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PRODUCTION AND CHARACTERIZATION OF BIOACTIVE EXTRACT OF CAMU-CAMU (*Myrciaria dubia*)

PRODUÇÃO E CARACTERIZAÇÃO DE EXTRATO BIOATIVO DE CAMU-CAMU (*Myrciaria dubia*)

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Abstract

The present study aims to describe the production and characterization of the antioxidant activity of camu-camu extract (*Myrciaria dubia*), from fruit collected in Rio Branco, Acre, Western Brazilian Amazon Region. A study was carried out based on the production process of the ethanolic extract, with the extraction steps, along with the main analyzes of the antioxidant capacity, with the results of DPPH, FOLIN, FRAP and ORAC.

Keywords: Extract; Fruit; Antioxidants; Camu-camu

Resumo

O presente estudo tem como objetivo descrever a produção e a caracterização da atividade antioxidante do extrato de camu-camu (*Myrciaria dubia*), a partir da fruta coletada em Rio Branco, Acre, Região Amazônica Ocidental Brasileira. Foi realizado um estudo descrito a partir do processo de produção do extrato etanólico, com as etapas de extração. E as principais análises da capacidade antioxidante, com os resultados de DPPH, FOLIN, FRAP e ORAC.

Palavras-chave: Extrato; Fruta; Antioxidantes; Camu-camu

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Camu-camu (*Myrciaria dubia*) is a fruit tree, belonging to the Mirtaceae family, widely distributed in the Amazon basin [1]. This plant has a high vitamin C content, found mainly in its fruit, which can reach 6,000 mg of ascorbic acid per 100g of fruit (fresh weight) [2]. It is a small fruit with a diameter of 1 to 3.2cm, dark red to black purple and weighs approximately 10g. It contains one to four seeds per fruit, these being reniform, ellipsoid and covered by a layer of fibrin.

Due to the high acidity, the fruits are rarely eaten fresh. Among the various elements that can be found in the fruit are: vitamin A, glucose, fructose, starch, pectin, anthocyanins, fiber, minerals (nitrogen, phosphorus and potassium) and several phenolic compounds with antioxidant activity [3].

The fruit of *M. dubia* is rich in natural antioxidants, ascorbic acid, vitamin E, β-carotene and several phenolic compounds, such as flavonoids, tannins and phenolic acids [4]. Phenolic compounds have excellent antioxidant activity because of their reducing properties and chemical structure that play an important role in neutralizing and scavenging free radicals and chelating transition metals, being able to act in the initiation and propagation stages of the oxidative process [5].

The present study aims to describe the production and characterization of the antioxidant activity of camu-camu (*Myrciaria dubia*) extract, from fruit collected in Rio Branco, Acre, Western Brazilian Amazon Region. This is a descriptive study about the production process and analysis of the antioxidant capacity of the ethanolic extract of camu-camu (*Myrciaria dubia*).

For the production of the ethanolic extract, the fruits were collected in the rural area of Rio Branco, Acre – Brazil, after the collection process they were sanitized according to the protocol established by ANVISA [6], and subsequently frozen and wrapped in aluminum foil.

To start the production process of the ethanolic extract, the fruits were fully mechanically macerated with a glass rod, and subsequently stored in an amber glass bottle with a lid and stopper, properly wrapped with aluminum foil in a place without lighting, with the proportion of 50g of fruit for 150ml of 70% alcohol, for 7 days.

The mixture was filtered through filter paper, and subsequently the extract concentration was carried out in a rotary evaporator under reduced pressure. After this

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process, the extract was frozen for 24 hours in a -80ºC freezer, and lyophilized for 24 hours. Figure 01 determines the flowchart of the ethanolic extract production process.

