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DETERMINAÇÃO DOS COMPOSTOS QUÍMICOS, ATIVIDADE ANTIOXIDANTE E CITOTOXICIDADE DO FRUTO DA PALMEIRA *AIPHANES ACULEATA*

LAIZA BERGAMASCO BELTRAN

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Dissertação apresentada aoprograma de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos paraobtenção do título de mestre em Ciência de Alimentos.

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LAIZA BERGAMASCO BELTRAN

“DETERMINAÇÃO DOS COMPOSTOS QUÍMICOS, ATIVIDADE ANTIOXIDANTE E CITOTOXICIDADE DO FRUTO DA PALMEIRA *Aiphanes aculeata*”.

Dissertação apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós- graduação em Ciência de Alimentos, para obtenção do grau de Mestre em Ciência de Alimentos.

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BIOGRAFIA

Laiza Bergamasco Beltran nasceu no Paraná na cidade de Maringá. Possui graduação em Tecnologia em Alimentos pela Universidade Tecnológica Federal do Paraná. Tem experiência nas áreas de análises de alimentos e extração de compostos bioativos atuando principalmente nos seguintes temas: Avaliação da atividade antioxidante, caracterização e análises fisico-quimicas de alimentos.

Dedico

A todos que influenciaram direta e indiretamente para a conclusão deste trabalho!

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APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de um artigo científico.

Laiza Bergamasco Beltran, Karine Campos Nunes, Raquel Gutierres Gomes, Celso Vataru Nakamura, Angélica Marquetotti Salcedo Vieira, Determination of chemical composition, antioxidant activity and cytotoxicity of the *Aiphanes aculeata* palm fruit, Food Chemistry – Qualis A1.

GENERAL ABSTRACT

INTRODUCTION: The Brazilian territory represents an immense heritage of natural resources, with an emphasis on exotic fruit species, many of which remain unexplored, such as potential sources for the food industry. The fruits of Arecaceae palms are considered rich in bioactive compounds, especially carotenoids, tocopherols, vitamin C and vitamin A. They also contain phenolic compounds, fibers and minerals. However, the trade of native Brazilian species is not very representative and most palms are exclusively destined for the local and regional market or are grown specifically for ornamental and landscape purposes. Therefore, the discovery and knowledge of the bioactive compounds content and antioxidant capacity of native fruits aims to give commercial and industrial value to these fruits, whose potential is still unexplored, in addition to contributing to the conservation of the Brazilian biome.

AIMS: Due to its little-known potential, this study aimed to analyze the composition, physicochemical properties, micronutrients, antioxidant activity and cytotoxicity of the pulp of *Aiphanes aculeata* palm.

MATERIALS AND METHODS: Fruits were harvested directly from several palms between June and September at the State University of Maringá, UEM/PR, Brazil. After harvesting, fruits were sorted and cleaned, packed in plastic bags and stored in a freezer at -18 °C until analysis. To obtain the pulp, the mesocarp (pulp) and epicarp (peel) were ground in a blender until completely homogenized. The average weight of the fruit was 2.5 g, and the quantity required to perform all analyzes was 500 g on a wet basis. The following analyzes were performed to determine the proximate composition: Moisture, ash, protein, lipids, fiber and carbohydrates. The physicochemical parameters were determined by analyzing the pH, soluble solids, titratable acidity, and reducing and non-reducing sugars. In the analysis of micronutrients, the mineral elements calcium (Ca), magnesium (Mg), potassium (K), iron (Fe) and phosphorus (P) were determined by atomic absorption spectrophotometric method. Vitamin C was determined by titration with potassium iodate. For the analysis of antioxidant activity and total phenols, the bioactive compounds were extracted using water, ethyl alcohol, methyl alcohol and acetone as solvents. The aqueous extract was carried out at three temperatures: 25, 40 and 90 ºC. Antioxidant activity was determined by DPPH, FRAP and ABTS method and total phenolic content by Folin-Ciocalteu method. Cytotoxicity was evaluated by MTT method in non-tumor (L-929) and tumor cells (Caco-2).

RESULTS AND DISCUSSION: In terms of macronutrients analyzed, moisture content was 77.13 %, ash 0.51 %, lipids 0.44 %, proteins 1.03 %, crude fiber 2.52 % and carbohydrates 18.37 %, of which 11.14 % were reducing sugars and 8.06 % were non-reducing. The pH of the fruit was in the range of 5.01, which is considered as a medium acidity fruit as reported in the literature. A value of 0.38 g citric acid/100 g of the fruit was determined for titratable acidity. In the literature, citric acid values are reported between 0.08 and 1.95 %, which are good for consumption. Of the micronutrients analyzed, potassium had the highest value (993.04 mg/100 g). Potassium-rich fruits and vegetables are considered a non-pharmacological source for the prevention and control of hypertension. The other mineral elements analyzed follow in descending order: Ca (26.61 mg/100 g), Mg (18.3 mg/100 g), P (15.2 mg/100 g), Zn (0.30 mg/100 g), Fe (0.07 mg/100 g). Consumption of vitamin C is of great importance as it is not produced by the body itself and is essential for its proper functioning. Therefore, it is necessary to take it in with food, fruits and vegetables being the main sources. The recommended

daily allowance of vitamin C for an adult is 45 mg. The pulp of *Aiphanes aculeata* contained 46.97 mg/100 g of vitamin C. With respect to the extractions performed, the acetonic and aqueous extracts at 90 °C showed greater antioxidant activity by the methods studied (DPPH, FRAP and ABTS), indicating a positive correlation with the content of total phenols (Folin-Ciocalteau). The extracts showed no cytotoxic activity in the non-tumor cell line (L-929) and there was a decrease in cell viability in the tumor line (Caco-2) above a concentration of 500 µg/mL.
CONCLUSION: The present study proved to be very important as the pulp of *Aiphanes aculeata* palm is poorly studied and has a rich and diverse nutrient composition. The extracts showed no cytotoxic activity in non-tumor cells (L-929) at the concentrations studied, which is a positive indicator for their use in food formulations with functional properties. Thus, the results obtained show the importance of research and knowledge of native species, as they are a source of bioactive compounds and have great nutritional and economic potential.
Keywords: *Aiphanes aculeata*, Brazilian fruits, Bioactive compounds, Antioxidant activity, Cytotoxicity.

