Phenolic Derivatives from Fruits of *Dipteryx lacunifera* DUCKE and Evaluation of Their Antiradical Activities

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The fruits of *Dipteryx lacunifera*, known as 'fava de morcego' and 'garampara', comprise pleasant tasting kernels that contain high amounts of fatty acids (mainly oleic acid) and are commonly consumed by inhabitants of the northeast of Brazil. In the present study, the crude EtOH extract of the fruit kernels was separated into hexane-, Et_2O-, AcOEt-, and H_2O-soluble fractions. The Et_2O fraction was found to exhibit the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity *in vitro*, and was subjected to further fractionation. Column chromatography over silica gel and *Sephadex LH-20*, followed by preparative HPLC-C_{18}, afforded (−)-eriodictyol (**1**), (−)-butin (**2**), luteolin (**3**), 3′,4′,7-trihydroxyflavone (**4**), butein (**5**), and sulfuretin (**6**). The antiradical activities of compounds **1**, **2**, **4**, and **6**, together with the positive controls rutin, butylated hydroxy toluene (BHT), and *tert*-butylhydroquinone (TBHQ), were evaluated with the DPPH assay and were found to decrease in the order rutin > **4** > **1** > **6** > **2** > TBHQ > BHT.

Introduction. – Various classes of metabolites have been isolated from members of the genus *Dipteryx* (family Leguminosae-Papilionoideae) including coumarins, isoflavones, fatty acids, triterpenoids, and furanocassane diterpenoids [1–7]. Kernels of the fruits of *Dipteryx lacunifera* DUCKE, a species that is widely distributed in the northeast of Brazil, where it is popularly known as 'fava de morcego' or 'garampara', are prized for their pleasant flavor, high energy content, and anti-oxidant activities [8][9]. In previous studies, the fruits of this species have been found to contain sesquiterpenoids [1], furanocassane diterpenoids and fatty acids [1][9], but no information is available concerning the occurrence of the polar constituents that are associated with its antiradical properties.

Free radicals are known to play a key role in biological damage and are implicated in the etiology of several degenerative conditions such as rheumatoid arthritis, coronary arterial disease, diabetes, and cancer. The consumption of various plant products, including vegetables, fruits, and herbal medicines, possessing radical-scavenging activities is apparently associated with low risk for these diseases [10–13]. In the present study, an activity-guided fractionation of an Et_2O-soluble fraction obtained from a crude ethanolic extract of fruit kernels of *D. lacunifera* was conducted, and four 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging flavonoids were isolated and identified. Since the fruits are already accepted for human consumption in Brazil, the

results reported herein will be of value in developing a functional product from the plant material by exploiting its antioxidant activity.

Results and Discussion. – The phenolic derivatives **1**–**6** were isolated by column chromatography and preparative HPLC from the DPPH-active Et_2O fraction of a crude EtOH extract of the fruit kernels of *D. lacunifera* (*Fig. 1*). The structures of **1**–**6** were deduced by interpretation of their ^{1}H- and ^{13}C-NMR (BBD and DEPT 135°), HMQC, and HMBC spectra, and by comparison of the spectral data with those reported in the literature.

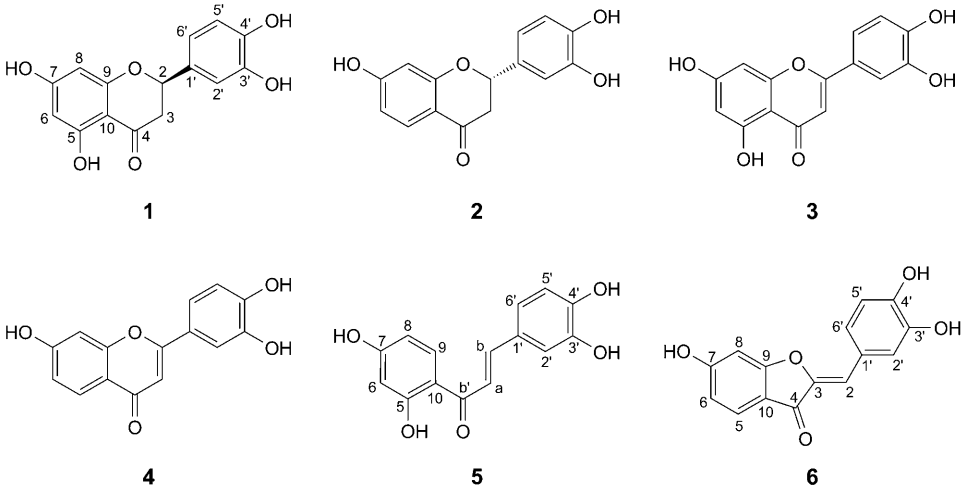

Fig. 1. *Phenolic derivatives* **1**–**6** *isolated from the fruit kernels of* Dipteryx lacunifera

