Journal of Life Sciences 10 (2016) 391-404
doi: 10.17265/1934-7391/2016.08.005

Açai Fruit as a Source of Bioactive Phytochemicals

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Abstract: The fruit of the açai palm (*Euterpe oleracea* Mart.) is a rich source of potential bioactive phytochemicals, however neither its precise composition nor the putative benefits to health from its consumption have been fully characterised. This study aims to elucidate the composition of açai fruit pulp and to explore the potential of these extracts to confer protection in a cell culture based model of oxidative DNA damage. Extractions of açai pulps were dissolved in a 80% methanol (methanol: water, 80: 20 v/v) based solvent and the phytochemicals present in the extract were analysing by HPLC detector. Lipids were assessed via GC. The Folin-Ciocalteau assay was used to determine the purposes of providing a phytochemical rich food based comparator with established health benefits. Finally, protection against oxidative DNA damage was assessed in HT-29 cell lines exposed to hydrogen peroxide via the comet assay. Açai pulp extracts were found to be rich in flavonoids, anthocyanins and carotenoids. These extracts were found to protect against oxidative DNA damage (anti-genotoxicity). Açai extracts were more effective at preventing oxidative DNA damage than the blueberry extracts, although their antioxidant capacities as assessed by the FRAP assay were similar. These data shown that açai is a rich source of plant phytochemicals and that these chemicals may protect against oxidative stress in human colon cells (HT-29). Further work is needed to establish the digestive fate of these phytochemicals and to prove the beneficial effects in human.

Key words: Açai pulp, antioxidant activity, anti-genotoxicity, polyphenols.

1. Introduction

Açai (*Euterpe oleracea* Mart.) is native to the Amazon basin where its fruit is a popular staple in the diet; it is most often consumed as a dessert or snack blended with other fruits. In recent years açai has appeared in the US and European food markets where consumers associate its consumption with unspecified benefits to health. Traditionally, açai has been used as a natural remedy for a number of disorders, including the treatment of influenza, cold and inflammatory conditions [1, 2]. As a food, the açai fruit is a good source of energy (81 Kcal/100 g), nutrients, insoluble fiber (15.0 g/100 g DW) and protein (10.3 g/100 g DW). Moreover, açai is reported to be rich in non-nutritive phenolics, including flavonoids, phenolics and hydroxycinnamic acids, all of which may be biologically active and are suggested to be beneficial to human health [3]. Despite the presence of these potentially bioactive compounds, the effects of consuming them in açai have been poorly characterized [4, 5]. However, the health benefits of consuming other foods, also rich in similar blends of plant polyphenols, have been well studied; these include established antioxidant, anti-cancer and anti-inflammatory benefits [6-9]. Açai is often marketed towards the health-conscious consumer, exploiting both public assumptions regarding health benefits and compositional characteristics similar to other healthy plant foods, there is however relatively little scientific evidence to substantiate any specific health benefits in human consumers [10].

In this study, we aimed to i/ characterize the composition of non-nutritive bioactives present in açai pulp, ii/ to determine the antioxidant capacity of these non-nutritives, and iii/ importantly, to establish protection against oxidative stress in a biological system conferred by the presence of these non-nutritives. We make comparisons with the effects of extracts of blueberry, as blueberry is a well-studied

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berry food with established health benefits [11, 12].

2. Materials and Methods

2.1 Chemicals and Reagents

The solvents used for HPLC and GC were purchased from Fisher Scientific (HPLC grade water, methanol, HCl, formic acid and acetonitrile). Standards for polyphenols, 3,4-dihydroxybenzioic acid, 4-hydroxybenzoic acid, gentisic acid, caffeic acid, syringic acid, chlorogenic acid, salicylic acid, trans-cinnamic acid, kaempferol, vanillic acid, gallic acid, quercetin, ferulic acid, isoferulic acid, p-coumaric acid, sinapic acid, catechin and acerbic acid were obtained from Sigma-Aldrich (UK). Cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-glucoside and peonidin-3-rutinoside were obtained from Extrasynthese (Genay, France) as anthocyanin standards for HPLC analysis. Reagents for the assessment of total phenolics and total flavonoids, ferric reducing antioxidant power (FRAP), Folin-Ciocalteu reagent, sodium carbonate, aluminium chloride, sodium nitrite, sodium hydroxide, and sodium acetate trihydrate, glacial acetic acid, 2,4,6-tripyridyl-S-triazin (TPTZ), ferric chloride hexahydrate and ascorbic acid (as standards) were obtained from Sigma-Aldrich. HT-29 cells were obtained from the Department of Food and Nutritional Sciences at the University of Reading.

2.2 Preparation of Fruit Extracts

Pure açai pulp was harvested from fully ripe fruit in 2012 and was kindly supplied by Sublime food (UK). Blueberries (*Vaccinium corymbosum*) were obtained in September, 2012 from a local supermarket in Reading (Berkshire, UK). Samples were prepared as described previously [3]. In brief, açai pulp (100 g) and whole blueberries (100 g) were freeze-dried upon receipt. Freeze-dried foods are stable against the oxidation process and the powdered form facilitates efficient extraction [13]. The samples were frozen overnight at -80 °C and then dried in an IEC Lyoprep-3000 freeze dryer (Dunstable, UK) for one week before the samples were ground to powder.

All samples were stored at -20 °C until further analysis Figure 17. The freeze-dried samples of açai and blueberry (0.5 g in triplicate) were homogenized in 80% methanol (methanol: water, 80: 20 v/v) using a vortex mixer for 5 min and kept at room temperature for 1 h [14]. The supernatants were collected following centrifugation at 1,500 g for 10 min at room temperature, before a second and third extraction. The supernatants were pooled and filtered through a 0.45-μm filter (sterile) (Fisher Scientific, UK). The extracts were protected from light by foil and used immediately to avoid the oxidation of polyphenols [15].

