

Myeloperoxidase Inhibitory and Radical Scavenging Activities of Flavones from *Pterogyne nitens*

Daniara Cristina FERNANDES,^a Luis Octávio REGASINI,^a José Carlos Rebuglio VELLOSA,^b Patrícia Mendonça PAULETTI,^a Ian CASTRO-GAMBOA,^a Vanderlan Silva BOLZANI,^a Olga Maria Mascarenhas OLIVEIRA,^b and Dulce Helena Siqueira SILVA^{*,a}

^aNuBBE-Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais, Department of Organic Chemistry, Institute of Chemistry, UNESP-São Paulo State University; Araraquara, SP, CP 355, CEP 14801-970, Brazil; and ^bDepartment of Biochemistry and Chemical Technology, Institute of Chemistry, UNESP-São Paulo State University; Araraquara, SP, CP 355, CEP 14801-970, Brazil.

Received December 22, 2007; accepted February 12, 2008; published online February 18, 2008

Two new flavone glucosides, nitenosides A and B (1, 2), together with four known compounds, sorbifolin (3), sorbifolin 6-*O*- β -glucopyranoside (4), pedalitin (5), and pedalitin 6-*O*- β -glucopyranoside (6) were isolated from *Pterogyne nitens*. Their structures were elucidated from 1D and 2D NMR analysis, as well as by high resolution mass spectrometry. All the isolated flavones were evaluated for their myeloperoxidase (MPO) inhibitory activity. The most active compound, pedalitin, exhibited IC₅₀ value of 3.75 nM on MPO. Additionally, the radical-scavenging capacity of flavones 1–6 was evaluated towards ABTS and DPPH radicals and compared to standard compounds quercetin and Trolox[®].

Key words flavone; *Pterogyne nitens*; myeloperoxidase; antioxidant; radical scavenging

The vast knowledge accumulated in recent years about inflammatory diseases has permitted a better understanding of the mechanism of action of the several mediators. In this context, some enzymes such as cyclooxygenases, nitric oxide synthase and myeloperoxidase (MPO) have been widely recognized as powerful targets for the action of anti-inflammatory agents.^{1–3} MPO is a leukocyte-derived heme peroxidase which has long been considered as a microbial enzyme centrally linked to the unspecific immune defense system. MPO plays an important role in oxidants production by polymorphonuclear neutrophils (PMNs). It uses hydrogen peroxide (H₂O₂) and chloride gas to catalyze the production of hypochlorous acid (HOCl), which contributes to both microbial killing, and subsequent oxidative injury of host tissue triggering severe inflammatory disorders,⁴ including rheumatoid arthritis,⁵ inflammatory bowel disease (IBD),⁶ cystic fibrosis,⁷ neonatal lung injury,⁸ and adult respiratory distress syndrome.⁹ Thus, the search for new MPO inhibitors from natural sources can provide prototypes for the anti-inflammatory drug discovery, and design of novel therapeutic strategies.

Pterogyne nitens TULASNE (Fabaceae) popularly known as “cocal”, “yvira-ró”, “amendoinzeiro”, “bálsamo” is a highly branched and ca. 5–12 m high tree.¹⁰ Its wood is used for house building, for furniture and to make bows.¹¹ In folk medicine, stem barks have been used as antiparasitic agent e.g., for treatment of ascariasis.¹² As previously reported, the leaves of *P. nitens* yielded five guanidine alkaloids, which exhibited cytotoxic activity against the DNA-repair-deficient yeast mutant RS 321 and moderate cytotoxicity against to CHO Aux B1 cells.¹³ Preliminary study on additional biological activities of *P. nitens* indicated a strong inhibition of rat paw edema suggesting the presence of anti-inflammatory compounds in its leaves EtOH extract which prompted us to investigate its chemical composition and evaluate the antioxidant and MPO inhibitory properties of the isolates.

Experimental

Plant Material Leaves of *Pterogyne nitens* were collected in the Botanic Garden of São Paulo, SP, Brazil, in May 2003, and a voucher specimen (SP204319) has been deposited in the herbarium of the Botanic Institute (SP, Brazil).

