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PREDICTION STUDY AND *IN VITRO* SAFETY PROFILE OF BIOACTIVE MOLECULES DERIVED FROM *Euterpe oleracea* Mart. EXTRACT[1]

ESTUDO DE PREDIÇÃO E PERFIL DE SEGURANÇA IN VITRO DE MOLÉCULAS BIOATIVAS DERIVADAS DO EXTRATO DE Euterpe oleracea Mart.

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ABSTRACT

Natural products have been highlighted for their bioactive properties. *Euterpe oleracea* Mart. (açaí) is a fruit that has been extensively investigated due to its significant effects on human health. However, due to the heterogeneous chemical matrix of açaí, its biological effects may be attributed to the bioactive molecules present in the extract. The objective of this study was to evaluate the biological properties of the 5 more abundant molecules of açaí extract, developing a study of prediction. Also, it was analyzed the catechin, apigenin and epicatechin *in vitro* safety profile. The prediction study was performed using the PASS online tool. The *in vitro* safety profile was conducted by using BV-2 cell line in different periods of incubation. The obtained results shown that catechin, epicatechin, epigallocatechin, apigenin and taxifolin were the most important elements at açaí's chemical matrix. These molecules were capable to positively modulate different parameters related to the oxidative metabolism and inflammatory pathways. Finally, most of the tested bioactive molecule's concentrations presented satisfactory *in vitro* safety profile. The results suggest that the known effects of the extract may occur as a result of the most abundant molecules in the chemical matrix of this natural product.

Keywords: *Euterpe oleracea* Mart.; flavonoids; computational tools.

RESUMO

Os produtos naturais têm se destacado por suas propriedades bioativas. O Euterpe oleracea Mart. (açaí) é uma fruta que tem sido amplamente investigada devido aos seus importantes efeitos na saúde humana. No entanto, devido a matriz química heterogênea do açaí, os efeitos biológicos podem estar atribuídos às moléculas bioativas presentes no extrato. O objetivo deste estudo foi avaliar as propriedades biológicas das cinco moléculas mais abundantes no extrato de açaí, desenvolvendo um estudo de predição. Além disso,

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foi analisado o perfil de segurança in vitro da catequina, apigenina e epicatequina. O estudo de predição foi realizado utilizando a ferramenta online PASS. O perfil de segurança in vitro foi avaliado utilizando a linhagem celular BV-2 em diferentes períodos de incubação. Os resultados obtidos mostraram que a catequina, epicatequina, epigalocatequina, apigenina e taxifolina foram os elementos mais importantes na matriz química do açaí. Essas moléculas foram capazes de modular positivamente diferentes parâmetros relacionados ao metabolismo oxidativo e às vias inflamatórias. Por fim, a maioria das concentrações das moléculas bioativas testadas apresentou um perfil de segurança in vitro satisfatório. Os resultados sugerem que os efeitos conhecidos do extrato podem ocorrer como resultado das moléculas mais abundantes na matriz química desse produto natural.

Palavras-chave: *Euterpe oleracea Mart., flavonoides, ferramentas computacionais.*

INTRODUCTION

There are many diseases that are characterized as worldwide public health problems. These diseases range from infectious problematic to highly complex multifactorial diseases, both acute and chronic. Cancer, diabetes, respiratory and cardiovascular diseases have been thoroughly studied for the investigation of new treatments (Choy *et al.*, 2019; Li *et al.*, 2019; Orhan; Senol Deniz, 2020; Ma *et al.*, 2021). However, despite the numerous scientific advances that involve understanding the etiological and pathophysiological aspects of diseases, there are still many health problems that challenge researchers and healthcare professionals in terms of pathophysiology (Makhoba *et al.*, 2020).

The advancement of disease-related studies has driven the search for a more in-depth understanding of the mechanisms involved. This has led to scientific investigations worldwide, aiming at the development of effective and personalized therapeutic methods to reduce possible side effects (Zhang *et al.*, 2018). In this context, naturally occurring products have emerged as a promising area of research.

Natural products have been widely used throughout history for the treatment and prevention of various diseases and infections. Ancient civilizations such as China, Greece and Egypt have shown interest in the healing properties of plants for thousands of years (Jamshidi-Kia; Lorigooini; Amini-Khoei, 2018). Likewise, indigenous communities have employed mixtures of medicinal herbs to relieve pain and treat wounds for centuries (Viegas Jr; Bolzani, 2006). This popular knowledge has become the basis for scientific research to prove the biological properties.

Research with products of natural source has been highlighted due to the numerous therapeutic activities they can present, such as antioxidant activities (Batista *et al.,* 2018), antitumor (Choi *et al.*, 2022; Fidelis *et al.*, 2021), anti-inflammatory (Wu *et al.*, 2022; Machado *et al.*, 2019) and neuroprotective (D'Amico *et al.*, 2022).