Compound **1** was determined to be a flavanone on the basis of peaks in the ^{1}H-NMR spectrum at δ(H) 5.17 (*dd*, $J = 12.5, 3.0$), 2.96 (*dd*, $J = 17.0, 12.5$), and 2.61 (*dd*, $J = 17.0, 3.0$), assigned to H_{ax}–C(2), H_{ax}–C(3) and H_{eq}–C(3), respectively, together with the presence of an intense band at 287 nm in the UV spectrum [14]. Substitution patterns in the *A* and *B* rings were deduced from the signals at δ(H) 5.79 (*d*, $J = 2.0$, H–C(6)), 5.80 (*d*, $J = 2.0$, H–C(8)), 6.83 (br. *s*, H–C(2′)), and 6.68–6.71 (*m*, H–C(5′), H–C(6′)) in the ^{1}H-NMR spectrum. The ^{13}C-NMR (BBD and DEPT 135°) spectra of **1** showed 15 peaks associated with two aliphatic C-atoms at δ(C) 80.4 (C(2)) and 44.0 (C(3)), twelve aromatic C-atoms at δ(C) 103.3–168.3 (attributed to C(5)–C(10) and C(1′)–C(2′)), and a conjugated C=O group at δ(C) 197.7 (assigned to C(4)). Important HMBC correlations (*Fig. 2*) were observed between H–C(2) and C(4)/C(2′)/C(6′), H–C(6) and C(8)/C(10), and H–C(2′) and C(2)/C(4′)/C(6′), and these confirmed the proposed structure. Finally, HR-ESI-MS showed a *pseudo*-molecular ion peak at *m*/*z* 287.0701 ($[M-H]^-$), which is in accord with a molecular formula of $C_{15}H_{12}O_6$. Comparison of spectral and optical rotation data with those reported in the literature [15–17] allowed the identification of **1** as (2*R*)-2-(3,4-dihydroxyphenyl)-2,3-dihydro-5,7-dihydroxy-4*H*-chromen-4-one ((−)-eriodictyol).

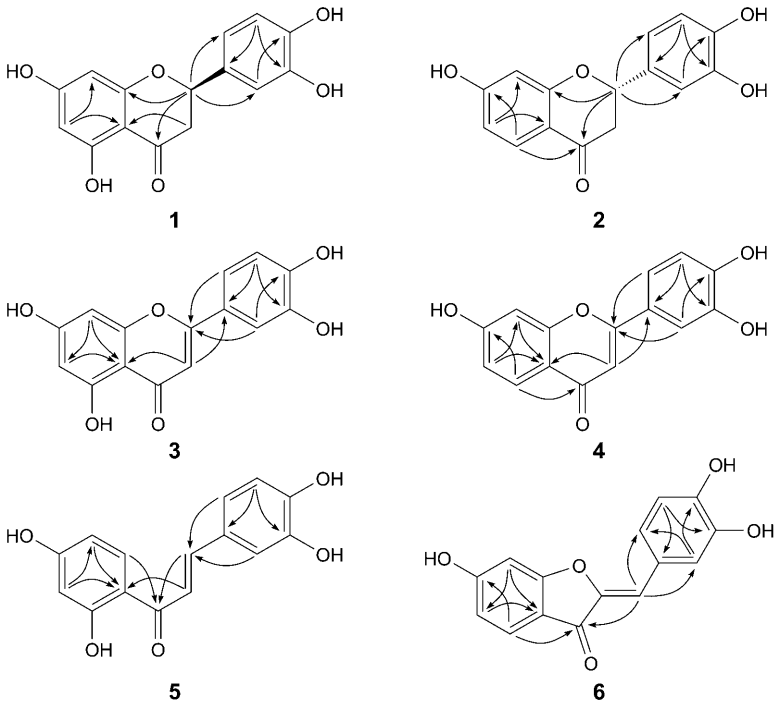

Fig. 2. *Important HMBC* (H → C) *data in the structures of phenolic derivatives* **1**–**6** *isolated from the fruit kernels of* Dipteryx lacunifera

