

Article

Quantification of Caffeic Acid as Well as Antioxidant and Cytotoxic Activities of Ucuuba (*Virola surinamensis*) Co-Product Extract to Obtain New Functional and Nutraceutical Foods

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Abstract

Ucuuba (*Virola surinamensis*) is a fruit of Amazonian origin with anti-inflammatory, nutritional, and phenolic substances. This study aimed to prepare and characterize the ucuuba co-product extract as well as to evaluate its antioxidant and cytotoxic activities, proximate composition, and water activity. For this purpose, the co-product and its extract were analyzed by Fourier-transform infrared (FTIR) spectroscopy, and their thermal behavior was investigated by thermogravimetry (TG). The ucuuba co-product extract was also evaluated for its contents of total polyphenols and flavonoids, antioxidant activity by the DPPH and ABTS assays, and cytotoxicity in normal J774.A1 macrophages by the MTT technique. The co-product proved to have important macronutrient contents from a nutritional point of view, i.e., $11.67 \pm 0.731\%$ fiber, $16.67 \pm 0.36\%$ lipids, $38.32 \pm 0.19\%$ proteins, and 30.56% carbohydrates, as well as low moisture content ($6.73 \pm 0.05\%$) and water activity (0.403). FTIR spectra showed characteristic absorption peaks of phenolic compounds. The ucuuba co-product (pressed seeds) and the extract obtained from the ucuuba seed co-product were stable at around $100\text{ }^{\circ}\text{C}$ and showed two mass loss events typical of natural products. The extract contained total polyphenols and flavonoids amounting to $806.45\text{ mg}/100\text{ g}$ and $62\text{ mg RE}/100\text{ g}$, respectively, and its antioxidant activity according to the DPPH and ABTS assays was 374.33 and $258.15\text{ }\mu\text{M Trolox/g}$, respectively. Caffeic acid was identified as an abundant phenolic compound ($5.17\text{ }\mu\text{g}/\text{mL}$) by high-performance liquid chromatography (HPLC-DAD), and its quantification method was validated. Furthermore, there was no cytotoxicity in the macrophage cell line at concentrations up to $100\text{ }\mu\text{g}/\text{mL}$. These results indicate that the ucuuba co-product could be reused to develop new functional and nutraceutical foods.

Keywords: biological properties; functional foods; ucuuba; bioactive compounds; chromatography



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1. Introduction

Virola surinamensis, popularly known as ucuuba, ucuuba-da-várzea, ucuuba-branca, and ucuuba-verdadeira, is a tree native to the Amazon floodplain that can be found in Brazil, Peru, Venezuela, Suriname, and even in Central American countries [1]. Ucuuba seeds and their fat have been the focus of interest of the pharmaceutical and cosmetics industries [2]. The ucuuba seed is rich in fat (60 to 70%) that is used by the local population to produce candles and soaps and consists mainly of myristic acid (68.23%) and lauric acid (18.32%). The use of ucuuba fatty products by the cosmetics industry encourages the oil and fat extraction sector to use it as a raw material and, as a result, a large quantity of co-products are produced and released into the environment without proper treatment [3].

There is growing concern worldwide regarding the creation of measures to reduce or eliminate co-products produced by the processing of various plant species [4,5]. The food sector has stood out in this regard by implementing initiatives that ensure sustainability in industrial production. Recycling these co-products arouses interest at technological, scientific, and economic levels, as it aims to recover raw materials and energy, preserving natural resources. In addition, this practice helps to minimize environmental impacts such as soil contamination resulting from their inadequate disposal, increases the income of food processors, and adds value to the production chain [6,7].

Until recently, these materials were classified as by-products (items that have less relevance in terms of use) and waste (products that do not have an established market). However, several studies have shown that these secondary products, now called co-products, can be used as raw materials for extraction and transformation into other products with greater added value [3,8]. Thus, the concept of co-product has gained relevance, as these products can be as significant to industry and commerce as the main product sought in the manufacturing process [3].

The use of co-products among researchers and their application in the development of new products in the pharmaceutical and cosmetic sectors has become a trend of growing interest [9]. This is because they can be purchased at low cost, in addition to possessing matrices rich in macronutrients and bioactive compounds, such as phenolics (tannins, gallic acid, vanillin, ferulic acid, caffeic acid, cinnamic acid, tannic acid, and epicatechin), as well as coumarin, mangiferin, and carotenoids, which, due to their antioxidant potential, are essential for promoting health [6,10–13]. These benefits give great value to antioxidant-rich foods, which are classified as functional foods. In addition, it is possible to use ingredients rich in antioxidants for the production of nutraceuticals and use as functional foods [14,15].

