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Lignans and Other Constituents of the Fruits of *Euterpe oleracea* (Açai) with Antioxidant and Cytoprotective Activities

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Using a hydroxyl radical scavenging assay, bioactivity-guided fractionation of a methanol-soluble extract of the fruits of *Euterpe oleracea* (açai) led to the isolation of 22 compounds of previously known structure. Altogether, 14 of these isolates were found to be active in an in vitro hydroxyl radical scavenging assay and seven of these isolates in a 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay. Dihydroconiferyl alcohol, (+)-lariciresinol, (+)-pinoresinol, (+)-syringaresinol, and protocatechuic acid methyl ester exhibited cytoprotective activity in cultured MCF-7 cells stressed by H_2O_2. Lignans have not been previously reported as constituents of this species and were found to be representative of the aryltetrahydronaphthalene, dihydrobenzofuran, furofuran, 8-*O*-4′-neolignan, and tetrahydrofuran structural types.

KEYWORDS: *Euterpe oleracea*; lignans; benzenoid; phenylpropanoid; hydroxyl radical scavenging activity; 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity; cytoprotection

INTRODUCTION

Euterpe oleracea Mart. (Arecaceae), of which the fruits are commonly known as açai, is distributed in northern regions of South America and is especially abundant in flood plains of the Amazon River (*1*). The fruits of *E. oleracea* are used to make various beverages and food preparations such as juices, ice cream, and desserts in Brazil, Colombia, and Suriname, and are used medicinally for the treatment of diarrhea (*2–6*). Açai products are currently being exported to Asia, Europe, and North America (*6*) and represent a major U.S. botanical dietary supplement according to a recent report (*8*). Previous phytochemical investigations have shown that anthocyanidins and flavonoids are constituents of *E. oleracea* fruits (*2, 5*). Antioxidant activities (*3, 5, 9, 10*), a vasodilatory effect (*7*), and inhibition of the COX-1 and -2 enzymes (*5*), NO production and iNOS expression (*11*), and testosterone 5α-reductase (*12*), as well as apoptosis induction by açai extracts or their individual constituents (*13*), have been reported so far. Of these biological evaluations, antioxidant effects have been most frequently investigated for this plant, but the responsible constituents have not been fully resolved.

As part of an ongoing investigation of potential cancer chemopreventive agents from botanical dietary supplements (*14–16*), an initial screening procedure using a hydroxyl radical scavenging assay was performed, and a methanol-soluble extract of the fruits of *E. oleracea* (açai) was chosen for phytochemical workup and biological testing due to its observed antioxidant activity.

Activity-guided fractionation of the fruits of *E. oleracea* led to the isolation of 22 known compounds including nine lignans (**1**−**4**, **6**−**8**, **13**, and **14**), four simple benzenoids (**9**−**11** and **15**), three flavonoids (**16**−**18**), a benzoquinone (**12**), three monoterpenoids (**19**−**21**), and two norisoprenoids (**5** and **22**). This is the first report of lignan constituents of *E. oleracea* fruits. Compounds active in the initial bioassay were evaluated for their cytoprotective activity in cultured MCF-7 cells mediated by H_2O_2.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Perkin-Elmer UV/vis lambda 10 spectrometer. Circular dichroism (CD) spectra were recorded on a JASCO J-810 spectropolarimeter. NMR spectroscopic data were recorded at room temperature using a Bruker DRX-400 spectrometer with tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) mass spectrometric analyses were obtained on a 3-T Finnigan FTMS-2000 Fourier transform mass spectrometer. A SunFire PrepC_{18}OBD column (5 μm, 150 mm × 19 mm i.d., Waters, Milford, MA) and a SunFire PrepC_{18} guard column (5 μm, 10 mm × 19 mm i.d., Waters) were used for preparative high-performance liquid chromatography (HPLC), along with two Waters 515 HPLC pumps and a Waters 2487 dual λ

