Flavonoids, Coumarins, and Cinnamic Acids as Antioxidants in a Micellar System. Structure−Activity Relationship†

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The antioxidant activity of selected representatives of flavonoids, coumarins, and cinnamic acids was examined by measuring their protective action toward linoleic acid peroxidation in micelles of sodium dodecyl sulfate in buffer solution, pH 7.4. Results are expressed as relative antioxidant efficiency (RAE), defined as the ratio of the antioxidant efficiency (AE) of the tested compound to that of α-tocopherol. The best RAE values were observed for flavonoids, followed by coumarins and cinnamic acids. From the results, within each class of compounds a structure−activity relationship can be deduced.

Keywords: *Antioxidant; flavonoids; coumarins; cinnamic acids and esters; relative antioxidant efficiency (RAE); lipid peroxyl radicals;* α*-tocopherol*

Oxidation of cellular constituents by free radicals has been recognized as the cause of numerous diseases. The paper by Steinberg *et al.* (1989), for instance, describes brilliantly the link between atherosclerosis and peroxidation of low-density lipoproteins (LDL), and many further correlations have been suggested in the literature (Barclay, 1993; Breccia *et al.*, 1986; Deutcke *et al.*, 1987; Girotti, 1990; Grossweiner and Goyal, 1983; Halliwell, 1987; Johnson, 1988; Kantor and Ritter, 1983; Rice-Evans *et al.*, 1987; Stadtman, 1992; Sun, 1990).

Oxidation reactions are a concern also for the food industry, in view of the qualitative decay they cause (Graf, 1994; Mehta *et al.*, 1994; Ramanathan and Das, 1992; Tsuda *et al.*, 1994). Since it is well-known that the use of antioxidants hampers the chemical modifications implied in these adverse processes, a vast amount of work has been devoted to the search for "powerful" nontoxic, natural antioxidants able to quench active free radicals such as $O_2^{\bullet-}$, $OH^{\bullet}$, or lipid peroxyl radicals $LOO^{\bullet}$. These natural products may be valuable for increasing the shelf life of foodstuffs and replacing synthetic compounds commonly used for this purpose, the innocuosness of which has still to be proved (Fauré *et al.*, 1990; Tsuda *et al.*, 1994; Yen *et al.*, 1993). In model systems many natural products show antioxidant activity, for instance, tocopherols, flavonoids, coumarins, and more generally phenolic compounds widespread in the plant kingdom (Foti *et al.*, 1994; Larson, 1988; Lewis, 1989). Among them, flavonoids are a particularly attractive class of polyphenols, as they occur often in significant concentrations (0.5−1.5%). Although the antioxidant activity of flavonoids has been the subject of some investigations (Benov and Georgiev, 1994; Fauré *et al.*, 1990; Husain *et al.*, 1987; Huguet *et al.*, 1989; Moroney *et al.*, 1988; Jovanovic *et al.*, 1994; Sichel *et al.*, 1991; Yuting *et al.*, 1990), no systematic work on structure−activity relationship has been reported until now. Occasionally it has been observed that ring B is more reactive than ring A, but no explanation of this fact has been given. This and the scanty work on the antioxidant properties of these compounds in micellar phase prompted us to study the relative antioxidant efficiency (RAE) of selected representatives of natural phenols in this model system.

In the present paper we describe the antioxidant activity of flavonoids **1−10**, coumarins **11−17** and cinnamic acids **18−23** (Chart 1) as protectors of linoleic acid subjected to peroxidation in aqueous micelles of sodium dodecyl sulfate (SDS) in buffer solution at pH 7.4 and 50 °C.

EXPERIMENTAL PROCEDURES

Linoleic acid and SDS were purchased from Sigma; 6,7- and 5,7-dihydroxy-4-methylcoumarin, 6,7-dihydroxyflavon, daphnetin, and 4-methyldaphnetin were from Extrasynthèse. Other flavonoids were purchased from Aldrich, and 2,2′-azobis(2-amidinopropane) dihydrochloride was from Wako. Other chemicals were from Fluka. Compounds were used as received. (+)-α-Tocopherol was obtained by hydrolysis of its acetate (Sigma). Methyl cinnamates were prepared by standard esterification of the acids. Kinetics were recorded on a Beckman DU-65 spectrophotometer equipped with a thermostated cuvette house and a PC to process the data.