This research group has carried out several studies that, by demonstrating the antioxidant potential and the presence of phenolic substances such as phenolic acids and flavonoids in co-products from different Amazonian plant species, suggest great potential for their exploitation. Furthermore, they have shown that these components make co-products excellent sources of natural antioxidants [16] and characterize them as food inputs, capable of functioning as nutraceuticals or even as food additives [17]. Examples of them are the co-products of cocoa [18], cupuaçu [19], pracaxi [20], and tucumã [17].

Therefore, aiming even more at the exploitation of these co-products, this study aimed to obtain and characterize the extract of the ucuuba co-product, identify its phenolic compounds by high-performance liquid chromatography, and evaluate its antioxidant activity and cytotoxicity, with a view to its use in the development of new functional foods and nutraceuticals.

2. Materials and Methods

2.1. Reagents, Solvents and Standards

Sodium hydroxide was purchased from Dinâmica (São Paulo, Brazil), while potassium hydroxide, sodium carbonate, and aluminum chloride were purchased from Synth (São Paulo, Brazil). Gallic acid, caffeic acid, hydrated rutin, catechin, epicatechin, quercetin, vanillic acid, shikimic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6) sulfonic acid (ABTS) $\geq 98\%$, ($\pm$)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) 98%, and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Obtainment and Treatment of the Ucuuba Co-Product

The co-product, obtained after cold pressing ucuuba almonds for the fat extraction process, was provided by the Amazon Oil factory (Ananindeua, Brazil). The raw ucuuba co-product was stored at $-20\text{ }^{\circ}\text{C}$ before analysis. It was then dried in an air circulation oven at a temperature of $40 \pm 2\text{ }^{\circ}\text{C}$ and weighed every 24 h until a constant weight was obtained. At the end of the process, the sample was crushed in a knife mill to reduce particle size.

2.3. Proximate Composition and Water Activity of the Co-Product

The proximate composition of the ucuuba co-product was determined as the contents of moisture, ash, lipids, fiber, and proteins, following the procedures of Instituto Adolf Lutz [21]. Moisture content was determined as the mass loss upon direct drying of the samples (2 g) in an oven at $105\text{ }^{\circ}\text{C}$, while ash content was determined by incineration of the organic content of the samples at $600\text{ }^{\circ}\text{C}$ in a muffle furnace. Protein content was determined by the Kjeldahl method, lipid content by the Soxhlet method in the ether extract, total dietary fiber by the enzymatic gravimetric method, and the percentage of carbohydrates as the difference between 100 and the sum of the percentages of moisture, protein, total lipids, ash, and fiber [21].

Water activity (Aw) was determined in a 7 mg sample of the co-product using a water activity meter, model 4TEV (Aqualab, São Paulo, Brazil) [22].

2.4. Extract Preparation

The tincture was obtained at room temperature by the percolation method. The plant raw material (200 g) was pulverized in a knife mill and placed in contact with the extracting liquid (70:30 (ethanol/water)) at the drug/solvent (1:10) ratio, totaling 2.0 L of tincture. Percolation was performed by constant dripping of the solvent (20 drops per minute) until complete exhaustion. The extract was concentrated in a rotary evaporator, model Laborota 4000 (Heidolph, São Paulo, Brazil), under monitored temperature ($40\text{ }^{\circ}\text{C}$) and rotation (40 rpm) conditions for ethanol evaporation. Aliquots (5 mL) of the concentrated extract were transferred to glass containers, subjected to freezing ($-20\text{ }^{\circ}\text{C}$) and subsequently freeze-dried in a lyophilizer, model L-101 (Liotop, São Carlos, Brazil) for a period of 72 h [23].

2.5. Characterization of the Ucuuba Extract and Co-Product

2.5.1. Thermal Behavior (TG/DTG)

The TG/DTG curves of the ucuuba co-product and its extract were obtained using a TGA-50 thermobalance (Shimadzu, Kyoto, Japan). Nine milligrams of the sample was weighed in an aluminum crucible and subjected to a nitrogen atmosphere at a flow rate of 50 mL/min, in the temperature range of 25 to $600\text{ }^{\circ}\text{C}$ and at a heating rate of $10\text{ }^{\circ}\text{C}/\text{min}$. The results were analyzed using TA-60W Shimadzu software [24].

2.5.2. Fourier-Transform Infrared (FTIR) Spectroscopy Profiles

The FTIR spectra of the ucuuba co-product and its extract were obtained on a Prestige-21 IR spectrometer (Shimadzu). Tablets were prepared by physically mixing 1 mg of the sample and 99 mg of potassium bromide (KBr), pressed and inserted into the equipment. The parameters used in the analysis were: absorption range of 4000 to 400 cm^{-1} , resolution of 2 cm^{-1} , and scan number of 20 [24].

