JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Capacity and Cytotoxicity of Essential Oil and Methanol Extract of *Aniba canelilla* (H.B.K.) Mez

JOYCE KELLY R. DA SILVA,[†] PERGENTINO JOSÉ C. SOUSA,[§] ELOÍSA HELENA A. ANDRADE,[#] AND JOSÉ GUILHERME S. MAIA*[,#]

Departamento de Química, Departamento de Farmácia, and Departamento de Engenharia Química e de Alimentos, Universidade Federal do Pará, 66075-900 Belém, PA, Brazil

The leaves and fine stems, bark, and trunk wood oils of *Aniba canelilla* showed yields ranging from 0.2 to 1.3%. The main volatile constituent identified in the oils was 1-nitro-2-phenylethane (70.2–92.1%), as expected. The mean of DPPH radical scavenging activity (EC_{50}) of the oils (198.17 ± 1.95 μg mL^{-1}) was low in comparison with that of wood methanol extracts (4.41 ± 0.12 μg mL^{-1}), the value of which was equivalent to that of Trolox (4.67 ± 0.35 μg mL^{-1}), used as antioxidant standard. The mean amount of total phenolics (TP) (710.53 ± 23.16 mg of GAE/g) and this value calculated as Trolox equivalent antioxidant capacity (TEAC) (899.50 ± 6.50 mg of TE/g) of the wood methanol extracts confirmed the high antioxidant activity of the species. On the other hand, in the brine shrimp bioassay the values of lethal concentration (LC_{50}) for the oils (21.61 ± 1.21 μg mL^{-1}) and 1-nitro-2-phenylethane (20.37 ± 0.99 μg mL^{-1}) were lower than that of the wood methanol extracts (91.38 ± 7.20 μg mL^{-1}), showing significant biological activities.

KEYWORDS: ***Aniba canelilla*** **(H.B.K.) Mez; essential oil composition; 1-nitro-2-phenylethane; antioxidant capacity; citotoxicity**

INTRODUCTION

Aniba canelilla (H.B.K.) Mez [syn. *Aniba elliptica* A. C. Sm., *Cryptocarya canelilla* Kunth], known as "casca-preciosa" (precious bark), is an important and historical species in the Amazon region. It was confused with cinnamon trees (*Cinnamomum zeylanicum* Blume) during the 1540 voyage of Pizzaro and Orellana from the Andes to the Amazon estuary and during Humbolt and Bonpland's 1800 expedition in the Orinoco River to find the "famous Amazon cinnamon".

The trunk wood, fine stems, and leaves of casca-preciosa are used as spices and ingredients for local dishes, fragrances, and cloth aromatization sachets. The bark decoction is commonly used in folk medicine for its antispasmodic, digestive, stimulating, and carminative properties (*1*). We observed that the *A. canelilla* bark oil exerts relaxant effects on intestinal smooth muscle, justifying the plant usage for gastrointestinal disorders (*2*). Recently, cardiovascular effects of the plant bark oil have been reported in normotensive rats, causing hypotension and bradycardia (*3*).

The odoriferous principle of leaf, bark, and trunk wood of *A. canelilla* is 1-nitro-2-phenylethane (*4*), also responsible for the plant cinnamon scent. In the first chemical analysis of *A. canelilla* oil (*4*) the compounds methyleugenol and eugenol were also detected. 1-nitro-2-phenylethane was also reported in the essential oil of *Ocotea pretiosa* (*5*). The presence of the benzyltetrahydroisoquinoline and tetrahydroprotoberberine alkaloids and their fungistatic properties as well as the lethal doses (LD_{50}) of the stem bark oil of *A. canelilla* have been reported (*6*, *7*). We recently reported the seasonal essential oil variation in some specimens of *A. canelilla* occurring southeastern Pará state, Brazil (*8*).

