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Absorption and Biological Activity of Phytochemical-Rich Extracts from Açai (*Euterpe oleracea* Mart.) Pulp and Oil in Vitro

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Polyphenolic extracts from various fruits and vegetables have been shown to exert growth inhibitory effects in cell culture studies. Whereas individual polyphenolic compounds have been extensively evaluated, understanding of the biological activity of polyphenolic extracts from natural sources is limited and critical to the understanding of their potential effects on the human body. This study investigated the absorption and antiproliferative effects of phytochemical extracts from açai pulp and a polyphenolic-enriched açai oil obtained from the fruit pulp of the açai berry (*Euterpe oleracea* Mart.). Chemical composition, antioxidant properties, and polyphenolic absorption of phytochemical fractions in a Caco-2 monolayer were determined, along with their cytotoxicity in HT-29 human colon adenocarcinoma cells. Standardized extracts were characterized by their predominance of hydroxybenzoic acids, monomeric flavan-3-ols, and procyanidin dimers and trimers. Polyphenolic mixtures (0−12 μg of gallic acid equiv/mL) from both açai pulp and açai oil extracts inhibited cell proliferation by up to 90.7%, which was accompanied by an increase of up to 2.1-fold in reactive oxygen species. Absorption experiments using a Caco-2 intestinal cell monolayer demonstrated that phenolic acids such as *p*-hydroxybenzoic, vanillic, syringic, and ferulic acids, in the presence of DMSO, were readily transported from the apical to the basolateral side along with monomeric flavanols such as (+)-catechin and (−)-epicatechin. Results from this study provide further evidence for the bioactive properties of açai polyphenolics and offer new insight on their composition and cellular absorption.

KEYWORDS: Açai; polyphenolic; absorption; cell proliferation; in vitro

INTRODUCTION

The intake of fruits and vegetables has been associated with a lower incidence of chronic diseases, including cancer (*1*). In addition to their antioxidant properties and ability to protect vital cellular components from oxidative stress, polyphenolics have also been shown to inhibit cell proliferation, induce apoptosis, alter cell cycle kinetics, and interfere with intracellular signal transduction in several in vitro cancer cell models (*2–5*). Information on the inhibitory effects of individual polyphenolic compounds on carcinogenesis is abundant; however, it has been suggested that isolated compounds may not exhibit the same properties as complex mixtures of polyphenolics in whole foods or extracts (*6*). Thus, studies on phytochemical mixtures in specific commodities may provide information that more appropriately reflects the potential health benefits associated with the consumption of the entire fruit.

Açai (*Euterpe oleracea* Mart.), a palm fruit native to the Amazon estuary, has received much attention in recent years due to potential health benefits associated with its high antioxidant capacity and phytochemical composition (*7–11*). However, reports on its biological properties are limited (*12*). The present study evaluated the antiproliferative activities of phytochemical-rich extracts from açai fruit in HT-29 human colon adenocarcinoma cells. Polyphenolic extracts from a phytochemical-enriched açai oil, found to contain concentrated amounts of phenolic acids and flavonoids naturally present in açai fruit (*13*), were compared to non-anthocyanin polyphenolic fractions from açai pulp. Although some studies have evaluated the bioavailability of certain polyphenolics in vitro (*14–16*), information on the cellular absorption of polyphenolics, particularly mixtures, is still very limited. In this study, polyphenolic absorption from açai extracts was evaluated using a Caco-2 human intestinal cell monolayer as an in vitro model for intestinal absorption, as previously described (*14, 15*).

Results from this study will provide new data on the in vitro biological activity and cellular absorption of polyphenolics from açai pulp and açai oil.

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Table 1. Concentration and Relative Abundance of Polyphenolics Present in Açai Pulp and Açai Oil Extracts

polyphenolic	açai pulp[a] (mg/L)	rel abundance[b] (%)	açai oil[a] (mg/L)	rel abundance[b] (%)
protocatechuic acid	159 ± 12	2.3 ± 0.1 a	540 ± 30	7.8 ± 0.4 b
p-hydroxybenzoic acid	172 ± 14	2.4 ± 0.3 a	570 ± 31	8.2 ± 0.9 b
(+)-catechin	491 ± 25	7.0 ± 0.8 a	67 ± 6.0	1.0 ± 0.1 b
vanillic acid	577 ± 40	8.2 ± 0.5 a	1610 ± 64	23 ± 1.8 b
syringic acid	434 ± 23	6.1 ± 0.7 a	969 ± 47	14 ± 1.1 a
(−)-epicatechin	446 ± 37	6.3 ± 0.2		
ferulic acid	122 ± 13	1.7 ± 0.3 a	109 ± 10	1.6 ± 0.2 a
procyanidin dimers	2300 ± 150	33 ± 2.3 a	1030 ± 130	15 ± 1.2 b
procyanidin trimers	2370 ± 180	34 ± 3.1 a	2040 ± 59	29 ± 2.7 a

[a] Values represent non-anthocyanin polyphenolic concentrations equivalent to single-strength açai oil and correspond to a 300-fold concentrated açai pulp. [b] Values with different letters between columns represent a significant difference (paired samples *t* test, $p < 0.05$).

