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Antioxidant Activity and Total Phenolic Content of Some Brazilian Species

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Abstract

The antioxidant activity of extracts and fractions of six vegetal species from the Brazilian Atlantic Forest were determined. The total antioxidant activity was assessed based on the scavenging activity of the stable DPPH free radical. Eight extracts or fractions of plants showed significant DPPH scavenging activity ($IC_{50} \leq 10.0\,\mu g/mL$) compared with the values obtained for ascorbic acid ($IC_{50} = 8.4\,\mu g/mL$) and gallic acid ($IC_{50} = 2.6\,\mu g/mL$). The extracts or fractions were as follows: ethanol extracts of leaves, flowers, and stems of *Baccharis illinita* DC., ethanol extracts of leaves and stems of *B. platypoda* DC., hydroalcoholic extract and ethyl acetate fraction of leaves of *Cyathea phalerata* Mart. and hydroalcoholic extract of bark of *Trichilia catigua* A. Juss. Seven flavonoids present in the plant extracts were also investigated. The most active compounds were taxifolin, quercetin, and luteolin, which possess the catechol group 3′,4′-diOH. In addition, the total phenolic or flavonoid contents of these extracts and fractions were evaluated. The phenolic content of the sample was determined using Folin-Ciocalteu reagent and varied from 489.07 to 11.29 mg/g dry weight expressed as gallic acid equivalents (GAE). The total flavonoid concentrations, detected using 2% aluminum chloride, varied from 61.82 to 5.6 mg quercetin equivalents (QE)/g dry weight. These results suggest that the level of antioxidant activity in these plants varies by a great extent. They also suggest that the phenolic content in these plants provides substantial antioxidant activity. The flora of Brazil appears to be a rich and interesting source for supplementary ethnomedical and phytochemical studies.

Keywords: Antioxidant activity, Brazilian species, DPPH, flavonoids.

Introduction

During normal metabolism in aerobic cells, molecular oxygen is reduced to water; yet the stepwise transfer of electrons generates free reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet}$-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$). Other radicals such as alkyl ($R^{\bullet}$), alkoxyl ($RO^{\bullet}$), and peroxyl ($ROO^{\bullet}$) radicals may also be produced endogenously, lipid peroxidation being another significant source of ROS (Simic et al., 1989). In addition, ROS may be formed in the cell as a consequence of environmental stress. Excessive generation of ROS and other radicals can damage proteins, carbohydrates, polyunsaturated fatty acids, and DNA, and may thus lead to oxidative stress and to a variety of degenerative processes and diseases such as aging, immunodeficiencies, neurologic disorders, inflammation, arteriosclerosis, coronary heart disease, and certain cancers (Sies, 1991; Gutteridge, 1993; Kehrer, 1993; Aruoma, 1994; Scandalios, 1997; Halliwell & Gutteridge, 1999).

ROS are continuously produced during normal physiologic events and removed by antioxidant defense mechanisms (Halliwell et al., 1992). There is a balance between generation of ROS and their removal by the antioxidant system in organisms. Under pathologic conditions, ROS are overproduced and result in lipid peroxidation and oxidative stress. An imbalance between ROS and antioxidant defense mechanisms leads to oxidative

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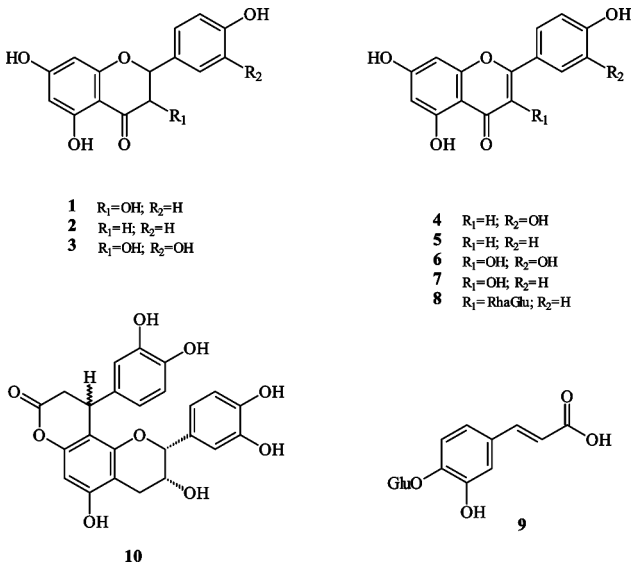

