Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 92 (2005) 349–355
Food Chemistry
www.elsevier.com/locate/foodchem

Structure–antioxidant activity relationships of flavonoids isolated from different plant species

Paola Montoro [a,*], Alessandra Braca [b], Cosimo Pizza [a], Nunziatina De Tommasi [a]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy*
[b] *Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Via Bonanno, 33, 56126 Pisa, Italy*

Received 9 December 2003; received in revised form 28 July 2004; accepted 28 July 2004

Abstract

In the course of our phytochemical studies of different plants from developing countries we isolated and structurally characterized several flavonoid derivatives (compounds **1**–**26**), both aglycones and glycosides, typical of the species investigated. The aim of this study was to evaluate varieties of medicinal plants that were growing in developing countries, known in traditional medicine as anti-inflammatory remedies, with respect to their flavonoidic isolated constituents, assuming that their anti-inflammatory activity could be explained, at least in part, by the presence of antioxidant principles. The antioxidant activities of compounds **1**–**26** were evaluated by measuring their ability to scavenge the radical cation 2,2′-azino-*bis* (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\cdot+}$) and the superoxide anion, to inhibit β-carotene oxidation in a lipid micelle system, and to inhibit xanthine oxidase activity, showing some structure–activity relationships.

Keywords: Flavonoids; Medicinal plants; Antioxidant activity; Free radicals

1. Introduction

Flavonoids are naturally occurring substances in plants that are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of biological actions such as antibacterial, antiviral, anti-inflammatory, anticancer, and antiallergic activities (Di Carlo, Mascolo, Izzo, & Capasso, 1999; Havsteen, 1983). Together with their biological activity, flavonoids are important components in the human diet, although they are generally considered as non-nutrients. Sources of flavonoids are foods, beverages, different herbal drugs, and related phytomedicines (Aisling Aherne & O'Brien, 2002).

The scientific evidence that plant-based diets, in particular those rich in vegetable and fruits, protect against cancer has been found to be strong and consistent by an expert panel (World Cancer Research Fund, 1997).

A number of flavonoids have been shown to suppress carcinogenesis in various animal models (Yang, Landau, Huang, & Newmark, 2001). The antioxidant property of flavonoids was the first mechanism of action studied, in particular with regard to their protective effect against cardiovascular diseases. Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals (Bravo, 1998) that are probably involved in several diseases. Mechanism

* Corresponding author. Fax: +39 089 962828.
E-mail address: pmontoro@unisa.it (P. Montoro).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.07.028

of antioxidant action can include suppressing reactive oxygen species formation, either by inhibition of enzymes or by chelating trace elements involved in free-radical production, scavenging reactive species, and upregulating or protecting antioxidant defences (van Acker et al., 1996).

In the course of our phytochemical and biological studies on several plants from developing countries we isolated and structurally characterized several flavonoid derivatives, both aglycones and glycosides, belonging to different chemical classes (flavones, flavanones, flavonols, and dihydroflavonols) (Braca et al., 2003; De Simone et al., 2001). The purpose of this study was to evaluate a variety of medicinal plants that were growing in developing countries, known in traditional medicine as anti-inflammatory remedies with respect to their flavonoidic isolated constituents, typical of the species investigated, assuming that their anti-inflammatory activity could be explained, at least in part, by the presence of antioxidant principles. The antioxidant activities of isolated flavonoids were evaluated by measuring their ability to scavenge the radical cation 2,2′-azino-*bis* (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) and the superoxide anion, to inhibit β-carotene oxidation in a lipid micelle system, and to inhibit xanthine oxidase (XOD) activity, showing some structure–activity relationships.

2. Materials and methods

2.1. Extraction and isolation

Kaempferol 3-*O*-rutinoside (**1**), kaempferol 3-*O*-α-L-(2″-β-D-xylosyl)rhamnoside (**2**), quercetin 3-*O*-α-L-arabinoside (**3**), myricetin 3-*O*-β-D-galactoside (**9**), taxifolin 3-*O*-α-L-rhamnoside (**23**), and dihydromyricetin 3-*O*-α-L-rhamnoside (**24**) were isolated from *Licania licaniaeflora* (Sagot) Blake (Chrysobalanaceae), as reported previously (Braca et al., 2003).