Isolation and Purification The shade-dried leaves (2.3 kg) of *P. nitens* were ground and defatted with *n*-hexane (1.8 l $\times$ 3, at room temperature) and exhaustively extracted by maceration with ethanol (2.7 l $\times$ 3) at room temperature. The ethanol extract was concentrated under reduced pressure to yield 9.2 g of a syrupy residue. The concentrate was then diluted with MeOH : H₂O (4 : 1) and successively partitioned with EtOAc and *n*-butanol. After solvent removal using a rotaevaporator, each partition phase yielded 3.0 and 4.2 g, respectively. The EtOAc residue (1.5 g) was chromatographed by gel permeation over Sephadex LH-20 eluted with methanol to afford eleven fractions (E1–E11). Chromatographic purification of fraction E5 (232 mg) by RP-HPLC [MeOH : H₂O : AcOH (46 : 53.5 : 0.5), UV detection at 274 nm; flow rate 10 ml/min] led to the isolation of compounds 1 (84 mg) and 6 (43 mg). Fraction E8 (112 mg) was purified by RP-HPLC [MeOH : H₂O : AcOH (55 : 44.5 : 0.5), UV detection at 274 nm; flow rate 13 ml/min], affording compound 3 (22 mg). TLC analysis of fraction E11 indicated it was pure, and led to its identification as compound 5 (130 mg). The butanol fraction (1.8 g) was dissolved in MeOH and submitted to gel filtration on Sephadex LH-20 eluted with MeOH, affording 14 fractions (B1–B14). After TLC comparison, fractions B4–B6 were combined (333 mg) and purified by RP-HPLC (MeOH : H₂O : AcOH (68 : 31.5 : 0.5), UV detection at 274 nm and flow rate 12 ml/min), to yield compounds 2 (66 mg) and 4 (89 mg).

Radical Scavenging Activity (RSA) Antioxidant activity of compounds 1–6 was determined using DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) and ABTS^{•+} [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] as reagents.^{14,15} Sample solutions of 1–6 and positive controls at various concentrations (33.3–1.7 μ M) in MeOH were individually added to 67.6 μ M DPPH in MeOH. The mixture was incubated in the dark at 25 °C for 30 min. Remaining DPPH was determined colorimetrically at 517 nm by comparing with methanol (negative control) in a microplate reader. ABTS^{•+} was prepared by reacting 5 ml of ABTS (7 mM) water solution with 88 μ l of 140 mM potassium persulphate (ratio 1 : 0.35) and the mixture allowed to stand in the dark at room temperature for 12–16 h before use. Prior to assay, ABTS^{•+} stock solution was diluted with KH₂PO₄/K₂HPO₄ (100 mM, pH 7.0 diluted 1 : 10 before use) buffer solution (ratio 1 : 88). ABTS^{•+} solution (1 ml) was added to glass test tubes containing different concentrations of compounds 1–6 and positive controls (0.5–7.0 μ g/ml) and mixed for 15 s. Tubes were incubated for 30 min at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 734 nm. A lower absorbance of the reaction mixture indicated higher free radical

* To whom correspondence should be addressed. e-mail: dhsilva@iq.unesp.br

scavenging activity. Anti-radical abilities against ABTS^{•+} and DPPH[•] species were expressed by using mean values obtained from triplicates as percentage of radical reduced (% inhibition) calculated from the equation: % inhibition = $[1 - (A_{\text{sample}}/A) \times 100]$, where A is test absorbance without sample (only solvent and free radicals) and A_{sample} is test absorbance with compounds **1–6** or positive controls.

Myeloperoxidase Inhibitory Activity Inhibitory effects of 7-methoxyflavones **1–6** on MPO were evaluated through guaiacol oxidation in presence of myeloperoxidase (8 nM) and hydrogen peroxide (0.3 mM).¹⁶ The reactions were spectrophotometrically followed at 470 nm, calculated from the initial linear rate of reaction using guaiacol (70 mM) as substrate for the peroxidase inhibitory activity, and were compared at different sample concentrations of compounds **1–6** (0.50–50 nM) and in the their absence. The IC₅₀ values were obtained from the rates calculated on the basis of % inhibition expressed in concentration-response curves.

