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In vitro antioxidant and antihypertensive compounds from camu-camu (*Myrciaria dubia* McVaugh, Myrtaceae) seed coat: A multivariate structure-activity study

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ABSTRACT

Camu-camu (*Myrciaria dubia*) pulp, seeds, and skin are widely known because of their nutritional properties. However, the seed coat has never been studied as a source of bioactive compounds. Herein, we characterized the phenolic composition, the antioxidant activity, and inhibition of angiotensin-converting enzyme (ACE) of three different extracts (water, propanone, and ethanol) from this residue and assessed the structure-activity using bivariate and multivariate statistical approaches. Phenolic acids and flavonoids were quantified by high-performance liquid chromatography while the ferric reducing antioxidant power (FRAP), inhibition of lipid peroxidation using egg yolk and Wistar rat brain, scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical, Folin-Ciocalteu reducing capacity (FCRC), and the inhibition of angiotensin-converting enzyme (ACE) by the extracts were also analyzed. *t*-Resveratrol was found in camu-camu seed coat for the first time. The aqueous extract had the highest total phenolic content, FRAP, DPPH•, FCRC, and inhibition of lipid oxidation using both chemical and biological assays, while the propanone extract showed the opposite behavior but it presented higher *in vitro* antihypertensive activity. The ethanolic extract exhibited intermediate values for the responses. The association between chemical composition and the functional properties of the camu-camu seed coat extracts were revealed using correlation analysis and principal component analysis.

1. Introduction

Tropical fruits are attractive to the food industry mainly because of their unique appearance, flavor, and nutritional value (Kaneshima et al., 2013). Among the fruits classified as berries, camu-camu has received attention due to its high content of bioactive compounds, such as phenolic compounds (flavonoids and ellagitannins), ascorbic acid, and β-carotene (Inoue et al., 2008; Gonçalves et al. al., 2010; Akter et al., 2011).

Camu-camu (*Myrciaria dubia* [H.B.K] McVaugh), also known as "caçari" or "araçá d'água", was discovered in 1958. It belongs to the Myrtaceae family and is spontaneously present on riverbanks and lakes of the Amazon basin, between Peru and Brazil (Zapata and Dufour, 1993; Silva and Andrade, 1997). Due to its high content of ascorbic acid and phenolic compounds, camu-camu reveals a bitter taste, which minimizes its consumption *in natura* (Myoda et al., 2010). As it is not widespread in the entire Brazilian territory, the commercialization is achieved on a small scale, basically in the form of frozen pulp. In Europe, Japan, Canada, and in the United States, there is great interest in the fruit, which is imported and its pulp transformed into sparkling drinks, vinegar, ice cream, and sweets (Yuyama, 2011).

The seeds and peels of camu-camu represent about 40% of the fruit (Rodrigues et al., 2001). However, these by-products are generally discarded without taking advantage of their chemical constituents. In this aspect, these residues may present higher antioxidant potential when compared to pulp because most bioactive compounds are retained on the fruit parts, which are treated as by-products by the food industry (Guo et al., 2003; Myoda et al., 2010; Azevêdo et al., 2014). Therefore, the processing of these by-products has the potential to become a segment of the agribusiness contributing to a better utilization,

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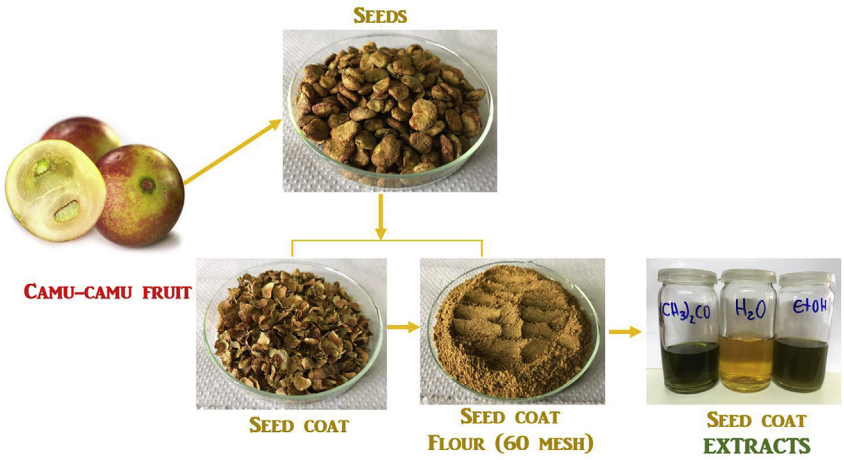

Fig. 1. Camu-camu fruit, its seeds, seed coat, and the extracts analyzed: $(CH_3)_2CO$ (propanone), H_2O (water), and EtOH (ethyl alcohol).

both in the food industry and cosmetics, thus allowing its economic valuation (Azevêdo et al., 2014; Nile et al., 2018).