Figure 1. Chemical structures of phenolic compounds **1**–**10**.

modification in the cellular membrane or intracellular molecules (El-Habit et al., 2000). Various endogenous antioxidant defense mechanisms play an important role in the elimination of ROS and lipid peroxides and therefore protect the cells against toxic effects of ROS and lipid peroxides (Halliwell, 1991; Halliwell et al., 1992; El-Habit et al., 2000).

Recently, interest in finding naturally occurring antioxidants has increased considerably, with a view to their use in foods or as medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenecity (Ito et al., 1983; Zheng & Wang, 2001). Natural antioxidants, the most prominent representatives of which are carotenoids, ascorbic acid, tocopherols, and flavonoids, can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods (Pryor, 1991; Kinsella et al., 1993; Lai et al., 2001). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free-radical generation (van Acker et al., 1996; Benavente-Garcia et al., 1997). Depending on their structure, flavonoids are able to scavenge practically all known ROS including superoxide anions, hydrogen peroxide, hydroxyl radicals, singlet oxygen, alkoxy, aroxy, and peroxyl radicals, as well as alkyl-, aryl-, and nitrogen-derived radicals. Hence, flavonoids may protect biosystems against free-radical attack, which may be involved in various cancers and coronary heart disease.

A method based on the scavenging of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) has been used extensively to predict the antioxidant activities of extracts of plants (Yen & Duh, 1994; Brand Williams et al., 1995; Kulisic et al., 2004). DPPH is a stable radical and it was used in this study for screening of the antioxidant antiradical activities of six plant extracts and its phenolic secondary metabolites, (Fig. 1) which are used in folk medicine for the treatment of some diseases.

Materials and Methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, gallic acid, quercetin, and Folin-Ciocalteu reagent were purchased from Fluka. All other chemicals were of analytic grade.

Plant material

Polygala cyparissias A. St.-Hill. & Moq., *Polygala sabulosa* A.W. Benn, and *Cyathea phalerata* Mart. were collected in the Santa Catarina State, Brazil, and identified by comparison with authenticated specimens. The vouchers are deposited at the herbarium of the Botany Department of Universidade Federal de Santa Catarina (*Polygala cyparissias* 22.744),Universidade Federal do Paraná (*Polygala sabulosa* 19.640), and Universidade Federal Rural do Rio de Janeiro (*Cyathea plalerata* RBR 4287), Brazil. *B. illinita* DC. (OUPR no 3450) and *B. platypoda* DC. (OUPR no. 4888) were collected in the vicinities of Ouro Preto, Minas Gerais, identified by Dr. José Badini, and vouchers are deposited at the herbarium of the Botany Department, Universidade Federal de Ouro Preto. The comercial sample of *Trichilia catigua* A. Juss. bark was supplied by Laboratório Catarinense S.A. (Joinville, SC, Brazil). In Table 1, the trivial names, families, and popular uses for different plants included in the study are indicated.

Preparation of plant extracts

The dried plant material samples (leaves, stems, and flowers) of *B. illinita* and *B. platypoda* were powdered separately and extracted first with chloroform and then with ethanol at room temperature for 15 days. The extracts were filtered and concentrated at reduced pressure, to yield the crude chloroform and ethanol extracts for each of the plant parts. Details of the partitioning, isolation procedures, and characterization of the substances are described in a previous paper (Verdi et al., 2004).

Aerial parts (150 g) of *P. sabulosa* and *P. cyparissias* were powdered and macerated at room temperature for 15 days in an alcohol/water mixture (4:1, v/v). After filtration, the solvent was removed by rotatory evaporation under reduced pressure to yield the hydroalcoholic crude extracts of both species. The crude hydroalcoholic

Table 1. Species screened for antioxidant activity.