MATERIALS AND METHODS

Phytochemical Extracts. Frozen, pasteurized açai pulp was kindly donated by Bossa Nova Beverage Group (Los Angeles, CA) and shipped overnight to the Department of Nutrition and Food Science at Texas A&M University. Prior to polyphenolic isolation, açai pulp was clarified according to a previously described procedure (*10*) to remove insoluble solids. Phenolic acids and non-anthocyanin flavonoids were isolated from the clarified açai pulp by repeated liquid/liquid extraction with ethyl acetate (1:1 ratio). The upper ethyl acetate fraction was recovered, passed through a 5 cm bed of sodium sulfate to remove residual water, evaporated under vacuum (<40 °C), and redissolved in dimethyl sulfoxide (DMSO). Açai oil was extracted from a water-insoluble filter cake that was commercially used to clarify açai pulp using a patent-pending process (*17*) from byproduct obtained during the clarification process. Polyphenolics present in the resultant açai oil were extracted (three times) by the addition of methanol and water (80:20 v/v mixture) and centrifuged at 5000*g* for 15 min. The methanolic extracts were then recovered, pooled, and concentrated under vacuum at <40 °C until complete solvent removal. The resultant extract was likewise reconstituted in DMSO to enhance solubility and used for further analyses.

Polyphenolic isolates from both açai oil and açai pulp were standardized to a total soluble phenolic content of 1200 mg of gallic acid equiv (GAE)/L, corresponding to the total soluble phenolic content of single-strength açai oil and equivalent to a 300-fold concentrate of the açai pulp. Total soluble phenolics were analyzed by using the Folin−Ciocalteu assay, according to a previously described procedure (*18*), and quantified against a gallic acid standard curve. All polyphenolic isolates were sterile-filtered prior to use in cell culture experiments and normalized to a final concentration of 0.1% DMSO when applied to the cells. A control with 0.1% DMSO was included in all assays.

Chemical Analyses. Polyphenolic isolates were analyzed by reversed phase HPLC with a Waters 2695 Alliance system (Waters Corp., Milford, MA), according to previously described chromatographic conditions (*11*). Identification and quantitation of polyphenolics were based on their spectral characteristics and retention time, as compared to authentic standards (Sigma Chemical Co., St. Louis, MO). Compound identities were further confirmed by mass spectrometric analyses, performed on a Thermo Finnigan LCQ Deca XP Max MS^n ion trap mass spectrometer equipped with an ESI ion source (Thermo Fisher, San Jose, CA). Separations were conducted using the Phenomenex (Torrance, CA) Synergi 4 μHydro-RP 80A (2 × 150 mm; 4 μm; S/N = 106273−106275) with a C18 guard column. Mobile phases consisted of 0.5% formic acid in water (phase A) and 0.5% formic acid in 50:50 methanol and acetonitrile (phase B) run at 0.25 mL/min. Polyphenolics were separated with a gradient elution program in which phase B changed from 5 to 30% in 5 min, from 30 to 65% in 70 min, and from 65 to 95% in 30 min and was held isocratic for 20 min. Ionization was conducted in the negative ion mode under the following conditions: sheath gas (N_2), 60 units/min; auxiliary gas (N_2), 5 units/min; spray voltage, 3.3 kV; capillary temperature, 250 °C; capillary voltage, 1.5 V; tube lens offset, 0 V. Total soluble phenolic contents were determined using the Folin−Ciocalteu assay (*18*) quantified in gallic acid equivalents (GAE) as was used to quantify normalized concentrations between açai pulp and açai oil extracts. Antioxidant capacity was determined by the oxygen radical absorbance capacity assay using a BMG Labtech FLUOstar fluorescent microplate reader (485 nm excitation and 538 nm emission), as previously described (*19*). Results were quantified in micromoles of Trolox equivalents per milliliter of extract.

Cell Proliferation. HT-29 human colon adenocarcinoma cells were obtained from American Type Culture Collection (ATCC, Manassas, VA), cultured in Dulbecco's modified Eagle's medium (1×) (DMEM) containing 5% fetal bovine serum, 1% nonessential amino acids, 100 units/mL penicillin G, 100 μg/mL streptomycin, 1.25 μg/mL amphotericin B, and 10 mM sodium pyruvate (Gibco BRL Life Technology, Grand Island, NY). Cells were incubated at 37 °C under 5% CO_2 and utilized between passages 10 and 20. Cells (5×10^4 cells/well) were seeded into each well of a 12-well tissue culture plate. After a 24 h incubation, the growth medium was replaced with 1000 μL of medium containing different concentrations of standardized polyphenolic extracts (from 0.04 to 12 μg of GAE/mL). Following incubation for 48 h, cell numbers were determined using a Beckman Coulter Particle Counter (Fullerton, CA). Cell numbers were expressed as a percentage of the 0.1% DMSO control. The extract concentration at which cell proliferation was inhibited by 50% (IC_{50}) was calculated by linear regression analyses on percentage cell inhibition as a ratio to the DMSO control.

Generation of Reactive Oxygen Species (ROS). The dichlorofluorescein (DCF) assay was performed according to a previously described procedure (*20*). HT-29 human colon adenocarcinoma cells (5×10^4/mL) were passed into 96-well plates and incubated for 24 h. Cells were washed twice with PBS and preloaded with dichlorofluorescein diacetate (DCFH-DA) substrate by incubation with 10 μM DCFH-DA for 30 min at 37 °C. Cells were subsequently washed and incubated with the standardized extract concentrations previously described. Fluorescence was determined after 30 min of incubation with polyphenolics using a BMG Labtech FLUOstar fluorescent microplate reader (485 nm excitation and 538 nm emission).

