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# Flavonols from *Pterogyne nitens* and their evaluation as myeloperoxidase inhibitors

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**Abstract**

A myeloperoxidase inhibitory kaempferol derivative, namely pterogynoside (**1**), was isolated from fruits of *Pterogyne nitens*, along with six known flavonols, kaempferol, afzelin, kaempferitrin, quercetin, isoquercetrin and rutin. The structures of all compounds were elucidated primarily from 1D and 2D NMR spectroscopic analyses, as well as by high resolution mass spectrometry. All flavonols were screened to identify secondary metabolites as potential myeloperoxidase (MPO) inhibitors, and at concentrations of 0.50–50 nM, quercetin (**5**), isoquercitrin (**6**) and rutin (**7**) exhibited strong inhibitory effects with $IC_{50}$ values of $1.22 \pm 0.01$, $3.75 \pm 0.02$ and $3.60 \pm 0.02$, respectively. The MPO activity detected for the new derivative **1** was markedly decreased ($IC_{50}$ $10.3 \pm 0.03$) when compared with known flavonols **5–7**, and interestingly increased when tested against ABTS scavenging activity.


*Keywords:* *Pterogyne nitens*; Fabaceae; Acylated flavonol; Myeloperoxidase; Antioxidant

## 1. Introduction

Myeloperoxidase (MPO) is a heme-enzyme present in human neutrophils and plays a role in infection and inflammation, converting hydrogen peroxide and chloride to hypochlorous acid and water (Winterbourn et al., 2000). Although hypochlorous acid has important roles in killing microorganisms, it also possesses high reactivity and the ability to damage macromolecules by oxidation (Teixeira et al., 2003). The excessive MPO activity and reactive oxygen species (ROS), especially HOCl, production are implicated in many inflammatory processes (Heinecke et al., 1993; Gurel et al., 2004), including atherosclerosis, cancer, and Alzheimer's disease; the search for new myeloperoxidase inhibitors from natural sources has become a staple in drug discovery.

*Pterogyne nitens* (Fabaceae) is popularly named in Brazil as "tipá", "viraró", "cocal", "amendoinzeiro", "amendoim-bravo", according to the region where this plant grows. It is a beautiful legume tree, and the sole member of the genus *Pterogyne*, which is distributed mainly in Brazil, Bolivia, Paraguay and Argentina, and can reach a height of 5–12 m. (Burkart, 1952). Its wood is also widely

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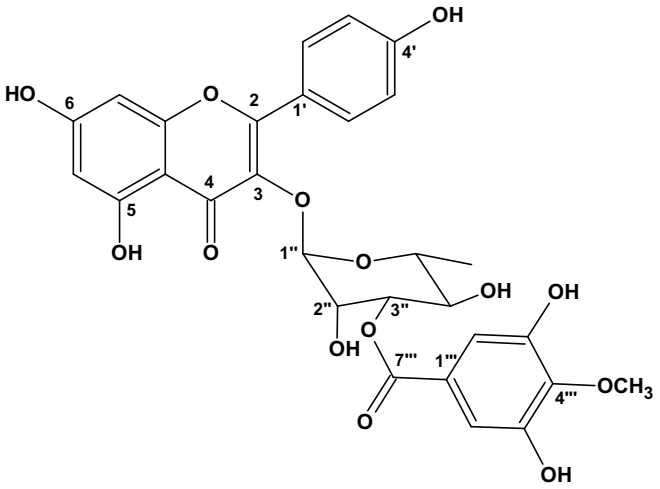


Fig. 1. Chemical structure of compound **1**.

used in building construction and, to our knowledge, there are no reports on ethnomedicinal uses for this species (Lorenzi, 1998).

Our previous studies on *P. nitens* have resulted in the isolation of flavones, and five guanidine alkaloids, which exhibited selective activity towards a DNA repair-deficient strain of yeast *Saccharomyces cerevisiae*, suggesting potential anti-cancer activity (Bolzani et al., 1995). In our continuing interest in searching for new biologically active metabolites from plants of the Cerrado and Atlantic Forest regions, we have investigated the fruits of this plant, collected at the Botanical Garden, São Paulo, in May 2003. We report herein the isolation, and structural elucidation of one new flavonol rhamnoside, namely pterogynoside (**1**) and six known flavonols: kaempferol (Mitscher et al., 1985), afzelin (Salama et al., 1981), kaempferitrin (Pizzolatti et al., 2003), quercetin, isoquercitrin and rutin (Markham et al., 1978). Additionally, these compounds were screened for MPO inhibitory activity, using a guaiacol oxidation method (Capeillère-Blandin, 1998). The new flavonol **1** was also analyzed for the scavenging of DPPH and ABTS free radicals activities and its antioxidant potential was evaluated (see Fig. 1).