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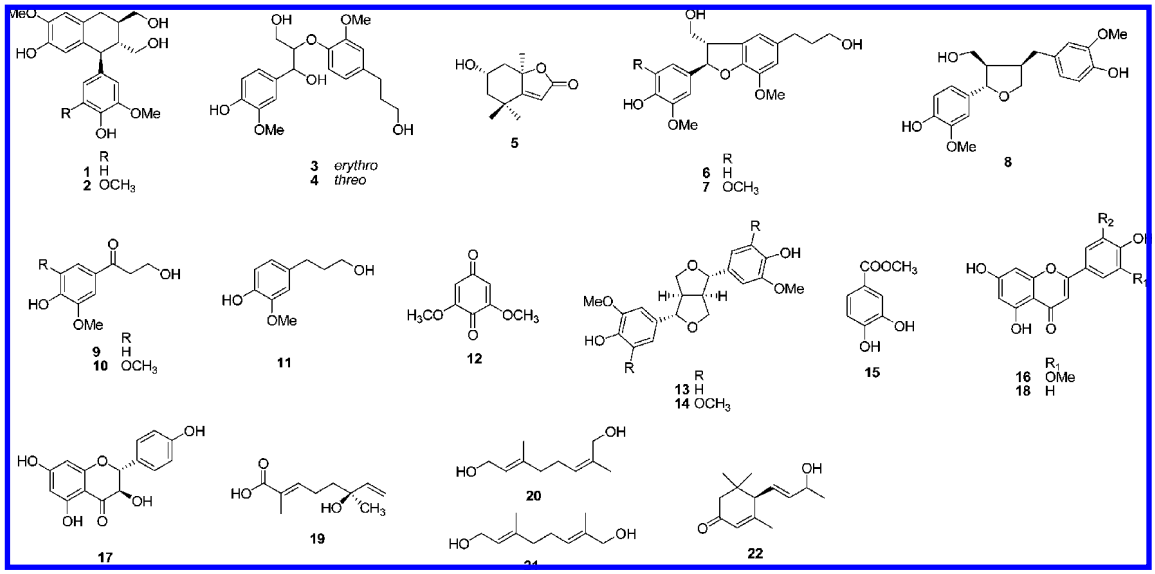

absorbance detector (Waters). Column chromatography was carried out with Purasil (230−400 mesh, Whatman, Clifton, NJ) and Sephadex LH-20 (Sigma, St. Louis, MO). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thick Partisil K6F (Whatman) glass plates while preparative TLC was conducted on precoated 20 cm × 20 cm, 500 μm thick Partisil K6F (Whatman) glass plates. All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ).

Chemicals. 2′,7′-Dichlorodihydrofluorescin diacetate (HDCF-DA), $FeSO_4$, dimethyl sulfoxide (DMSO), trichloroacetic acid, and sulforhodamine B were purchased from Sigma Chemicals Co. (St. Louis, MO). All other chemicals and solvents utilized were of the highest grade.

Plant Material. The dried and powdered flakes of the fruit pulp of *E. oleracea*, originally collected in the Amazon, were obtained through Nature's Sunshine Products, Inc. (Spanish Fork, Utah) (lot #0136700) from Dudas Roda Industrial [Jaraguá do Sul (SC), Brazil] (#4103). In their preparation, the fruits were washed with water, with the pulp sieved to separate the bark and seeds, pasteurized, and freeze-dried to form flakes. A representative sample (OSUADK-CCP0006) was deposited in the Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University.

Extraction and Isolation. The dried and powdered flakes of the fruits of *E. oleracea* (4 kg) were extracted by maceration with MeOH (3 L each) three times at room temperature, for up to 2 days each, and then evaporated in vacuo. The dried MeOH extract (154 g) was suspended with water and partitioned sequentially with hexanes (3 × 1000 mL), $CHCl_3$ (3 × 1000 mL), and EtOAc (3 × 1000 mL). All partitions were tested in a hydroxyl radical scavenging assay, and the $CHCl_3$ extract (11.5 g) was found to exhibit activity (4.8 μg/mL) in this assay. The $CHCl_3$-soluble fraction (11.0 g) was chromatographed over a silica gel column, using a gradient of increasing polarity with $CHCl_3$ and MeOH mixtures as solvents, and was fractionated into 10 subfractions (F01−F10). The antioxidant activity of these subfractions was monitored using the hydroxyl radical scavenging assay, and three fractions (F02, F03, and F05; 1.6, 1.9, and 0.5 μg/mL, respectively) were deemed to be active.