Quercetin 3-methoxy-7-*O*-β-D-glucoside (**4**), apigenin 7-*O*-gentiobioside (**18**), and luteolin 7-*O*-β-D-glucoside (**19**) were purified from *Befaria cinnamomea* Lindely (Ericaceae, Perù) with the following isolation procedure: 270 g of aerial parts were defatted with petroleum ether and then extracted at room temperature with $CHCl_3$, $CHCl_3$:MeOH, 9:1, and MeOH to give 10.0, 19.8, 3.5, and 21 g of the respective residues. Part of the methanolic extract was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain 10 major fractions. Fraction 6 (230 mg) was purified by RP-HPLC on a Waters C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O, 45:55, to yield compounds **4** (14.0 mg, t_R = 19 min), **19** (5.5 mg, t_R = 21 min), and **18** (25.0 mg, t_R = 24 min).

Quercetin 3-methoxy-7-*O*-gentiobioside (**5**), quercetin 3-*O*-(6‴-caffeoyl)gentiobioside (**6**), luteolin 7-*O*-gentiobioside (**20**), and luteolin 7-*O*-(4‴-caffeoyl)gentiobioside (**21**) were isolated from *Lonicera implexa* Aiton (Caprifoliaceae), as reported previously (Flamini, Braca, Cioni, Morelli, & Tomè, 1997).

Myricetin 7-*O*-gentiobioside (**7**) and myricetin 7-*O*-rutinoside (**8**) were purified from *Tachigalia paniculata* Aubl. (Leguminosae) (Cioffi et al., 2002).

Myricetin 3,4′-*O*-di-α-L-rhamnoside (**10**), myricetin 4′-methoxy-3-*O*-β-D-galactoside (**11**), myricetin 4′-methoxy-3-*O*-α-L-rhamnoside (**12**) were obtained from *Licania heteromorpha* var. *heteromorpha* Bentham (Chrysobalanaceae), as described in the literature (Braca et al., 2003).

3,7,3′,4′,5′-Pentahydroxyflavone (**13**), 3,7,3′,5′-tetrahydroxy-4′methoxyflavone (**14**), and 3,7,4′-trihydroxy-3′,5′-dimethoxyflavone (**15**) were isolated from the roots of *Entada africana* Guill. and Perr. (Leguminosae, Mali): 200 g of the roots were defatted with petroleum ether and then extracted at room temperature with $CHCl_3$, $CHCl_3$–MeOH 9:1, and MeOH to give 1.7, 4.7, 10.0, and 18.0 g of residues, respectively. Part of the methanolic extract was submitted to Sephadex LH-20 chromatography, using MeOH as eluent, to obtain compounds **13** (6.0 mg), **14** (13.0 mg), and **15** (15.0 mg).

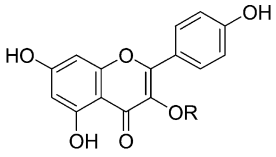
4′-Methoxy-apigenin (**16**) and apigenin 7-*O*-β-D-glucuronide (**17**) were purified from *Onopordum illyricum* L. (Compositae), as reported previously (Braca, De Tommasi, Morelli, & Pizza, 1999).

8-Hydroxy-naringenin (**25**) and naringenin 7-*O*-neohesperidoside (**26**) were purified from *Notholaena nivea* var. *nivea* (Pteridaceae, Perù) with the following isolation procedure: 400 g of the leaves were defatted with petroleum ether and then extracted at room temperature with $CHCl_3$, $CHCl_3$–MeOH 9:1, and MeOH to give 15.0, 13.8, 7.0, and 8.0 g of the respective residues. Part of the methanolic extract (2.5 g) was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain compound **25** (3.0 mg), together with 12 major fractions. Fraction 5 (198 mg) was purified by RP-HPLC on a Waters C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 ml min^{-1}) with MeOH–H_2O 35:65 to yield pure compound **26** (20.0 mg, t_R = 26 min).

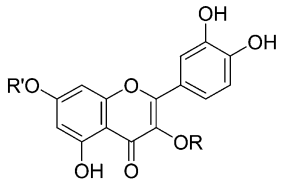
Purity of each compound was checked by HPLC-DAD and NMR immediately before testing them.

