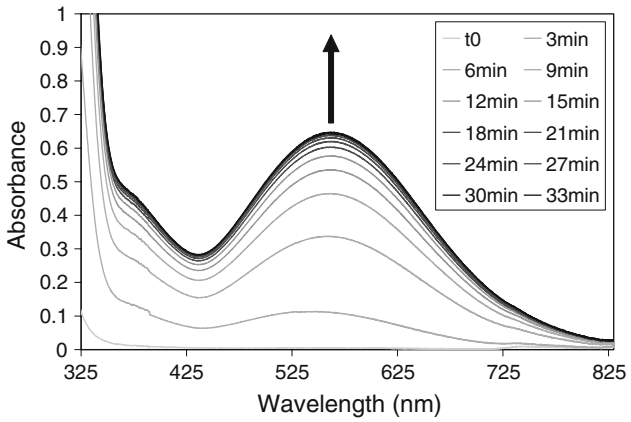

Fig. 8 Autooxidation of Fe^{2+} (145 μM) by O_2 upon binding of methyl-3,4-dihydroxybenzoate (MEPCA, 435 μM) as measured over time by UV–vis spectroscopy. Spectra were taken every 3 min after addition of Fe^{2+}. Sample was prepared in MES buffer (50 mM, pH 6.0)

bind iron in a 1:2 fashion in methanol, with a formula of $[Fe_2(Q)(H_2O)_8]Cl_2$ when synthesized from Fe^{2+} chloride tetrahydrate. However, rutin (Rut; quercetin-3-rutinoside) binds iron in a 2:3 ratio in methanol, with the formula $[Fe_3(Rut)_2(H_2O)_{12}]Cl_2$. Stoichiometry of products were confirmed by elemental analysis and ^1H NMR spectroscopy, with the Q complex showing iron bound not only at the catechol oxygens in the B ring, but also between the 3-OH and the 4-carbonyl oxygen of the C ring. ^1H NMR spectra of the rutin complex indicated iron ions bound to the two catechol groups of the B rings and an additional iron, likely bound at the 7-OH groups of ring A between two Rut molecules (Fig. 9) [186].

In contrast to the results reported by de Souza et al., Escandar and Sala reported only 1:1 and 2:1 metal-to-ligand complexes between rutin and iron (depending on pH), with no experimental evidence for the existence of the bridging iron at the 7-OH groups. Instead, Escandar and Sala proposed coordination of iron between the 4-carbonyl oxygen and the 5-OH group on the rutin molecule. However, they reached similar conclusions for the iron coordination of quercetin, stating that coordination occurred at both the catecholate group and between the 3-OH and 4-carbonyl oxygen in a 2:1 metal to ligand ratio, based on potentiometric and spectrophotometric results [187]. The conflicting results obtained for iron–rutin complexes illustrate the difficulty of determining iron coordination for polyphenol compounds with multiple iron binding sites.

Mechanisms of Polyphenol Antioxidant Activity

Cytoprotective Effects of Polyphenols Related to Iron-Binding

Iron is implicated in many oxidative-stress-related pathways and conditions, and is the primary generator of $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$ (reactions 1 and 3) that damages DNA and other biomolecules [48, 109, 110]. Therefore, understanding the biochemistry of iron has been the focus of many experiments aimed at preventing, inhibiting, or intercepting the harmful products of oxidative stress. Despite the strong iron-binding properties of polyphenols, whether the iron-chelating ability of catechol or gallol containing polyphenols actually plays a key role in their antioxidant activity is a matter of some debate. Kawabata et al. and Yoshino and Murakami have shown that iron chelation by several polyphenols protects rat microsomes from lipid peroxidation by blocking the Fenton reaction [142, 143]. Yet van Acker et al. have reported that iron chelation by several different polyphenols does not play a significant antioxidative role in microsomal lipid peroxidation using UV–vis spectroscopy, and they have noted that "there are, however, several contradictions in the literature, and the outcome largely depends on the experimental conditions and the type of assay used" [188]. Sugihara et al. [189] showed concentration-dependent antioxidant and prooxidant activity for several flavonoid compounds in an iron-induced lipid peroxidation system with cultured hepatocytes. Under the same conditions, however, they also found that catechins were antioxidants at all concentrations, and attributed the antioxidant behavior to iron chelation [190]. Generally, iron chelation by polyphenols is attributed solely to antioxidant rather than prooxidant effects. Morel et al. [191, 192] have shown that the ability of polyphenols to chelate and remove iron from iron-loaded hepatocytes correlates with cytoprotective effects of these compounds, and Ferrali et al. [193] have shown that quercetin (Q, Fig. 6) protects mouse erythrocytes from iron-mediated lipid peroxidation by binding iron. Similarly, Anghileri and Thouvenot [194] have also shown that polyphenols from mate tea, green tea, and red wine extracts protect against iron-induced lipid peroxidation of mouse liver tissue suspensions.

Sestili et al. [195] observed a dose-dependent cytoprotective effect for Q on human leukemic cells (U937) exposed to *t*-butyl hydroperoxide (*t*-BuOOH). They also determined an IC_{50} for Q of 12.67 ± 0.86 μM, for inhibition of H_2O_2-mediated cytotoxicity. Cells incubated with desferrioxamine (DFO, a known cell-permeable iron-

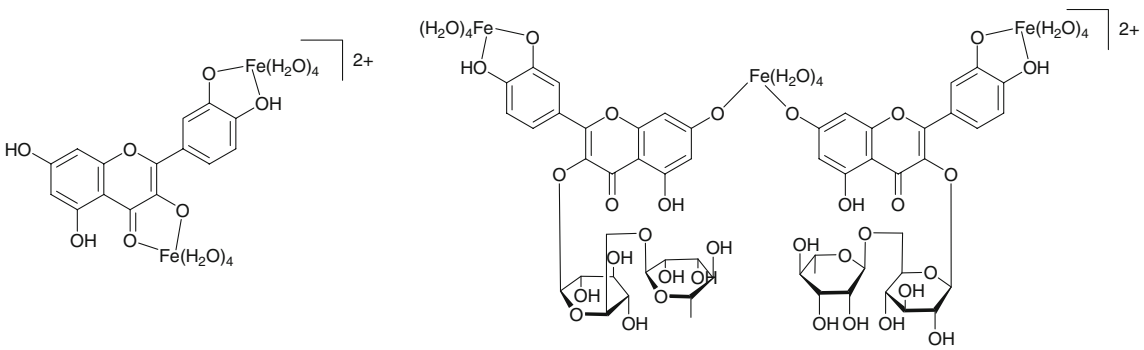

Fig. 9 Structures of the iron–quercetin (left) and iron–rutin (right) complexes proposed by de Souza et al.

chelator) showed the same inhibitory effect on DNA strand scission as Q, so Sestili et al. [195] inferred that iron-chelation was responsible for prevention of nuclear DNA damage by Q. Using similar methods, Sestili et al. [196] tested a variety of polyphenols and reported that only those compounds with catechol groups displayed both cytoprotective effects and DNA damage inhibition. Once again, these results suggested that iron binding contributes to the antioxidant activity of these compounds. Antioxidant activity of these compounds also correlated to their lipophilicity (*C*log*P*), suggesting that a combination of iron-binding ability and the ability to cross cell membranes contribute to the antioxidant activity of polyphenol compounds [196]. Similar correlations between polyphenol iron-chelating ability and lipophilicity on prevention of DNA damage in H_2O_2-treated Jurkat cells were also noted by Melidou et al. [197].

Recently, Garcia-Alonso et al. [10] also studied the ability of a polyphenol-rich fruit juice blend to protect U937 cells from cell death by *t*-BuOOH. The blend of grape, cherry, and berry juices inhibited DNA damage similarly to the iron chelator *ortho*-phenanthroline so iron-chelation by the juice polyphenols was inferred to be the cause of antioxidant activity [10]. Boato et al. [198] observed that fruit juices high in polyphenols (>1 mg/ml), such as red grape and prune juices, limited bioavailability of iron for human colon cancer cells (Caco-2) in iron-enriched medium. Because juices low in polyphenols (<1 mg/ml) increased iron bioavailability, this reduced bioavailability was attributed to iron–polyphenol coordination preventing absorption by the cells. The increase in iron bioavailability was correlated to high ascorbate/polyphenol ratio, since ascorbate is known to reduce Fe^{3+} to the more water-soluble Fe^{2+}. Thus, Boato et al. [198] suggested a dietary balance of juices containing high ascorbate concentrations (for proper iron uptake) and high polyphenol content (for their cancer preventative properties). Several other groups have shown that polyphenols have a significant inhibitory effect on the bioavailability of iron not only in vitro [199–201], but also in rats [202], and in humans [203, 204]. This effect is usually assumed to occur either pre-ingestion (i.e., due iron-binding in the food or beverage) or in the gut, not after iron and polyphenols are separately absorbed or metabolized. The general bioavailability of polyphenol compounds, not related to iron, is an active research area [205, 206], but is beyond the scope of this review.

Kuo et al. [207] also examined the effects of polyphenols on Caco-2 cells but from a different perspective: metallothionein (MT) expression. MT is a sulfur-rich protein, which helps to control oxidative stress and heavy metal toxicity in vivo by chelating metal ions, including lead, mercury, cadmium, copper, and zinc. Kuo et al. proposed that polyphenols might upregulate or downregulate MT expression, since polyphenol–metal chelation might prevent metal-mediated damage to biomolecules, resulting in lower MT levels. If the polyphenols themselves upregulate MT expression, increased MT levels could bind potentially damaging metal ions before they disrupt cellular function [207]. Using a ^{109}Cd-binding assay to determine MT expression, Kuo and coworkers observed that upon addition of the avonoid quercetin, expression of MT was decreased in a dose- and time-dependent manner. On the other hand, kaempferol, genistein and biochanin-A increased expression of MT, and avone, catechin, and rutin had no effect on MT levels (Fig. 10). Although these results were primarily attributed to the ability of these compounds to bind either Cu^{2+} or Zn^{2+} (two metals that increase MT expression) [208–210], UV–vis spectroscopy also indicated interactions with Mn^{2+}, Fe^{2+}, and Fe^{3+} for some of these polyphenols. Interestingly, none of the polyphenol compounds that increased MT expression were shown to bind to metal ions under their experimental conditions [207].

In addition, Anghileri and Thouvenot reported that mate tea and green tea extracts and, to a lesser extent, red wine polyphenols, prevented iron-dependent calcium uptake in mouse liver tissue suspensions. They reasoned that iron binding by polyphenols limited the bioavailability of iron, thus inhibiting iron-dependent calcium uptake [194].

It is clear that polyphenols have varied cellular effects due to their metal-binding properties. A substantial body of work suggests that iron-binding by polyphenol compounds results in cytoprotective effects, and that both iron binding and lipophilicity are important factors contributing to overall antioxidant activity. However, de nitive correlations between results from cell studies and prevention of oxidative stress-related diseases in animal or human clinical trials have not been established. Further investigation of indirect modes of polyphenol antioxidant activity, such as limitation of iron bioavailability and gene regulation, in animals or humans is also warranted.

Protective Effects of Polyphenols in Blood and Plasma

Although iron homeostasis in the blood is tightly regulated, red blood cells may become a target of oxidative damage in diseases disrupting normal hemoglobin function, such as β-thalassemia, sickle cell anemia, and other hemoglobinopathies, due in large part to their high non-protein-bound iron content [211–214]. Since plasma concentrations of polyphenols in humans commonly reach 1–10 μM [25–29], these compounds might be expected to have a protective effect on red blood cells. Grinberg, et al. have reported such protective effects for polyphenols in green and black tea against red blood cell lipid peroxidation, noting a

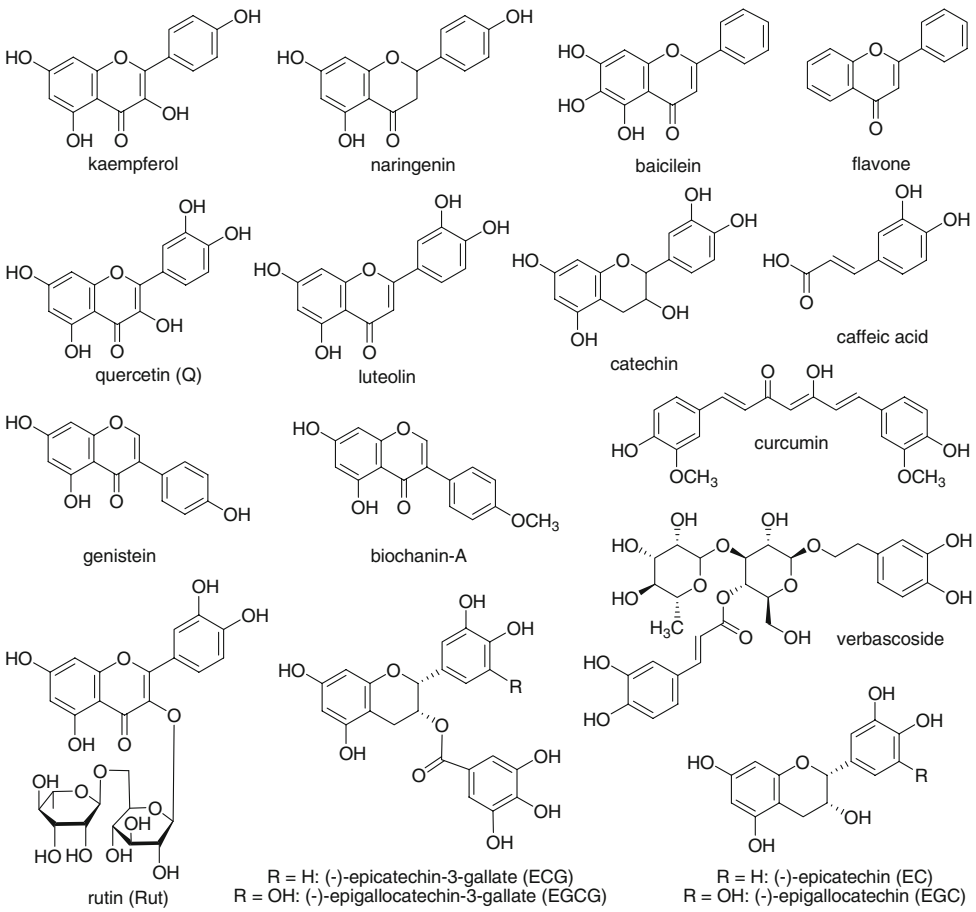

Fig. 10 Structures, names, and abbreviations of selected polyphenol compounds mentioned in this review that have been tested for antioxidant activity

concentration-dependent inhibitory effect on $^{\bullet}OH$ production, and cytoprotection of red blood cells in vitro from primaquine-induced oxidative stress due to $O_2^{\bullet-}$ and H_2O_2 generation. These effects were attributed to iron-chelation by the tea polyphenols [215].