Results and Discussion

Nitensoside A (**1**) was obtained as yellow amorphous powder. Its IR spectrum showed absorption bands at 3427 (OH), 1656 (α,β -unsaturated C=O), 1606, 1502, 1452 (C=C, aromatic), and a broad band at 1160 cm⁻¹, indicating the presence of a glucosyl unit (Fig. 1). The UV spectrum displayed two strong absorption bands at 274 nm and 338 nm. These results strongly suggested that compound **1** is a flavonoid glycoside.^{17,18} Analysis of the ¹H-NMR data (Table 1) revealed characteristic resonances of aromatic and glycosidic protons (including two anomeric protons) and one methoxy group. The signals at δ 4.92 (1H, d, $J=7.0$ Hz, H-1''), δ 4.45 (1H, d, $J=1.5$ Hz, H-1'''), δ 3.40 (1H, m, H-6a''), δ 3.70 (1H, dd, $J=10.0, 1.5$ Hz, H-6b''), and δ 1.04 (1H, d, $J=6.0$ Hz, H-6''') suggested the presence of one β -glucopyranosyl and one α -rhamnopyranosyl unit in compound **1**, which was con-

firmed after inspection of the ¹³C-NMR data set (Table 1) and observation of gHMQC correlations of sugar protons to their respective carbon signals, especially those at δ 102.4 (C-1''), δ 100.6 (C-1'''), δ 66.6 (C-6'') and δ 17.7 (C-6'''). In the aromatic moiety of compound **1**, signals at δ 7.96 (2H, d, $J=8.5$ Hz, H-2', 6'), 6.94 (2H, d, $J=8.5$ Hz, H-3', 5'), 6.87 (1H, s, H-8) and 6.81 (1H, s, H-3) suggested that the aglycone was an apigenin derivative (5,7,4'-trihydroxyflavone).^{17,19} The singlet at δ 6.87 was assigned to H-8 of the flavone A-ring on the basis of its characteristic ¹³C-NMR resonance at δ 91.5.¹⁷ The position of the methoxy group was assigned at C-7 on the basis of observed gHMBC and NOESY correlations (Fig. 2). The NOESY 1D spectrum showed a strong interaction between the methoxy proton signal at δ 3.90 and H-8 whereas a gHMBC correlation was observed between the signals at δ 158.7 (C-7) and δ 3.90, which confirmed the position of the methoxy group at C-7. A singlet observed at δ 13.0 was assigned to the C-5 hydroxy proton. These observations confirmed the aglycone unit as 5,6,4'-trihydroxy-7-methoxyflavone (sorbifolin), which was consistent with the protonated aglycone [sorbifolin+H]⁺ ion at m/z 301 observed in first-order LR-ESI-MS (positive mode) of compound **1**. A cross-peak between the signals for

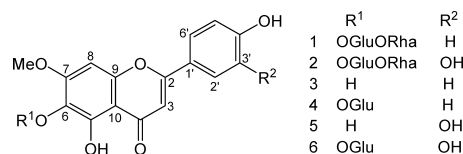


Fig. 1. Structures of Compounds **1–6**

Table 1. ¹H- (500 MHz) and ¹³C-NMR (125 MHz) Data for Flavone Glucosides **1** and **2** in DMSO-*d*₆