RESUMO GERAL

INTRODUÇÃO: O território brasileiro constitui um patrimônio imensurável de recursos naturais, com ênfase em espécies de frutas exóticas, sendo muitas ainda inexploradas, as quais são fontes em potencial para a indústria de alimentos. Os frutos das palmeiras Arecaceae são considerados ricos em compostos bioativos, principalmente carotenoides, tocoferóis, vitamina C e vitamina A. Além disso, possuem considerável teor de compostos fenólicos, fibras e minerais. No entanto, o comércio de espécies nativas brasileiras é pouco representativo e a maioria das palmeiras são destinadas exclusivamente ao mercado local e regional ou cultivada especificamente para fins ornamentais e paisagísticos. Portanto, a descoberta e o conhecimento do conteúdo de compostos bioativos e da capacidade antioxidante de frutas nativas visa agregar valor comercial e industrial a estas frutas que possuem seu potencial ainda inexplorado, além de contribuir com a conservação do bioma brasileiro.

OBJETIVO: Devido seu potencial ainda pouco conhecido, este estudo teve como objetivo realizar as análises de composição centesimal, físico-químicas, micronutrientes, atividade antioxidante e citotoxicidade da polpa do fruto da palmeira *Aiphanes aculeata*.

MATERIAIS E MÉTODOS: Os frutos foram coletados entre os meses de junho e setembro diretamente de diversas palmeiras localizadas na Universidade Estadual de Maringá, UEM/PR, Brasil. Após a colheita, procedeu-se com a seleção e higienização dos frutos e estes foram embalados em sacos plásticos e acondicionados em freezer à -18 ˚C até o momento da realização das análises.Para a obtenção da polpa, o mesocarpo (polpa) e epicarpo (casca) foram triturados em liquidificador até a total homogeneização. O peso médio dos frutos é 2,5 g e a quantidade necessária para realização de todas as análises foi de 500 g em base úmida. Realizou-se as seguintes análises para determinar a composição centesimal: Umidade, cinzas, proteína, lipidios, fibra e carboidratos. Os paramentros fisico-quimicos foram determinados através das análises: pH, sólidos solúveis, acidez titulável, açucar redutor e não redutor. Em relação a análise de micronutrientes os elementos minerais cálcio (Ca), magnésio (Mg), potássio (K), ferro (Fe) e fósforo (P) foram determinados utilizando a técnica espectrofotométrica de absorção atômica. A vitamina C foi determinada por titulação com iodato de potássio. Para as análises de atividade antioxidante e fenólicos totais, foi realizada a extração de compostos bioativos utilizando os solventes: água, álcool etílico, álcool metílico e acetona. O extrato aquoso foi realizado em três temperaturas 25, 45 e 90 ˚C. A atividade antioxidante foi realizada através dos métodos DPPH, FRAP e ABTS e o conteúdo fenólico total foi determinado utilizando o método de Folin-Ciocalteu. A citotoxidade foi avaliada pelo método MTT em células não-tumorais (L-929) e tumorais (Caco-2).

RESULTADOS E DISCUSSÃO: Em relação aos macronutrientes analisados obteve-se um teor de umidade de 77,13 %, cinzas 0,51 %, lipideos 0,44 %, proteínas 1,03 %, fibra bruta 2,52 % e carboidratos 18,37 %, sendo 11,14 % de açucar redutor e 8,06 % de não redutor. Em relação ao pH do fruto, este se apresentou na faixa de 5,01, sendo considerado uma fruta de acidez média conforme proposto pela literatura. Quanto a acidez titulável o valor obtido foi de 0,38 g ácido cítrico/100 g de fruta. Valores de ácido citríco em frutas variando de 0,08 a 1,95 % é reportado na literatura que são bem aceitos para consumo.

Dentre os micronutrientes analisados o potássio apresentou maior quantidade (993,04 mg/100 g). Frutas e vegetais ricos em potássio são considerados fontes

não-farmacológicas para a prevenção e controle da hipertensão. Segue em ordem decrescente os demais elementos minerais analisados: Ca (26,61 mg/100 g), Mg (18,3 mg/100 g), P (15,2 mg/100 g), Zn (0,30 mg/100 g), Fe (0,07 mg/100 g). O consumo de vitamina C é de grande importância, pois ela não é naturalmente produzida pelo organismo sendo imprescindível para seu bom funcionamento. Deste modo, é necessário ingeri-la, tendo as frutas e hortaliças como suas principais fontes. A recomendação diária de vitamina C para um adulto é de 45 mg. A polpa do fruto da *Aiphanes aculeata* apresentou 46,97 mg/100 g de vitamina C. Em relação aos extratos realizados, o acetônico e aquoso a 90 ºC apresentaram maior atividade antioxidante nas metodologias estudadas (DPPH, FRAP e ABTS), indicando correlação positiva com o teor de fenólicos totais (Folin-Ciocalteau). Os extratos não apresentaram atividade citotóxica na linhagem de células não tumorais (L-929) e houve diminuição da viabilidade celular na linhagem tumoral (Caco-2) a partir da concentração de 500 µg/mL.
CONCLUSÃO: O presente estudo se mostrou de grande importância, pois a polpa do fruto da palmeira *Aiphanes aculeata* é ainda pouco explorada e apresentou uma composição nutricional rica e variada. Os extratos não apresentaram atividade citotóxica nas concentrações estudadas em células não tumorais (L-929), o que é um indicador positivo para ser usado em formulações alimentícias com propriedades funcionais. Os resultados obtidos demonstram a necessidade de pesquisas e conhecimento sobre as espécies nativas, visto que são fonte de compostos bioativos e apresentam grande potencial nutricional e econômico.
Palavras-chave: *Aiphanes aculeata*, frutas brasileiras, compostos bioativos, atividade antioxidante, citotoxicidade.

Determination of chemical composition, antioxidant activity and cytotoxicity of the *Aiphanes aculeata* palm fruit

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Abstract

Berry-type fruits from Arecaceae palms have excellent nutritional, functional and economic characteristics. This study aimed to evaluate the pulp of the *Aiphanes aculeata* palm fruit through the analysis of proximate composition, physicochemical parameters, micronutrients, total phenolics, antioxidant activity and cytotoxicity. In relation to macronutrients, low levels of lipids, proteins and fibers, and high levels of carbohydrates and sugars were determined. Among the micronutrients analyzed (Ca, Mg, Zn, Fe, P and K), potassium (933.04 mg/100 g) was the most abundant element in the fruit. Regarding the analysis of vitamin C, the value determined was 46.97 mg/100 g. The DPPH, FRAP and ABTS assays were used to evaluate the antioxidant activity. The extracts showed antioxidant activity in the methods used, providing potential for their application in the composition of the food products formulations with functional properties since there was no cytotoxic potential in normal cells (L-929).