2.2. Chemicals

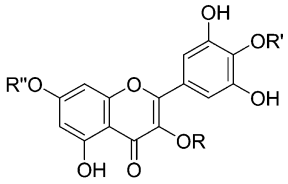
Taxifolin (**21**), 2,2′-azino-*bis* (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, linoleic acid, Tween 20, butylhydroxytoluene (BHT), β-carotene, EDTA, bovine serum albumin (BSA), nitro-



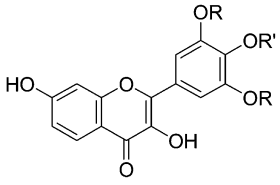
R = rutinoside **1**
R = α-L-(2"-β-D-xylosyl)rhamnoside **2**



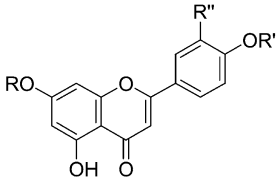
R = α-L-arabinoside R' = H **3**
R = CH_3 R' = β-D-glucoside **4**
R = CH_3 R' = gentiobioside **5**
R = CH_3 R' = (6"'-caffeoyl)gentiobioside **6**



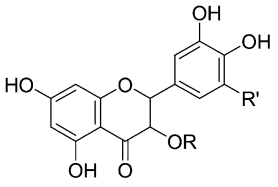
R = R' = H R"= gentiobioside **7**
R = R' = H R"= rutinoside **8**
R = β-D-galattoside R' = H R" = H **9**
R = R' = α-L-rhamnoside R" = H **10**
R = β-D-galactoside R' = CH_3 R" = H **11**
R = α-L-rhamnoside R' = CH_3 R" = H **12**



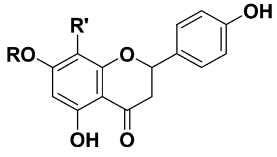
R = R' = H **13**
R = H R' = CH_3 **14**
R = CH_3 R' = H **15**



R = R" = H R' = CH_3 **16**
R = β-D-glucuronide R' = R" = H **17**
R = gentiobioside R' = R" = H **18**
R = β-D-glucoside R' = H R" = OH **19**
R = gentiobioside R' = H R" = OH **20**
R = (4"'-caffeoyl)gentiobioside R' = H R" = OH **21**



R = R' = H **22**
R = α-L-rhamnoside R' = H **23**
R = α-L-rhamnoside R' = OH **24**



R = H R ' = OH **25**
R = neohesperidoside R' = H **26**

blue-tetrazolium (NBT), xanthine, XOD, sodium carbonate, monobasic sodium phosphate, and dibasic sodium phosphate were obtained from Sigma Aldrich (Gillingam, Dorset, UK). Quercetin was purchased from Carl Roth KG. The solvents were obtained from Carlo Erba reagent (Milano, Italy). Nanopure water was prepared by Milli-Q apparatus.

2.3. TEAC test

Pure compounds were tested by using the trolox equivalent antioxidant capacity (TEAC) assay. The TEAC value is based on the ability of the antioxidant to scavenge the radical cation 2,2′-azino-*bis* (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) by spectrophotometric analysis

(Re et al., 1999). The $ABTS^{\cdot+}$ cation radical was produced by the reaction between 7 mM ABTS in H_2O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. The $ABTS^{\cdot+}$ solution was then diluted with PBS (pH 7.4) to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. Samples were diluted with methanol to produce solutions of 0.3, 0.5, 1, 1.5, and 2 mM concentration. The reaction was initiated by the addition of 1 ml of diluted ABTS to 10 μl of each sample solution. Determinations were repeated three times for each sample solution. The percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to a blank absorbance (methanol) and was plotted as a function of concentration of compound or standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). The antioxidant activities of compounds **1**–**26** are expressed as TEAC values in comparison with TEAC activity of the reported reference compound quercetin (Re et al., 1999). The TEAC value is defined as the concentration of standard trolox with the same antioxidant capacity as a 1 mM concentration of the antioxidant compound under investigation.

2.4. Auto-oxidation of β-carotene

Oxidation of linoleic acid was measured by the method described by Pratt (Pratt, 1992). Quantities of linoleic acid (20 mg) and Tween 20 (200 mg) were placed in a flask, and a solution of 2 mg of β-carotene in 10 ml of $CHCl_3$ was added. After removal of $CHCl_3$, 50 ml of distilled water saturated with oxygen for 30 min were added. Aliquots (200 μl) of each compound, dissolved in ethanol to give a 15 μg/ml solution, were added to each flask with shaking. Samples without test compounds were used as blanks, and a sample with 2,6-di-*tert*-butyl-4-methoxyphenol (BHT) was used as a control substance. Samples were subjected to oxidation by placing in an oven at 50 °C for 3 h. The absorbance was read at 470 nm at regular intervals. The antioxidant activity was expressed as AA and calculated with the equation

$$AA = [1 - (A_0 - A_t)/(A_{00} - A_{0t})] \times 100,$$

where A_0 is the absorbance at the beginning of the incubation, with test compound; A_t, absorbance at the time t, with test compound; A_{00}, absorbance at beginning of the incubation, without test compound and A_{0t}, absorbance at the time t, without test compound.