The most potent fraction F05 (612 mg) was subjected to column chromatography using Sephadex LH-20 with elution by MeOH and afforded nine subfractions (F0501−F0509). The constituents of subfraction F0503 (270 mg) were purified by HPLC, following preliminary silica gel column chromatography ($CHCl_3$−MeOH; 50:1, 30:1, 20:1, and 10:1; 500 mL each), to furnish four subfractions (F050301−04). The separation for F050303 was conducted by HPLC with MeCN−H_2O (16:84), 7.0 mL/min, by isocratic elution, to afford compounds **1** (t_R = 44.6 min, 8.9 mg, 0.00022%) and **3** (t_R = 34.1 min, 1.0 mg, 0.000025%) and a mixture of **2** and **4** (t_R = 39.4 min, 11.0 mg). This mixture (**2**, t_R = 38.9 min, 4.0 mg, 0.0001%; **4**, t_R = 40.2 min, 3.0 mg, 0.000075%) was separated using these HPLC experimental conditions. Subfraction F050302 (4 mg) was subjected to HPLC purification using MeCN−H_2O (20:80) as solvent, to afford compounds **6** (t_R = 46.6 min, 1.1 mg, 0.000028%) and **7** (t_R = 51.4 min, 1.6 mg, 0.00004%). From F0502 (18 mg), **5** (1.3 mg) was purified using silica gel column chromatography (hexanes−EtOAc, 3:1).

Fraction F02 (0.9 g) was chromatographed over Sephadex LH-20 (MeOH) to give nine subfractions (F0201−F0209). F0204 (174 mg) was further subjected to silica gel column chromatography ($CHCl_3$−MeOH, 30:1) and furnished compound **12** (2 mg, 0.00005%) and eight subfractions (F020401−F020408). Using preparative TLC, **8** (2.3 mg, 0.000058%, R_f 0.30, hexanes−EtOAc, 1:1) was purified from F020405. From F020404, compounds **9** (6.7 mg, 0.00017%, R_f 0.15), **10** (7.0 mg, 0.00018%, R_f 0.25), **11** (0.9 mg, 0.000023%, R_f 0.50), and **14** (9.7 mg, 0.00024%, R_f 0.10) were isolated using preparative TLC (hexanes−EtOAc, 1:1). F0205 was subjected to silica gel column chromatography ($CHCl_3$−MeOH, 30:1), to afford **13** (9 mg, 0.00023%) and **15** (3.7 mg, 0.000092%). F0209 (5 mg) was also purified by silica gel column chromatography ($CHCl_3$−MeOH, 30:1) and furnished **16** (1.0 mg, 0.000025%).

Fraction F03 (720 mg) was fractionated into 13 subfractions (F0301−F0313). Subfraction F0302 (25 mg) was subjected to HPLC separation (MeCN−H_2O, 27:73; 7 mL/min) and gave a mixture of **20** and **21** (t_R = 13.5 min) and compounds **19** (t_R = 19.9 min, 8.6 mg, 0.00022%) and **22** (t_R = 27.5 min, 1.3 mg, 0.000033%). This mixture (**20**, t_R = 24.0 min, 1.0 mg, 0.000025%; **21**, t_R = 25.3 min, 0.9 mg, 0.000023%) was purified by HPLC (MeCN−H_2O 25:75, 7 mL/min). From F0303 (190 mg), compounds **18** (1.5 mg, 0.000038%) and **19** (11 mg, 0.00028%) were isolated using silica gel column chromatography ($CHCl_3$−MeOH, 20:1). From F0309 (10 mg), compound **17** (12 mg, 0.0003%) was purified by silica gel column chromatography using a mixture of hexanes−EtOAc (1:1) as eluting solvents.

