DISCIPLINARUM SCIENTIA *Disciplinarum Scientia.* **Série: Ciências da Saúde**
Santa Maria, v. 25, n. 1, p. 313-325, 2024
Recebido em: 13.08.2024. Aprovado em: 24.02.2025.
ISSN 2177-3335 | DOI: doi.org/10.37777/dscs.v25n1-023

EVALUATION OF THE ANTIPROLIFERATIVE POTENTIAL OF AÇAÍ (*Euterpe oleracea* Mart.) ISOLATED OR IN ASSOCIATION TO 5-FLUOROURACIL AGAINST HeLa CELLS[1]

AVALIAÇÃO DO POTENCIAL ANTIPROLIFERATIVO DO AÇAÍ (Euterpe oleracea Mart.) ISOLADO OU EM ASSOCIAÇÃO COM 5-FLUOROURACIL CONTRA CÉLULAS HeLa[1]

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ABSTRACT

Cancer constitutes a major problem for society, the occurrence of this disease is associated with the aging of the population, as well as the increased prevalence of risk factors. Cervical cancer is the third most common type of cancer in the women, and fourth leading cause of cancer-related deaths among women in Brazil. Around 60% of the currently used antineoplastic drugs were discovered from natural products, Euterpe oleracea Mart. popularly known as açaí, has been the subject of many studies in recent years, due to its high consumption, and its rich content of phytochemicals, particularly polyphenols, which have shown antiproliferative and pro-apoptotic effects in tumor cells, as well as tumor suppression, prevention adipogenesis, reduction of oxidative stress, and strong anti-inflammatory activity. This study aimed to evaluate the antiproliferative activity of the fruit, using its hydroalcoholic extract either alone, or combined with the chemotherapeutic drug 5-Fluorouracil. HeLa cells were cultured in a CO_2 incubator and treated with different concentrations of the extract (0.01-1000 µg/mL). The cytotoxicity of the same was evaluated by the cell viability assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide and evaluation of cellular metabolism was performed by the total rate of nitric oxide production. The results showed potential for the reduction of cell proliferation, especially at the concentration of 1 µg /mL, and for cell metabolism, the cells exhibited similar rates to the negative control at the lowest concentrations.

Keywords: Cervical cancer; Chemotherapeutic drugs; Natural products.

RESUMO

O câncer constitui um amplo problema para a sociedade, sua ocorrência está associada ao envelhecimento da população, bem como ao aumento da prevalência de fatores de risco. O câncer cervical é o terceiro tipo de tumor mais frequente na população feminina, sendo a quarta causa morte de mulheres por câncer no Brasil. Cerca de 60% dos antineoplásicos atualmente utilizados foram descobertos a partir de produtos naturais, sabe-se que o Euterpe oleracea Mart. conhecido popularmente como açaí, tem sido objeto de muitos estudos

1 Research article from scientific initiation manuscript

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nos últimos anos, devido ao seu alto consumo e à sua grande quantidade de fitoquímicos, principalmente à alta taxa de polifenóis que apresentam efeitos antiproliferativos, pró-apoptóticos em células tumorais, supressão de tumores, prevenção de adipogênese, estresse oxidativo e alta atividade anti-inflamatória. Este estudo teve como objetivo avaliar a atividade antitumoral do fruto, a partir do extrato hidroalcoólico do mesmo, combinado ou não ao quimioterápico 5-Fluorouracil. As células HeLa foram cultivadas em incubadora de CO_2 e tratadas com diferentes concentrações do extrato (0.01-1000 µg/mL). A citotoxicidade do mesmo foi avaliada pelo ensaio de viabilidade celular utilizando brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difenil-tetrazólio e a avaliação do metabolismo celular foi realizada pela taxa total de produção de óxido nítrico. Os resultados mostraram um potencial para a redução da proliferação celular, especialmente na concentração de 1 µg/mL, e para o metabolismo celular, as células apresentaram taxas semelhantes às do controle negativo nas concentrações mais baixas.

Palavras-chave: *Câncer cervical; Produtos naturais; Quimioterápicos.*

INTRODUCTION

According to the World Health Organization (WHO), cancer comprises a large group of diseases characterized by the uncontrolled growth of abnormal cells. It is a serious public health issue, with its rising incidence linked to factors such as population growth, smoking, obesity, physical inactivity, and changing reproductive patterns - often associated with urbanization and economic development (Sung *et al*., 2021). If untreated, cancer is often fatal, which highlighting the critical importance of early, being essential, early treatment and diagnosis (Mao *et al*., 2021). In Brazil, the National Cancer Institute (INCA) has estimated that 704,000 new cases of cancer, across all types, will be expected for each year of the three-year period 2023-2025, showing that, as in the rest of the world, cancer is also a national public health issue (INCA, 2023).

Among the different types of cancer, cervical cancer (CC) deserves to be highlighted due to its prevalence and mortality. Cervical cancer is the fourth most common cancer among women worldwide and the third most common cancer in Brazil, behind only breast and colorectal cancer, being the fourth leading cause of death for women due to cancer (INCA, 2022).