Transepithelial Transport Model. Caco-2 colon carcinoma cells were obtained from ATCC, cultured in DMEM (1×) high glucose containing 10% fetal bovine serum, 1% nonessential amino acids, 100 units/mL penicillin G, 100 μg/mL streptomycin, 1.25 μg/mL amphotericin B, and 10 mM sodium pyruvate. Cells were incubated at 37 °C and 5% CO_2 (chemicals were obtained from Sigma-Aldrich Co.). Cells between passages 10 and 20 were seeded in 12 mm transparent polyester cell culture insert well plates (Transwell, Corning Costar Corp., Cambridge, MA) at 1.0×10^5 cells per insert with 0.5 mL of medium in the apical side and 1.5 mL of medium in the basolateral side. Cells were grown and differentiated to confluent monolayers for 21 days, as previously described (*21*). Transepithelial electrical resistance (TEER) values were monitored with an EndOhm Volt ohm-meter equipped with a STX-2 electrode (World Precision Instruments Inc., Sarasota, FL), and monolayers with TEER values >450 Ω cm^2 after correction for the resistance in control wells were used for transport experiments. TEER values were also obtained at the conclusion of transport experiments to ensure integrity of the monolayer. For transport studies, medium pH was adjusted to 6.0 on the apical side and 7.4 on the basolateral side using Hank's balanced salt solution (HBSS, Fischer Scientific, Pittsburgh, PA) containing 10 mM 2-(*N*-morpholino)ethanesulfonic acid solution (MES) and HBSS containing *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) buffer solution (1 M) (HEPES)