The essential oils have been proposed as natural antioxidants for food preservation. On the other hand, the biologically active natural compounds are of pharmaceutical interest for the control of human illness and the prevention of lipid peroxidative damage implicated in several pathological disorders, such as atherosclerosis, Alzheimer's disease, carcinogenesis, and aging processes (*9*–*12*).

Now we report the results obtained for the essential oil composition of two other *A. canelilla* specimens as well as the antioxidant capacity and the biological activity of the essential oils and wood methanol extracts using the DPPH radical scavenging and the brine shrimp bioassay, respectively.

MATERIALS AND METHODS

Plant Material. Specimen A of *A. canelilla* was collected in the area of Jatapu River, municipality of Novo Airão, Amazonas state, Brazil, in May 2004 and specimen B in the area of Cauaxi River, municipality of Ulinópolis, Pará state, Brazil, in May 2005, both during

* Corresponding author (telephone 55 91 32017836; fax 55 91 32353043; e-mail gmaia@ufpa.br).
[†] Departamento de Química.
[§] Departamento de Farmácia.
[#] Departamento de Engenharia Química e de Alimentos.

10.1021/jf071928e CCC: $37.00
Published on Web 10/17/2007

Table 1. Composition of Volatiles Identified in the Oils of *A. canelilla*

no.	component	RI[a]	LSOA (%)[b]	LSOB (%)[b]	TWOA (%)[b]	TWOB (%)[b]	BWOB (%)[b]
1	α-pinene	937	0.4	0.2			
2	benzaldehyde	961		1.1	0.4		
3	β-pinene	978	0.3	0.2			
4	myrcene	989	0.1	0.9			0.1
5	α-phellandrene	1003		0.1			
6	*p*-cymene	1026		0.3			
7	limonene	1029		1.2			
8	β-phellandrene	1030	0.6				
9	δ-3-carene	1031		0.2			
10	(*Z*)-β-ocimene	1037		0.2			
11	phenylacetaldeyde	1040	0.3				
12	(*E*)-β-ocimene	1050		0.7	0.5	0.5	1.2
13	linalool	1097	0.8	7.6			
14	*trans*-*p*-menth-2-en-1-ol	1141		0.4	0.6	0.3	0.3
15	α-terpineol	1189		0.2			
16	safrole	1286				0.5	
17	**1-nitro-2-phenylethane**	**1327**	**91.8**	**74.0**	**92.1**	**70.2**	**90.3**
18	eugenol	1359		0.9	1.2	0.8	1.7
19	α-copaene	1377	0.4	1.3			
20	β-elemene	1390	0.1				
21	**methyleugenol**	**1403**			**4.3**	**25.8**	**2.0**
22	longifolene	1405	0.4				
23	α-gurjunene	1410		0.3			
24	β-caryophyllene	1418	1.6	3.5			
25	aromadendrene	1441		0.1			
26	α-humulene	1454	0.3	0.4			
27	β-selinene	1488	0.1	0.8			
28	α-selinene	1497	0.1				
29	β-bisabolene	1505	0.3				
30	δ-cadinene	1522	0.1				
31	β-sesquiphellandrene	1523		0.6			
32	spathulenol	1578	0.1	0.3			
33	caryophyllene oxide	1583	0.3	1.6			
34	guaiol	1600	0.1				
35	humulene epoxide II	1607	0.1				
36	1-epi-cubenol	1627		0.3			
37	epi-α-muurulol	1642		0.2			
38	selin-11-en-4-α-ol	1660	1.3	1.7	0.4	1.2	3.5
39	bulnesol	1670	0.1				
total			**99.7**	**99.3**	**99.5**	**99.3**	**99.0**

[a] Retention indices calculated for all volatile constituents using a homologous series of *n*-alkanes. [b] Percentages are the mean of three runs and were obtained from FID electronic integration.

the rainy season. The specimens were identified by comparison with an authentic voucher (MG 174904) of *A. canelilla* that is deposited in the herbarium of Emílio Goeldi Museum, city of Belém, Pará state, Brazil.

