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# Malvidin and cyanidin derivatives from açai fruit (*Euterpe oleracea Mart.*) counteract UV-A-induced oxidative stress in immortalized fibroblasts

Ganna Petruk[a], Anna Illiano[a], Rita Del Giudice[a], Assunta Raiola[b], Angela Amoresano[a], Maria Manuela Rigano[b], Renata Piccoli[a,c], Daria Maria Monti[a,c,*]

[a] Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, via Cinthia 4, 80126 Naples, Italy
[b] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Naples, Italy
[c] Istituto Nazionale di Biostrutture e Biosistemi (INBB), Rome, Italy

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ABSTRACT

UV-A radiations are known to induce cellular oxidative stress, leading to premature skin aging. Consumption of açai fruit (*Euterpe oleracea Martius*) is known to have many health benefits due to its high level of antioxidants. Herein, we analyzed the ability of phenolic compounds extracted from this fruit to attenuate UV-A-induced oxidative stress in immortalized fibroblast.

A methanol/water açai extract was fractionated by HPLC and each fraction tested for anti-oxidant stress activity. Immortalized fibroblasts were pre-incubated with açai fractions and then exposed to UV-A radiations. Açai extract was found to be able to strongly protect cells from oxidative stress. In particular, reactive oxygen species (ROS) production, GSH depletion, lipid peroxidation and no increase in the phosphorylation levels of proteins involved in the oxidative stress pathway was observed in cells pre-incubated with the extract and then irradiated by UV-A. Mass spectrometry analyses of HPLC fractionated extract led us to the identification of malvidin and cyanidin derivatives as the most active molecules able to counteract the negative effects induced by UV-A irradiation.

Our results indicate, for the first time, that açai fruit is a valuable natural source for malvidin and cyanidin to be used as anti-stress molecules and represent good candidates for dietary intervention in the prevention of age related skin damage.

## 1. Background

In the last few years, an increasing attention has focused on age-related diseases, including skin aging. Oxidative stress caused by aging is considered a general initiating factor of neurodegeneration and carcinogenesis [1]. Indeed, it is known that the skin is constantly exposed to oxidative stress induced by reactive oxygen species (ROS), generated by endogenous (i.e. enzyme activities) or exogenous sources [2,3].

UV radiation from sunlight is one of the most important health-related environmental factors because of its hazardous effects, which include generation of skin cancer, suppression of the immune system, and premature skin aging [2]. In particular, UV-A (400–315 nm) radiations are weakly absorbed by DNA, but rather excite endogenous chromophores, leading to DNA damage. This occurs through the production of reactive singlet oxygen that specifically reacts with guanine within the DNA molecule [4,5]. UV-A radiations may also promote the formation of hydroxyl radicals via the photosensitized production of superoxide anions. Because of their high reactivity and low specificity, hydroxyl radicals likely induce a wide range of DNA damage [6]. Fibroblasts, cells of the dermis, are continuously exposed to UV-A radiations, which are able to penetrate deeply in the skin. The cellular antioxidant defense system is composed of endogenously produced antioxidant molecules, but the intrinsic mechanism for antioxidant defense is gradually impaired with aging, resulting in an inability to deal with ROS generation [7]. A continuous and regular

*Abbreviations:* ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); DCF, 2′,7′-dichlorofluorescein; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; EDTA, ethylene diamine tetra acetic acid; HAA, hydrophilic antioxidant activity; $H_2$-DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P-HSP-27, phosphorylated heat shock protein; P-p38, phosphorylated p38 MAP kinase; P-MAPKAPK-2, phosphorylated MAP kinase-activated protein kinase; ROS, reactive oxygen species; r.t., room temperature; TBA, thiobarbituric acid; TBARS, TBA reactive substances; TFA, trifluoroacetic acid; TNB, 5-thio-2-nitrobenzoic acid

* Corresponding author at: Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, via Cinthia 4, 80126 Naples, Italy.
*E-mail address:* mdmonti@unina.it (D.M. Monti).

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**Table 1**
Total phenolic acids, total flavonoids and hydrophilic antioxidant activity in açai extract.

| Açai antioxidant determination | Value |
|---|---|
| Total phenols (mg/100 g DW) | 192.41 ± 10.78 |
| Total flavonoid (mg/100 g DW) | 159.67 ± 7.65 |
| HAA (mmol TE/100 g DW) | 2.1 ± 0.17 |

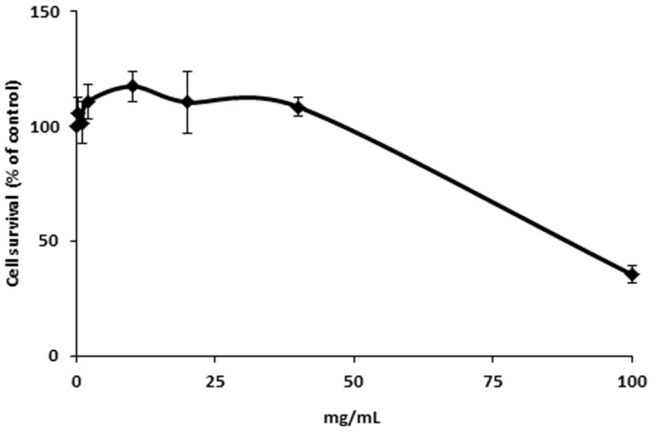


**Fig. 1.** Effect of açai extracts on the viability of BALB/3T3 fibroblasts. Dose-response curve of BALB/3T3 cells after 48 h incubation with increasing concentrations of açai extracts. Cell viability was assessed by the MTT assay and expressed as described in Materials and Methods section. Values are given as means ± S.D. (n ≥ 3).

intake of vitamins, trace metals, polyunsaturated fatty acids, and polyphenols from food sources contributes to counteract oxidative stress [8,9] and in preventing or retarding age-related diseases [10–12]. As an example, we recently report the beneficial effects of tomato extracts in counteracting oxidative stress in different cell lines [13,14]. Therefore, a continuous search for natural extracts highly rich in antioxidant molecules is needed to identify and provide novel natural drugs against aging-associated diseases.