With respect to compound **2**, an intense band at 277 nm in the UV spectrum, together with ^{1}H-NMR peaks associated with H_{ax}–C(2), H_{ax}–C(3), and H_{eq}–C(3) at δ(H) 5.33 (*dd*, J = 13.0, 3.0), 3.01 (*dd*, J = 17.0, 13.0), and 2.71 (*dd*, J = 17.0, 3.0), respectively, characterized the compound as a flavanone [14]. Aromatic H-atoms were observed at δ(H) 7.73 (*d*, J = 8.5, H–C(5)), 6.51 (*dd*, J = 8.5, 2.5, H–C(6)), and 6.37 (*d*, J = 2.5, H–C(8)), suggesting a 1,2,4-trisubstituted *A* ring, a pattern dissimilar from that observed in **1**. However, aromatic H-atoms of ring *B* were detected at δ(H) 6.94 (*d*, J = 1.5, H–C(2′)) and 6.79–6.82 (*m*, H–C(5′), H–C(6′)), indicating a pattern that was similar to **1**. The ^{13}C-NMR (BBD and DEPT 135°) spectra of **2** showed 15 peaks, including two associated with aliphatic C-atoms at δ(C) 81.0 (C(2)) and 44.9 (C(3)), and one conjugated C=O group at δ(C) 193.5 (C(4)). However, the multiplicities of the aromatic C-atoms, the chemical shift of the signal attributed to C(5) (δ(C) 129.8), and data from HMBC spectral analysis (*Fig. 2*), indicated the absence of a OH group at C(5). A molecular formula of $C_{15}H_{12}O_5$ was confirmed for **2** by the HR-ESI-MS, in which a *pseudo*-molecular ion peak at *m*/*z* 271.0765 ($[M-H]^-$) was observed. Comparison of spectral and optical rotation data with those reported in the literature [17–20] allowed the identification of **2** as (2*S*)-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-4*H*-chromen-4-one ((−)-butin).

The UV spectrum of **3** exhibited bands at 256 and 396 nm, which could be assigned, respectively, to benzoyl and cafeoyl chromophores of a flavone derivative [14]. The

^{1}H-NMR spectrum of **3** showed aromatic H-atoms attributed to a flavone at δ(H) 7.37–7.40 (*m*, H–C(2′), H–C(6′)), 6.92 (*d*, J = 8.5, H–C(5′)), 6.23 (*d*, J = 2.0, H–C(6)), and 6.46 (*d*, J = 2.0, H–C(8)), together with a *singlet* at δ(H) 6.56, which could be assigned to H–C(3). The ^{13}C-NMR (BBD and DEPT 135°) spectra showed 15 sp^2 C-atoms, one of which (δ(C) 183.8, C(4)) was characteristic of a conjugated C=O group, and six were characteristic of oxygenated C-atoms at δ(C) 165.9 (C(2)), 163.2 (C(5)), 166.3 (C(7)), 159.4 (C(9)), 147.0 (C(3′)), and 150.9 (C(4′)). All of the other C-atom signals could be assigned from the HSQC and HMBC spectra (*Fig. 2*). The HR-ESI-MS presented a *pseudo*-molecular ion peak at *m*/*z* 285.0400 ([M – H]$^-$), which was consistent with a molecular formula of $C_{15}H_{10}O_6$. This, taken together with the other spectral data, allowed the identification of **3** as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one (luteolin) [17].

Bands at 235 and 340 nm in the UV spectrum of **4** could be assigned to benzoyl and cafeoyl chromophores of a flavone derivative [14]. The ^{1}H-NMR spectrum of **4** showed a profile similar to that observed for **3** with aromatic H-atoms at δ(H) 6.64 (*s*, H–C(3)), 6.93–6.95 (*m*, H–C(6), H–C(8), H–C(5′)), and 7.40–7.42 (*m*, H–C(2′), H–C(6′)). However, the spectrum of **4** showed an additional *doublet* at δ(H) 7.98 (J = 8.1), which could be assigned to H–C(5) because of the coupling with the signal of H–C(6), which was observed in the DQ-COSY spectrum. The ^{13}C-NMR (BBD and DEPT 135°) spectra of **4** showed 15 sp^2 C-atoms, one of which was characteristic of a conjugated C=O group at δ(C) 180.2 (C(4)), and five were characteristic of oxygenated C-atoms at δ(C) 166.0 (C(2)), 165.0 (C(7)), 159.7 (C(9)), 147.0 (C(3′)), and 150.8 (C(4′)). Cross-peaks between H–C(3) and C(10)/C(1′), H–C(5) and C(4)/C(7), H–C(8) and C(6)/C(10), and H–C(2′) and C(2)/C(4′)/C(6′) observed in the HMBC spectrum, and a HR-ESI-MS *pseudo*-molecular ion peak at *m*/*z* 269.0626 ([M – H]$^-$) corresponding to a molecular formula of $C_{15}H_{10}O_5$, allowed **4** to be identified as 2-(3,4-dihydroxyphenyl)-7-hydroxy-4*H*-chromen-4-one. Comparison of the spectroscopic data with those reported in the literature [21] confirmed the proposed structure of **4**.