## 2. Results and discussion

Shade-dried, powdered fruits of *P. nitens* were first defatted with *n*-hexane and exhaustively extracted by remaceration with ethanol. The solvent was evaporated at low temperature under reduced pressure to yield a syrup which was suspended in methanol–water (4:1) and then successively partitioned with ethyl acetate and *n*-butyl alcohol. The resulting fractions were subjected to successive column chromatography separation steps to yield pterogynoside (**1**) along with six known flavonols.

Pterogynoside (**1**) was isolated as yellow prisms, m.p. 272–273 °C, and gave a positive reaction with ferric chloride, indicating it as a phenol derivative. The IR spectrum exhibited strong absorption bands due to phenolic hydroxyl (3280 $cm^{-1}$), chelated hydroxy group (3265 $cm^{-1}$), conjugated ester (1695 $cm^{-1}$), conjugated ketone (1650 $cm^{-1}$) and aromatic C=C absorptions (1612, 1593, 1550 and 1508 $cm^{-1}$). The UV spectrum displayed absorptions ($\lambda_{max}$) ($\log\varepsilon$) at 316 (4.53) and 297 (4.29), indicating the existence of oxy-aromatic systems, compatible with a flavonol structure (Mabry et al., 1970; Agrawal et al., 1989). The UV spectra recorded in EtOH and shift reagents such as KOH, NaOAc and $AlCl_3$ suggested the presence of free hydroxy groups at C-4′, C-7 and C-5, respectively. Its elemental composition $C_{29}H_{26}O_{14}$, with 17° of unsaturation, was deduced from its HRESIMS, which showed a deprotonated molecular ion ($[M-H]^-$) at *m/z* 597.5087 (calcd for $C_{29}H_{26}O_{14}$, 597.5081) consistent with its $^{13}C$ NMR data (Table 1, Supporting Information). The CID (collision induced dissociation) experiments using the product ion scan mode ($MS^2$) allowed characterization and establishment of the fragmentation pathway of pterogynoside, with an intense (64%) kaempferol peak at *m/z* 285 in the negative ion mode. The positive ion mode also gave an intense kaempferol peak at *m/z* 287. Further MS analysis showed a deprotonated ion peak at *m/z* 597, and a product ion at *m/z* 431 indicating the presence of one hexose residue in compound **1**. Its $^1H$ and $^{13}C$ NMR and DEPT spectra evi-

Table 1
$^1H$ (500 MHz) and $^{13}$ C NMR (126 MHz) spectroscopic data for flavonol **1** in methanol-$d_4$

| Position | $\delta_C$ (mult.)[a,b] | $\delta_H$ [(mult.) *J* (Hz)] | *g*HMBC |
|---|---|---|---|
| 2 | 159.2 (*s*) | – | H-2′ |
| 3 | 136.0 (*s*) | – | H-1″ |
| 4 | 179.5 (*s*) | – | – |
| 5 | 163.1 (*s*) | – | H-6 |
| 6 | 99.9 (*d*) | 6.10 (*d*, 2.0) | H-8, H-10 |
| 7 | 165.9 (*s*) | – | H-6, H-8 |
| 8 | 94.4 (*d*) | 6.29 (*d*, 2.0) | H-6, H-10 |
| 9 | 158.5 (*s*) | – | H-8 |
| 10 | 106.9 (*s*) | – | – |
| 1′ | 112.6 (*s*) | – | – |
| 2′,6′ | 131.9 (*d*) | 7.74 (*d*, 9.0) | – |
| 3′,5′ | 116.6 (*d*) | 6.87 (*d*, 9.0) | – |
| 4′ | 161.5 (*s*) | – | H-2′,H-3′ |
| 1″ | 103.6 (*d*) | 5.38 (*d*, 1.5) | H-3, H-3″ |
| 2″ | 69.8 (*d*) | 4.38 (*dd*, 3.2, 1.5) | – |
| 3″ | 75.5 (*d*) | 5.11 (*dd*, 9.8, 3.2) | H-1″, H-4″ |
| 4″ | 70.6 (*d*) | 3.58 (*dd*, 9.8, 9.5) | H-6″ |
| 5″ | 72.3 (*d*) | 3.37 (*dd*, 9.5, 6.5) | H-6″, H-3″ |
| 6″ | 17.7 (*q*) | 0.89 (*d*, 6.5) | H-5″ |
| 1‴ | 126.8 (*s*) | – | – |
| 2‴, 6‴ | 110.5 (*d*) | 7.06 (*s*) | – |
| 3‴, 5‴ | 151.5 (*s*) | – | H-2‴ |
| 4‴ | 141.2 (*s*) | – | H-2‴, $OCH_3$-4‴ |
| 7‴ | 167.7 (*s*) | – | H-3″, H-2‴ |
| $OCH_3$-4‴ | 60.7 (*q*) | 3.77 (*s*) | – |