2.5.3. Total Polyphenol Content of the Extract

The total polyphenol content of the extract was determined in a UV 1800 spectrophotometer (Shimadzu) using the Folin–Ciocalteu reagent and a gallic acid standard curve. The extract at a concentration of 100 $\mu\text{g}/\text{mL}$ was diluted 10 times in distilled water and mixed with hexane at a ratio of 1:2 to remove the fat. The mixture led to two phases, the precipitate and the supernatant. The latter was transferred to a test tube containing 2.4 mL of distilled water. The reaction medium consisted of 100 μL of the sample, 250 μL of Folin–Ciocalteu reagent (2 N), 500 μL of saturated sodium carbonate solution, and 4150 μL of distilled water, totaling a final volume of 5 mL. After 1 h of reaction protected from light, the blank consisting only of water and the samples were subjected to reading in the spectrophotometer at a wavelength of 725 nm, with the results being expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/100 g) [25].

2.5.4. Total Flavonoid Content of the Extract

The total flavonoid content of the extract was determined using the same spectrophotometer described above. After 70 times dilution of the ucuuba extract, which was performed because its high initial concentration exceeded the highest point of the rutin calibration curve range [26], an aliquot of the diluted sample (800 μL) was transferred to a 5 mL volumetric flask, 1000 μL of a 2.5% aluminum chloride solution was mixed with the reaction medium, the volume was completed (5000 μL) with distilled water, and the reaction was allowed to occur for 15 min. Then, readings were performed on the spectrophotometer at 510 nm using water as a blank. A standard curve of rutin was prepared at concentrations of 5, 10, 15, 20, and 25 $\mu\text{g}/\text{mL}$ in a 10 mL volumetric flask. The assays were performed in triplicate, and the results were expressed as milligrams of rutin equivalent per gram of dry extract (mg RE/100 g) [27].

2.5.5. Quantification of Majority Compounds in the Extract by High-Performance Liquid Chromatography (HPLC)

The analysis of majority compounds in the extract was performed in a high-performance liquid chromatograph (Agilent 1260 Infinity, Wilmington, DE, USA) coupled to a photodiode array UV/Vis detector with an Agilent 1260 Infinity electrochemical cell (Wilmington, DE, USA) and a XDB C18 column (4.6 mm $\times$ 250 mm) with 5 μm particle size. The separation of samples was performed at a constant flow rate of 0.8 mL/min, column temperature of 40 $^{\circ}\text{C}$, and injection volumes of 10 and 20 μL for standard solution and sample, respectively. The eluent system was of the gradient type with acidified water (Solvent A) and methanol (Solvent B), according to the following variation in Solvent B proportion: 10% for 5 min, increments to 25% in 7 min and to 50% in 9 min, maintenance at this value until 24 min, and return to 10% in 27 min. Peaks were recorded at wavelengths in the range of 210 to 600 nm. The standards used were catechin, epicatechin, quercetin, rutin, vanillic acid, shikimic acid, and caffeic acid [28]. These standards were selected based on the comparison and similarity of the spectra of the detected substances with those of these standards, which are available, together with their retention times, in the chromatograph library.

2.6. Validation of the Caffeic Acid Quantification Method

The method of quantification of caffeic acid, detected as the major component of the extract, was validated according to the Resolution of the Collegiate Board—RDC No. 166 of the Brazilian Health Regulatory Agency (ANVISA) [29], which establishes criteria for the validation of analytical methods. The parameters evaluated were linearity, selectivity, precision, accuracy, robustness, and detection and quantification limits. The linearity of the method was investigated using caffeic acid solutions in methanol at concentrations of 2.00, 3.12, 6.25, 12.5, 25.0, and 50.0 µg/mL prepared from a 1 mg/mL standard solution. The tests were performed in triplicate in order to evaluate the linear relationship between the analyte concentration and the absorbances obtained. The results were plotted on a graph of concentration versus absorbance of the sample, obtaining the coefficient of determination (R^2) of the analytical curve. The selectivity was determined by injection into the HPLC of the extract, the caffeic acid standard, and the sample contaminated with the standard, in order to confirm the presence of caffeic acid in the sample. The precision was evaluated by repeatability from 6 determinations of the theoretical concentration of 50 µg/mL, and the results were expressed using the coefficient of variation (CV) (Equation (1)). The accuracy (R%) was determined for 3 concentrations (2.00, 12.5, and 50 µg/mL) in triplicate (Equation (2)). The robustness was checked by reading the concentration of 50 µg/mL in triplicate, changing the assay temperature to 30 °C. Finally, the detection (DL) and quantification (QL) limits were calculated according to Equations (3) and (4), respectively:

$$CV = \frac{\text{Standard deviation}}{\text{Mean concentration determined}} \times 100 \quad (1)$$

$$R\% = \frac{\text{Experimental mean concentration}}{\text{Theoretical concentration}} \times 100 \quad (2)$$

$$DL = \frac{SDa \times 3}{IC} \quad (3)$$

$$QL = \frac{SDa \times 10}{IC} \quad (4)$$

where SDa is the standard deviation of the y-intercept of three calibration curves and IC is the slope of the analytical curve.