2.3 Determination of Total Phenolics

The total phenolic content was assessed using the Folin-Ciocalteu method [16]. In brief, 5-μL aliquots of gallic acid (0-1,000 mg/L) were prepared as standards and added to the wells of a 96-well plate to obtain data for generating a standard curve, while 5-μL aliquots of solvent extracts of açai or blueberry were added to the other wells. 145 μL of distilled water and 25 μL of Folin-Ciocalteu reagent was then added to each well.

After incubation for 3 min at room temperature, 100 μL of sodium carbonate was added and the plate was placed on a shaker for 25 min at room temperature. Absorbance was read at 650 nm using a GENios Pro^{TM} microplate reader with Magellan^{TM} software. The total phenolic content was calculated using the calibration curve constructed with gallic acid as a standard and the result was expressed as gallic acid equivalent (GAE) mg/g freeze-dried açai or blueberry (mean ± SD; n = 3, triplicate analysis).

2.4 Determination of the Total Flavonoid Content in Fruit Extracts

The total flavonoid content was measured using the

aluminium chloride assay [17]. In brief, 1 mL of a solvent extract of açai or blueberry or a catechin standard was added to 4 mL of distilled water in a 15-mL tube. This preparation was mixed with 0.3 mL of sodium nitrite (5%). After incubation for 5 min at room temperature, 0.3 mL of 10% $AlCl_3$ was added. After a further 6 min, 2 mL of 1 M sodium hydroxide was added and the total volume was made with 10 mL with distilled water. The optical density was assessed at 510 nm using a microplate reader (TECAN). All samples were run in triplicate and compared with a blank The results were expressed as catechin equivalent (CHE) mg/g freeze-dried Açai (mean ± SD; n = 3, triplicate analysis).

2.5 Determination of the Antioxidant Activity of Fruit Extracts Using the FRAP Assay

The antioxidant activity of the fruit extracts was determined using the FRAP assay, which is based on the ferric iron reducing power where the formation of a deep blue complex is readily discernible (Fe^{2+}/TPTZ) [18]. In this assay, 10 µL of serial dilutions of ascorbic acid standards (0-1,000 µmol) or 10 µL of samples were added to the wells of a 96-well plate, followed by 300 µL of FRAP reagent. To prepare the reagent, 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L HCl solution and 2.5 mL of 20 mmol/L ferric chloride hexahydrate solution was added to 25 mL of 0.3 M acetate buffer (pH 3.6; prepared fresh prior to the test). Absorbance was measured after 30 min incubation in the dark at 600 nm using a GENios reader at room temperature with Magellan™ software. The antioxidant capacity was calculated relative to the ascorbic acid serial dilution (0-1,000 µM Trolox equivalent) and expressed as µM ascorbic acid equivalent/L freeze-dried açai [19].

2.6 Analysis of Phenolic Compounds by HPLC

Açai polyphenols were extracted immediately prior to analysis from dried. A freeze-dried sample (0.5 g) was extracted 3 times in 5 mL of solvent [acidified methanol with 0.1% formic acid for anthocyanin compounds and other phenols]. The supernatants were collected, pooled and vortexed for 5 min and then centrifuged for 10 min at 1,500× g at room temperature. Next, 50 µL of the extract was filtered through a 0.45-µm filter (Fisher Scientific, UK) [20]. HPLC was performed using an Agilent 1,100 series liquid chromatograph with a quaternary pump and a photodiode array detector (Hewlett-Packard Agilent, Bracknell, UK). A Nova Pak C18 4-µm column (4.6 × 250 mm) (Waters, Elstree, UK) was used to separate the phenolic constituents at a solvent flow rate of 0.4 mL/min and the column was allowed to equilibrate for 15 min between each injection. Added 50 µL of each sample or standard solution was injected for each analysis. The mobile phase A was 95% HPLC water, 5% methanol and 0.1% HCl; mobile phase B was 50% HPLC water, 50% acetonitrile and 0.1% HCl. The identification of phenolic compounds from açai was based on the fragmentation patterns and retention times in the UV spectra compared with the standards. The detection wavelengths were 254, 280, 320 and 520 nm. A standard curve was constructed to quantify the amount of each compound identified in açai extracts.

2.7 Analysis of Carotenoids

Carotenoids were identified by HPLC using available standards for β-carotene, all trans- retinal, retinal, β-cryptoxanthin, xanthophyll, lycopene (Sigma-Aldrich, UK) lutein and zeaxanthin (Extrasynthese, France) according to the method of Oliveira et al. [21]. In brief, 2 g of freeze-dried açai fruit (triplicate samples) was extracted in 10 mL methanol: tetrahydrofuran (60: 40, v/v) and then homogenized for 3 min using a vortex homogenizer. The sample was centrifuged for 5 min at 1,500 g and the supernatant collected. The pellet was extracted twice more or until the supernatant was colourless. The supernatants were transferred to a rotary evaporator at 30 °C. The residues were reconstituted

in 5 mL of methanol: hexane and the sample was passed through a 0.45-µm filter, followed by injection of 100 µL into HPLC vials. Individual carotenoids were separated using HPLC with an analytical LUNA C18 (2) ODS, (Phenomenex, detector, Torrance, CA, USA). The mobile phase was acetonitrile: tetrahydrofuran: methanol: 1% ammonium acetate in water (684: 220: 68: 28) and the sample was filtered using Millipore Type HA 0.45-µm- and 47-µm-diameter membrane filters. The flow rate of the mobile phase was set to 3 mL/min and the detection wavelength was 450 nm for carotenoids. Chromatography was performed at room temperature and the chromatograms were integrated using the DELTA chromatography data system (Data WorX Pty Ltd., Kangaroo Point, QLD Australia). Quantification was based on comparison with standard curves.