[a] Multiplicities were deduced from DEPT 90° and DEPT 135° experiments.
[b] Assignments were deduced from HMQC correlations and comparison with literature data (Agrawal et al., 1989).

denced the presence of a 5,7,4″- trisubstituted flavonol moiety, in addition to 4‴-methylgallate and α-L-rhamnopyranosyl residues. The assignment of all proton and carbon signals followed from extensive analysis of $^{1}H$–$^{1}H$ *g*COSY, *g*HMQC, and *g*HMBC spectra (Table 1). The $^{13}C$ NMR spectrum of **1** presented 29 carbons deduced from DEPT 90°, DEPT 135° and *g*HMQC techniques as one methyl, thirteen methines, one methoxyl and fourteen quaternary carbons, including two carbonyl groups at $\delta$ 167.7 and $\delta$ 179.5, attributed to ester and ketone groups, respectively. The $^{1}H$ NMR and $^{1}H$–$^{1}H$ *g*COSY spectra (Table 1) showed an $A_2M_2$ spin system assigned to the hydrogen signals at $\delta$ 7.74 (2H, *d*, $J = 9.0$ Hz, H-2″, 6′) and 6.87 (2H, *d*, $J = 9.0$ Hz, H-3′, 5′) attributed to a 1,4-disubstituted aromatic ring; and a pair of doublets at $\delta$ 6.10 (1H, *d*, $J = 2.0$ Hz, H-6) and 6.29 (1H, *d*, $J = 2.0$ Hz, H-8) for the trioxygenated A ring of compound **1**, suggesting a kaempferol moiety for its aglycone. Additionally, the $^{1}H$ NMR resonances at $\delta$ 5.38 and 0.89 evidenced an α-rhamnopyranosyl moiety, which was confirmed by observation of correlated signals at $\delta$ 103.6, $\delta$ and $\delta$ 17.7 in the HMQC spectrum. A cross-peak between the signals for C-3 ($\delta$136.0) and the anomeric hydrogen H-1″ ($\delta$ 5.38) in the *g*HMBC spectrum indicated the position of the rhamnosyl unit at C-3 of the kaempferol moiety. Further analysis of 1D and 2D NMR spectroscopic data (Table 1) indicated an additional methylgallate unit in compound **1**, which was placed at C-3″ of the rhamnopyranosyl moiety due to the downfield chemical shift observed for H-3″ ($\delta$ 5.11), and a *g*HMBC cross-peak of H-3″ with the carbonyl ester C-7‴ ($\delta$ 167.7). An additional *g*HMBC correlation was observed between the signal at $\delta$ 141.2 (C-4‴) and $\delta$ 3.77 (OMe) which confirmed the position of the methoxy group at C-4‴. Hence, the structure of pterogynoside (**1**) was completely elucidated, and established as kaempferol-3-*O*-(3″-*O*-4‴-methylgallate-α-L-rhamnoside. Additionally, six known flavonols were isolated and identified as kaempferol (**2**) (Mitscher et al., 1985), afzelin (**3**) (Salama et al., 1981), kaempferitrin (**4**) (Pizzolatti et al., 2003), quercetin (**5**), isoquercitrin (**6**) and rutin (**7**) (Markham et al., 1978). Their identification was based on analyses of $^{1}H$, $^{13}C$ NMR and MS data, as well as by comparison with authentic material available in our laboratory. Furthermore, this is the first report of flavonol glycosides from *P. nitens*.