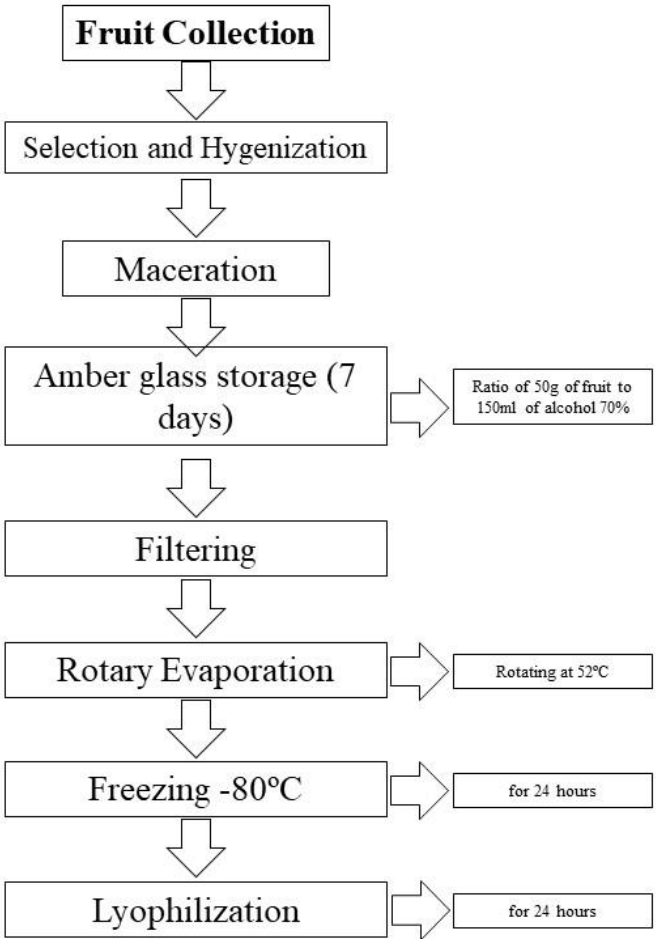

Figure 01 - Production flowchart of camu-camu ethanolic extract (*Myrciaria dubia*)
Source: Authors (2023)

DPPH Method Protocol - DPPH solutions diluted in P.A methanol (10µM, 20µM, 30µM, 40µM, 50µM and 60µM) were used for the standard curve. The ethanolic extract sample was separated in an aliquot of 0.01ml/ml and transferred to a 10ml flask completed with 70% ethanol. From the extract obtained, in a dark environment, an aliquot of 75 µL of the extract dilution was transferred to the 96-well plate with 225 µL of the DPPH radical (0.06mM) in triplicate. For the reagent blank, 75µL of the control solution (50% ethanol and water) with 225µL of the DPPH radical was used. The readings were analyzed with an absorbance of 515nm, with a reaction time of 30 minutes in a microplate reader (SpectraMax i3x) [7] .

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ORAC Method Protocol - for the standard curve, Trolox solutions diluted in PBS buffer (2.5µL/mL, 5µL/mL, 7.5µL/mL, 10µL/mL, 12.5µL/mL, 15µL/mL, 17 .5µL/mL and 20µL/mL). The ethanolic extract sample was separated in an aliquot of 0.01ml/ml and transferred to a 10ml flask completed with 70% ethanol. Afterwards, an aliquot of 1mL of the sample with 1mL of DMSO was transferred to a 10mL flask and completed with PBS buffer [8].

From the extracts obtained, solutions were prepared in eppendorfs of different concentrations (33.3µL/mL, 50µL/mL, 100µL/mL, 142.85µL/mL, 200µL/mL and 500µL/mL). In the 96-well plate, 20µL of sample + 120µL of fluroscein were added in triplicate for each dilution. For the blank, 20µL of PBS + 120µL of fluroscein were added in duplicate, as well as for the trolox curve. The plate was incubated for 15 minutes at 37°C and after adding 60µL of AAPH in each well. The plate was placed for serial readings every 5 minutes in a microplate reader (SpectraMax i3x) for 2 hours.

The ORAC value (µM Trolox/g) was calculated by the area under the fluorescence emission curve, simultaneously using the time intensity information.

