Indian Journal of Experimental Biology
Vol. 62, February 2024, pp. 112-118
DOI: 10.56042/ijeb.v62i02.2539

Preliminary *in vitro* evaluation of the cytoprotective effects of clarified *açaí* (*Euterpe oleracea* MART.) pulp on African green monkey kidney cells (VERO) treated with the antifungal fluconazole

Pereira ALT[1], Santos TS[1], Guimarães AC[1], Muto NA[2], Rogez H[2], Burbano RMR[1] & Bahia M de O[1]*

[1]Laboratório de Citogenética Humana e Genética Toxicológica, Instituto de Ciências Biológicas, Universidade Federal do Pará (UFPA), Belém–Pará, Brazil

[2]Centro de Valorização de Compostos Bioativos da Amazônia, Universidade Federal do Pará (UFPA), Belém–Pará, Brazil

Received 10 June 2023; *revised 28 September 2023*

Açaí (*Euterpe oleracea* MART.) has been the target of several studies due to its strong antioxidant potential and possible cytoprotective effect, thus providing health benefits to the population. Fluconazole is a widely known and used drug in the treatment of fungal diseases. However, the literature shows that this drug can show cytotoxic effects, as well as cause DNA damage. In this regard, the present study aims to assess the putative cytoprotective effect of clarified *açaí* (CA) pulp extract on VERO cells exposed to fluconazole. It was observed in the MTT assay that CA was able to increase the viability of cells exposed to the antifungal agent in three different times of exposure (24, 48 and 72 h). Similarly, the results of the comet assay demonstrated that CA was able to decrease the genotoxic effects of fluconazole significantly. Thus, we conclude that, under the assessed conditions, CA shows cytoprotective effects against the damage caused by fluconazole.

Keywords: Antioxidant, Comet assay, Cyanidin 3-glucoside, Cytoprotective, Fluconazole, Fungal diseases, Genotoxic, Isoorientin, Microgel electrophoresis (MGE), Single cell gel assay (SCG), Water palm

The *açaizeiro* (*Euterpe oleracea* MART.) is a palm tree endemic to the Amazon region and is of great commercial importance and nutritional value in the diet of the Brazilian population. The state of Pará is the largest national producer of *açaí* (the fruit from the *açaizeiro*) and because of its cultural importance, it has become a symbolic fruit in the diet of the state, contributing to the economic development of the region[1]. The fruit of the *açaízeiro* is used to make juice, pulp or simply *açaí*, as it is known in the region. The word *açaí* comes from the indigenous Tupi language "yasa'y"(i), which means "water palm". Açaí pulp or juice is usually eaten with manioc flour, which is combined with fish, shrimp, or beef, and is the staple food of riverside communities[2]. In addition to providing *açaí*, which is widely consumed as a food, the *açaizeiro* is also commonly used in folk medicine[3]. Its roots are used to treat malaria[4] and leishmaniasis[5]; the oil extracted from the fruit is also used to treat diarrhea[6]. In this respect, this fruit has been the target of several studies due to these beneficial effects on health in general since its juice/pulp is consumed almost daily in some regions of the country. Among the studies that support the beneficial effects of regular *açaí* intake are those that show the strong antioxidant potential of the fruit, mainly due to the presence of polyphenols in its constitution[7,8].

Other studies show the anticarcinogenic effect of *açaí* in animal models of bladder, esophageal, and colon cancers, among several others[9]. *Açaí* has also been shown to be a potential photosensitizer in photodynamic therapy against cancer[10] and proved effective in protecting against arterial stiffness in individuals with excess weight[11]. In addition, *açaí* seems to have no genotoxic effects on normal cells *in vitro* or in *in vivo* models[12]. Besides showing no cytotoxic or genotoxic effects, it is suggested that *açaí* may act as a cytoprotective agent. Ribeiro *et al.*[13] demonstrated that the fruit, while not inducing genotoxicity in Swiss albino rats, protected their cells against the genotoxic effects of doxorubicin, an antitumor drug used in clinical and experimental oncology.

The triazole antifungal fluconazole (2-(2,4-difluoro phenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol) is a

*Correspondence:
Phone: +55 91 32017102
E-Mail: mbahia@ufpa.br

synthetic compound containing three nitrogen atoms in the azol ring[14]. Its mechanism of action is based on the inhibition of the oxidative enzyme lanosterol 14-α-demethylase, which is associated with cytochrome P450 and is essential in the bioregulation of cell membrane fluidity, asymmetry, and integrity[15]. Fluconazole has a broad spectrum and is used to treat infections caused by various pathogenic fungi[16].