2.5. Superoxide anion enzymatic generation assay

Superoxide anion was generated in an enzymatic system by preparing a mixture of xanthine and XOD. The reaction mixture included 0.1 mM EDTA, 50 μg/ml of BSA, 25 μM nitroblue tetrazolium (NBT), 0.1 mM xanthine and 3.3×10^{-3} units XOD in 40 mM sodium carbonate buffer (pH 10.2) in a final volume of 3 ml. After incubation at 25 °C with increasing concentrations of samples, the absorbance of formazan produced was determined at 560 nm. The inhibitory effect of samples on the generation of superoxide anion were estimated by the equation: inhibitory ratio = $(A_0 - A_1) \times 100/A_0$, where A_0 is the absorbance with no addition of sample; A_1 is the absorbance with addition of sample. Inhibitory ratio for each sample was plotted as a function of the concentration; then the IC_{50} value was calculated by the statistical method of linear regression (Robak & Gryglewski, 1988). The influence of superoxide dismutase (SOD) on enzymatic reduction of NBT was determined in order to have a reference value.

2.6. Xanthine oxidase activity assay

Xanthine oxidase activity was evaluated by the spectrophotometric measurement of the formation of uric acid by xanthine. An aliquot of a 100 μM solution of xanthine in 0.1 M phosphate buffer (pH 7.8) with 0.04 units/ml of XOD was incubated for 10 min at room temperature (total volume 1 ml) and read at 295 nm against a blank sample which did not contain the enzyme. Different concentrations of tested compounds were added to samples before the enzyme had been added and their effect on the generation of uric acid was used to calculate regression lines and IC_{50} values (Robak & Gryglewski, 1988). Quercetin was used as reference compound.

3. Results and discussion

The antioxidant activities of compounds **1**–**26** were first studied in the TEAC assay (see Section 2 and Tables 1–4). TEAC values of quercetin derivatives **3**–**6** were higher than those of kaempferol **1**–**2** and myricetin **7**–**15**, that still have the unsaturation in the C ring and the hydroxyl group at C-3. In any case, the activities of compounds **3**–**6** were lower than that of quercetin because the hydroxyl group at C-3 is glycosidated and/or methoxylated, while the glycosidation at the C-7 hydroxyl group, as in compounds **4** and **5**, further reduced the activity (Rice-Evans, Miller, & Paganga, 1996). It is noteworthy that myricetin derivatives **13**–**15** had activities comparable to that of myricetins **7** and **8**, despite the absence of the hydroxyl group at C-5. This result established that the hydroxyl group at C-5 did not influence the radical-scavenger activity. Between flavones, luteolin derivatives were clearly more active than apigenin ones; the absence of the *ortho*-diphenolic arrangement in the B ring of compounds **16**–**18** produced a decrease of activity. Results obtained from dihydroflavonols **22**–**24** and flava-

Table 1
Antioxidant activities of flavonols **1**–**15** in the TEAC, auto-oxidation, inhibition of xanthine oxidase, and reduction of superoxide level assays[a]