The UV spectrum of **5** showed bands at 260 and 379 nm, which were assigned to benzoyl and cafeoyl chromophores of a chalcone derivative [14]. The ^{1}H-NMR spectrum exhibited *doublet*s at δ(H) 7.54 (J = 15.0) and 7.73 (J = 15.0), assigned to the α and β H-atoms of a chalcone, and three aromatic H-atoms at δ(H) 6.30 (*d*, J = 2.0), 6.43 (*dd*, J = 8.5, 2.0), and 7.95 (*d*, J = 8.5), assigned to H–C(6), H–C(8), and H–C(9), respectively, of ring *A*. Three other aromatic H-atoms were observed at δ(H) 7.20 (*d*, J = 1.5), 6.83 (*d*, J = 8.0), and 7.12 (*dd*, J = 8.0, 1.5), and were attributed to H–C(2′), H–C(5′), and H–C(6′), respectively, of ring *B*. The ^{13}C-NMR (BBD and DEPT 135°) spectra of **5** exhibited 15 signals corresponding to twelve aromatic C-atoms at δ(C) 103.8–167.4, and two signals associated with non-cyclic C=C bond C-atoms at δ(C) 118.3 (CH) and 146.8 (CH). These two latter signals, together with the C=O signal at δ(C) 193.5, indicated the presence of a chalcone, which was identified as (2*E*)-1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxyphenyl)prop-2-en-1-one (butein). Comparison of the spectral data with those reported in [18] indicated that the signals assigned to C(α), C(β), C(5), C(6), C(8) and C(9) should be corrected. Complete assignment of the C- and H-atom signals of **5** was possible following analysis of the HMBC spectrum, which revealed cross-peaks between H–C(2′) and C(β)/C(4),

H–C(α) and C(1′)/C(10), and H–C(9) and C(5)/C(7)/C(β′). The HR-ESI-MS showed a *pseudo*-molecular ion peak at *m*/*z* 271.0612 ([*M* – H]$^-$) corresponding to a molecular formula of $C_{15}H_{12}O_5$ thus confirming the complete structure of **5**.

With regard to compound **6**, the UV spectrum showed bands at 256 and 393 nm characteristic of an aurone derivative [14]. In the ^{1}H-NMR spectrum, a *singlet* at δ(H) 6.58 could be assigned to H–C(2), whilst signals of H-atoms in the *A* ring were observed at δ(H) 7.51 (*d*, *J* = 8.5), 6.59 (*dd*, *J* = 8.5, 2.0), and 6.61 (*d*, *J* = 2.0), and assigned to H–C(5), H–C(6), and H–C(8), respectively. Three further aromatic H-atoms were observed at δ(H) 7.42 (*d*, *J* = 2.0, H–C(2′)), 6.75 (*d*, *J* = 8.5, H–C(5′)), and 7.14 (*dd*, *J* = 8.5, 2.0, H–C(6′)). The ^{13}C-NMR (BBD and DEPT 135°) spectra showed 15 signals, one being associated with a conjugated C=O group at δ(C) 184.3 (C(4)), and five attributed to oxygenated C-atoms at δ(C) 147.7 (C(3)), 169.8 (C(7)), 168.3 (C(9)), 146.7 (C(3′)), and 149.4 (C(4′)). Analysis of the HMBC spectrum (^3J) showed that there was a strong correlation between H–C(2) and C(4)/C(2′)/C(6), and this could only occur with an aurone structure as proposed. A molecular formula of $C_{15}H_{10}O_5$ was determined for **6** on the basis of the HR-ESI-MS analysis, which showed a *pseudo*-molecular ion peak at *m*/*z* 269.0630 ([*M* – H]$^-$). On the basis of this information, and by comparison with published spectral data [22][23], the structure of **6** was determined as (2*Z*)-2-(3,4-dihydroxybenzylidene)-6-hydroxy-1-benzofuran-3(2*H*)-one (sulfuretin).

This is the first report of the occurrence of flavanone, flavone, chalcone, and aurone derivatives in a species of *Dipteryx*, and the occurrence of flavonoids **1**–**6** in *D. lacunifera* is reported here for the first time.