Plant species	Family	Trivial name	Part used	Popular use[a]
Baccharis illinita DC.	Asteraceae	Carqueja	Stems Leaves Flowers	Hepatoprotective agent and gastric diseases
Baccharis platypoda DC.	Asteraceae	Carqueja	Stems Leaves Flowers	Antiparasitic
Polygala cyparissias A. St.-Hill & Moq.	Polygalaceae	Pinheirinho-da-praia	Aerial parts	Kidney infections, parasitic diseases, local anesthetic
Polygala sabulosa A. W. Benn.	Polygalaceae	Timutu-pinheirinho	Aerial parts	General pain, parasitic diseases
Cyathea phalerata Mart.	Cyatheaceae	Xaxim	Stems leaves	Expectorant and kidney diseases
Trichilia catigua A. Juss.	Meliaceae	Catuaba	Bark	Mental tonic, sexual stimulant

[a]Bandoni et al. (1972); Korbes (1995).

extract of *P. sabulosa* was suspended in water and separated by liquid-liquid partitioning using ethyl acetate and *n*-butanol.

Fresh wood samples (9.18 kg) of *C. phalerata* were cut into small pieces and extracted with 96% EtOH (15 days room temperature). After evaporation, the extract containing water was left to stand for 1 day, allowing the formation of an insoluble precipitate. This extract was filtered to obtain the solid residue (48.0 g) and aqueous phase. The aqueous phase was partitioned between *n*-hexane, ethyl acetate, and *n*-butanol yielding respective fractions. The ethyl acetate fraction (2.64 g) was chromatographed on successive normal and flash silica gel 60 columns with hexane–ethyl acetate–ethanol and methanol-chloroform gradients, respectively, and subsequent recrystallization of fractions with acetone yielded a yellow solid (1.35 g), kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, a white solid crystalline (74.0 mg), 4-*O*-β-D-glucopyranosylcaffeic acid, and kaempferol (21.0 mg). The structure of the substance was determined by IR, MS, and ^{1}H and ^{13}C NMR (COSY, HETOR, APT) spectroscopies and by comparison with literature data (Dauguet et al., 1993; Bringmann & Günther, 1999). The ethanol extract from the leaves of *C. phalerata* was prepared in a similar manner as that of the stems.

The dried powdered bark of *T. catigua* was extracted with ethanol for 15 days. The ethanol extract was concentrated under vacuum, and 3 g of the residue were subjected to repeated silica gel column chromatography, eluted with a gradient of ethyl acetate–acetone–hexane, to yield epimeric flavolignans (Pizzolatti et al., 2004).

Determination of total phenolic compounds in the extracts

The amount of total phenolics was determined using the Folin-Ciocalteu method (Gutfinger, 1981). In this method, the reaction mixture was composed of 0.5 mL of extract, 5.0 mL of distilled water, and 0.5 mL of the Folin-Ciocalteu reagent. After a period of 3 min, 1.0 mL of saturated sodium carbonate solution was added. This mixtures were shaken and allowed to stand for 1 h. The absorbance was measured at 725 nm (each measurement was repeated three-times) in a Hitachi UV-Vis U 3000 spectrophotometer (Tokyo, Japan). A calibration curve of gallic acid was prepared, and the results were expressed as mg GAE (gallic acid equivalents)/g dry extract.

Determination of total flavonoid content in the extracts

The total flavonoid content was determined spectrophotometrically according to Lamaison and Carnat (Quettier-Deleu et al., 2000). Briefly, 0.5 mL of 2% aluminum chloride ($AlCl_3$) ethanol was mixed with the same volume of vegetal extracts (0.1–1.0 mg/mL). Absorption readings at 415 nm were taken after 1 h against a blank (ethanol). The total flavonoid content was determined using a standard curve with quercetin (0–50 mg/L). The mean of three readings was used and expressed as milligrams of quercetin equivalents (QE/g of dry extract).