2.7. Antioxidant Activity of the Extract

2.7.1. Capture of the ABTS⁺ Radical Cation

The reagent solution was prepared by mixing 25 mL of the ABTS aqueous solution (7 mM) and 440 µL of the potassium persulfate aqueous solution (140 mM) in an Erlenmeyer flask. After keeping the reagent solution in the dark for 16 h, it was diluted in ethanol until obtaining an absorbance at 734 nm of approximately 0.7 using the spectrophotometer mentioned above. The extract was diluted 50 times with distilled water. The reaction medium prepared in a test tube consisted of 150 µL of the sample diluted in 3000 µL of the ABTS reagent solution, which remained protected from light. After 2 minutes of reaction, the absorbances of the samples were read using distilled water as a blank. To construct the standard curve, concentrations of 75 to 175 µM/L of the Trolox standard were prepared in a 10 mL volumetric flask. The analysis was performed in triplicate, and the results were expressed in Trolox equivalent (µM) per gram of extract [30].

2.7.2. Capture of the DPPH• Radical

This analysis was performed on extract samples diluted 50 times, using the same spectrophotometer mentioned above. The reaction medium prepared in a test tube consisted of 75 µL of diluted sample and 2925 µL of DPPH• radical solution in methanol, totaling a

volume of 3000 μL . After a reaction time of 30 min protected from light, samples were read at a wavelength of 515 nm using methanol as a blank for equipment reset. The assay was performed in triplicate, and the results were expressed in Trolox equivalent (μM) per gram of extract [31]. The inhibition percentage was calculated according to Equation (5):

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample})}{\text{Absorbance of the control}} \times 100 \quad (5)$$

2.8. In Vitro Cytotoxicity Assay

2.8.1. Obtaining and Cultivating Cells

The mouse peritoneal macrophage cell line J774.A1 was provided by the Laboratory of Molecular and Cellular Neurochemistry of the Institute of Biological Sciences of the Federal University of Pará. The macrophages were maintained in cell culture bottles in DMEM medium supplemented with 10% FBS, penicillin (100 IU/mL), and streptomycin (100 $\mu\text{g}/\text{mL}$) in an incubator at 37 °C under 5% CO_2 . The subculture was performed when 80% cell confluence was observed using a 0.05% trypsin/EDTA solution. After the addition of this solution, the macrophage-containing bottles were placed in an incubator at 37 °C under 5% CO_2 for 5 min to trypsinize the adherent cells. Then, serum was added to inactivate the trypsin, and the macrophages were transferred to 15 mL centrifuge tubes and centrifuged for 10 min at 2000 rpm. After centrifugation, the supernatant was discarded, and the pellet was resuspended in 1 mL of DMEM medium complemented with 10% fetal bovine serum (SBF), penicillin (100 IU/mL), and streptomycin (100 $\mu\text{g}/\text{mL}$). The counting was performed in a Neubauer chamber, and the cells were plated at the appropriate concentration for the experiment [32].

2.8.2. Cell Viability

To perform the cell viability test in 96-well plates, 1×10^3 macrophages were plated per well and subjected to treatment with ucuuba extracts at concentrations of 0.5, 5, 10, 50, and 100 $\mu\text{g}/\text{mL}$ for 24 h in an oven at 37 °C under 5% CO_2 . After removal of the supernatant, the wells were washed with phosphate-buffered saline (PBS) of pH 7.2, and then 50 μL (0.5 mg/mL) of MTT diluted in the same buffer was added. After incubation at 37 °C in an oven containing 5% CO_2 for 3 h, the supernatant was removed, and 200 μL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan crystals with incubation on a shaker plate for 10 min. Subsequently, the final solution was read in a spectrophotometer (model 450 Microplate Reader, Bio-Rad, São Paulo, Brazil) at a wavelength of 570 nm [32].

2.9. Statistical Analysis

The results of total polyphenol and flavonoid contents were analyzed in triplicate and expressed as the mean and standard deviation using Excel Office 365, while those of cytotoxicity and antioxidant activity were analyzed using Graphpad 5.0 software. Analysis of variance (ANOVA) and Tukey's test were used, considering results with $p \leq 0.01$ as statistically significant.

3. Results

3.1. Proximate Composition, Moisture Content, and Water Activity of the Co-Product

The proximate composition shows the nutrient content of any material or food. In the case of food, the content of macronutrients with reference to 100 g of food is conventionally evaluated. Regarding the nutritional composition of the ucuuba co-product, there was a predominance of fibers, followed by carbohydrates, lipids, and, to a lesser extent, proteins.

The moisture and ash contents and the water activity found in the co-product can be considered low (Table 1).

Table 1. Proximate composition and water activity of the ucuuba co-product.