Similarly, Srichairatanakool et al. [216] found protective effects for green tea polyphenols against iron-overload symptoms in β-thalassemia patients and correlated these effects with both non-protein-bound iron chelation in thalassemic plasma, as well as radical scavenging, as measured by the TEAC assay. They also noted that polyphenols do not result in the adverse affects of typical iron-overload treatments, as DFO or deferiprone do. The individual green tea polyphenols (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG) (Fig. 10) also prevented oxidative stress in iron-treated erythrocytes similarly to the results observed for green tea [217].

Polyphenols Protect Against Neurodegenerative Diseases

A number of studies have linked iron homeostasis disruption to neurodegenerative diseases. Iron has been shown to accumulate in degenerating neurons [218], and also induces aggregation and deposition of peptides such as amyloid beta ($A\beta$) [219] and α-synuclein [220, 221] in the brain, linking this metal to both Alzheimer's and Parkinson's diseases. Some comprehensive reviews of the protective effects and challenges of using green tea polyphenols for prevention or treatment of neurodegenerative diseases have been published by Singh et al. and Pan et al. [222, 223].

Green tea catechins are able to cross the highly selective blood-brain barrier (BBB) and protect against iron-induced neurodegeneration in mice [40, 224], as measured by their down-regulation of the amyloid precursor protein (APP) in the hippocampus region of the brain. APP can be converted to $A\beta$ in the presence of divalent metal ions (such as Fe^{2+} and Cu^{2+}), generating neurotoxic amyloid brils [225]. Mandel et al. [226] have written an excellent review summarizing the neuroprotective effects of green tea polyphenols related to iron chelation.

Guo et al. [227] reported that green tea polyphenols protect brain synaptosomes against iron-induced lipid peroxidation. Both green tea and wine polyphenols were shown to inhibit aggregation and accumulation of β A brils [228], and to protect against βA neurotoxicity [229], perhaps as an indirect result of their iron-chelation ability.

Curcumin (Fig. 10), a polyphenol from turmeric, inhibits neurodegeneration in a mouse model, likely through a similar mechanism to green tea catechins [230, 231].

Additional work in the area of iron-mediated neurodegeneration prevention comes from Oboh and Rocha [232], who have used the thiobarbituric acid reactive species (TBARS) assay on homogenates of brain and liver cells of rats. TBARS is a common antioxidant assay that uses UV–vis spectroscopy to monitor the products of lipid peroxidation as they react with thiobarbituric acid and generate a colored species [232]. Oboh and Rocha's results show that polyphenols isolated from red pepper inhibit Fe^{2+}-mediated lipid peroxidation in both brain and liver cells by an iron-chelating mechanism, although iron chelation by the polyphenols was measured separately by UV–vis spectroscopy. In addition to iron binding, $^{\bullet}OH$ and $NO^{\bullet}$ scavenging were implicated as additional protective mechanisms for pepper polyphenols [233].

The interest in using antioxidant properties of polyphenols to prevent and treat neurodegenerative diseases [237] is relatively new, and only a small number of compounds have been tested for this activity. Additional polyphenols should be tested for their effects on prevention of oxidative stress-induced neurodegeneration by chelating iron and copper ions as well as their ability to cross the BBB. These compounds should also be examined for their ability to inhibit APP aggregation. Identifying specific polyphenol compounds that have BBB permeability and metal chelating activity may lead to new treatments for neurodegenerative diseases.

Assays Quantifying the Inhibition of Iron-Mediated DNA Damage by Polyphenols

Whole-cell assays involve many variables and can make it difficult to definitively attribute polyphenol antioxidant activity to metal binding. For this reason, many in vitro methods have been used to examine the iron-binding antioxidant mechanism of polyphenols in order to correlate the results to those observed in biological systems. Two common methods for assessing inhibition of iron-mediated DNA damage by polyphenols are DNA gel electrophoresis and the deoxyribose assay, an assay that uses UV–vis spectroscopy to quantify malonaldehyde (a product formed from $^{\bullet}OH$ degradation of 2-deoxyribose) by its condensation reaction with thiobarbituric acid [182, 234, 235]. The major benefit of the deoxyribose assay is that it allows for faster screening of compounds, but its conditions do not closely resemble those of biological systems: the substrate is not truly DNA, but only 2-deoxyribose. In addition, many Fe^{3+}-polyphenol complexes absorb at or near the wavelength of the deoxyribose degradation product (532 nm), making this technique inherently problematic for measurements of polyphenol inhibition of iron-mediated damage. Therefore, gel electrophoresis is perhaps the best technique because it both directly determines DNA damage and closely simulates biologically relevant conditions (such as pH, buffering capacity, and ionic strength).

Romanová et al. [236] have shown prevention of iron-mediated DNA damage for three flavonoid compounds (including quercetin), citing their iron-chelation ability, measured by UV–vis spectroscopy, as the most likely cause for DNA protection. In addition, Lopes et al. [182] used the deoxyribose assay to show that tannic acid prevents the Fenton reaction and protects 2-deoxyribose from oxidation by chelating Fe^{2+}. In contrast, Moran et al. observed prooxidant activity for polyphenol compounds, including gallic acid and methyl gallate (Fig. 6), using a modification of the deoxyribose assay. However, they used Fe^{3+}-EDTA in place of aqueous Fe^{2+}, and cited both the ability of polyphenols to reduce Fe^{3+} and EDTA blocking polyphenol chelation as likely causes of this prooxidant activity [237]. Prooxidant activity was also observed for polyphenols in a DNA damage assay using the Fe^{3+}-bleomycin complex as the iron source. Again, reduction of Fe^{3+}-bleomycin by polyphenols was described as the mechanism for prooxidant activity [237]. Laughtin et al. [165] also reported prooxidant activity for polyphenols in the presence of Fe^{3+}-EDTA/H_2O_2 and with the Fe^{3+}-bleomycin complex.

Using gel electrophoresis, both Zhao et al. and Perron et al. [181, 238] have studied the effects of polyphenols on plasmid DNA damage resulting from Fe^{2+} and H_2O_2. Zhao et al. [238] observed that verbascoside (Fig. 10) inhibited DNA damage from the Fenton reaction in a dose-dependent manner, and attributed this antioxidant activity to iron binding by the polyphenol. In an extension of this work, Perron et al [181] assayed 12 different phenolic compounds, showing that all of the compounds with catechol or gallol groups inhibited 50% of the DNA damage from Fe^{2+} and H_2O_2 (IC_{50}) at concentrations between 1–5 μM. Notably, the IC_{50} value for quercetin (10.8 ± 1 μM) was identical within error to the IC_{50} reported by Sestili et al. for inhibition of H_2O_2-mediated cytotoxicity in U937 cells. Perron et al. [181] also directly confirmed the necessity of iron binding for DNA damage prevention by showing that EGCG (Fig. 10) did not inhibit DNA damage when Fe^{2+} was coordinated with EDTA prior to addition of the polyphenol, although the Fe^{2+}-EDTA complex generated hydroxyl radical in the presence of H_2O_2.

Generally, polyphenols protect DNA from damage in systems involving Fe^{2+} at biologically relevant, low micromolar concentrations. While some experiments with Fe^{3+} have shown prooxidant activity of polyphenol compounds, it is expected that most non-protein-bound iron in cells is in the Fe^{2+} oxidation state [113]. Because research

in vitro is extremely promising, it will be important to con rm iron binding as a primary mechanism of antioxidant activity in animal models. The next logical step would be testing of polyphenol compounds in clinical trials for treatments or prevention of diseases attributed to iron-mediated DNA damage and other oxidative damage, such as cancer, cardiovascular diseases, and neurodegenerative diseases. One such clinical trial by the Mayo Clinic, in collaboration with the National Cancer Institute, is currently recruiting participants; their goal is to test green tea extract with high EGCG content in patients with chronic lymphocytic leukemia to observe the effects on this form of cancer [239].

In Vitro Iron-Binding Structure–Activity Relationships of Polyphenols

Because of the immense variety and many different classes of polyphenol compounds, determining structure–activity relationships (SARs) for antioxidant properties of polyphenols is a challenging undertaking. For identi cation of effective polyphenol antioxidants, determining SARs for these compounds is required to realize their potential to treat and prevent diseases caused by oxidative damage.

Cheng and Breen have used cyclic voltammetry to show that four polyphenol compounds, baicilein, naringenin, luteolin, and quercetin (Fig. 10) effectively suppress reduction of H_2O_2 by the Fe^{2+}-ATP complex. The two compounds with catechol moieties on the B ring (luteolin and quercetin) are more potent inhibitors of the Fenton reaction than the two compounds without catechols (baicilein and naringenin) [240]. Based on the large stability constants for iron–catecholate complexes, it would seem, therefore, that iron binding at the catecholate group may be responsible for the greater antioxidant activity observed for luteolin and quercetin. However, baicilein has a gallol group on the A ring rather than the B ring, which could also bind iron. Since baicilein was a weaker antioxidant in this system, Cheng and Breen concluded that substituents on the B ring more signi cantly affect antioxidant activity.

The higher antioxidant activity of phenol substituents on the B ring as compared to the A ring was con rmed by Jovanovich et al. [241], as well as Arora et al. [242], who showed that a catechol group on the B ring gives rise to iron binding and antioxidant activity. In addition to reporting that compounds with no phenol groups had negligible antioxidant activity in a lipid peroxidation model evaluating antioxidant activities of polyphenols, Arora et al. stated that phenol substituents on the A ring contribute little to the antioxidant activity of polyphenols. However, in this study they also tested one compound (7,8-dihydroxy avone) with a catechol substituent on the A ring that had similar antioxidant activity to compounds with B ring catechol substituents. Thus, the lower antioxidant activity of A ring polyphenols compared to B ring polyphenols may not apply universally.

Khokar and Apenten [243] compared polyphenol compounds with catechol and gallol moieties on the B ring (as well as tannic acid), and concluded that these structural motifs are optimal for iron binding and antioxidant activity in vitro. They also proposed that the presence of a hydroxy-keto group (a 3-OH or 5-OH plus a 4-C=O), as well as a large number of catechol/gallol groups (as in the case of tannic acid, Fig. 11), also contributes to iron-binding and antioxidant activity [243].

By quantifying a representative selection of polyphenol compounds for prevention of iron-mediated DNA damage, Perron et al. [181] determined that compounds with gallol groups were more potent antioxidants than those with only catechol groups. Furthermore, the IC_{50} values for twelve polyphenols reported by Perron and coworkers correlated to the pK_a value of the most acidic phenolic hydrogen of the polyphenol compounds (Table 2), representing the first predictive model of antioxidant potency as a function of iron-binding ability (Fig. 12). This correlation, along with additional experiments, directly established iron binding as the mechanism of the observed antioxidant activity [181, 244]. This predictive model allows the library of polyphenols to be efficiently screened for those with the highest iron-binding antioxidant activity.

The SARs of polyphenols related to iron binding have generally been established for catechol and gallol containing compounds. In addition to these iron-binding groups, hydroxy-keto moieties may also contribute to antioxidant activity by binding iron. Although the

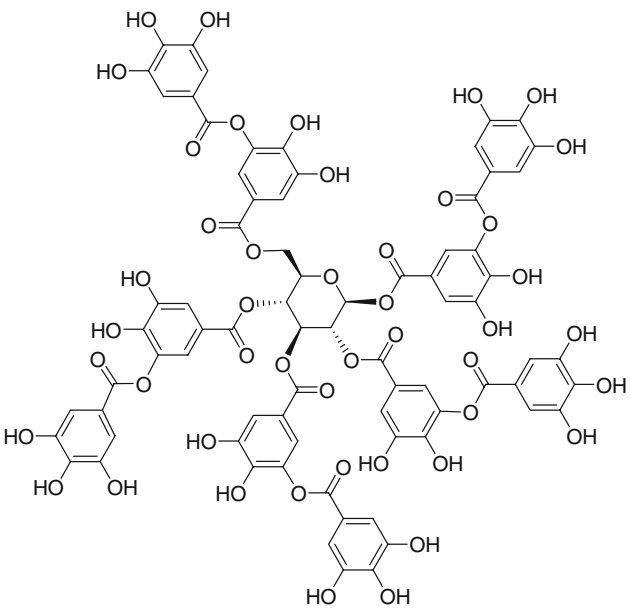

Fig. 11 Structure of tannic acid

important iron-binding functional groups are known, better predictive models must now be developed to screen the enormous number of polyphenol compounds based on their physical and chemical properties (lipophilicity/bioavailability, molecular weight, shape, π-acidity, etc.) for those with optimal antioxidant activity in vitro. After selecting the best candidates, the in vitro experiments should be followed by cell and animal studies, and eventually clinical trials for the most promising polyphenol antioxidants.

Polyphenols as Preservatives

Iron-mediated oxidative damage is not limited to living organisms. Due to the presence of iron in the environment, iron generated $^{\bullet}OH$ is also responsible for food spoilage and wood rotting [245, 246], and conflicting reports exist about whether polyphenols are suitable for use as preservatives of food and wood. Contreras et al. [247] have reported increased degradation of wood after treatment with several catechol polyphenols, including catechol, protocatechuic acid, and 2,3-dihydroxybenzoic acid (Fig. 6), and similar to compounds that have been isolated from brown-rot fungi [248]. Under mildly acidic conditions, these compounds were able to reduce the Fe^{3+} to Fe^{2+} in their mono (catecholate) complex forms [247]. Tiron (Fig. 6), however, was not shown to participate in the redox-cycling mechanism, as this catechol compound cannot reduce Fe^{3+} [249]. In contrast, Binbuga et al. [140] have shown preservation of wood using *n*-propyl gallate (Fig. 6), citing iron chelation as a likely mechanism even under the mildly acidic conditions present in wood. This preservative effect of *n*-propyl gallate was attributed primarily to its antifungal properties, since it may interfere with the Fenton reaction and redox cycles that wood-decaying brown rot fungi use to decompose wood [248, 250, 251].

In addition to wood preservation, polyphenol compounds have been widely studied for use as preservatives for food, cosmetics, and pharmaceuticals, with many patents for these applications both nationally and internationally related to their antioxidant properties [252–258]. The FDA has also approved *n*-propyl gallate for use as a food preservative [259]. However, most patents discussing polyphenol preservative mechanisms do not cite metal chelation as a cause of their antioxidant activity, instead claiming the radical scavenging ability often attributed to these compounds.