Radical-scavenging effect of extracts in DPPH radicals

The free radical scavenging activity of the plants was measured using the method described by BrandWilliams et al. (1995) with some modifications. One milliliter of the methanol extracts of the plants (200, 100, 80, 40, 20, 10, and 5 μg/mL) was added to 2 mL of a solution of DPPH radicals in methanol (0.004%). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance (Abs_{sample}) of the resulting solution was measured at 517 nm and converted into percentage of antioxidant activity (AA) using the following formula: $AA\% = 100–\{[(Abs_{sample}–Abs_{blank}) \times 100]/Abs_{control}\}$. A methanol (2.0 mL) and plant extract (1.0 mL) solution was used as the blank (Abs_{blank}). A DPPH (2.0 mL) and methanol (1.0 mL) solution was used as the control ($Abs_{control}$). Ascorbic and gallic acids were used as standards. The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50%

(IC_{50}). The IC_{50} value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration.

Results and Discussion

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997).

Extracts of plants are allowed to react with the stable radical, DPPH, in methanol solution. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm, induced by an antioxidant (AH) after 30 min, as follows:

$$DPPH^{\cdot} + AH \longrightarrow DPPH - H + A^{\cdot}$$

The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity (Sanchez-Moreno et al., 1998). A lower IC_{50} value corresponds with a higher antioxidant power.

The eight plant extracts showed significant DPPH scavenging activity ($IC_{50} \leq 10.0\,\mu g/mL$) compared with the values obtained for ascorbic acid ($IC_{50} = 8.4\,\mu g/mL$) and gallic acid ($IC_{50} = 2.6\,\mu g/mL$) standards. All results are shown in Tables 2 and 3.

Natural extracts with proven antioxidant activity usually contain compounds with a phenolic moiety, for example coumarins, flavonoids, tocopherols, and cathechins. Organic acids, carotenoids, protein hydrolysates, and taninns can also be present and act as antioxidants or have a synergistic effect with phenolic compounds (Dapkevicius et al., 1998).

The extracts of *B. illinita*, *B. platypoda*, *T. catigua*, and *C. phalerata* , with significant DPPH scavenging activity, also had a higher quantity of total phenolics, for example, 351.95 and 230.98 mg GAE/g extract for the ethanol extracts of the leaves and stems of *B. illinita*, respectively, and 486.07 mg GAE/g extract for the hydroalcoholic extract of *T. catigua*. These extracts, which have a high antioxidant activity, also had a great quantity of flavonoids, between 61.82 and 26.68 mg QE/g extract, as summarized in Table 2. However, exceptions were observed for the leaves of *C. phalerata* and the hydroalcoholic extract of *T. catigua*, where concentrations of total phenolics and flavonoids, respectively, were low.

A high antioxidant activity (greater than the standard) was found in the extract of *T. catigua* bark ($IC_{50} = 2.1\,\mu g/mL$). For this extract, a high total phenolic content was determined, this comprising a small fraction of flavonoids, only 5.61 mg QE/g extract. From the crude extract of this species, an epimeric mixture of flavolignans has been isolated and identified (Pizzolatti et al., 2004), with an IC_{50} value of 0.9 μg/mL (Table 3).

The extracts of the *B. illinita* leaves showed a higher level of free-radical sequestering than the respective extracts of *B. platypoda*. The former *Baccharis* species

Table 2. The phenolic and flavonoid contents and IC_{50} values of plants in relation to DPPH inhibition

