Free Radical Research, 2003 Vol. 37 (5), pp. 555–559

Taylor & Francis healthsciences

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

Antioxidant Activity of *Baccharis articulata* Extracts: Isolation of a New Compound with Antioxidant Activity

SIMONE Q. DE OLIVEIRA[a], FELIPE DAL-PIZZOL[b,c], GRACE GOSMANN[a,*], DOMINIQUE GUILLAUME[d], JOSÉ CLÁUDIO F. MOREIRA[b] and ELOIR P. SCHENKEL[e]

[a]*Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752, Porto Alegre, RS, 90610-000, Brazil;* [b]*Departamento de Bioquímica, Laboratório de Estresse Oxidativo, Universidade Federal do Rio Grande do sul, Porto Alegre, RS, Brazil;* [c]*Departmento de Medicina, Universidade do Extremo Sul Catarinense, Criciúma, Brazil;* [d]*Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, UPJV, Amiens, France;* [e]*Centro de Ciências da Saúde, Universidade Federal de Santa Catarnia, Florianópolis, Brazil*

Accepted by Professor B. Halliwell

(Received 25 October 2002; In revised form 2 December 2002)

Baccharis articulata is traditionally used as diuretic and digestive in local folk medicine of south of Brazil, Uruguay and Argentina. The antioxidant activity of crude ethanol and aqueous extracts, together with dichloromethane, ethyl acetate and *n*-butanol fractions obtained from aqueous extract of *B. articulata* was determined using TRAP and TBARS assays. The *n*-butanol fraction was found to be the most active and its major compound (**BaII**) was isolated, identified and assayed. The structure of the phenolic compound **BaII** was established by means of spectroscopic and chemical methods as 4′-*O*-β-D-glucopyranosyl-3′,5′-dimethoxybenzyl-caffeate. This previously unreported metabolite presented similar antioxidant capacity when compared to Trolox.

Keywords: *Baccharis*; *Baccharis articulata*; Antioxidant; Free radicals; Plant phenols; *n*-Butanol fraction

INTRODUCTION

Baccharis articulata, a member of Asteraceae, is a shrub native of South Brazil, Paraguay, Uruguay and Argentina. Its aerial parts has been used in folk medicine as diuretic and digestive.[1] Whereas previous studies have reported the identification of flavonoids and diterpenes from its chloroformic extract,[2–4] the chemical composition of other extracts of *B. articulata* has not been investigated yet.

Clinicians and biomedical scientists are interested in antioxidants because they could retard the oxidative damage of a tissue by increasing natural defenses. There is an increasing interest in the antioxidant effects of compounds derived from herbs which could be relevant in relation to their nutritional incidence and their role in health and disease.[5] Several disease of the gastrointestinal tract seems to be induced by oxidative stress. The role of oxygen-derived free radicals has been studied in acute gastric and esophageal mucosal injury caused by ischemia, anti-inflammatory drugs, or ethanol.[6] Administration of free radical scavengers has been found to prevent esophageal mucosal damages.[6] These suggest that the folk use of *B. articulata* could be associated with antioxidant properties. Despite the extensive history and popular acceptance of products containing *B. articulata*, however, no articles in the literature have discussed its antioxidant properties and their physiological effects in biological systems.

This paper reports the antioxidant activity of crude extracts, dichloromethane, ethyl acetate and *n*-butanol fractions obtained from *B. articulata* aerial parts through the evaluation of total antioxidant potential (TRAP), the prevention of formation of thiobarbituric acid reactive species (TBARS) induced by hydrogen peroxide and by the determination of the protection of Sertoli cells against hydrogen peroxide induce cell damage. The *n*-butanol fraction was found to be the most active and its major compound (**BaII**) was isolated, identified and assayed.