Açai (*Euterpe oleracea Martius*) tree is a large palm found in the Amazon flood plain. The fruit of this palm is a small purple-black berry which reaches about 10 mm in diameter and is usually consumed in all states of Brazil since it is rich in α-tocopherol, fibers, lipids, polyphenols (including anthocyanins), and mineral ions [15,16]. The high polyphenol content, mostly composed of anthocyanins and flavones, is thought to confer to açai fruit several health-promoting effects, including anti-inflammatory, immunomodulatory, antinociceptive, and antioxidant properties [17–25]. In particular, açai extracts were shown to increase plasma antioxidant capacity [21], to decrease oxidative stress in endothelial cells [26], to attenuate tumor growth in mice affected by esophageal cancer [27] and to lower the level of blood cholesterol in animal models for hypercholesterolemia [28].

A positive role of açai in modulating ROS production and activating antioxidant genes expression in rat liver was also reported [29] and, more recently, Peixoto and colleagues demonstrated the beneficial effect of açai extract in counteracting oxidative stress and aging in *C. elegans* [18].

Although several reports on the spectrum of health benefits of açai have been reported, only few studies are available so far on the identification of the antioxidant molecules responsible for the reported beneficial effects on human health.

Here, a methanol/water extract from *Euterpe oleracea* fruits was analyzed for its antioxidant activity on fibroblasts exposed to UV-A-induced insults. A combined approach of bioassays and mass spectrometry analyses led to the identification of açai bioactive compounds.

## 2. Methods

### 2.1. Açai Extracts

Methanolic extracts from açai fruit were obtained as reported by Rigano et al. [30], starting from commercially available dried powder (2 g, Tuialimentos, Brasil). The mixture was dried in a rotovapor (R-210, Buchi), and dissolved in 5% dimethyl sulfoxide (DMSO) in PBS (1 mL).

### 2.2. Antioxidant Compounds Determination and Antioxidant Activity Analysis

Total phenols content was determined in the whole açai extract according to the method of Singleton et al. [31] modified as reported by Rigano et al. [30]. Briefly, an equal volume of Folin-Ciocalteu's phenol reagent and two volumes of $ddH_2O$ were added to the hydrophilic extract. After 6 min, $Na_2CO_3$ was added (7% final concentration). After 90 min the absorbance was read at 760 nm. A standard curve was obtained by using gallic acid in the range 0–70 μg/mL. The total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100 g dry weight (DW) of açai. Three independent analyses

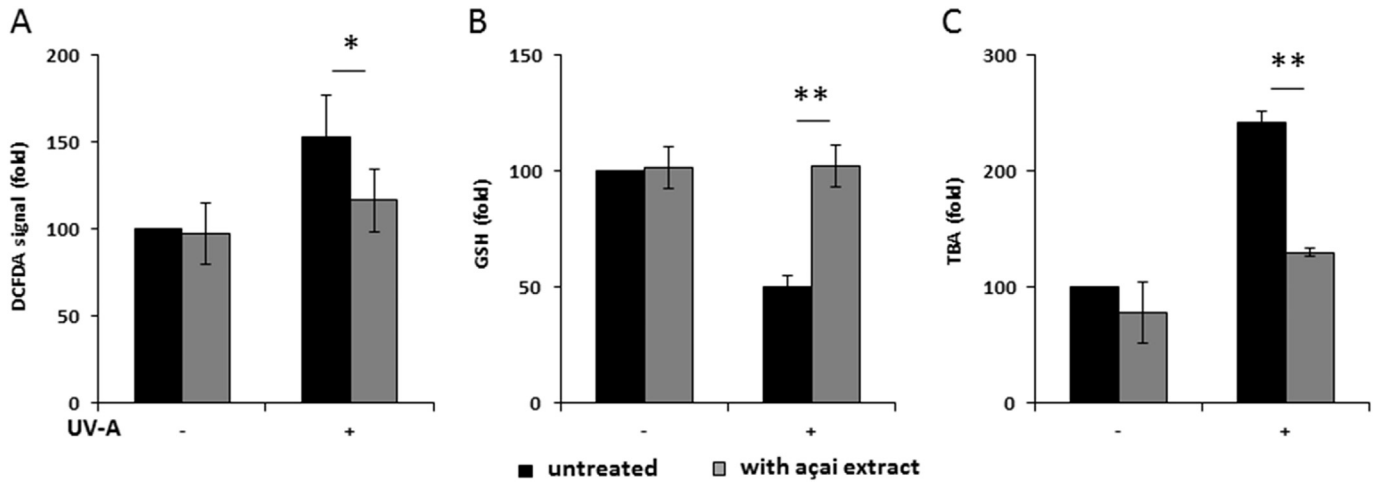


**Fig. 2.** ROS production, GSH oxidation and lipid peroxidation in BALB/3T3 cells irradiated by UV-A in the presence of açai extracts. Cells were pre-incubated in the presence of 10 mg/mL açai extract (grey bars) for 2 h and then irradiated by UV-A (100 J/cm$^2$). A, intracellular ROS levels were determined by DCFDA assay; B, intracellular GSH levels determined by DTNB assay; C, lipid peroxidation levels determined by TBARS assay. Values are expressed as fold increase with respect to control (i.e. untreated) cells. Data shown are the means ± S.D. of three independent experiments. * indicates $p < 0.01$; ** indicates $p < 0.001$.