The development of CC is associated with several risk factors such as early onset of sexual activity, multiple sexual partners, multiparity, active and passive smoking, alcoholism, poor sexual hygiene, prolonged use of birth control pills, and especially viral infections, including herpes virus type 2, cytomegalovirus and in particular human papillomavirus (Andrade; Souza; Prates, 2022). Genital HPV infection is quite common and does not cause CC most of the time. However, in some cases, when infection with high-risk serotypes occurs, cell damage may occur that may progress to cancer.

These cell lesions are easily discovered on the preventative exam (most commonly known as the Pap smear) and are curable most of the time. This is due to the importance of periodic/annual repetition of the examination (INCA, 2016). The types of treatments most commonly used for CC are

surgery, radiotherapy, and chemotherapy. Treatment will vary with each patient's stage, tumor size, and individual factors (Carvalho *et al.,* 2021).

However, the treatments available for CC are expensive and the expected result is not always obtained, so the search for new therapy methods is of great relevance and scientific interest. 5-Fluorouracil is one of the most widely used chemotherapeutic drugs for the treatment of different cancers, including cervical cancer. It acts by blocking the cell cycle, especially in the synthesis phase (S phase) of DNA, which in turn acts by inhibiting tumor growth. However, this drug, as well as any chemotherapy, causes unwanted side effects such as nausea, alopecia, leukopenia, anorexia, and thrombocytopenia among others (Hertz *et al*., 2015). In this field of search for new therapeutic alternatives, studies with natural products have been gaining special attention.

The *Euterpe oleracea* Mart. fruit, better known popularly as Açaí, originating in Central and South America, is very abundant in wetlands of the Amazon (Kang *et al*., 2010). Such fruit has been studied for its high macronutrient content, and for its bioactive functional properties and pharmacological potential, due to its antioxidant, anti-inflammatory, hypocholesterolemic and even anticancer activity (Machado *et al*., 2022; Neto da Silva *et al.,* 2023; Borges *et al.,* 2024).

Positive results were pointed, for example, in the study by Borges *et al.* (2024), who found the antitumor effect of açaí extract in vitro using human breast carcinoma cell lines. According to Silva *et al.* (2014), açaí extract has potential antiproliferative capacity through activation of apoptotic pathways and suppression of tumor genes. Additionally, Silva *et al.* (2014) described that açaí acts on tumor suppression by inducing the formation of cellular vacuoles that occur by overexpression of genes linked to autophagosome formation. However, despite the existence of some studies that demonstrate the antiproliferative activity of açaí, there is still no research to elucidate whether the açaí berry hydroalcoholic extract has antiproliferative capacity in cervical cancer cells.

In view of this, the use of natural products as a therapeutic alternative is a promising strategy. The aim of this study is to investigate the antiproliferative potential of the hydroalcoholic extract of açaí (*Euterpe oleracea* Mart), a plant known for its antioxidant, anti-inflammatory and antiproliferative properties, in HeLa cells and to evaluate oxidative damage in this process. In addition, the study evaluates the combination of the extract with the chemotherapy drug 5-FLU, with the aim of improving the effectiveness of the treatment and minimizing side effects.

METHODOLOGY

EXPERIMENTAL DESIGN

This was an experimental study, which was developed using cell culture protocols with HeLa cervical cancer cell line and different treatments with açaí extract alone or in combination with

5-Fluorouracil chemotherapy. This research was developed with the purpose of investigating the anti-proliferative potential of açaí berry extract against this cell line due, especially to the large number of women affected by this multifactorial disease and also by the growing commercial and consumption scale of the fruit in question.

HYDROALCOOLIC FRUIT EXTRACT PRODUCTION *Euterpe oleracea* Mart (Açaí) AND CHEMICAL MATRIX CHARACTERIZATION

The fruits were obtained from a harvest region near the city of Manaus, AM, being transported to the Cell Culture and Bioactive Effects Laboratory at Franciscan University (UFN) under adequate refrigeration conditions so that the fruits did not lose their functional characteristics. Then the fruits were completely macerated and deposited in amber jars at a concentration of 300 mg/mL according to Bittencourt *et al*. (2013), using 70% ethanol as a solvent. The complete extraction process comprised 21 days, and each week the material was filtered and stored in a refrigerator. (Then, the filtered material was rotary evaporated and lyophilized to obtain the extract powder. After extract preparation, high-performance liquid chromatography was performed (HPLC). The extract concentration used for the characterization was 10 mg/mL, using the model handset SIL-20^{A} Shimadzu Auto Sampler and separation column Phenomenex C_{18}, according to Boligon *et al*. (2015). Quantifications of the chemical matrix were conducted by the integration of the peaks using external standards, such as gallic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid, epicatechin, orientin, cyanide-3-0-glycoside, luteolin, and apigenin, the complete characterization of the extract is described in the study by Machado *et al.* (2016). All standards were acquired through the company Sigma-Aldrich® (São Paulo, SP, Brazil).