Proximate Composition	Mean Value $\pm$ SD
Fibers	41.42 $\pm$ 0.73%
Lipids	15.54 $\pm$ 0.36%
Proteins	11.07 $\pm$ 0.19%
Carbohydrates *	20.61%
Ash	4.66 $\pm$ 0.02%
Moisture	6.73 $\pm$ 0.05%
Water activity	0.403 $\pm$ 0.01

* Calculated by difference. Data expressed as the mean of triplicate runs $\pm$ standard deviation (SD).

3.2. Thermogravimetric Analysis (TG/DTG) of the Ucuuba Co-Product and Its Extract

The thermal behavior curves (TG/DTG) of the co-product and its extract (Figure 1) showed two mass loss events. In the co-product, the first event occurred in the range of 40.14 to 200.69 °C, with a mass loss of 4.60%, while the second event was between 250.20 and 400.50 °C, with a mass loss of 62.34%. The thermal decomposition of the extract showed the first event in the range of 85.62 to 104.23 °C, with a mass loss of 6.62%, and the second event between 150.51 and 475.96 °C, with a mass loss of 41.17%.

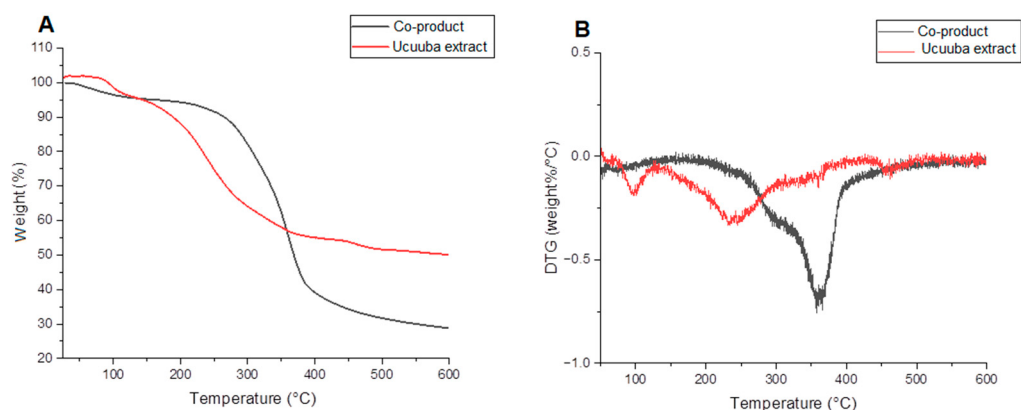


Figure 1. (A) Thermogravimetric (TG) curves of the ucuuba co-product (black line) and extract (red line). (B) Derivative thermogravimetric (DTG) curves of the ucuuba co-product (black line) and extract (red line).

3.3. Fourier-Transform Infrared (FTIR) Spectroscopic Profile of Ucuuba Co-Product and Its Extract

Figure 2 shows the FTIR spectra obtained for the ucuuba co-product and its extract. Absorption bands at wavenumbers of 2920 cm^{-1} and 2851 cm^{-1} were observed in the co-product and at 2932 cm^{-1} and 2851 cm^{-1} in the extract, referring to hydrocarbon stretching (-C-H). Both presented an absorption band at 2357 cm^{-1} , which is characteristic of the amino group. The band at 1742 cm^{-1} present in the co-product can be attributed to carbonyl stretching, which is possibly related to polyphenols and carbohydrates. The stretching at 1660 cm^{-1} and 1600 cm^{-1} in the co-product and extract, respectively, is characteristic of axial deformation of the aromatic ring (C=C). The extract showed absorption at 1245 cm^{-1} , characteristic of angular harmonic vibration (=C-H). The co-product and the extract exhibited weak intensity bands at 1180 cm^{-1} and 1037 cm^{-1} , respectively, which can be ascribed to the axial deformation of -C-O and C(=O)-O in alcohols and phenols [33].