*Corresponding author. Tel.: +55-51-3316-5362. Fax: +55-51-3316-5313. E-mail: graceg@farmacia.ufrgs.br

ISSN 1071-5762 print/ISSN 1029-2470 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1071576031000076259

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

MATERIALS AND METHODS

Reagents

Thiobarbituric acid, hydrogen peroxide, luminol were purchased from Sigma, St. Louis, MO. 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) and Trolox were purchased from Aldrich Chemical Co., Milwaukee, WI.

Instrumentation

FAB-MS analysis was performed on a VG ZAB HS spectrometer. Ultraviolet absorbance measurements were obtained on a Shimadzu UV-2201 spectrophotometer. Infrared spectrum was recorded on a Shimadzu DR-800 spectrophotometer. Optical rotation was measured on a Perkin Elmer 341 polarimeter. NMR spectra were recorded on a Bruker Avance 500 spectrometer. Scintillation counting was performed on a Beckman instrument.

Plant Material

Aerial parts of *Baccharis articulata* (Lam.) Person were collected in Porto Alegre, State of Rio Grande do Sul, Brazil, in March 2000. A herbarium specimen is on deposit at the Herbarium of the Botany Department of Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (Voucher leg. Sobral 9057). Plant material was air-dried and powdered.

Extraction and Isolation

Evaluation of the antioxidant activity was performed on ethanol, dichloromethane and water extracts. Plant material (1.5 kg) was macerated in ethanol (plant–solvent, 1:10, w/v) (2 × 10 days). The crude ethanol extract was obtained after filtration and evaporation of the ethanol under vacuum. The aqueous extract was obtained by decoction of the plant material (1 g, 2 × 100 ml) during 30 min. The dichloromethane extract was obtained by refluxing the plant material (1 g, 2 × 100 ml) during 4 h. After preliminary assays, the aqueous extract was partitioned with dichloromethane, ethyl acetate and *n*-butanol yielding three the fractions (20, 3 and 178 mg, respectively) together with an aqueous residue (6 mg). Each extract or fraction was evaporated to dryness under reduced pressure.

For phytochemical analysis, the ethanol extract was prepared as mentioned above. The ethanol extract was suspended in water and extracted successively with dichloromethane, ethyl acetate and *n*-butanol. The *n*-butanol fraction (12 g) was column chromatographed on silica gel (Merck®, 70-230 mesh) using a gradient of ethyl acetate–ethanol as a solvent and yielding **BaII** (150 mg) that was further purified by precipitation with methanol.

BaII. 4′-*O*-β-D-glucopyranosyl-3′,5′-dimethoxybenzyl-caffeate. White amorphous powder. $[\alpha]_D = -14°$ (c = 0.4, pyridine, 26°C). UV λ_{max}: 330, 212 (MeOH); 376, 205 (MeOH/NaOH); 360, 261, 225, 207 ($AlCl_3$); 330, 206 ($AlCl_3$/HCl). IR in KBr (cm^{-1}): 3900, 3273, 1870, 1840, 1770, 1500, 1400, 1160. FAB MS (positive mode) m/z 531 $[M + Na]^+$. ^1H and ^{13}C NMR data: see Table I and Fig. 1.

Alkaline Hydrolysis of BaII

Thirty milligram were hydrolyzed using sodium hydroxide 2% at room temperature during 2 h. Then, the solvent was evaporated by reduced pressure and the residue, suspended in water. After neutralization, the solution was extracted with ethyl acetate yielding two compounds. One was identified as caffeic acid through thin-layer co-chromatography using a reference substance (caffeic acid from Aldrich®, Si gel, dichloromethane–ethanol, 80:20, v/v), the other (**BaII-H**, 5 mg) was isolated through column chromatography using Lichroprep® RP-18 (Merck®, 40–63 μm) and water–methanol as eluent.