CELL CULTURE AND TREATMENTS

HeLa lineage cells (ATCC® CCL-2^{TM}) cervical cancer and fibroblasts HFF-1 (ATCC® SCRC-1041) were obtained from cells bank the Rio de Janeiro (BCRJ, Rio de Janeiro, RJ, Brasil). Cell cultures were maintained at 37 °C in a humidified atmosphere of 5%, in the cell culture laboratory from UFN. The culture medium used was the *Dulbecco's Modified Eagle Medium* (DMEM) (Sigma-Aldrich, D5796, São Paulo, SP, Brasil), containing 10% fetal bovine serum (SFB) (Sigma-Aldrich, F2442, São Paulo, SP, Brasil) and supplemented with 1% penicillin/streptomycin antibiotics (100 U/mL e 100 mg/mL, respectively) (Gibco® Thermo Fisher, 15140122, São Paulo, SP, Brasil) and 1% amphotericin B antifungal (Gibco® Thermo Fisher, 15290018, São Paulo, SP, Brasil), in CO_2 incubators, with 5% saturation of CO_2, 37 °C and humidified environment. After obtaining the number of cells required for the development of all experiments, the cells were plated on ELISA 96

well for cell culture and conduction of treatments with different concentrations of açai hydroalcoholic extract (0.001-1000 µg/mL) incubation periods of 24, 48 e 72 h, therefore the effective concentration able to reduce cell viability by 50% can be determined (EC50) and the best incubation period. Made these determinations, the EC50 was then combined with chemotherapeutic drug 5-Fluorouracil at the concentration of 50 µM according to Zhang *et al.* (2017), to investigate the potential synergistic effect between the natural product and the chemical. After incubation periods, colorimetric and fluorimetric assays were developed, consistent with the objectives of this study.

AÇAÍ EFFECTS IN NORMAL CELLS

We analyzed the potential cytotoxic effect of açaí in Peripheral Blood Mononuclear Cells (PBMCs) and HFF1 cells since PBMCs are systemic cells and HFF-1 are precursor cells that are considered as mesenchymal stem cells (MSCs). The use of PBMC was approved by the Research Ethics Committee according to number 31211214.4.0000.5306. Cells were exposed to the same açaí concentrations used in cancer cell lines and incubated at 37 quand°C in a humidified atmosphere of 5% CO_2, for 48 h. After treatments adherent cells (HFF-1) were trypsinized to be detached and were washed twice with cold Phosphate Buffered Saline (PBS). HFF-1 and PBMCs were then resuspended at a concentration of $1x10^5$ cells/ mL. Cytotoxicity was also evaluated by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay. Cells were exposed to different açaí hydroalcoholic extract concentrations (0.001-1000 µg/mL) and incubated for 48 h. After the treatments, tests were carried out to assess cell proliferation, determine the total rate of nitric oxide and the release of reactive oxygen species.

DETERMINING CELL VIABILITY AND PROLIFERATION

After appropriate treatments, with different concentrations of isolated açaí extract or EC50 extract in association with 5-Fluorouracil, cells were evaluated for viability (24 h de incubation) and proliferation (48 h e 72 h of incubation) phone using the test MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), following the instructions described by Kang *et al.* (2010). This is a colorimetric test where the reagent MTT is yellow and penetrates cells, being metabolized by viable cell mitochondrial enzymes to form intracellular formazan ridges that have a violet color. Such crystals were solubilized with dimethyl sulfoxide (DMSO) and the absorbance reading was performed in plate reader equipment Anthos 2010 (Biochrom® Anthos 2010, Londres, Inglaterra) at 570 nm.

To complement the determination of cell viability of cells exposed to EC50 Açaí extract alone or in combination with the chemotherapy, the quantification of double stranded DNA was conducted (ds) extracellular as Cadoná *et al.* (2016), using reagent DNA PicoGreen® (Quant-It™ PicoGreen™

dsDNA Assay Kit, Thermo Fisher, São Paulo, SP, Brasil). The reagent DNA PicoGreen® can intercalate between molecules of dsDNA and emit fluorescence. Therefore, as the quantification of dsDNA is performed on cell supernatants, the higher the intensity of fluorescence emitted, the higher the cell mortality rate. Thus, the fluorescence emitted at 480 nm of excitement and 520 nm emission using plate reader apparatus SpetraMax i3 (Molecular Devices, San Jose, CA, USA).

DETERMINATION OF TOTAL NITRIC OXIDE RATE

The concentration-effect curves of açaí extract at different incubation times, such as EC50 isolated or in combination with 5-fluorouracil, were tested for the total rate of nitric oxide production by the colorimetric assay described by Choi *et al.* (2012). This assay is based on the use of Greiss which enables the determination metabolic nitrate and nitrite. The intensity of the color was quantified in a plate reader Anthos 2010 (Biochrom® Anthos 2010, Londres, Inglaterra) at 570 nm.