Identification of Isolated Compounds. *(+)-(6R,7S,8S)-Isolariciresinol* (**1**). $[\alpha]_D$ +33.0 (*c* 0.49, MeOH) [lit. $[\alpha]_D$ +34 (MeOH) (*17*)]. CD (MeOH) $\Delta\epsilon_{239}$ +0.2 $\Delta\epsilon_{274}$ +1.3 $\Delta\epsilon_{292}$ −0.2. ESIMS *m/z* 383 [M + Na]. ^{1}H and ^{13}C NMR data were consistent with the literature (*18*).

(+)-(6R,7S,8S)-5-Methoxyisolariciresinol (**2**). $[\alpha]_D$ +43.1 (*c* 0.20, MeOH) [lit. $[\alpha]_D$ +7.0 (MeOH) (*17*)]. CD (MeOH) $\Delta\epsilon_{240}$ +0.3 $\Delta\epsilon_{272}$ +0.3 $\Delta\epsilon_{290}$ −0.3. ESIMS *m/z* 413 [M + Na]. ^{1}H and ^{13}C NMR data were consistent with the literature (*17, 18*).

erythro-1-(4-Hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxy-phenoxy]-1,3-propanediol (**3**). $[\alpha]_D$ −24.0 (*c* 0.1, MeOH). ESIMS *m/z* 401 [M + Na]. ^{1}H and ^{13}C NMR data were consistent with the literature (*19–21*).

threo-1-(4-Hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**4**). $[\alpha]_D$ −14.3 (*c* 0.3, MeOH). ESIMS *m/z* 401 [M + Na]. ^{1}H and ^{13}C NMR data were consistent with the literature (*20–22*).

*(−)-Loliolide (**5**).* $[\alpha]_D$ −122.2 (*c* 0.09, MeOH) [lit. $[\alpha]_D$ −97.2 ($CHCl_3$) (*23*)]. CD (MeOH) $\Delta\epsilon_{218}$ −20.5. ESIMS *m/z* 219 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*23–25*).

*(−)-7R,8S-Dihydrodehydroconiferyl Alcohol (**6**).* $[\alpha]_D$ −25.0 (*c* 0.10, MeOH) [lit. $[\alpha]_D$ −22.2 (MeOH) (*26*)]. CD (MeOH) $\Delta\epsilon_{209}$ +1.6 $\Delta\epsilon_{290}$ −0.42 ESIMS *m/z* 383 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*26, 27*).

*(+)-7R,8S-5-Methoxydihydrodehydroconiferyl Alcohol (**7**).* $[\alpha]_D$ +32.2 (*c* 0.11, MeOH) [lit. $[\alpha]_D$ +4.7 (MeOH) (*17*)]. CD (MeOH) $\Delta\epsilon_{213}$ +1.5 $\Delta\epsilon_{295}$ −0.08. ESIMS *m/z* 413 [M + Na]. ^1H NMR (400 MHz, CD_3OD): δ 6.83 (2H, s, H-2 and 6), 6.67 (2H, s, H-2′ and 6′), 5.49 (1H, d, J = 6.3 Hz, H-7), 3.86 (3H, s, OCH_3-3′), 3.83 (1H, m, H-9a), 3.75 (1H, dd, J = 9.2, 7.3 Hz, H-9b), 3.80 (6H, s, 2 × OCH_3-3 and 5), 3.56 (2H, t, J = 6.5 Hz, H-9′), 3.46 (1H, m, H-8), 2.62 (2H, t, J = 7.7 Hz, H-7′), 1.81 (2H, pentet, J = 7.0 Hz, H-8′). ^{13}C NMR (100 MHz, CD_3OD): δ 149.3 (C-3 and 5), 147.5 (C-3′), 145.2 (C-4′), 137.0 (C-4), 134.0 (C-1 and C-1′), 129.8 (C-5′), 117.9 (C-6′), 114.1 (C-2′), 104.1 (C-2 and C-6), 89.1 (C-7), 65.0 (C-9), 62.2 (C-9′), 56.5 (C-8), 56.4 (OCH_3-3 and 5), 55.6 (OCH_3-3′), 35.8 (C-8′), 32.9 (C-7′). ^1H and ^{13}C NMR data in the literature (*28*) were not available and, hence, were assigned based on 2D NMR spectroscopic measurements.