Among the various natural products that have bioactive activities, açaí (*Euterpe oleracea* Mart.) is a fruit from the Amazonia region, widely consumed in Brazil and worldwide, may be mentioned. Numerous studies have demonstrated the varied actions of açaí against aspects that are

intimately related to some diseases. D'Amico *et al.* (2022) demonstrated that açaí supplementation was able to neutralize the neuroinflammatory and oxidative effects found in people with Parkinson's disease, for example. This action seems to occur through the decrease in neuronal death and the improvement of the physiological antioxidant defense system in rats. In a study by Fragoso *et al.* (2018), it was shown that the freeze-dried pulp of açaí presented cellular protection capacity in rats with colon cancer induced by colitis.

Additionally, studies by our research group also describe interesting effects of the hydroalcoholic extract of açaí. Machado *et al.* (2016) first described the neuroprotective effect of such an extract in a model of neuronal-like cells induced to mitochondrial dysfunction by the use of rotenone. Later, in 2019, Machado *et al.* (2019) described the potential peripheral anti-inflammatory effect of the açaí extract in an experimental *in vitro* model of human macrophage activation. The anti-inflammatory profile of açaí was also evaluated in the research of de Souza *et al.* (2020) and Cadoná *et al.* (2021), showing that such a natural product can modulate the NLRP3 inflammasome and reducing pro-inflammatory cytokines in microglial cells.

An important aspect is that many of the observed and proven biological effects of natural products are directly related to the composition and chemical structure of these products, due to the presence of bioactive molecules as components of the chemical matrix of such products, which present biological activities, i.e., they can interact positively with the body's tissues (Fakri Mustafa *et al.*, 2021). The chemical matrix of açaí is vast and presents substances with high pharmacological capacity, being of great interest in research and the pharmaceutical industry (Cedrim; Barros; Nascimento, 2018).

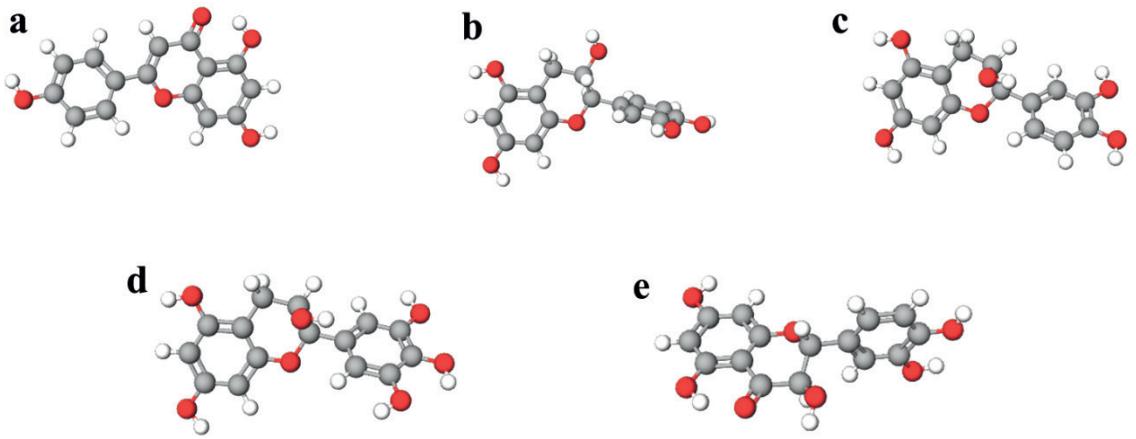
The chemical composition of açaí presents a class of polyphenols known as flavonoids. Several studies demonstrate the importance of flavonoids in human health due to their numerous bioactive effects (Ginwala *et al.*, 2019). Examples of flavonoids present in açaí are molecules such as apigenin, epicatechin, epigallocatechin, taxifolin and catechin.

Apigenin (Figure 1a) is one of the most widespread flavonoids among plants and belongs to the flavone class (Salehi *et al.*, 2019). It has been thoroughly studied for being an antioxidant, anti-inflammatory, anti-allergic, neuroprotective agent, as well as other beneficial activities for human health (Dourado *et al.*, 2020; Park *et al.*, 2020). Epigallocatechin (Figure 1d), on the other hand, is a flavonoid that has been studied for its benefits to cardiovascular health (Chen *et al.*, 2013), control of obesity (Kim *et al.*, 2019) and for inducing apoptosis in cancer cells (Vergote *et al.*, 2002). The molecule taxifolin (Figure 1e) has been widely studied due to its potential antioxidant (Topal *et al.*, 2016) and anti-inflammatory (Park *et al.*, 2023) effects. Regarding catechin (Figure 1b), several studies show its antioxidant (Grzesik *et al.*, 2018), anti-inflammatory (Hodges *et al.*, 2020), antimicrobial (Sinsinwar; Vadivel, 2020), and other important activities.

Epicatechin (Figure 1c) is a molecule that also belongs to the group of flavonoids and is part of the chemical matrix of açaí. This molecule can be found in other natural products such as

cocoa (*Theobroma cacao*) and green tea (*Camellia sinensis*), for example (Prakash; Basavaraj; Murthy, 2019). Epicatechin has been extensively investigated for its numerous therapeutic properties, such as improved cardiovascular function (Alañón *et al.*, 2020), high free radical-scavenging capacity (Qing *et al.*, 2023; Shanmugam *et al.*, 2017), and potential antitumor activity (Pereyra-Vergara *et al.*, 2020).