Keywords: *Aiphanes aculeata*, Brazilian fruits, Bioactive compounds, Antioxidant activity, Cytotoxicity.

1. Introduction

In Brazil, there are different types of geographical and climatic conditions that favor fruit cultivation, resulting in a great diversity of small fruit species called berries. They are distributed in six biomes (Caatinga, Amazon Forest, Pampa, Pantanal, Cerrado and Atlantic Forest). Indeed, the Brazilian territory represents an immense heritage of natural resources, with an emphasis on exotic fruit species, many of which are still unexplored and are potential sources for the food industry seeking healthy products and technological innovations (Nile & Park, 2014).

Arecaceae palm fruits are considered rich in vitamin C, vitamin A, especially carotenoids and tocopherols. They also have a considerable content of minerals, fibers and

phenolic compounds (De Souza, Araújo, Farias, Zanotto, Neri-Numa & Pastore, 2020). However, the trade of native Brazilian species is not very representative, and most palms are exclusively destined for the local and regional market or are grown specifically for ornamental and landscape purposes (Campos, De Lima Araújo, Gaoue, & Albuquerque, 2019).

Recently, studies have shown that several fruits of the Arecaceae family have important biological activities, such as anti-inflammatory properties (*Cocos nucifera* L.), preventive effect on hypoglycemia (*Syagrus romaziffiana*) (Ribeiro, Bieski, Balogun, & Martins, 2017) and photochemoprotective effect (oil from the pulp of *Mauritia flexuousa*) (Zanatta, Mitjans, Urgatondo, Rocha-Filho, & Vinardell, 2010). However, no data on the nutrient composition and antioxidant potential of the palm fruits of *Aiphanes aculeata* were found in the literature.

Fruits are generally considered to be a rich source of nutrients. Several studies have found a positive correlation between phenolic compound content and antioxidant potential (Santos-Buelga, González-Paramás, Oludemi, Ayuda-Durán, & González-Manzano, 2019). A high consumption of foods with phenolic compounds in their composition has been associated with a lower incidence of diseases such as inflammation, cancer, cardiovascular dysfunction, and a decrease in the immune system. In addition, they play an important role in the prevention of neurodegenerative diseases and antiviral activity (Liu, Wang, & Wang, 2018).

The antioxidant activity of phenolic compounds is attributed to their ability to scavenge free radicals by donating hydrogen atoms, electrons, or chelating metal cations, thus inhibiting the initiation or even the continuation chain of free radical-induced oxidative reactions in the human organism. The action of these compounds is thus associated with the attenuation of oxidative processes, which may contribute to the protection of body cells from damage by providing a protective effect against reactive oxygen species (Santos-Buelga, González-Paramás, Oludemi, Ayuda-Durán, & González-Manzano, 2019).

The discovery and knowledge of the bioactive compounds content and antioxidant capacity of indigenous fruits aims to give commercial and industrial value to these fruits, which have an unexplored potential. In addition, it could contribute to the conservation of the Brazilian biome by providing nutritional alternatives and reducing the harmful effects of free radicals due to their antioxidant properties (Teixeira et al. 2019). With this in mind, this work aimed to perform analyzes of proximate composition, physicochemical properties, micronutrients, antioxidant activity and cytotoxicity of *Aiphanes aculeata* palm fruit, since its potential has not yet been explored.

2. Materials and methods

2.1 Chemical reagents

The respective SYNTH brand reagents were used: hexane, acetone, ethyl alcohol, methyl alcohol, acetone, sulfuric acid, boric acid, hydrochloric acid, gallic acid, dimethylsulfoxide (DMSO), potassium sulfate, phenolphthalein, potassium iodide , potassium iodate, starch, sodium carbonate. The respective DYNAMIC brand reagents were used: copper sulfate, methyl red, sodium hydroxide, fehling solution A and B, methylene blue. The respective SIGMA-ALDRICH brand reagents were used: ABTS (2.2′-azino- bis-(3-ethylbenzenethiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-Tri-(2-Pyridyl)-1,3,5-Triazine 1:3 with p-Toluenesulfonic acid), Folin-Ciocalteu reagent, quercetin, 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT). The respective FBS - Life Technologies/Gibco Laboratories brand reagents were used: Dulbecco's Modified Eagle's medium (DMEM); fetal bovine serum.

Fig. 1. *Aiphanes aculeata* palm trees located at the State University of Maringá.

2.2 Aiphanes aculeata palm tree fruit

The fruits were collected between June and September, 2019 (Figure 1), directly from the palm trees located at the State University of Maringá (UEM), Paraná, Brazil, (-23.4051563, -51.93876817195696). After harvesting, fruits selection and cleaning were carried out, being further packaged in plastic bags and stored in a freezer at -18 ºC until the analysis performance.

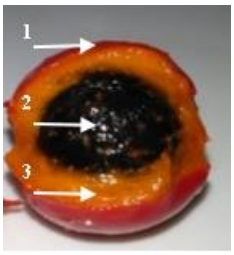

Fig. 2. Overview of *Aiphanes aculeata* fruit;1. epicarp; 2. endocarp; 3. mesocarp.

2.3 Fruit pulp obtaining

To obtain the fruit pulp used in the analysis, we proceeded with the fruits thawing and pulping, in which the epicarp and mesocarp (Figure 2) were placed in a blender until (Britânia 700w) total homogenization. The average weight of the fruits was 2.5 g and the amount needed to carry out all analyses was 500 g of the pulp *in natura*.

2.4 Proximate composition analysis

The following analyses were performed in triplicate. Moisture and ash contents were determined by drying in an oven with circulating air at 105 ºC (934.06 - AOAC, 2016) and incineration in a muffle at 550 ºC (940.26 - AOAC, 2016) respectively; crude protein was determined by the Kjeldahl method (960.52 - AOAC, 2016), using the conversion factor 5.75 for vegetable proteins according to the RDC ANVISA Resolution No 360, of December 23, 2003; the lipid content was performed by Soxhlet extraction using hexane as an extracting solvent (920.39 - AOAC, 2016). Crude fiber analysis was determined by acid hydrolysis following the method (958.06 - AOAC, 2005). Carbohydrate was calculated by difference using the formula: 100 - (moisture + lipid + protein + ash + crude fiber).