*(+)-(7S,8R,8′R)-Lariciresinol (**8**).* $[\alpha]_D$ +33.4 (*c* 0.23, MeOH) [lit. $[\alpha]_D$ +30 (MeOH) (*29*)]. CD (MeOH) $\Delta\epsilon_{244}$ −0.42 $\Delta\epsilon_{290}$ −0.26. ESIMS *m/z* 383 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*29*).

*3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**9**).* ESIMS *m/z* 219 [M + Na]. ^1H and ^{13}C NMR data consistent with the literature (*30*).

*3,4′-Dihydroxy-3′-methoxypropiophenone (**10**).* ESIMS *m/z* 249 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*31*).

*Dihydroconiferyl Alcohol (**11**).* ESIMS *m/z* 205 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*32*).

*2,6-Dimethoxy-1,4-benzoquinone (**12**).* ESIMS *m/z* 191 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*33*).

*(+)-Pinoresinol (**13**).* $[\alpha]_D$ +10.0 (*c* 0.44, MeOH) [lit. $[\alpha]_D$ +27.2 ($CHCl_3$) (*34*)]. ESIMS *m/z* 381 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*34*).

*(+)-Syringaresinol (**14**).* $[\alpha]_D$ +10.6 (*c* 0.47, MeOH) [lit. $[\alpha]_D$ +10.4 (MeOH) (*35*)]. ESIMS *m/z* 441 [M + Na]. ^1H and ^{13}C NMR data consistent with the literature (*35*).

*Protocatechuic Acid Methyl Ester (**15**).* ESIMS *m/z* 191 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*36*).

*Chrysoeriol (**16**).* ESIMS *m/z* 323 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*37*).

*(2R,3R)-Dihydrokaempferol (**17**).* $[\alpha]_D$ +22.1 (*c* 0.60, MeOH) [lit. $[\alpha]_D$ +25.9 (MeOH) (*38*)]. CD (MeOH) $\Delta\epsilon_{290}$ −11.6 $\Delta\epsilon_{329}$ +3.2. ESIMS *m/z* 311 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*38*).

*Apigenin (**18**).* ESIMS *m/z* 293 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*38*).

*(S)-Menthiafolic Acid (**19**).* $[\alpha]_D$ +14.8 (*c* 0.25, $CHCl_3$) [lit. $[\alpha]_D$ +18.2 (MeOH) (*39*)]. ESIMS *m/z* 207 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*39*).

*(E,Z)-2,6-Dimethyl-2,6-octadiene-1,8-diol (**20**).* ESIMS *m/z* 193 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*40*).

*(E,E)-2,6-Dimethyl-2,6-octadiene-1,8-diol (**21**).* ESIMS *m/z* 193 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*40*).

*(4R)-4-[(1E)-3-Hydroxy-1-butenyl]-3,5,5-trimethyl-2-cyclohexen-1-one (**22**).* $[\alpha]_D$ +200 (*c* 0.09, MeOH) [lit. $[\alpha]_D$ +292 (CH_2Cl_2)]. CD (MeOH) $\Delta\epsilon_{244}$ +6.3 $\Delta\epsilon_{318}$ −3.2. ESIMS *m/z* 231 [M + Na]. ^1H and ^{13}C NMR data were consistent with the literature (*41*).

Evaluation of Hydroxyl Radical Scavenging Activity. Hydroxyl radical (•OH) scavenging activity was conducted according to a previously described method (*15, 42*). The final assay volume (250 μL) contained 160 μL of freshly prepared mixed solution (1.2 mM H_2O_2 and 0.2 mM $FeSO_4$ in 50 mM phosphate buffer at pH 7.4) with 10 μL of test sample in 25% DMSO solution, which was incubated at 37 °C for 5 min. Then, 80 μL of esterase (1.0 units/mL)-treated HDCF-DA (2 μM) in 50 mM phosphate buffer (pH 7.4) was added and mixed well. Changes in •OH radicals were measured using an FLx800 fluorescence spectrophotometer (Bio-Tek) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm after 30 min.