Fluconazole is used in the clinic for prophylaxis and treatment of oropharyngeal and esophageal candidiasis in AIDS patients, as well as in neutropenic patients, or those who have undergone surgical procedures and developed invasive candidiasis[17]. Despite its wide use, Correa *et al.*[18] reported that fluconozale increases the DNA damage index (DI) and the frequency of micronucleus of VERO cells. The authors also demonstrated that fluconazole is both a necrosis and a reactive oxygen species (ROS) inducer. Fluconazole also induced genotoxicity, mutagenicity, and cytotoxicity in human peripheral blood mononuclear cells (PBMCs)[19].

Since this antifungal is widely used, it is of interest to develop studies that evaluate compounds that protect cells against the aforementioned effects. Thus, in the present study, we explored whether clarified *açaí* (CA) pulp extract can act as a cytoprotective agent against fluconazole-induced cytotoxicity and genotoxicity in VERO cells.

Material and Methods

Cell line cultures and treatments

VERO cells, obtained from African green monkey (*Cercopithecus aethiops*)[20] kidney epithelial cells, were purchased from the *Banco de Células do Rio de Janeiro* (BCRJ - Rio de Janeiro Cell Bank). Cells were cultured in DMEM supplemented with 10% SBF, 0.1 mg/mL streptomycin, and 99 U/mL penicillin and maintained in a 5% CO2 incubator at 37°C. Cells were subcultured 2-3 times per week. Pure grade fluconazole (CAS: 86386-73-4) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). The chemical structure of fluconazole (2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol) is shown in Fig. 1. The drug was dissolved in pure dimethyl sulfoxide (DMSO) (CAS 67-68-5, Sigma Chemical Co, St. Louis, MO, USA) in order to achieve less than 1% (v/v) final concentration of DMSO in the cultures. The experimental groups were as follows: Gr. I: negative control where cells grown only in the presence of DMEM culture medium; Gr. II: positive control where cells were treated with a single concentration of 1306 µM fluconazole; Gr. III-V: cells treated only with CA at three different concentrations (50 µg/mL; 100 µg/mL and 200 µg/mL); Gr. VI-VIII: simultaneous CA + fluconazole treatment (50 µg/mL CA + fluconazole 1306 µM; 100 µg/mL CA + fluconazole 1306 µM and 200 µg/mL CA + fluconazole 1306 µM), and the experiments were performed in triplicate. The fluconazole concentration used was taken from Correa *et al.*[18], while the CA concentrations were chosen from Martinez *et al.*[21].

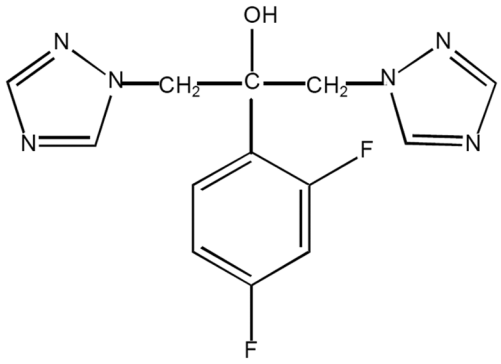

Fig. 1 — Chemical structure of fluconazole

Clarified *açaí* (CA) pulp extract

Amazon Dreams (Belém - Pará - Brazil) kindly provided the *açaí* (clarified pulp extract) used in this study. The process to produce the pulp was licensed by the company and by the Federal University of Pará (PI 8 1003060-3) and includes microfiltration and centrifugation of the juice prepared with fresh fruit[22]. In order to quantify the anthocyanins and the main flavonoids present in the clarified pulp, two validated UHPLC-DAD methods were used[23,24] with the standard compounds (orientin, homoorientin, taxifolin, cyanidin 3-glucoside, and cyanidin 3-rutinoside) purchased from Extrasynthèse.

Cell viability assay (MTT)

For the analysis of cell viability by the yellow tetrazolium salt [(3-(4,5-dimethylthiazol-2-yl)-2, 5-di phenyltetrazolium bromide] (MTT) assay, 96-well plates with 5×10^3, 4×10^3 and 3×10^3 cells per well were used at 24 h, 48 h and 72 h of treatment, respectively. After treatment, 100 µL of MTT (5000 µg/mL) was added to the cells for 3 h. Then, the MTT was removed and 100 µL of DMSO (Sigma Chemical Co, St. Louis, MO, USA) was added to the wells. Subsequently, the DMSO was read in a spectro-

photometer (λ=562 nm). Cell survival was calculated according to the percentage of absorbance relative to the absorbance of the control.