Although a broad spectrum of biological activities has already been demonstrated for flavonoids (Middleton et al., 2000; Cuzzocrea et al., 2001; Havsteen, 2002; Mollace et al., 2005), few studies have been devoted to the myeloperoxidase inhibitory activity of this class of natural polyphenols (Kato et al., 2003).

Myeloperoxidase production is triggered by inflammatory mediators resulting in acceleration of superoxide anion ($O_2^-$) conversion to hydrogen peroxide an other ROS especially HOCl, which reacts further to generate singlet oxygen ($^{1}O_2$) and hydroxyl radical ($HO^{\bullet}$) as powerful oxidizing agents. Such species are involved in oxidative stress-related diseases as atherosclerosis, cancer, Alzheimer disease etc., which evidences the importance of research towards new MPO inhibitors.

Table 2
Myeloperoxidase (MPO) inhibitory activity and free radical scavenging activity (FRSA) towards ABTS and DPPH of flavonols **1-7**

| Compound | MPO inhibition $IC_{50}$ (nM)[a] | Free radical scavenging activity | |
|---|---|---|---|
| | | ABTS $IC_{50}$ (μg/mL)[a] | DPPH $IC_{50}$ (μg/mL)[a] |
| Pterogynoside (**1**) | 10.3 ± 0.03 | 8.10 ± 0.1 | >80.0 |
| Kaempferol (**2**) | 8.30 ± 0.03 | 18.0 ± 0.5 | 17.8 ± 0.8 |
| Afzelin (**3**) | 13.9 ± 0.04 | 17.0 ± 0.7 | >80.0 |
| Kaempferitrin (**4**) | 15.8 ± 0.05 | 18.5 ± 0.2 | 55.8 ± 0.8 |
| Quercetin (**5**)[b] | 1.22 ± 0.01 | 4.12 ± 0.2 | 2.60 ± 0.1 |
| Isoquercitrin (**6**) | 3.75 ± 0.02 | 4.37 ± 0.2 | 2.77 ± 0.4 |
| Rutin (**7**) | 3.60 ± 0.02 | 4.85 ± 0.2 | 3.15 ± 0.3 |
| Trolox® | – | 0.63 ± 0.3 | 4.72 ± 0.4 |

[a] $IC_{50}$ values were determined by regression linear and expressed as means ± SD of tree replicates.
[b] Quercetin was used as positive control (Kato et al., 2003).

All isolates were assayed for their MPO inhibitory activity (Table 2), evidencing a key role of the B ring oxygenation pattern on the level of activity. Compounds **5**–**7**, presenting a catechol derived B-ring, exhibited $IC_{50}$ values of 1.22, 3.75 and 3.60 nM, respectively, indicating a higher MPO inhibitory activity than compounds **1**–**4**, which are kaempferol derivatives, thus presenting a monohydroxy phenolic B ring. The aglycone quercetin (**5**) was used as positive control (Kato et al., 2003) and presented higher activity than its glucosides isoquercitrin (**6**) and rutin (**7**), suggesting an important role of the free hydroxyl at C-3 for enhancement of the MPO inhibitory activity. This trend has also been observed for kaempferol derivatives **1**–**4**, as the glucosides pterogynoside (**1**), afzelin (**3**) and kaempferitrin (**4**) exhibited $IC_{50}$ values of 10.3, 13.9 and 15.8 nM, respectively, and were less active than the aglycone kaempferol (**2**, $IC_{50}$ 8.3 nM). These results indicate a clear positive correlation between MPO inhibitory activity and antioxidant properties of flavonoids as shown in Table 2. The catechol on B ring and the α,β-unsaturated carbonyl moieties have been extensively pointed out as the main features in flavonoids associated with enhanced free radical stabilization after one hydrogen radical donation for the antioxidant activity (Van Acker et al., 1996). The presence of a galloyl group, e.g. in flavonoids, has also been associated with increased antioxidant capacity (Heijnen et al., 2002). In this respect, the presence of an additional galloyl unit in compound **1** might explain its higher MPO inhibitory activity, when compared with compounds **3** and **4**.

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. Quercetin derivatives **5**–**7** showed higher scavenging activity towards ABTS radical, with values of $IC_{50}$ (μg/mL) of 4.12, 4.37 and 4.85, and DPPH with values of 2.60, 2.77 and 3.15, respectively, than kaempferol derivatives **1**–**4** (Table 2), indicating a major role of the catechol moiety on the B-ring

of flavonoids for enhancement of the free radical scavenging activity. Additionally, among compounds **1–4**, increasing activities were observed for pterogynoside (**1**) and kaempferol (**2**), which confirms the importance of the free hydroxyl groups on C-3 (in **2**) and the enhancement of RSA due to the galloyl group (in **1**) in both assays. Among compounds **5–7**, quercetin (**5**) exhibited higher activity than its 3-*O*-glucosyl derivatives **6** and **7**, which confirmed the influence of the free-hydroxy group at C-3 for potentiating the free radical scavenging activity of flavonoids.