EVALUATION OF TOTAL RATE OF OXYGEN REACTIVE SPECIES

HeLa cells previously treated with extract concentrations alone or EC50 in association with chemotherapy were tested for cellular oxidative metabolism profile by 2´7 dichlorofluorescein diacetate (DCFH-DA), according to instructions described by Costa *et al.* (2012). This is a fluorimetric assay based on the initial metabolism of the DCFH-DA by intracellular enzymes, forming dichlorofluorescein (DCFH). This molecule in turn when in contact with ERO, especially the H_2O_2, forms dichlorodihydrofluorescein (DCF) which emits fluorescence at 525 nm when stimulated to 488 nm. Readings were determined on a plate reader SpetraMax i3 (Molecular Devices, San Jose, CA, USA).

STATISTICAL ANALYSIS

All results obtained were tabulated in the program table Microsoft Excel, version 2010, being converted to a percentage relative to the negative control. The data were then statistically analyzed by analysis of variance (ANOVA) one or two way, depending on the situation, followed by of post hoc de Tukey, using the statistical analysis and graphing program GraphPad Prism (GraphPad Prism, La Jolla, CA, USA), version 5,0. The results with $p<0,05$ were considered significant.

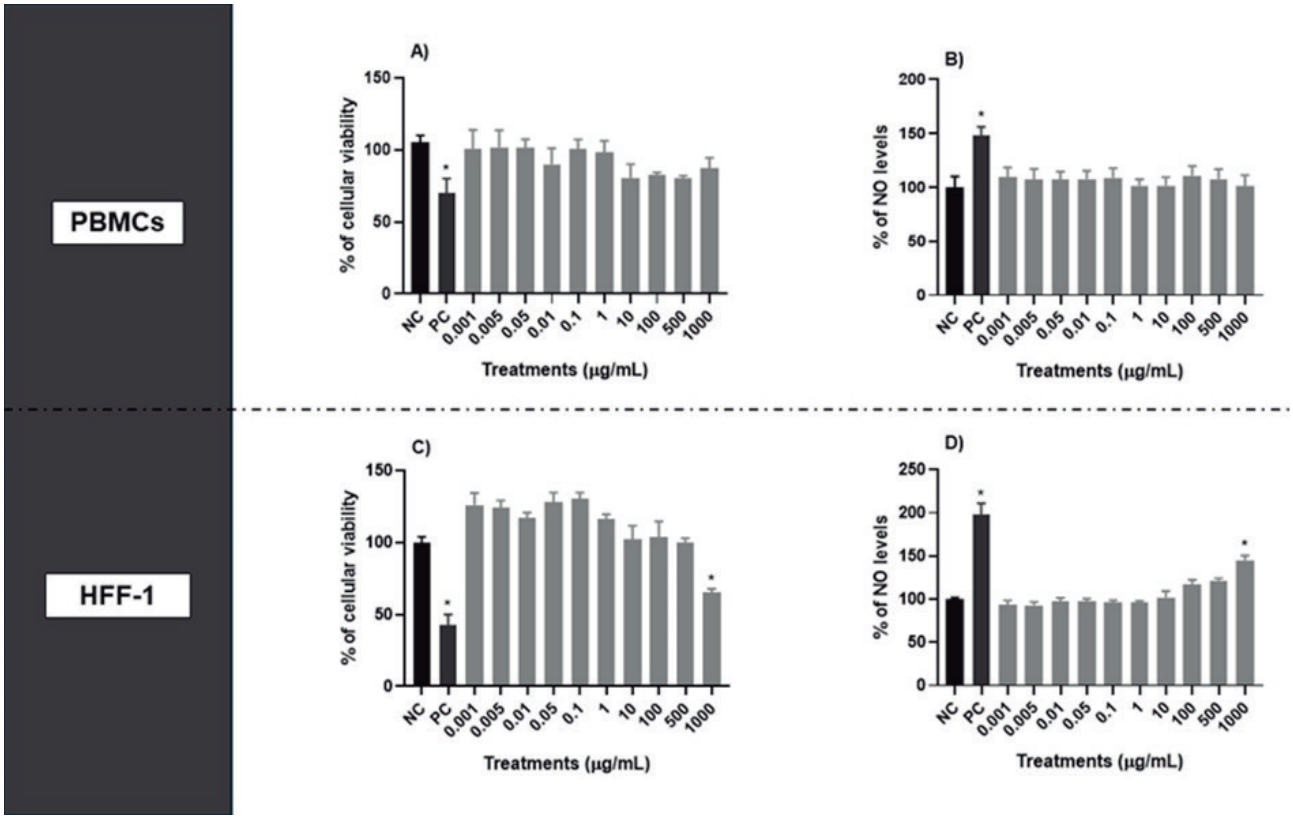
RESULTS AND DISCUSSION

To evaluate the safety profile of the hydroalcoholic açaí extract on normal PBMC and HFF-1 cell lines, MTT and NO release assays were performed. Regarding the determination of the safety

profile for açaí extract at concentrations of 0.001 to 1000 µg/mL in PBMC cells over a period of 24 h, no significant changes in cell viability were observed compared to the negative control (NC). Similarly, nitric oxide (NO) production remained unchanged relative to the negative control.