Evaluation of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity. The scavenging activity of the stable DPPH free radical was measured using a established method (*43*). Reaction mixtures containing test samples (dissolved in DMSO) and 316 μM DPPH ethanolic solution in 96-well microtiter plates were incubated at 37 °C for 30 min and then were measured at 515 nm.

Evaluation of Cytoprotective Activity. A cytoprotective assay was performed using a published method with 96-well plates (*16, 43*). Cells (MCF-7; human breast cancer) were seeded in 96-well plates at a density of 7200 cells per well. After they were incubated for 24 h, the cells were treated with the test samples at a concentration of 20 μg/mL and then incubated for 40 min at 37 °C. After incubation for 40 min, 10 μL of H_2O_2 (0.4 mM, final concentration) were added to each well, and the plates were incubated for 5 h at 37 °C. The cytoprotective activity of the test samples was determined based on their cellular protein content by staining with sulforhodamine B. The group without any treatment was used for the control. The absorbance was measured at 515 nm on an enzyme-linked immunosorbent assay (ELISA) reader. The samples were prepared in 10% aqueous DMSO. The relative cytoprotection to the control was calculated by the following method:

$$\text{cytoprotection (\%)} = 100 \times (\text{sample-treated group} - \text{hydrogen peroxide-treated group})/(\text{no treatment group} - \text{hydrogen peroxide-treated group})$$

Evaluation of Cytotoxicity. A cytotoxicity assay was performed using an established method with 96-well plates (*44*). The harvested cells (MCF-7), after appropriate dilutions (7×10^4 cells/mL), were added to 96-well plates containing the test samples dissolved in DMSO, and negative control wells contained DMSO only. The plates were incubated for 3 days at 37 °C in 5% CO_2. On the third day, the cells were fixed to the plates by the addition of cold 20% trichloroacetic acid and incubated for 30 min. The plates were washed with tap water and dried overnight. The fixed cells were dyed with a dilute acetic acid solution of sulforhodamine B, an anionic protein stain, and incubated for 30 min. The plates were washed with dilute acetic acid and allowed to dry. Then, the dye was solubilized with dilute Tris base, and the plates were read at 515 nm with an ELISA reader. The ED_{50} values of test samples were calculated using nonlinear regression analysis (TableCurve2DV4; AISN Software Inc., Mapleton, OR).

RESULTS AND DISCUSSION

Bioactivity-guided fractionation for the chloroform-soluble extract of the fruits of *E. oleracea* (açai) led to the isolation and characterization of 22 known compounds. The structures of the known compounds were confirmed by comparing their physical and spectroscopic data ([α], CD, ^1H and ^{13}C NMR, 2D NMR, and MS) with literature values. These were identified as (+)-isolariciresinol (**1**), (+)-5-methoxy-isolariciresinol (**2**), *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**3**), *threo*-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**4**), (−)-loliolide (**5**), (−)-(7*R*,8*S*)-dihydrodehydroconiferyl alcohol (**6**), (+)-(7*R*,8*S*)-5-methoxy-dihydrodehydroconiferyl alcohol (**7**), (+)-lariciresinol (**8**), 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**9**), 3,4′-dihydroxy-3′-methoxypropiophenone (**10**), dihydroconiferyl alcohol (**11**), 2,6-dimethoxy-1,4-benzoquinone (**12**), (+)-pinoresinol (**13**), (+)-syringaresinol (**14**), protocatechuic acid methyl ester (**15**), chrysoeriol (**16**), (2*R*,3*R*)-dihydrokaempferol (**17**), apigenin (**18**), (+)-menthiafolic acid (**19**), (*E*,*Z*)-2,6-dimethyl-2,6-octadiene-1,8-diol (**20**), (*E*,*E*)-2,6-dimethyl-2,6-octadiene-1,8-diol (**21**), and (4*R*)-4-[(1*E*)-3-hydroxy-1-butenyl]-3,5,5-trimethyl-2-cyclohexen-1-one (**22**). Except for compound **15**, all of these isolates were isolated and characterized from