2.5 Physicochemical analysis

The pulp was evaluated for pH using a calibrated digital potentiometer (AOAC, 1992); soluble solids were determined by direct reading in a bench-top refractometer (Abbe, 2WAJ) (AOAC, 1992); determination of the titratable acidity, this method is based on the potentiometric titration of the sample with a 0.1 M sodium hydroxide solution (942.15b – AOAC, 1995); the content of reducing and non-reducing sugars was determined using the Fehling titration technique, and the results were expressed in percentages of glucose and sucrose, respectively (958.06 – AOAC, 1995). All analyses were performed in triplicate.

2.6 Micronutrient Analysis

The mineral micronutrients analyses were carried out after the solubilization of the fixed mineral residue. It was conducted according to the method No 975.03 described by the Association of Official Analytical Chemists (AOAC, 2005), using the spectrophotometric technique of atomic absorption (Varian, Spectra AA 50B), which analyzed the calcium (Ca), magnesium (Mg), potassium (K), iron (Fe) and phosphorus (P) mineral elements.

2.7 Vitamin C analysis

Vitamin C content was determined by the procedure described by Association of Official Analytical Chemists, method 43.064 (AOAC, 1992). The method used was based on the vitamin C oxidation by titration with potassium iodate. The analyses were performed in triplicate and the results were expressed in mg of pulp vitamin C/100g.

2.8 Extraction of bioactive compounds

For the analysis of antioxidant activity and total phenolics, bioactive compounds were extracted using the following solvents: water, ethyl alcohol, methyl alcohol and acetone. All extractions were performed following the methods proposed by Roesler et al. (2007), with some modifications.

2.8.1 Aqueous extract

As regards the aqueous extract, the pulp was homogenized for 20 minutes with distilled water at a ratio of 1: 3 (m/m) pulp: water in a magnetic stirrer (MS-H-Pro) at 420 rpm. The material was filtered with a Whatman No1 paper filter and the residue was re-extracted with water under the same conditions. This procedure was performed at 25, 45 and 90 ºC. The extracts obtained were lyophilized (Alpha 1-4 LO plus) and stored in amber vials at -18 °C.

2.8.2 Ethanol, methanol and acetonic extract

In an Erlenmeyer flask, the pulp was added at a ratio of 1:3 (m/m) pulp: extracting solution for both solvents with a concentration of 3:7 (v/v) water: solvent. The sample was homogenized for 20 minutes at room temperature (25 ± 2 ºC) in a magnetic stirrer (MS-H-Pro) at 420 rpm and filtered with a Whatman No. 1 paper filter. The residue was washed with 30 mL of extractor solution. The filtrate was rota-evaporated (Tecnal TE-211) at 50 °C to recover

158 the solvent. The extracts obtained were lyophilized (Alpha 1-4 LO plus) and stored in amber
159 vials at -18 °C.
160
161 *2.9 Antioxidant capacity evaluation*
162 *2.9.1 DPPH· (free radical scavenging) assay*
163 The ability of extracts to scavenge the DPPH radical was evaluated according to the
164 methods proposed by Rufino et al. (2007), with some modifications. To prepare the DPPH
165 reagent, 1 mg of DPPH was diluted in 1 mL of methanol, obtaining a solution with a
166 concentration of 130 µM. The extracts were diluted in different concentrations (1000 - 31
167 µg/mL). Thereafter, 100 µl of each dilution in the 96-well transparent microplate and 100 µl of
168 the 130 µM DPPH solution were added. The microplate was incubated at room temperature
169 protected from light for 30 minutes. The reading was performed at 517 nm in a microplate
170 reader (BioTek, Power Wave XS). Antioxidant capacity was expressed as the sample
171 concentration needed to reduce the initial number of free radicals by 50 % (EC_{50}). The results
172 were expressed in µg/mL.
173
174 *2.9.2 Ferric reducing antioxidant power (FRAP) assay*
175 The following solvents were used to prepare the FRAP reagent: 0.3 mM of acetate
176 buffer (pH 3.6), TPTZ 10 mM reagent in HCl 40 mM and $FeCl_3$ 20 mM solution. These
177 solutions were mixed in a 10:1:1 ratio, resulting in the final solution of the FRAP reagent. In a
178 96-well transparent microplate, 30 µL of the previously prepared extracts (1000 – 31 µg/mL)
179 and 150 µL of the prepared FRAP reagent were added. This solution was incubated for 30
180 minutes at 37 ºC in an oven with air circulation. After stabilization at room temperature, the
181 reading was performed at 595 nm in a microplate reader (BioTek, Power Wave XS). Trolox
182 was used as the standard antioxidant, and the results were expressed as micromoles of Trolox

equivalents per gram of sample (μmol of TE / g sample). The test was carried out using the methods proposed by Rufino et al. (2010) with modifications.

2.9.3 ABTS$^{\cdot+}$ assay

The capacity of the extracts to scavenge the cationic radical ABTS$^{\cdot+}$ was evaluated in accordance with the procedures proposed by Rufino et al. (2010), with some modifications. The ABTS$^{\cdot+}$ radical was obtained by mixing 5 mL of the ABTS solution (7.0 mmol/L) and 88 μL of the potassium persulfate solution (2.45 mmol/L) for 12 h, in the absence of light. Subsequently, ethyl alcohol was added to this solution up to an absorbance of 0.700 ± 0.05 at 754 nm in a microplate reader. In a 96-well transparent microplate, 7 μL of the previously prepared dilutions (1000 – 31 μg/mL) and 200 μL of the ABTS solution were added, and the mixture was homogenized. After 6 minutes of reaction, the reading was performed at 734 nm in a microplate reader (BioTek, PowerWave XS). Trolox was used as the standard antioxidant. The results were expressed as micromoles of Trolox equivalents per gram of sample (μmol of TE / g of sample).

2.9.4 Total Phenolics determination

The total phenolic content was determined according to the methods proposed by Pires et al. (2017) with modifications. Accordingly, 20 μL of the previously prepared dilutions (5 - 0.31 mg/mL) were added in the following solutions: 180 μL of deionized water, 20 μL of methanol, 20 μL of Folin-Ciocalteu reagent (1N) and 60 μL of carbonate solution sodium (10 %) in a 96-well clear microplate. The microplate was incubated for 20 minutes at room temperature and protected from direct light. The reading was performed at 760 nm in a microplate reader (BioTek, PowerWave XS). The results were expressed in mg of equivalents in gallic acid (GAE) per 100 g of fresh fruit (mg GAE/100 g of fresh fruit).