The formation of iron–polyphenol complexes has been attributed to negative aspects in foods as well, such as off-color development or browning of bruised or sliced fruits [260]. Similar polyphenol oxidation pathways occur in polyphenol oxidase and tyrosinase, two enzymes that utilize both a metal ion and O_2 to oxidize phenolic compounds [261, 262]. While these enzymes typically

Table 2 pK_a values for phenolic compounds and IC_{50} values for iron(II)/H_2O_2-mediated DNA damage inhibition by phenolic compounds[a]

Compound	IC_{50} (μM)	pK_{a1}^{b}	pK_a reference
(−)-Epigallocatechin-3-gallate (EGCG)	1.1	7.55 ± 0.03	[306]
(−)-Epicatechin-3-gallate (ECG)	2.3	7.6	[306]
		7.74	[307]
Methyl 3,4,5-trihydroxybenzoate (MEGA)	4.0	7.92	[62]
		8.03	[308]
Propyl gallate (PrEGA)	5.1	7.77 ± 0.04	[140]
(−)-Epigallocatechin (EGC)	9.83	8.51 ± 0.04	[306]
		7.87	[307]
Gallic acid (GA)	14.0	8.80 ± 0.02	[309]
		8.70 ± 0.02	[310]
		8.45 ± 0.06	[310]
Methyl 3,4-dihydroxybenzoate (MEPCA)	15.6	8.12 ± 0.18	[311]
Protocatechuic acid	34.4	8.90 ± 0.03	[309]
		8.79 ± 0.05	[136]
		8.64 ± 0.05	[310]
		8.81 ± 0.01	[312]
(−)-Epicatechin (EC)	59.1	8.76 ± 0.02	[306]
		8.72 ± 0.01	[312]
		8.91 ± 0.23	[313]
Vanillic acid (VA)	140.0	9.39	[314]
		9.391	[315]
		9.17	[316]

[a] IC_{50} values are taken from Perron et al. (reference [181]) and are all ± 1 μM

[b] pK_a values are for the most acidic phenolic hydrogen

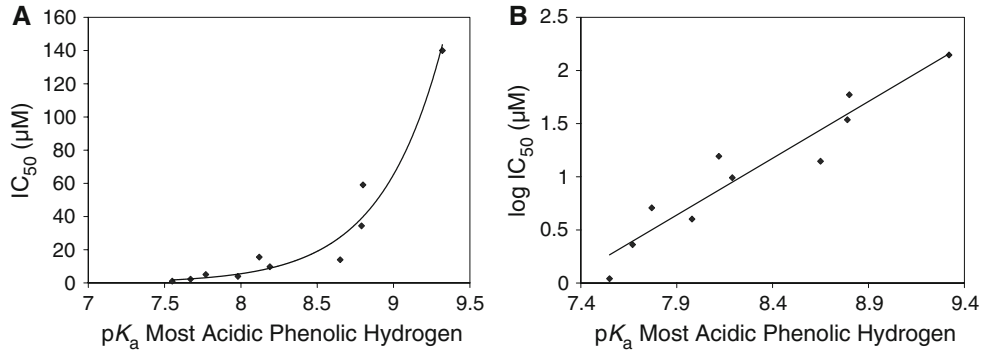

Fig. 12 a Graph of IC_{50} versus pK_a for the most acidic phenolic hydrogen showing the best- t exponential correlation to the data for polyphenols (*solid line*, $R^2 = 0.91$). The data points for myricetin and quercetin were omitted from the data set because of their non-gallol and non-catechol binding sites, respectively. Error bars for IC_{50} values are within the size of the data points. b Linear representation of the correlation between IC_{50} and polyphenol pK_a, shown by plotting log IC_{50} versus pK_a (*solid line*, $R^2 = 0.91$). pK_a values used in this gure are the averages of the literature values listed in Table 2

have copper-containing active sites, an iron-containing tyrosinase has been isolated from tea leaves [263].

Based on the limited research in this area, the use of polyphenol compounds as preservatives for food and wood seems promising, although selection of speci c polyphenol compounds with antioxidant properties will be important, since some appear to actually accelerate rotting processes under certain conditions. Therefore, as with polyphenol antioxidants for use in vivo, further testing is needed to determine both the conditions under which polyphenols have preservative effects, and the SARs of polyphenol compounds as related to antioxidant, preservative, and antibiotic properties.

Prooxidant Activity of Polyphenols Related to Iron Binding

Hydroxyl Radical Production by Iron–Polyphenol Complexes

Although the focus of this review is on the antioxidant activity of polyphenol compounds, several reports have described prooxidant behavior for polyphenols, and this activity must not be overlooked. Prooxidant activity may arise from the ability of polyphenol compounds to bind and reduce Fe^{3+} to the hydroxyl radical generating Fe^{2+}. EGCG and ECG, with two gallol groups or one gallol and one catechol group, respectively, have been reported to reduce up to four equivalents of Fe^{3+} in this manner [154].

In addition to the prooxidant activity observed for polyphenol reduction of Fe^{3+} (discussed in Reduction of Fe^{3+} by Polyphenol Ligands and Assays Quantifying the Inhibition of Iron-Mediated DNA Damage by Polyphenols sections), Hiramoto et al. [264], Moran et al. [237], and Ohashi et al. [144] have observed DNA strand scission by Fe^{3+}-polyphenol complexes in vitro, for gallic acid and the green tea compounds EGC and EGCG, among many others. Puppo [164] also observed an increase in $^{\bullet}OH$ production from the Fenton-type reaction of the Fe^{3+}-EDTA complex in the presence of polyphenols myricetin, quercetin, and catechin, among others. A review of the prooxidant and toxic effects of polyphenols both in vitro and in vivo was published by Schweigert et al. [166], primarily addressing copper-mediated prooxidant activity but also prooxidant activity with iron as well.

Prooxidant activity of polyphenol compounds is believed to arise from the ability of polyphenols to reduce Fe^{3+} or Cu^{2+} and the prevention of polyphenol binding by EDTA or other chelating ligand already present. Thus, experiments exploring the prooxidant properties of polyphenol compounds are typically performed either in the presence of metal chelators (such as EDTA, bleomycin, or ADP) [164, 165, 237, 265], that block chelation of iron by the polyphenol ligand, and/or often use oxidized metal ions, such as Fe^{3+} or Cu^{2+}. In these cases, the polyphenol compound can reduce the metal ion via an outer-sphere electron transfer, making it available for reaction with H_2O_2 or other sources of radicals.

In contrast to the prooxidant effects of polyphenols with Fe^{3+}-EDTA or Fe^{3+}-bleomycin complexes, Inoue et al. [266] have shown by gel electrophoresis that the polyphenol caffeic acid (Fig. 10) does not damage DNA in the presence of aqueous Fe^{3+} and H_2O_2. Once again, this implies that iron binding is required for polyphenol antioxidant activity, or at the very least, prevention of prooxidant activity.

It must be noted that the intracellular cytoplasmic environment is known to be quite reducing, due to the many reductants present inside the cell, such as NADH, glutathione, thioredoxin, ascorbic acid, and citric acid [114, 115, 267]. Thus, any non-protein-bound metal ions would

most likely be present in their reduced forms in vivo [13]. Due to the deleterious effects of prooxidant activity, however, it is essential to understand both the antioxidant and prooxidant behavior of polyphenol compounds.

It is interesting that polyphenol compounds can display both antioxidant and prooxidant activity under very similar conditions. Often these conditions are quite similar to those in biological systems, however, there are not usually ligands as strongly chelating as EDTA other than proteins in cells, and these are highly specialized and prevent iron-mediated prooxidant activity. Plus, iron is not usually found as Fe^{3+} in any appreciable intracellular concentration. Therefore, the conditions for polyphenol prooxidant activity are actually quite limited, often not biologically relevant, and therefore may be of less concern to humans who ingest these compounds in food sources. However, this body of work showing prooxidant activity for polyphenols when they cannot directly chelate iron does stress the essentiality of iron-binding as a viable antioxidant mechanism for these compounds.

Superoxide Dismutase-Like Activity of Polyphenols and Iron-Polyphenol Complexes

Polyphenol compounds and their complexes with Fe^{2+} are reported to react with superoxide ($O_2^{\bullet-}$) to form H_2O_2 and a semiquinone radical, resulting in superoxide dismutase (SOD)-like behavior [66, 186, 259, 268, 269]. The reactivity is similar to SOD, an enzyme present in all aerobic organisms, which catalyzes the dismutation of two $O_2^{\bullet-}$ anions under physiological pH into H_2O_2 and O_2 (reaction 7) [270].

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (7)$$

Since these reactions form H_2O_2, polyphenol compounds may contribute to cellular oxidative stress. An example of this proposed SOD-like activity was reported by de Souza et al. for the Fe^{2+}-quercetin complex. Upon deprotonation and iron binding, the oxidation potential of the flavonoid is decreased so that it is oxidized in the presence of $O_2^{\bullet-}$ to yield a Fe^{2+}-quercetin semiquinone radical complex and H_2O_2 [186] (Fig. 13). This mechanism was proposed from cyclic voltammetry measurements based on the absence of normally observed oxidation waves for the Fe^{2+}-quercetin complex in the presence of $O_2^{\bullet-}$, suggesting that the Fe^{2+}-quercetin complex scavenges $O_2^{\bullet-}$ radicals. However, the products of this reaction were not isolated or characterized, therefore, such reaction products must be further characterized to confirm the SOD-like mechanism for iron-polyphenol complexes.

Since Fe^{3+} from enzymes such as ferritin [88], hydrolyases [87], or dehydratases [87, 89], can be reduced by $O_2^{\bullet-}$ to generate Fe^{2+} (reactions 3–5), it has been hypothesized that H_2O_2 generated from SOD-like reactions could react with Fe^{2+} to form the more reactive hydroxyl radical ($^{\bullet}OH$) via the Fenton reaction (reaction 1) [271, 272]. If polyphenol compounds decompose $O_2^{\bullet-}$, they may directly prevent $O_2^{\bullet-}$ from reducing iron and subsequent iron release from proteins. Polyphenol compounds can also chelate Fe^{2+}, so the iron released from proteins may be bound by polyphenol compounds, resulting in an additional antioxidant mechanism as proposed by Reddan et al. [259]. This polyphenol-bound iron would not be available to react with H_2O_2, and thus the H_2O_2 would be decomposed by catalase or peroxidase enzymes, such as glutathione peroxidase [273–275].

The interactions between iron, polyphenols, and ROS and RNS are extremely complex, so it is necessary to explore these systems carefully, using diverse and complementary experimental techniques (studying the structure of iron–polyphenol complexes, for example, combined with their ROS reactivity as examined using EPR spectroscopy), to positively identify the mechanisms and products of these reactions.

Interactions Between Polyphenols and Copper

Although the purpose of this review is to highlight the iron binding properties of polyphenol compounds, due to similarities in copper-mediated ROS generation, the interactions between polyphenols and copper should not be ignored. Copper also generates $^{\bullet}OH$ in a Fenton-like reaction with H_2O_2, and this copper-generated $^{\bullet}OH$ can damage DNA (Fig. 14) [86, 276]. Superoxide also reduces Cu^{2+} to form H_2O_2 and Cu^+ (reaction 8) [277].

$$Cu^{2+} + O_2^{\bullet-} + 2H_2O \rightarrow Cu^+ + H_2O_2 + O_2 \quad (8)$$

Polyphenols have fairly strong binding interactions with Cu^{2+}, a borderline-hard Lewis acid similar to Fe^{2+}; in fact, stability constants for Cu^{2+} catecholate complexes are larger than for Fe^{2+} [136, 278]. However, polyphenol ligands are widely reported to reduce Cu^{2+} to Cu^+, a soft Lewis acid for which polyphenols have little affinity [133]. For this reason, stability constants for Cu^+-polyphenol complexes have not been reported. This weak interaction between polyphenols and Cu^+ may also explain why so little has been published on the antioxidant effects of polyphenols related to copper chelation. In contrast with other metal ions found in biological systems, Cu^{2+} has a positive reduction potential in aqueous solution, facilitating reduction of Cu^{2+} to Cu^+ and promoting Cu^{2+} binding to electron-rich ligands such as oxygen atoms [279]. This tendency toward copper reduction, coupled with the tendency of polyphenol compounds to oxidize results in

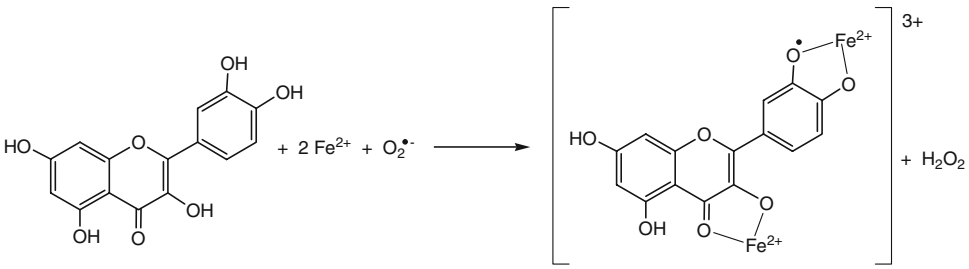

Fig. 13 SOD-like reactions of the Fe^{2+}-quercetin complex, as proposed by de Souza et al.

complex copper–polyphenol interactions, especially in the presence of ROS [280–282].

To the best of our knowledge, only Andrade et al. and Perron, et al. [283, 284] have reported antioxidant effects for protection of copper-induced DNA damage by polyphenols. Andrade et al. examined the antioxidant activity of tannic acid (Fig. 10) using the deoxyribose assay with Cu^{+}/H_2O_2 (see the Assays Quantifying the Inhibition of Iron-Mediated DNA Damage by Polyphenols section). From their results, they calculated an IC_{50} value of $5.3 \pm 0.8\ \mu M$ for tannic acid [283].

Using gel electrophoresis, Perron et al. tested 12 phenolic compounds for their effects on Cu^{+}/H_2O_2-mediated DNA damage and observed both antioxidant and prooxidant behaviors. They showed inhibition of copper-mediated DNA damage for EGCG (Fig. 10) resulting in an IC_{50} of $249 \pm 1\ \mu M$. In contrast, EC and EGC displayed prooxidant activity under the same conditions, whereas ECG displayed both prooxidant activity and antioxidant activity at low (0.1–4 μM) and high (10–1,000 μM) concentrations, respectively. Very similar experiments with Fe^{2+} instead of Cu^{+} resulted in an IC_{50} of $1.1 \pm 1\ \mu M$ for inhibition of iron-mediated DNA damage: nearly 250 times more potent. The greatly diminished antioxidant potency for polyphenols in the copper system was attributed to the weak interactions between polyphenols and Cu^{+}. Based on these experiments, a copper redox-cycling mechanism was proposed for the prooxidant activity observed for some polyphenols under these conditions [132, 276, 285, 286].