Compound	TEAC	Auto-oxidation	Superoxide	Xanthine oxidase
	(mM) ± SD[b]	t = 60 min	IC_{50} (μM) ± SD[b]	IC_{50} (μM) ± SD [b]
Kaempferol 3-*O*-rutinoside (**1**)	1.14 ± 0.02	n.d.[c]	>50	32.1 ± 3.30
Kaempferol 3-*O*-α-L-(2″-β-D-xylosyl)rhamnoside (**2**)	1.06 ± 0.01	43.7	20.3 ± 1.62	14.1 ± 0.66
Quercetin 3-*O*-α-L-arabinoside (**3**)	1.50 ± 0.05	33.3	0.12 ± 0.02	4.47 ± 0.28
Quercetin 3-methoxy-7-*O*-β-D-glucoside (**4**)	1.28 ± 0.012	6.3	19.1 ± 1.65	16.2 ± 1.16
Quercetin 3-methoxy-7-*O*-gentiobioside (**5**)	1.09 ± 0.002	24.1	21.1 ± 0.86	12.1 ± 1.02
Quercetin 3-*O*-(6‴-caffeoyl)gentiobioside (**6**)	1.65 ± 0.021	26.8	12.9 ± 2.41	7.88 ± 0.24
Myricetin 7-*O*-gentiobioside (**7**)	1.62 ± 0.01	n.d.	>50	6.52 ± 0.1
Myricetin 7-*O*-rutinoside (**8**)	1.42 ± 0.05	n.d.	>50	6.13 ± 1.3
Myricetin 3-*O*-β-D-galactoside (**9**)	0.810 ± 0.001	27.9	>50	2.37 ± 0.56
Myricetin 3,4′-*O*-di-α-L-rhamnoside (**10**)	0.977 ± 0.021	9.3	>50	8.10 ± 1.32
Myricetin 4′-methoxy-3-*O*-β-D-galactoside (**11**)	1.04 ± 0.008	20.2	39.2 ± 3.15	6.57 ± 0.42
Myricetin 4′-methoxy-3-*O*-α-L-rhamnoside (**12**)	0.967 ± 0.010	7.4	24.9 ± 0.93	4.02 ± 0.49
3,7,3′,4′,5′-pentahydroxyflavone (**13**)	1.61 ± 0.005	44.4	12.8 ± 0.54	13.3 ± 0.46
3,7,3′,5′-tetrahydroxy-4′methoxyflavone (**14**)	1.52 ± 0.002	45.8	5.08 ± 0.30	9.48 ± 0.02
3,7,4′-trihydroxy-3′,5′-dimethoxyflavone (**15**)	1.36 ± 0.012	44.2	3.73 ± 0.193	9.16 ± 0.24
Quercetin	2.60 ± 0.02			3.00 ± 0.13
BHT[d]		71.3		
SOD[e]			69.5 ± 0.07 mU/ml	

[a] For protocols used, see Section 2.
[b] n = 3.
[c] n.d., not detected.
[d] BHT, 2,6-di-*tert*-butyl-4-methoxyphenol; standard control substance.
[e] SOD, superoxide dismutase.

Table 2
Antioxidant activity of flavones **16**–**21** in the TEAC, auto-oxidation, inhibition of xanthine oxidase, and reduction of superoxide level assays[a]

compound	TEAC	Auto-oxidation	Superoxide	Xanthine oxidase
	(mM) ± SD[b]	t = 60 min	IC_{50} (μM) ± SD[b]	IC_{50} (μM) ± SD[b]
4′-Methoxy-apigenin (**16**)	0.612 ± 0.010	n.d.[c]	7.93 ± 3.206	0.77 ± 0.02
Apigenin 7-*O*-β-D-glucuronide (**17**)	0.921 ± 0.030	n.d.	14.84 ± 1.87	16.6 ± 1.08
Apigenin 7-*O*-gentiobioside (**18**)	0.419 ± 0.011	9.4	39.1 ± 0.08	6.89 ± 1.59
Luteolin 7-*O*-β-D-glucoside (**19**)	0.985 ± 0.015	14.0	1.27 ± 0.14	15.7 ± 3.35
Luteolin 7-*O*-gentiobioside (**20**)	0.990 ± 0.003	n.d.	16.6 ± 3.66	3.69 ± 0.01
Luteolin 7-*O*-(4‴-caffeoyl)gentiobioside (**21**)	1.10 ± 0.012	n.d.	28.6 ± 1.55	11.0 ± 0.86

[a] For protocols used, see Section 2.
[b] n = 3.
[c] n.d., not detected.