208 *2.10 Cell viability assay*

209 L-929 fibroblasts (ATCC®CCL-1.3™) and human colon adenocarcinoma cell line Caco-
210 2 (ATCC, #HTB-37) were cultured in DMEM with 10 % fetal bovine serum (FBS) and 1 %
211 penicillin-streptomycin in a 5 % atmospheric CO_2 at 37 °C. The cultured cell suspension was
212 plated in a 96-well plate (2.5×10^5cells/well) and incubated for 24 hours. Afterwards, the
213 medium was removed and the cells were treated with different samples concentrations: 31.25-
214 250 μg/mL for L-929 and 31.25-500 μg/mL for Caco-2. After 24 hours, the cells were washed
215 with PBS and received 50 μL of MTT (2 mg/mL), being incubated for 4 hours. Finally, 200 μL
216 of DMSO were added and the absorbance was determined at 570 nm (BioTek, PowerWave XS
217 microplate spectrophotometer) (Berridge, Herst & Tan, 2005)

218

219 *2.11 Statistical analysis*

220 Statistical analysis was performed using GraphPad Prism software v. 5.00 (San Diego,
221 CA, USA). Data were analyzed using the ANOVA (one-way or two way), followed by Tukey
222 post hoc or Bonferroni correction, considering $p \leq 0.05$ as statistically significant. Data were
223 expressed as mean ± standard deviation of at least three independent experiments.

224

225 **3. Results and discussion**

226 Table 1 shows the results of the analyses of proximate composition, physicochemical
227 characterization, and Vitamin C of the pulp of the *Aiphanes aculeata* palm fruit.

228

229 **Table 1**
230 Results of the proximate composition, physicochemical characterization and
231 Vitamin C analyses of the *Aiphanes aculeata* fruit pulp.[a]

Analyses	*Aiphanes aculeata* pulp *in natura*
Moisture (%)	77.13 ± 0.22
Ash (%)	0.51 ± 0.01
Lipids (%)	0.44 ± 0.04
Protein (%)	1.03 ± 0.20

Fiber (%)	2.52 ± 0.01
Carbohydrates (%)	18.37 ± 0.09
pH	5.01 ± 0.04
Soluble solids (º Brix)	18.42 ± 0.03
Reducing sugar (%)	11.14± 0.68
Non-reducing sugar (%)	8.06 ± 0.30
Titratable Acidity (g acid citric /100 g)	0.38 ± 0.09
Vitamin C (mg/100 g of fresh pulp)	46.97 ± 5.08

[a] Mean value ± standard deviation based of fruit pulp weight; n=3.

Moisture content is a measure of the total amount of water in the food. It is usually expressed as a percentage from the total weight. The moisture content in the *Aiphanes aculeata* fruit (77.13 %) is in the expected range for fresh fruit since the water content can vary from 65 to 95 % (Cecchi, 2003). The moisture content found was higher than that presented by Lescano et al. (2018) for *Syagrus romanzoffiana* (69.62 %) and *Mauritia flexuosa* (73.45 %), which are fruits from the same botanical family.

The minerals or ash are obtained through the incineration process using high temperatures, burning the sample's organic matter. Thus, through the resulting content, it is possible to obtain the number of specific components such as minerals Ca, Mg, Fe, Zn, P, K, among other micronutrients. These elements are necessary for the proper functioning of vital functions, as well as for the good health of the human body (Zambiazi, 2010). The ash content found in the *Aiphanes aculeata* pulp (0.51 %) is similar to the content of other fruits from the Brazilian cerrado such as *Psidium cattleianum* (0.43 %), *Mauritia flexuosa* (1.01 %), *Stenocalyx dysentericus* (0.30 %), *Hancornia speciosa* (0.53 %) e *Annona crassiflora* (0.68 %), reported by Schiassi, Souza, Lago, Campos and Queiroz (2018).

The *Aiphanes aculeata* fruits can be considered a low source of lipids (0.44 %). When analyzing fruits from the Brazilian cerrado, Schiassi, Souza, Lago, Campos and Queiroz (2018) found a low lipid value for *Eugenia dysenterica* DC. (0.49 %) and *Spondias mombin* L. (0.48 %) fruits, with similar values from those found in the present work.

253 The protein content found in the *Aiphanes aculeata* fruit (1.03 %) presented a value
254 close to that described by Hamacek, Della Lucia, Silva, Moreira and Pinheiro-Sant'ana (2018)
255 for the *Mauritia flexuosa* L pulp (1.00 %) and a higher value than that found by Becker, Chagas,
256 Marty, Mendes and Nunes (2018) for *Maximiliana maripa* pulp (0.49 %) and *Euterpe oleracea*
257 Mart. (0.72 %), both fruits from the Arecaceae family.

258 Regarding the total fiber content, the value found for the *Aiphanes aculeata* pulp was
259 lower than those reported for fruits from the same species, as presented by Hameck, Della
260 Lucia, Silva, Moreira and Pinheiro-Sant'ana (2018) for the *Mauritia flexuosa* L pulp (9.23 %),
261 and for the *Attalea phalerata* fruit pulp (6.33 %), as reported by Lescano et al. (2018).

262 Amongst the macronutrients, fruits have a higher carbohydrate content in relation to
263 proteins and lipids (Lescano et al. 2018). The pulp of *Aiphanes aculeata* had a higher
264 carbohydrate content (18,37 %) in comparison to *Mauritia flexuosa* pulp from different biomes.
265 A content of 9.70 % and 11.31 % were reported from the Cerrado and Amazonian fruits,
266 respectively (Cândido & Silva, 2017). The sugar content in fruits is influenced by the amount
267 of light the plants receive. As photosynthesis occurs for a longer time, it is possible to transport
268 sugar reserves to the fruits, therefore, the greater the degree of maturation of the fruit, the
269 greater its sweetness (Taiz & Zeiger, 2006).

270 According to the classification proposed by the Food and Drug Administration (FDA,
271 2016), the *Aiphanes aculeata* fruits are classified as medium acidity fruits (pH 5.01), in which
272 the pH ranges from 4.6 to 5.3. Acidic fruits have a pH range between 3.7 and 4.6 and very
273 acidic fruits have a pH < 3.7. For the *Mauritia vinifera* fruit pulp, which is from the same
274 family as *Aiphanes aculeata*, Hamacek, Della Lucia, Silva, Moreira and Pinheiro-Sant'ana
275 (2018) reported a pH of 3.59.