2.8 Fatty Acid Analysis

Freeze-dried açai samples (1 g) were extracted in duplicate using diethyl ether and hexane (5: 4, v/v) and then evaporated to dryness at 60 °C for 1 h under nitrogen. The samples were dissolved in hexane methyl esters using sodium methoxide in methanol. Fatty acids were identified and quantified by gas chromatography using a Bruker 450-GC (Bruker Daltonik GmbH, Bremen, Germany) equipped with a CP-Sil 88 column (100 m × 0.25 mm) coated with a 0.2-µm film of cyanopropyl siloxane (CP-SIL 88, Varian Inc., Palo Alto, CA); 20 µL of the sample was passed through a temperature gradient with hydrogen as the carrier gas (30 psi/207 kPa). The column temperature was held at 70 °C for 4 min, increased at a rate of 8 °C/min to 110 °C, then increased to 170 °C and held at 170 °C for 10 min, increased to a final temperature of 240 °C at a rate of 3 °C/min and held at 240 °C for 8 min. The injector and detector temperatures were maintained at 255 °C. Identification and quantification of fatty acids in açai extracts were based on comparisons with the retention times and standard curves of known spectra [22].

2.9 Cell lines and Cell Culture

The human HT-29 colorectal adenocarcinoma cell line was used in the cytotoxicity and anti-genotoxicity experiments. Cells were cultured in Dulbecco's modified Eagle's medium (Lonza, UK) supplemented with 10% foetal bovine serum, 2 mM L-glutamine and 100 IU/mL penicillin/streptomycin (Lonza, UK). The cells were passaged 21-35 times with 2 passages per week in T75 cell culture flasks (Fisher Scientific, UK) [23].

2.10 Cytotoxicity

HT-29 cells were seeded in a 96-well plate at a concentration of 1.6×10^5 (250 µL) cells/mL and incubated for 24 h at 37 °C prior to the assay. Dilutions of açai or blueberry extracts [0%, 1%, 2.5%, 5% and 10% (v/v) in medium] were prepared in carrier control and used to treat the cells, followed by incubation for 24 h at 37 °C. Thereafter, the medium was removed and 100 µL of DAPI (3 mM) was added for 4 h at 37 °C. Subsequently, DAPI was removed and absorbance was measured at 540 nm using a microplate reader (TECAN). The experiment was performed in triplicate in independent experiments. The results were expressed as the mean percentage cell survival, which was normalized against the control (without treatment).

2.11 Genotoxicity (Comet Assay)

The method used was based on that reported by Brown [24]. In brief, HT-29 cells were grown for 3 days and treated with açai or blueberry extracts for 30 min at 37 °C before harvesting. The cell concentration was adjusted to 3.2×10^6 cells/mL (450 µL) to study exogenously induced DNA damage, where cells were treated with 75 µmol H_2O_2 (100 µL) (Sigma-Aldrich, UK) and incubated on ice (4 °C) for 5 min. PBS was used as the negative control and H_2O_2-treated cells not exposed to the sample extracts were included as the positive control. Cell suspensions were centrifuged for 5 min at 250 g and 4 °C and the supernatants were discarded. The cells were resuspended in 85 µL of 0.85%

low-melting- point agarose (Sigma-Aldrich, UK). The suspension was mixed well and aliquots were dispensed onto frosted slides (1% normal agarose gel with 150 μL of PBS on each slide and large cover slips) (Sigma-Aldrich, UK). These slides were incubated in the fridge for 10 min before lysis buffer [pH 10; 1% Triton X-100 (2.5 NaCl, 0.1 M EDTA, 0.01 M Tris); Sigma-Aldrich, UK] for 1 h at 4 °C. The slides were washed with 100 mL of enzyme buffer (pH 8.0; 400 mM HEPES, 1 M KCl, 5 mM EDTA, 2 mg/mL BSA; Sigma-Aldrich, UK) in a staining jar 3 times for 5 min at 4 °C and compared with the controls. To assess the oxidative damage, endonuclease III (Endo III) or formamidopyrimidine-DNA glycosylase (FPG) (100 μL) was applied to a subset of slides post-treatment, whereas the control slides were exposed to enzyme reaction buffer in the absence of enzymes. The slides were incubated at 37 °C for 45 min with Endo III or 30 min with FPG. DNA was allowed to unwind in electrophoresis buffer (10 M NaOH, 0.2 M EDTA, pH 13.5; Sigma-Aldrich, UK) at 4 °C for 20 min before electrophoresis (20 min at 26 V, 300 mA, 0.037 V/cm). The slides were then washed 3 times with neutralizing buffer (0.4 M Tris, pH 7.5 with HCl; Sigma-Aldrich, UK) at 4 °C for 5 min. All slides were stained with 45 μL of ethidium bromide (20 μg/mL in PBS) and stored in a dark moist box at 4 °C for ≤ 48 h.

Comets were visualized at 400× magnification using an epifluorescence microscope (Olympus, Bx51). 50 cells per sample were counted and percentage tail were quantified using Komet 5.5 image analysis software (Kinetic Imaging Ltd., Liverpool, UK). Mean values were calculated for 50 cells from each slide (each sample in triplicate) and the data were expressed as the mean percentage tail DNA compared with the controls [23].

2.12 Statistical Analysis

All experiments were analysed using PASW statistics 18 (SPSS) software. Independent samples *t*-tests were used to assess significant differences in the total polyphenol, flavonoid contents and antioxidant activity (FRAP) of the 2 samples (açai and blueberry). One-way ANOVA with Dunnett's post-hoc test was used to analyse the comet assay data. The data were expressed as the mean of 3 individual analyses for each sample (mean ± SD; n = 3).