Solutions. A 0.1 M solution of SDS was prepared in aqueous 0.01 M NaH_2PO_4 and adjusted to pH 7.4 with concentrated aqueous NaOH. Linoleic acid was added, immediately before each experiment, to a concentration 0.0026 M. A stock solution (0.07 M) of 2,2′-azobis(2-amidinopropane) dihydrochloride in water, stored at 5−10 °C, was used within a week. Solutions of compounds to be tested (5×10^{-3}−10^{-5} M) in methanol were prepared immediately before use.

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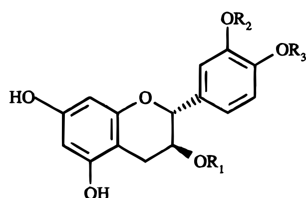
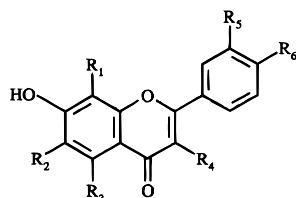
Chart 1



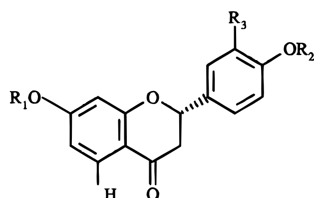
Flavan-3-ols

1 R_1=R_2=R_3= H (Catechin)
2 R_1=R_2=R_3= $COCH_3$ (Triacetylcatechin)



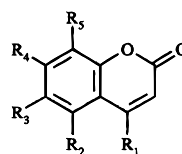
Flavonols and Flavones

3 R_1=R_2= H; R_3=R_4=R_5=R_6= OH (Quercetin)
4 R_1=R_2=R_4=R_5=R_6= H; R_3= OH (Chrysin)
5 R_1=R_2=R_3=R_4=R_5=R_6= H (7-Hydroxyflavone)
6 R_1=R_3=R_4=R_5=R_6= H; R_2= OH (6,7-Dihydroxyflavone)
7 R_1=OH; R_2=R_3=R_4=R_5=R_6= H (7,8-Dihydroxyflavone)



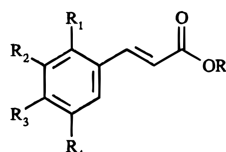
Flavanones

8 R_1=R_2=R_3= H (Naringenin)
9 R_1= 2-*O*-rhamnoglucose; R_2=R_3= H (Naringin)
10 R_1= 6-*O*-rhamnoglucose; R_2= CH_3; R_3= OH (Hesperidin)



Coumarins

11 R_1=OH; R_2=R_3=R_4=R_5= H (4-Hydroxycoumarin)
12 R_1=R_2=R_3=R_5= H; R_4= OH (7-Hydroxycoumarin / Umbelliferon)
13 R_1=R_2=R_5= H; R_3=OCH_3; R_4=OH (6-Methoxy-7-Hydroxycoumarin / Scopoletin)
14 R_1=CH_3; R_2=R_4= OH; R_3=R_5= H (5,7-Dyhydroxy-4-Methylcoumarin)
15 R_1=CH_3; R_2=R_5= H; R_3=R_4= OH (6,7-Dihydroxy-4-Methylcoumarin)
16 R_1=R_2=R_3= H; R_4=R_5= OH (7,8-Dihydroxycoumarin / Daphnetin)
17 R_1=CH_3; R_2=R_3= H; R_4=R_5= OH (7,8-Dihydroxy-4-Methylcoumarin /4-Methyldaphnetin)

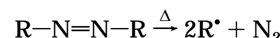


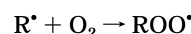
Cinnamic acids

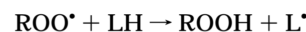
18 R_1= OH; R_2=R_3=R_4=R_5= H (*o*-Coumaric acid)
19 R_1= R_3=R_4=R_5= H; R_2=OH (*m*-Coumaric acid)
20 R_1=R_2=R_4= R_5= H; R_3= OH (*p*-Coumaric acid)
21 R_1=R_2=R_5= H; R_3= OH; R_4=OCH_3 (Ferulic acid)
22 R_1=R_2=R_5= H; R_3=R_4= OH (Caffeic acid)
23 R_1= OH; R_2=R_3=R_4= H; R_5= CH_3 (*o*-Coumaric acid methyl ester)
24 R_1= R_3=R_4= H; R_2=OH; R_5= CH_3 (*m*-Coumaric acid methyl ester)
25 R_1=R_2=R_4= H; R_3= OH; R_5= CH_3 (*p*-Coumaric acid methyl ester)
26 R_1=R_2= H; R_3= OH; R_4=OCH_3; R_5= CH_3 (Ferulic acid methyl ester)
27 R_1=R_2=H; R_3=R_4= OH; R_5= CH_3 (Caffeic acid methyl ester)