The prooxidant activity for polyphenol compounds under certain conditions has prompted some cautionary advice on consuming large amounts of these compounds [287, 288], but copper homeostasis is even more tightly regulated than iron; it has been estimated that the intracellular concentration of non-protein-bound Cu^{+} is less than 10^{-18} M in unstressed yeast cells, corresponding to less than one non-protein-bound Cu^{+} ion per cell [289]. Furthermore, compounds such as glutathione (1–15 mM) [122, 290] are present at much higher concentrations in vivo than those attainable by polyphenols (1–10 μM), and have much higher affinity for Cu^{+} than polyphenols. Recently, however, non-protein-bound copper pools have been found in the mitochondria and neuronal cells of higher organisms [291, 292], and mis-regulation of copper homeostasis results in higher cellular concentrations and increased oxidative stress [7, 293–296]. Therefore, elucidating the antioxidant and prooxidant mechanisms of polyphenol–copper interactions will be important for a complete understanding of polyphenol activity in vivo.

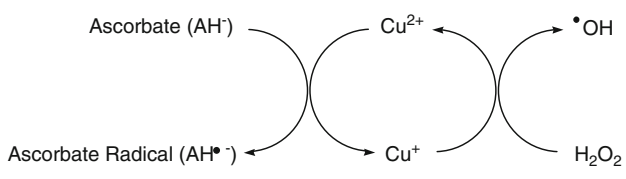

Fig. 14 The Fenton-like reaction with copper

Conclusions and Future Directions

Although both Fe^{2+} and Cu^{+} perform Fenton-like reactions with H_2O_2, polyphenol compounds containing metal binding catechol and gallol groups have very different activities, depending on the metal ion. Antioxidant activity is commonly observed for polyphenols in Fe^{2+}/H_2O_2 systems using a number of different assays, including cell studies and in vitro DNA damage inhibition experiments. In contrast, when testing polyphenols in Cu^{+}/H_2O_2 systems, greatly diminished antioxidant potency or even prooxidant activity is often observed from the interactions between polyphenols and copper. These findings stress the need for caution in experimental design: in addition to the polyphenol compounds, the metal ion must be specifically chosen to examine the metal–antioxidant interactions. Since results are highly dependent on experimental conditions, it should be the goal of researchers to design the most biologically relevant experiments possible.

Because iron-mediated damage to biomolecules such as lipids and DNA is implicated in disease development, the iron-chelating mechanism of polyphenol antioxidant activity must be fully explored in addition to radical scavenging to understand polyphenol antioxidant behavior. Polyphenols with gallol or catechol groups are generally the most potent antioxidants, primarily because of the large iron-binding stability constants for these groups. Also, compounds with the hydroxy-keto moiety can chelate iron, giving rise to antioxidant activity. As compared to radical

scavenging, however, the iron-binding mechanism for polyphenol antioxidant activity is relatively underdeveloped. Therefore, additional research is needed in several areas, including stability constant measurements for iron binding, pK_a measurements of polyphenols, and additional cell studies and DNA damage prevention experiments to correlate in vitro and in vivo antioxidant activity of these compounds. The goal of this work should be to develop biologically relevant predictive models and SARs for polyphenol compounds, as well as high-throughput screening methods for determining antioxidant (or prooxidant) behavior. These further studies will enable identi cation of highly effective polyphenol antioxidants for clinical trials to prevent or treat diseases caused by oxidative stress.

References

1. Kühnau, J. (1976). The avonoids: A class of semi-essential food components: Their role in human nutrition. *World Review of Nutrition and Dietetics, 24*, 117–191.
2. Sutherland, B. A., Rahman, R. M. A., & Appleton, I. (2006). Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. *The Journal of Nutritional Biochemistry, 17*, 291–306.
3. Cabrera, C., Artacho, R., & Gimenez, R. (2006). Bene cial effects of green tea—A review. *Journal of the American College of Nutrition, 25*, 79–99.
4. Gardner, E. J., Ruxton, C. H. S., & Leeds, A. R. (2007). Black tea—Helpful or harmful? A review of the evidence. *European Journal of Clinical Nutrition, 61*, 3–18.
5. Vinson, J. A. (1998). Flavonoids in foods as in vitro and in vivo antioxidants. *Advances in Experimental Medicine and Biology, 439*, 151–164.
6. Nardini, M., Cirillo, E., Natella, F., & Scaccini, C. (2002). Absorption of phenolic acids in humans after coffee consumption. *Journal of Agricultural and Food Chemistry, 50*, 5735–5741.
7. Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001). Phenol antioxidant quantity and quality in foods: Fruits. *Journal of Agricultural and Food Chemistry, 49*, 5315–5321.
8. Mertens-Talcott, S. U., Jilma-Stohlawetz, P., Rios, J., Hingorani, L., & Derendorf, H. (2006). Absorption, metabolism, and antioxidant effects of pomegranate (*Punica granatum* L.) polyphenols after ingestion of a standardized extract in healthy human volunteers. *Journal of Agricultural and Food Chemistry, 54*, 8956–8961.
9. Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Feng, L., Dreher, M., et al. (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of Agricultural and Food Chemistry, 56*, 1415–1422.
10. Garcia-Alonso, F. J., Guidarelli, A., & Periago, M. J. (2007). Phenolic-rich juice prevents DNA single-strand breakage and cytotoxicity caused by tert-butylhydroperoxide in U937 cells: The role of iron chelation. *The Journal of Nutritional Biochemistry, 18*, 457–466.
11. Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry, 48*, 4581–4589.
12. Vinson, J. A., Hao, Y., Su, X., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: Vegetables. *Journal of Agricultural and Food Chemistry, 46*, 3630–3634.
13. Oboh, G., & Rocha, J. B. T. (2007). Polyphenols in red pepper [*Capsicum annuum var. aviculare* (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. *European Food Research and Technology, 225*, 239–247.
14. Gutiérrez, F., Arnaud, T., & Garrido, A. (2001). Contribution of polyphenols to the oxidative stability of virgin olive oil. *Journal of the Science of Food and Agriculture, 81*, 1463–1470.
15. Visioli, F., Bellomo, G., & Galli, C. (1998). Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications, 247*, 60–64.
16. Lodovici, M., Guglielmi, F., Casalini, C., Meoni, M., Cheynier, V., & Dolara, P. (2001). Antioxidant and radical scavenging properties in vitro of polyphenolic extracts from red wine. *European Journal of Nutrition, 40*, 74–77.
17. Makris, D. P., Psarra, E., Kallithraka, S., & Kefalas, P. (2003). The effect of polyphenolic composition as related to antioxidant capacity in white wines. *Food Research International (Ottawa, Ont.), 36*, 805–814.
18. Vinson, J. A., Proch, J., & Zubik, L. (1999). Phenol antioxidant quantity and quality in foods: Cocoa, dark chocolate, and milk chocolate. *Journal of Agricultural and Food Chemistry, 47*, 4821–4824.
19. Arts, I. C. W., Van de Putte, B., & Hollman, P. C. H. (2000). Catechin contents of foods commonly consumed in the Netherlands. Part 2. Tea, wine, fruit juices, and chocolate milk. *Journal of Agricultural and Food Chemistry, 48*, 1752–1757.
20. Arts, I. C. W., Van de Putte, B., & Hollman, P. C. H. (2000). Catechin contents of foods commonly consumed in the Netherlands. Part 1. Fruits, vegetables, staple foods, and processed foods. *Journal of Agricultural and Food Chemistry, 48*, 1746–1751.
21. de Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2000). Quantitative analysis of avan-3-ols in Spanish foodstuffs and beverages. *Journal of Agricultural and Food Chemistry, 48*, 5331–5337.
22. USDA. (2004). *USDA database for the proanthocyanidin content of selected foods*. Accessed December 18, 2008, from http://www.nal.usda.gov/fnic/foodcomp/Data/PA/PA.pdf
23. "5 A Day". (2008). Accessed April 9, 2008, from www.cdc.gov/nccdphp/dnpa/5aday/index.htm
24. Graham, H. N. (1992). Green tea composition, consumption, and polyphenol chemistry. *Preventive Medicine, 21*, 334–350.
25. Reddy, V. C., Sagar, G. V. V., Sreeramulu, D., Venu, L., & Raghunath, M. (2005). Addition of milk does not alter the antioxidant activity of black tea. *Annals of Nutrition and Metabolism, 49*, 189–195.
26. Sugisawa, A., & Umegaki, K. (2002). Physiological concentrations of (–)-epigallocatechin-3-O-gallate (EGCg) prevent chromosomal damage induced by reactive oxygen species in WIL2-NS cells. *Journal of Nutrition, 132*, 1836–1839.
27. Van het Hof, K. H., Kivits, G. A. A., Weststrate, J. A., & Tijburg, L. B. M. (1998). Bioavailability of catechins from tea: The effect of milk. *European Journal of Clinical Nutrition, 52*, 356–359.
28. Yang, C. S., Chen, L., Lee, M.-J., Balentine, D., Kuo, M. C., & Schantz, S. P. (1998). Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiology, Biomarkers and Prevention, 7*, 351–354.
29. Yamamoto, T., Hsu, S., Lewis, J., Wataha, J., Dickinson, D., Singh, B., et al. (2003). Green tea polyphenol causes differential

oxidative environments in tumor versus normal epithelial cells. *The Journal of Pharmacology and Experimental Therapeutics, 307*, 230–236.
30. Erlund, I., Silaste, M. L., Alfthan, G., Rantala, M., Kesaniemi, Y. A., & Aro, A. (2002). Plasma concentrations of the avonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. *European Journal of Clinical Nutrition, 56*, 891–898.
31. Hollman, P. C. H., Gaag, M. V. D., Mengelers, M. J. B., van Trijp, J. M. P., de Vries, J. H. M., & Katan, M. B. (1996). Absorption and disposition kinetics of the dietary antioxidant quercetin in man. *Free Radical Biology and Medicine, 21*, 703–707.
32. Nitta, Y., Kikuzaki, H., & Ueno, H. (2007). Food components inhibiting recombinant human histidine decarboxylase activity. *Journal of Agricultural and Food Chemistry, 55*, 299–304.
33. Macheix, J.-J., Fleuriet, A., & Billot, J. (1990). *Fruit Phenolics* (pp. 272–273). Boca Raton: CRC Press, Inc.
34. Romero, C., Medina, E., Vargas, J., Brenes, M., & De Castro, A. (2007). In vitro activity of olive oil polyphenols against *Helicobacter pylori*. *Journal of Agricultural and Food Chemistry, 55*, 680–686.
35. Song, J.-M., Lee, K.-H., & Seong, B.-L. (2005). Antiviral effect of catechins in green tea on in uenza virus. *Antiviral Research, 68*, 66–74.
36. Lorenz, M., Jochmann, N., von Krosigk, A., Martus, P., Baumann, G., Stangl, K., et al. (2007). Addition of milk prevents vascular protective effects of tea. *European Heart Journal, 28*, 219–223.
37. Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., & Kromhout, D. (1993). Dietary antioxidant avonoids and risk of coronary heart disease. The Zutphen elderly study. *Lancet, 342*, 1007–1011.
38. Naasani, I., Oh-hashi, F., Oh-hara, T., Feng, W. Y., Johnston, J., Chan, K., et al. (2003). Blocking telomerase by dietary polyphenols is a major mechanism for limiting the growth of human cancer cells in vitro and in vivo. *Cancer Research, 63*, 824–830.
39. He, Q., Lv, Y., & Yao, K. (2006). Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. *Food Chemistry, 101*, 1178–1182.
40. Mandel, S. A., Amit, T., Zheng, H., Weinreb, O., & Youdim, M. B. H. (2006). The essentiality of iron chelation in neuroprotection: A potential role of green tea catechins. *Oxidative Stress and Disease, 22*, 277–299.
41. Lambert, J. D., & Yang, C. S. (2003). Mechanisms of cancer prevention by tea constituents. *Journal of Nutrition, 133*(Suppl), 3262S–3267S.
42. Fresco, P., Borges, F., Diniz, C., & Marques, M. P. M. (2006). New insights on the anticancer properties of dietary polyphenols. *Medicinal Research Reviews, 26*, 747–766.
43. Garg, A. K., Buchholz, T. A., & Aggarwal, B. B. (2005). Chemosensitization and radiosensitization of tumors by plant polyphenols. *Antioxidants and Redox Signaling, 7*, 1630–1647.
44. Stoner, G. D., & Casto, B. C. (2004). Chemoprevention by fruit phenolic compounds. In G. J. Kelloff, E. T. Hawk, & C. C. Sigman (Eds.), *Cancer chemoprevention* (pp. 419–435). Totowa, NJ: Humana Press, Inc.
45. Horvathova, K., Novotny, L., Tothova, D., & Vachalkova, A. (2004). Determination of free radical scavenging activity of quercetin, rutin, luteolin and apigenin in H_2O_2-treated human ML cells K562. *Neoplasma, 51*, 395–399.
46. Inoue, M., Suzuki, R., Koide, T., Sakaguchi, N., Ogihara, Y., & Yabu, Y. (1994). Antioxidant, gallic acid, induces apoptosis in HL-60RG cells. *Biochemical and Biophysical Research Communications, 204*, 898–904.
47. Orrenius, S., Gogvadze, V., & Zhivotovsky, B. (2007). Mitochondrial oxidative stress: Implications for cell death. *Annual Review of Pharmacology and Toxicology, 47*, 143–183.
48. Huang, X. (2003). Iron overload and its association with cancer risk in humans: Evidence for iron as a carcinogenic metal. *Mutation Research, 533*, 153–171.
49. Hajiliadis, N. D. (Ed.). (1997). *Cytotoxic, mutagenic, and carcinogenic potential of heavy metals related to human environment*. Netherlands: Kluwer Academic Press.
50. Markesbery, W. R., & Lovell, M. A. (2006). DNA oxidation in Alzheimer's disease. *Antioxidants and Redox Signaling, 8*, 2039–2045.
51. Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases. *Drugs and Aging, 18*, 685–716.
52. Markesbery, W. R. (1997). Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biology and Medicine, 23*, 134–147.
53. Markesbery, W. R. (1999). Oxidative alterations in Alzheimer's disease. *Brain Pathology, 9*, 133–146.
54. Drew, B., & Leeuwenburgh, C. (2002). Aging and the role of reactive nitrogen species. *Annals of the New York Academy of Sciences, 959*, 66–81.
55. Vokurkova, M., Xu, S., & Touyz, R. M. (2007). Reactive oxygen species, cell growth, cell cycle progression and vascular remodeling in hypertension. *Future Cardiology, 3*, 53–63.
56. Steinberg, D. (1997). Low density lipoprotein oxidation and its pathobiological signi cance. *The Journal of Biological Chemistry, 272*, 20963–20966.
57. Ide, T., Tsutsui, H., Hayashidani, S., Kang, D., Suematsu, N., Nakamura, K.-I., et al. (2001). Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. *Circulation Research, 88*, 529–535.
58. Chevion, M., Berenshtein, E., & Zhu, B.-Z. (1999). The role of transition metal ions in free radical-mediated damage. In D. L. Gilbert & C. A. Colton (Eds.), *Reactive oxygen species in biological systems* (pp. 103–131). New York: Plenum Publishers.
59. SciFinder search on May 5, 2008 using the terms "polyphenol" and "radical scavenging", re ned by year 1995–current (duplicate entries removed).
60. Hanasaki, Y., Ogawa, S., & Fukui, S. (1994). The correlation between active oxygens scavenging and antioxidative effects of avonoids. *Free Radical Biology and Medicine, 16*, 845–850.
61. Bors, W., Heller, W., Michel, C., & Saran, M. (1990). Flavonoids as antioxidants: Determination of radical-scavenging ef ciencies. *Methods in Enzymology, 186*, 343–355.
62. Muzolf, M., Szymusiak, H., Swiglo, A. G., Rietjens, I. M. C. M., & Tyrakowska, B. (2008). pH-dependent radical scavenging capacity of green tea catechins. *Journal of Agricultural and Food Chemistry, 56*, 816–823.
63. Nanjo, F., Goto, K., Seto, R., Suzuki, M., Sakai, M., & Hara, Y. (1996). Scavenging effects of tea catechins and their derivatives on 1, 1-diphenyl-2-picrylhydrazyl radical. *Free Radical Biology and Medicine, 21*, 895–902.
64. Borkowski, T., Szymusiak, H., Gliszczynska-Swiglo, A., Rietjens, I. M. C. M., & Tyrakowska, B. (2005). Radical scavenging capacity of wine anthocyanins is strongly pH-dependent. *Journal of Agricultural and Food Chemistry, 53*, 5526–5534.
65. van Acker, S. A. B. E., Tromp, M. N. J. L., Haenen, G. R. M. M., van der Vijgh, W. J. F., & Bast, A. (1995). Flavonoids as scavengers of nitric oxide radical. *Biochemical and Biophysical Research Communications, 214*, 755–759.
66. Furuno, K., Akasako, T., & Sugihara, N. (2002). The contribution of the pyrogallol moiety to the superoxide radical scavenging activity of avonoids. *Biological and Pharmaceutical Bulletin, 25*, 19–23.