Comet assay (alkaline version)

For this assay, 0.18×10^6 cells/well were seeded in 12-well culture plates (Corning) for 20 h. The cells were subsequently incubated for 3 h with CA and fluconazole as described above. After 3 h of treatment, a sample (450 µL) was collected from each group and centrifuged (900 g/5 min) in a microcentrifuge (Eppendorf). The remaining pellet was mixed with 300 µL of low melting point agarose (0.8%) and placed on slides pre-coated with normal melting point agarose (1.5%). The slides were then covered with coverslips. The slides were kept at 4°C for 5 min until the agarose polymerization. After this period, the coverslips were removed, and the slides were immersed in ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% DMSO; pH: 10) for 5 min and kept at 4°C protected from light. After lysis, the slides were subjected to electrophoresis with a freshly prepared electrophoresis solution (1 mM EDTA, 300 mM NaOH; pH ≥13) at 4°C. Electrophoresis was performed at 34 V (0.8 V/cm^{-1}) and 300 mA for 25 min. After electrophoresis, the slides were placed in distilled water at 4°C for 5 min for neutralization and fixed in ethanol (3 min). The slides were stained with ethidium bromide (20 µg/mL). One hundred cells were analyzed per sample (50 cells from two different slides) using a fluorescence microscope (OLYMPUS BX41). The Damage Index (DI) was determined by visualization under the microscope according to the size and intensity of the comet tail (halo). Considering these criteria, 5 categories (0-4) were obtained, namely, 0 = no damage (<5%); 1 = low level of damage (5-20%); 2 = medium level of damage (20-40%); 3 = high level of damage (40-95%) and 4 = total damage (95%)[25,26]. The formula used for DI was: DI (*au*): $[(N1 * 1 + N2 * 2 + N3 * 3 + N4 * 4)]/100$ (total number of cells analyzed), where DI is the damage index, *au* is arbitrary unit and N1-N4 are the cells in categories 1, 2, 3 and 4.

Statistical analysis

Statistical analysis was performed using BIOESTAT 5.0 software considering *P*-values <0.05 as significant. For parametric data sets, statistical analysis was performed using ANOVA followed by the Tukey test. For non-parametric data sets, the Kruskal-Wallis test followed by the Dunn test was used.

Results and Discussion

According to the UHPLC-DAD techniques cited in the *Material and Method* section, CA presented five main phenolic compounds, all expressed in 100 mL of pulp: 38 mg orientin, 25 mg homoorientin, 31 mg taxifolin deoxyhexose, 18 mg cyanidin 3-glucoside and 45 mg cyanidin 3-rutinoside.

The cell viability assay (MTT) (Fig. 2), carried out at three different exposure times, showed that CA increases the viability of VERO cells exposed to fluconazole. At the 24 h treatment, this increased cell viability was observed to occur at the 100 µg/mL CA + fluconazole (P <0.05) and 200 µg/mL CA + fluconazole (P <0.01) co-treatments. Also, in the 24 h treatment, a significant decrease in cell viability was observed at the 200 µg/mL CA concentration (P <0.05) when compared to the negative control. At the 48 h treatment, increased cell viability was observed only at the 200 µg/mL CA + fluconazole co-treatment (P <0.05). On the other hand, at this same treatment time, a significant decrease in cell viability was observed at the 100 µg/mL CA (P <0.05) concentration compared to the negative control. The 72 h treatment demonstrated a significant increase in cell viability only at the 200 µg/mL CA + fluconazole co-treatment (P <0.05). All single concentrations of fluconazole significantly decreased the viability of VERO cells when compared to their respective controls (24 h, P <0.01; 48 h, P <0.05; 72 h, P <0.05).

The data obtained in the comet assay showed that as the concentrations of CA increased in the co-treatments, the lower the DI was (Fig. 3). The differences were significant in the three co-treatments (50 µg/mL CA + fluconazole (P <0.01; DI=2.45), 100 µg/mL CA + fluconazole (P <0.01; ID=2.14) and 200 µg/mL CA + fluconazole (P <0.01; DI=1.89) compared to treatment with fluconazole alone (DI=3.42). Fluconazole DI (3.43) was significantly different (P <0.01) from the control (DI=0.65). A significant decrease in damage indices was also observed for the 100 µg/mL CA (P <0.05; DI=0.46) and 200 µg/mL CA (P <0.01; DI=0.36) concentrations compared to control (DI=0.65). A significantly high percentage of cells with damage 4 was observed in the fluconazole treatment (P <0.01) compared to negative control. There was also a significant decrease in the number of cells with damage 4 in the three co-treatments (p<0.01) when compared to the fluconazole treatment (Table 1).