Figure 1 - Chemical structures of the molecules apigenin (a), catechin (b), epicatechin (c), epigallocatechin (d) and taxifolin (e).



Developed by the authors through the website MolView.

However, despite the numerous studies highlighting the biological activity of açaí extract, further investigation is still needed regarding the effect of the bioactive molecules identified in the chemical matrix of this extract. Therefore, it is crucial to understand the biological and pharmacological activities of the main isolated substances found in açaí in order to unravel the synergistic mechanism of action of these components.

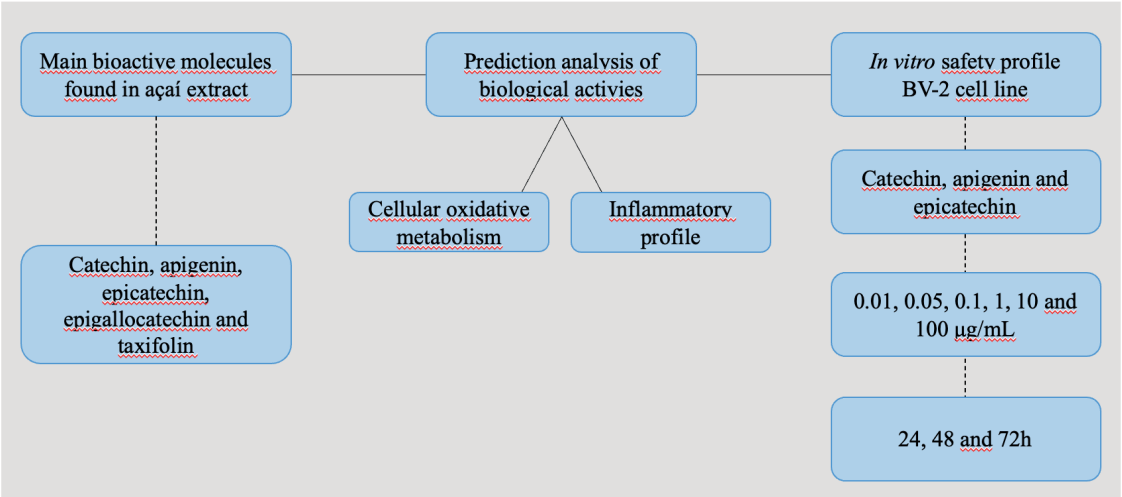
Thus, the use of computational tools for the prediction of biological activities of substances becomes of great importance, as these tools play a fundamental role in understanding the properties of these compounds of interest (Dorneles *et al.*, 2021). Therefore, this study aims to analyze the prediction of biological activities of the main substances present in açaí, as well as to perform *in vitro* safety profile assays of catechin, apigenin and epicatechin.

METHODOLOGY

RESEARCH DESIGN

This study has a theoretical and experimental nature, as shown in Figure 2.

Figure 2 - Graphical diagram of the study design.



PREDICTION ANALYSIS OF BIOLOGICAL ACTIVITIES

The study of prediction of biological and pharmacological activities of molecules was performed using open access platforms, such as the PubChem website, which provides information about the substances of interest and their chemical structures. From the canonical SMILES format obtained from the site database, the prediction of activity was carried out through the PASS Online tool (http://www.way2drug.com/passonline/). This tool allows the prediction of biological activities of individual molecules according to their chemical structures. Thus, the tool makes it possible to estimate the biological and pharmacological activity of a specific molecule before conducting experimental analysis or cell culture tests, for example.

The classification of the results obtained was demonstrated according to the information provided by the PASS Online system considering the estimated possibility of a molecule showing the probability of being active (Pa) or inactive (Pi) regarding the cell oxidative metabolism and the inflammatory profile, through the similarity of the chemical structure with other known substances that present such activity.

CELL CULTURE AND TREATMENTS

The BV-2 cell line (microglial cells) (BCRJ code #0356) was used for the *in vitro* safety profile evaluation of the bioactive molecules: catechin, apigenin and epicatechin. The cells were obtained from the Banco de Células do Rio de Janeiro (BCRJ) and were cultivated using RPMI cell culture medium containing 10% fetal bovine serum, 1% penicillin and streptomycin antibiotics and 1% HEPES 0.1M. The cells were kept in an incubator with 5% CO_2, 37°C, and a humid environment.

After obtaining the ideal number of cells for the *in vitro* tests, the cells were plated in 96-well plates at a density of $1x10^5$ cells/mL. After 24 hours of stabilization, the cells were treated with the catechin, apigenin and epicatechin using a concentration curve (0.01; 0.05; 0.1; 1; 10 and 100 µg/mL) for 24, 48, and 72h of incubation. The negative control (NC) consisted of cells only with culture medium, while the positive control (PC) consisted of cells exposed to 200 µM hydrogen peroxide (H_2O_2). All tests were performed in triplicate.