Ball-H. 4-*O*-β-D-glucopyranosyl-3,5-dimethoxybenzylalcohol (5 mg). ^1H NMR (500 MHz, methanol-d_4) δ: 3.00 (m, 1H, glc-5), 3.20 (m, 2H, glc-3, glc-4), 3.25 (m, 1H, glc-2), 3.45 (m, 1H, glc-H6b); 3.55 (dd, 1H, $J = 2.5$; 12.0 Hz, glc-H6a), 3.70 (s, 6H, 2 × CH_3–O–), 4.35 (s, 2H, CH_2–O–), 4.65 (1H, H-1, under HDO signal), 6.55 (s, $2H_{arom}$). ^{13}C NMR (500 MHz, methanol-d_4) δ: 57.0 (2 × CH_3O–), 62.2 (glc-6), 64.4 (CH_2O–), 71.1 (glc-4), 75.5 (glc-2), 77.8 (glc-3), 78.5 (glc-5), 104.4 (glc-1), 105.2 (2 × CH_{arom}), 152.0 (2 × Cq).

Antioxidant Activity

The *in vitro* antioxidant potential of *B. articulata* extracts were estimated by the total radical-trapping antioxidant parameter (TRAP). The principle of TRAP measurement has been described previously.[7] Briefly, the reaction was initiated by injecting luminol and AAPH in glycine buffer that resulted in steady luminescence emission. The addition of different concentration of plant extracts, **BaII** (0.1, 0.2 and 2.0 mM), and Trolox (0.1, 0.2 and 2.0 mM, as the standard antioxidant) decreases the luminescence proportionally to its antioxidant potential. The luminescence emission was followed for 70 min after the addition of plant extracts or Trolox. Results are expressed as percentage of solvent control.

The *ex vivo* antioxidant potential of *B. articulata* was estimated by the prevention of formation of TBARS, and by the determination of cell viability in

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

TABLE I ^{1}H and ^{13}C NMR assignments (125–500 MHz) and their correlations of **BaII** in pyridine-d_5

Carbon	DEPT	^{13}C (ppm)	^{1}H (ppm)	^{1}H^{1}H-COSY (ppm)	HMBC (ppm)
1	Cq	127.0	–	–	–
2	CH	116.6	7.70 s (1H)	H7.20	C148.0; C151.0; C123.0; C147.0
3	Cq	148.0	–	–	–
4	Cq	151.0	–	–	–
5/6	CH (2X)	117.0 123.0	7.20 m (2H)	H7.70	C127.0; C117.0; C116.6; C148.0; C151.0; C147.0
7	CH=	147.0	8.20 d (1H) $J = 15.8$ Hz	H6.70	C123.0; C116.6; C168.0; C127.0
8	CH=	115.4	6.70 d (1H) $J = 15.8$ Hz	H8.20	C127.0; C168.0
9	CO	168.0	–	–	–
1′	Cq	133.0	–	–	–
2′/6′	CH (2X)	108.0	6.90 brs (2H)	H 5.30; H3.80	–
3′/5′	Cq (2X)	154.0			
4′	Cq	136.0	–	–	–
7′	H_2C–O	67.0	5.30 brs (2H)	H6.90	C168.0; C133.0; C108.0
8′/9′	H_3C–O– (2X)	57.3	3.80 s (6H)	H6.90	C154.0
Glc-1″	CH	105.6	5.80 d (1H) $J = 7.0$ Hz	H4.38	C79.1
Glc-2″	HC–O	76.8	4.38 m (4H)	H3.90; H4.41; H5.80	C105.6; C79.4; C79.1; C76.8; C72.3; C63.4
Glc-3″	HC–O	79.4	4.38 m (4H)	H3.90; H4.41; H5.80	C105.6; C79.4; C79.1; C76.8; C72.3; C63.4
Glc-4″	HC–O	72.4	4.38 m (4H)	H3.90; H4.41; H5.80	C105.6; C79.4; C79.1; C76.8; C72.3; C63.4
Glc-5″	HC–O	79.1	3.90 m (1H)	H4.38; H4.41	
Glc-6″	H_2C–O	63.4	4.38 m (4H) 4.41 m (1H)	H4.38; H3.90	