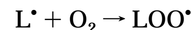
Procedure. An aliquot (2 mL) of the micellar suspension of linoleic acid in a UV cell was stirred in the sample compartment of the spectrophotometer at 50 °C. The buffered SDS solution was used as blank. After thermal equilibration (20 min), 10 μL of the radical initiator solution was added and the progress of the peroxidation monitored by recording the absorbance at 234 nm for 15 min. Then scalar amounts of antioxidant solution (1−20 μL) were added and the kinetics followed for a further 20−30 min. The slope of the linear plot of absorbance *vs* time after the addition of the antioxidant gives d*A*/d*t*. From the plot d*A*/d*t* *vs* $[InH]^{-1}$ the slope S_{inh} was obtained and the RAE value calculated (Pryor *et al.*, 1993).

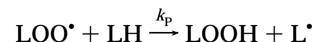
Kinetic Treatment. Recently a new method for determining the antioxidant efficiency of a compound relative to α-tocopherol in aqueous micelles of SDS containing linoleic acid (LH) has been developed (Pryor *et al.*, 1993). The method is based on the spectrophotometric determination of the rate of conjugated diene formation from LH, in the presence of either α-tocopherol or a potential antioxidant. Conjugated diene hydroperoxides (LOOH) are the end products of LH peroxidation induced into the micellar phase by a radical initiator which, on thermolysis, provides radicals at constant rate. These hydroperoxides have strong UV absorption, in micelles of SDS in buffer solution at pH 7.4, with a maximum at 234 nm and a molar extinction coefficient of 26 100 M^{-1} cm^{-1}. No significant interference from the tested compounds was observed at the low concentration used. The method is extremely sensitive and allows the study of the oxidation process at low conversions (less than 10%), at which steady state kinetic analysis better applies. The following scheme summarizes the reactions involved:


$$R{-}N{=}N{-}R \xrightarrow{\Delta} 2R^{\bullet} + N_2$$

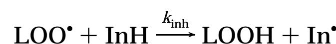

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$


$$ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet}$$


$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$


$$LOO^{\bullet} + LH \xrightarrow{k_p} LOOH + L^{\bullet}$$

$$2LOO^{\bullet} \xrightarrow{k_t} \text{nonradical products}$$


$$LOO^{\bullet} + InH \xrightarrow{k_{inh}} LOOH + In^{\bullet}$$

$$LOO^{\bullet} + In^{\bullet} \rightarrow \text{nonradical products}$$

R−N=N−R is the radical initiator, LH linoleic acid, $L^{\bullet}$ a linoleic radical, $LOO^{\bullet}$ a linoleic peroxy radical, and InH the antioxidant (inhibitor); k_p, k_t and k_{inh} are rate constants of the propagation, termination, and inhibition steps, respectively.

The rate of oxygen uptake in the lipid autoxidation process, in homogeneous solution under sufficient oxygen pressure and in the presence of an antioxidant, is given by the equation

$$-\frac{d[O_2]}{dt} = \frac{k_p}{nk_{inh}}\frac{[LH]}{[InH]}R_i \quad (1)$$

where n is the stoichiometric factor of the antioxidant, i.e. the number of radicals trapped by a single molecule of antioxidant (for α-tocopherol $n = 2$), and R_i the radical production rate from the radical initiator (Burton and Ingold, 1981; Pryor *et al.*, 1993).

The above equation, derived by assuming the long-chain approximation in a homogeneous system, is also valid for micellar systems (Pryor *et al.*, 1993).