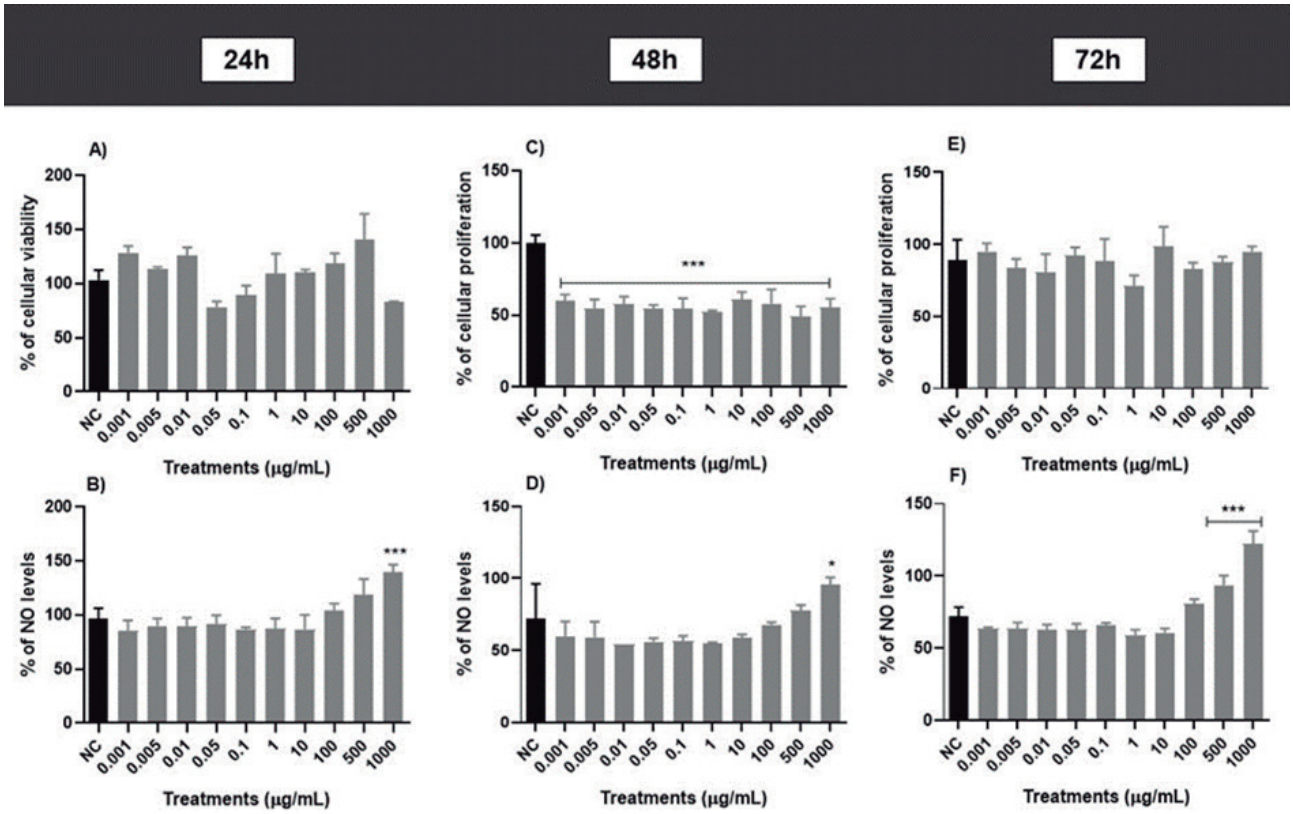
In HFF-1 cells, an increase in cell viability was identified in all tested concentrations of the extract, except for 1000 µg/mL, which showed a significant reduction. With regard to the indirect assessment of NO production, only the concentration of 1000 µg/mL promoted a statistically significant increase in NO release. The results are presented in Figure 1.

Figure 1 - Safety profile of açaí hydroalcoholic extract. Analysis of the safety profile of açaí hydroalcoholic extract on PBMCs and HFF-1 cell lines treated with different concentrations of açaí hydroalcoholic extract. (A and C) analysis of cell viability and (B and D) analysis of NO levels. The results were expressed in percentage in relation to the negative control (100%) and the statistical analysis was performed via one-way ANOVA followed by Tukey's post hoc. * Represents comparison with the negative control, where *p<0.05. NC refers to negative control (cells with cell culture medium only). CP: Positive cell death control DMSO 15%.



The reaction to MTT occurs exclusively in viable cells, and the absorbance values inversely reflect the levels of cell death. Thus, the results are compared to the absorbance value of the negative control, showing that the extract does not induce a reduction in cell viability (Figure 1 A and B). The results demonstrated that the extract exhibited no cytotoxic effects on normal cells, and did not affect the viability of non-tumor cells, in line with the findings of Barros *et al.* (2015).