cultured rat Sertoli cells exposed to hydrogen peroxide as previously described.[8] Briefly, Sertoli cells were isolated and cultured for 48 h. After this cells were treated with crude ethanol, and aqueous extracts (1.0 and 10 mg/ml), ethyl acetate fraction, *n*-butanol fraction of aqueous extracts and aqueous residue (1 mg/ml), **BaII** (0.2 and 2.0 mM) and Trolox (0.2 and 2.0 mM) for 24 h. Control cells received the same volume of solvent. After treatment, the medium was removed, cells were washed with phosphate-buffered saline and exposed to hydrogen peroxide (400 μm) for 15 min. Cells were then washed with phosphate-buffered saline, scraped and sonicated. TBARS production was estimated by absorbance at 532 nm, and expressed as malondialdehyde (MDA) equivalents (nm/mg protein).[8] Cell viability was determined by the Trypan blue exclusion test.[9] Briefly, cells were treated with plant extracts and H_2O_2 as described above. After this cells were incubated at 34°C for 10 min with trypan blue 0.4% and the number of trypan blue permeable cells and no-stained cells were counted in six randomly chosen fields. By counting the cells in the field and calculating the ratio blue/white, we can express the viability of the culture as the percentage of viable cells.

Results are expressed as means and p values were considered significant when $p < 0.05$. Differences in experimental groups were determined by ANOVA. Comparison between means was carried out using a Newman–Keuls test.

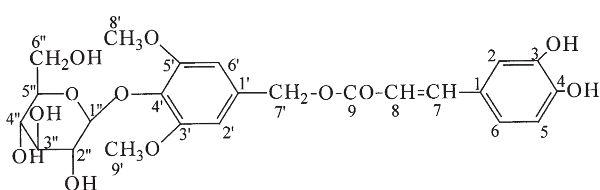

FIGURE 1 Compound **BaII** (4′-*O*-β-D-glucopyranosyl-3′,5′-dimethoxybenzyl-caffeate). Its structural elucidation was established on the basis of spectrometric methods (mass, UV, NMR) and alkaline hydrolysis of the *n*-butanol fraction from aerial parts of *B. articulata*.

RESULTS AND DISCUSSION

In this work, *B. articulata* extracts were studied for their activity as inhibitors of lipid peroxidation and scavengers of free radicals *in vitro*, by using different systems. The TRAP, the TBARS and the cell viability were evaluated in different extracts and fractions from aerial parts of *B. articulata*.

In evaluating TRAP levels, crude ethanol and aqueous extracts exhibited a dose-dependent antioxidant activity in concentrations of 0.1, 1.0 and 10 mg/ml (data not shown). Considering that this plant is prepared by infusion or decoction to be used as tea, it was decided to search for the active(s) compound(s) through partition of the crude aqueous extracts using different organic solvents.

The *n*-butanol fraction and the aqueous residue, in concentrations of 1 mg/ml exhibited a similar antioxidant activity that was higher than that of the ethyl acetate fraction, in relation to TRAP values (data not shown). Crude extracts (ethanol and aqueous) and fractions (*n*-butanol fraction and the aqueous residue) were capable of inhibiting TBARS

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

formation and cell mortality induced by hydrogen peroxide (data not shown). None of these extracts include a decreased in cell viability assessed by trypan blue test (data not shown). The crude dichloromethane extract and dichloromethane fraction did not exhibit significant antioxidant activity (data not shown).