The rate of conjugated diene formation is proportional to the rate of oxygen uptake, i.e.

$$-\frac{d[LOOH]}{dt} = K\frac{d[O_2]}{dt} \quad (2)$$

If it is assumed that the same value of K applies both to the noninhibited and to the antioxidant-inhibited process (Pryor *et al.*, 1993), the rate of conjugated diene formation for the inhibited oxidation is

$$\frac{d[LOOH]}{dt} = K\frac{k_p[LH]}{nk_{inh}[InH]}R_i \quad (3)$$

The value of d[LOOH]/dt is determined by measuring the absorbance A as function of time at 234 nm in a micellar system

$$\frac{d[LOOH]}{dt} = \frac{1}{\epsilon l}\frac{dA}{dt} \quad (4)$$

where ϵ is the molar extinction coefficient of the conjugated diene (26 100 M^{-1} cm^{-1} at 234 nm) and l the width of the cell in centimeters. Equation 4 can be rewritten as follows:

$$\frac{dA}{dt} = \frac{\epsilon lKk_pR_i[LH]}{nk_{inh}[InH]} \quad (5)$$

Since [LH] is almost constant during the experiment, the plot of dA/dt *vs* $[InH]^{-1}$ is a straight line having as slope S

$$S_{inh} = \frac{\epsilon lKk_pR_i[LH]}{nk_{inh}} \quad (6)$$

When α-tocopherol is used as the inhibitor, the preceding equation becomes

$$S_{\alpha\text{-toc}} = \frac{\epsilon lKk_pR_i[LH]}{2k_{\alpha\text{-toc}}} \quad (7)$$

The ratio of $S_{\alpha\text{-toc}}$ over S_{inh} for a different antioxidant gives the RAE value for the latter, providing the experimental conditions are identical for both sets of experiments:

$$RAE_{inh} = \frac{S_{\alpha\text{-toc}}}{S_{inh}} = \frac{nk_{inh}}{2k_{\alpha\text{-toc}}} \quad (8)$$

Measurement of Partition Coefficient (log *P*). A solution (10^{-4}–10^{-5} M) of the compound of choice in *n*-octanol was kept at 50 °C for 1 h. A UV spectrum was then run, and the value of the absorbance at the maximum was measured (A_0). Equal volumes of the organic solution and water were mixed at 50 °C. UV spectra of the organic layer were run at regular intervals until the absorbance value became constant (A_x). The partition coefficient (log P) was calculated according to the relationship

$$P = \frac{C_{n\text{-octanol}}}{C_{water}} = \frac{A_x}{A_0 - A_x}$$

where $C_{n\text{-octanol}}$ and C_{water} are the concentrations of the compound in *n*-octanol and water, respectively, and A_0 and A_x the initial and final absorbance values of the organic layer. A solution of *n*-octanol saturated with water was used as blank.

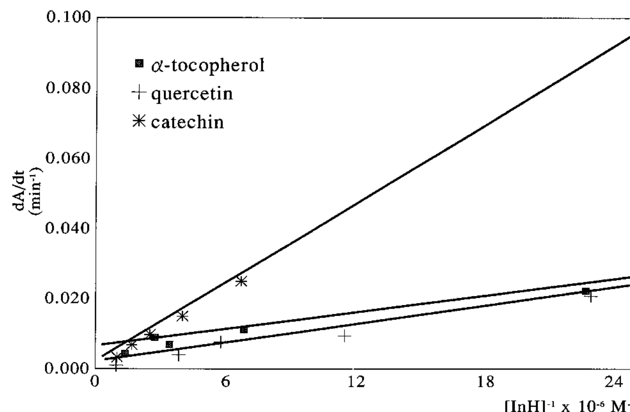


Figure 1. Calculation of AE values for α-tocopherol (■), quercetin (+), and catechin (*) in 0.10 M SDS/0.05 M phosphate buffer (pH 7.4), at 50 °C (SD = ±12%).

Table 1. Relative Antioxidant Efficiency (RAE) Values of Compounds 1–27[a]