TOTAL PHENOLIC COMPOUNDS Method Protocol - For the standard curve, solutions of gallic acid diluted in water (10µM, 20µM, 30µM, 40µM, 50µM and 60µM) were used. Based on these concentrations, 2.7 µL of each dilution of gallic acid, 150 µL of 10% Folin-Ciocalteu and 120 µL of 4% sodium carbonate were added to the well. For the ethanolic extract sample, an aliquot of 0.01ml/ml was separated and transferred to a 10ml flask completed with 70% ethanol [9].

From the extracts obtained, in a dark environment, an aliquot of 30µL of the dilution of each extract was transferred to the 96-well plate with 150µL of 10% Folin-Ciocalteu and 120 µL of 4% sodium carbonate in triplicate. For the reagent blank, 75µL of the control solution (50% ethanol and water) with 150µL of 10% Folin-Ciocalteu and 120µL of 4% sodium carbonate were used. The readings were analyzed with an absorbance of 750nm, with a reaction time of 2 hours in a dark environment in a microplate reader (SpectraMax i3x).

FRAP Method Protocol - For the standard curve, solutions of ferrous sulfate diluted in water (1µM, 5µM, 10µM, 25µM, 50µM and 100µM) were used. From these concentrations, 35µL of each ferrous sulfate dilution and 265µL of FRAP reagent were

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added to the well. The ethanolic extract sample was separated in an aliquot of 0.01ml/ml and transferred to a 10ml flask completed with 70% ethanol [8].

From the extracts obtained, in a dark environment, an aliquot of 10µL of the dilution of each extract was transferred to the 96-well plate with 25µL of solvent and 265µL of FRAP in triplicate. For the reagent blank, 35 µL of the control solution (50% ethanol and water) with 265 µL of FRAP were used. The readings were analyzed with an absorbance of 595 nm, with a reaction time of 30 minutes at a temperature of 37ºC in a dark environment in a microplate reader (SpectraMax i3x).

Table 01 – Determination of the antioxidant activity of ethanolic extract of camu-camu (*Myrciaria dubia*)

ANALYSIS	FOLIN (mgEAG/g)	DPPH (µmol Trolox/g)	FRAP (µmol ferrous sulfate/g)	ORAC (µmol Trolox/g)
Ethanolic extract of camu-camu	9,51±0,28	2571,34±31,54	192,54 ±33,66	485,01±55,85

Nature has always been, and still is, a source of foods and ingredients that are beneficial to human health. Nowadays, plant extracts are increasingly becoming important additives in the food industry due to their content in bioactive compounds such as polyphenols [10] and carotenoids [11], which have antioxidant activity, especially against low-density lipoprotein (LDL) and deoxyribonucleic acid (DNA) oxidative changes [12]. The efficient extraction of these compounds from their natural sources and the determination of their activity in commercialized products have been great challenges for researchers and food chain contributors to develop products with positive effects on human health. Moreover, it is important to consider that individual bioactive compounds exist in different proportions in whole natural matrices.

Total phenolic content in the extract produced is higher than that in a range of other tropical fruits, with a higher content in seeds and peel. [13] reported for the first time on the anthocyanin profile of camu-camu in fruits from two regions of Sao Paulo, Brazil. The major anthocyanins were cyaniding-3-glucoside, which was the major pigment, followed by delphinidin-3-glucoside. In addition to their light attenuating role, anthocyanins act as powerful antioxidants. The antioxidant capacity of camu-camu was reported to be the highest among the Brazilian fruits evaluated by [14]. Our results confirmed previous study that camu-camu exhibited significant and almost identical antioxidant properties through use of the total oxidant scavenging capacity assay

against peroxyl radicals and peroxynitrite, demonstrated by different methods and protocols.

The results of this work open the perspectives to a potentially substantial role for extract of camu-camu as a supplement, and an alternative in medicine products, including dietary supplements.

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