Plant Processing. The leaf and fine stems and trunk wood (samples A and B) and the bark wood (sample B) were air-dried separately, ground, and submitted to hydrodistillation (100 g, 4 h) using a Clevenger-type apparatus. The powdered trunk wood was also submitted to solvent extraction (75 g, 4 h) using a Sohxlet extractor and methanol. The oils were dried over anhydrous sodium sulfate, and their percentage content was calculated on the basis of the plant dry weight. The moisture content of samples was calculated after the phase separation in a Dean–Stark trap (5 g, 30 min) using toluene. The methanol extracts were submitted to vacuum evaporation to eliminate the solvent and their yields calculated. The oils were codified as LSOA and LSOB (leaf and fine stems of samples A and B, respectively), TWOA and TWOB (trunk wood of samples A and B, respectively), and BWOB (bark wood of sample B). The methanol extracts were codified as TWEA and TWEB (trunk wood of samples A and B, respectively).

Oil Fractionation. The essential oil of trunk wood of sample A (TWOA, 15 g) was submitted to fractionation in a silica gel chromatographic column using petroleum ether (isocratic elution) and thin-layer chromatography to purify 1-nitro-2-phenylethane, the main constituent of TWOA and TWOB. The percentage contents of 1-nitro-2-phenylethane in the oil and in the purified fractions were obtained by GC-FID.

Oil Composition Analysis. Qualitative analysis of the volatile compounds was performed on a Finnigan Mat INCOS XL GC-MS instrument, with the following conditions: WCOT DB-5 ms (30 m × 0.25 mm; 0.25 μm film thickness) fused silica capillary column; temperature programmed from 60 to 240 °C (3 °C/min); injector temperature, 220 °C; carrier gas, helium, adjusted to a linear velocity of 32 cm/s (measured at 100 °C); injection types, (1) SPME device coupled directly to GC injector and (2) splitless (2 μL of a 1:1000 hexane solution); split flow was adjusted to give a 20:1 ratio; septum sweep was a constant 10 mL/min; EIMS, electron energy, 70 eV; ion source temperature and connection parts, 180 °C. The quantitative data of oils and SPME concentrates were obtained by peak area normalization using a HP 5890 GC/FID operated under the same GC-MS conditions, except for the carrier gas that was hydrogen produced by a Packard hydrogen generator and a WCOT CP-Sil CB (25 m × 0.25 mm; 0.25 μm film thickness) fused silica capillary column.

Individual components of oils were identified by comparison of both mass spectrum and their GC retention data with those of authentic compounds previously analyzed and stored in the data system. Other identifications were made by comparison of mass spectra with those existing in the data system libraries and cited in the literature (*13*). The retention index was calculated for all volatile constituents using an *n*-alkane homologous series.

NMR Spectral Data of 1-Nitro-2-phenylethane. The ^{1}H NMR spectrum was obtained in a Varian Mercury NMR at 300 MHz using $CDCl_3$ as solvent.

Antioxidant Capacity Evaluation. A stock solution of DPPH radical (0.5 mM) in methanol was prepared. The solution was diluted in methanol (60 μM, approximately) by measuring an initial absorbance of 0.62 ± 0.02 in 517 nm and room temperature. The reaction mixture was composed by 1950 μL of DPPH solution and 50 μL of the samples diluted in different methanol portions. For each sample a methanol blank was also measured. The absorbance was measured in the reaction starting (time zero), each 5 min during the first 20 min and then at continuous intervals of 10 min up to constant absorbance. All experiments were triplicate. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard antioxidant. The radical scavenging activity of each sample was calculated by the DPPH inhibition percentage according to the equation $IP_{DPPH} = 100(A - B)/A$ (where *A* and *B* are the blank and sample absorbance values in the end reaction). The radical scavenging activity, expressed as milligrams of Trolox equivalent per gram of each sample, was also calculated by means of the equation $TE = (A - B)/(A - C) \times 25/1000 \times 250.29/1000 \times 1000/10 \times D$ (where *A*, *B*, and *C* are the blank, sample, and Trolox absorbance values in the end reaction and *D* is the dilution factor) (*14–16*). The concentration of antioxidant required for 50% scavenging of DPPH radicals (EC_{50}) was determined by linear regression using Windows/Excel.