Figure 2 - Analysis of antiproliferative capacity of açaí hydroalcoholic extract in HeLa cell line treated. (A) analysis of cell viability; (C and E) analysis of cell proliferation, (B, D and F) analysis of NO levels. The results were expressed in percentage to the negative control (100%) and the statistical analysis was performed via one-way ANOVA followed by Tukey's post hoc. (the most effective concentration was 1ug/mL for 48h IC 50= 464,54 ug/mL). * Represents comparison with the negative control, being: *p<0.05; **p<0.01; ***p<0.001. NC refers to negative control (cells with cell culture medium only).



To analyze the antiproliferative potential of the extract, the tests were carried out using HeLa cells. To verify the most effective concentration for cell death-inducing activity or antiproliferative potential, HeLa cells were exposed to 24, 48 and 72 h different concentrations of açaí hydroalcoholic extract, which were evaluated by the colorimetric assay MTT, as well as determining the total production rate of NO. The results showed that there was a slight variability in cell viability at the concentrations used during 24 h (Figure 2A), however, these results were not significant. On the other hand, all concentrations of the extract used during 48 h (Figure 2C) showed potential capacity to reduce cell proliferation, especially the concentration of 1 µg/mL. Already the cells exposed to the extract during 72h (Figure 2E) presented reduction of cell proliferation from the concentration of 100 µg/mL.

Although the focus of this study is the evaluation of the antiproliferative activity of the açaí extract, research into other plant extracts with similar properties is relevant. The study by Matic *et al.* (2013) showed that the extract of Helichrysum zivojinii has antitumor activity against HeLa cells, inducing changes in the cell cycle, activation of caspases and promoting apoptosis, characteristics similar to those observed in the açaí extract. Furthermore, it was described in the study that the extract was highly cytotoxic to malignant cells but declared very low toxicity to PBMCs.

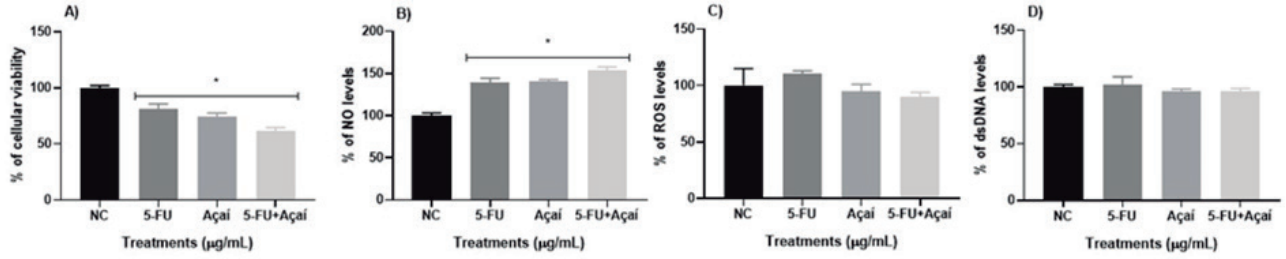
This finding is in line with the study by Filho *et al.* (2023) which also highlighted the antitumor potential of açaí seed extract, showing its efficacy in reducing the viability of prostate cancer cells (LNCaP) and in in vivo models of solid tumors, emphasizing its therapeutic potential.

In addition to cell viability and proliferation assessments, cells exposed to different açaí extract concentration-effect curves were tested for total NO production. (figures 2B, 2D e 2F). At all exposure times, cells showed a total NO rate like the negative control at lower concentrations. However, from the concentration of 10 µg/mL an increase in the amount of NO can be visualized in a dose-dependent manner, these values being significant in the concentrations of 1000 µg/mL at all exposure times and the concentration of 500 µg/mL after 72h of treatment. NO is a physiological soluble gas that is involved in various body processes, but also unbalanced in some pathological processes, even influencing cellular oxidative metabolism (Giordano; Verde; Corti, 2022, Lundberg; Weitzberg, 2022). Therefore, it is believed that the effects of açaí extract on NO levels are linked to its antioxidant profile. Açaí extract has been described in several scientific studies as a product of high antioxidant capacity in different cell lines. In the study of Cadoná *et al.* (2016), for example, the antioxidant activity of the extract in neurons with mitochondrial dysfunction has been described.

From the results of viability, proliferation and total cell rate of NO, the concentration of 1 µg/mL and the incubation period of 48h were selected as the ideal exposure parameters for the development of the other treatments and tests, due to the effectiveness and constancy of the oxidative profile observed. Therefore, new treatments were performed considering such determinations.

5-Fluorouracil (5-FU) is a chemotherapy drug used to treat different types of cancer, including CC. This drug acts by blocking the cell cycle in the synthesis phase (phase S) of DNA which acts by inhibiting tumor growth. However, this drug, like any chemotherapy, causes unwanted side effects (Hertz *et al*., 2015). To continue the proposed analyses, 5-FU and its combination with açaí extract were used. After the appropriate treatments, the in vitro test was carried out again (Figure 3). In the MTT test (Figure 3A), the results obtained again demonstrated the antiproliferative capacity of açaí on HeLa cells, which is similar to the antitumor standards of 5-Fu. In addition, the antiproliferative activity of the combination of the reference drug and the natural extract was also observed. Although no significant difference was observed between the treatment groups, all the groups showed statistically significant values when compared to the NC.