Table 3
Antioxidant activity of dihydroflavonols **22**–**24** in the TEAC, auto-oxidation, inhibition of xanthine oxidase, and reduction of superoxide level assays[a]

Compound	TEAC	Auto-oxidation	Superoxide	Xanthine oxidase
	(mM) ± SD[b]	t = 60 min	IC_{50} (μM) ± SD[b]	IC_{50} (μM) ± SD[b]
Taxifolin (**22**)	1.32 ± 0.010	16.1	0.88 ± 0.02	9.65 ± 0.19
Taxifolin 3-*O*-α-L-rhamnoside (**23**)	0.757 ± 0.019	13.6	5.02 ± 0.41	5.43 ± 0.82
Dihydromyricetin 3-*O*-α-L-rhamnoside (**24**)	0.761 ± 0.016	8.4	15.5 ± 0.01	13.2 ± 0.08

[a] For protocols used, see Section 2.
[b] n = 3.

nones **25**–**26** showed that the lack of the 2,3-unsaturation decreased the activity, as reported in the literature (Rice-Evans et al., 1996). The decrease of activity between aglycone and glycosylated derivatives was confirmed also by the comparison of TEAC values of compounds **22** and **23**.

Table 4
Antioxidant activity of flavanones **25**–**26** in the TEAC, auto-oxidation, inhibition of xanthine oxidase, and reduction of superoxide level assays[a]

compound	TEAC	Auto-oxidation	Superoxide	Xanthine oxidase
	(mM) ± SD[b]	t = 60 min	IC_{50} (μM) ± SD[b]	IC_{50} (μM) ± SD[b]
8-Hydroxy-naringenin (**25**)	0.428 ± 0.011	4.1	45.4 ± 3.46	9.09 ± 0.86
Naringenin 7-*O*-neohesperidoside (**26**)	0.782 ± 0.012	16.3	>50	14.4 ± 1.09

[a] For protocols used, see Section 2.
[b] n = 3.

The results of the antioxidative effect of compounds **1**–**26** on the autoxidation of linoleic acid are reported in Tables 1–4. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Especially, linoleic acid and arachidonic acid are the target of lipid peroxidation. It is generally thought that the inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. The data obtained showed that compounds **13**–**15** had better activities then all other tested flavonoids. Also, in this test, the absence of the hydroxyl group at C-5 did not influence the antioxidant activity, that is preserved in these flavonoids without the 5-OH group (rare in nature). However, the differences in solubilities of flavonoids, both aglycones, glycosides, and methoxylated derivatives, in a micellar water–lipid system may influence results obtained from this test, and the partition of the compounds between the two phases can influence oxidation results (Burda & Oleszek, 2001).

The enzyme XOD plays a crucial role in the production of uric acid, catalyzing the oxidation of hypoxanthine and xanthine. During the reoxidation of XOD, molecular oxygen acts as an electron acceptor, producing superoxide radical and hydrogen peroxide. Consequentely, XOD is considered to be an important biological source of superoxide radicals. Both, inhibition of XOD and the scavenging effect on the superoxide anion, were measured in one assay. Inhibition of XOD results in a decreased production of uric acid, which can be measured spectrophotometrically, and a decreased production of superoxide. For each flavonoid tested, two IC_{50} values (50% inhibitory concentrations) can be calculated by linear regression analysis: 50% inhibition of XOD (=50% decrease of uric acid production) and 50% reduction of the superoxide level. From our results, several tested compounds, such as **3**, **6**–**8**, **11**, **12**, **16**, and **23**, showed a high inhibition of XOD with $IC_{50} < 10$ μM. Compounds **13**–**15**, characterized by the absence of the 5-OH group, revealed less action than those with the hydroxyl function at C-5 (**7**–**12**). Comparing the results obtained from the inhibition of XOD and the scavenging of the superoxide anion, it is clear that compounds **3**, **6**, **16**, and **23** showed activity in both tests, so that the inhibition of the XOD system is strengthened by the simultaneous action on the superoxide scavenger. From our results, it appears that, for the inhibition of XOD activity by flavonoids, the hydroxyl groups at C-5 and C-7 and the 2,3-double bond are important: between flavonols, quercetins **3** and **6** and myricetins **11** and **12** were the most active, while myricetin derivatives **13**–**15**, without the hydroxyl group at C-5, were less active. Structure–activity relationships of flavonoids in the inhibition of XOD and in the scavenging of superoxide anion appeared similar. Structural differences between flavonols, flavones, flavanones, and dihydroflavonols influenced the inhibitory effect on XOD and superoxide anion. The unsaturation in the C ring and the free hydroxyl group at C-7 enhanced the activity. A reduction in uric acid production automatically resulted in an equivalent reduction in superoxide. In the case of both inhibition of XOD activity and superoxide-scavenging activity, the superoxide concentration reduction is higher so that the corresponding IC_{50} values of the flavonoid for superoxide are lower than those of uric acid.