3. Results

3.1 Phytochemical Composition of Fruit Extracts

The Folin-Ciocalteu method was used to determine the total phenolic content in the 2 different fruits. As expected, the total phenolic content was very high in both fruits (Açai 105.1 GAE mg/g and blueberry 182.6 GAE mg/g) although the dried blueberry extract had a higher phenolic content compared with the açai pulp extract ($P < 0.001$). In contrast, the aluminium chloride assay Table 7 showed that the freeze-dried açai pulp extract contained significantly higher levels of flavonoids compared with blueberry (Açai 54.27 CHE mg/g and blueberry 32.04 CHE mg/g) ($P < 0.001$). This may explain the comparable antioxidant capacities of both fruits observed in the FRAP assay (Açai 1,048.76 μM/L ascorbic acid and blueberry 1,033.11 μM/L ascorbic acid).

Table 1 Total polyphenol and flavonoid contents and antioxidant capacity (FRAP assay) freeze-dried fruit extracts.

Samples	Total Phenolic content (GAE mg/g)	Total flavonoid content (CHE mg/g)	Antioxidant activity (Ascorbic acid μM/L)
Açai	105.1 ± 2.4	54.27 ± 0.28	1,048.76 ± 57.25
Blueberry	182.6 ± 10.2	32.04 ± 2.30	1,033.11 ± 37.73
P value for comparison	0.001	0.001	0.073

*The data presented for the total phenolics are the mean values ± SD of 4 replicates (n = 4) expressed as mg/g of gallic acid equivalent (GAE) per g. The total flavonoid content is the mean value of 3 replicates (n = 3) ± SD expressed as catechin equivalents mg/g. The total antioxidant activity is the mean values from the FRAP assay (n = 3) ± SD and expressed as μmol/L of ascorbic acid. *P*-value are from *t*-test comparisons of açai and blueberry extracts.

Table 2 Identification and quantification of phenolic compounds in açai fruit extracts by HPLC.

Compound	Açai extract
Gallic acid	701.60 ± 4.48
3,4-Dihydroxybenzoic acid	5.03 ± 0.56
4-Hydroxybenzoic acid	3.37 ± 0.36
2,5-Dihydroxybenzoic acid	28.18 ± 0.80
Chlorogenic acid	37.65 ± 1.55
Caffeic acid	8.12 ± 0.32
Syringic acid	11.23 ± 0.09
Ferulic acid	2.46 ± 0.24
Trans-cinnamic acid	1.65 ± 0.02
Quercetin	39.02 ± 0.58
Vanillic acid	1.02 ± 0.24
Cyanidin-3-*O*-glucoside	998.74 ± 7.47
Cyanidin-3-*O*-rutinoside	433.98 ± 11.61
Pelargonidin-3-*O*-glucoside	17.58 ± 0.35
Peonidin-3-*O*-rutinoside	544.50 ± 77.60

Table 3 Fatty acids present in açai pulp extracts (mg/g Dry weight total fatty acids).

Compounds Identified	Açai extracts
Palmitic acid	134.1
Oleic acid	321.5
Linoleic acid	65.9
Total fatty acids	521.5

3.2 Polyphenol Profile

Table 8 provides a summary of the phytochemicals identified in açai pulp extracts. The major polyphenols in the açai extract were phenolic acids (gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, syringic acid and vanillic acid), flavonols (quercetin), hydroxycinnamic acids (chlorogenic acid, caffeic acid and ferulic acid) and anthocyanins, including cyanidin-3-*O*-glucoside (998.7 mg/100 g DW), cyanidin-3-*O*-rutinoside (434.0 mg/100 g), pelargonidin-3-*O*-glucoside (17.6 mg/100 g) and peonidin-3-*O*-rutinoside (544.5 mg/100 g) Figure 18.

3.3 Carotenoid Profile

Figure 19 summarizes the carotenoids identified in the freeze-dried açai pulp extract. The major carotenoids identified were lutein (43.9 mg/ 100 g) and β-carotene (439.1 mg/100 g). Standards were included for other carotenoids (trans-retinal, retinal, β-cryptoxanthin, xanthophyll, lycopene and zeaxanthin). However, these compounds were not detected in the freeze-dried açai pulp extract.

3.4 Fatty Acid Profile

Gas chromatography was used to elucidate the fatty acid composition of açai pulp extracts. The total fatty acid content was 521.5 mg/g DW and 3 principal fatty acids were identified. Oleic acid comprised 61.6% of the total fatty acids (321.5 mg/g DW), followed by palmitic acid (25.7%; 134.1 mg/g DW) and linoleic acid (12.6%; 65.9 mg/g DW) Table 9. These data are consistent with the results of the only other previous study to report on the fatty acid content in açai pulp [5].

3.5 Cytotoxicity of Açai Berry in HT-29 Cells

No appreciable cytotoxicity was observed when açai or blueberry extracts were applied to HT-29 cells at concentrations of 0%, 1%, 2.5% and 5% (data not shown). Thus, an extract concentration of 2.5% [2.5 mg/mL in medium (v/v)] was considered suitable for the genotoxicity experiments.