Position	1			2		
	δ_c (mult.)	δ_H , mult., J (Hz)	gHMBC	δ_c (mult.)	δ_H , mult., J (Hz)	gHMBC
2	164.0 (s)	—	H-3, H-2', H-6'	164.1 (s)	—	H-3, H-2', H-6'
3	102.7 (s)	6.81 (s)	—	102.7 (s)	6.67 (s)	—
4	182.2 (s)	—	H-3	182.1 (s)	—	H-3
5	151.7 (s)	—	5-OH	152.8 (s)	—	—
6	128.1 (s)	—	H-8, H-1'', 5-OH	128.0 (s)	—	H-8, H-1''
7	158.7 (s)	—	H-8, 7-OMe	158.7 (s)	—	H-8, 7-OMe
8	91.5 (d)	6.87 (s)	—	91.4 (d)	6.79 (s)	—
9	152.8 (s)	—	H-8	151.7 (s)	—	H-8
10	104.9 (s)	—	H-3, H-8, 5-OH	104.8 (s)	—	H-3, H-8
1'	121.2 (s)	—	H-3, H-3', H-5'	121.5 (s)	—	H-3, H-2', H-5', H-6'
2'	128.5 (d)	7.96 (d, $J=8.5$)	H-6'	113.5 (d)	7.50 (d, $J=2.0$)	H-6'
3'	115.9 (d)	6.94 (d, $J=8.5$)	H-5'	145.7 (s)	—	H-2', H-5'
4'	161.2 (s)	—	H-2', H-6'	149.7 (s)	—	H-2', H-6'
5'	115.9 (d)	6.94 (d, $J=8.5$)	H-3'	115.9 (d)	6.88 (d, $J=8.5$)	—
6'	128.5 (d)	7.96 (d, $J=8.5$)	H-2'	119.0 (d)	7.42 (dd, $J=8.5, 2.0$)	H-2', H-5'
1''	102.4 (d)	4.92 (d, $J=7.0$)	H-2''	102.4 (d)	4.89 (d, $J=7.0$)	H-2''
2''	74.0 (d)	3.24 (m)	H-3''	74.0 (d)	3.22 (m)	H-3''
3''	75.7 (d)	3.22 (m)	H-2''	75.7 (d)	3.19 (m)	H-2''
4''	70.0 (d)	3.10 (m)	H-3''	70.0 (d)	3.08 (m)	H-3'', H-4''
5''	76.4 (d)	3.12 (m)	—	76.4 (d)	3.10 (m)	—
6''	66.6 (t)	3.40 (m); 3.70 (dd, $J=11, 1.5$)	H-1'''	66.5 (t)	3.39 (m); 3.68 (d, $J=11$)	H-1'''
1'''	100.6 (s)	4.45 (d, $J=1.5$)	H-6a'', H-6b''	100.5 (s)	4.43 (br s)	H-6''a
2'''	70.3 (d)	3.42 (m)	—	70.3 (d)	3.40 (m)	H-3'''
3'''	70.6 (s)	3.30 (m)	H-1'''	70.5 (s)	3.26 (m)	H-1'''
4'''	71.8 (s)	3.10 (m)	H-6'''	71.8 (s)	3.08 (m)	H-6'''
5'''	68.1 (s)	3.26 (m)	H-6'''	68.1 (s)	3.24 (m)	H-6'''
6'''	17.7 (q)	1.04 (d, $J=6.0$)	H-4'''	17.7 (q)	1.01 (d, $J=6.5$)	H-4'''
7-OMe	56.4 (q)	3.90 (s)	—	56.4 (q)	3.88 (s)	—
5-OH	—	13.0 (br s)	—	—	13.0 (br s)	—

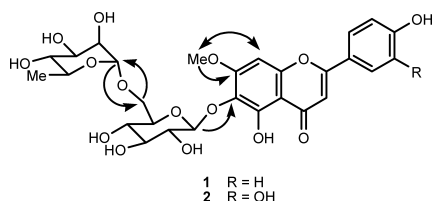


Fig. 2. Selected gHMBC (Single Arrow) and NOESY (Double Arrow) Correlations for Compounds **1** and **2**

C-6 (δ 128.1) and the anomeric proton (δ 4.92) in the gHMBC spectrum indicated the position of the glucopyranosyl unit at C-6 of the flavone moiety. Assignment of the inner sugar sugar resonances was achieved by using the anomeric proton resonance at δ 4.92 as a starting point for the interpretation of ^1H - ^1H -COSY and gHMQC datasets. The coupling constant of the anomeric proton ($J=7.0$ Hz) confirmed the β -linkage of the first glucosyl moiety. The downfield shift of C-6'' (+5.5 ppm) and upfield shift of C-5'' (-1.4 ppm) glucopyranosyl moiety resonances, respectively, compared to sorbifolin-6- O - β -glucopyranoside,¹⁹ indicated that the rhamnopyranosyl moiety was attached at C-6''. Moreover, the gHMBC correlation between the signals at δ 100.6 (C-1''') and δ 3.70 (H-6'') confirmed the C-6''- O -C-1''' interglycosidic linkage of compound **1**. The high resolution ESI mass spectrum of compound **1** showed a deprotonated molecular ion peak $[\text{M}-\text{H}]^-$ at 607.1657 m/z , in agreement with the calculated mass for the molecular formula $\text{C}_{28}\text{H}_{31}\text{O}_{15}$ (607.1657).

