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Chemical composition, antioxidant and antimicrobial activity of guavirova (*Campomanesia xanthocarpa* Berg) seed extracts obtained by supercritical CO_2 and compressed *n*-butane

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ABSTRACT

This work reports the chemical characterization, antioxidant and antimicrobial activity of *Campomanesia xanthocarpa* seed extracts obtained from compressed *n*-butane and supercritical CO_2 extraction. For supercritical CO_2, the extraction condition was 40 °C and 250 bar, while for *n*-butane 35 °C and 10 bar. The total phenolic compound content was 68.58 ± 1.15 mg g^{-1} for *n*-butane extract and 17.18 ± 2.44 mg g^{-1} for supercritical CO_2. Both extracts were rich in terpenoids, especially β-caryophyllene, while *n*-butane extract had a larger content of flavonoids than supercritical CO_2. The antioxidant activity was tested with FRAP, DPPH and deoxyribose assay, with *n*-butane extracts exhibiting better results. Antimicrobial activity was verified for both extracts against *Pseudomonas aeruginosa*, however, supercritical CO_2 extract afforded a better performance, reaching 5.0 mg mL^{-1} for MIC and 10.0 mg mL^{-1} for MBC, while *n*-butane extract showed 40.0 mg mL^{-1} in both antimicrobial tests. Higher extraction yields were obtained using *n*-butane, which also presented higher antioxidant activity, while supercritical CO_2 extracts afforded the highest antimicrobial activity.

1. Introduction

Campomanesia xanthocarpa Berg, popularly known as "guavirova", is a native species belonging to Myrtaceae family. Fruits from these trees present a nice acidic-sweet taste, and have been recently studied as a raw material not only by the food industry but also for pharmacological applications, due to the fact that the extract presents a high antioxidant potential [1,2], and has been demonstrated to be efficient in hypoglycemic and antithrombotic treatments, as well as for diarrhea and gastric ulcers [3].

Among the extraction methods, supercritical fluid extraction (SFE) has been widely employed to extract target compounds from a variety of natural matrices [4]. Carbon dioxide is the elected supercritical solvent in the extraction of flavor and fragrance compounds due to its well-known properties [5–7].

Nevertheless, propane and *n*-butane can also be a good choice because their critical pressures are relatively low compared to that of carbon dioxide and present dielectric constants of around 1.7, quite similar to carbon dioxide [8–11]. Since liquid *n*-butane exhibits low compressibility and very low solubility in water, the literature indicates that they have a hydrostatic behavior working as mechanical press or piston fluid that increases the system pressure changing favorably the extraction of non-polar components [10,12]. Besides, *n*-butane is plenty available, cheaper and it can be used in much lower pressures compared to carbon dioxide. In addition, as with any pressurized gas, *n*-butane has the advantage over liquid organic solvents of being easily separated from the final product by system depressurization. Thus, besides the mild temperature and pressure operating conditions, the use of short-chain hydrocarbons, like *n*-butane, allows reduction of extraction time, while it may improve the quality of the extract obtained. However, literature is scarce on the vegetable matrices extraction using pressurized *n*-butane, though some tests have been reported and proved profitable and efficient [12–14].

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In this context, the aim of this work is to report the extraction of *C. xanthocarpa* seeds using two pressurized fluids, CO_2 and *n*-butane, with a focus on the antioxidant potential of the extracts obtained along with the phytochemical characterization of the extracts, total phenolics, and antimicrobial activity against *Enterococcus faecalis* – ATCC 29212 (gram-positive) and two gram-negative bacteria, *Escherichia coli* – ATCC 11229 and *Pseudomonas aeruginosa* – ATCC 27283.

2. Experimental

2.1. Raw material

C. xanthocarpa ripened fruit samples were collected on November 2013 (spring) from the native plants of the city of Quilombo, which is located approximately 688 m above sea level, in the South Region of Brazil (26°47′23.6″ S, 52°45′42.41″ W). This region is characterized as having a humid subtropical mesothermal climate. Type specimens were deposited in the Herbarium at Universidade Comunitária de Chapecó (Unochapecó Herbarium, SC, Brazil) under the access number 3153. After collection, the fruits were stored in glass flasks under nitrogen atmosphere, protected from the light, in refrigeration (8 °C) until the extraction. Seeds were manually separated from the fruits just before the extraction assays and ground in a home blender.