276 The soluble solids content relates to the total sugar content, an important aspect when
277 correlated with the sweetness of the fruit, since sweet fruits are usually more accepted by

consumers. The *Aiphanes aculeata* pulp had a total solids content of 18.42 ºBrix. In view of this, De Souza et al. (2020) reported total solids contents for the *Mauritia flexuosa* (13.40 ºBrix), *Euterpe oleracea* Mart. (6.46 ºBrix) and *Acrocomia aculeata* (29.70 ºBrix), all fruits from the Arecaceae family. Thus, the *Aiphanes aculeata* fruit has a high content of soluble solids, being superior to several fruits from the same botanical family, which are already known and accepted by consumers.

Regarding titratable acidity, the value obtained for the *Aiphanes aculeata* fruit (0.38 g citric acid/100 g) was lower than those reported by Hamacek, Della Lucia, Silva, Moreira and Pinheiro-Sant'ana (2018) for the *Mauritia vinífera* fruit pulp (1.42 g citric acid/100 g), which also belongs to the Arecaceae family.

One of the most important quality factors of fruits is the flavor, assessed by the content of soluble sugars and organic acids in it. During ripening, the organic acid content decreases and the sugar content increases. The reducing sugar refers to the glucose content and the non-reducing sugar represents the sucrose content in the fruit (Batista-Silva et al. 2018). Regarding the content of reducing and non-reducing sugars, values of 11.14 % and 8.06 % were found for the *Aiphanes aculeata* fruit pulp, respectively. The contents of reducing and non-reducing sugars differ among the fruits as they depend on the interaction between several variables, such as age, climatic conditions, maturation and cultivation.

Ascorbic acid is a water-soluble vitamin, essential for collagen synthesis and tissue repair. It plays a significant role in the metabolism of carbohydrates and in the synthesis of lipids and proteins. Additionally, it supports the immune system by means of its antioxidant property, helping to neutralize free radicals in cells. The consumption of vitamin C is of great importance, as it is not naturally produced by the human body, being essential for its proper functioning. Thus, it is necessary to acquire it by ingesting fruits and vegetables since they are its main sources (Garcia, Lima & Bomfim, 2017). According to the Dietary Reference Intakes

(DRIs) (2000), the recommended daily intake of vitamin C is 45 mg for adults. Therefore, a 100 mg portion of *Aiphanes aculeata* fruit provides the recommended amount of vitamin C. The value reported for the *Aiphanes aculeata* fruit (46.97 mg/100g) was higher than those from the *Mauritia flexuosa* L. palm tree (17.4 mg/100g) (Morais et al. 2019).

Fruits and vegetables are important sources of nutrients. Minerals play a vital role in the development and proper functioning of the human body. Table 2 shows the results obtained in the mineral composition analyzes (Ca, Mg, Zn, Fe, P and K) of the *Aiphanes aculeata* fruit pulp. From these results, we were able to calculate the mineral contribution considering the Dietary Reference Intake (DRI) for a healthy adult, as a percentage (%) per 100g of pulp (Institute of Medicine, 1999-2011).

Table 2
Mineral contents and contribution according to the Dietary Reference Intake (DRI) per 100 g of fruit pulp.[a]

Minerals	*Aiphanes aculeata* pulp *in natura*
Ca (mg/100 g)	26.61 ± 0.22
% DRI	2.67
Mg (mg/100 g)	18.3 ± 0.14
% DRI	7.07
Zn (mg/100 g)	0.30 ± 0.10
% DRI	4.28
Fe (mg/100 g)	0.07 ± 0.16
% DRI	0.50
P (mg/100 g)	15.2 ± 0.15
% DRI	2.17
K (mg/100 g)	993.04 ± 0.11
% DRI	21.13

[a] Mean value ± standard deviation; n = 3. DRI (Dietary Reference Intake), Ca (Calcium), Mg (Magnesium), Zn (Zinc), Fe (Iron), P (Phosphorus), K (Potassium).

It follows in decreasing order the content of minerals obtained in 100 grams of the pulp of the fruit of *Aiphanes aculeata* Potassium > Calcium > Magnesium > Phosphorus > Zinc > Iron. The consumption of foods that have several minerals in their composition is of great importance for health, as they have important factors in the prevention of various diseases, and fruits are potential sources of them. Among the mineral contents analyzed, potassium (933.04

mg/100 g) was the most abundant element present in the *Aiphanes aculeata* fruit. The value found was higher than those reported by De Souza et al. (2020) for *Mauritia flexuosa* (183.55 mg/100 g) and *Acrocomia aculeata* (2.36 mg/100 g), fruits from the Arecaceae family. Potassium plays an important role in intracellular and extracellular water distribution. It is an important factor in lowering blood pressure and in cardiovascular disease. The most recent recommendations for the detection, prevention and control of hypertension in adults recommend increased potassium intake through fresh foods as one of the most effective non-pharmacological measures for the prevention and treatment of hypertension (Turck et al. 2016).

The analyses of total phenolic compounds and antioxidant activity were performed using different solvents, once the extraction of bioactive compounds maximum extraction is important to evaluate the antioxidant capacity, which have different polarity. Thus, solubility in a particular solvent is a distinctive feature of the phytochemistry present in the fruit, which explains the lack of a universal extraction procedure (Santos, Feitosa, Rodrigues, & Santana, 2019). Table 3 shows the results of the aqueous extract subjected to temperatures of 25, 45 and 90 °C, as well as the ethanolic, methanolic and acetonic extracts of the *Aiphanes aculeata* fruit by the DPPH, FRAP, ABTS and Total Phenolics tests.