Table 1 — Distribution of damage classes observed in VERO cells by comet assay after 3 h of treatment

Treatments	Damage classes[≠] by Comet assay (mean ± standard deviation) %					No. of cells
	0	1	2	3	4	
Gr. I (negative control)	70.33±1.53	12.00±1.00	6.33±1.53	4.67±0.58	6.67±0.58	300
Gr. II Fluc (1306 µM) (positive control)	5.00±2.65	3.67±2.08	6.00±3.00	14.67±2.08	70.67±1.53*	300
Gr. III 50 µg/mL CA	70.33±3.51	15.33±5.51	7.00±1.00	4.00±1.00	3.33±0.58	300
Gr. IV 100 µg/mL CA	73.00±1.00	16.67±3.06	5.00±1.00	3.67±1.53	2.67±0.58	300
Gr. V 200 µg/mL CA	80.67±3.21	10.33±2.31	3.00±1.00	2.67±0.58	2.67±0.58	300
Gr. VI 50 µg/mL CA+fluc	12.33±0.58	13.00±1.73	23.00±3.00	21.00±4.00	30.67±1.15[#]	300
Gr. VII 100 µg/mL CA+fluc	16.67±1.53	21.33±1.53	18.00±1.73	19.00±2.00	25.00±1.00[#]	300
Gr. VIII 200 µg/mL CA+fluc	20.33±1.53	29.00±2.65	15.67±1.15	11.67±0.58	23.33±2.08[#]	300

[[≠]Damage classes: 0 = no damage; 1 = low level of damage; 2 = medium level of damage; 3 = high level of damage; 4 = total damage. CA: clarified *açaí*; Fluc: fluconazole. **P* <0.01 compared to negative control. [#]*P* <0.01 compared to positive control (fluconazole) (ANOVA/Tukey post-test). Mean of three experiments]

The MTT colorimetric test is a widely used cell viability indicator that assesses the ability of mitochondria to metabolize and reduce, via mitochondrial dehydrogenases, the MTT tetrazolium salt into a blue/purple product called formazan. This product can be quantified by spectrophotometry in viable cells[27]. Our results with the MTT assay demonstrated that CA increases the viability of cells exposed to fluconazole, especially at the concentration of 200 µg/mL (Fig. 2). Similar results were reported by Monteiro *et al.*[28]. These authors found that the hydroalcoholic extract of the *açaí* seed, rich in polyphenols, could protect human umbilical vein endothelial cells (HUVEC) from cell death induced by indoxyl sulfate, an oxidative stress-inducing uremic toxin. Another similar study was carried out by Al-Nasser *et al.*[29]. Using the MTT assay, the authors showed that the aqueous and ethanolic extracts of *açaí* can protect the human neuroblastoma cell line SH-SY5Y against oxidative stress induced by the neurotransmitter L-glutamate (L-Glu), indicating a neuroprotective effect of the fruit.

According to Correa *et al.*[18], fluconazole is cytotoxic, inducing decreased cell viability and necrosis in VERO cells at the same concentration used in the present study. In the same study, the authors showed that fluconazole is an inducer of oxidative stress. Thus, we suggest that the increase in cell viability induced by CA in VERO cells exposed to fluconazole may be related to the antioxidant effects of phenolic compounds[30] present in our extract. By decreasing the number of reactive oxygen species (ROS) CA prevents them from damaging cell membranes[31,32], thus decreasing the induction of necrosis.

Cyanidin 3-glucoside (C3G) is among the components with antioxidant capacity that are found

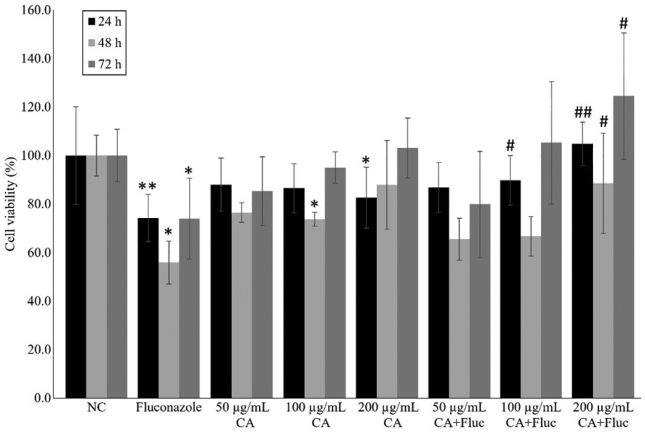

Fig. 2 — Cell viability observed after 24, 48 and 72 h of treatment according to the protocol described in Methodology section. [CA: clarified *açaí*; Fluc: fluconazole. **P* <0.05; ***P* <0.01 compared to their respective negative control. [#]*P* <0.01; [##]*P* <0.01 compared to their respective positive control (fluconazole) (ANOVA/Tukey post-test) (Kruskal-Wallis/Dunn post-test). Mean of three experiment]

in CA. An MTT study by Yang *et al.*[33] showed that C3G increases the viability of hippocampal cells from Sprague-Dawley rats exposed to beta-amyloid Aβ (25-35), a protein that importantly forms senile plaques in the brains of Alzheimer's disease patients. This increase in viability was followed by a significant reduction in the ROS rate induced by the protein. Also using MTT, Tan *et al.*[34]. found that C3G increases the viability of HepG2 cells exposed to H_2O_2. As reported by Yang *et al.*[33], this increase was followed by a significant decrease in the ROS rate.