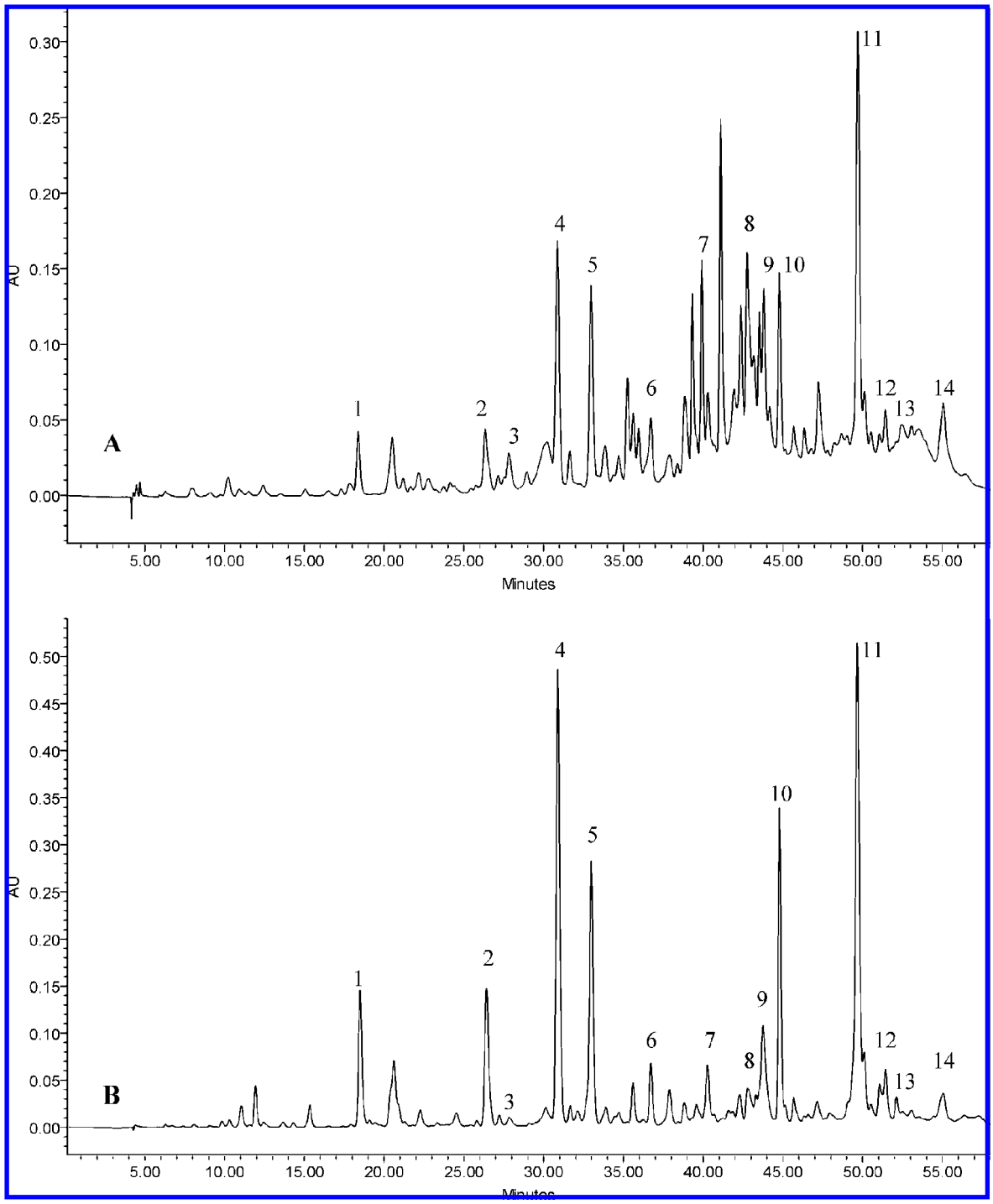

Figure 1. HPLC chromatogram of polyphenolics in phytochemical-rich extracts from açai pulp (**A**) and açai oil (**B**). Peak assignments: 1, protocatechuic acid; 2, *p*-hydroxybenzoic acid; 3, (+)-catechin; 4, vanillic acid; 5, syringic acid; 6−7, procyanidin dimers; 8, ferulic acid; 9−10, procyanidin dimers; 11−14, procyanidin trimers.

to create a pH gradient similar to the absorption sites in the small intestine environment (chemicals obtained from Gibco BRL Life Technology). Standardized polyphenolic extract solutions were diluted in HBSS (from 2.4 to 36 μg of GAE/mL) and loaded into the apical side of the cells. Transepithelial transport was followed over time, and sample aliquots (200 μL) were taken at 30, 60, and 120 min from the basolateral compartment, as previously described (*22, 23*). Samples were filtered prior direct injection into the HPLC.

Statistical Analysis. Data from in vitro experiments were analyzed by one-way analysis of variance (ANOVA) using SPSS version 15.0 (SPSS Inc., Chicago, IL). Mean separations were conducted using posthoc Tukey−Kramer HSD ($p < 0.05$) pairwise comparisons. Correlation and linear regression analyses were conducted using a significance level of 0.05.

RESULTS AND DISCUSSION

Chemical Analyses. Characterizations of the predominant polyphenolics present in açai fruit were included in our previous papers (*10, 11*). Açai fruits are especially high in the anthocyanins cyanidin-3-rutinoside and cyanidin-3-glucoside, but in the manufacture of açai oil these compounds are not retained, whereas numerous phenolic acids and flavan-3-ols are solubilized in the açai oil following isolation from açai pulp

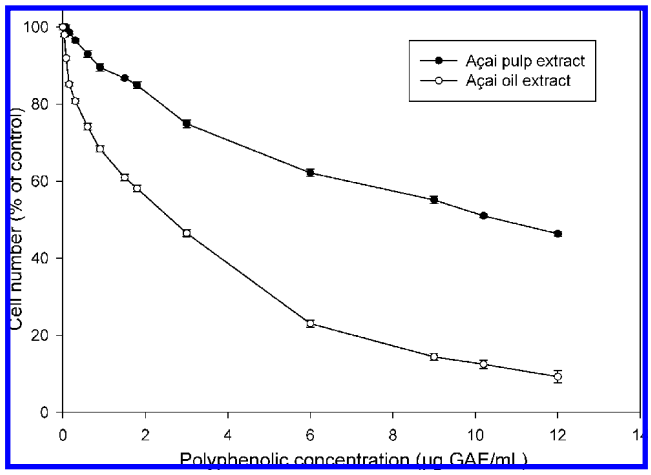

Figure 2. Percent changes in total HT-29 cell numbers expressed as a ratio to control cells following treatment of cells with açai pulp or açai oil polyphenolic extracts adjusted to different concentrations (expressed as μg of gallic acid equivalents, GAE/mL) for 48 h. Error bars represent the standard error of the mean ($n = 6$).

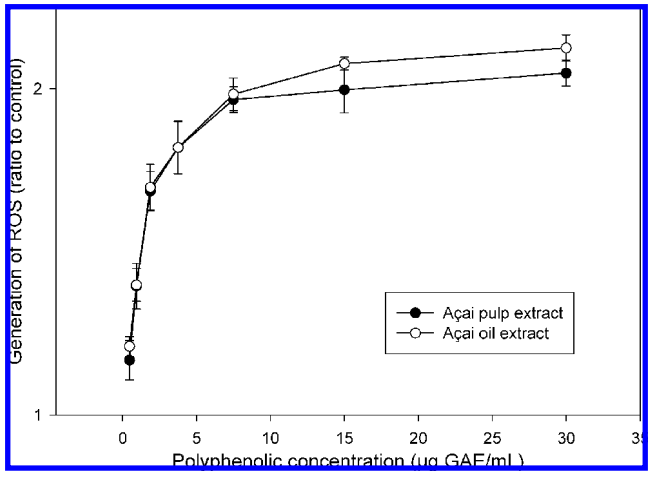

Figure 3. Intracellular levels of reactive oxygen species in HT-29 cells following treatment with açai pulp or açai oil polyphenolic extracts adjusted to different concentrations (expressed in micrograms of gallic acid equivalents, GAE/mL). Error bars represent the standard error of the mean ($n = 6$).