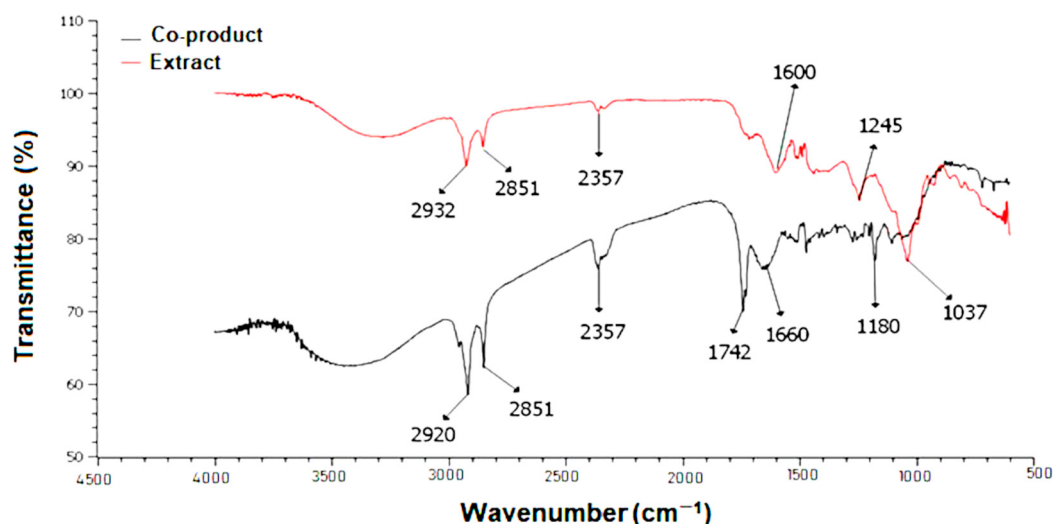


Figure 2. FTIR spectra of the ucuuba co-product (black line) and its extract (red line).

3.4. Total Polyphenol and Total Flavonoid Contents of the Extract

In the extract of the ucuuba co-product, the values found for total polyphenols and total flavonoids were 806.45 ± 1.9 mg GAE/100 g and 62.0 ± 1.27 mg RE/100 g, respectively.

3.5. Quantification of Caffeic Acid in the Extract by HPLC

Chromatographic analyses were performed to identify the compounds present in the ucuuba co-product extract. A comparison of the chromatograms and retention times of the extract compounds and the standards (catechin, epicatechin, quercetin, rutin, vanillic acid, shikimic acid, and caffeic acid) allowed to identify only caffeic acid in the sample, whose peak area was in fact the only one increased by co-elution (Figure 3).

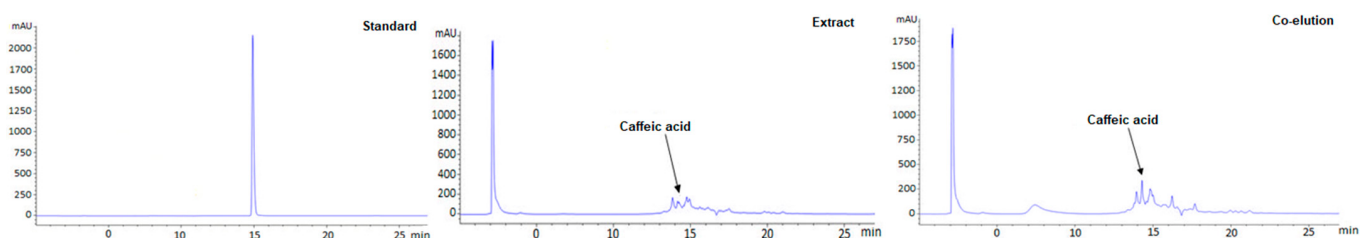


Figure 3. Chromatograms obtained in HPLC-DAD analysis at a wavelength of 330 nm of the caffeic acid standard (15.01 min), of the extract (14.13 min), and after co-elution (standard + extract, 14.38 min).

After confirming the presence of caffeic acid in the extract, the method was validated for its quantification according to Brazilian legislation (Resolution of the Collegiate Board—RDC No. 166 of the Brazilian Health Regulatory Agency (ANVISA) and ICH–Harmonised Tripartite Guidance. Validation of Analytical Procedures Q2(R1) (Supplementary Material, Figure S1) [29,34,35].

3.6. Total Antioxidant Activity of the Extract

The antioxidant potential of the ucuuba co-product extract evaluated by the DPPH method was 374.33 ± 2.5 μ M Trolox/g, corresponding to an inhibition potential of $89.67 \pm 0.13\%$, while the one evaluated by the ABTS method was 258.15 ± 1.8 μ M Trolox/g, with statistically significant variations ($p < 0.01$). These results demonstrate that the extract had good antioxidant activity according to both assays.

3.7. In Vitro Cytotoxicity Assay

The cytotoxicity assay was performed on macrophage cell lines with different concentrations of ucuuba co-product extract (0.5–100 µg/mL). After 24 h of treatment, no significant difference in cell viability ($p > 0.01$) was observed in the macrophage cell line compared to that in the control group (Figure 4), which indicates low cytotoxicity of the ucuuba extract against the macrophage cell line at the different tested concentrations.

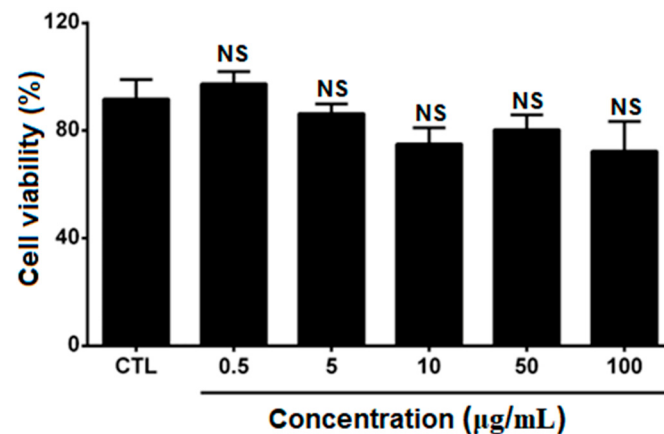


Figure 4. Cytotoxicity assay performed on ucuuba co-product extract against macrophage cell lines at different concentrations. NS: non-significant difference compared to the control group.