Total Phenolics (TP) Evaluation. The amount of TP of wood extracts was determined according to the Folin–Ciocalteu procedure (*17, 18*). The experimental calibration curve was prepared using 500 μL of aqueous solution of gallic acid mixed with 250 μL of Folin–Ciocalteu reagent (1.0 N) and 1250 μL of sodium carbonate (75 g/L), resulting in final gallic acid concentrations of 0.57, 1.14, 2.28, 3.42, 4.56, 5.70, and 6.84 mg L^{-1}. The absorbance was measured after 30 min at 760 nm and 25 °C (UV–vis spectrophotometer, ULTROSPEC 2000). The extracts were dissolved in methanol (2 mg mL^{-1}), diluted in water (1:99), and submitted to the same procedure. The TP content was expressed as gallic acid equivalents (GAE) in milligrams per gram of extract, using the equation $GAE\ (mg/L) = A \times D \times 7.93 \times d$ (where A = sample absorbance, D = sample dilution, 7.93 = angular coefficient, and d = reaction dilution).

Brine Shrimp Bioassay. A brine shrimp lethality bioassay was carried out to investigate the cytotoxicity (biological activity) of the oils and extracts. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a glass rectangular vessel (5 L), filled with sterile artificial seawater, prepared using water (2 L), NaCl (46 g), $MgCl_2 \cdot 6H_2O$ (22 g), Na_2SO_4(8 g), $CaCl_2 \cdot 2H_2O$ (2.6 g), and KCl (1.4 g), with a pH of 9.0 adjusted with Na_2CO_3, under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from the brighter portion of the hatching chamber and used for the

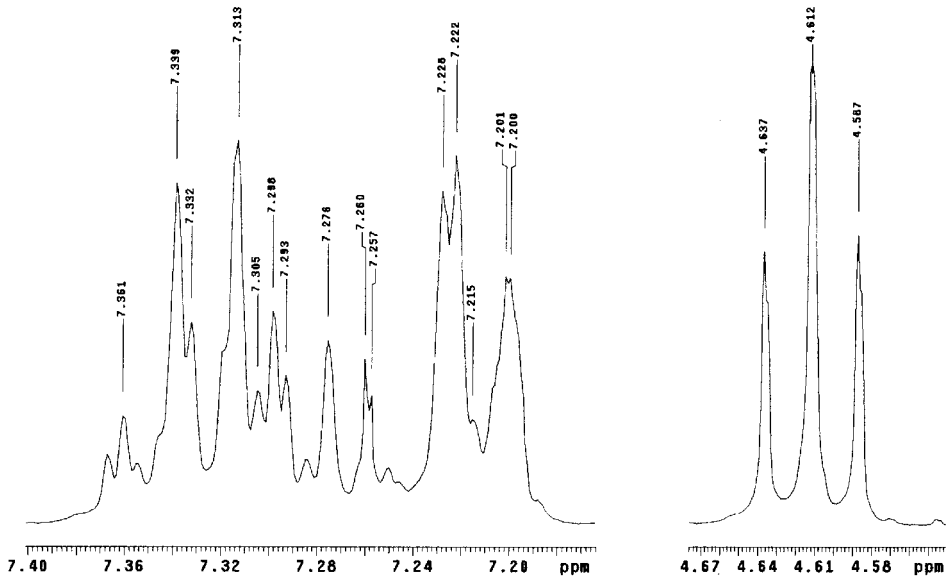

Figure 1. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of 1-nitro-2-phenylethane.