Table 1. DPPH Hydroxyl Radical Scavenging Activities of Isolated Compounds **1**−**22**

compound	DPPH (IC_{50}, μg/mL[a])	hydroxyl radical (IC_{50}, μg/mL[a])	compound	DPPH (IC_{50}, μg/mL[a])	hydroxyl radical (IC_{50}, μg/mL[a])
1	37.4 ± 0.9	0.68 ± 0.02	**12**	>40	1.7 ± 0.12
2	33.0 ± 2.2	0.56 ± 0.02	**13**	34.7 ± 5.0	1.8 ± 0.20
3	>40	3.7 ± 0.50	**14**	29.7 ± 2.0	0.40 ± 0.13
4	>40	3.5 ± 0.23	**15**	7.5 ± 0.4	1.1 ± 0.11
5	>40	>5	**16**	>40	1.4 ± 0.16
6	>40	3.3 ± 0.24	**17**	>40	2.7 ± 0.31
7	NT[b]	0.98 ± 0.05	**18**	NT	>5
8	22.4 ± 3.0	0.70 ± 0.13	**19**	<40	>5
9	>40	>5	**20**	>40	>5
10	>40	>5	**21**	>40	>5
11	32.6 ± 1.3	0.91 ± 0.04	**22**	NT	>5
quercetin		0.31 ± 0.02	gallic acid	3.7 ± 0.4	

[a] Each value represents the mean ± SD ($n = 3$). [b] Not tested due to the limited samples available.

this plant for the first time. The most abundant of these identified constituents were the lignans (**1**−**4**, **6**−**8**, **13**, and **14**), a compound class that has not been reported from açai fruits previously.

All of these constituents of *E. oleracea* fruits obtained in the present study were tested in an in vitro hydroxyl radical scavenging assay, and the results are summarized in the **Table 1**. Lignans **1**−**4**, **6**−**8**, **13**, and **14** and 2,6-dimethoxy-1,4-benzoquinone (**12**) exhibited potent activities in the IC_{50} value range 0.6−3.7 μg/mL. Also, dihydroconiferyl alcohol (**11**), protocatechuic acid methyl ester (**15**), chrysoeriol (**16**), and dihydrokaempferol (**17**) showed antioxidant effects ($IC_{50} = 0.91$, 1.1, 1.4, and 2.7 μg/mL, respectively) in this radical scavenging assay. The isolated compounds were also tested in DPPH radical scavenging assay (**Table 1**). Of these, it was found that compounds **1**, **2**, **8**, **11**, and **13**−**15** were active in this antioxidant assay.

Of five lignan type compounds (**1**, **2**, **8**, **13**, and **14**) obtained, pinoresinol (**13**) has been reported frequently as an antioxidant principle of various plants, using thiocyanate antioxidant (*45*), Cu^{2+}-induced low density lipoprotein oxidation (*46*), lipid peroxidation in rat liver (*47, 48*), DPPH radical (*49*), and peroxy radical assays (*48*). Similarly, syringaresinol (**14**) has been demonstrated as antioxidative in Cu^{2+}-induced low-density lipoprotein oxidation (*46*) and DPPH radical assays (*50–52*). In turn, isolariciresinol (**1**) was reported to exhibit antioxidant activity in a Trolox equivalent antioxidant capacity (TEAC) test (*36*). Interestingly, the antioxidant effect of 5-methoxyisolariciresinol (**2**) does not seem to have been investigated previously. Antioxidant activities of lariciresinol (**8**) have been observed in the lipid peroxidation (*48, 53, 54*), peroxy radical (*47, 52*), and superoxide radical assay systems (*53*). There have been no reports on the antioxidant activities of neolignans **3**, **4**, **6**, and **7**, as well as benzoquinone **12**. Recently, dihydroconiferyl alcohol (**11**) and some derivatives were studied for DPPH radical scavenging activities, and this compound was found to be active (*56*). Protocatechuic acid methyl ester (**15**) was tested in DPPH radical (*52, 57–59*), linoleic acid autoxidation (*60*), and TEAC (*36, 61*) methods and found to have activity in all assay systems used.