3.6 Comet Assay

HT-29 cells were pre-treated with 2.5% (v/v) açai or blueberry extracts for 30 min prior to the induction of oxidative DNA damage with H_2O_2 Figure 21A, or they were treated with açai or blueberry extracts without peroxide challenge to assess the effects of the extracts on endogenous DNA damage. Following treatment with peroxide DNA strand breaks were observed to increase in the HT29 cells, from background levels of under 6% tail DNA rising to closer to 11% Figure 20 (Contro 11%, açai 6.6% and blueberry 9.3%). In addition to strand breaks, the Endo III and FPG modified assays demonstrate increased levels of both oxidised purine and pyrimidine bases. Pre-incubation with extracts of açai, but not blueberry, protected against peroxide-induced DNA strand breaks. And results from the modified comet assays showed protection against peroxide induced oxidation of both pyrimidines and purines Endo III (Control 12.6%, Açai 8% and blueberry 11.6%) FPG (Control 14%, açai 7.3% and blueberry 10.3%). In the absence of peroxide neither the extract of açai or the extract of blueberry was shown to induce DNA strand breaks or oxidative damage and neither extract conferred protection against the endogenous DNA damage Figure 21B.

4. Discussion

It is hypothesized that non-nutritive plant polyphenols and carotenoids contribute, in part, to the reduced risk of chronic disease associated with consuming a diet rich in fruits and vegetables [25]. One putative beneficial mode of action for plant polyphenols in the body is their antioxidant effect. In this study, we determined the phytochemical profile of açai fruit pulp and we showed that it is a very rich source of flavonoids. The relative abundance of secondary metabolites in most fruits varies according to the ripening and harvest time and growing conditions [26]. A drawback to this study is the limited regional and seasonal variety of açai samples studied, that said, our polyphenol data are consistent with the results of previous studies [13, 27]. In the present work, we identified phenolic acids (gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, gentisic acid, syringic acid and vanillic acid), flavonols (quercetin), hydroxycinnamic acids (chlorogenic acid, caffeic acid and ferulic acid) and an abundance of anthocyanins in açai extracts Figure .

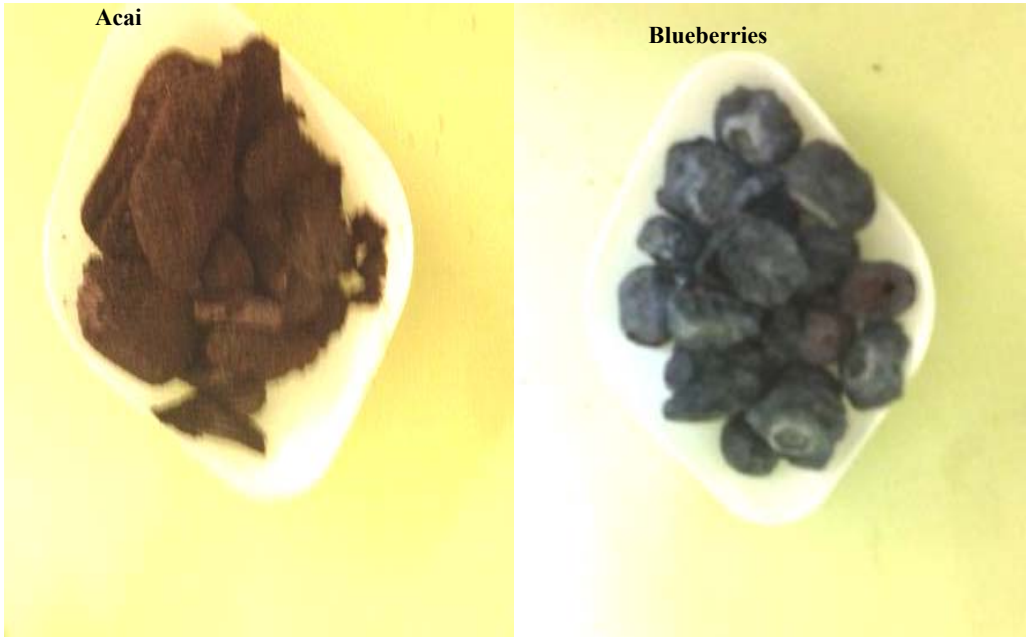

Fig. 1 Samples after freeze -dried; Açai pulp and Blueberries.