Our results with the MTT assay also demonstrated that CA decreases the viability of VERO cells in two specific situations: the 48 h treatment with 100 µg/mL CA and the 24 h treatment with 200 µg/mL CA (Fig. 2). Silva *et al.*[35] observed that similarly to CA, the hydroalcoholic extract of *açaí* pulp decreases the viability of MCF-7 human breast adenocarcinoma

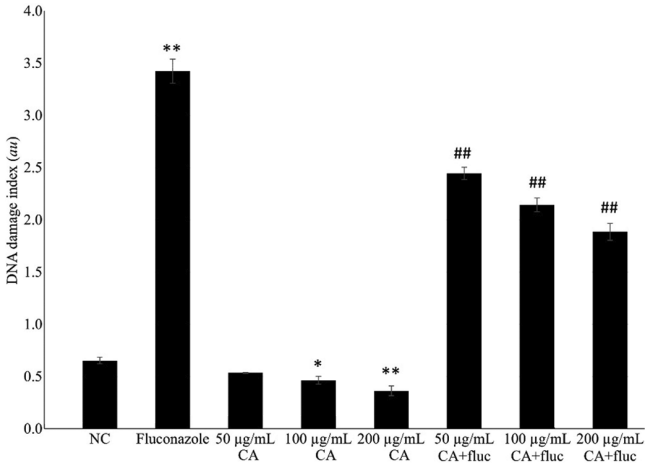

Fig. 3 — DNA damage index observed in VERO cells after 3 h of treatment, according to the protocol described in Methodology section. [CA: clarified *açaí*; Fluc: fluconazole; *au*: arbitrary unit. **P* <0.05; ***P* <0.01 compared to negative control. $^{\#\#}P$ <0.01 compared to positive control (fluconazole) (ANOVA/Tukey post-test). Mean of three experiments]

cells, without, however, clarifying or suggesting which component of the extract would be responsible for this result. On the other hand, Marques *et al.*[36] found that *açaí* oil was not able to decrease the viability of lymphocytes. In general, neoplastic cells seem to be more sensitive to *açaí* than non-transformed cells[37], thus further studies are needed to clarify this CA-induced decrease in viability in our experimental conditions.

Regarding the MTT assay, some considerations should be made. Although the test is widely used to detect what we call cell viability, the tetrazolium reduction reflects cell metabolism, not cell number or cell death[38]. Thus, we cannot claim whether the results obtained with the test are related to an effect of CA on cell metabolism, since *açaí* is highly energetic[39], or on cell viability itself. To clarify this question, it would be important to perform tests that detect apoptosis/necrosis, either by flow cytometry[40] or by staining with fluorescent dyes[41], or tests that detect cell membrane damage, such as the trypan blue test[40].

The comet assay, also called individualized single cell gel assay (SCG) or microgel electrophoresis (MGE) is a test that evaluates DNA lesions involving applications of electric current to cells, on agarose gel slides, stained with specific dyes and visualized by light microscopy[42]. In our results of the comet assay it was observed that CA significantly decreases the DI of VERO cells exposed to fluconazole (figure 3). These findings are in agreement with the study by Ribeiro *et al.*[13], who investigated the genotoxic and antigenotoxic activities of *açaí* pulp in peripheral blood, kidney, and liver cells of Swiss mice treated with doxorubicin (DXR), an oxidative stress-inducing antitumor. The authors observed that *açaí* pulp inhibited DNA damage induced by the antitumor agent DXR in all organs tested. The authors attributed this effect to the antioxidant compounds contained in *açaí* pulp that are also present in our CA, e.g. the phenolic compounds cyanidin 3-glucoside (discussed earlier) and cyanidin 3-rutinoside. Isoorientin (homoorientin) is also one of the flavonoids found in CA. Chen *et al.*[43], investigated the effects of isoorientin against oxidative injury induced by heavy metal cadmium in rat proximal tubular cell line NRK-52E and primary rat proximal tubular (rPT) cells. The authors observed that the treatment with 2.5 µM cadmium for 12 h resulted in DNA damage and cell cycle arrest in G0/G1, while isoorientin significantly decreased such effects when in co-treatment with the metal. Therefore, in the same way that the antioxidant compounds found in CA protect cells against the cytotoxic effects of fluconazole by increasing their viability, they probably also protect against DNA damage, which would explain the antigenotoxic effects observed in our experiments.