compound	RAE[b] × 100
α-tocopherol	100
flavonoids	
3,3′,4′,5,7-flavanpentol (catechin) (**1**)	22
3,3′,4′-triacetoxy-5,7-dihydroxyflavanone (**2**)	1.70
3,3′,4′,5,7-pentahydroxyflavone (quercetin) (**3**)	90
5,7-dihydroxyflavone (chrysin) (**4**)	0.51
7-hydroxyflavone (**5**)	na[c]
6,7-dihydroxyflavone(**6**)	70
7,8-dihydroxyflavone (**7**)	63
4′,5,7-trihydroxyflavanone (naringenin) (**8**)	0.61
naringin (naringenin 7-rhamnoglucoside) (**9**)	0.01
hesperidin (hesperetin 7-ramnoglucoside) (**10**)	1
coumarins	
4-hydroxycoumarin (**11**)	na
7-hydroxycoumarin (umbelliferon) (**12**)	0.03
6-methoxy-7-hydroxycoumarin (scopoletin) (**13**)	0.18
5,7-dihydroxy-4-methylcoumarin (**14**)	0.72
6,7-dihydroxy-4-methylcoumarin (**15**)	2.42
7,8-dihydroxycoumarin (daphnetin) (**16**)	3.30
4-methyldaphnetin (**17**)	3.02
cinnamic acids	
o-coumaric acid (**18**)	na
m-coumaric acid (**19**)	na
p-coumaric acid (**20**)	0.06
ferulic acid (**21**)	0.04
caffeic acid (**22**)	0.06
o-coumaric acid methyl ester (**23**)	na
m-coumaric acid methyl ester (**24**)	na
p-coumaric acid methyl ester (**25**)	0.25
ferulic acid methyl ester (**26**)	0.10
caffeic acid methyl ester (**27**)	0.30

[a] See Experimental Procedures for experimental protocols. [b] Values are the average of four measurements (SD = ±15%). [c] na, not active.

RESULTS AND DISCUSSION

Figure 1 illustrates typical examples of the plot dA/dt *vs* $[InH]^{-1}$, while Table 1 summarizes the RAE values for compounds **1–27** obtained from the slopes of the plots. The radical-trapping efficiency is modulated by the aptitude of the compounds to transfer an H atom to LOO•, that is, by the stability of the radical form of the inhibitor In•.

Among the flavonoids **1–10**, 7-hydroxyflavone, 5,7-dihydroxyflavone (chrysin) and 4′,5,7-trihydroxyflavone have very low activity, if any, and the same applies to the glycosides naringin and hesperidin. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is the most active fla-

Table 2. Partition Coefficent (Log *P*) and RAE of Selected Compounds[a]

compound	log P^b	RAE[c]
flavonoids		
3,3′,4′,5,7-flavanpentol (catechin) (**1**)	1.31	22
3,3′,4′,5,7-pentahydroxyflavone (quercetin) (**3**)	1.26	90
7,8-dihydroxyflavone (**7**)	1.43	63
4′,5,7-trihydroxyflavanone (naringenin) (**8**)	1.60	0.61
naringin (naringenin 7-rhamnoglucoside) (**9**)	0.11	0.01
hesperidin (hesperetin 7-ramnoglucoside) (**10**)	0.32	1
coumarins		
4-hydroxycoumarin (**11**)	0.93	na
7,8-dihydroxycoumarin (daphnetin) (**16**)	0.71	3.30
cinnamic acids		
o-coumaric acid (**18**)	0.62	na
ferulic acid (**21**)	0.55	0.04
caffeic acid (**22**)	0.56	0.06
ferulic acid methyl ester (**26**)	1.20	0.10

[a] See Experimental Procedures for experimental protocols. [b] Values are the average of three measurements (SD = ±5%). [c] See Table 1 for details.

vone (RAE = 90), while the flavanol with the same hydroxylation pattern, (+)-catechin (3,3′,4′,5,7-flavanpentol), is about 4-fold less active. The activity of 3,3′,4′-triacetylcatechin is an order of magnitude lower than that of the parent flavanol. These data show that in determining the level of antioxidant activity of the flavonoids in a micellar system the number of hydroxyl groups is of negligible importance, as is the fact that they are located on ring A or ring B. The true difference is made by the presence or not of two ortho phenolic functions, as is readily apparent by a comparison of the RAE values for catechin and its triacetate. This conclusion is strengthened by the behavior of the synthetic compounds 6,7- and 7,8-dihydroxyflavones with an "unnatural" ortho-hydroxylation pattern on ring A, both having a remarkable antioxidant efficiency, not far from that of quercetin. The difference between quercetin and catechin, which possess the same hydroxylation pattern, can be referred to the increased stability of the aryloxyl radical of the former compound due to the presence of the carbonyl group in conjugation with ring B. Analogously, the greater activity of the synthetic dihydroxyflavones **6** and **7** in comparison to catechin can be explained by the presence of a carbonyl in the central ring of their molecules, which takes part in the stabilization of the intermediate radical.

The great reactivity of the ortho-dihydroxy system is possibly due to the smaller dissociation energy of the O−H bond in comparison with the 1,3-system, owing to the greater stability of the transient radical involved, in which the oxygen-centered unpaired p-orbital is conjugated with a lone pair on the adjacent oxygen atom. Moreover, one cannot ignore the presence in the central ring of the carbonyl and the 2,3-double bond, which partecipate in the radical stabilization, resulting in an increased antioxidant activity.