Currently, the extraction of bioactive compounds from by-products (i.e., skin and seeds) of fruits and vegetables has been increasingly studied in order to avoid significant losses and wastes, besides representing potential benefits for applications in the industry (Sagar et al., 2018; Lu et al., 2018; Fan et al., 2018). Studies have reported that camu-camu seeds are composed of different classes of phenolic compounds, which are widely known for their functional and biological effects. Among these substances, phenolic acids (ellagic and syringic acids), and flavonoids, such as quercetin, myricetin, rutin, and catechin stand out (Reynertson et al., 2008; Akter et al., 2011; Azevêdo et al., 2014).

Phenolic compounds from camu-camu have attracted commercial and scientific interest mainly because of their potential to be used as nutraceuticals (isolated compounds) and functional food (camu-camu extracts), acting to reduce the risk of non-communicable diseases (Azevêdo et al., 2015; Grigio et al., 2017; Donado-Pestana et al., 2018). Myoda et al. (2010) found that the total phenolic content of camu-camu seed aqueous and propanone extracts was three times higher than that of acelora, demonstrating the functional potential of camu-camu seeds as sources of bioactive compounds. Therefore, the high levels of phenolic compounds combined with the antioxidant capacity identified show that the camu-camu residue may represent a new source of functional compounds for the improvement of human nutrition (Souza et al., 2017).

In order to avoid food waste, in addition to the fact that the Brazilian fruit exploitation may be of industrial interest, the objectives of this work were to evaluate different extracts (water, propanone, and ethyl alcohol) of camu-camu seed coat with respect to their phenolic composition, antioxidant activity, and inhibition of angiotensin-converting enzyme (ACE) and to assess the structure-activity using bivariate and multivariate statistical methods.

2. Materials and methods

2.1. Chemicals

Gallic, chlorogenic, syringic, *p*-coumaric, ferulic, rosmarinic, and ellagic acids, quercetin-3-rutinoside (rutin), quercetin, (+)-catechin, (−)-epicatechin, Folin-Ciocalteu's phenol reagent 2 N, 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), pyrocatechol violet (3,3′,4-trihydroxyfuchsone- 2″-sulfonic acid), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), vanillin, angiotensin I-converting enzyme from rabbit lung (EC 3.4.15.1), hippuryl-L-histidyl-L-leucine substrate, and acetonitrile were obtained from Sigma-Aldrich (São Paulo, Brazil). *trans*-Resveratrol was obtained from Extrasynthese (France). 2,4-Dihydroxybenzoic and 2,5-dihydroxybenzoic acids were purchased from Carl Roth (Karlsruhe, Germany). Ascorbic acid and propanone were obtained from Biotec (Paraná, Brazil). Sulfuric acid and sodium carbonate were obtained from Vetec (Rio de Janeiro, Brazil). Anhydrous sodium acetate and methyl alcohol were obtained from Anidrol (São Paulo, Brazil) while potassium hexacyanoferrate (III) was obtained from Merck (Darmstadt, Germany). Ethyl alcohol was purchased from Neon (São Paulo, Brazil). Formic acid was acquired from Reagen (Rio de Janeiro, Brazil), and Milli-Q (São Paulo, Brazil) ultrapure water was used in the experiments.

2.2. Camu-camu seeds and extraction

Camu-camu fruit was cultivated in Iguape in the state of São Paulo/Brazil, geographical coordinates 24° 41′51" south, 47° 34′16" west at 6 m altitude, and harvested in March 2017. The fruits were sanitized (NaClO at 200 mg/L for 15 min) and the seeds removed manually. The seeds were dried in an oven with air circulation at 35 °C for 31 h (~12% moisture) and then the seed coat was removed and ground in a universe hard metal cutter mill (Ika Werke, Model M 20, USA). The camu-camu seed coat flour was standardized with 42 Tyler mesh sieve and stored in a glass vessel at 8 °C. The extractions were performed using the ratio 1:20 (sample:solvent, m/v), i.e., 10 g of flour obtained from the camu-camu seed coats were mixed with 200 mL of solvent mixture. Overall, samples were extracted with continuous magnetic agitation under temperature control (45 °C) in three different solvents; ultrapure water, ethyl alcohol or propanone (Fig. 1) for 45 min and the extracts were finally vacuum-filtered using a Büchner's funnel and immediately analyzed for their phenolic composition and functional properties.

2.3. Phenolic composition

The total phenolic content (TPC) of extracts was determined using a colorimetric method that uses K_3 $[Fe(CN)_6]$ and $FeCl_3{\cdot}6H_2O$ at 0.5 mmol/L (Margraf et al., 2015). Results were expressed as mg of gallic acid equivalent per 100 g of seed coat (mg GAE/100 g). The condensed tannins content (CT) was quantified using the vanillin-H_2SO_4 method (Horszwald and Andlauer, 2011) and data were expressed as mg of (+)-catechin equivalent per 100 g of seed coat (mg CE/100 g). The non-tannin phenolic content was estimated by subtracting the CT from the TPC and results were expressed as mg/100 g.

The quantification of individual phenolic compounds was performed using a high-performance liquid chromatograph (HPLC)