Compound **2** was obtained as amorphous yellow powder and its HR-ESI-MS (negative mode) showed a deprotonated molecular ion at m/z 623.1600 (Calcd 623.1657) associated to the molecular formula $\text{C}_{28}\text{H}_{31}\text{O}_{16}$. The UV and IR spectra of **2** were similar to those of compound **1** suggesting a 6- O -glucosylflavone derivative. The IR of **2** showed a broad hydroxyl band at 3427 cm^{-1} , and an α,β -unsaturated carbonyl absorption at 1656 cm^{-1} , in addition to a C-O stretching band at 1045 cm^{-1} , indicating a glycosidic substance. The UV spectrum of **2** exhibited absorption maxima typical of a flavone at 270 nm and 349 nm. The ^1H - and ^{13}C -NMR data (Table 1) suggested that compound **2** had the same 7-methoxyflavone skeleton as compound **1**, except for the presence of a hydroxy group at C-3'. In fact, the major difference between compounds **1** and **2** was the aromatic spin system displayed by the B ring hydrogens, which were resolved as one ABX spin system at δ 7.42 (1H, dd, $J=8.5$, 2.0 Hz, H-6'), δ 7.50 (1H, d, $J=2.0$ Hz, H-2'), and δ 6.88 (1H, d, $J=8.5$ Hz, H-5') for compound **2**. The ^{13}C -NMR spectrum of compound **2** presented 28 signals, deduced from distortionless enhancement by polarization transfer (DEPT) 90°, DEPT 135° and gHMQC experiments as one methyl, one methylene, fifteen methines, one methoxyl and ten quaternary carbons, including one α,β -unsaturated ketone at δ 182.1, which confirmed the flavone core. Similarly as for nitenoside A, the position of methoxy group and sugar units, as well as the interglycosidic linkage were deduced from gHMBC and NOESY 1D experiments. A gHMBC correlation of signals at δ 158.7 (C-7) and δ 3.88 (OMe) in addition to NOESY interaction of signals at δ 3.88 and δ 6.79 (H-8) indicated the position of the methoxy group at C-7. Cross-peaks between signals at δ 128.0 (C-6) and δ 4.89 (H-1''), and between signals at δ 66.5 (C-6'') and δ 4.43

(H-1''') led to the establishment of glucosylation at C-6 and the C-6''- O -C-1''' interglycosidic linkage in compound **2**. Further gHMBC correlations gave additional support to the structure of this new pedaltin glucoside derivative (Fig. 2). Thus based on spectrometric data analysis, the structures of flavones **1** and **2** were elucidated as sorbifolin 6- O - α -rhamnopyranosyl(1''' $\rightarrow$ 6'')- β -glucopyranoside, and pedaltin 6- O - α -rhamnopyranosyl(1''' $\rightarrow$ 6'')- β -glucopyranoside, two new naturally occurring compounds named as nitenoside A and B, respectively. In addition, four known 7-methoxyflavones were isolated and identified as sorbifolin (**3**),²⁰ sorbifolin 6- O - β -glucopyranoside (**4**),²⁰ pedaltin (**5**),²⁰ and pedaltin 6- O - β -glucopyranoside (**6**).¹³

The antioxidant activity of the isolates was evaluated by measuring free radical scavenging effects using two different assays, the ABTS radical cation decolorization assay and the DPPH radical scavenging activity assay.^{14,15} Pedaltin derivatives had better radical scavenging activity than sorbifolin derivatives, probably due to presence of the *ortho*-dihydroxy group (catechol residue on ring B), which is responsible for enhanced free radical stabilization after one hydrogen radical donation for the antioxidant capacity.^{21,22} Pedaltin derivatives **2**, **5** and **6** showed higher scavenging activity towards ABTS radical, with IC_{50} (μM) values of 5.02, 1.36 and 4.07, respectively; and towards DPPH with IC_{50} values of 11.0, 5.22, and 8.19, respectively, than sorbifolin derivatives **1**, **3** and **4** (Table 2), evidencing a major role of the catechol moiety on the B-ring of flavonoids for enhancement of the free radical scavenging activity. Additionally, among pedaltin derivatives, increased activities were observed for pedaltin (**5**) when compared to its glucosides **2** and **6** in both assays, evidencing the influence of the free hydroxy group at C-6, to generate an additional catechol group (on ring A), and therefore potentiating its free radical scavenging activity. The presence of two catechol moieties on pedaltin provided even higher activity towards ABTS than for the reference compound quercetin (IC_{50} 4.12 μM), as observed previously for glucosyl-xanthones.²³ Similarly, this trend has also been observed for sorbifolin, which showed lower IC_{50} (5.7 μM towards ABTS, and 13.5 μM towards DPPH) than its 6- O -glucosyl derivatives **1** and **4**, thus confirming the importance of the 5,6-dihydroxy moiety on ring A for enhanced antiradical activity of 7-methoxyflavones.