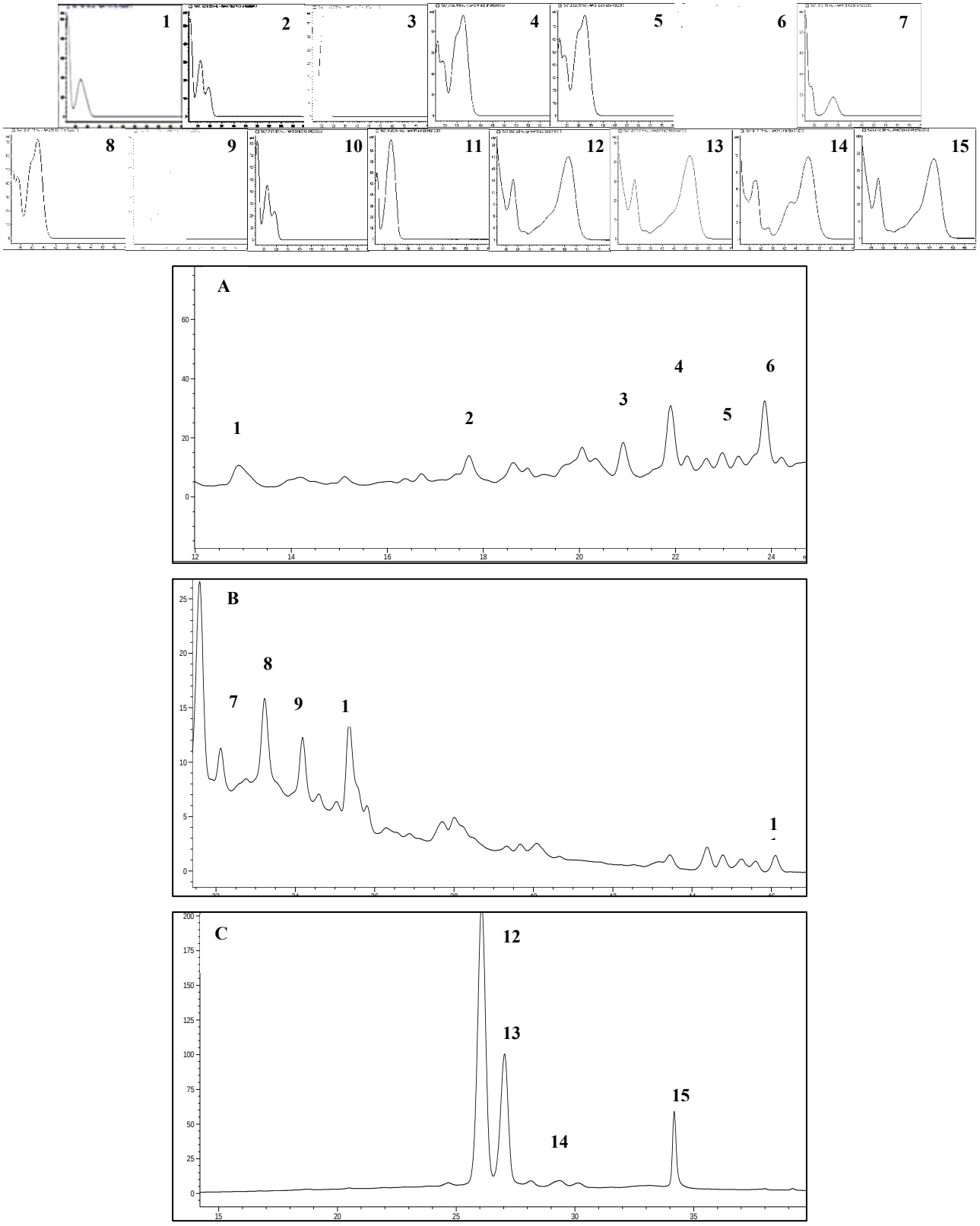

Fig. 2 Chromatograms obtained by HPLC using freeze-dried acai pulp. Polyphenol compounds detected (A) at 280 nm: 1, gallic acid; 2, dihydroxybenzonic acid; 3, 4-hydroxybenzonic acid; 4, chlorogenic acid; 5, caffeic acid; 6, syringic acid; (B) at 254 nm: 7, 2,5-dihydroxybenzoic acai; 8, ferulic acid; 9, quercetin; 10, vanillic acid; 11, trans-cinnamic acid and (C) at 520 nm: 12, cyanidin-3-O-glucoside; 13, cyanidin-3-O- rutinoside; 14, pelargonidin-3-O-glucoside; 15, peonidin-3-O-rutinoside.

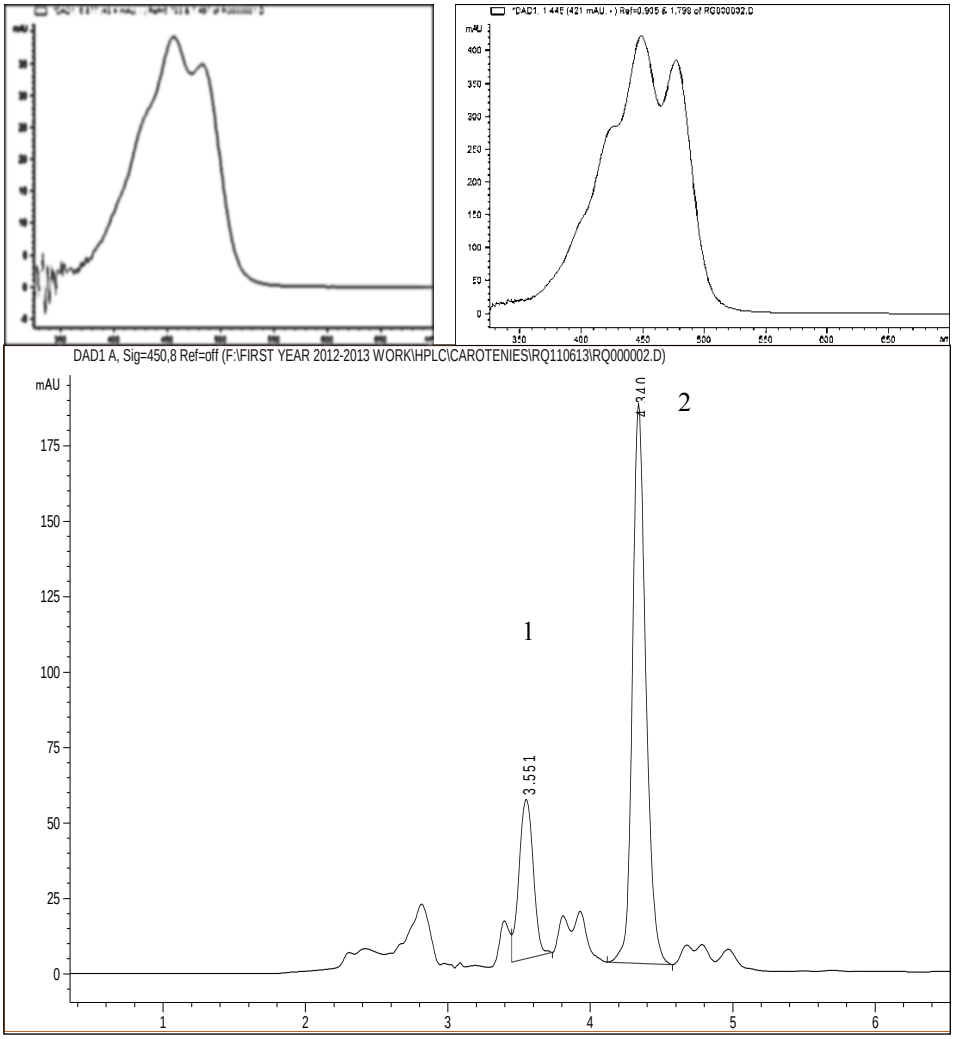

Fig. 3 HPLC spectra of the freeze-dried açai pulp extract to detect carotenoid compounds at 450 nm: 1, β-carotene and 2, lutein.