4. Discussion

The use of co-products from agro-industrial processes, especially of those from the food and cosmetics industries, has been highlighted. After obtaining the pulp and extracting the oil from seeds, the co-products are usually discarded, which represents a source of serious environmental pollution. However, they have in their composition chemical constituents with different biological activities, such as antioxidant and antimicrobial ones, and research has demonstrated their great potential for use as a food input [17,20,36].

As is known, the quality of a food is determined by its nutritional value and the quantity that can be consumed. This value is influenced by the chemical composition, which includes crude protein, crude fiber, minerals, vitamins, and the characteristics of digested products [37]. The characteristics of co-products resulting from the extraction of seed oil appear to be influenced not only by the properties and quality of the industrialized fruit, but also by the processing method and the storage period until its use, which often occurs inadequately due to the lack of knowledge about its nutritional potential [38].

Macronutrients are nutrients that the body needs in large quantities and that are widely found in foods, which should be ingested daily to ensure a healthy diet. In particular, proteins, carbohydrates, lipids, and fibers are the components of foods that contribute to the supply of energy (calories) for the diet [39]. In this regard, the evaluation of the proximate composition of the ucuuba co-product showed evidence of its potential use in the food area, both for human and animal nutrition, especially thanks to its high fiber content (highest percentage) likely resulting from the lignans present in ucuuba seeds. It is worth remembering, in fact, that lignans are insoluble fibers that exert some important actions on the body, being involved in the prevention of intestinal constipation, in the increase of fecal mass, and even in the reduction of the risk of cardiovascular diseases [40,41].

In general, the predominant class of fatty acids was the saturated ones, especially myristic acid and lauric acid [2], which are associated with an increased risk of cardiovascular disease, resulting in few biological benefits. However, they are valuable from a technological point of view, having greater resistance to oxidation and temperature and re-

maintaining solid above 20 °C, which facilitates the molding of solid foods that can be prepared from the co-product [42].

Water activity is a parameter that measures the amount of water available for the proliferation of microorganisms and for chemical and biochemical reactions [43]. In particular, the higher its value, which can vary from 0 to 1, the greater the availability of water for microbial growth [44]. Since pathogenic microorganisms cannot develop in environments with water activity below 0.6, it is assumed that, in order to preserve the microbiological integrity of the product, water activity below 0.5 is acceptable [45]. It is important to remember that water activity differs from moisture content: while moisture content refers to the total amount of water present in a food, water activity is a ratio between two water vapor pressures, i.e., that of the food and that of pure water at the same temperature [45]. The value of the latter reveals the amount of unbound water in the food and, therefore, available for biochemical reactions and microbial growth [46]. In this study, the low values of both water activity (0.403 ± 0.01) and moisture content ($6.73 \pm 0.05\%$) (Table 1) indicate that the ucuuba co-product may be free from microbial contamination and possible degradation of its constituents.

An alternative way to assess the stability of a product is through its thermal analysis using thermogravimetry (TG/DTG). In this work, this technique was applied to investigate the thermal stability of both the ucuuba co-product and its extract. The former event of mass loss observed in both samples was ascribed to the evaporation of surface water, resulting from moisture and volatility of some components. This event reinforces the result of co-product moisture content. The latter event, which implied a significant mass loss, may be linked to successive decomposition reactions of carbohydrates, lipids, and other organic components. In the extract, the latter event of mass loss was also associated with decomposition, thermodegradation, and evaporation of substances with higher molecular weight [24].

The chemical characterization of the co-product and the extract by Fourier-transform infrared (FTIR) spectroscopy allowed an initial study on the chemical composition of both. The FTIR spectra revealed bands of functional groups characteristic of hydrocarbons, alcohols, and phenols that typically make up the structure of phenolic compounds [17]. However, these data are still preliminary and require the adoption of other techniques to confirm that the functional groups identified in the extract actually belong to phenolic compounds.

In order to further investigate the chemical composition of the extract, a spectrophotometric analysis was performed regarding phenolic compounds, which play a crucial role in the biological, pharmacological, and antioxidant activities of raw plant materials. Within this vast class of compounds, flavonoids and polyphenols stand out. Relevant contents of total polyphenols (TP) and total flavonoids (TF) were identified in the extract of Amazonian tucumã co-product ($TP = 135.1 \pm 0.078$ mg GAE/100 g; $TF = 32.73 \pm 0.009$ mg QE/100 g) [17]. In the current study, high concentrations of both TP (806.45 ± 1.9 mg GAE/100 g) and TF (62.0 ± 1.27 mg RE/100 g) were observed. These findings are quite encouraging, especially taking into account that the analyzed sample came from a co-product.