CELL VIABILITY AND PROLIFERATION ANALYSIS

For the evaluation of cell viability (24h) and proliferation (48 and 72h), the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test was performed. When incubated with viable cells, the tetrazolium salt, which has a yellow coloration, is reduced by the action of the succinate dehydrogenase enzyme, present in mitochondria. After reduction, MTT is converted into a compound called formazan (formazan crystals), stored in the cytoplasm of cells, with a purple-blue coloration. When dimethylsulfoxide (DMSO) is added, the formazan compound is solubilized, and subsequently a colorimetric quantification was performed through spectrophotometry, with a wavelength of 570 nm. The absorbance value is proportional to the number of viable cells (Fukui; Yamabe; Zhu, 2010).

DETERMINATION OF NITRIC OXIDE LEVELS

Nitric oxide (NO) levels were determined via an indirect colorimetric assay based on the use of the Griess reagent. This assay allowed the measurement of organic nitrite in cells, which is a metabolite of nitric oxide. Upon adding the Griess reagent to the cells, nitrite is detected through the formation of pink coloration, which occurs due to the presence of sulfanilamide in the Griess reagent, which is responsible for the formation of diazonium salts from nitrite present in cells. After incubation of the cell supernatant with the Griess reagent for 15 minutes at room temperature, the absorbance was determined at 540 nm (Noh *et al.*, 2015). In this assay, the PC was composed of 10 µM sodium nitroprusside.

EVALUATION OF REACTIVE OXYGEN SPECIES LEVELS

To evaluate the levels of reactive oxygen species (ROS), the 2,7-dichlorofluorescein diacetate (DCFH-DA) technique was performed. This test consists of the ability of this substance to be deacetylated by cytosolic esterases to dichlorodihydrofluorescein (DCFH), which does not produce fluorescence. Upon interacting with reactive species, DCFH is converted into dichlorofluorescein (DCF), which emits fluorescence and can be measured at 525 nm when excited at 488 nm (Halliwell; Whiteman, 2004).

STATISTICAL ANALYSIS

The data obtained in the in vitro model were initially tabulated in Microsoft Excel spreadsheets, version 16.65. Then, the statistical analysis was performed using GraphPad Prism version 8.0, via a one-way ANOVA followed by Tukey's post hoc. Results where $p < 0.05$ were considered significant.

RESULTS

PREDICTION ANALYSIS OF BIOLOGICAL ACTIVITIES

Initially, the prediction analysis related to the oxidative cell metabolism of the molecules apigenin, catechin, epicatechin, epigallocatechin and taxifolin, the majority molecules present in the extract of açaí was carried out. Thus, it was possible to observe important bioactive effects presented by the molecules (Table 1).

Table 1 - Prediction analysis of biological activities related to cellular oxidative metabolism of the five most abundant molecules of açaí extract.

Property	Pa/Pi	Apigenin	Catechin	Epicatechin	Epigallocatechin	Taxifolin
Nitric Oxide inhibitor	Pa	0,444	0,541	0,541	0,453	0,418
	Pi	0,006	0,004	0,004	0,005	0,006
Antioxidant	Pa	0,732	0,810	0,810	0,814	0,938
	Pi	0,004	0,003	0,003	0,003	0,002
Free radical scavenger	Pa	0,719	0,842	0,842	0,934	0,877
	Pi	0,004	0,002	0,002	0,001	0,002
NOS2 expression inhibitor	Pa	0,732	0,631	0,631	0,573	0,657
	Pi	0,002	0,003	0,003	0,005	0,003
Oxidoreductase inhibitor	Pa	0,692	0,571	0,571	0,460	0,528
	Pi	0,016	0,047	0,047	0,088	0,064
Lipid peroxidase inhibitor	Pa	0,695	0,888	0,888	0,946	0,915
	Pi	0,005	0,003	0,003	0,002	0,002
Membrane permeability inhibitor	Pa	0,946	0,790	0,790	0,707	0,850
	Pi	0,002	0,011	0,011	0,036	0,005

Legend: The blue color represents a probability "to be active" (Pa) greater than 0,500, while the red color representing a Pa less than 0,500. Source: Edited by the authors.

Epicatechin and catechin demonstrated a high antioxidant potential, as the probability of being active (Pa) for this parameter was 0,810 in both. In addition, the two molecules had a strong activity to eliminate free radicals (Pa: 0,842) and to inhibit lipid peroxidase. (Pa: 0,888). Apigenin has a high potential to inhibit membrane permeability (Pa: 0,946), as well as demonstrating high antioxidant potential and inhibiting nitric oxide expression. (Pa: 0,732). With regard to epigallocatechin, it has been shown that such a molecule has a strong activity to eliminate free radicals (Pa: 0,934), inhibit

lipid peroxidase (PA: 0,946) and inhibit membrane permeability (Pa: 0,707). Taxifolin demonstrated important antioxidant capacity (Pa: 0,938), ability to inhibit lipid peroxidase (Pa: 0,915) and inhibit membrane permeability (Pa: 0,850).

In addition to the analysis of oxidative cell metabolism, the prediction of potential anti-inflammatory activities of the molecules present in the chemical matrix of açaí was also performed, as shown in Table 2.