In addition to the structural features, surely the heterogeneous system used plays a role, owing to the different partition of the tested substances between the two phases (water/micelles). Therefore, the partition coefficients (log *P*) of selected compounds in an octanol/water two-phase system were measured (Table 2) in order to evaluate the role of their lipophilicity on the antioxidant effectiveness. From a comparison of the log *P* and RAE values (Table 2) it is apparent that within the class of flavonoids the most active compounds (**1**−**3**) have high log *P* values, whereas the same does not hold true for naringenin (**8**), which has low activity but high log *P* value. This suggests that, in the presence of comparable lipophilicity, the structural factors are essential and predominate in determining the activity of a specific compound. A further analysis of data in Table 2 shows that the lower activity of the glycoside naringin (**9**) in comparison with the corresponding aglycon (naringenin) can be related to the different lipophilicities.

The seven coumarins tested, **11**−**17** [4-hydroxycoumarin, 7-hydroxycoumarin (umbelliferon), 6-methoxy-7-hydroxycoumarin (scopoletin), 5,7-dihydroxy-4-methylcoumarin, 6,7-dihydroxy-4-methylcoumarin, 7,8-dihydroxycoumarin (daphnetin), and 4-methyldaphnetin], all have very low antioxidant activity, with RAE values in the range 3.30−0.03. The only information that emerges from consideration of the relevant data in Table 1 is that again the most active compounds are those possessing an ortho-dihydroxy phenolic system, namely 6,7-dihydroxy-4-methylcoumarin, 7,8-dihydroxy-4-methylcoumarin (daphnetin), and 4-methyldaphnetin. Finally, we examined the activity of the cinnamic acids **18**−**22**. Of the five acids tested, *p*-coumaric, caffeic, and ferulic acids showed very low activities, while *o*- and *m*-coumaric acids were completely inactive. Since at pH 7.4 these compounds are present largely as anions, they may not reach the inside of the SDS micelles owing to the repulsion exerted by the negative charges at their outer surface. To test this hypothesis, we measured the activity of the corresponding methyl cinnamates, **23**−**27**. As can be seen from the data in Table 1, while methyl *o*-coumarate and methyl *m*-coumarate are devoid of activity, the esters of caffeic, *p*-coumaric, and ferulic acids are decidedly more active than the free acids.

The present study confirms the potential of the micellar system in the rapid determination of the antioxidant activity. However, the interpretation of the results requires some caution. Compounds ionizable at the pH of the medium (for instance, cinnamic acids) or highly hydrophilic (glydosides) due to the difficult access to the inside of the micelles gave results that require careful examination. The different activities of the three classes of phenolic compounds considered here (flavonoids, coumarins, and cinnamic acids), as well as the differences within each class, can be ascribed primarily to their intrinsic capability of transferring a hydrogen atom to the peroxy radical LOO•. However, the hydrophilicity also plays a role in determining the values of the relative antioxidant activity as measured in that heterogeneous system, and this seems to be the major limitation of the method.

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LITERATURE CITED

Barclay, L. R. C. Model biomembrane: quantitative studies of peroxidation, antioxidant action, partitioning, and oxidative stress (1992 Syntex Award Lecture), *Can. J. Chem.* **1993**, *71*, 1−16, and references cited therein.

Benov, L.; Georgiev, N. The antioxidant activity of flavonoids isolated from *Corylus colurna*. *Phytother. Res.* **1994**, *8*, 92−94.

Breccia, A., Rodgers, M. A. J., Semeraro, Eds. *Oxygen and Sulfur Radicals in Chemistry and Medicine, Oxygen Radicals in Chemistry and Biology;* Edizioni Scientifiche Lo Scarabeo: Bologna, 1986; Chapter 1, pp 20−52.

Burton, G. W.; Ingold, K. U. Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related

chain-breaking phenolic antioxidants *in vitro*. *J. Am. Chem. Soc.* **1981**, *103*, 6472−6477.

Deuticke, B.; Heller, K. B.; Haest, C. W. M. Progressive oxidative damage in erythrocytes after pulse treatment with *t*-butylhydroperoxide. *Biochim. Biophys. Acta* **1987**, *899*, 113−124.