Table 2. Antioxidant Capacity Data of the Trunk Wood Oils and Extracts of *A. canelilla*

sample	concn (μg mL^{-1})	DPPH inhibition[a] (%)	DPPH EC_{50}[a] (μg mL^{-1})	TEAC[a] (mg of TE/g)	TP[a] (mg of GAE/g)
TWOA	1400	93.06 ± 0.54	223.81 ± 1.88	17.45 ± 0.25	
	1125	84.12 ± 0.02			
	445	76.88 ± 1.12			
	225	48.29 ± 0.79			
	110	32.38 ± 1.08			
TWOB	1400	93.31 ± 0.81	172.52 ± 2.02	16.95 ± 0.07	
	1125	83.60 ± 0.32			
	445	79.13 ± 0.20			
	225	59.47 ± 1.03			
	110	34.64 ± 0.80			
TWEA	10	92.64 ± 0.44	4.37 ± 0.14	893.99 ± 4.30	717.41 ± 24.78
	4	49.69 ± 2.44			
	2	29.81 ± 1.35			
TWEB	10	93.78 ± 0.64	4.44 ± 0.10	905.01 ± 8.69	703.66 ± 21.55
	4	52.49 ± 2.01			
	2	25.47 ± 0.51			
1-nitro-2-phenylethane	1000	63.19 ± 2.32	792.50 ± 9.97	5.88 ± 0.07	
	500	30.54 ± 1.75			
	200	11.47 ± 0.96			
Trolox	10	96.72 ± 0.52	4.67 ± 0.35		
	4	53.17 ± 1.63			
	2	21.46 ± 2.25			

[a] Mean ± standard deviation.

assay. Ten nauplii were drawn through a glass capillary and placed in vials containing 5 mL of brine solution. In each experiment the oil (1%) and extract (5%) solutions were prepared using the brine solution and DMSO. From them were obtained four other solutions at different concentrations, which were then added to the 5 mL brine solution. The vials were maintained at room temperature for 24 h under light, and the surviving larvae were counted. Experiments were conducted along with control and different concentrations (1, 10, 100, and 1000 μg mL^{-1}) in a set of three tubes per dose. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. Lethal concentration (LC_{50}) values were obtained from the best-fit line plotting concentration versus percentage lethality (*19*–*21*).

RESULTS AND DISCUSSION

The following oil yields of leaf and fine stems and bark and trunk woods of samples A and B of *A. canelilla* were obtained: LSOA, 1.3%; TWOA, 0.2%; LSOB, 1.2%; TWOB, 0.2%; BWOB, 0.5%; respectively. These values are in agreement with that previously reported by us (*8*). The oil chemical composition of the analyzed samples (A and B) of *A. canelilla* is similar to that of other specimens previously studied, and the main component identified was 1-nitro-2-phenylethane (*4*, *7*, *8*), as expected. In the leaf and fine stems oils of samples A (LSOA) and B (LSOB) this compound ranged from 91.8 to 74.0%. In the trunk wood oils of samples A (TWOA) and B (TWOB) was observed a variation from 92.8 to 70.2%. In our previous work it was seen that the percentage content of 1-nitro-2-phenylethane is larger during the rainy season (*8*). The volatiles identified in leaf and fine stems and trunk and bark woods oils are listed in **Table 1**.

1-Nitro-2-phenylethane was isolated from the trunk wood oil by silica column chromatography, reaching 99% purity. The hydrogen NMR spectrum furnished the signals δ 7.27 (m, 5H, monosubstituted aromatic ring), δ 4.61 (t, 2H, $J = 7.5$ Hz, α-position to nitro group), and δ 3.33 (t, 2H, $J = 7.5$ Hz, α-position to aromatic ring) as can seen in **Figure 1**. This agrees with the information previously described (*7*).