Figure 3 - Analysis of the antiproliferative capacity of 5-Fu, the hydroalcoholic extract of açaí (1 µg/mL) free and when associated with chemotherapy (5-Fu) against HeLa cells. (3A) analysis of cell viability, (3B) analysis of NO levels; (3C) Analysis of ROS levels and (3D) analysis of extracellular dsDNA levels. The results were expressed in percentage in relation to the negative control (100%) and the statistical analysis was performed via one-way ANOVA followed by Tukey's post hoc. * Represents comparison with the negative control, being: *p<0.05. NC refers to negative control (cells with cell culture medium only).



A fluorimetric assay was carried out to quantify the total levels of dsDNA in the extracellular medium (Figure 3D), wich showed no significant difference in increase when using the Picogreen® reagent. However, in the MTT analysis, an increase in the induction of HeLa cell death was observed in relation to the chemotherapy, the açaí extract and when the tumor cells were exposed to the combination of both variables. The results show that the açai extract, in the concentration of 1 µg/mL, does not reduce the antiproliferative activity of 5-FU, does not cause any negative pharmacological interaction, on the contrary, it is believed that the extract can act synergistically with the chemotherapy used as a reference. These results corroborate the research of Hertz *et al.* (2015) which demonstrated synergistic effect between 5-FU and guarana hydroalcoholic extract on breast cancer cells.

Additionally, HeLa celss exposed to açaí extract, 5-Fu, and the association of both were tested for ON production levels (Figure 3B) and the total rate of ERO (Figure 3C) and no significant cell modulation results were observed for any of the variables tested. Therefore, it is believed that the mechanism by which treatments induce cell death is not directly related to cellular oxidative metabolism but through some modification of the cell cycle.

Finally, based on the results found and according to research conducted by Silva (2015), açaí's antiproliferative activity is linked to its high concentration of polyphenols, which demonstrate actions in tumor suppression, preventing the proliferation of cells in certain cancers, such as colon cancer as well as pro-apoptotic and antiproliferative activity in leukemic cells. The results of this study also corroborate the data found by other studies, such as the study that evaluated the extract of Açaí seeds, which has high levels of catechin/epicatechin and showed significant antitumor activity against LNCaP cells, mediated mainly by mitochondrial and nuclear alterations (Filho *et al.,* 2023).

Thus, regarding the present study, it is necessary to carry out complementary tests that explore all possible routes of efficacy of the extract. In addition, because it is an in vitro study, limitations are present. However, it is believed that the results obtained are promising and significantly corroborate the scientific progress of the area.

CONCLUSION

The results obtained suggest that açaí extract acts as a antiproliferative potential agent against HeLa cervical cancer cells, particularly in the concentration of 1 µg/mL and after 48h of incubation. Furthermore, the extract was not able to negatively affect the activity of the 5-Fluorouracil chemotherapeutic agent, but it is suggested that there is a synergistic antiproliferative effect between the commercial drug and the natural agent. However, modulations of cellular oxidative metabolism were not evidenced through treatments with the extract, which ends up discarding the possibility of extract acting via oxidative imbalance induction. Therefore, it is believed that further studies could be developed on açaí extract as an antitumor agent, particularly to elucidate its mechanism of action and to confirm the efficacy and safety of this natural product.

REFERENCES

ANDRADE, J. V. ; SOUZA, J. C. M. DE; PRATES, J. G. **Pesquisas e abordagens educativas em ciências da saúde - Volume III**. [s.l.] Amplla Editora, 2022.

BARROS, L.C. *et al.* The powerful in vitro bioactivity of Euterpe oleracea Mart. seeds and related phenolic compounds. **Industrial Crops and Products**. v. 76, p. 318-322, 2015.

BITTENCOURT, L. S. *et al*. The protective effects of guaraná extract (Paullinia cupana) on fibroblast NIH-3T3 cells exposed to sodium nitroprusside. **Food Chemistry Toxicology**. v. 53, p. 119-125, 2013.

BOLIGON, A. A. *et al*. HPLC analysis and antimicrobial, antimycobacterial and antiviral activities of Tabernaemontana catharinensis. **Journal of Applied Biomedicine**. v. 3, p. 7-18, 2015.

BORGES, K. *et al*. Açaí (Euterpe oleracea Mart.) Seed Oil and Its Nanoemulsion: Chemical Characterisation, Toxicity Evaluation, Antioxidant and Anticancer Activities. **Current Issues in Molecular Biology**, v. 46, n. 5, p. 3763-3793, 23 abr. 2024.