Fauré, M.; Lissi, E.; Torres, R.; Videla, L. A. Antioxidant activities of lignans and flavonoids. *Phytochemistry* **1990**, *29*, 3773−3775.

Foti, M.; Piattelli, M.; Amico, V.; Ruberto, G. Antioxidant activity of phenolic meroditerpenoids from marine algae. *J. Photochem. Photobiol. B* **1994**, *26*, 159−164.

Girotti, A. W. Photodynamic peroxidation in biological systems. *Photochem. Photobiol.* **1990**, *51*, 497−509.

Graf, E. Copper(II) acorbate: a novel food preservation system. *J. Agric. Food Chem.* **1994**, *42*, 1616−1619.

Grossweiner, L. I.; Goyal, G. C. Photosensitized lysis of liposomes by hematoporphyrin derivative. *Photochem. Photobiol.* **1983**, *37*, 529−532.

Halliwell, B. Oxidant and human disease: some new concepts. *FASEB J.* **1987**, *1*, 358−364.

Huguet, A. I.; Manez, S.; Alcaraz, M. J. Superoxide scavenging properties of flavonoids in a non-enzymic system. *Z. Naturforsch.* **1989**, *45C* , 19−24.

Husain, S. R.; Cillard, J.; Cillard, P. Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* **1987**, *26*, 2489−2491.

Johnson, B. E. Skin and related photoreactions. In *Photosensitation;* Moreno et al., Eds.; NATO ASI Series H 15; Springer-Verlag: Berlin, 1988; pp 253−264.

Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. Flavonoids as antioxidants. *J. Am. Chem. Soc.* **1994**, *116*, 4846−4851.

Kantor, G. J.; Ritter, C. Sunlight-induced killing of nondividing human cells in culture. *Photochem. Photobiol.* **1983**, *37*, 533−538.

Larson, R. A. The antioxidants from higher plants. *Phytochemistry* **1988**, *27*, 969−978.

Lewis, D. A. *Anti-inflammatory Drugs from Plant and Marine Source;* Birkäuser-Verlag: Basel, 1989; pp 95−283.

Mehta, R. L.; Zayas, J. F.; Yang, S.-S. Ajowan as a source of natural lipid antioxidant. *J. Agric. Food Chem.* **1994**, *42*, 1420−1422.

Moroney, M. A.; Alcaraz, M. J.; Forder, R. A.; Carey, F.; Hoult, R. S. Selectivity of neutrophil 5-lipoxygenase and cyclooxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J. Pharm. Pharmacol.* **1988**, *40*, 787−792.

Pryor, W. A.; Cornicelli, J. A.; Dervall, L. J.; Tait, B.; Trivedi, B. K.; Witiak, D. T.; Wu, M. A rapid screening test to determine the antioxidant potencies of natural and synthetic antioxidants. *J. Org. Chem.* **1993**, *58*, 3521−3532.

Ramanathan, L.; Das, N. P. Studies on the control of lipid oxidation in ground fish by some polyphenolic products. *J. Agric. Food Chem.* **1992**, *40*, 17−21.

Rice-Evans, C.; Hochstein, P. Alterations in erythrocyte membrane fluidity by phenylhydrazine-induced peroxidation of lipids. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1537−1542.

Sichel, G.; Corsaro, C.; Scalia, M.; Di Bilio, A. J.; Bonomo, R. *In vitro* scavenger activity of some flavonoids and melanins against $O_2^{\bullet -}$. *Free Radicals Biol. Med.* **1991**, 10.

Stadtman, E. R. Protein oxidation and aging. *Science* **1992**, *257*, 1220−1224.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Modification of low density lipoprotein that increase its atherogenicity. *New Engl. J. Med.* **1989**, *320*, 915−924.

Sun, Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radicals Biol. Med.* **1990**, *8*, 583−599.

Tsuda, T.; Ohshima, K.; Kawakishi, S.; Osawa, T. Antioxidative pigments isolated from the seeds of *Phaseolus vulgaris* L. *J. Agric. Food Chem.* **1994**, *42*, 248−251.

Yen, G. C.; Duh, P. D.; Tsai C. L. Relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem.* **1993**, *41*, 67−70.

Yuting, C.; Rongliang, Z.; Zhongjian, J.; Yong, J. Flavonoids as superoxide scavengers and antioxidants. *Free Radicals Biol. Med.* **1990**, *9*, 19−21.

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