The radical-scavenging capacity of a compound is commonly evaluated by reduction of the stable free radical DPPH in MeOH or EtOH solution to form $DPPH_2$ [10]. As presented in the *Table*, the crude EtOH extract of kernels of *D. lacunifera* fruits showed weak scavenging activity (485.91 ± 77.30 μg/ml). However, the Et_2O-soluble fraction of the ethanolic extract, obtained by serial extraction with hexane, Et_2O, AcOEt, and H_2O, showed moderate activity (141.86 ± 7.92 μg/ml), and

Table. *Antiradical Properties, as Determined by DPPH Assay, of a Crude EtOH Extract of the Fruit Kernels of* Dipteryx lacunifera, *and an Et_2O-Soluble Fraction and Flavonoids* **1**, **2**, **4**, *and* **6** *Derived therefrom, together with Rutin, BHT, and TBHQ as Positive Controls*

Extracts/compounds	EC_{50} [μg/ml ± SD][a])	$EC_{50} \times 10^{-2}$ [μM ± SD][a])
EtOH extract	485.91 ± 77.30	n.d.[b])
Et_2O fraction	141.86 ± 7.92	n.d.
1	31.39 ± 1.45	10.90 ± 0.50
2	33.54 ± 2.58	12.33 ± 0.94
4	28.19 ± 1.74	10.44 ± 0.64
6	32.12 ± 1.43	11.90 ± 0.52
Rutin[c])	31.93 ± 0.52	5.20 ± 0.85
BHT[c])	67.19 ± 6.04	30.54 ± 2.74
TBHQ[c])	30.01 ± 0.87	18.07 ± 0.52

[a]) $n = 3$. [b]) n.d. = not determined. [c]) Positive controls.

was subjected to purification procedures with the aim of isolating the active components. Following chromatographic separation, six phenolic derivatives (**1**–**6**) were obtained, and four of them (**1**, **2**, **4**, and **6**) were subjected to the DPPH assay together with the positive controls rutin, butylated hydroxy toluene (BHT) and *tert*-butylhydroquinone (TBHQ). The results obtained (*Table*) revealed that **4** possessed the highest hydrogen-donating capacity, with an EC_{50} value of 28.19 ± 1.74 μg/ml ($10.44 \pm 0.64 \times 10^{-2}$ μM) showing an activity superior to those of BHT and TBHQ but lower than that of rutin. The antiradical activities of the isolated compounds and the positive controls decreased in the following order: rutin > **4** > **1** > **6** > **2** > TBHQ > BHT.

Fig. 3 shows that, similar to rutin, compounds **1**, **2**, **4**, and **6** exhibited concentration-dependent DPPH radical-scavenging activities in the concentration range 25–100 μg/ml. At concentrations > 100 μg/ml, the DPPH radical-scavenging activity showed no significant change with respect to the concentration of the analyte. Statistical analyses (ANOVA and Tukey) revealed that the radical-scavenging activity of flavonoid **4** was not significantly different ($p < 0.05$) from that of the positive control rutin within the range 25–250 μg/ml.