Species	Part used	Extract/fractions	mg GAE/g dry extract	mg QE/g dry extract	IC_{50} (μg mL^{-1})
Baccharis illinita	Leaves	Hydroalcoholic	89.06 ± 2.99	25.96 ± 0.13	21.5
		Ethanol	351.95 ± 1.48	61.82 ± 0.02	4.0
		Chloroform	19.91 ± 1.72	8.22 ± 0.41	*
	Flowers	Ethanol	188.15 ± 2.63	53.10 ± 0.10	7.0
		Chloroform	15.56 ± 0.09	5.22 ± 0.07	*
	Stems	Ethanol	230.98 ± 1.48	48.11 ± 1.45	9.0
		Chloroform	35.16 ± 0.45	27.93 ± 0.20	85.0
Baccharis platypoda	Leaves	Hydroalcoholic	31.62 ± 0.18	10.24 ± 0.23	38.0
		Ethanol	149.13 ± 3.91	28.88 ± 0.02	9.0
		Chloroform	11.29 ± 0.46	7.71 ± 0.07	*
	Stems	Ethanol	127.63 ± 1.49	35.87 ± 0.01	9.0
		Chloroform	49.13 ± 2.02	18.87 ± 0.06	*
Polygala cyparissias	Aerial parts	Hydroalcoholic	33.97 ± 0.72	12.08 ± 0.23	*
Polygala sabulosa	Aerial parts	Hydroalcoholic	64.92 ± 0.63	24.68 ± 0.56	57.0
		Fraction: Ethyl acetate	102.94 ± 1.27	26.32 ± 0.88	15.0
		n-Butanolic	82.80 ± 0.73	23.69 ± 1.14	30.0
Cyathea phalerata	Leaves	Hydroalcoholic	37.97 ± 1.27	26.68 ± 0.79	9.0
	Wood	Hydroalcoholic	54.85 ± 1.44	9.03 ± 0.72	20.0
		Fraction: Ethyl acetate	305.57 ± 2.98	58.64 ± 0.32	7.0
		Aqueous	82.98 ± 1.72	13.09 ± 0.39	17.0
		Residue	119.00 ± 2.90	18.84 ± 0.51	13.0
Trichilia catigua	Bark	Hydroalcoholic	486.07 ± 3.90	5.61 ± 0.03	2.1

*No antioxidant activity observed at the concentrations tested.

Table 3. IC_{50} values of isolated compounds of *B. illinita*, *C. phalerata*, and *T. catigua* in relation to DPPH inhibition

Species	Compound	IC_{50} (μg/mL)	IC_{50} (μM)
B. illinita	Aromadendrin (**1**)	19.0	65.9
	Naringenin (**2**)	18.0	66.2
	Taxifolin (**3**)	2.0	6.6
	Luteolin (**4**)	4.5	15.7
	Apigenin (**5**)	31.0	114.8
	Quercetin (**6**)	2.1	6.9
C. phalerata	Kaempferol (**7**)	15.0	52.4
	Kaempferol-3-*O*-α-L-rham(1→2)-β-D-glc (**8**)	425.3	673.0
	4-*O*-β-D-glucopyranosylcaffeic acid (**9**)	150.0	438.0
T. catigua	Cinchonain (**10**)	0.9	2.0
	Ascorbic acid	8.4	15.3
	Gallic acid	2.6	47.7
	BHT	17.3	84.8

also showed a greater content of total phenolics and of flavonoids. The ethanol extracts of different parts of both *Baccharis* species were more active than the chloroform extracts (Table 2). From the ethanol extracts of the *B. illinita* flowers, nine flavonoids of previously known structure (Verdi et al., 2004) were isolated, and these are generally responsible for the high antioxidant activity of these extracts. Of these flavonoids, only six were evaluated in relation to their antioxidant activity: aromadendrin, naringenin, taxifolin, luteolin, apigenin, and quercetin. The IC_{50} values are given in Table 3. Radical scavenging activity of flavonoids toward DPPH radicals is dependent on the number of hydroxyl groups in the B ring. The greater the number of hydroxyl groups in the B ring, the greater the radical-scavenging potency of flavonoids. However, the C2-C3 double bond apparently does not contribute to the hydrogen-donating ability of flavonoids in the absence of a polyhydroxylated structure in the B ring. These hydroxyl groups help to stabilize the aryloxyl radical after hydrogen donation in the process of scavenging the free radicals (Cao et al., 1997). Of the polyhydroxylated flavonoids, the most notable are those that have a 3′,4′-dihydroxy catechol system in the B ring, as in the case of luteolin ($IC_{50} = 15.7\,\mu M$), quercetin ($IC_{50} = 6.9\,\mu M$), and taxifolin ($IC_{50} = 6.6\,\mu M$), which showed excellent inhibitory activity against DPPH radicals when compared with ascorbic acid ($IC_{50} = 47.7\,\mu M$), and gallic acid ($IC_{50} = 15.3\,\mu M$). In addition, some flavonoids with only one hydroxyl group (4′-hydroxyl), such as naringenin and aromadendrin, were also found to have inhibitory activity. This fact indicates that *ortho*-hydroxyl substitutions, whether on the B ring or the A ring, are the most important features for the antiradical activity of flavonoids, while additional substitution seems to have no obvious influence (Yokozawa et al., 1998).