67. Keyer, K., Gort, A. S., & Imlay, J. A. (1995). Superoxide and the production of oxidative DNA damage. *Journal of Bacteriology, 177*, 6782–6790.
68. Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Poel, B. V., et al. (1998). Structure–activity relationship and classification of avonoids as inhibitors of xanthine oxidase and superoxide scavengers. *Journal of Natural Products, 61*, 71–76.
69. Arts, M. J. T. J., Dallinga, J. S., Voss, H.-P., Haenen, G. R. M. M., & Bast, A. (2004). A new approach to asses the total antioxidant capacity using the TEAC assay. *Food Chemistry, 88*, 567–570.
70. Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity of phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.
71. Sharma, A., Bhardwaj, S., Mann, A. S., Jain, A., & Kharya, M. D. (2007). Screening methods of antioxidant activity: An overview. *Pharmacognosy Reviews, 1*, 232–238.
72. Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.
73. Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., & Freeman, B. A. (1990). Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 1620–1624.
74. Gilbert, D. L., & Colton, C. A. (Eds.). (1999). *Reactive oxygen species in biological systems*. New York: Plenum Publishers.
75. Adler, V., Yin, Z., Tew, K. D., & Ronai, Z. (1999). Role of redox potential and reactive oxygen species in stress signaling. *Oncogene, 18*, 6104–6111.
76. Forman, H. J., & Torres, M. (2002). Reactive oxygen species and cell signaling. *American Journal of Respiratory and Critical Care Medicine, 166*, 54–58.
77. Bredt, D. S., & Snyder, S. H. (1994). Nitric oxide: A physiologic messenger molecule. *Annual Review of Biochemistry, 63*, 175–195.
78. Suzuki, Y. J., Forman, H. J., & Sevanian, A. (1997). Oxidants as stimulators of signal transduction. *Free Radical Biology and Medicine, 22*, 269–285.
79. Rhee, S. G. (1999). Redox signaling: Hydrogen peroxide as intracellular messenger. *Experimental and Molecular Medicine, 31*, 53–59.
80. Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews, 82*, 47–95.
81. Koppenol, W. H., Moreno, J. J., Pryor, W. A., Ischiropoulos, H., & Beckman, J. S. (1992). Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chemical Research in Toxicology, 5*, 834–842.
82. Squadrito, G. L., & Pryor, W. A. (1995). The formation of peroxynitrite in vivo from nitric oxide and superoxide. *Chemico-Biological Interactions, 96*, 203–206.
83. Henle, E. S., & Linn, S. (1997). Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. *The Journal of Biological Chemistry, 272*, 19095–19098.
84. Imlay, J. A., & Linn, S. (1988). DNA damage and oxygen radical toxicity. *Science, 240*, 1302–1309.
85. Henle, E. S., Han, Z., Tang, N., Rai, P., Luo, Y., & Linn, S. (1999). Sequence-specific DNA cleavage by Fe^{2+}-mediated Fenton reaction has possible biological implications. *The Journal of Biological Chemistry, 274*, 962–971.
86. Battin, E. E., Perron, N. R., & Brumaghim, J. L. (2006). The central role of metal coordination in selenium antioxidant activity. *Inorganic Chemistry, 45*, 499–501.
87. Flint, D. H., Tuminello, J. F., & Emptage, M. H. (1993). The inactivation of Fe–S cluster containing hydro-lyases by superoxide. *The Journal of Biological Chemistry, 268*, 22369–22376.
88. Keyer, K., & Imlay, J. A. (1996). Superoxide accelerates DNA damage by elevating free-iron levels. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 13635–13640.
89. Benov, L. (2001). How superoxide radical damages the cell. *Protoplasma, 217*, 33–36.
90. Haber, F., & Weiss, J. (1932). Über die katalyse des hydroperoxydes. *Naturwiss, 51*, 948–950.
91. Koppenol, W. H. (2001). The Haber–Weiss cycle—70 years later. *Redox Report, 6*, 229–234.
92. George, P. (1947). Some experiments on the reactions of potassium superoxide in aqueous solutions. *Discussions of the Faraday Society, 2*, 196–205.
93. Nakagawa, O., Ono, S., Tsujimoto, A., Li, Z., & Sasaki, S. (2007). Selective uorescence detection of 8-oxoguanosine with 8-oxoG-CLAMP. *Nucleosides, Nucleotides, and Nucleic Acids, 26*, 645–649.
94. Weimann, A., Belling, D., & Poulsen, H. E. (2002). Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. *Nucleic Acids Research, 30*, e7/1–e7/8.
95. Shigenaga, M. K., Aboujaoude, E. N., Chen, Q., & Ames, B. N. (1994). Assays of oxidative DNA damage biomarkers 8-oxo-2′-deoxyguanosine and 8-oxoguanine in nuclear DNA and biological uids by high-performance liquid chromatography with electrochemical detection. *Methods in Enzymology, 234*, 16–33.
96. Shigenaga, M. K., Gimeno, C. J., & Ames, B. N. (1989). Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 9697–9701.
97. Zheng, L.-F., Dai, F., Zhou, B., Yang, L., & Liu, Z.-L. (2008). Prooxidant activity of hydroxycinnamic acids on DNA damage in the presence of Cu(II) ions: Mechanism and structure-activity relationship. *Food and Chemical Toxicology, 46*, 149–156.
98. Ohshima, H., Gilibert, I., & Bianchini, F. (1999). Induction of DNA strand breakage and base oxidation by nitroxyl anion through hydroxyl radical production. *Free Radical Biology and Medicine, 26*, 1305–1313.
99. Fisher, G. R., & Gutierrez, P. L. (1991). Free radical formation and DNA strand breakage during metabolism of diaziquone by NAD(P)H quinone-acceptor oxidoreductase (DT-diaphorase) and NADPH cytochrome c reductase. *Free Radical Biology and Medicine, 11*, 597–607.
100. Kashige, N., Yamaguchi, T., Ohtakara, A., Mitsutomi, M., Brimacombe, J. S., Miake, F., et al. (1994). Structure-activity relationships in the induction of single-strand breakage in plasmid pBR322 DNA by amino sugars and derivatives. *Carbohydrate Research, 257*, 285–291.
101. Bhat, R., & Hadi, S. M. (1994). DNA breakage by tannic acid and Cu(II): Sequence speci city of the reaction and involvement of active oxygen species. *Mutation Research, 313*, 39–48.
102. Rai, P., Cole, T. D., Wemmer, D. E., & Linn, S. (2001). Localization of Fe^{2+} at an RTGR sequence within a DNA duplex explains preferential cleavage by Fe^{2+} and hydrogen peroxide. *Journal of Molecular Biology, 312*, 1089–1101.
103. Gao, Y. G., Sriram, M., & Wang, A. H. (1993). Crystallographic studies of metal ion–DNA interactions: Different binding modes of cobalt(II), copper(II) and barium(II) to N7 of guanines in Z-DNA and a drug–DNA complex. *Nucleic Acids Research, 21*, 4093–4101.
104. Rai, P., Wemmer, D. E., & Linn, S. (2005). Preferential binding and structural distortion by Fe^{2+} at RGGG-containing DNA sequences correlates with enhanced oxidative cleavage at such sequences. *Nucleic Acids Research, 33*, 497–510.

105. Lu, A.-L., Li, X., Gu, Y., Wright, P. M., & Chang, D.-Y. (2001). Repair of oxidative DNA damage. *Cell Biochemistry and Biophysics, 35*, 141–170.
106. Kennedy, L. J., Moore, K., Jr., Caul eld, J. L., Tannenbaum, S. R., & Dedon, P. C. (1997). Quantitaion of 8-oxoguanine and strand breaks produced by four oxidizing agents. *Chemical Research in Toxicology, 10*, 386–392.
107. Aruoma, O. I., Halliwell, B., Gajewski, E., & Dizdaroglu, M. (1991). Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. *The Biochemical Journal, 273*, 601–604.
108. Macomber, L., Rensing, C., & Imlay, J. A. (2007). Intracellular copper does not catalyze the formation of oxidative DNA damage in *Escherichia coli*. *Journal of Bacteriology, 189*, 1616–1626.
109. Mello-Filho, A. C., & Meneghini, R. (1991). Iron is the intracellular metal involved in the production of DNA damage by oxygen radicals. *Mutation Research, 251*, 109–113.
110. Hoffmann, M. E., Mello-Filho, A. C., & Meneghini, R. (1984). Correlation between cytotoxic effect of hydrogen peroxide and the yield of DNA strand breaks in cells of different species. *Biochimica et Biophysica Acta, 781*, 234–238.
111. Andrews, N. C. (2004). Probing the iron pool. Focus on "detection of intracellular iron by its regulatory effect". *American Journal of Physiology. Cell Physiology, 287*, C1537–C1538.
112. Yamamoto, Y., Fukui, K., Koujin, N., Ohya, H., Kimura, K., & Kamio, Y. (2004). Regulation of the intracellular free iron pool by Dpr provides oxygen tolerance to *Streptococcus mutans*. *Journal of Bacteriology, 186*, 5997–6002.
113. Woodmansee, A. N., & Imlay, J. A. (2002). Quantitation of intracellular free iron by electron paramagnetic resonance spectroscopy. *Methods in Enzymology, 349*, 3–9.
114. Jacobs, A. (1977). Low molecular weight intracellular iron transport compounds. *Blood, 50*, 433–439.
115. Miller, J. P. G., & Perkins, D. J. (1969). Model experiments for the study of iron transfer from transferrin to ferritin. *European Journal of Biochemistry, 10*, 146–151.
116. Touati, D., Jacques, M., Tardat, B., Bouchard, L., & Despied, S. (1995). Lethal oxidative damage and mutagenesis are generated by iron in Δ*fur* mutants of *Escherichia coli*: Protective role of superoxide dismutase. *Journal of Bacteriology, 177*, 2305–2314.
117. Biemond, P., Swaak, A. J. G., van Eijk, H. G., & Koster, J. F. (1988). Superoxide dependent iron release from ferritin in in ammatory diseases. *Free Radical Biology and Medicine, 4*, 185–198.
118. Ke, Y., & Qian, Z. M. (2003). Iron misregulation in the brain: a primary cause of neurodegenerative disorders. *Lancet Neurology, 2*, 246–253.
119. Selima, M. H., & Ratan, R. R. (2004). The role of iron neurotoxicity in ischemic stroke. *Ageing Research Reviews, 3*, 345–353.
120. Wood, R. J. (2004). The iron–heart disease connection: Is it dead or just hiding? *Ageing Research Reviews, 3*, 355–367.
121. Brewer, G. J. (2007). Iron and copper toxicity in diseases of aging, particularly atherosclerosis and Alzheimer's disease. *Experimental Biology and Medicine, 232*, 323–335.
122. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions, 160*, 1–40.
123. Luxford, C., Dean, R. T., & Davies, M. J. (2000). Radicals derived from histone hydroperoxides damage nucleobases in RNA and DNA. *Chemical Research in Toxicology, 13*, 665–672.
124. Liang, Q., & Dedon, P. C. (2001). Cu(II)/H_2O_2-induced DNA damage is enhanced by packaging of DNA as a nucleosome. *Chemical Research in Toxicology, 14*, 416–422.
125. Midorikawa, K., Murata, M., & Kawanishi, S. (2005). Histone peptide AKRHRK enhances H_2O_2-induced DNA damage and alters its site speci city. *Biochemical and Biophysical Research Communications, 333*, 1073–1077.
126. Turro, N. J. (2002). Damage control of DNA in nucleosome core particles: When a histone's loving embrace is just not good enough. *Chemistry and Biology, 9*, 399–401.
127. Weissman, L., de Souza-Pinto, N. C., Stevnsner, T., & Bohr, V. A. (2007). DNA repair, mitochondria, and neurodegeneration. *Neuroscience, 145*, 1318–1329.
128. Berneburg, M., Kamenisch, M., Krutmann, J., & Roecken, M. (2006). 'To repair or not to repair—No longer a question': Repair of mitochondrial DNA shielding against age and cancer. *Experimental Dermatology, 15*, 1005–1015.
129. Birch-Machin, M. A. (2005). Using mitochondrial DNA as a biosensor of early cancer development. *British Journal of Cancer, 93*, 271–272.
130. Chatterjee, A., Mambo, E., Zhang, Y., DeWeese, T., & Sidransky, D. (2006). Targeting of mutant hogg1 in mammalian mitochondria and nucleus: effect on cellular survival upon oxidative stress. *BMC Cancer, 6*, 235.
131. de Grey, A. D. N. J. (1997). A proposed re nement of the mitochondrial free radical theory of aging. *BioEssays, 19*, 161–166.
132. Hider, R. C., Liu, Z. D., & Khodr, H. H. (2001). Metal chelation of polyphenols. *Methods in Enzymology, 335*, 190–203.
133. Pearson, R. G. (1963). Hard and soft acids and bases. *Journal of the American Chemical Society, 85*, 3533–3539.
134. Loomis, L. D., & Raymond, K. N. (1991). Solution equilibria of enterobactin and metal–enterobactin complexes. *Inorganic Chemistry, 30*, 906–911.
135. Avdeef, A., Sofen, S. R., Bregante, T. L., & Raymond, K. N. (1978). Coordination chemistry of microbial iron transport compounds. 9. Stability constants for catechol models of enterobactin. *Journal of the American Chemical Society, 100*, 5362–5370.
136. Martell, A. E., & Smith, R. M. (1977). *Critical stability constants* (Vol. 3, pp. 200–201). New York: Plenum Press.
137. Kipton, H., Powell, J., & Taylor, M. C. (1982). Interactions of iron(II) and iron(III) with gallic acid and its homologues: A potentiometric and spectrophotometric study. *Australian Journal of Chemistry, 35*, 739–756.
138. Erdogan, G., Karadag, R., & Dolen, E. (2005). Potentiometric and spectrophotometric determination of the stability constants of quercetin (3, 3′, 4′, 5, 7-pentahydroxy avone) complexes with aluminium(III) and iron(II). *Reviews in Analytical Chemistry, 24*, 247–261.
139. Buf e, J., & Martell, A. E. (1977). Metal ion catalyzed oxidation of o-dihydroxy aromatic compounds by oxygen. 2. Complexes of 1, 2-dihydroxynaphthalene-4-sulfonate with iron(III) and iron(II). *Inorganic Chemistry, 16*, 2225–2229.
140. Binbuga, N., Chambers, K., Henry, W. P., & Schultz, T. P. (2005). Metal chelation studies relevant to wood preservation. 1. Complexation of propyl gallate with Fe^{2+}. *Holzforschung, 59*, 205–209.
141. Chvátalová, K., Slaninová, I., Brezinová, L., & Slanina, J. (2008). In uence of dietary phenolic acids on redox status of iron: Ferrous iron autoxidation and ferric iron reduction. *Food Chemistry, 106*, 650–660.
142. Yoshino, M., & Murakami, K. (1998). Interaction of iron with polyphenolic compounds: Application to antioxidant characterization. *Analytical Biochemistry, 257*, 40–44.
143. Kawabata, T., Schepkin, V., Haramaki, N., Phadke, R. S., & Packer, L. (1996). Iron coordination by catechol derivative antioxidants. *Biochemical Pharmacology, 51*, 1569–1577.
144. Ohashi, Y., Yoshinaga, K., Yoshioka, H., & Yoshioka, H. (2002). Kinetic analysis of the effect of (−)-epigallocatechin gallate on the DNA strand scission induced by Fe(II). *Bioscience, Biotechnology, and Biochemistry, 66*, 770–776.