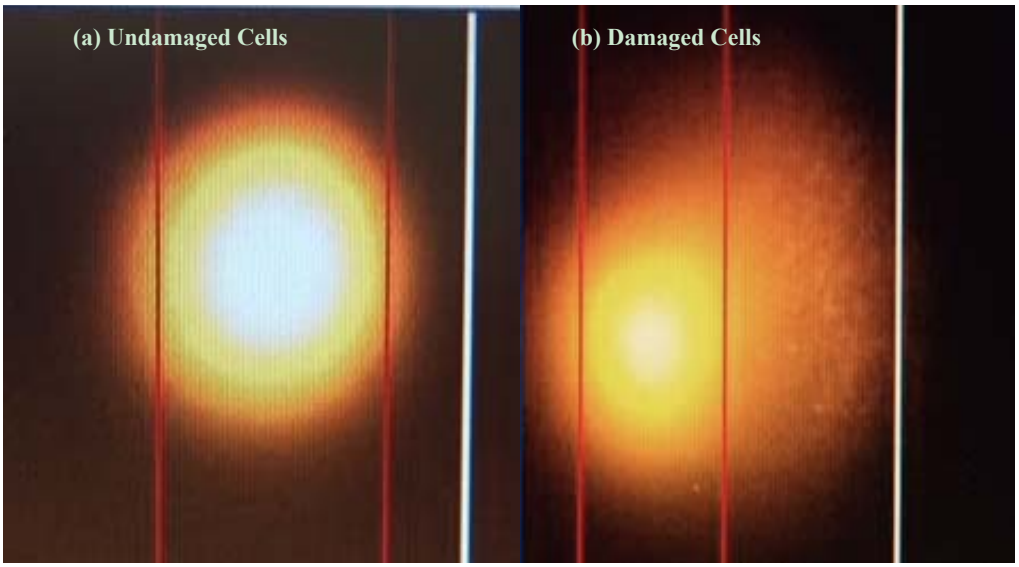

Fig. 4 Comet assay images.

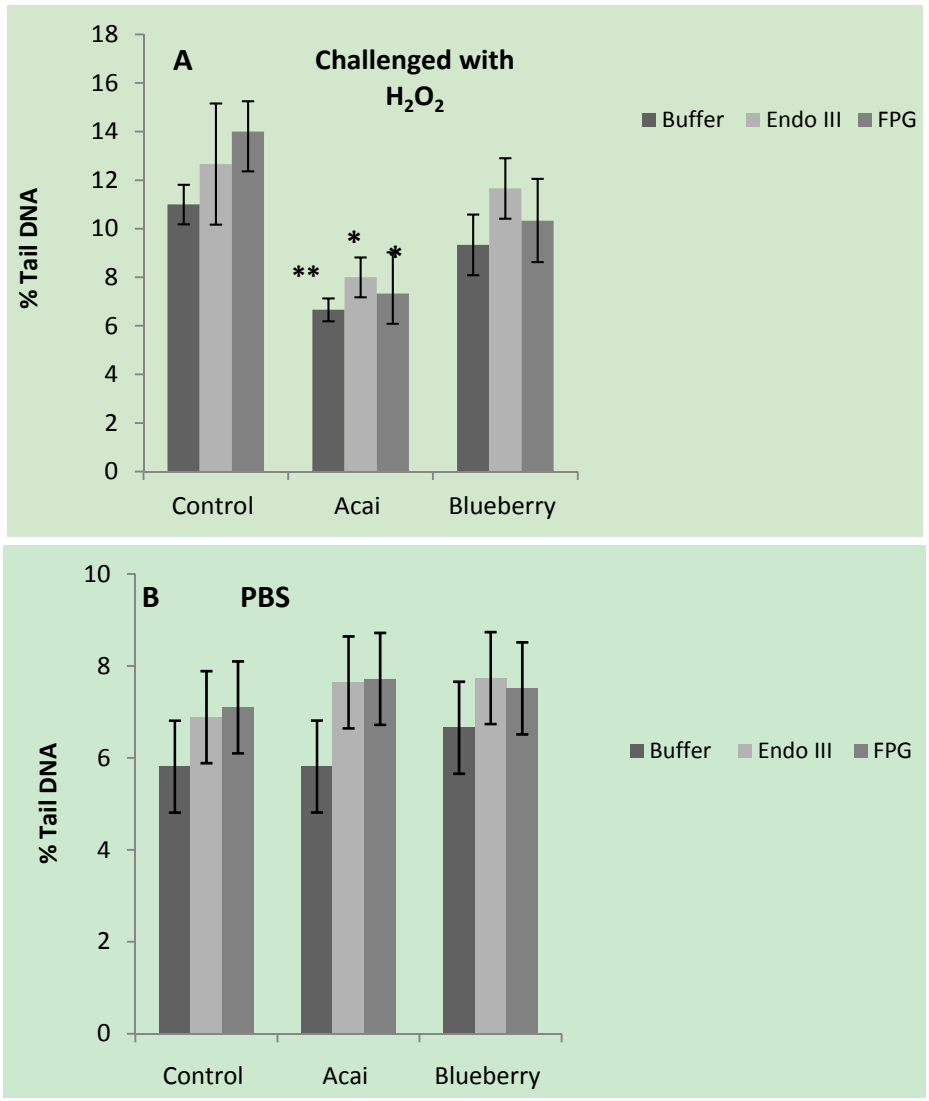

Fig. 5 Anti-genotoxic effects of fruit extracts on HT-29 cells (comet assay). HT-29 cells were treated with extracts (2.5 mg/mL in MeOH) for 30 min before the experiment. (A) The effect of the extracts on tail DNA when cells were challenged with H_2O_2 (75 µM for 5 min), representing an exogenous source of free radical damage. (B) Cells were challenged with PBS, demonstrating the effects of the fruit extracts on endogenous oxidative DNA damage. Buffer represents global DNA damage. Endo III enzyme was used to assess oxidized pyrimidines. FPG enzyme was used to assess oxidized purines. The data represent the mean of 3 experiments ± SD. One-way ANOVA and Dunnett's test: *P < 0.01, **P < 0.001, significant differences compared with the control.