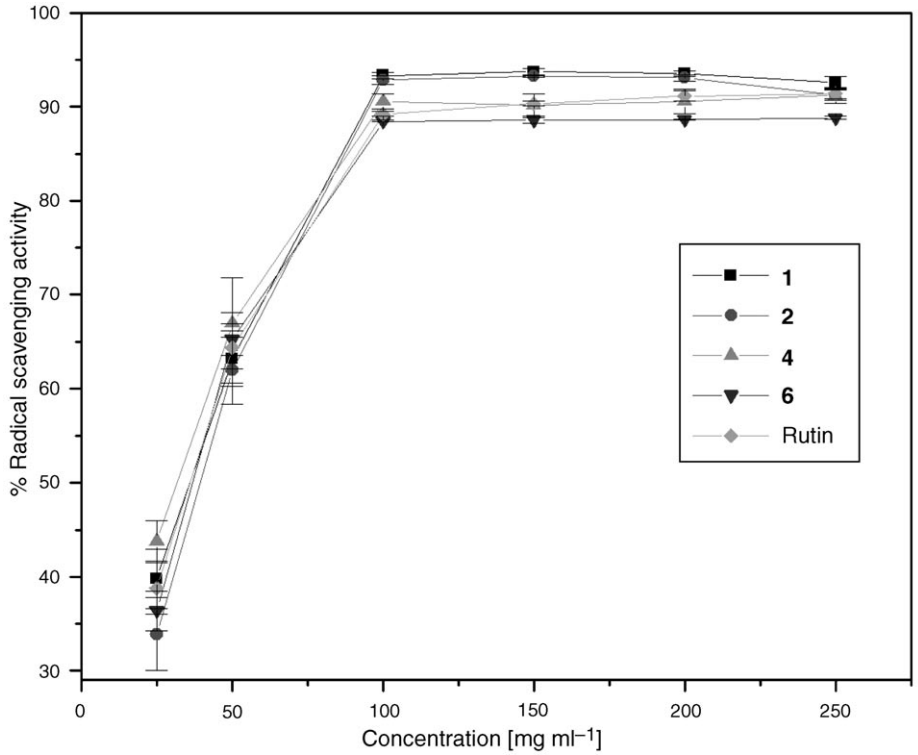

Fig. 3. *Estimation of the percentage free radical-scavenging activities of the flavonoids* **1**, **2**, **4**, *and* **6** *from fruit kernels of* Dipteryx lacunifera, *together with the positive control* (rutin), *measured after 30 min of reaction*

In conclusion, the phenolic compounds isolated from fruit kernels of *D. lacunifera* exhibited strong radical-scavenging activities indicating that this plant may represent a valuable source of natural antiradical derivatives.

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Experimental Part

General. DPPH and BHT from *Sigma*; TBHQ from *BASF*; other chemicals were of analytical reagent grade. Column chromatography: silica gel *60* (SiO_2, 70–230 mesh; *Merck*) column (38 × 5.5 cm, 250 g); *Sephadex® LH-20* (*Sigma*) column (65 × 1.5 cm). Prep. HPLC: *Varian PrepStar SD-1* chromatograph, *Varian 320* UV-VIS detector, *Rheodyne 7725i* injector, *Phenomenex Luna* C_{18} column (250 × 21 mm; 10 μm), at 11 ml/min. UV Spectra: *Hitachi U-3000* spectrometer, in MeOH (pure compounds) or methanolic DPPH soln. (radical-scavenging assay). ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), HMQC, and HMBC Spectra: *Varian Inova 500* spectrometer, in CD_3OD, δ in ppm rel. Me_4Si, J in Hz. HR-ESI-MS: *Bruker Daltonics ultrOTOFq* (ESI/TOF), negative mode.

Plant Material. Fruits of *D. lacunifera* (1532 g) were collected during August 2003 in the district of Bom Jesus, Piaui State, Brazil. The plant material was identified by Dr. *Haroldo Cavalcante Lima*, Instituto de Pesquisa Jardim Botânico do Rio de Janeiro, Brazil. A voucher specimen is deposited with the Herbarium Graziela Barroso, Universidade Federal de Piauí, Brazil under reference TEPB 18246.

Extraction and Isolation Procedures. The fruits of *D. lacunifera* were separated into seeds (122 g) and kernels (1410 g) and exhaustively extracted with hexane to remove waxy material. Following removal of solvent, the dried kernels were ground and extracted consecutively with hexane and EtOH. The resulting EtOH soln. was concentrated under vacuum to yield 79 g of a crude extract, which was fractionated by serial extraction with hexane, Et_2O, AcOEt, and H_2O to yield residues of 23, 33, 2.5, and 11.5 g, resp., after solvent removal. Part of the DPPH-active Et_2O fraction (10 g) was subjected to CC over SiO_2 with gradient elution from $CHCl_3$ to MeOH to yield 30 fractions. DPPH-active fractions *16* (429 mg), *19* (326 mg), and *22* (209 mg) were submitted to CC over *Sephadex LH-20*, eluted with MeOH to yield **1** (94 mg, 0.02%), **6** (174 mg, 0.04%), and **6** (19 mg, 0.004%). The DPPH-active fraction *18* (400 mg) afforded sub-fractions *I–X* following chromatography over *Sephadex LH-20* with MeOH as eluent. Sub-fraction *II* (197 mg) comprised pure **2** (0.05% yield), whilst sub-fraction *V* (35 mg), after prep. HPLC eluted with MeOH/AcOH 0.5% in H_2O (53:47), afforded **1** (7 mg, 0.002%), **5** (12 mg, 0.003%), and **3** (10 mg, 0.002%). DPPH-active fraction *20* (756 mg) was subjected to CC over *Sephadex LH-20* with MeOH as eluent to yield 9 sub-fractions (*I–IX*). Sub-fraction *VII* (100 mg) comprised pure **6** (0.02%) whilst sub-fraction *IV* (81 mg), after prep. HPLC eluted with MeOH/AcOH 0.5% : H_2O (1:1), afforded **4** (52 mg, 0.01%).