## 3. Concluding remarks

In summary, flavonoids with either the catechol moiety or α,β-unsaturated carbonyl with the free hydroxyl group at C-3 have shown best anti-radicalar and MPO inhibitory properties, which corroborates the involvement of MPO in redox processes. The absence of such features in pterogynoside (**1**) has been counteracted by the galloyl moiety on C-3″ leading to MPO inhibitory activity comparable to kaempferol (**2**), and higher free radical scavenging ability than **2** or its glucosides **3** and **4**.

Flavonols from *P. nitens* presented potent inhibition towards the pro-oxidant and pro-inflammatory enzyme myeloperoxidase and potent radical scavenging effects, which may be particularly important because they are easily available in the plant kingdom (Middleton et al., 2000) and are able to protect biological systems against stress phenomena such as lipid peroxidation, as shown for a variety of kaempferol derivatives exhibiting high antioxidant selectivity (Gebre-Mariam et al., 2005), and for quercetin and its derivatives, which have been shown to protect cells against $H_2O_2$-induced oxidative stress and calcium dysregulation (Wang and Joseph, 1999).

## 4. Experimental

### 4.1. General methods

Melting points were recorded on a Microquimica MQAPF-301 melting point apparatus. The 1D – ($^1$H, $^{13}$C and DEPT) and 2D – ($^1$H – $^1$H *g*COSY, *g*HMQC and *g*HMBC) NMR experiments were recorded on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz ($^1$H) and 126 MHz ($^{13}$C), using the solvents as an internal standard. Negative-ion high resolution mass spectra were recorded on HRMS ultrOTOFQ-ESI-TOF instrument (Bruker Daltonics). IR spectra absorptions were measured on a Perkin Elmer 1600 FT-IR spectrometer using KBr discs, whereas UV spectra were acquired on a Perkin Elmer UV–vis Lambda 14P spectrophotometer. Optical rotations were measured on a Polamat A Carl Zeiss Jena polarimeter. Analytical HPLC was performed on a Varian Pro Star 230 using a Phenomenex C-18 column (250 mm × 4.6 mm). Column chromatography (CC) was performed over reversed-phase silica gel 230–400 mesh (Merck). TLC was performed using Merck silica gel 60 (>230 mesh) and precoated silica gel 60 $PF_{254}$ plates. Spots on TLC plates were visualized under UV light and by spraying with anisaldehyde-$H_2SO_4$ reagent followed by heating at 120 °C. Preparative HPLC was carried out using a Varian Prep-Star 400 system using a Phenomenex C-18 (250 mm × 21.2 mm) preparative column. Sephadex LH-20 was purchased from Pharmacia Biotech, Sweden. All enzymatic or scavenging free radical assays were performed using a Spectrophotometer HP 8543, Diode Array. Guaiacol, ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Chemical®. All other reagents were analytical grade and commercially available. MPO was purchased from Planta Natural Products®.

### 4.2. Plant material

Fruits of *P. nitens* were collected in the Botanic Garden of São Paulo, São Paulo State, Brazil, in May 2003. A voucher specimen **(SP204319)** has been deposited in the herbarium of the Botanic Institute (São Paulo State, Brazil).

### 4.3. Extraction and isolation

The shade-dried fruits (2 kg) of *P. nitens* were ground and defatted with *n*-hexane (15 L × 5, at room temperature, for two weeks) and exhaustively extracted by maceration with EtOH (25 L × 5) at room temperature. The combined ethanol extracts were concentrated in vacuo (< 40 °C) to yield 8.0 g of a syrupy residue. The concentrate was then diluted with $CH_3OH$:$H_2O$ (4:1) and successively partitioned with EtOAc and *n*-BuOH. After removal of the solvent, each extract yielded 2.5 and 3.8 g, respectively. The EtOAc residue was subjected to silica gel (230–400 mesh) CC eluted with EtOAc/MeOH gradient (0–100% MeOH) to afford eight fractions (E1–E8) and free aglycones **2** (220.9 mg) and **5** (150.8 mg). Fraction E6 (397.8 mg) was further purified by RP-HPLC [MeOH:-$H_2O$:AcOH (58:42:0.1), UV detection at 254 nm; flow rate 10 mL/min], affording acylated flavonol **1** (115.9 mg) and two flavonol monoglycosides **3** (82.9 mg) and **6** (78.7 mg).