145. Hajji, H. E., Nkhili, E., Tomao, V., & Dangles, O. (2006). Interactions of quercetin with iron and copper ions: Complexation and autoxidation. *Free Radical Research, 40*, 303–320.
146. Cooper, S. R., McArdle, J. V., & Raymond, K. N. (1978). Siderophore electrochemistry: Relation to intracellular iron release mechanism. *Proceedings of the National Academy of Sciences of the United States of America, 75*, 3551–3554.
147. McBryde, W. A. E. (1964). A spectrophotometric reexamination of the spectra and stabilities of the iron (III)—Tiron complexes. *Canadian Journal of Chemistry, 42*, 1917–1927.
148. Perron, N. R., DeGuire, S. M., & Brumaghim, J. L. (2008). Kinetics of iron oxidation upon polyphenol binding (in preparation).
149. Stumm, W., & Lee, G. F. (1961). Oxygenation of ferrous iron. *Industrial and Engineering Chemistry, 53*, 143–146.
150. King, J., & Davidson, N. (1958). Kinetics of the ferrous iron-oxygen reaction in acidic phosphate–pyrophosphate solutions. *Journal of the American Chemical Society, 80*, 1542–1545.
151. Posner, A. M. (1953). The kinetics of autoxidation of ferrous ions in concentrated HCl solutions. *Transactions of Faraday Society, 49*, 382–388.
152. Huffman, R. E., & Davidson, N. (1956). Kinetics of the ferrous iron-oxygen reaction in sulfuric acid solution. *Journal of the American Chemical Society, 78*, 4836–4842.
153. George, P. (1954). The oxidation of ferrous perchlorate by molecular oxygen. *Journal of the Chemical Society*, 4349–4359.
154. Ryan, P., & Hynes, M. J. (2007). The kinetics and mechanisms of the complex formation and antioxidant behaviour of the polyphenols EGCg and ECG with iron(III). *Journal of Inorganic Biochemistry, 101*, 585–593.
155. Jameson, G. N. L., & Linert, W. (2001). The oxidation of 6-hydroxydopamine in aqueous solution. Part 3. Kinetics and mechanism of the oxidation with iron(III). *Journal of the Chemical Society. Perkin Transactions, 2*, 569–575.
156. Hynes, M. J., & Coinceanainn, M. O. (2001). The kinetics and mechanisms of the reaction of iron(III) with gallic acid, gallic acid methyl ester and catechin. *Journal of Inorganic Biochemistry, 85*, 131–142.
157. Ryan, P., & Hynes, M. J. (2008). The kinetics and mechanisms of the reactions of iron(III) with quercetin and morin. *Journal of Inorganic Biochemistry, 102*, 127–136.
158. El-Ayaan, U., Herlinger, E., Jameson, R. F., & Linert, W. (1997). Anaerobic oxidation of dopamine by iron(III). *Journal of the Chemical Society, Dalton Transactions*, 2813–2818.
159. Basolo, F., & Pearson, R. G. (1967). *Mechanism of inorganic reactions, a study of metal complexes in solution* (2nd ed.). New York: Wiley.
160. Hider, R. C., Mohr-Nor, A. R., Silver, J., Morrison, I. E. G., & Rees, L. V. C. (1981). Model compounds for microbial iron-transport compounds. Part 1. Solution chemistry and Mössbauer study of iron(II) and iron(III) complexes from phenolic and catecholic systems. *Journal of the Chemical Society, Dalton Transactions*, 609–622.
161. Hider, R. C., Howlin, B., Miller, J. R., Mohr-Nor, A. R., & Silver, J. (1983). Model compounds for microbial iron-transport compounds. Part IV. Further solution chemistry and Mössbauer studies on iron(II) and iron(III) catechol complexes. *Inorganica Chimica Acta, 80*, 51–56.
162. Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 48*, 3396–3402.
163. Zhang, L., Bandy, B., & Davison, A. J. (1996). Effects of metals, ligands and antioxidants on the reaction of oxygen with 1, 2, 4-benzenetriol. *Free Radical Biology and Medicine, 20*, 495–505.
164. Puppo, A. (1992). Effect of avonoids on hydroxyl radical formation by Fenton-type reactions; in uence of the iron chelator. *Phytochemistry, 31*, 85–88.
165. Laughton, M. J., Halliwell, B., Evans, P. J., & Hoult, J. R. S. (1989). Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myricetin. Effects on lipid peroxidation, hydroxyl radical generation and bleomycin-dependent damage to DNA. *Biochemical Pharmacology, 38*, 2859–2865.
166. Schweigert, N., Zehnder, A. J. B., & Eggen, R. I. L. (2001). Chemical properties of catechols and their molecular modes of toxic action in cells, from microorganisms to mammals. *Environmental Microbiology, 3*, 81–91.
167. Yamahara, R., Ogo, S., Masuda, H., & Watanabe, Y. (2002). (Catecholato)iron(III) complexes: Structural and functional models for the catechol-bound iron(III) form of catechol dioxygenases. *Journal of Inorganic Biochemistry, 88*, 284–294.
168. Wunderlich, C. H., Weber, R., & Bergerhoff, G. (1991). On iron gallic ink. *Zeitschrift für Anorganische Und Allgemeine Chemie, 598*(599), 371–376.
169. Feller, R. K., & Cheetham, A. K. (2006). Fe(III), Mn(II), Co(II), and Ni(II) 3, 4, 5-trihydroxybenzoate (gallate) dihydrates; a new family of hybrid framework materials. *Solid State Sciences, 8*, 1121–1125.
170. Higuchi, M., Hitomi, Y., Minami, H., Tanaka, T., & Funabiki, T. (2005). Correlation of spin states and spin delocalization with the dioxygen reactivity of catecholatoiron (III) complexes. *Inorganic Chemistry, 44*, 8810–8821.
171. Floquet, S., Simaan, A. J., Rivière, E., Nierlich, M., Thúery, P., Ensling, J., Gütlich, P., Girerd, J.-J., Boillot, M.-L. (2005). Spin crossover of ferric complexes with catecholate derivatives. Single-crystal X-ray structure, magnetic and Mössbauer investigations. *Dalton Transactions*, 1734–1742.
172. Chiou, Y.-M., & Que, L. (1995). Structure of a mononuclear iron(II)-catecholate complex and its relevance to the extradiol-cleaving catechol dioxygenases. *Inorganic Chemistry, 34*, 3577–3578.
173. Velusamy, M., Mayilmurugan, R., & Palaniadavar, M. (2004). Iron(III) complexes of sterically hindered tetradentate monophenolate ligands as functional models for catechol 1, 2-dioxygenases: The role of ligands stereoelectronic properties. *Inorganic Chemistry, 43*, 6284–6293.
174. Jo, D.-H., Chiou, Y.-M., & Que, J. L. (2001). Models of extradiol cleaving catechol dioxygenases: Syntheses, structures, and reactivities of iron(II)-monoanionic catecholate complexes. *Inorganic Chemistry, 40*, 3181–3190.
175. Grillo, V. A., Hanson, G. R., Wang, D., Hambley, T. W., Gahan, L. R., Murray, K. S., et al. (1996). Synthesis, X-ray structural determination, and magnetic susceptibility, Mössbauer, and EPR studies of $(Pr_4N)_2[Fe_2(Cat)_4(H_2O)_2]\cdot 6H_2O$, a catecholato-bridged dimer of iron(III). *Inorganic Chemistry, 35*, 3568–3576.
176. Caulder, D. L., Powers, R. E., Parac, T. N., & Raymond, K. N. (1998). The self-assembly of a predesigned tetrahedral M_4L_6 supramolecular cluster. *Angewandte Chemie (International ed. in English), 37*, 1840–1843.
177. Jewett, S. L., Eggling, S., & Geller, L. (1997). Novel method to examine the formation of unstable 2:1 and 3:1 complexes of catecholamines and iron(III). *Journal of Inorganic Biochemistry, 66*, 165–173.
178. Jovanovic, S. V., Simic, M. G., Steenken, S., & Hara, Y. (1998). Iron complexes of gallocatechins. Antioxidant action or iron regulation? *Journal of the Chemical Society. Perkin Transactions, 2*, 2365–2369.
179. Ackermann, V. G., & Hesse, D. (1970). Über eisen(III)-komplexe mit phenolen. III. *Zeitschrift für Anorganische Und Allgemeine Chemie, 375*, 77–86.