clarification. Therefore, biological activities of phytochemical-rich extracts from açai oil were compared to non-anthocyanin polyphenolic extracts from açai pulp and their respective chemical compositions contrasted under identical chromatographic conditions. Both açai oil and açai pulp contained similar phenolic acids, flavonoids, and procyanidins, but these compounds were present in different ratios in their respective matrices. Phenolic acids present were confirmed by LC-MS in negative ionization mode and included protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, and ferulic acid along with the monomeric flavonols (+)-catechin and (−)-epicatechin. Each was identified on the basis of UV and mass spectrometric characteristics as compared to authentic standards. In addition, several procyanidin dimers and trimers were identified on the basis of their distinctive fragmentation patterns and spectral similarities to (+)-catechin and (−)-epicatechin. Procyanidin dimers were characterized by signals at *m/z* 577.1 and major fragments at *m/z* 425.0 and 289.2. Procyanidin trimers (*m/z* 865.1) were additionally characterized by predominant product ions at *m/z* 577.2, 425.0, and 289.2. Individual polyphenolic concentrations in açai pulp and açai oil extracts are presented herein (**Table 1**). Similar polyphenolic profiles were observed in the açai oil extracts and non-anthocyanin polyphenolic extracts of açai pulp (**Figure 1**); however, their absolute and relative ratios differed markedly, which was likely a contributing factor to their various bioactivities. Individual phenolic acid concentrations were up to 3.4-fold higher in açai oil extracts ranging from 540 to 1607 mg/L compared to acai pulp extracts at 159−577 mg/L. Ferulic acid was the one exception with nearly equivalent concentrations in açai oil and pulp. In contrast, flavanols such as (+)-catechin and (−)-epicatechin were abundant in pulp extracts but only sparingly present in extracts from açai oil. Likewise, procyanidin dimers in açai pulp extracts were twice those present in açai oil extracts, yet equivalent concentrations of procyanidin trimers were present in both extracts. Differences in composition between the açai extracts were attributed in part to extraction protocols, but primarily to the difference in the matrices (aqueous versus lipophilic) from which the polyphenolics were derived. Due to these differences in polyphenolics, the açai extracts were normalized to an equivalent concentration of total soluble phenolics (1200 mg of GAE/L) for their subsequent use in cell culture experiments. This concentration was comparable to concentrations originally present in the açai oil but a 300 -fold concentration of polyphenolics present in açai pulp following anthocyanin removal. Resultant antioxidant capacity of the normalized extracts was 17.2 ± 0.16 μmol of Trolox equiv/mL for the açai pulp extract and 15.3 ± 0.11 μmol of Trolox equiv/mL for the açai oil extract due to differences in individual polyphenolics present.

Cell Proliferation. The antiproliferative activities of açai pulp and açai oil extracts were evaluated in a cell culture model using HT-29 colon carcinoma cells. Total cell numbers were indicative of the proliferative activity of HT-29 cells and the cytotoxic effects of açai extracts. Both polyphenolic extracts caused significant ($p < 0.01$) decreases in total cell numbers in a concentration-dependent manner (**Figure 2**). However, polyphenolic extracts from açai oil were more than twice as effective in reducing total cell numbers across all dilutions, with an IC_{50} of 4.5 μg of GAE/mL compared to 10.2 μg of GAE/mL for açai pulp extracts. Previous studies have reported synergistic effects among polyphenolic mixtures in cell culture models (*24–26*), and interactions among phenolic acids and flavanols may play an important role in the growth inhibitory action of açai pulp and açai oil extracts on cell proliferation observed in this study. Differences in the inhibitory effects by açai pulp and açai oil extracts may be due to their polyphenolic composition, because standardized açai oil extracts had 3.4-fold higher phenolic acid and 2−14-fold lower monomeric and dimeric flavanol concentrations than standardized extracts from pulp. Thus, results from this study suggest that cancer cell growth inhibition by these extracts is associated with phenolic acids and their interactions among polyphenolic components. Previous studies on cranberries in HT-29 cell models have suggested nonadditive interactions involving flavanol derivatives (*27, 28*), whereas potent growth inhibitory effects of cloudberry, bilberry, raspberry, black currant, strawberry, and lingonberry extracts were attributed to synergistic interactions among non-anthocyanin polyphenolics, including phenolic acids and flavanols, that enhanced expression of $p21^{WAF1}$ as an inducer of cell cycle arrest and apoptosis (*29*).

Cell growth inhibition by polyphenolic-rich açai extracts were related to total soluble phenolic concentrations originally present in açai pulp (4 μg of GAE/mL) and in the açai oil (1200 μg of GAE/mL). Therefore, 10 μg of GAE/mL of açai pulp extract