DPPH Radical-Scavenging Assay. The DPPH-scavenging activities were determined using the method of *Valco et al.* [12][13]. Aliquots (0.3 μl) of the positive control, or of solns. containing 250, 200, 150, 100, 50, and 25 μg/ml of pure **1**, **2**, **4**, and **6** individually dissolved in MeOH, were incubated at 25° with 100 μM DPPH in MeOH (2.7 ml). Absorbance values at 516 nm were recorded every 10 min until the reaction was complete (typically 60 min). The percentage of antiradical activity was calculated from $[A_{DPPH} - (A_{sample} - A_{blank})] \times 100/A_{DPPH}$ (where A_{DPPH} is the absorbance of DPPH, A_{sample} is the absorbance of DPPH plus sample and A_{blank} is the absorbance of the blank). Activities were determined in triplicate and the results reported as mean values ± standard deviation (SD). Statistical analyses of the results were carried out using Microcal Origin® 7.5 and SPSS® 13.0. The antiradical activities of **3** and **5** could not be measured since the compounds were obtained in amounts that were only sufficient for spectral analysis.

(−)-Eriodictyol (=*(2*R*)-2-(3,4-Dihydroxyphenyl)-2,3-dihydro-5,7-dihydroxy-4*H*-chromen-4-one*; **1**). Yellow amorphous solid. $[\alpha]_D = -17.8$ ($c = 0.1$, MeOH). UV (MeOH): 287. ^{1}H-NMR (500 MHz, CD_3OD): 6.83 (br. *s*, H–C(2′)); 6.68–6.71 (*m*, H–C(5′), H–C(6′)); 5.80 (*d*, $J = 2.0$, H–C(8)); 5.79 (*d*, $J = 2.0$, H–C(6)); 5.17 (*dd*, $J = 12.5, 3.0$, H–C(2)); 2.96 (*dd*, $J = 17.0, 12.5$, H_{ax}–C(3)); 2.61 (*dd*, $J = 17.0, 3.0$, H_{eq}–C(3)). ^{13}C-NMR (75 MHz, CD_3OD): 197.7 (C(4)); 168.3 (C(7)); 165.4 (C(5)); 164.8 (C(9));

146.8 (C(4′)); 146.4 (C(3′)); 131.7 (C(1′)); 119.2 (C(6′)); 116.2 (C(5′)); 114.7 (C(2′)); 103.3 (C(10)); 97.0 (C(6)); 96.2 (C(8)); 80.4 (C(2)); 44.0 (C(3)). HR-ESI-MS (neg.): 287.0701 ($[M-H]^-$, $C_{15}H_{11}O_6^+$; calc. 287.0554).

(−)-Butin (=*(2*S*)-2-(3,4-Dihydroxyphenyl)-2,3-dihydro-7-hydroxy-4*H*-chromen-4-one*; **2**). Yellow amorphous solid. $[\alpha]_D = -59.6$ ($c = 0.1$, MeOH). UV (MeOH) 277. ^{1}H-NMR (500 MHz, CD_3OD): 7.73 (d, $J = 8.5$, H−C(5)); 6.94 (d, $J = 1.5$, H−C(2′)); 6.79–6.82 (m, H−C(5′), H−C(6′)); 6.51 (dd, $J = 8.5, 2.5$, H−C(6)); 6.37 (d, $J = 2.5$, H−C(8)); 5.33 (dd, $J = 13.0, 3.0$, H−C(2)); 3.01 (dd, $J = 17.0, 13.0$, H_{ax}−C(3)); 2.71 (dd, $J = 17.0, 3.0$, H_{eq}−C(3)). ^{13}C-NMR (75 MHz, CD_3OD): 193.5 (C(4)); 166.9 (C(7)); 165.5 (C(9)); 146.8 (C(4′)); 146.4 (C(3′)); 132.0 (C(1′)); 129.8 (C(5)); 119.2 (C(6′)); 116.2 (C(5′)); 114.9 (C(10)); 114.7 (C(2′)); 111.7 (C(6)); 103.8 (C(8)); 81.0 (C(2)); 44.9 (C(3)). HR-ESI-MS (neg.): 271.0765 ($[M-H]^-$, $C_{15}H_{11}O_5^+$; calc. 271.0605).

Luteolin (=*2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one*; **3**). Yellow amorphous solid. UV (MeOH) 256, 396. ^{1}H-NMR (500 MHz, CD_3OD): 7.37–7.40 (m, H−C(2′), H−C(6′)); 6.92 (d, $J = 8.5$, H−C(5′)); 6.56 (s, H−C(3)); 6.46 (d, $J = 2.0$, H−C(8)); 6.23 (d, $J = 2.0$, H−C(6)). ^{13}C-NMR (75 MHz, CD_3OD): 183.8 (C(4)); 166.3 (C(7)); 165.9 (C(2)); 163.2 (C(5)); 159.4 (C(9)); 150.9 (C(4′)); 147.0 (C(3′)); 123.7 (C(6′)); 120.3 (C(1′)); 116.8 (C(5′)); 114.2 (C(2′)); 105.3 (C(10)); 103.9 (C(3)); 100.1 (C(6)); 95.0 (C(8)). HR-ESI-MS (neg.): 285.0400 ($[M-H]^-$, $C_{15}H_9O_6^+$; calc. 285.0398).