180. Maqsood, Z. A. T., & Kazmi, S. A. (1993). Formation of iron gallic acid complexes at different pH and determination of their stability constants. *Pakistan Journal of Scienti c and Industrial Research, 6*, 511–516.
181. Perron, N. R., Hodges, J. N., Jenkins, M., & Brumaghim, J. L. (2008). Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. *Inorganic Chemistry, 47*, 6153–6161.
182. Lopes, G. K. B., Schulman, H. M., & Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochimica et Biophysica Acta, 1472*, 142–152.
183. Mentasti, E., Pelizzetti, E., & Saini, G. (1976). Interactions of Fe(III) with adrenaline, L-Dopa, and other catechol derivatives. *Journal of Inorganic and Nuclear Chemistry, 38*, 785–788.
184. Mentasti, E., & Pelizzetti, E. (1973). Reactions between iron(III) and catechol (1,2-dihydroxybenzene). Part I. Equilibria and kinetics of complex formation in aqueous acid solution. *Journal of the Chemical Society. Dalton Transactions*, 2605–2608.
185. Kennedy, J. A., & Powell, H. K. J. (1985). Aluminium(III) and iron(III) 1, 2-diphenolato complexes: A potentiometric study. *Australian Journal of Chemistry, 38*, 659–667.
186. de Souza, R. F. V., Sussuchi, E. M., & De Giovani, W. F. (2003). Synthesis, electrochemical, spectral, and antioxidant properties of complexes of avonoids with metal ions. *Synthesis and Reactivity in Inorganic and Metal-Organic Chem, 33*, 1125–1144.
187. Escandar, G. M., & Sala, L. F. (1991). Complexing behavior of rutin and quercetin. *Canadian Journal of Chemistry, 69*, 1994–2001.
188. van Acker, S. A. B. E., van Balen, G. P., van den Berg, D.-J., Bast, A., & van der Vijgh, W. J. F. (1998). In uence of iron chelation on the antioxidant activity of avonoids. *Biochemical Pharmacology, 56*, 935–943.
189. Sugihara, N., Arakawa, T., Ohnishi, M., & Furuno, K. (1999). Anti- and pro-oxidative effects of avonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with -linolenic acid. *Free Radical Biology and Medicine, 27*, 1313–1323.
190. Sugihara, N., Ohnishi, M., Imamura, M., & Furuno, K. (2001). Differences in antioxidative ef ciency of catechins in various metal-induced lipid peroxidations in cultured hepatocytes. *Journal of Health Science, 47*, 99–106.
191. Morel, I., Lescoat, G., Cogrel, P., Sergent, O., Pasdeloup, N., Brissot, P., et al. (1993). Antioxidant and iron-chelating activities of the avonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochemical Pharmacology, 45*, 13–19.
192. Morel, I., Lescoat, G., Cillard, P., & Cillard, J. (1994). Role of avonoids and iron chelation in antioxidant action. *Methods in Enzymology, 234*, 437–443.
193. Ferrali, M., Signorini, C., Caciotti, B., Sugherini, L., Ciccoli, L., Giachetti, D., et al. (1997). Protection against oxidative damage of erythrocyte membrane by the avonoid quercetin and its relation to iron chelating activity. *FEBS Letters, 416*, 123–129.
194. Anghileri, L. J., & Thouvenot, P. (2000). Natural polyphenols–iron interaction. *Biological Trace Element Research, 73*, 251–258.
195. Sestili, P., Guidarelli, A., Dacha, M., & Cantoni, O. (1998). Quercetin prevents DNA single strand breakage and cytotoxicity caused by *tert*-butylhydroperoxide: Free radical scavenging versus iron chelating mechanism. *Free Radical Biology and Medicine, 25*, 196–200.
196. Sestili, P., Diamantini, G., Bedini, A., Cerioni, L., Tommasini, I., Tarzia, G., et al. (2002). Plant-derived phenolic compounds prevent the DNA single-strand breakage and cytotoxicity by *tert*-butylhydroperoxide via an iron-chelating mechanism. *The Biochemical Journal, 364*, 121–128.
197. Melidou, M., Riganakos, K., & Galaris, D. (2005). Protection against nuclear DNA damage offered by avonoids in cells exposed to hydrogen peroxide: The role of iron chelation. *Free Radical Biology and Medicine, 39*, 1591–1600.
198. Boato, F., Wortley, G. M., Liu, R. H., & Glahn, R. P. (2002). Red grape juice inhibits iron availability: Application of an in vitro digestion/Caco-2 cell model. *Journal of Agricultural and Food Chemistry, 50*, 6935–6938.
199. Matuschek, E., & Svanberg, U. (2002). Oxidation of polyphenols and the effect on in vitro iron accessibility in a model food system. *Journal of Food Science, 67*, 420–424.
200. Matuschek, E., Towo, E., & Svanberg, U. (2001). Oxidation of polyphenols in phytate-reduced high-tannin cereals: Effect on different phenolic groups and on in vitro accessible iron. *Journal of Agricultural and Food Chemistry, 49*, 5630–5638.
201. Gaffney, S., Williams, V., Flynn, P., Carlino, R., Mowry, C., Dierenfeld, E., et al. (2004). Tannin/polyphenol effects on iron solubilization in vitro. *Bios, 75*, 43–52.
202. Brown, R., Klein, A., & Hurrell, R. F. (1989). Effect of polyphenols on iron bioavailability in rats. *Special Publication (Royal Society of Chemistry), 72*, 152–154.
203. Das, P., Raghuramulu, N., & Rao, K. C. (2003). Effect of organic acids and polyphenols on in vitro available iron from foods. *Journal of Food Science and Technology, 40*, 677–681.
204. Tuntawiroon, M., Sritongkul, N., Brune, M., Rossander-Hulten, L., Pleehachinda, R., Suwanik, R., et al. (1991). Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in men. *The American Journal of Clinical Nutrition, 53*, 554–557.
205. Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioef cacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition, 81*, 230S–242S.
206. Williamson, G., & Manach, C. (2005). Bioavailability and bioef cacy of polyphenols in humans. II. Review of 93 intervention studies. *The American Journal of Clinical Nutrition, 81*, 243S–255S.
207. Kuo, S.-M., Leavitt, P. S., & Lin, C.-P. (1998). Dietary avonoids interact with trace metals and affect metallothionein level in human intestinal cells. *Biological Trace Element Research, 62*, 135–153.
208. Davis, S. R., & Cousins, R. J. (2000). Metallothionein expression in animals: A physiological perspective on function. *The Journal of Nutrition, 130*, 1085–1088.
209. Riggio, M., Filosa, S., Parisi, E., & Scudiero, R. (2003). Changes in zinc, copper and metallothionein contents during oocyte growth and early development of the teleost *Brachydanio rerio* (zebra sh). *Comparitive Biochemistry and Physiology C. Comparitive Pharmacology and Toxicology, 135*, 191–196.
210. Eiichi, T., Shin-Ichi, O., Kumiko, I., Akira, N., Yoshihisa, I., & Takashi, S. (2007). Metallothionein proteins expression, copper and zinc concentrations, and lipid peroxidation level in a rodent model for amyotrophic lateral sclerosis. *Toxicology, 229*, 33–41.
211. Rachmilewitz, E. A., Lubin, B. H., & Shohet, S. B. (1976). Lipid membrane peroxidation in -thalassemia major. *Blood, 47*, 495–505.
212. Hebbel, R. P., Eaton, J. W., Balasingam, M., & Steinber, M. H. (1982). Spontaneous oxygen radical generation by sickle erythrocytes. *The Journal of Clinical Investigation, 70*, 1253–1259.
213. Scott, M. D., van den Berg, J. J. M., Repka, T., Rouyer-Fessard, P., Hebbel, R. P., Beuzard, Y., et al. (1993). Effect of excess hemoglobin chains on cellular and membrane oxidation in model -thalassemic erythrocytes. *The Journal of Clinical Investigation, 91*, 1706–1712.

214. Vives Corrons, J. L., Pujades, M. A., Miguel-Garcia, A., Miguael-Sosa, A., Cambiazzo, S., Dibarrart, M. T., et al. (1995). Increased susceptibility of microcytic red blood cells to in vitro oxidative stress. *European Journal of Haematology, 55*, 327–331.
215. Grinberg, L. N., Newmark, H., Kitrossky, N., Rahamim, E., Chevion, M., & Rachmilewitz, E. A. (1997). Protective effects of tea polyphenols against oxidative damage to red blood cells. *Biochemical Pharmacology, 54*, 973–978.
216. Srichairatanakool, S., Ounjaijean, S., Thephinlap, C., Khansuwan, U., Phisalpong, C., & Fucharoen, S. (2006). Iron-chelating and free-radical scavenging activities of microwave processed green tea in iron overload. *Hemoglobin, 30*, 311–327.
217. Thephinlap, C., Ounjaijean, S., Khansuwan, U., Fucharoen, S., Porter, J. B., & Srichairatanakool, S. (2007). Epigallocatechin-3-gallate and epicatechin-3-gallate from green tea decrease plasma non-transferrin bound iron and erythrocyte oxidative stress. *Medicinal Chemistry, 3*, 289–296.
218. So c, E., Paulus, W., Jellinger, K., Riederer, P., & Youdim, M. B. H. (1991). Selective increase of iron in substantia nigra zona compacta of Parkinsonian brains. *Journal of Neurochemistry, 56*, 978–982.
219. Atwood, C. S., Obrenovich, M. E., Liu, T., Chan, H., Perry, G., Smith, M. A., et al. (2003). Amyloid β: A chameleon walking in two worlds: A review of the trophic and toxic properties of amyloid-β. *Brain Research Reviews, 43*, 1–16.
220. Turnbull, S., Tabner, B. J., El-Agnaf, O. M. A., Moore, S., Davies, Y., & Allsop, D. (2001). α-Synuclein implicated in Parkinson's disease catalyses the formation of hydrogen peroxide in vitro. *Free Radical Biology and Medicine, 30*, 1163–1170.
221. Ostrerova-Golts, N., Petrucelli, L., Hardy, J., Lee, J. M., Farer, M., & Wolozin, B. (2000). The A53T α-synuclein mutation increases iron-dependent aggregation and toxicity. *The Journal of Neuroscience, 20*, 6048–6054.
222. Singh, M., Arseneault, M., Sanderson, T., Murthy, V., & Ramassamy, C. (2008). Challenges for research on polyphenols from foods in Alzheimer's disease: Bioavailability, metabolism, and cellular and molecular mechanisms. *Journal of Agricultural and Food Chemistry, 56*, 4855–4873.
223. Pan, T., Jankovic, J., & Le, W. (2003). Potential therapeutic properties of green tea polyphenols in Parkinson's disease. *Drugs and Aging, 20*, 711–721.
224. Mandel, S. A., Avramovich-Tiorsh, Y., Reznichenko, L., Zheng, H., Weinreb, O., Amit, T., et al. (2005). Multifunctional activities of green tea catechins in neuroprotection. *Neurosignals, 14*, 46–60.
225. Bush, A. I. (2003). The metallobiology of Alzheimer's disease. *Trends in Neurosciences, 26*, 207–214.
226. Mandel, S. A., Amit, T., Reznichenko, L., Weinreb, O., & Youdim, M. B. H. (2006). Green tea catechins as brain-permeable, natural iron chelators-antioxidants for the treatment of neurodegenerative disorders. *Molecular Nutrition and Food Research, 50*, 229–234.
227. Guo, Q., Zhao, B., Li, M., Shen, S., & Xin, W. (1996). Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. *Biochimica et Biophysica Acta, 1304*, 210–222.
228. Ono, K., Yoshiike, Y., Takashima, A., Hasegawa, K., Naiki, H., & Yamada, M. (2003). Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. *Journal of Neurochemistry, 87*, 172–181.
229. Levites, Y., Amit, T., Mandel, S., & Youdim, M. B. H. (2003). Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (–)-epigallocatechin-3-gallate. *FASEB Journal, 17*, 952–954.
230. Baum, L., & Ng, A. (2004). Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. *Journal of Alzheimer s Disease, 6*, 367–377.
231. Lim, G. P., Chu, T., Yang, F., Beech, W., Frautschy, S. A., & Cole, G. M. (2001). The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *The Journal of Neuroscience, 21*, 8370–8377.
232. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351–358.
233. Oboh, G., & Rocha, J. B. T. (2007). Distribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). *Journal of Food Biochemistry, 31*, 456–473.
234. Hermes-Lima, M., Wang, E. M., Schulman, H. M., Storrey, K. B., & Ponka, P. (1994). Deoxyribose degradation catalyzed by Fe(III)EDTA: Kinetic aspects and potential usefulness for submicromolar iron measurements. *Molecular and Cellular Biochemistry, 137*, 65–73.
235. Winterbourn, C. C. (1987). The ability of scavengers to distinguish $^{\bullet}$OH production in the iron-catalyzed Haber–Weiss reaction: Comparison of four assays for $^{\bullet}$OH. *Free Radical Biology and Medicine, 3*, 33–39.
236. Romanová, D., Vachálková, A., Cipák, L., Ovesná, Z., & Rauko, P. (2001). Study of antioxidant effect of apigenin, luteolin, and quercetin by DNA protective method. *Neoplasma, 48*, 104–107.
237. Moran, J. F., Klucas, R. V., Grayer, R. J., Abian, J., & Becana, M. (1997). Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: Prooxidant and antioxidant properties. *Free Radical Biology and Medicine, 22*, 861–870.
238. Zhao, C., Dodin, G., Yuan, C., Chen, H., Zheng, R., Jia, Z., et al. (2005). "In vitro" protection of DNA from Fenton reaction by plant polyphenol verbascoside. *Biochimica et Biophysica Acta, 1723*, 114–123.
239. Web site: www.clinicaltrials.gov/ct/show/NCT00166335?order=00166331 "Green tea extract in treating patients with stage 00166330, stage I, or stage II chronic lymphocytic leukemia". Accessed June 00166326, 00162008.
240. Cheng, I. F., & Breen, K. (2000). On the ability of four avonoids, baicilein, luteolin, naringenin, and quercetin, to suppress the Fenton reaction of the iron-ATP complex. *BioMetals, 13*, 77–83.
241. Jovanovic, S. V., Steenken, S., Hara, Y., & Simic, M. G. (1996). Reduction potentials of avonoid and model phenoxyl radicals. Which ring in avonoids is responsible for antioxidant activity? *Journal of the Chemical Society. Perkin Transactions, 2*, 2497–2504.
242. Arora, A., Nair, M. G., & Strasburg, G. M. (1998). Structure–activity relationships for antioxidant activities of a series of avonoids in a liposomal system. *Free Radical Biology and Medicine, 24*, 1355–1363.
243. Khokhar, S., & Apenten, R. K. O. (2003). Iron binding characteristics of phenolic compounds: Some tentative structure–activity relations. *Food Chemistry, 81*, 133–140.
244. Perron, N. R., & Brumaghim, J. L. (2008). *Method for predicting the antioxidant potency of polyphenol compounds*, US patent pending.
245. Oviedo, C., Contreras, D., Freer, J., & Rodriguez, J. (2003). A screening method for detecting iron reducing wood-rot fungi. *Biotechnology Letters, 25*, 891–893.
246. Stadler, R. H., Richoz, J., Turesky, R. J., Welti, D. H., & Fay, L. B. (1996). Oxidation of caffeine and related methylxanthines in