BRASIL. Ministério da Saúde. Instituto Nacional de Câncer José Alencar Gomes da Silva - INCA. Controle do Câncer do Colo do Útero. **Diretrizes Brasileiras para o Rastreamento do Câncer do Colo do Útero**. 2022. Disponível em: https://www.gov.br/inca/pt-br/assuntos/gestor-e-profissional-de-saude/controle-do-cancer-do-colo-do-utero/dados-e-numeros/incidencia

BRASIL. Ministério da Saúde. Instituto Nacional de Câncer José Alencar Gomes da Silva - INCA. Serviço de Edição e Informação Técnico-Científica. **ABC do Câncer**: Abordagens Básicas para o Controle do Câncer. 2011. Disponível em: http://bvsms.saude.gov.br/bvs/publicacoes/abc_do_cancer.pdf. Acesso em: 1 set. 2017.

CADONÁ, F.C. *et al.* Guaraná a caffeine-rich food increase oxaliplatin sensitivity of colorectal HT-29 cells by apoptosis pathway modulation. Anti-Cancer. **Agents and Medicinal Chemistry**. v. 16, n. 8, p. 1055-1065, 2016.

CARVALHO, N. S. DE *et al.* Protocolo Brasileiro para Infecções Sexualmente Transmissíveis 2020: infecção pelo papilomavírus humano (HPV). **Epidemiologia e Serviços de Saúde**, v. 30, n. spe1, 15 mar. 2021.

CHOI, Woo-Suk *et al.* The regulatory effect of veratric acid on NO production in LPS-stimulated RAW264. 7 macrophage cells. **Cellular immunology**, v. 280, n. 2, p. 164-170, 2012.

COSTA, F. *et al.* Influence of Val16Ala SOD2 polymorphism on the in-vitro effect of clomiphene citrate in oxidative metabolism. **Reproductive biomedicine online**. v. 24, n. 4, p. 474-481, 2012.

FILHO, W. E. M. et al. Antitumor Effect of Açaí (Euterpe oleracea Mart.) Seed Extract in LNCaP Cells and in the Solid Ehrlich Carcinoma Model. **Cancers**, v. 15, n. 9, p. 2544-2544, 28 abr. 2023.

GIORDANO, D.; VERDE, C.; CORTI, P. Nitric Oxide Production and Regulation in the Teleost Cardiovascular System. **Antioxidants**, v. 11, n. 5, p. 957, 12 maio 2022.

HERTZ, E. *et al.* Effect of Paullinia cupana on MCF-7 breast cancer cell response to chemotherapeutic drugs. **Molecular and Clinical Oncology**.v. 3, n. 1, p. 37-43, 2015.

INSTITUTO NACIONAL DE CÂNCER (INCA). *Diretrizes para o rastreamento do câncer do colo do útero*. 2016. Disponível em: https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/diretrizesparaorastreamentodocancerdocolodoutero_2016_corrigido.pdf. Acesso em: 9 mar. 2024.

KANG, J. *et al*. Anti-Oxidant Capacities of Flavonoid Compounds Isolated from Acai Pulp (Euterpe Oleracea Mart.). **Food Chemistry**. v. 122, n. 3, p. 610-617, 2010.

LUNDBERG, J. O.; WEITZBERG, E. Nitric oxide signaling in health and disease. **Cell**, v. 185, n. 16, p. 2853-2878, 4 ago. 2022.

MACHADO, A. K. *et al*. Neuroprotective Effects of Açaí (Euterpe oleracea Mart.) against Rotenone in Vitro Exposure. **Oxidative Medicine and Cellular Longevity**, v. 2016, 2016.

MACHADO, A. P. DA F. *et al.* Antiproliferative effects and main molecular mechanisms of Brazilian native fruits and their by-products on lung cancer. **Food research international**, v. 162, p. 111953-111953, 1 dez. 2022.

MAO, J. J. *et al*. Integrative oncology: Addressing the global challenges of cancer prevention and treatment. **CA: A Cancer Journal for Clinicians**, v. 72, n. 2, p. 144-164, 9 nov. 2021.

MATIĆ*, I. Z. et al. In vitro antitumor actions of extracts from endemic plant Helichrysum zivojinii.* **BMC Complementary and Alternative Medicine**, v. 13, p. 13-36, fev. *2013.*

NETO DA SILVA, M. A. C. *et al.* Açaí (Euterpe oleracea Mart.) byproduct reduces tumor size and modulates inflammation in Ehrlich mice model. **Journal of Functional Foods**, v. 103, p. 105474, abr. 2023.

SILVA, D.F. *et al*. Cytotoxic Effects of Euterpe Oleracea Mart. in Malignant Cell Lines. **BMC Complementary and Alternative Medicine**. v. 14, n. 175, p. 1-9, 2014.

SUNG, H. *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: **A Cancer Journal for Clinicians**, v. 71, n. 3, p. 209-249, 2021.

WORLD HEALTH ORGANIZATION. **Cancer**. Disponível em: https://www.who.int/health-topics/cancer#tab=tab_1.

ZHANG, F. *et al*. Anti-Cancerous Potential of Polysaccharide Fractions Extracted from Peony Seed Dreg on Various Human Cancer Cell Lines Via Cell Cycle Arrest and Apoptosis. **Frontiers in Pharmacology**. v. 8, p. 1-13, 2017.7