The *n*-butanol fraction from aerial parts of *B. articulata* yielded **BaII** (Fig. 1) as the main component (2.5% of total constituents). Its structural elucidation was established on the basis of spectrometric methods (mass, UV, NMR) and alkaline hydrolysis. FAB MS (positive ion mode) of compound **BaII** displayed a quasi-molecular ion peak at m/z 531 $[M + Na]^+$. This information together with the presence of 24 signals in ^{13}C NMR spectrum of **BaII** suggested the molecular formula $C_{24}H_{28}O_{12}$. The UV spectrum of **BaII** in methanol (λ_{max} 212 and 310 nm) and bathochromic effects observed after addition of aluminum chloride and aluminum chloride/hydrochloric acid provided preliminary evidence for an ortho diphenol.[10] The DEPT spectrum revealed two methyl, two methylene, twelve methane and eight quaternary carbon atoms (Table I). The chemical shift of the two methylene groups (δ 67.0 and 63.4) evidenced that they were substituted by an oxygen atom.

^{1}H NMR spectrum exhibited an AM system (δ 6.70, 1H and 8.20, 1H, $J = 15.8$ Hz) attributed to *trans* olefinic protons. Together with the UV data, this observation suggested the presence of a caffeic acid derivative.[11–13] In addition, one singlet (6H) at δ 3.80 indicated the presence of two methoxy groups. Two broad singlets at δ 6.90 (2H) and δ 7.70 (1H) and one multiplet at δ 7.20 (2H) also suggested the presence of two aromatic nuclei.[14] Analysis of the remaining signals suggested the presence of a sugar residue whose anomeric proton would appear at δ 5.80 (d, $J = 7.5$ Hz, 1H) and whose H2″–H6″ NMR pattern was typical of β-D-glucose.[14–16] The ^{13}C NMR spectrum confirmed the presence of one carbonyl function (δ 168.0), one aliphatic double bond (δ 147.0 and 115.0), two methoxy groups (δ 57.3), three aromatic methine carbons (δ 123.0, 117.0 and 116.6) attributed to the catechol nucleus and, two additional aromatic methine carbons (δ 108) attributed to a symmetric nucleus substituted with two methoxy groups.[14]

The final structural assignment was established by heteronuclear 2D NMR experiments which showed long range correlations between the methoxy hydrogens (δ 3.80) and quaternary carbon of the tetrasubstituted aromatic nucleus (δ 154.0). Long range correlations between *trans* olefinic protons at δ 6.70 and 8.20 with the C-1 at δ 127.0 of the catechol nucleus were also observed as well as a correlation between H-2′/H-6′ and the methylene atom of the benzyl alcohol moiety establishing the location of the methoxy groups on positions 3 and 5.

Nevertheless, the structural assignment was further confirmed by alkaline hydrolysis of **BaII** that led to caffeic acid identified by thin layer chromatography and to **BaII-H** whose structure was established by NMR spectroscopy (^{1}H, ^{13}C NMR, HMQC and HMBC) to be 4-*O*-β-D-glucopyranosyl-3,5-dimethoxy-benzylalcohol. Therefore, **BaII** was identified as 4′-*O*-β-D-glucopyranosyl-3′,5′-dimethoxybenzyl-caffeate, a novel compound.

BaII was tested in concentration of 0.1, 0.2 and 2.0 mM (Fig. 2) for TRAP and exhibited an antioxidant activity in a dose-dependent profile and similar to the equal molar concentration of Trolox. In addition, **BaII** at 0.2 and 2.0 mM diminished the formation of TBARS and cell mortality induced by H_2O_2 (Fig. 3). This protection against H_2O_2 induced oxidative stress was higher than equal concentrations of Trolox (Fig. 3). **BaII** did not induce a decrease in cell viability assessed by trypan blue test (Fig. 3).