Table 3
Antioxidant capacity by DPPH, FRAP, ABTS and Total Phenolic methods. [a]

Extracts	DPPH EC_{50} [µg/mL] [b]	FRAP (µmol TE/g sample)	ABTS (µmol TE/g sample)	Total Phenolics (mg GAE/100 g of fresh fruit)
Aqueous 25 °C	352.74 ± 28,73[a]	10.13 ± 1.07[ad]	208.79 ± 5,59[a]	393.89 ± 1.34[a]
Aqueous 45 °C	274.10 ± 24,54[b]	13.04 ± 2.12[a]	561.97 ± 1,17[b]	347.45 ± 3.87[a]
Aqueous 90 °C	64.20 ± 4.18[c]	41.16 ± 2.84[b]	712.89 ± 1,37[c]	1419.44 ± 2.16[b]
Acetone 70 %	43.70 ±1.97[c]	84.27± 3.69[c]	829.09 ± 8,97[d]	1074.58 ± 5.93[c]
Ethanol 70 %	146.05 ± 10.19[b]	15.27 ± 1.28[d]	703.19 ± 1,18[b]	309.63 ± 1.70[a]
Methanol 70 %	157.71 ± 16.48[b]	30.62 ± 5.06[b]	616.54 ± 9,52[bc]	344.72 ± 2.10[a]

[a] Mean value ± standard deviation; n=3. Different letters in the same column indicate a significant difference by Tukey's test ($p < 0.05$). GAE (gallic acid equivalents), TE (trolox equivalents).
[b] Concentration of antioxidant required to reduce the original number of free radicals by 50%.

In order to obtain maximum yield of phenols, several researchers have studied extraction with different solvents. Methanol, acetone, ethanol, and water are the most common solvents used for extraction due to their polar properties, which provide affinity for phenolic compounds in general (Santos, Feitosa, Rodrigues, & Santana, 2019). These compounds are associated with the adaptation and resistance mechanisms of plants to the environment and can influence the taste, technological properties, and nutritional and functional potential of fruit species (Rocha et al. 2011).

The aqueous extract, in which a temperature of 90 °C was used, had the highest content of total phenolics (1419.44 mg GAE/100 g of fresh fruit). Therefore, it is evident that the compounds in the *Aiphanes aculeata* fruit have greater polarity, being more water-soluble (i.e., have greater affinity with water). The efficiency of water in the extraction process is important because it has a more environmentally friendly extraction and low toxicity compared to organic solvents. Moreover, the use of water is a good alternative for the extraction of phenolic compounds due to its safety, accessibility and low cost. Garcia-Mendoza et al (2017) observed that by increasing the temperature from 40 °C to 80 °C, the extraction of phenolic compounds from *Euterpe edulis* fruit residues increased by almost 62 %. These results indicate that high temperatures have a positive effect on the solubility of phenolic compounds, which increases the transfer of these compounds to the solvent and improves the efficiency of extraction.

Vasco, Ruales & Kamal-Eldin (2008) classified total phenolic content in fruits into three categories: low (100 mg GAE /100g), medium (100-500 mg GAE /100g) and high (500 mg GAE /100g) for samples from fresh material. According to this classification, the palm fruit of *Aiphanes aculeata* is classified with high total phenolic content considering the aqueous and acetonic extract at 90 °C (1074.58 mg GAE /100 g fresh fruit). The value for total phenolic content of *Aiphanes aculeata* fruit determined in the present study was higher than the values reported by Seraglio et al. (2015) for two species of *Bactris gasipaes* palm fruits, where the

values reported for red peach palm and yellow peach palm were 93.35 and 87.36 mg GAE /100 g of fresh fruit, respectively.

Currently, there is no official method for determining antioxidant activity in foods because there are different mechanisms and a variety of bioactive compounds. Therefore, more than one method is used to evaluate the antioxidant activity of a compound (Lima-Neto et al. 2017).

As for the extracts analyzed, the antioxidant activity for DPPH free radical capture was, in decreasing order, acetone > 90 ºC aqueous > ethanol > methanol > 45 ºC aqueous > 25 ºC aqueous. According to Villaño, Fernández-Pachón, Moyá, Troncoso, & García-Parrilla (2007), the EC_{50} value is inversely proportional to the antioxidant capacity of a compound, since the EC_{50} value indicates the number of antioxidants required to reduce the concentration of the radical by 50 %. This means that the lower the EC_{50} value, the greater the antioxidant activity.

Amongst the extracts analyzed, there was no significant difference ($p < 0.05$) between acetonic (43.70 µg/mL) and aqueous at 90 ºC (64.20 µg/mL). Roesler et al. (2007), when evaluating the antioxidant capacity of fruits from the Brazilian cerrado, found EC_{50} values for the aqueous and ethanol extracts of the following fruits: *Eugenia dysenterica* DC (879.93 and 387.47 µg/mL), *Annona montana* (1321, 93 and 148.82 µg/ml) and *Solanum lycocarpum* (1328.98 and 182.16 µg/ml). Superior results are identified when comparing acetonic and aqueous extracts at 90 ºC of the *Aiphanes aculeata* fruit, since it was only necessary 43.70 and 64.20 µg / mL, respectively, to capture 50 % of the radicals.

In terms of Fe^{+3} reducing power, the acetone extract had the highest activity (84.24 µmol TE/g sample), followed by the extracts (in decreasing order): aqueous 90 ºC > methanol > ethanol > aqueous 45 ºC > 25 ºC aqueous. Pásko et al. (2021) studied the aqueous and methanolic extract from three species of pitaya fruits, *Hylocereus costaricensis*, *Hylocereus*

394 *undatus* and *Hylocereus megalanthus*. and obtained an activity of 17 and 13 µmol TE/g sample,
395 15.3 and 13.2 µmol TE/g of sample and 16.6 and 14 µmol TE/g sample, respectively.

396 As for $ABTS^{\cdot+}$ radical capture, the antioxidant activity of the acetone extract showed
397 higher activity (829.09 µmol TE/g sample) compared to the other extracts (e.g., aqueous 90 ºC
398 > ethanol > methanol > 45 ºC aqueous > 25 ºC aqueous). Rufino, Alves, de Brito et al. (2010)
399 analyzed the combined extract of methanol and acetone of *Euterpe oleracea*, *Copernicia*
400 *prunifera*, and *Euterpe edulis*, three fruits of the Arecaceae family. They found an activity of
401 15.1, 10.7 and 78.3 µmol TE / g sample, respectively.

402 Based on the results, it can be noted that the extracts studied showed a relationship
403 between phenolic compounds and antioxidant activity, once those with the highest
404 concentration of polyphenols were the ones with the highest antioxidant activity regardless of
405 the method used.

406 The cytotoxic concentration, i.e., the amount of a product (natural or synthetic) that is
407 toxic to a given cell, must be determined in order to establish a safety margin for use in
408 products, taking into account their biological activities (Santos et al. 2013). Figure 4 shows the
409 results of the cytotoxicity assay by means of the MTT assay to analyze the cell viability of the
410 L-929 (A) and Caco-2 (B) cell lines in the 90 ºC aqueous, ethanolic and acetonic extracts of
411 the *Aiphanes aculeata* fruit pulp. They were selected for this experiment on the grounds that
412 they showed a better antioxidant activity. The method has the principle of evaluating cell
413 viability based on damage induced in mitochondria, which are quantified by reducing MTT (a
414 yellow-colored salt in water) to formazan (purple-colored crystals, insoluble in water). The
415 reduction of MTT to formazan is directly proportional to mitochondrial activity and cell
416 viability (Li & Song, 2007).