2.2. Pressurized fluid extraction

The experimental apparatus consists basically of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 130 cm^3 jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of 0.12 bar, a collector vessel with a glass tube, and a cold trap [15,16]. Amounts of around 30 g (±0.05 g) of seeds, first dried in an oven at 40 °C to constant weight and then comminuted in a blender (average particle size of 0.14 mm), were charged into the extraction vessel. The solvent was pumped at a constant flow rate of 2 mL min^{-1} into the bed, which was supported by two 300 mesh wire disks at both ends and was kept in contact with the herbaceous matrix for at least 30 min to allow system stabilization. Afterward, the extract was collected by opening the micrometering valve, and the solvent flow rate was accounted for by the pump recordings, which means the use of around 70 g of *n*-butane and 300 g in the case of carbon dioxide. After that, the mass of the extract was weighed, and the glass tube was reconnected to the equipment. This procedure was performed until no significant mass was extracted.

For *n*-butane (White Martins, 99.5% purity), after preliminary tests, the total extraction time was defined as 60 min, temperature 35 °C and pressure 10 bar. Solvent density was estimated using the HBT (P-V-T) correlation for compressed liquids [17] or taken from experimental literature values [18], making possible to estimate the mass of solvent charged into the reaction vessel.

For carbon dioxide (White Martins, 99.9% purity), after preliminary tests, the extraction time was fixed at 150 min, at 40 °C and 250 bar. Carbon dioxide densities were estimated using Angus correlation [19]. In both cases, experiments were accomplished isothermally at constant pressure. A whole experimental run lasted in general 6 h, including all steps involved: sample weighing, temperature stabilization (baths, extractor), extraction, and depressurization. Based on triplicate experiments carried out for all the experimental conditions for both compressed solvents, the overall average standard deviation of the yields noticed was about 0.2 wt%.

2.3. GC and GC/MS analysis

The components of *C. xanthocarpa* seed extracts (CO_2 and *n*-butane) were determined by gas chromatography. GC analyses were performed on a Varian 3800 Gas Chromatograph using an HP-Innowax column (25 m × 0.25 mm, 0.5 mm film thickness). Injector and detector temperature was set at 250 °C; and the oven temperature was programmed from 60 °C (8 min) to 180 °C at 4 °C min^{-1}, 180 to 230 °C at 20 °C min^{-1} and subsequently holding at 230 °C (20 min). Helium was employed as carrier gas (3.4 bar). Percentage compositions were obtained from electronic integration measurements using flame ionization detection. GC-MS analyses were performed on an HP 5973 – 6890 GC-MSD system operating in the EI mode at 70 eV, equipped with an HP-5 cross-linked capillary column (30 m × 0.25 mm). The temperature of the column and the injector were the same as those from GC. Helium was employed as a carrier gas (5.6 bar, 1 mL min^{-1}).

Identification of the constituents of *C. xanthocarpa* samples was based on the retention index (RI), determined with reference to the homologous series of *n*-alkanes, C7-C30, under identical experimental conditions, comparing the mass spectra with those of NBS Library and those described in the literature [20]. The relative amounts of individual components were calculated based on the GC peak area (FID response).

2.4. Quantification of total phenolic and phytochemical profile

To contribute with the chemical aspect of *C. xanthocarpa* seed extracts, the phytochemical profile was determined using chromatographic analyses *via* thin-layer chromatography on Silica gel (Merck – Germany). The solvent system for alkaloids, coumarins and flavonoids consisted of 92:8 (v/v) (chloroform; methanol), while for terpenes, sterols and tannins 70:30 (v/v) (hexane; ethyl acetate) was used. The methods were based on Wagner and Bladt report with some modification [21].

The total phenolic compounds for both extracts of *C. xanthocarpa* seed were analyzed using the Folin-Ciocalteu colorimetric method described by Shahidi and Naczk [22] with some modification. Triplicate extract solutions protected from light were prepared at concentration of 0.5 mg mL^{-1} by adding water and the Folin-Ciocalteu reagent after 3 min, together with 0.2 mL of a saturated solution of sodium carbonate. After one hour, absorbance at 765 nm was read in a spectrophotometer. Quantitation was performed based on a gallic acid standard curve where the total phenolic compounds were expressed as mg gallic acid equivalent (GAE) per g of extract.

2.5. Quantification of total flavonoids

The quantification of total flavonoids was assessed by incubating the diluted (50 mg mL^{-1}) *C. xanthocarpa* seed extract with an ethanolic solution of aluminum chloride (1.33 mg mL^{-1}) and potassium acetate (4.0 mg mL^{-1}). The reaction mixture was kept at room temperature (~25 °C) for 30 min and the absorbance was measured against blank at 415 nm (Scinco SUV-2120 spectrophotometer). Quercetin was used as the standard for the calibration curve, and the total flavonoid content was expressed as milligrams of quercetin equivalents per g of extract (mg g^{-1}).

2.6. Analysis of antioxidant activity

2.6.1. FRAP assay for antioxidant capacity

The ferric reducing antioxidant power (FRAP) assay was performed in triplicate, as described by Benzie and Strain [23] with some modifications. Different dilutions of seed extract (0–160 μg mL^{-1}) were incubated with the working solution of