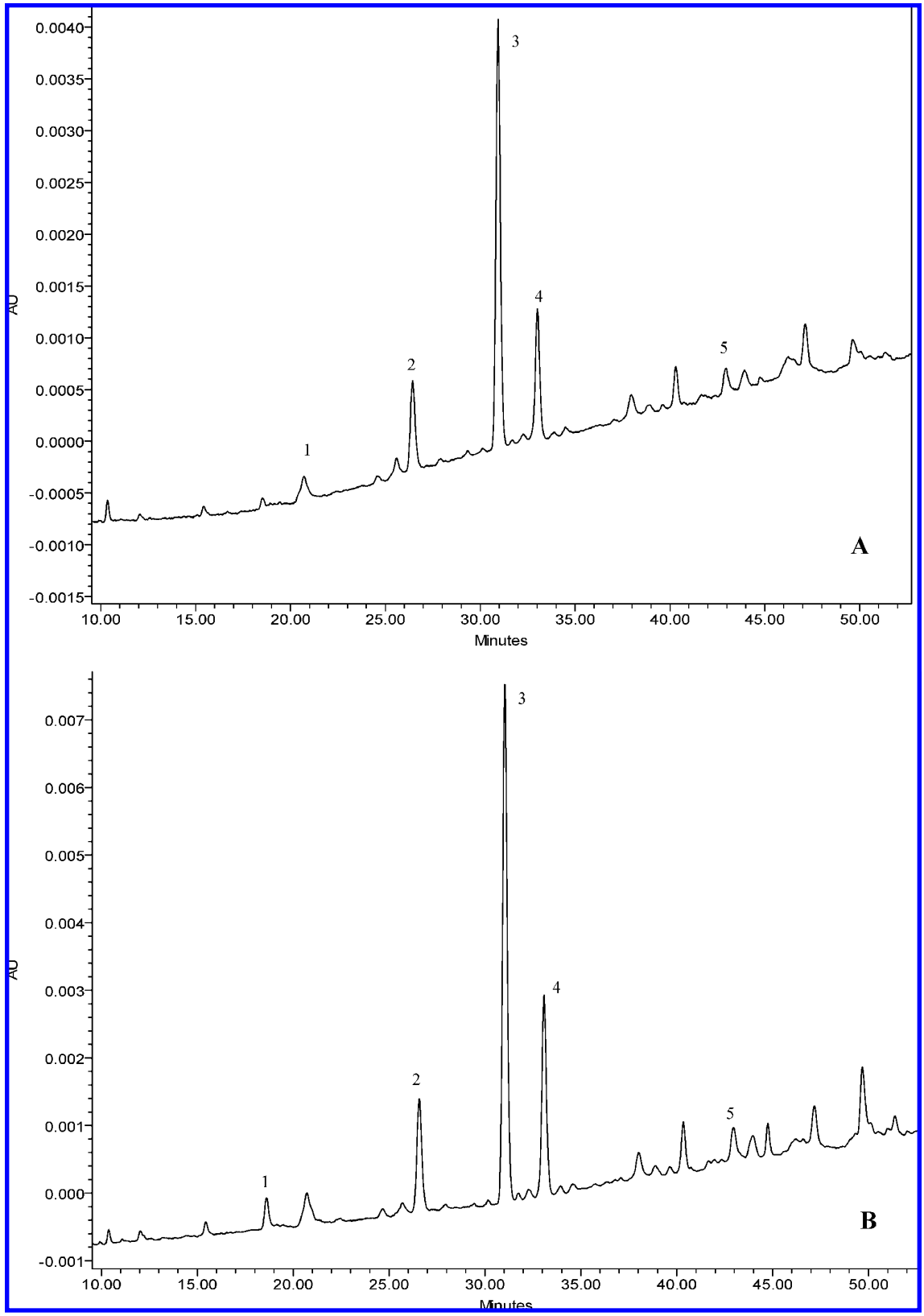

Figure 4. Typical HPLC chromatogram of polyphenolics present in the basolateral compartment of Caco-2 cell monolayers following incubation with açai pulp (**A**) and açai oil (**B**) extracts for 2 h. Peak assignments: 1, protocatechuic acid; 2, *p*-hydroxybenzoic acid; 3, vanillic acid; 4, syringic acid; 5, ferulic acid.

resulted in 50% reduction of cell proliferation, whereas comparable inhibition was observed for only 3 μg of GAE/mL of the açai oil extract (**Figure 2**). Therefore, 10 μg of GAE of açai pulp extract, which corresponded to the phenolic equivalent present in 2.5 mL of açai pulp, and 3 μg GAE of açai oil extract, which corresponded to the equivalent of 2.5 μL of açai oil, were both equivalent in terms of their inhibitory effects on cell proliferation. These observations suggest that the polyphenolic-enriched açai oil, obtained from a water-insoluble filter cake used commercially to clarify açai pulp, was >3 times more

Table 2. Average Transport Rates of Polyphenolics from Açai Pulp and Açai Oil Extracts from the Apical to the Basolateral Side of Caco-2 Cell Monolayers, as a Function of Total Soluble Phenolic Contents

	transport (μg/mL·h) of polyphenolics from açai pulp[a]					transport (μg/mL·h) of polyphenolics from açai oil[a]				
polyphenolic[b] (μg)	12 min	30 min	60 min	120 min	180 min	12 min	30 min	60 min	120 min	180 min
p-hydroxybenzoic acid	0.02 ± 0.003a	0.24 ± 0.03c	0.36 ± 0.04d	0.49 ± 0.05e	0.73 ± 0.06f	0.06 ± 0.01b	0.77 ± 0.06f	1.77 ± 0.10g	3.42 ± 0.30h	4.04 ± 0.40h
vanillic acid	0.05 ± 0.004a	0.50 ± 0.06c	0.64 ± 0.06c	1.30 ± 0.10d	2.00 ± 0.14e	0.11 ± 0.01b	2.07 ± 0.20e	5.75 ± 0.50f	8.23 ± 0.70g	10.4 ± 0.90h
syringic acid	0.03 ± 0.003a	0.16 ± 0.03b	0.28 ± 0.03c	0.57 ± 0.04d	0.76 ± 0.06e	0.13 ± 0.01b	0.62 ± 0.05d	1.44 ± 0.10f	4.91 ± 0.50g	7.38 ± 0.80h
ferulic acid		0.03 ± 0.002a	0.10 ± 0.01c	0.14 ± 0.01d	0.15 ± 0.01d			0.02 ± 0.003a	0.06 ± 0.005b	0.11 ± 0.01c
(+)-catechin	0.02 ± 0.003a	0.27 ± 0.04d	0.29 ± 0.03d	0.50 ± 0.04e	0.91 ± 0.08f			0.02 ± 0.003a	0.04 ± 0.003b	0.09 ± 0.01c
(−)-epicatechin	0.03 ± 0.004a	0.36 ± 0.05b	0.37 ± 0.03b	0.66 ± 0.05c	1.05 ± 0.09d					