Although a broad spectrum of the effects on mammalian enzyme systems has already been demonstrated for flavonoids,²⁴ few studies have been devoted to the MPO inhibitory activity of this class of natural polyphenols.^{25,26} MPO inhibitory activity has been proven to be dependent on the redox status of biological systems and has been evaluated in this study by a reference method with modifications.¹⁶ The results have been summarized in Table 2 and evidence the importance of the catechol system on rings A and/or B for increased MPO inhibitory activity. Compounds **2**, **5** and **6**, displaying a 3',4'-dihydroxy phenolic moiety, exhibited IC_{50} values of 19.2, 3.8, and 15.8 nM, respectively, indicating a higher MPO inhibitory activity than compounds **1**, **3** and **4**, which are sorbifolin derivatives, thus presenting a monohydroxy phenolic B ring. Glucosides **1**, **2**, **4** and **6** showed slightly lower activities than their free aglycones, suggesting an important role of the free hydroxyl at C-6. Altogether, these results indicate a clear positive correlation between

Table 2. MPO Inhibition and ABTS and DPPH Radical Scavenging Activity of Flavones 1–6^{a)}

Compounds	MPO (nM)	ABTS radical (μ M)	DPPH radical (μ M)
1	25.9 $\pm$ 0.5	15.3 $\pm$ 0.3	14.7 $\pm$ 0.5
2	19.2 $\pm$ 0.1	5.0 $\pm$ 0.5	11.0 $\pm$ 0.3
3	19.2 $\pm$ 0.1	5.7 $\pm$ 0.4	13.5 $\pm$ 0.5
4	22.0 $\pm$ 0.3	6.3 $\pm$ 0.4	14.0 $\pm$ 0.7
5	3.8 $\pm$ 0.1	1.4 $\pm$ 0.3	5.2 $\pm$ 0.1
6	15.8 $\pm$ 0.1	4.1 $\pm$ 0.5	8.2 $\pm$ 0.2
Quercetin ^{b)}	1.2 $\pm$ 0.1	4.2 $\pm$ 0.5	2.6 $\pm$ 0.5
Trolox ^{c)}	—	0.6 $\pm$ 0.1	4.7 $\pm$ 0.7

a) DPPH=2,2-diphenyl-1-picrylhydrazyl; ABTS=2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonic acid); IC₅₀ values were determined by linear regression and expressed as means $\pm$ S.D. of triplicate experiments. b) Quercetin was used as positive control for MPO inhibition.²⁰⁾ c) Trolox[®] and quercetin were used as positive controls for RSA assays.

MPO inhibitory activity and radical scavenging properties of flavonoids as shown on Table 2, which might be important for treating inflammation derived diseases.

Conclusion

The association of antiradical properties and MPO inhibitory activity observed for flavones 1–6, tested in this study, indicates this might be a rational approach in the search for bioactive natural products. In conclusion, this study has demonstrated that the isolated flavones might be considered as potential candidates for the development of anti-inflammatory agents, since they present antiradical properties and inhibit myeloperoxidase, a key enzyme involved in inflammatory processes.

Acknowledgements This work was funded by grants from the “Fundação de Amparo à Pesquisa do Estado de São Paulo”, FAPESP (Brazil), as part of the Biota-FAPESP—The Biodiversity Virtual Institute Program (www.biota.org.br); Grant No. 04/07932-7 awarded to D.H.S.S., PI. D.C.F., L.O.R., J.C.R.V., P.M.P., V.S.B. and D.H.S.S. also acknowledge FAPESP and CNPq for researcher and students fellowships.