ascorbate and polyphenol-driven Fenton-type oxidations. *Free Radical Research, 24*, 225–240.
247. Contreras, D., Rodríguez, J., Freer, J., Schwederski, B., & Kaim, W. (2007). Enhanced hydroxyl radical production by dihydroxybenzene-driven Fenton reactions: Implications for wood biodegradation. *Journal of Biological Inorganic Chemistry, 12*, 1055–1061.
248. Paszczynski, A., Crawford, R., Funk, D., & Goodell, B. (1999). De novo synthesis of 4, 5-dimethoxycatechol and 2, 5-dimethoxyhydroquinone by the brown rot fungus *Gloeophyllum trabeum*. *Applied and Environmental Microbiology, 65*, 674–679.
249. Kennedy, J. A., & Powell, H. K. J. (1985). Polyphenol interactions with aluminium(III) and iron(III): Their possible involvement in the podalization process. *Australian Journal of Chemistry, 38*, 879–888.
250. Kerem, Z., Jensen, K. A., & Hammel, K. E. (1999). Biodegradative mechanism of the brown rot basidiomycete *Gloeophyllum tradbeum*: Evidence for an extracellular hydroquinone-driven Fenton reaction. *FEBS Letters, 446*, 49–54.
251. Wang, W., & Gao, P. J. (2003). Function and mechanism of a low-molecular-weight peptide produced by *Gloeophyllum trabeum* in biodegradation of cellulose. *Journal of Biotechnology, 101*, 119–130.
252. Richards, M. P., Lee, C.-H., & Reed, J. D. (2003). *Methods and lipophilic antioxidant compositions to inhibit lipid oxidation in food products*. US patent 2005175762.
253. Furukawa, J., Shibuta, T., & Takamine, K. (2003). *Food additives for improving shelf life*. Japan patent 2003088345.
254. Richards, G. N. (1999). *Polyphenol-containing wood extract as antibacterial for food.* US patent 19970924.
255. Schur, J. P. (1998). *Preservative for foods and cosmetics.* Germany patent 97-19726429 19970623.
256. Levy, M.-C., & Andry, M.-C. (1995). *Microcapsules with walls made of cross-linked plant polyphenols, for foods, pharmaceuticals or cosmetics*. PCT Int. Appl. patent 94-1146 19940202.
257. Hara, M., & Ishigami, T. (1990). *Complex of tea polyphenols and protein for uses in medicine and food industries.* Japan patent 02202900.
258. Mai, J., Chambers, L. J., & McDonald, R. E. (1985). *Yucca extract used for food preservation.* Brit. UK Pat. Appl. patent 2151123.
259. Reddan, J. R., Giblin, F. J., Sevilla, M., Padgaonkar, V., Dziedzic, D. C., Leverenz, V. R., et al. (2003). Propyl gallate is a superoxide dismutase mimic and protects cultured lens epithelial cells from H_2O_2 insult. *Experimental Eye Research, 76*, 49–59.
260. Mellican, R. I., Li, J., Mehansho, H., & Nielsen, S. S. (2003). The role of iron and the factors affecting off-color development of polyphenols. *Journal of Agricultural and Food Chemistry, 51*, 2304–2316.
261. Martinez, M. V., & Whitaker, J. R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology, 6*, 195–200.
262. Mayer, A. M. (2006). Polyphenol oxidases in plants and fungi: Going places? A review. *Phytochemistry, 67*, 2318–2331.
263. Singh, D. V., & Mukherjee, P. P. (1973). Tyrosinase, an iron(II)-containing enzyme from tea leaves. *Current Science, 42*, 391.
264. Hiramoto, K., Ojima, N., Sako, K., & Kikugawa, K. (1996). Effect of plant phenolics on the formation of spin-adduct of hydroxyl radical and the DNA strand breaking of hydroxyl radical. *Biological and Pharmaceutical Bulletin, 19*, 558–563.
265. Kawanishi, S., Oikawa, S., & Murata, M. (2005). Evaluation for safety of antioxidant chemopreventive agents. *Antioxidants and Redox Signaling, 7*, 1728–1739.
266. Inoue, S., Ito, K., Yamamoto, K., & Kawanishi, S. (1992). Caffeic acid causes metal-dependent damage to cellular and isolated DNA through H_2O_2 formation. *Carcinogenesis, 13*, 1497–1502.
267. Malaisse, W. J., Hutton, J. C., Kawazu, S., Herchuelz, A., Valverde, I., & Sener, A. (1979). The stimulus-secretion coupling of glucose-induced insulin release. *Diabetologia, 16*, 331–341.
268. Kostyuk, V. A., Potapovich, A. I., Strigunova, E. N., Kostyuk, T. V., & Afanas'ev, I. B. (2004). Experimental evidence that avonoid metal complexes may act as mimics of superoxide dismutase. *Archives of Biochemistry and Biophysics, 428*, 204–208.
269. Afanas'ev, I. B., Ostrakhovitch, E. A., Mikhal'chik, E. V., Ibragimova, G. A., & Korkina, L. G. (2001). Enhancement of antioxidant and anti-in ammatory activities of bio avonoid rutin by complexation with transition metals. *Biochemical Pharmacology, 61*, 677–684.
270. McCord, J. M., Keele, J. B. B., & Fridovich, I. (1971). An enzyme-based theory of obligate anaerobiosis: The physiological function of superoxide dismutase. *Proceedings of the National Academy of Sciences of the United States of America, 68*, 1024–1027.
271. Fenton, H. J. H. (1894). Oxidation of tartaric acid in presence of iron. *Journal of the Chemical Society, Transactions, 65*, 899–910.
272. Fenton, H. J. H., & Jones, H. O. (1900). The oxidation of organic acids in presence of ferrous iron. Part I. *Journal of the Chemical Society, Transactions, 77*, 69–76.
273. Deisseroth, A., & Dounce, A. L. (1970). Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. *Physiological Reviews, 50*, 319–369.
274. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1972). Selenium: Biochemical role as a component of glutathione peroxidase. *Science, 179*, 588–590.
275. Mugesh, G., & du Mont, W.-W. (2001). Structure-activity correlation between natural glutathione peroxidase (GPx) and mimics: A biomimetic concept for the design and synthesis of more ef cient GPx mimics. *Chemistry – A European Journal, 7*, 1365–1370.
276. Gunther, M. R., Hanna, P. M., Mason, R. P., & Cohen, M. S. (1995). Hydroxyl radical formation from cuprous ion and hydrogen peroxide: A spin-trapping study. *Archives of Biochemistry and Biophysics, 316*, 515–522.
277. Kadiiska, M. B., & Mason, R. P. (2002). In vivo copper-mediated free radical production: an ESR spin-trapping study. *Spectrochimica Acta. Part A: Molecular Spectroscopy, 58*, 1227–1239.
278. Bhattacharya, P. K., & Patel, V. K. (1985). Effect of substitution on the catecholate ring on ternary complex stability. *Proceedings of the Indiana Academy of Science (Chemical Science), 94*, 495–500.
279. Blanco, C. A., & Hynes, M. J. (1992). Catalysis of the deprotonation of β-diketones during formation of the 1:1 metal complexes. *Canadian Journal of Chemistry, 70*, 2285–2289.
280. Esparza, I., Salinas, I., Santamaria, C., Garcia-Mina, J. M., & Fernandez, J. M. (2005). Electrochemical and theoretical complexation studies for Zn and Cu with individual polyphenols. *Analytica Chimica Acta, 543*, 267–274.
281. Mahal, H. S., Kapoor, S., Satpati, A. K., & Mukherjee, T. (2005). Radical scavenging and catalytic activity of metal–phenolic complexes. *The Journal of Physical Chemistry B, 109*, 24197–24202.
282. Bendini, A., Cerretani, L., Vecchi, S., Carrasco-Pancorbo, A., & Lercker, G. (2006). Protective effects of extra virgin olive oil phenolics on oxidative stability in the presence or absence of copper ions. *Journal of Agricultural and Food Chemistry, 54*, 4880–4887.
283. Andrade, R. G., Jr., Dalvi, L. T., Silva, J. J. M. C., Lopes, G. K. B., Alonso, A., & Hermes-Lima, M. (2005). The antioxidant effect of tannic acid on the in vitro copper-mediated formation

of free radicals. *Archives of Biochemistry and Biophysics, 437*, 1–9.
284. Perron, N. R., Chaur, M., Echegoyen, L., & Brumaghim, J. L. (2008). Antioxidant and prooxidant effects of polyphenols on copper-mediated DNA damage (submitted).
285. Li, Y., & Cao, Z. (2002). The neuroprotectant ebselen inhibits oxidative DNA damage induced by dopamine in the presence of copper ions. *Neuroscience Letters, 330*, 69–73.
286. Rahman, A., Shahabuddin, Hadi, S. M., Parish, J. H., & Ainley, K. (1989). Strand scission in DNA induced by quercetin and Cu(II): Role of Cu(I) and oxygen free radicals. *Carcinogenesis, 10*, 1833–1839.
287. Elbling, L., Weiss, R.-M., Teufelhofer, O., Uhl, M., Knasmueller, S., Schulte-Hermann, R., et al. (2005). Green tea extract and (−)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities. *FASEB Journal, 19*, 807–809.
288. Lambert, J. D., Sang, S., & Yang, C. S. (2007). Possible controversy over dietary polyphenols: Benefits vs risks. *Chemical Research in Toxicology, 20*, 583–585.
289. Rae, T. D., Schmidt, P. J., Pufahl, R. A., Culotta, V. C., & O'Halloran, T. V. (1999). Undetectable intracellular free copper: The requirement of a copper chaperone for superoxide dismutase. *Science, 284*, 805–808.
290. Masella, R., Benedetto, R. D., Vari, R., Filesi, C., & Giovannini, C. (2005). Novel mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione-related enzymes. *The Journal of Nutritional Biochemistry, 16*, 577–586.
291. Yang, L., McRae, R., Henary, M. M., Patel, R., Lai, B., Vogt, S., et al. (2005). Imaging of the intracellular topography of copper with a fluorescent sensor and by synchrotron X-ray fluorescence microscopy. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 11179–11184.
292. Que, E. L., Domaille, D. W., & Chang, C. J. (2008). Metals in neurobiology: Probing their chemistry and biology with molecular imaging. *Chemical Reviews, 108*, 1517–1549.
293. Turnlund, J. R., Jacob, R. A., Keen, C. L., Strain, J. J., Kelley, D. S., Domek, J. M., et al. (2004). Long-term high copper intake: Effects on indexes of copper status, antioxidant status, and immune function in young men. *The American Journal of Clinical Nutrition, 79*, 1037–1044.
294. Bremner, I. (1998). Manifestations of copper excess. *The American Journal of Clinical Nutrition, 67*, 1069S–1073S.
295. Koizumi, M., Fujii, J., Suzuki, K., Inoue, T., Inoue, T., Gutteridge, J. M. C., et al. (1998). A marked increase in free copper levels in the plasma and liver of LEC rats: An animal model for Wilson disease and liver cancer. *Free Radical Research, 28*, 441–450.
296. Sarkar, B. (1999). Treatment of Wilson and Menkes diseases. *Chemical Reviews, 99*, 2535–2544.
297. Heller, J., & Schwarzenbach, G. (1952). 102. Metallindikatoren V. Die eisenkomplexe der 2, 3-dioxy-naphtalin-6-sulfonsäure. *Helvetica Chimica Acta, 35*, 812–817.
298. Raymond, K. N., McMurry, T. J., & Garrett, T. M. (1988). Macrocyclic catechol-containing ligands. *Pure and Applied Chemistry, 60*, 545–548.
299. Harris, W. R., & Raymond, K. N. (1979). Ferric ion sequestering agents. 3. The spectrophotometric and potentiometric evaluation of two new enterobactin analogues: 1, 5, 9-N, N′, N″-Tris(2, 3-dihydroxybenzoyl)-cyclotriazatridecane and 1, 3, 5-N, N′, N″-tris(2, 3-dihydroxybenzoyl)triaminomethylbenzene. *Journal of the American Chemical Society, 101*, 6534–6541.
300. Engelmann, M. D., Hutcheson, R., & Cheng, I. F. (2005). Stability of ferric complexes with 3-hydroxy avone (avonol), 5, 7-dihyroxy avone (chrysin), and 3′,4′-dihydroxy avone. *Journal of Agricultural and Food Chemistry, 53*, 2953–2960.
301. Elhabiri, M., Carrer, C., Marmolle, F., & Traboulsi, H. (2007). Complexation of iron(III) by catecholate-type polyphenols. *Inorganica Chimica Acta, 360*, 353–359.
302. Maqsood, Z. T., & Kazmi, S. A. (1993). Determination and comparison of stability constants, enthalpy and entropy of formation of iron(III) complexes of gallic acid and methyl ester of gallic acid. *Journal of the Chemical Society of Pakistan, 15*, 30–35.
303. Tsobuchi, A., Kanno, C., & Akiyama, M. (1997). Enterobactin model compounds (LYSCAMs): Iron transport to microorganisms and iron removal from transferrin. *Journal of Inorganic Biochemistry, 67*, 31.
304. Rodgers, S. J., Lee, C.-W., Ng, C. Y., & Raymond, K. N. (1987). Ferric ion sequestering agents. 15. Synthesis, solution chemistry, and electrochemistry of a new cationic analogue of enterobactin. *Inorganic Chemistry, 26*, 1622–1625.
305. Thomas, F., Beguin, C., Pierre, J.-L., & Serratrice, G. (1999). Thermodynamic and kinetic studies of the sulfonated derivative of the iron chelator TRENCAM, an analog of enterobactin. *Inorganica Chimica Acta, 291*, 148–157.
306. Inoue, M. B., Inoue, M., Fernando, Q., Valcic, S., & Timmermann, B. N. (2002). Potentiometric and ^{1}H NMR studies of complexation of Al^{3+} with (−)-epigallocatechin gallate, a major active constituent of green tea. *Journal of Inorganic Biochemistry, 88*, 7–13.
307. Kumamoto, M., Sonda, T., Nagyama, K., & Tabata, M. (2001). Effects of pH and metal ions on antioxidative activities of catechins. *Bioscience, Biotechnology, and Biochemistry, 65*, 126–132.
308. Jovanovic, S. V., Hara, Y., Steenken, S., & Simic, M. G. (1995). Antioxidant potential of gallocatechins. A pulse radiolysis and laser photolysis study. *Journal of the American Chemical Society, 117*, 9881–9888.
309. Beltrán, J. L., Sanli, N., Fonrodona, G., Barrón, D., Özkan, G., & Barbosa, J. (2003). Spectrophotometric, potentiometric and chromatographic pK_a values of polyphenolic acids in water and acetonitrile-water media. *Analytica Chimica Acta, 484*, 253–264.
310. IUPAC. (1979). *IUPAC stability constants of metal ion complexes, part B, organic ligands*. Oxford, UK: Pergamon Press.
311. Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris.
312. Kennedy, J. A., Munro, M. H. G., Powell, H. K. J., Porter, L. J., & Foo, L. Y. (1984). The protonation reactions of catechin, epicatechin and related compounds. *Australian Journal of Chemistry, 37*, 885–892.
313. Herrero-Martínez, J. M., Sanmartin, M., Rosés, M., Bosch, E., & Ràfols, C. (2005). Determination of dissociation constants of flavonoids by capillary electrophoresis. *Electrophoresis, 26*, 1886–1895.
314. Ragnar, M., Lindgren, C. T., & Nilvebrant, N.-O. (2000). pK_a values of guaiacyl and syringyl phenols related to lignin. *Journal of Wood Chemistry and Technology, 20*, 277–305.
315. Nordström, C.-G., Lindberg, J. J., & Karumaa, L. J. (1963). Thermodynamic ionization constants of phenolic carboxylic acids related to guaiacol. *Soumen Kem B, 36B*, 105–109.
316. Ermakova, M. I., Kiryushina, M. F., & Zarubin, M. Y. (1985). OH-acidity of phenols related to lignin in dimethyl sulfoxide, dioxane and their mixtures with water. *Koksnes Kimija, 6*, 61–64.