The most active compounds in the radical scavenging assay were then evaluated for cytotoxicity against MCF-7 cells to select active compounds without cytotoxicity for a cytoprotective experiment. Among those compounds tested in the cytotoxic assay, only one isolate, 2,6-dimethoxy-1,4-benzoquinone (**12**), exhibited a cytotoxic effect (ED_{50}, 0.8 μg/mL) against MCF-7 cells, in a manner consistent with previous literature (*62, 63*). Thus, except for this compound, all of the active compounds in the hydroxyl radical assay were used for the cytoprotective evaluation using the MCF-7 cell line stressed by H_2O_2. As shown in **Figure 1**, three lignans (**8**, **13**, and **14**) at 44, 38, and 53%, respectively, as well as the phenylpropanoid, dihydroconiferyl alcohol (**11**, 31%), and the benzenoid, protocatechuic acid methyl ester (**15**, 74%), were found to display significant cytoprotective effects in this assay as compared to the control.

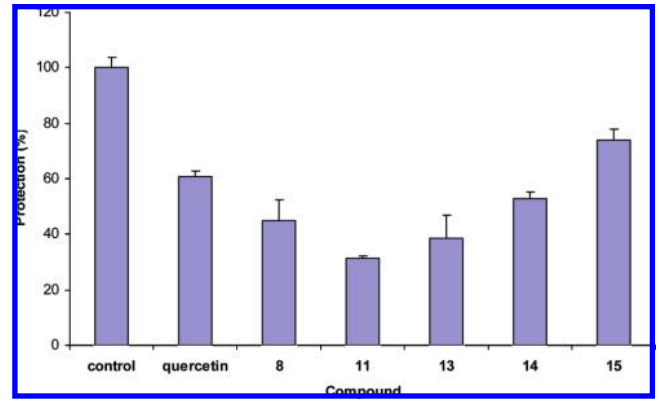

Figure 1. Cytoprotective effects of the isolates (20 μg/mL) in cultured MCF-7 cells stressed by H_2O_2. Each value represents the mean ± SD ($n = 3$). Significantly different from control ($p < 0.01$).

Several independent groups (*3, 5, 9, 10*) have found that açai extracts exhibit antioxidant effects. Lichtenthaler et al. (*9*) analyzed the correlations between the antioxidant effects (peroxy radicals, peroxynitrites, and hydroxyl radicals) and the two major anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, and came to the conclusion that the antioxidant capacity of these anthocyanidins in the fruits may be responsible for 10% of the overall antioxidant activity of the açai extract investigated. Similar results were also observed for a seed extract of *E. oleracea* by the same group in 2006 (*3*). Thus, beside anthocyanins, other so far undetermined secondary metabolites of açai have been proposed as antioxidant constituents. In the present study using a bioactivity-guided fractionation procedure, a benzenoid, a benzoquinone, a phenylpropanoid, two flavonoids, and nine lignans were identified using an in vitro hydroxyl radical assay as new antioxidant constituents of açai fruits. The lignans are of the aryltetrahydronaphthalene (**1** and **2**), dihydrobenzofuran (**6** and **7**), furofuran (**13** and **14**), 8-*O*-4′ neolignan (**3** and **4**), and tetrahydrofuran (**8**) structural types. Moreover, in a cell-based assay, procatechuic acid methyl ester (**15**) was found to be the most cytoprotective compound (74%), comparable to the positive control used, quercetin (60%). Therefore, the bioactive constituents found in the present investigation may account, in part, for the antioxidant activities of *E. oleacea* fruits and serve as standard markers for açai preparations with regard to antioxidant activities.

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