The *C. phalerata* leaves show a greater free radical sequestering activity than the stem, although there was no parallelism in relation to the phenolic compounds content. However, a higher quantity of flavonoids was observed in the extract with greater antioxidant activity. On the other hand, in the ethyl acetate fraction, a greater antioxidant activity was found and also a higher percentage of total phenolics and of flavonoids (Table 2). From this fraction, the compounds kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosylcaffeic, 4-*O*-β-D-glycopyranosylcaffeic acid, and kaempferol, were isolated, with only kaempferol showing notable levels of antioxidant activity (Table 3).

The effect of quercetin and kaempferol on DPPH radical scavenging is well-known. The values of $IC_{50} = 9.8\,\mu M$ (Torres et al., 2006), $IC_{50} = 7.94\,\mu M$ (Yokozawa et al., 1998), and $IC_{50} = 8.1\,\mu g/mL$ (Choi et al., 2002) for quercetin have been established. These values are close to the values we determined. For kaempferol, the value of $IC_{50} = 22.81\,\mu M$ was reported (Yokozawa et al., 1998).

In relation to the two *Polygala* species, it was observed that *P. sabulosa* is more active than *P. cyparissias*, for which no antioxidant activity was observed until the highest concentration of extract tested (200 μg/mL). For the ethyl acetate fraction of *P. sabulosa*, a greater activity in relation to sequestering of free radicals was found (Table 2).

These results indicate that all plant extracts have a noticeable effect on the scavenging of free radicals. This activity also increases with increasing concentration. The extracts of these plants can be regarded as promising candidates for a plant-derived antioxidant compound.

This study reveals that Brazilian species offer an interesting source of new antioxidative plant extracts, such as those of *T. catigua* and *B. illinita*, there being a potential for their use in different fields (foods, cosmetics, pharmaceuticals). Flavonoids, phenolic acids, and flavolignans may be the compounds responsible for the antioxidant activity in these plants.

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References

Acker SV, Balen GPV, Berg DJV, Vijgh WJF (1996): Influence of iron chelation on the antioxidant activity of flavonoids. *Biochem Pharmacol 56*: 935–943.

Aruoma OI (1994): Nutrition and health aspects of free radicals and antioxidants. *Food Chem Toxicol 62*: 671–683.

Bandoni AL, Rondina RVD, Coussio JD, Mendiond ME (1972): Survey of Argentine medicinal plants—Plants 1. Folklore and phytochemical screening. *Lloydia 35*: 69–80.

Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA (1997): Uses and properties of *Citrus* flavonoids. *J Agric Food Chem 45*: 4505–4515.

Brand Williams W, Cuvelier ME, Berset C (1995): Use of a free-radical method to evaluate antioxidant activity. *Food Sci Technol-Lebens Wissens Technol 28*: 25–30.

Bringmann G, Günther JDN (1999): Isolation of 4-*O*-β-D-glucopyranosylcaffeic acid and gallic acid from *Cyathea dregei* Kunze (Cyatheaceae). *Pharm Pharmacol Lett 9*: 41–43.

Cao G, Sofic E, Prior RL (1997): Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radic Biol Med 22*: 749–760.

Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK (2002): Antioxidat activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Sci 163*: 1161–1168.

Dapkevicius A, Venskutonis R, Beek TA, Linssen PH (1998): Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J Sci Food Agric 77*: 140–146.