The wood oils of *A. canelilla* (TWOA and TWOB) were assayed at 110, 225, 445, 1125, and 1400 μg mL^{-1} concentrations to determine the DPPH scavenging activity. The kinetic reaction was slow in all concentrations with a mean of 113 min for the mentioned wood oils. The resulting DPPH inhibition percent varied from 32.38 to 93.06% in trunk wood oil of sample A (TOWA) and from 34.64 to 93.31% in trunk wood oil of sample B (TOWB). 1-Nitro-2-phenylethane was evaluated against the DPPH radical for concentrations at 200, 500, and 1000 μg mL^{-1}, and the inhibition percent varied from 11.47 to 63.19%. The wood extracts of *A. canelilla* (TWEA and TWEB) were assayed at 2, 4, and 10 μg mL^{-1} to determine the DPPH scavenging activity. The kinetic reaction was slow in all tested concentrations with a mean of 85 min for the mentioned wood extracts. The resulting DPPH inhibition percent varied from 29.81 to 92.64% in the wood extract of sample A (TWEA) and from 25.47 to 93.78% in the wood extract of sample B (TWEB).

The EC_{50} values obtained for the *A. canelilla* wood extracts (TWEA, 4.37 ± 0.14 μg mL^{-1}; TWEB, 4.44 ± 0.10 μg mL^{-1}), defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals, were equivalent to that of Trolox (4.67 ± 0.35 μg mL^{-1}), signifying a high antioxidant activity for the species.

The amounts of total phenolics (TWEA, 717.41 ± 24.78 mg of GAE/g; TWEB, 703.66 ± 21.55 mg of GAE/g) and the TEAC equivalent (TWEA, 893.99 ± 4.30 mg of TE/g; TWEB, 905.01 ± 8.69 mg of TE/g) for the methanol wood extracts were also very significant. This noteworthy antioxidant capacity could be attributed to the presence of benzyltetrahydroisoquinoline and tetrahydroprotoberberine alkaloids previously reported for the bark wood of *A. canelilla* (*6*). The antiradical and antioxidant activities of protoberberine and aporphine alkaloids have been described (*22*–*24*). We suppose that the N–H groups and the phenolic hydroxyls existing in these alkaloid structures have contributed to the extracts' antioxidant efficiency, as previously observed for phenolic compounds cited in the literature.

The data of DPPH inhibition, DPPH radical activity (EC_{50}), Trolox equivalent antioxidant capacity (TEAC), and total phenolics (TP) for the trunk wood oils and methanol extracts of samples (A and B) of *A. canelilla* are shown in **Table 2**.

For the brine shrimp bioassay performed with the trunk wood oils (TWOA or TWOB) and 1-nitro-2-phenylethane isolated from *A. canelilla* the lethal concentrations (LC_{50}) were 21.61 ± 1.21 and 20.37 ± 0.99 μg mL^{-1}, respectively, suggesting a high cytotoxicity. On the other hand, for the wood extracts (TWEA or TWEB) the value was 91.38 ± 7.20 μg mL^{-1}, showing a cytotoxicity 4 times lower. Oils and extracts from plants presenting LC_{50} values below 1000 μg mL^{-1} are considered to be bioactives (*20*). In the case of *A. canelilla* the oil samples presented higher biological activity than the methanol extract samples.

LITERATURE CITED

(1) Maia, J. G. S.; Zoghbi, M. G. B.; Andrade, E. H. A. *Plantas Aromáticas da Amazônia e Seus Óleos Essenciais*; Museu Paraense Emílio Goeldi: Belém, Brazil, 2001.

(2) Maia, J. G. S.; Sousa, P. J. C.; Fontes Junior, E. A.; Santos, M. A. S. Volatile compounds and antispasmodic activity of the stem bark oil of *Aniba canelilla*. Presented at the XII Congresso Italo-latinoamericano de Etnomedicina, Rio de Janeiro, Brazil, **2003**.

(3) Lahlou, S.; Magalhães, P. J. C.; Siqueira, R. J. B.; Figueiredo, A. F.; Interaminense, L. F.; Maia, J. G. S.; Sousa, P. J. C. Cardiovascular effects of the essential oil of *Aniba canelilla* bark in normotensive rats. *J. Cardiovasc. Pharmacol.* **2005**, *46*, 412–421.