The *n*-BuoH residue was dissolved into MeOH and submitted to gel filtration on Sephadex LH-20 eluted with MeOH, affording 23 fractions (B1–B23). After TLC comparison, fractions B12–B19 were combined (488.3 mg) and purified by RP-HPLC using MeOH:$H_2O$:AcOH (68:32:0.1) as mobile phase, with UV detection at 254 nm and flow rate of 12 mL/min, affording flavonol diglycosides **4** (150.7 mg) and **7** (299.0 mg).

### 4.4. Kaempferol-3-O-(3″-O-4‴-methylgallate-α-L-rhamnoside) (*1*)

Yellowish prisms (MeOH); m.p. 272–273 °C; $[\alpha]_D^{22}$ – 131 (MeOH, *c*, 1.0); UV (EtOH) $\lambda_{max}$ (log ε); 316 (4.53), 297

(4.29). IR $\nu_{max}$ (KBr) $cm^{-1}$: 3280, 3265, 1695, 1650, 1612, 1593, 1550, 1508. ESIMSMS spectra showed $[M+H]^+$ and $[M-H]^-$ peaks at *m/z* 599 and *m/z* 597, respectively. ESIMSMS gave product ion from $[M+H]^+$ at *m/z* 287 $(C_{15}H_{10}O_6$, kaempferol$)^+$. ESIMSMS product ions from $[M-H]^-$ were observed at *m/z* 431 (M−H–4‴-*O*-methylgallate)$^-$ $(C_{21}H_{20}O_{10})^-$, *m*/z 285 $(C_{15}H_{10}O_6$, kaempferol$)^-$. HRESIMS (negative ion mode) (*m/z*) 597.5087; (M−H, calcd for $C_{29}H_{26}O_{14}$: 597.5079). For $^1$H NMR (500 MHz, $CD_3OD$) and $^{13}$C NMR (126 MHz, $CD_3OD$) spectroscopic data, see Table 1.

### 4.5. Assay for MPO inhibitory activity

MPO inhibitory effects of flavonols **1**–**7** were evaluated through guaiacol oxidation in presence of myeloperoxidase (8 nM) and hydrogen peroxide (0.3 mM). 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**–**7** (0.50–50 nM) and in the their absence (Kato et al., 2003). The $IC_{50}$ values were calculated from the rates calculated on the basis of inhibition percentage expressed in concentration–response curves.

### 4.6. Evaluation of radical scavenging activity (RSA)

Radical scavenging activity (RSA) of compounds **1**–**7** was determined using DPPH$^\cdot$ (2,2-diphenyl-1-picrylhydrazyl) and ABTS$^{\cdot+}$ [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] as reagents. Sample solutions of **1**–**7** and Trolox® at various concentrations (10–100 μg/ml) in EtOH were individually added to 0.6 mM DPPH in EtOH. The mixture was incubated in the dark at 25 °C for 30 min (Soares et al., 1997; Duarte-Almeida et al., 2006). Remaining DPPH was determined colorimetrically at 531 nm by blanking against absolute ethanol. ABTS$^{\cdot+}$ was prepared by reacting 5 mL of ABTS (7 mM) water soln. with 88 μL 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$^{\cdot+}$ stock soln. was diluted with $KH_2PO_4$/$K_2HPO_4$ (100 mM, pH 7.0 diluted 1:10 before use) buffer soln. (ratio 1:88). One milliliter ABTS$^{\cdot+}$ was added to glass test tubes containing different concentrations of compound **1** and Trolox® (0.5–7.0 μg/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 scavenging activity (Pellegrini et al., 1999). Anti-radical abilities against ABTS$^{\cdot+}$ and DPPH$^\cdot$ species were expressed by using mean values obtained from triplicates as percentage of radical reduced (inhibition%) calculated from the equation: Inhibition % = $[1 - (A_{sample}/A) \times 100]$, where $A$ is test absorbance without sample (only solvent and free radicals) and $A_{sample}$ is test absorbance with compounds **1**–**7** or Trolox®.

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