[a] Values with different letters within rows are significantly different (LSD test, $p < 0.05$). [b] Total soluble phenolic contents (μg of GAE), which represent the absolute polyphenolic amount loaded into the apical side of cell monolayers. These amounts are equivalent to 3, 7.5, 15, 30, and 45 mL of açai pulp and to 10, 25, 50, 100, and 150 μL of açai oil, respectively.

Table 3. Transport of Polyphenolics from Açai Pulp and Açai Oil Extracts, from the Apical to the Basolateral Side of Caco-2 Cell Monolayers following Incubation (2 h, 37°C), as a Function of Total Soluble Phenolic Contents

	% transport of polyphenolics from açai pulp[a]					% transport of polyphenolics from açai oil[a]				
polyphenolic[b] (μg)	12 min	30 min	60 min	120 min	180 min	12 min	30 min	60 min	120 min	180 min
p-hydroxybenzoic acid	1.5 ± 0.13a	1.9 ± 0.18b	2.0 ± 0.18b	2.0 ± 0.17b	2.0 ± 0.19b	1.9 ± 0.20b	5.7 ± 0.60c	6.8 ± 0.57d	6.4 ± 0.71d	6.4 ± 0.55d
vanillic acid	1.07 ± 0.10a	1.1 ± 0.11a	1.1 ± 0.10a	1.1 ± 0.10a	1.1 ± 0.10a	1.1 ± 0.10a	5.2 ± 0.51b	6.3 ± 0.60c	8.1 ± 0.77d	7.9 ± 0.81d
syringic acid	0.55 ± 0.08a	0.61 ± 0.55a	0.64 ± 0.05a	0.63 ± 0.07a	0.62 ± 0.05a	1.0 ± 0.10b	2.5 ± 0.22c	3.2 ± 0.31d	5.5 ± 0.48e	5.5 ± 0.51e
ferulic acid		0.21 ± 0.02b	0.52 ± 0.18c	0.62 ± 0.05c	0.59 ± 0.06c			0.11 ± 0.10a	0.10 ± 0.01a	0.13 ± 0.01a
(+)-catechin	0.16 ± 0.02a	0.67 ± 0.05b	0.69 ± 0.07b	0.72 ± 0.06b	0.69 ± 0.07b			0.11 ± 0.10a	0.14 ± 0.01a	0.15 ± 0.01a
(−)-epicatechin	0.18 ± 0.01a	0.91 ± 0.10b	1.1 ± 0.10b	1.1 ± 0.10b	1.0 ± 0.10b					

[a] Values with different letters within rows are significantly different (LSD test, $p < 0.05$). [b] Total soluble phenolic contents (μg of GAE), which represent the absolute polyphenolic amount loaded into the apical side of cell monolayers. These amounts are equivalent to 3, 7.5, 15, 30, and 45 mL of açai pulp and to 10, 25, 50, 100, and 150 μL of açai oil, respectively.

effective than the non-anthocyanin polyphenolics isolated from açai pulp for inhibition of colon carcinoma cell proliferation when present at equal concentrations. Although in vitro antioxidant activity of polyphenolic compounds has been associated with potential health benefits (*30*), results from this study suggest that inhibition of cancer cell proliferation in vitro by phenolic acids and flavonoids in açai extracts may be independent of their antioxidant activity. Potential mechanisms for this chemopreventive activity may be due to inhibition of carcinogen formation, immunoregulation, disruption of cell cycle, enzyme regulation, and enhancement of DNA repair levels (*31*).

Generation of ROS. The generation of ROS was evaluated by the DCF assay. Both açai pulp and açai oil extracts induced a significant, concentration-dependent increase in the generation of ROS (**Figure 3**). However, unlike the antiproliferative effect, no significant differences between açai pulp or açai oil extracts were detected at any of the concentrations tested. Both extracts induced the generation of ROS at concentrations between 0.4 and 30 μg of GAE/mL. Their inductive effects were more pronounced at lower concentrations (<5 μg of GAE/mL) and decreased markedly above 7.5 μg of GAE/mL. Similarly, low polyphenolic concentrations (<5 μg of GAE/mL) of açai pulp or açai oil extracts were effective at increasing average ROS generation rates. Decreased generation of ROS by higher polyphenolic extract concentrations may be attributable to the presence of sufficient polyphenolics in the model to reduce the generation of ROS through their antioxidant potential; however, due to the complex nature of these reactions, this influence can only be hypothesized. The observed differences between açai pulp and açai oil extracts in the inhibition of HT-29 cell proliferation were not explained by their similarities in the rate and extent of generation of ROS, suggesting that induction of ROS was not the primary mechanism responsible.