In literature, we found only one similar study, where the authors evaluated the genotoxicity and antigenotoxicity of *açaí in vitro*[36]. Here, the authors have observed that *açaí* oil does not show genotoxicity and, unlike our results, also does not protect DNA of peripheral lymphocytes and HepG2 cells against the effects of methylmethanosulfonate (MMS). This difference between our results and those of Marques *et al.*[36], may be attributed to the inducer of DNA damage used in their study (i.e., MMS) which is not a classical oxidant agent, but rather an alkylating agent[44].

Conclusion

Results obtained in our study using the comet assay and MTT assay for cell viability demonstrated that the clarified *açaí* extract can act as an antigenotoxic and anticytotoxic agent, protecting VERO cells from DNA damage caused by the antifungal fluconazole and recovering their viability. We suggest that these effects are related to the phenolic compounds present in CA that with their antioxidant capacity are able to neutralize the reactive oxygen species induced by

fluconazole. However, our study is preliminary, thus, we cannot rule out other cytoprotective pathways. Further studies are needed to better clarify the mechanisms of action of CA that cause this protection.

Conflict of interest

Authors declare no competing interests.

References

1 Ribeiro MJG & Lisboa SPP, A importância da extração vegetal do açaí (*Euterpe oleraceae* MART.) no estado do Pará. *Revista Multidisciplinar De Educação E Meio Ambiente*, 2 (2021) 38. DOI: https://doi.org/10.51189/rema/1719.

2 Leila M, História e natureza: do açaí ao palmito. *Revista Territórios e Fronteiras*, 3 (2010) 74. DOI: https://doi.org/10.22228/rtf.v3i2.69.

3 Yamaguchi KK, Pereira LF, Lamarão CV, Lima ES & da Veiga-Junior VF, Amazon acai: chemistry and biological activities: a review. *Food Chem*, 179 (2015) 137.

4 Vigneron M, Deparis X, Deharo E & Bourdy G, Antimalarial remedies in French Guiana: a knowledge attitudes and practices study. *J Ethnopharmacol*, 98 (2005) 351.

5 Odonne G, Berger F, Stien D, Grenand P & Bourdy G, Treatment of leishmaniasis in the Oyapock basin (French Guiana): A K.A.P. survey and analysis of the evolution of phytotherapy knowledge amongst Wayãpi Indians. *J Ethnopharmacol*, 137 (2011) 1228.

6 Ulbricht C, Brigham A, Burke D, Costa D, Giese N, Iovin R, Grimes Serrano JM, Tanguay-Colucci S, Weissner W & Windsor R, An evidence-based systematic review of acai (*Euterpe oleracea*) by the Natural Standard Research Collaboration. *J Diet Suppl*, 9 (2012) 128.

7 Oliveira NKS, Almeida MRS, Pontes FMM, Barcelos MP, Silva CHTP, Rosa JMC, Cruz RAS & Hage-Melima LIS, Antioxidant effect of flavonoids present in *Euterpe oleracea* Martius and neurodegenerative diseases: A Literature Review. *Cent Nerv Syst Agents Med Chem*, 19 (2019) 75.

8 Martins GR, Mattos MMG, Nascimento FM, Brum FL, Mohana-Borges R, Figueiredo NG, Neto DFM, Domont GB, Nogueira FCS, de Paiva Campos FA & Sant'Ana da Silva A, Phenolic Profile and Antioxidant Properties in Extracts of Developing Açaí (*Euterpe oleracea* Mart.) Seeds. *J Agric Food Chem*, 70 (2022) 16218.

9 Alessandra-Perini J, Rodrigues-Baptista KC, Machado DE, Nasciutti LE & Perini JA, Anticancer potential, molecular mechanisms andtoxicity of *Euterpe oleracea* extract (açaí): A systematic review. *PLoS ONE*, 13 (2018) 7.

10 Silva Junior ZS, Dos Santos LAD, Gonçalves MLL, Gallo JMAS, da Silva T, Motta LJ, Santos EM, Horliana ACRT, Fernandes KPS, Mesquita-Ferrari RA & Bussadori SK, Photodynamic therapy with acai (*Euterpe oleracea*) and blue light in oral cells: A spectroscopic and cytotoxicity analysis. *J Biophotonics*, 16 (2023) e202200259. doi: 10.1002/jbio.202200259.

11 Arisi TOP, Gorski F, Eibel B, Barbosa E, Boll L, Waclawovsky G & Lehnen AM, Dietary intake of anthocyanins improves arterial stiffness, but not endothelial function, in volunteers with excess weight: A randomized clinical trial. *Phytother Res*, 37 (2023) 798.