(4) Gottlieb, O. R.; Magalhães, M. T. Essential oil of the bark and wood of *Aniba canelilla*. *Perfum. Essent. Oil Rec.* **1960**, *51*, 69–70.

(5) Gottlieb, O. R.; Magalhães, M. T. Occurrence of 1-nitro-2-phenylethane in *Ocotea pretiosa* and *Aniba canelilla*. *J. Org. Chem.* **1959**, *24*, 2070–2071.

(6) Oger, J. M.; Fardeau, A.; Richomme, P.; Fournet, A.; Guinaudeau, H. Nouveaus alcaloïdes isoquinoléiques isolés d'une Lauraceae bolivienne *Aniba canelilla* H.B.K. *Can. J. Chem.* **1993**, *71*, 1128–1135.

(7) Oger, J. M.; Richomme, P.; Guinaudeau, H.; Bouchara, J. P.; Fournet, A. *Aniba canelilla* (H.B.K.) Mez essential oil: analysis of chemical constituents, fungistatic properties. *J. Essent. Oil Res.* **1994**, *6*, 493–497.

(8) Taveira, F. S. N.; Lima, W. N.; Andrade, E. H. A.; Maia, J. G. S. Seasonal essential oil variation of *Aniba canelilla*. *Biochem. Syst. Ecol.* **2003**, *31*, 69–75.

(9) Ruberto, G.; Baratta, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* **2000**, *69*, 167–174.

(10) Smith, M. A.; Perry, G.; Pryor, W. A. Causes and consequences of oxidative stress in Alzheimer's disease. *Free Radical Biol. Med.* **2002**, *32*, 1049–1054.

(11) Mímica-Durik, N.; Bozin, B.; Sokovic, M.; Mihajlovic, B.; Matavulj, M. Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Med.* **2003**, *69*, 413–419.

(12) Mimica-Durik, N.; Bozin, B.; Sokovic, M.; Simin, N. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. *J. Agric. Food Chem.* **2004**, *52*, 2485–2489.

(13) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*; Allured Publishing: Carol Stream, IL, 2001.

(14) Choi, H. S.; Song, H. S.; Ukeda, H.; Sawamura, M. Radical scavenging activities of *Citrus* essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. *J. Agric. Food Chem.* **2000**, *48*, 4156–4161.

(15) Hu, Q.; Xu, J.; Chen, S.; Yang, F. Antioxidant activity of extracts of black sesame seed (*Sesamum indicum* L.) by supercritical carbon dioxide extraction. *J. Agric. Food Chem.* **2004**, *52*, 943–947.

(16) Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, J. HPLC method for evaluation of free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1201–1204.

(17) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

(18) Kähkönen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J. P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954–3962.

(19) Finney, D. J. *Probits Analysis*; Cambridge University Press: Cambridge, U.K., 1971.

(20) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* **1982**, *45*, 31–34.

(21) Lewan, L.; Anderson, M.; Morales-Gomez, P. The use of *Artemia salina* in toxicity test. *Alternatives Lab. Anim.* **1992**, *20*, 297–301.

(22) Rackova, L.; Stefek, M.; Majekova, M. Structural aspects of antioxidant activity of substituted pyridoindoles. *Redox Prep.* **2002**, *7*, 207–214.

(23) Rackova, L.; Majekova, M.; Kost′alova, D.; Stefek, M. Antiradical and antioxidant activities of alkaloids isolated from *Mahonia aquifolium*: structural aspects. *Bioorg. Med. Chem.* **2004**, *12*, 4709–4715.

(24) Ubeda, A.; Montesinos, C.; Paya, M.; Terencio, C.; Alcaraz, M. J. Antioxidant action of benzylisoquinoline alkaloids. *Free Radical Res. Commun.* **1993**, *18*, 167–175.

Received for review June 28, 2007. Revised manuscript received September 15, 2007. Accepted September 21, 2007. We are grateful to the Biodiversity Program (PPBio), Ministry of Science and Technology (MCT/CNPq), Brazilian government, for financial support.

JF071928E