Considering the promising content of TP of the ucuuba co-product extract, the identification and quantification of the predominant compound were started by means of HPLC-DAD. The study by Ferreira et al. [17] using UHPLC-DAD demonstrated the presence of caffeic acid with a retention time of 18.51 min (325 nm) in the extract of the co-product of tucumã almonds. During the development phase of the analytical method, the optimization of the analysis conditions is essential for the accurate identification of the metabolite of interest. The selected conditions were effective in quantifying one of the most relevant phenolic acids of this class, caffeic acid, in the extract of the ucuuba co-product. This result suggests that such an extract may have beneficial properties for human health.

Phenolic acids are known for their antioxidant properties, both in food and in the body, in addition to their anti-inflammatory, antiviral, and anticancer actions, which make them recommended for the prevention and treatment of cancer and cardiovascular diseases, among other illnesses [47,48].

In this context, among the various properties of secondary metabolites produced by plant species, the antioxidant activity present in many of them stands out. The detection of secondary metabolites is essential in natural products, as they are responsible for numerous biological activities, such as the anti-inflammatory, antimicrobial, antifungal, anticancer, and antioxidant ones [49,50]. Phenolic compounds are one of the groups of metabolites that stand out most in terms of antioxidants [51,52]. This is because antioxidants are compounds that, in reduced quantities in relation to the oxidizable substrate, can significantly delay or even prevent the onset or propagation of oxidation reactions [47]. These compounds act by inhibiting not only lipid peroxidation, but also the oxidation of other molecules [48].

Research on co-products of Amazonian species has demonstrated antioxidant potential for the co-products of pracaxi (910.82 ± 7.33 μM Trolox/g by the ABTS assay and 906.68 ± 1.20 μM Trolox/g by the DPPH one) [20] and tucumã (1094.01 – 1247.88 μM Trolox/g by the ABTS assay and 8.29 – 60.22 μM Trolox/g by the DPPH one) [17]. Even though the extract of the ucuuba co-product showed good antioxidant activity using both methods, the result obtained by the DPPH method was superior, with the antioxidants inhibiting the radical by almost 90%. This result corroborates the hypothesis that the ucuuba co-product has promising potential for use as a functional food and ingredient in the production of nutraceuticals.

From this perspective, after performing the physicochemical characterization and analysis of the biological properties, the challenge arose to investigate whether the ucuuba co-product extract had any cytotoxic effect. This effect was investigated through in vitro tests, which are typically performed on fibroblast, keratinocyte, or macrophage cells. Macrophages play a crucial role in the immune system, being stimulated by the presence of bacteria, fungi, and protozoa [53]. When activated, they exhibit cytoplasmic projections, increase their adhesion, spread, produce cytokines, and intensify the response against pathogens, generating nitric oxide and reactive oxygen species.

Since these cell types are essential in the initial defense of the organism, it is essential that any extract used does not cause damage to them. Thus, their ingestion through food or their inclusion in topical or oral formulations must be safe for the body [53]. The results of cytotoxicity tests showed that the extract, even at the highest concentration investigated (100 $\mu\text{g/mL}$), did not cause any significant variation in the viability ($p > 0.01$) of macrophages compared to that in the control group.

The use of co-products is a sustainable way to make the most of Amazonian resources, especially ucuuba, whose almond fat is currently used in the cosmetics industry. In this context, this work contributed to showing that the ucuuba seed co-product and its derivatives have promising potential for obtaining new products aimed at application in the food sector.

5. Conclusions

The ucuuba co-product, thanks to its high concentration of macronutrients, proved to be a promising raw material for possible applications in the food industry. The co-product and its extract displayed thermal stability around 100 $^{\circ}\text{C}$, while the FTIR spectra showed characteristic bands of functional groups present in the chemical structure of phenolic substances, and UV–Vis spectroscopy showed significant levels of total polyphenols and total flavonoids. HPLC allowed the identification and quantification of one of the main phenolic acids found in plant species, caffeic acid. The extract was, in fact, characterized as

a quality input, having shown promising antioxidant properties. Furthermore, it did not exhibit any cytotoxic effect on a macrophage lineage. The results of this study reinforce the idea that the ucuuba seed co-product, if suitably exploited, can constitute an excellent natural source of secondary metabolites with antioxidant properties and be applied as a functional food and/or nutraceutical ingredient, in addition to adding value to the production chain of this species.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app151810291/s1>, Figure S1: Calibration curve of caffeic acid used for its quantification in the extract. mAU = milli absorbance unit.

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