Dauguet JC, Bert M, Dolley J, Bekaert A, Lewin G (1993): Methoxykaempferol 3-neohesperidoside and other flavonoids from bee pollen of *Crataegus monogyna*. *Phytochemistry 33*: 1503–1505.

El-Habit OH, Saada HN, Azab KS (2000): The modifying effect of β-catotene on gamma radiation-induced elevation of oxidative reactions and genotoxicity in male rats. *Mutat Res 466*: 179–186.

Gutfinger T (1981): Polyphenols in olive oils. *J Am Oil Chem Soc 58*: 966–968.

Gutteridge JMC (1993): Free radicals in disease processes: A compilation of cause and consequence. *Free Radic Res Commum 19*: 141–1583.

Halliwell B (1991): Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. *Am J Medicine 91*: 14–22.

Halliwell B, Gutteridge JMC (1999): *Free Radicals in Biology and Medicine*, 3rd ed. Oxford: Clarenson Press, pp. 416–494.

Halliwell B, Gutteridge JMC, Cross CE (1992): Free radicals antioxidants, and human disease: Where are we now? *J Lab Clin Med 119*: 598–620.

Ito N, Kukushima S, Hasegawa A, Shibata M, Ogiso T (1983): Carcinogenecity of buthylated hydroxyanisole in F344 rats. *J Natl Cancer Inst 70*: 343–347.

Kehrer JP (1993): Free radicals as mediators of tissue injury and disease. *CRC Crit Rev Toxicol 23*: 21–48.

Kinsella JE, Frankel E, German B, Kanner J (1993): Possible mechanism for the protective role of the antioxidant in wine and plant foods. *Food Technol 47*: 85–89.

Korbes CV (1995): *Plantas Medicinats*, Francisco Beltrão, Paraná, Associação de Estudos, Orientação e Assistência Rural.

Kulisic T, Radonic A, Katalinic V, Milos M (2004): Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem 85*: 633–640.

Lai LS, Chou ST, Chao WW (2001): Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. *J Agric Food Chem 49*: 963–968.

Pizzolatti MG, Venson AF, Smania A, Smania EDA, Braz Filho R (2002): Two epimeric flavalignans from *Trichilia catigua* (Meliaceae) with antimicrobial activity *Z Naturforsch C 57*: 483–488.

Pryor WA (1991): The antioxidant nutrient and disease prevention—What do we know and what do we need to find out? *Am J Clin Nutr 53*: 391–393.

Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, Cazin JC, Bailleul F, Trotin F (2000): Phenolic compounds and antioxidant activities of buckweat (*Fagopyrum esculentum* Moench) hulls and flour. *J Ethnopharmacol 72*: 35–42.

Sanchez-Moreno C, Larrauri JA, Saura-Calixto F (1998): A procedure to measure the antiradical efficiency of polyphenols. *J Agric Food Chem 47*: 425–431.

Scandalios JG (1997): *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*. New York, Cold Spring Harbor Laboratory Press.

Sies H (1991): *Oxidative Stress: Introduction*. London, Academic Press, pp. 650.

Simic MG, Bergtold DS, Karam LR (1989): Generation of oxy radicals in biosystems. *Mutat Res 214*: 3–12.

Soares JR, Dins TCP, Cunha AP, Almeida LM (1997): Antioxidant activity of some extracts of *Thymus zygis*. *Free Radic Res 26*: 469–478.

Torres R, Faini F, Modak B, Urbina F, Labbé C, Guerrero J (2006): Antioxidant activity of coumarins and flavonols from the resinous exudate of *Haplopappus multifolius*. *Phytochemistry 67*: 984–987.

Verdi LG, Brighente IMC, Schripsema J, Braz Filho R, Pizzolatti MG (2004): Kaurene diterpenes and flavonoids from *Baccharis illinita* flowers. *Biochem Syst Ecol 32*: 837–840.

Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka G-I, Nishioka I (1998): Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl. *Biochem Pharmacol 56*: 213–222.

Zheng W, Wang SY (2001): Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem 49*: 5165–5170.

Yen GC, Duh PD (1994): Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *J Agric Food Chem 42*: 629–632.