Table 2 - Prediction analysis of the inflammatory profile of the five most abundant molecules of the açaí extract.

Property	Pa/Pi	Apigenin	Catechin	Epicatechin	Epigallocatechin	Taxifolin
Interferon gamma antagonist	Pa	0,148	0,138	0,138	0,123	0,143
	Pi	0,046	0,056	0,056	0,080	0,051
Caspase 3 stimulant	Pa	0,577	0,661	0,661	0,509	0,708
	Pi	0,019	0,013	0,013	0,026	0,011
Caspase 8 stimulant	Pa	0,450	0,469	0,469	0,372	0,484
	Pi	0,030	0,024	0,024	0,070	0,021
Transcription factor NF kappa B stimulant	Pa	0,280	0,302	0,302	0,269	0,304
	Pi	0,132	0,110	0,110	0,145	0,108
TNF expression inhibitor	Pa	0,609	0,517	0,517	0,573	0,544
	Pi	0,012	0,026	0,026	0,016	0,021
ATPase inhibitor	Pa	0,286	0,186	0,186	0,228	0,190
	Pi	0,016	0,093	0,093Pa	0,048	0,088

Legend: The blue color represents a probability "to be active" (Pa) greater than 0,500, while the red color representing a Pa less than 0,500. Source: Edited by the authors.

Epicatechin and catechin showed potential capacity to stimulate caspase 3 (Pa: 0,661), in addition to the ability to inhibit expression of tumor necrosis factor (TNF) (Pa: 0,517). Apigenin also demonstrated the ability to inhibit TNF expression (Pa: 0,609) and stimulate caspase 3 (Pa: 0,577). Epigallocatechin showed the ability to stimulate caspase 3 with Pa equal to 0,509 and to inhibit TNF expression with Pa equivalent to 0,573. The taxifolin showed greater capacity to stimulate caspase 3 with Pa of 0,708. In relation to the ability to inhibit TNF expression, taxifolin presented Pa equal to 0,544. In the other parameters evaluated, the molecules presented a probability of being active less than 50%.

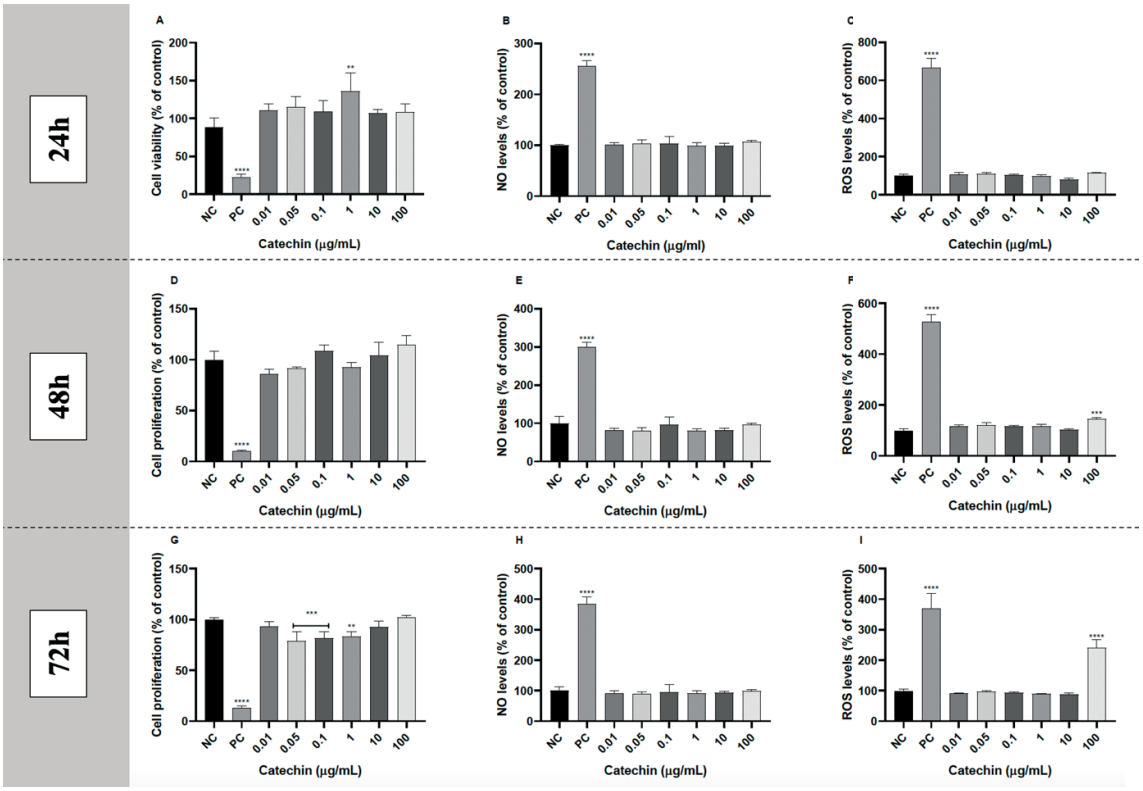
IN VITRO SAFETY PROFILE OF BIOACTIVE MOLECULES

Three bioactive molecules were chosen to conduct the experimental tests: catechin, apigenin and epicatechin. All bioactive molecules were evaluated for their safety profile in BV-2 cells during 24, 48 and 72h of exposure.