3′,4′,7-Trihydroxyflavone (=*2-(3,4-Dihydroxyphenyl)-7-hydroxy-4*H*-chromen-4-one*; **4**): Yellow amorphous solid. UV (MeOH) 235, 340. ^{1}H-NMR (500 MHz, CD_3OD): 7.98 (d, $J = 8.1$, H−C(5)); 7.40–7.42 (m, H−C(2′), H−C(6′)); 6.93–6.95 (m, H−C(6), H−C(8), H−C(5′)); 6.64 (s, H−C(3)). ^{13}C-NMR (75 MHz, CD_3OD): 180.2 (C(4)); 166.0 (C(2)); 165.0 (C(7)); 159.7 (C(9)); 150.8 (C(4′)); 147.0 (C(3′)); 127.7 (C(5)); 124.0 (C(1′)); 117.2 (C(10)); 120.1 (C(6′)); 116.8 (C(5′)); 116.3 (C(6)); 114.1 (C(2′)); 105.2 (C(3)); 103.4 (C(8)). HR-ESI-MS (neg.): 269.0626 ($[M-H]^-$, $C_{15}H_9O_5^+$; calc. 269.0449).

Butein (=*3′,4′,3,5-Tetrahydroxychalcone* = *(2*E*)-1-(2,4-Dihydroxyphenyl)-3-(3,4-dihydroxyphenyl)-prop-2-en-1-one*; **5**): Yellow amorphous solid. UV (MeOH) 260, 379. ^{1}H-NMR (500 MHz, CD_3OD): 7.95 (d, $J = 8.5$, H−C(9)); 7.73 (d, $J = 15.0$, H−C(β)); 7.54 (d, $J = 15.0$, H−C(α)); 7.20 (d, $J = 1.5$, H−C(2′)); 7.12 (dd, $J = 8.0, 1.5$, H−C(6′)); 6.83 (d, $J = 8.0$, H−C(5′)); 6.43 (dd, $J = 8.5, 2.0$, H−C(8)); 6.30 (d, $J = 2.0$, H−C(6)). ^{13}C-NMR (75 MHz, CD_3OD): 193.5 (C(β')); 167.4 (C(5)); 166.3 (C(7)); 149.9 (C(4′)); 146.8 (C(β)); 146.0 (C(3′)); 133.2 (C(9)); 128.4 (C(1′)); 123.5 (C(6′)); 118.3 (C(α)); 116.6 (C(2′)); 115.8 (C(5′)); 114.7 (C(10)); 109.1 (C(8)); 103.8 (C(6)). HR-ESI-MS (neg.): 271.0612 ($[M-H]^-$, $C_{15}H_{11}O_5^+$; calc. 271.0605).

Sulfuretin (=*3′,4′,6-Trihydroxyaurone* = *(2*Z*)-2-(3,4-Dihydroxybenzylidene)-6-hydroxy-1-benzofuran-3(2*H*)-one*; **6**): Yellow amorphous solid. UV (MeOH) 256, 393. ^{1}H-NMR (500 MHz, CD_3OD): 7.51 (d, $J = 8.5$, H−C(5)); 7.42 (d, $J = 2.0$, H−C(2′)); 7.14 (dd, $J = 8.5, 2.0$, H−C(6′)); 6.75 (d, $J = 8.5$, H−C(5′)); 6.61 (d, $J = 2.0$, H−C(8)); 6.59 (dd, $J = 8.5, 2.0$, H−C(6)); 6.58 (s, H−C(2)). ^{13}C-NMR (75 MHz, CD_3OD): 184.3 (C(4)); 169.8 (C(7)); 168.3 (C(9)); 149.4 (C(4′)); 147.7 (C(3)); 146.7 (C(3′)); 126.8 (C(5)); 126.4 (C(6′)); 125.6 (C(1′)); 118.9 (C(2′)); 116.8 (C(5′)); 114.9 (C(10)); 114.7 (C(6)); 114.1 (C(2)); 99.4 (C(8)). HR-ESI-MS (neg.): 269.0630 ($[M-H]^-$, $C_{15}H_9O_5^+$; calc. 269.0450).

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