Results on flavonoids from plants, used for a long time in developing countries as folk medicine, showed that these compounds have the ability to protect against lipid peroxidation and also show scavenging activity on the ABTS and superoxide radicals; the antioxidant activity of most of the compounds is here reported for the first time, also, because they were new secondary metabolites, isolated only from the species reported above. The role of oxygen-derived free radical anti-inflammatory process is well known. Free radicals are important in the inflammatory process because they are implicated in the activation of nuclear factor κB, which induces the transcription of inflammatory molecules (Sahnoun, Jamoussi, & Zeghal, 1998; Winrow, Winyard, Morris, & Blake, 1993).

The results of the present work suggested that the anti-inflammatory activities of plants could be explained, at least in part, by their antioxidant properties. These plants, rich in flavonoids, could be a good source of compounds that would help to increase the overall antioxidant capacity of an organism and protect it against lipid peroxidation, induced by oxidative stress.

Acknowledgment

The Ministero dell'Istruzione, dell'Università e della Ricerca, Roma (MIUR) is gratefully acknowledged for financial support.

References

Aisling Aherne, S., & O'Brien, N. M. (2002). Dietary flavonols: Chemistry, food content and metabolism. *Nutrition, 18*, 75–81.

Braca, A., De Tommasi, N., Morelli, I., & Pizza, C. (1999). New metabolites from *Onopordum illyricum*. *Journal of Natural Products, 62*, 1371–1375.

Braca, A., Bilia, A. R., Mendez, J., Pizza, C., Morelli, I., & De Tommasi, N. (2003). Chemical and biological studies on *Licania* genus. In Atta-ur-Rahman (Ed.). *Studies in natural products chemistry,* (Vol. 28, pp. 35–67). Amsterdam: Elsevier.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Reviews, 56*, 317–333.

Burda, S., & Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry, 49*, 2774–2779.

Cioffi, G., D'Auria, M., Braca, A., Mendez, J., Castillo, A., Morelli, I., De Simone, F., & De Tommasi, N. (2002). Antioxidant and free radical scavenging activity of constituents from the leaves of *Tachigalia paniculata*. *Journal of Natural Products, 65*, 1526–1529.

De Simone, F., Aquino, R., De Tommasi, N., Mahamood, N., Piacente, S., & Pizza, C. (2001). Anti-HIV aromatic compounds from higher plants in bioactive compounds from natural sources. Corrado Tringali (Ed.), Taylor & Francis, pp. 305.

Di Carlo, G., Mascolo, N., Izzo, A. A., & Capasso, F. (1999). Flavonoids: Old and new aspects of a class of natural therapeutic drugs. *Life Science, 65*, 337–353.

Flamini, G., Braca, A., Cioni, P. L., Morelli, I., & Tomè, F. (1997). Three new flavonoids and other constituents from *Lonicera implexa*. *Journal of Natural Products, 60*, 449–452.

Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. *Biochemical Pharmacology, 32*, 1141–1148.

Pratt, D. E. (1992). Natural antioxidants from plant material. II. In M. T. Huang & C. Y. Lee (Eds.), *Phenolic compounds in food and their effects on health*. *Symposium series* (507, pp. 54–71). Washington, DC, USA: American Chemical Society.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*, 933–956.

Robak, J., & Gryglewski, R. J. (1988). Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology, 37*, 837–841.

Sahnoun, Z., Jamoussi, K., & Zeghal, K. M. (1998). Free radicals and antioxidants: Physiology, human pathology and therapeuthic aspects. *Therapie, 53*, 315–339.

van Acker, S. A. B. E., van den Berg, D. J., Tromp, M. N. J. L., Griffioen, D. H., van Bennekom, W. P., van der Vijgh, W. J. F., & Bast, A. (1996). Structural aspects of antioxidant activity of flavonoids. *Free Radical and Biological Medicine, 20*, 331–432.

Winrow, V. R., Winyard, P. G., Morris, C. J., & Blake, D. R. (1993). Free radicals in inflammation: Second messengers and mediators of tissue destruction. *British Medicinal Bulletin, 49*, 506–522.

World Cancer Research Fund/American Institute for Cancer Research. (1997). Food, nutrition and the prevention of cancer: A global perspective.

Yang, C. S., Landau, J. M., Huang, M. T., & Newmark, H. L. (2001). Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual Review of Nutrition, 21*, 381–406.