Microglial cells were not affected by exposure to the tested concentration curve of catechin after 24h (Figure 3A-3C). However, after 48 and 72h of incubation, the concentration of 100 µg/mL

increased ROS generation (Figure 3F and 3I, respectively), and after 72h of incubation the concentrations of 0.05 to 1 µg/mL of catechin impacted cell proliferation (Figure 3G).

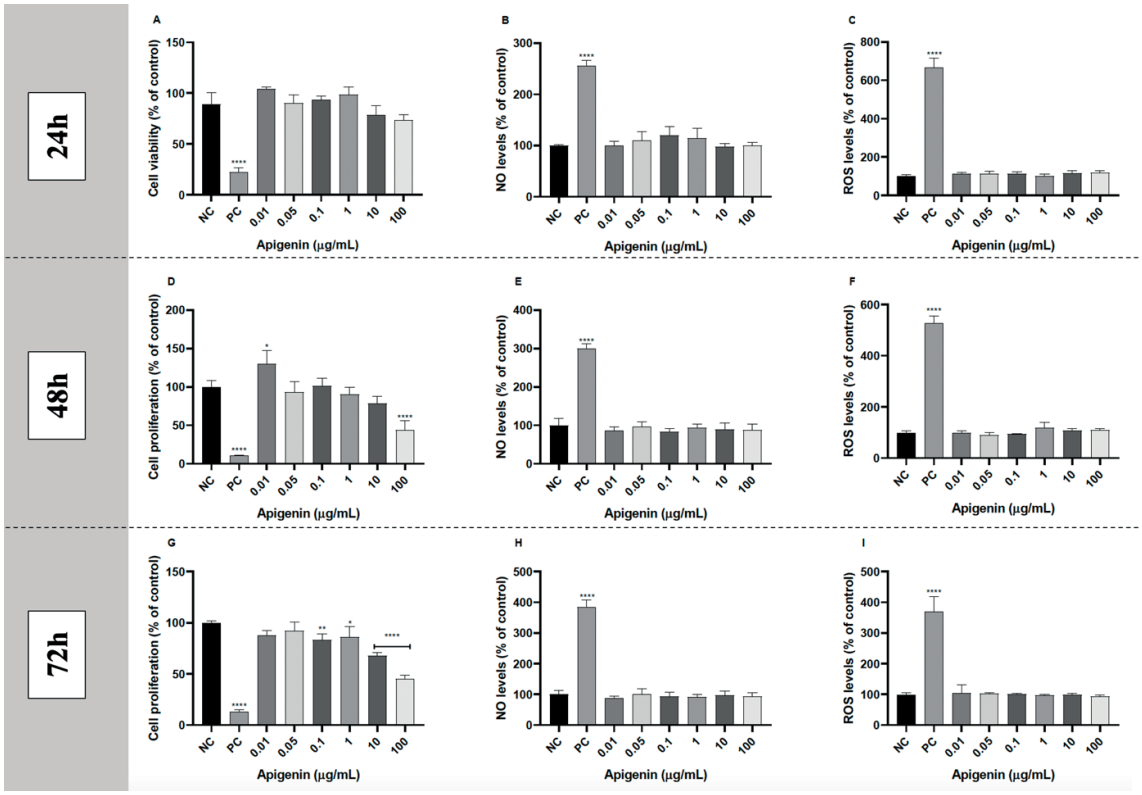
Figure 3 - *In vitro* safety profile of catechin.



Legend: Catechin concentration-response curve - Safety profile evaluation. BV-2 cells were exposed to different concentrations of catechin during 24, 48 and 72h of incubation. A, D and G) Assessment of cellular viability (24h) and proliferation (48 and 72h) indexes by MTT assay; B, E and H) Measurement of NO levels after 24, 48 and 72h of incubation, respectively; C, F and I) Measurement of ROS levels after 24, 48 and 72h of incubation, respectively. NC: negative control (cells under conventional cell culture condition); PC: cells exposed to 200 µM of H_2O_2 for MTT and DCFH-DA, and 10 µM of sodium nitroprusside for NO determination assay; Statistical analysis was performed by One-way Anova followed by Tukey *post hoc*. Results with $p<0.05$ were considered significant. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

Most of the tested concentrations of apigenin did not alter the cellular parameters evaluated in microglia, however, as in the treatments with catechin, the highest concentrations of apigenin reduced cell proliferation after 48 and 72h of incubation compared to the negative control (Figure 4D and 4G).