Transepithelial Transport Model. Transport of non-anthocyanin polyphenolics from açai pulp and açai oil extracts was also evaluated using Caco-2 cell monolayers as an in vitro intestinal absorption model. Caco-2 cells are the most extensively characterized and functional in vitro model in the field of drug absorption and permeability (*32*) and were previously used to evaluate intestinal absorption and transport of various phenolic acids (*14–16*), flavonoids (*33, 34*), and procyanidins (*35*). Transport of polyphenolics across the Caco-2 monolayers was studied in the apical to basolateral direction. Extracts were loaded into the apical side of the cell monolayers, and individual polyphenolic concentrations appearing in the basolateral side were evaluated over time by HPLC, after incubation for 0.5, 1, and 2 h. Analytical HPLC chromatograms of polyphenolics present in the basolateral solutions after incubation for 2 h are presented in **Figure 4**. Phenolic acids such as *p*-hydroxybenzoic, vanillic, syringic, and ferulic acids were transported from the apical to the basolateral side of Caco-2 cell monolayers, along with monomeric flavanols such as (+)-catechin and (−)-epicatechin, when present in complex polyphenolic mixtures. Average transport rates (μg/mL·h) of non-anthocyanin polyphenolics from açai pulp and açai oil extracts adjusted to different concentrations (μg of GAE/mL), from the apical to the basolateral side of Caco-2 cell monolayers were given in time (**Table 2**). Individual polyphenolic transport rates (0.02−10.4 μg/mL·h) increased in a concentration-dependent manner (24−360 μg of GAE/mL) for both extracts; however, absolute phenolic acid transport rates were significantly higher ($p < 0.05$) for açai oil extracts than for their açai pulp counterparts at equivalent concentrations. The opposite was observed for (+)-catechin and (−)-epicatechin monomers, as indicative of the polyphenolic profiles of açai pulp and açai oil extracts. Variations in individual polyphenolic transport rates were negligible at low phenolic extract concentrations (~24 μg/mL·h) with the exception of vanillic and syringic acid, for which transport efficiencies were enhanced at higher extract concentrations. Higher transport for these two compounds may be related to their methylated structures, previously shown to enhance transport of anthocyanins (*36*) and other flavonoids (*37, 38*) in comparable Caco-2

cell models. Whereas the presence of methyl groups increased polyphenolic transport rates, the presence of polar hydroxyl groups was associated with increased membrane retention from hydrogen-bond formation with polar functional groups on lipids at the lipid/water interface (*38, 39*). In contrast, flavanols are primarily transported via paracellular diffusion (*14*); therefore, higher (+)-catechin and (−)-epicatechin transport rates in cells loaded with açai pulp polyphenolic extracts may be due to higher initial concentrations in açai pulp extracts compared to açai oil extracts.

Relative transport of açai polyphenolics from açai pulp and açai oil extracts following incubation for 2 h is summarized in **Table 3**. Transport efficiencies were expressed as the percentage of the initial polyphenolic concentration (loaded in the apical side) detected on the basolateral side of Caco-2 cell monolayers following incubation for 2 h. In contrast to previous observations on polyphenolic transport rates, relative transport efficiencies of individual polyphenolics did not vary as a function of initial polyphenolic concentrations and showed signs of saturation above polyphenolic concentrations of about 30 μg of GAE. Transport efficiencies for *p*-hydroxybenzoic (~2.0%), vanillic (~1.0%), and syringic (~0.6%) acids were not affected ($p < 0.05$) by initial polyphenolic concentrations in açai pulp extracts nor were efficiencies for ferulic acid (~0.1%) and (+)-catechin (~0.1%) in açai oil extracts. Similar transport efficiencies were observed for ferulic acid, (+)-catechin, and (−)-epicatechin at açai pulp extract concentrations above 60 μg/mL. Transport of phenolic acids such as *p*-hydroxybenzoic, vanillic, and syringic from açai oil extracts increased proportionally to the amount originally loaded into the apical compartment, up to a concentration of 240 μg/mL, after which no further changes in transport were observed. This effect might be attributed in part to the presence of concentrated amounts of other polyphenolic components at higher extract concentrations, which may interfere with both active and passive absorption (*14, 34*).

Results from this study suggest that non-anthocyanin polyphenolic extracts from açai pulp and from a polyphenolic-enriched açai oil obtained from a commercial açai pulp clarification process are sources of biologically active phenolic acids and flavan-3-ols. Polyphenolic-rich extracts from açai pulp and açai oil significantly inhibited cell proliferation and increased the generation of ROS in a concentration-dependent manner. Despite the generation of ROS from these compounds in açai fruit, further mechanisms are likely responsible for the potent cytotoxicity of açai oil extracts on HT-29 colon cancer cells. Transepithelial transport of these phenolic compounds was also evaluated in Caco-2 monolayers as a model for intestinal absorption and demonstrated significant transport from the apical to the basolateral side of the monolayer. These data demonstrate that the chemical composition has an appreciable influence on the cell proliferation and absorption properties of phenolic acids and flavonoids from açai fruit and açai oil. Results from this study provide further evidence of the antiproliferative properties of açai fruit polyphenolics in cultured cancer cells and offer new insights on their absorption.

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