Herbal extracts have been used for centuries around the world to treat different diseases and there is a long history of the use of plants in many cultures. The beneficial effects of antioxidant nutrients are supported by many studies, and epidemiological evidence suggests an association between diets rich in vegetables, fruits and red wine and a decreased risk of degenerative disease.[17] The supplementation of the natural antioxidant defense system of the body through a balanced diet containing fruits, vegetables or natural beverages derived from them, could protect the body against various oxidative stresses. Nowadays, *Baccharis* species continue to play an important role in the traditional medicine of many modern cultures, being used for treatment of gastrointestinal disorders and hepatic alterations. The established use of *B. articulata* preparations in

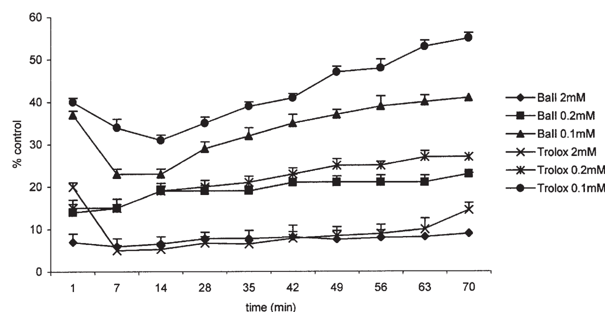

FIGURE 2 TRAP of compound **BaII**. **BaII** isolated from the *n*-butanol fraction of *B. articulata* was evaluated for its total radical-trapping potential in different doses (0.1, 0.2 and 2 mM). **BaII** and Trolox (0.1, 0.2 and 2 mM) were added to a solution containing AAPH and luminol and the luminescence was followed by 70 min as described under "Material and Methods" section. The decrease in the luminescence is proportional to the antioxidant potential. Values are expressed as means ± S.D. ($n = 4$ each group). Values are expressed as percentage of control (extract solvent) = 100%.

RIGHTSLINK

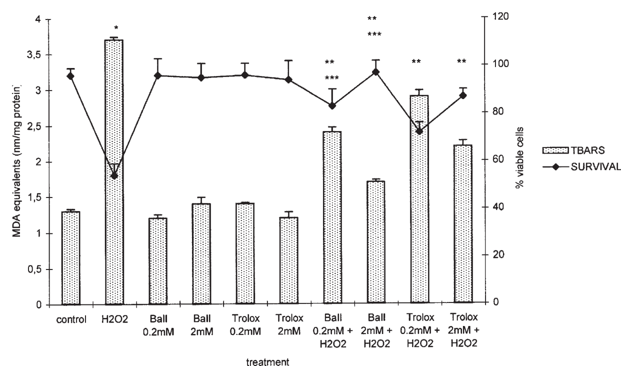

FIGURE 3 **BaII** prevention of the formation of thiobarbituric acid reactive species (TBARS) and cell damage induced by hydrogen peroxide. **BaII** isolated from the *n*-butanol fraction of *B. articulata* were evaluated for the prevention of hydrogen peroxide induced TBARS formation and cell damage. Cultured Sertoli cells were treated with **BaII** (0.2 and 2.0 mM) or Trolox (0.2 and 2.0 mM) for 24 h. After this period cells were exposed to hydrogen peroxide (400 μM) by 15 min as described under "Material and Methods" section. The formation of TBARS and the determination of cell viability were evaluated as described under "Material and Methods" section. Values are expressed as means ± S.D. ($n = 3$ each group). *Different from control, $p < 0.05$; **different from hydrogen peroxide group; $p < 0.05$; **different from Trolox group; $p < 0.05$.

traditional medicine for treatment of a variety of disorders might be attributed, to some degree, to the antioxidant activity of compounds such as **BaII**. Further investigations of purified *B. articulata* components, therefore, are needed to discover information about the biological basis for the efficacy of *B. articulata* preparations.

Acknowledgements

We are grateful to Marcos Sobral from Universidade Federal do Rio Grande do Sul, Brazil, for collecting and identifying the plant material. This work was supported by fellowships to S.Q. de Oliveira from CAPES (Brazil) and to G. Gosmann, F. Dal-Pizzol, J.C.F. Moreira and E.P. Schenkel from CNPq (Brazil).

References

[1] Zardini, E.M. (1984) "Etnobotánica de Compuestas Argentinas com especial referencia a su uso farmacológico (primera parte)", *Acta Farm. Bonaer.* **3**, 77–99.