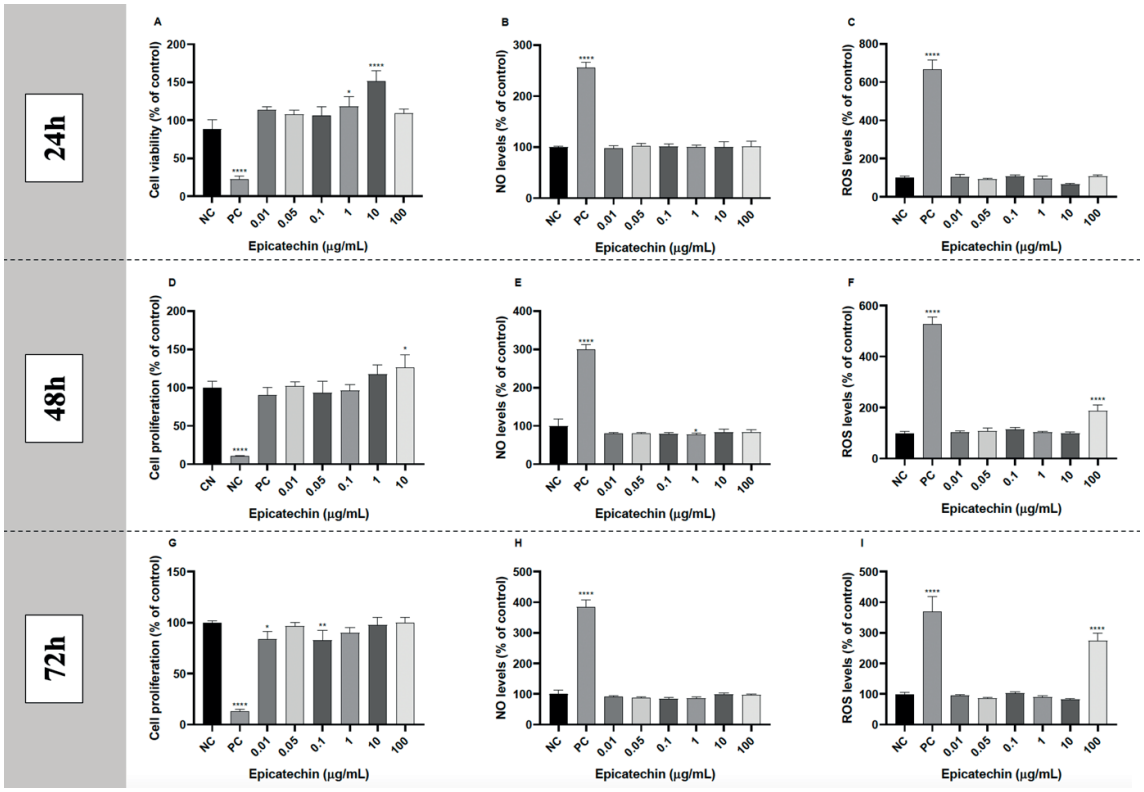
Figure 4 - *In vitro* safety profile of apigenin.



Legend: Apigenin concentration-response curve - Safety profile evaluation. BV-2 cells were exposed to different concentrations of apigenin during 24, 48 and 72h of incubation. A, D and G) Assessment of cellular viability (24h) and proliferation (48 and 72h) indexes by MTT assay; B, E and H) Measurement of NO levels after 24, 48 and 72h of incubation, respectively; C, F and I) Measurement of ROS levels after 24, 48 and 72h of incubation, respectively. NC: negative control (cells under conventional cell culture condition); PC: cells exposed to 200 µM of H_2O_2 for MTT and DCFH-DA, and 10 µM of sodium nitroprusside for NO determination assay; Statistical analysis was performed by One-way Anova followed by Tukey *post hoc*. Results with $p<0.05$ were considered significant. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

Regarding the epicatechin concentration curve tested, it was observed that there were no statistically significant changes in the evaluated cellular parameters. Only the concentration of 10 µg/mL promoted an increase in cell viability compared to the untreated cell control, while the other concentrations maintained viability at the basal level (Figure 5A). Additionally, only the highest concentration of epicatechin increased ROS generation after 48 and 72h of exposure (Figure 5F and I).

Figure 5 - *In vitro* safety profile of epicatechin.



Legend: Epicatechin concentration-response curve - Safety profile evaluation. BV-2 cells were exposed to different concentrations of epicatechin during 24, 48 and 72h of incubation. A, D and G) Assessment of cellular viability (24h) and proliferation (48 and 72h) indexes by MTT assay; B, E and H) Measurement of NO levels after 24, 48 and 72h of incubation, respectively; C, F and I) Measurement of ROS levels after 24, 48 and 72h of incubation, respectively. NC: negative control (cells under conventional cell culture condition); PC: cells exposed to 200 µM of H_2O_2 for MTT and DCFH-DA, and 10 µM of sodium nitroprusside for NO determination assay; Statistical analysis was performed by One-way Anova followed by Tukey *post hoc*. Results with $p<0.05$ were considered significant. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

DISCUSSION

The present study developed a prediction analysis based on an open access platform that allowed to investigate the biological effects as to the antioxidant and anti-inflammatory profile of different molecules derived from the chemical matrix of the fruit. Additionally, *in vitro* analyses have been conducted to analyze the safety profile of catechin, apigenin and epicatechin, a molecule that is part of the açaí matrix. These bioactive molecules were selected based on the characterization of the chemical matrix of the açaí extract in a previously published study by our research group (Davidson *et al.*, 2024).