12 Marques ES, Froder JG, Carvalho JCT, Rosa PCP, Perazzo FF & Maistro EL, Evaluation of the genotoxicity of *Euterpe oleraceae* Mart. (Arecaceae) fruit oil (açaí), in mammalian cells *in vivo*. *Food Chem Toxicol*, 93 (2016) 13.

13 Ribeiro JC, Antunes LMG, Aissa AF, Darin JAC, Rosso VV, Mercadante AZ & Pires BMLP, Evaluation of the genotoxic and antigenotoxic effects after acute and subacute treatments with açaí pulp (*Euterpe oleracea* Mart.) on mice using the erythrocytes micronucleus test and the comet assay. *Mutat Res*, 695 (2010) 22. doi: 10.1016/j.mrgentox.2009.10.009.

14 Groppelli S, Zega G, Biggiogero M, Bernardi F, Sotgia C & Pennati R, Fluconazole induces teratogenic effects in the tunicate *Phallusia mammillata*. *Environ Toxicol Appl*, 3 (2007) 71.

15 Catalán M & Montejo JC, Antifúngicos sistémicos: Farmacodinamica y farmacocinética. *Rev Iberoam Micol*, 23 (2006) 39.

16 Bergold AM & Georgiadis S, Novidades em fármacos antifúngicos: uma revisão. *Visão Acadêmica*, 5 (2004) 2.

17 Loeffler J & Stevens DA, Antifungal drug resistance. *Clin Infect Dis*, 36 (S1) (2003) S31. https://doi.org/10.1086/344658.

18 Correa RMDS, Mota TC, Guimarães AC, Bonfim LT, Burbano RR & Bahia MO, Cytotoxic and genotoxic effects of fluconazole on African green monkey kidney (Vero) cell line. *Biomed Res Int*, 2018 (2018) 7.

19 Silva GS, Zuravski L, Duarte MMMF, Machado MM & Oliveira LFS, Fluconazole induces genotoxicity in cultured human peripheral blood mononuclear cells via immunomodulation of TNF-α, IL-6, and IL-10: new challenges for safe therapeutic regimens. *Immunopharmacol Immunotoxicol*, 41 (2019) 123.

20 Yasumura Y, The research for the SV40 by means of tissue culture technique. *Nippon Rinsho*, 21 (1963) 1201.

21 Martinez RM, Guimarães DAB, Berniz, CR, Abreu JP, Rocha APMD, Moura RS, Resende AC & Teodoro AJ, Açai (*Euterpe oleracea* Mart.) seed extract induces cell cycle arrest and apoptosis in human lung carcinoma cells. *Foods*, 11 (2018) 178.

22 Souza-Monteiro JR, Hamoy M, Santana-Coelho D, Arrifano GP, Paraense RS, Costa-Malaquias A, Mendonça JR, da Silva RF, Monteiro WS, Rogez H, de Oliveira DL, do Nascimento JL & Crespo-López ME, Anticonvulsant properties of *Euterpe oleracea* in mice. *Neurochem Int*, 90 (2015) 20.

23 Dias ALS, Rozet E, Larondelle Y, Hubert P, Rogez, H. & Quetin-Leclercq J, Development and validation of an UHPLC-LTQ-Orbitrap MS method for non-anthocyanin flavonoids quantification in *Euterpe oleracea* juice. *Anal Bioanal Chem*, 405 (2013) 28.

24 Dias ALS, Rozet E, Chataigne G, Oliveira AC, Rabelo CAS, Hubert P & Quetin-Leclercq J, A rapid validated UHPLC–PDA method for anthocyanins quantification from *Euterpe oleracea* fruits. *J Chromatogr. B*, 907 (2012) 108.

25 Singh NP, Mccoy MT, Tice RR & Schneider ELA, Single technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*, 175 (1988) 184.

26 Hartmann A & Speit G, The contribution of cytotoxicity to DNA effects in the single cell gel test (comet assay). *Toxicol Lett*, 90 (1997) 183.