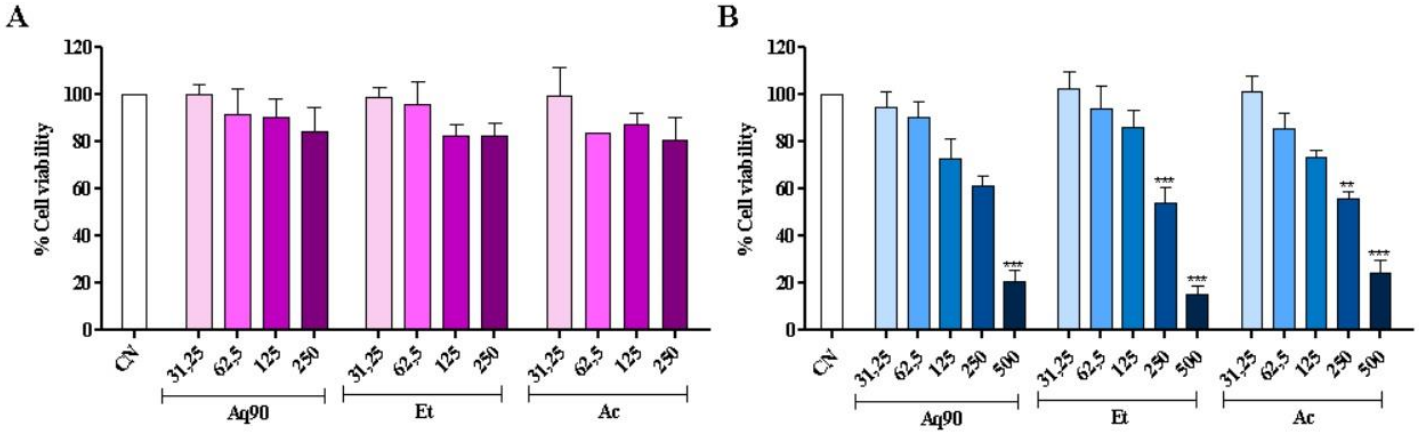

Fig. 3. Effect of *Aiphanes aculeata* extract treatment on L-929 (A) and Caco-2 (B) cell viability. The effect of the aqueous extract at 90 ºC, (Et) ethanol extract and (Ac) acetonic extract, at concentrations of 31.25-500 µg/mL on cell viability were evaluated using the MTT assay. Values are expressed as mean ± standard deviation (n = 3) and are representative of three independent experiments with similar results. ***p < 0.001, significant difference compared to CN (one-way ANOVA, post hoc Tukey). There were no significant differences between groups (two-way ANOVA, Bonferroni test), CN (Negative Control).

Cytotoxicity assessment using non-tumor cell lines is an important tool for risk prediction and safety assessment of the potential toxicity of bioactive compounds. *In vitro* systems are used primarily for screening purposes, for example, to assess cell selectivity and to establish more comprehensive toxicological profiles (Eisenbrand et al. 2002). A sample is considered cytotoxic when cell death occurs and the percentage of viability of exposed cells is < 70 % (Fraga et al. 2021). Therefore, the more toxic the compound, the lower the cell viability.

When evaluating the extracts using ethanol and hexane solvents from the *Talisia esculenta* fruit pulp and peel using the L-929 cell line to evaluate the cytotoxic potential of the extracts, Fraga et al. (2021) determined that there is no cytotoxic potential at both tested concentrations (12.5 - 200 µg/mL) in relation to the negative control, which are cells without any treatment, since there was no cell death, with cell viability above 70 %.

Having this in mind, the extracts were evaluated by 2 cell lines, being the fibroblasts L-929 non-tumor cells and Caco-2 tumor cells, aiming to evaluate their behavior and selectivity against different cell lines. Regarding the L-929 lineage, cell viability was higher than 70 % at

all concentrations of both extracts, with no significant difference compared to the control condition ($p < 0.05$), showing no cytotoxic potential. However, for the Caco-2 cells, the ethanolic and acetonic extracts showed cytotoxic potential at the concentration of 250 µg/mL, even decreasing cell viability at the concentration of 500 µg/mL (20.43, 15,13 and 24, 26 %) for the aqueous 90 °C, ethanolic and acetonic extracts, respectively. Pásko et al. (2021) reported a cell viability of 31.7 and 51.6 % when evaluating the cytotoxicity of aqueous and methanolic extracts of the *Hylocereus costaricensis* fruit pulp in Caco-2 cells at a concentration of 500 µg/mL, respectively.

4. Conclusion

The present study proved to be of great importance as the *Aiphanes aculeata* fruit pulp presented a rich and varied nutritional and mineral composition. Concerning the total phenolics content and antioxidant activity, the results indicate a strong correlation, since the aqueous 90 °C and acetonic extract showed a greater antioxidant activity in the methods performed. The active concentrations of these extracts did not show cytotoxicity to fibroblast cells. There were no significant differences between the extracts and the negative control (untreated) in the entire concentration range tested. This result is positive evidence for the extracts to be used in food formulations with functional properties. Regarding the human colon adenocarcinoma cell line (Caco-2), the two highest extracts concentrations significantly decreased cell viability. Nevertheless, further studies are needed, or even different forms of extraction, in order to isolate and evaluate possible compounds with antitumor potential. The results from the present study demonstrate the importance of a proper exploration and knowledge of native species, as many have great nutritional and economic potential. However, they remained to be underexplored.

Conflict of interest

The authors declare that they have no conflicts of interest.

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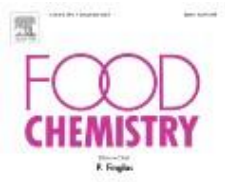

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Food Chemistry has two open access companion journals *Food Chemistry: X* and Food Chemistry: Molecular Sciences.

The Aims and Scope of *Food Chemistry* are assessed and modified on an annual basis to reflect developments in the field. This means that research topics that have been deemed in scope previously may now fall outside of the scope of the journal as our scientific and technical understanding of the fields evolve and topics become less novel, original or relevant to *Food Chemistry.*

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