References and Notes

- 1) Barnes P., Chung K. F., Page C. P., *Pharmacol. Rev.*, **50**, 515–596 (1998).
- 2) Di Rosa M., Lalenti A., Ianaro A., Sautebin L., *Prostaglandins Leukot. Essent. Fatty Acids*, **54**, 229–238 (1996).
- 3) Sautebin L. A., Lalenti A., Ianaro A., Di Rosa M., *Br. J. Pharmacol.*, **114**, 323–328 (1995).
- 4) Winterbourn C. C., Vissers M. C. M., Kettle A. J., *Curr. Opin. Hematol.*, **7**, 53–58 (2000).
- 5) Edwards S. W., Hallet M. B., *Immunol. Today*, **18**, 320–324 (1997).
- 6) Dhaneshwar S. S., Kandpal M., Vadnerkar G., Rath B., Kadam S. S., *Eur. J. Med. Chem.*, **42**, 885–890 (2007).
- 7) Regelman W. E., Siefferman C. M., Herron J. M., Elliot G. R., Clawson C. C., Gray B. H., *Pediatr. Pulmonol.*, **19**, 1–9 (1995).
- 8) Buss I. H., Darlow B. A., Winterbourn C. C., *Pediatr. Res.*, **47**, 640–645 (2000).
- 9) Chabot F., Mitchell J. A., Gutteridge J. M., Evans T. W., *Eur. Respir. J.*, **11**, 745–757 (1998).
- 10) Lorenzi H., “Árvores brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas do Brasil,” Vol. 1, Plantarum, Nova Odessa, 1998, p. 162.
- 11) Burkart A., “Las Leguminosas Argentinas,” Aemé Agency, Buenos Aires, 1952, p. 156.
- 12) Crivos M., Martinez M. R., Pochettino M. L., Remorini C., Sy A., Teves L., *J. Ethnobiol. Ethnomed.*, **2**, 1–12 (2007).
- 13) Bolzani V. S., Gunatilaka A. A. L., Kingston D. G. I., *J. Nat. Prod.*, **58**, 1683–1688 (1995).
- 14) Soares J. R., Dinis T. C., Cunha A. P., Almeida L. M., *Free Rad. Res.*, **26**, 469–478 (1997).
- 15) Pellegrini N., Re R., Yang M., Rice-Evans C., *Methods Enzymol.*, **299**, 379–389 (1999).
- 16) Capeillère-Blandin C., *Biochem. J.*, **336**, 395–404 (1998).
- 17) Mabry T. J., Markham K. R., Thomas M. B., “The Systematic Identification of Flavonoids,” Springer-Verlag, New York, 1970, pp. 84–120.
- 18) Agrawal P. K., Thakur R. S., Bansal M. C., “Carbon-13 NMR of Flavonoids,” Vol. 39, ed. by Agrawal P. K., Elsevier, New York, 1989, pp. 95–182.
- 19) Markham K. R., Geiger H., “The Flavonoids: Advances in Research Since 1986,” ed. by Harborne J. B., Chapman and Hall, London, 1993, pp. 441–497.
- 20) Zhang X. F., Hu B. L., Wang S. X., *Acta Bot. Sin.*, **36**, 645–648 (1994).
- 21) Van Acker S. A. B. E., Vanden Berg D.-J., Tromp M. N. J. L., Griffioen D. H., Van Bennekom W. P., Van der Vijgh W. J. F., Bast A., *Free Rad. Biol. Med.*, **20**, 331–342 (1996).
- 22) Seyoum A., Asres K., El-Fiky F. K., *Phytochemistry*, **67**, 2058–2070 (2006).
- 23) Pauletti P. M., Castro-Gamboa I., Silva D. H. S., Young M. C. M., Tomazela D. M., Eberlin M. N., Bolzani V. D., *J. Nat. Prod.*, **66**, 1384–1387 (2003).
- 24) Middleton E., Kandaswami C., Theoharides T. C., *Pharmacol. Rev.*, **52**, 673–751 (2000).
- 25) Kato Y., Nagao A., Terao J., Osawa T., *Biosci. Biotechnol. Biochem.*, **67**, 1136–1139 (2003).
- 26) Rosso R., Vieira T. O., Leal P. C., Nunes R. J., Yunes R. A., Creczynski-Pasa T. B., *Bioorg. Med. Chem.*, **14**, 6409–6413 (2006).