27 Mosmann T, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65 (1983) 1.
28 Monteiro EB, Soares EDR, Trindade PL, de Bem GF, Resende AC, Passos MMCDF, Soulage CO & Daleprane JB, Uraemic toxin-induced inflammation and oxidative stress in human endothelial cells: protective effect of polyphenol-rich extract from açaí. *Exp Physiol*, 105 (2020) 542.
29 Al-Nasser MN, AlSaadi AM, Whitby A, Kim DH, Mellor IR & Carter WG, Acai Berry (*Euterpe* sp.) Extracts Are Neuroprotective against L-Glutamate-Induced Toxicity by Limiting Mitochondrial Dysfunction and Cellular Redox Stress. *Life* (*Basel*), 13 (2023) 1.
30 Dias MM, Martino HS, Noratto G, Roque-Andrade A, Stringheta PC, Talcott S, Ramos AM & Mertens-Talcott SU, Anti-inflammatory activity of polyphenolics from açai (*Euterpe oleracea* Martius) in intestinal myofibroblasts CCD-18Co cells. *Food Funct*, 6 (2015) 10.
31 Vanlangenakker N, Vanden BT, Krysko DV, Festjens N & Vandenabeele P, Molecular mechanisms and pathophysiology of necrotic cell death. *Curr Mol Med*, 8 (2008) 207.
32 Ni CH, Yu CS, Lu HF, Yang JS, Huang HY, Chen PY, Wu SH, Ip SW, Chiang SY, Lin JG & Chung JG, Chrysophanol-induced cell death (necrosis) in human lung cancer A549 cells is mediated through increasing reactive oxygen species and decreasing the level of mitochondrial membrane potential. *Environ Toxicol*, 29 (2014) 740.
33 Yang JS, Jeon S, Yoon KD & Yoon SH, Cyanidin-3-glucoside inhibits amyloid β25–35-induced neuronal cell death in cultured rat hippocampal neurons. *Korean J Physiol Pharmacol*, 22 (2018) 689.
34 Tan J, Li P, Xue H & Li Q, Cyanidin-3-glucoside prevents hydrogen peroxide (H2O2)-induced oxidative damage in HepG2 cells. *Biotechnol Lett*, 11 (2020) 2453.
35 Silva DF, Vidal FC, Santos D, Costa MC, Morgado-Díaz JA, Nascimento MDSB & de Moura RS, Cytotoxic effects of *Euterpe oleracea* Mart. in malignant cell lines. *BMC Complement Altern Med*, 14 (2014) 175. doi: 10.1186/1472-6882-14-175.
36 Marques ES, Tsuboy MSF, Carvalho JCT, Rosa PCP, Perazzo FF, Gaivão IOM & Maistro EL, First cytotoxic, genotoxic, and antigenotoxic assessment of *Euterpe oleracea* fruit oil (açaí) in cultured human cells. *Genet Mol Res*, 16 (2017) 1.
37 Filho WEM, Almeida-Souza F, Vale AAM, Victor EC, Rocha MCB, Silva GX, Teles AM, Nascimento FRF, Moragas-Tellis CJ, Chagas MDSDS, Behrens MD, Hardoim DJ, Taniwaki NN, Lima JA, Abreu-Silva AL, Gil da Costa RM, Calabrese KDS, Azevedo-Santos APS & Nascimento MDDSB, Antitumor Effect of Açaí (*Euterpe oleracea* Mart.) Seed Extract in LNCaP Cells and in the Solid Ehrlich Carcinoma Model. *Cancers* (Basel), 15 (2023) 2544.
38 Berridge MV, Herst PM & Tan AS, Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. *Biotechnol Ann Rev*, 11 (2005) 127.
39 Yuyama LKO, Aguiar JPL, Silva Filho DFS, Yuyama K, Varejão MJ, Fávaro DIT, Vasconcelos MBA, Pimentel SA & Caruso MSF, Caracterização físico-química do suco de açaí de *Euterpe* precatória Mart. oriundo de diferentes ecossistemas amazônicos. *Acta Amazon*, 41 (2011) 545.
40 Lin KN, Zhao W, Huang SY & Li H, Grape seed proanthocyanidin extract induces apoptosis of HL-60/ADR cells via the Bax/Bcl-2 caspase-3/9 signaling pathway. *Transl Cancer Res*, 10 (2021) 3939.
41 Silva EL, Mesquita FP, Ramos INF, Gomes CBSMR, Moreira CDS, Ferreira VF, da Rocha DR, Bahia MO, Moreira-Nunes CA, de Souza CRT, Burbano RMR & Montenegro RC, Antitumoral effect of novel synthetic 8-hydroxy-2-((4-nitrophenyl)thio)naphthalene-1,4-dione (CNN16) via ROS-mediated DNA damage, apoptosis and anti-migratory effect in colon cancer cell line. *Toxicol Appl Pharmacol*, 456 (2022) 116256.
42 Di Paolo C, *Aplicação do ensaio cometa a estudo de danos ao DNA de robalos*, *Centropomus parallelus (Poey*, *1860)*, *expostos à ß-naftoflavona*, (Biblioteca Digital de Teses e Dissertações, Universidade de São Paulo, São Paulo), 2006.
43 Chen S, Yu Q, Dong W, Zhang H & Zou H, Isoorientin plays an important role in alleviating Cadmium-induced DNA damage and G0/G1 cell cycle arrest. *Ecotoxicol Environ Saf*, 187 (2020) 109851.
44 Beranek DT, Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. *Mutat Res*, 231 (1990) 11.