[2] Stapel, G., MenßEn, H.G. and Snatzke, G. (1980) "Isolierung und Strukturaufklärung von Zwei Diterpenen aus *Baccharis articulata*", *Planta Med.* **39**, 366–374.

[3] Dai, J., Suttisri, R., Bordas, E., Soejarto, D.D. and Kinghorn, A.D. (1993) "Clerodane diterpenoids from *Baccharis articulata*", *Phytochemistry* **34**, 1087–1090.

[4] Gianello, J.C. and Giordano, O.S. (1984) "Examen quimico en seis especies del genero *Baccharis*", *Rev. Latinoam. Quim.* **15**, 84–86.

[5] Sarkar, A. and Bhaduri, A. (2001) "Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species: comparison with its individual catechin constituents and green tea", *Biochem. Biophys. Res. Commun.* **284**, 173–178.

[6] Oh, T.Y., Lee, J.S., Ahn, B.O., Cho, H., Kim, W.B., Surch, Y.J., Cho, S.W. and Hahm, K.B. (2001) "Oxidative damages are critical in pathogenesis of reflux esophagitis: implication of antioxidants in its treatment", *Free Radic. Biol. Med.* **30**, 905–915.

[7] Lissi, E., Salim-Hanna, M., Pascual, C. and Del Castillo, M.D. (1995) "Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements", *Free Radic. Biol. Med.* **18**, 153–158.

[8] Dal-Pizzol, F., Klamt, F., Benfato, M.S., Bernard, E.A. and Moreira, J.C.F. (2001) "Retinol supplementation induces oxidative stress and modulates antioxidant enzyme activities in rat Sertoli cells", *Free Radic. Res.* **34**, 395–404.

[9] Dal-Pizzol, F., Klamt, F., Dalmolin, R.J.S., Bernard, E.A. and Moreira, J.C.F. (2001) "Mitogenic signaling mediated by oxidants in retinol treated Sertoli cells", *Free Radic. Res.* **35**, 749–755.

[10] Mabry, T.J., Markham, K.R. and Thomas, M.B. (1970) *The Systematic Identification of Flavonoids* (Springer, New York).

[11] Ravn, H. and Brimer, L. (1988) "Structure and antibacterial activity of plantamajoside, a caffeic acid sugar ester from *Plantago major subsp. Major*", *Phytochemistry* **27**, 3433–3437.

[12] Merfort, I. (1992) "Caffeoylquinic acids from flowers of *Arnica montana* and *Arnica chamissonis*", *Phytochemistry* **31**, 2111–2113.

[13] Kamiyama, T., Watanabe, N., Sakata, K., Yagi, A. and Ina, K. (1993) "4(*S*)-(6-*O*-caffeoyl-β-D-glucopyranosyl)-2-pentanone from young leaves of *Photinia glabra*", *Phytochemistry* **32**, 353–356.

[14] Kijima, H., Ide, T., Otsuka, H., Ogimi, C., Hirata, E., Takushi, A. and Takeda, Y. (1997) "Water-soluble phenolic glycosides from leaves of *Alangium premnifolium*", *Phytochemistry* **44**, 1551–1557.

[15] Shoyama, Y., Hatano, K., Nishioka, I. and Yamagishi, T. (1986) "Phenolic glycosides from *Lilium longiflorum*", *Phytochemistry* **26**, 2965–2968.

[16] Ekabo, O.A., Farnsworth, N.R., Santisuk, T. and Reutrakul, V. (1993) "Phenolic, iridoid and ionyl glycosides from *Homalium ceylanicum*", *Phytochemistry* **32**, 747–754.

[17] Schinella, G.R., Troiani, G., Dávila, V., de Buschiazzo, P.M. and Tournier, V.H. (2000) "Antioxidant effects of an aqueous extract of *Ilex paraguariensis*", *Biochem. Biophys. Res. Commun.* **269**, 357–360.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/26/11
For personal use only.

RIGHTSLINK