According to the computational prediction analysis, all the molecules evaluated have an expressive antioxidant effect, with Pa above 0,700, with taxifolin being highlighted with a Pa of 0,938. A study by Topal *et al.* (2016) demonstrated the immense antioxidant capacity of taxifolin by different mechanisms. Regarding the ability to eliminate free radicals, all molecules showed a high potential,

with epigallocatechin being the molecule with the highest Pa (Pa = 0,934). Another parameter analyzed was the potential of molecules to inhibit the expression of nitric oxide synthase (NOS2), with all molecules showing a Pa higher than 0,500 for this parameter. NOS2 is capable of synthesizing nitric oxide (Barros *et al.*, 2014), therefore, the ability to inhibit the expression of NOS2 is important to prevent cells from damage caused by excess nitric oxide, as well as this effect can act to avoid oxidative stress or even uncontrolled inflammatory activation (Guzik *et al.*, 2003).

Furthermore, the potential of molecules to inhibit lipid peroxidase was evaluated and it was observed that all molecules have a high Pa for this parameter, meaning that they are capable of protecting cellular membranes. In addition, with regard to the inhibition of membrane permeability, all molecules showed a high Pa, which confirms the protection of cell membranes. These findings can be correlated with the antioxidant capacity of such molecules, since membrane damage and lipid peroxidation can be a consequence of oxidative stress (Ghezzi *et al.*, 2017). Therefore, antioxidant molecules may also be capable of inhibiting this effect induced by cellular imbalance.

Regarding the evaluations related to the anti-inflammatory profile, it was found that among the parameters evaluated, caspase 3 stimulation and inhibition of TNF expression were the most prominent for the molecules tested. All molecules tested have a Pa higher than 0,500 for the caspase 3 activation parameter, with taxifolin standing out with a value of 0,708. In addition, all molecules have also shown the ability to inhibit TNF expression, with apigenin standing out with a Pa of 0,609. Apoptosis is an essential process for maintaining bodily homeostasis. Among the complex mechanisms involved in apoptosis, the effect of caspases stands out. Caspases can be classified as initiator and effector. Initiating the apoptosis process, caspase 8 works by performing an intracellular signaling process that culminates in the activation of effector caspases, such as 3 and 7 (Julien; Well, 2017). Therefore, molecules with the ability to activate initiator or effector caspases may be interesting candidates for the treatment of neoplasms, for example. Additionally, between apoptosis and inflammatory activation processes, there is TNF. TNF, especially alpha, works by activating various intracellular proinflammatory activation processes, acting as a proinflammatory cytokine (Aram *et al.*, 2017). Therefore, inhibitors of this cytokine can be important candidates with an anti-inflammatory profile.

In this study, BV-2 cell line was used to investigate the *in vitro* safety profile of different concentrations of catechin, apigenin and epicatechin. BV-2 cell line are microglial cells derived from mouse brain tissue and are widely used in scientific research due to their representativeness as a model of microglia, the immune cells of the central nervous system. In this context, the study by de Souza *et al.* (2020) verified the anti-neuroinflammatory potential of whole açaí extract in BV-2 cells through an *in vitro* inflammatory activation model. Therefore, it is interesting to investigate the potential biological effect of the bioactive molecules identified in the açaí extract. In addition to efficacy evaluations, when it comes to new molecules and/or extracts, the development of safety profile investigations is essential.

It was observed that most concentrations of the bioactive molecules tested did not significantly alter the cellular parameters evaluated in microglia, suggesting a satisfactory safety profile in this cell type. Davidson *et al.* (2024) verified a similar safety profile of the bioactive molecules catechin, apigenin and epicatechin using VERO cells. The concentration of 100 µg/mL of catechin and epicatechin appears to have a pro-oxidant effect, while the remaining concentrations seem to maintain the antioxidant profile of this substance. In the prediction analysis, these molecules showed the ability to inhibit lipid peroxidase with a Pa of 0,888 and to inhibit nitric oxide release with a Pa of 0,541. Therefore, most of the concentrations tested *in vitro* are in accordance with the prediction findings.

Overall, the findings of this study highlight the promising antioxidant and anti-inflammatory potential of catechin, apigenin, and epicatechin, reinforcing their relevance as bioactive compounds derived from açaí. The consistency between in vitro and computational prediction analyses strengthens the reliability of these results, suggesting that these molecules could contribute to cellular protection mechanisms. However, further studies are necessary to explore their full therapeutic potential to better understand their pharmacokinetics, bioavailability, and potential applications in neuroprotection and inflammation-related disorders.

CONCLUSION

The results obtained through this theoretical and experimental study are suggestive that the most abundant molecules in açaí extract may be responsible for the biological effects associated with this natural product, as they are capable of positively modulating different parameters related to oxidative and inflammatory metabolism. In addition, most of the tested concentrations of catechin, apigenin and epicatechin were safe *in vitro.* Additional *in vitro* assays are needed to verify the potential anti-neuroinflammatory effect of each molecule.

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