Furthermore, we showed that the extracts of açai have an *in vitro* antioxidant capacity similar to that of blueberry extracts, this capacity may be a function of both the phenolics and the carotenoids identified. Previous reports have established powerful anti-oxidant effects for açai, however here we go a step further, demonstrating that açai extracts protect against oxidative DNA damage in a biological system to a greater extent than extracts of blueberry. This finding may be much more relevant to human health than purely chemical measures of antioxidant function as cellular oxidative stress is implicated in the development of cardiovascular disease and cancer [28].

In this study, we have identified the fat composition in açai pulp and we found that açai has content high amount of fatty acids contains mainly oleic acid (MUFA) and linoleic acid (PUFA). The lipid fraction of

açai is similar to that of olive oil, which contain mainly of oleic acid found in animals and vegetables oils and is the major component of the mediterranean diet that high in fruit and vegetable which is responsible for health benefits such as breast cancer and other chronic diseases [29, 30]. Also with high antioxidant capacity of Acai pulp, which may be associated with the production, lipid fraction from oxidation in the plant and fruit. The effects of dietary antioxidants in the human body are unclear and phytochemicals with powerful antioxidant effects as assessed by purely chemical measures may not necessarily retain these properties in living systems [31]. Inferences based on *in vitro* data assume that antioxidants survive the digestive environment of the gastrointestinal tract and that the compounds are taken up into the bloodstream or at least into the cells lining the gastrointestinal tract where they may act. Returning to the polyphenol profile of açai, *in vitro* studies suggest that anthocyanins, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside are associated with the inhibition of amylase activity [32], anti-mutagenic effects [33], suppression of proliferation and induction of apoptosis [34], protection against LDL oxidation [35] and protection against endothelial dysfunction [36]. Similarly, high antioxidant and anti-mutagenic capacities have been reported for peonidin-*O*-rutinoside [37], which also inhibits the growth of cultured cancer cells [34]. The hydroxycinnamate, caffeic acid has previously been shown to exert a variety of anti-inflammatory and anti-cancer effects *in vitro* [38] and has also been shown to help control blood glucose and vascular dysfunction in rats [39]. The carotenoid lutein may protect against oxidative stress [40], although evidence of protection against cardiovascular disease is equivocal [41]. The combined effects of these phytochemicals within the body, as part of a consumed food, also assumes their bioavailability, and the more general benefits claimed for açai appear to have been inferred on the basis of established health benefits associated with consuming other polyphenol-rich berry foods in areas such as cardiovascular and metabolic disease risk [42].

Here we report a similar polyphenol content in açai relative to blueberry, but we would caution against making inferences on the basis of phenolic equivalents. Interventions with very high doses of antioxidants have not always equated to protection against disease and in some cases these interventions have been harmful [43]. Many of the noted benefits of polyphenols are often not associated with a classic linear dose-response relationship during *in vitro* studies [42] and some phenolics may actually act as pro- oxidants at high concentrations. Thus, it is important to independently characterize the health benefits of consuming açai as a food, very few studies have addressed this. Anti- mutagenic effects have been reported on the basis of in vivo models [44], as well as anti-hypercholesterolemic effects [45]. We only identified 4 small dietary intervention studies in human volunteers. The highest quality study was a 4-way crossover trial with açai pulp and juice where apple sauce and a polyphenol-free beverage were used as controls. This study established the bioavailability of açai polyphenols on the basis of urinary recovery and also demonstrated an increase in the post-consumption plasma antioxidant capacity in 12 volunteers [10]. Similarly, Jensen et al. observed an increase in serum antioxidant levels and a reduction in lipid peroxidation following acute consumption of a 'polyphenol-enriched' açai—based beverage in a double-blind crossover [46]. More recently, the same authors reported slight improvements in the range of motion in arthritic patients following chronic consumption of the same product in a small non-controlled chronic study [47]. Udani et al. observed significant reductions in plasma glucose, insulin and total cholesterol levels in a small non-controlled pilot study based on the chronic consumption of açai pulp in overweight volunteers [48]. Each of these studies demonstrates the bioavailability of polyphenols and suggested *in vivo*

antioxidant effects. However, only two of the studies used pure açai pulps and only one of these followed a blinded controlled design and in this study the principal outcome measure (plasma antioxidant status) would not satisfy the EFSA criteria for a health claim on food.

Our study is limited in that, it is *in vitro* work and uses experimental exposure levels of açai phytochemicals that may not be achievable through diet, the results are however comparable with the effects of blueberry in these assays. Furthermore, the findings here support those of others and are suggestive of potential health benefits for açai, more research is needed to establish the digestive fate of açai polyphenols, to determine their actual effects on the human body, and to determine the levels of exposure required to confer any benefits to health.

Acknowledgments

We thank the Saudi Arabian Ministry of Education for funding this study. We also thank Dr. Kirsty Kliem and Paulina Erceg for helping us in this study.

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