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Study of the supercritical extraction of *Pterodon* fruits (Fabaceae)

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ABSTRACT

In this study, the extraction of sucupira fruits (*Ptedoron* spp.) was conducted by using the supercritical carbon dioxide as solvent and the mass percent yield, chemical profile and the antioxidant activity of the extracts obtained were evaluated. The extractions were conducted in 313–333 K and 10–22 MPa. Extractions conditions were defined by phase transition measurements for the system CO_2 (1) + sucupira extract (2). The phase equilibrium data were modeled using the Peng-Robinson equation with Wong-Sandler quadratic mixing rule. Sovová model was applied to fit the kinetic extraction curves. The extractions were conducted in 313–333 K and 10–22 MPa. The highest mass percent yield (21.2%) and the highest content of vouacapan diterpenes (35.66%) were obtained from the supercritical extraction on the conditions of 313 K and 22 MPa. Yet, the highest antioxidant activity (77.59%) was obtained at 323 K and 16 MPa.

1. Introduction

Pterodon spp. is a tree species of *Fabaceae* family which includes four species: *Pterodon abruptus* Benth, *Pterodon apparicioi* Pederdoli, *Pterodon polygalaeflorus* Benth and *Pterodon emarginatus* Vogel (synonym *Pterodon pubescens* Benth) [1]. Native Brazilian trees, typically of the Cerrado, are popularly known as sucupira, sucupira-branca and faveira [2]. The seeds, peels and the oil of the sucupira have been used in folk medicine for presenting potential pharmacological effect with antirheumatic, anti-inflammatory, and antinociceptive properties [3].

Studies about the pharmacological properties of the sucupira show that the ethanolic extract of the seeds [4] and hexane and methanoloic extract of the fruits of *P. polygalaeflorus* show considerable larvicide activities against the *Aedes aegypti* mosquito [5] and antinociceptive activities [6]. The resin-oil has antispasmodic and vascular relaxing effects [7]. The hydroethanolic extract of the leaves of *P. emarginatus* [8] and the oilseed extract [9] and ethanolic [10] of the fruits of *P. pubescens* have antinociceptive activities in animals. The essential oil of seeds of P. *emarginatus* shows high citotoxity in cancer cells, demonstrating antiproliferative activity [11], reduces and limits the severity and the development of autoimmune diseases such as multiple sclerosis [12].

Phytochemical studies show that the essential oil of *P. emarginatus* is composed of sesquiterpene hydrocarbons and oxygenated sesquiterpenes, whose major components are β-caryophillene, β-elemene, spathulenol, α-humulene and γ-muurolene [13,14]. The vouacapan diterpenes existing in the extracts of the *Pterodon* species obtained by organic solvents are directly related to the biological activities [15].

The works found in the literature focus on the production of the oils (steam-distillation) and/or the extracts obtained from organic solvents (maceration, percolation and Soxhlet) extracted from the fruits, from the leaves and from the stems of the *Pterodon* plants to evaluate its pharmacological potential, phytochemical profile, biological activities and antioxidant activities [16]. However, studies involving the use of supercritical technology to obtain the extract from the fruit of *Pterodon* plants are limited. Santos et al. [17] produced nanoemulsions of extract of *Pterodon* fruits obtained via supercritical carbon dioxide ($scCO_2$) extraction to evaluate the Antileishmanial activity. The application of the supercritical technology to obtain the extracts through plant sources with pharmacological potentials is preferred because the extraction by applying the carbon dioxide in supercritical state is characterized by a fast process, it does not need any further separation processes and the possibility to adjust the solvation power with changing the temperature and pressure conditions [18].

The knowledge of the phase system behavior of carbon dioxide and the plant extract is important for the project and for the definition of the operating conditions of temperature and pressure on the extraction process with $scCO_2$. However, there are few studies which use this

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information to optimize extraction experiments using pressurized or supercritical fluids [19].

In this context, the objetive of this work was the study of the extraction of bioactive compounds of sucupira fruits using $scCO_2$ as solvent. The performance of the extraction with $scCO_2$ was assigned considering the mass percent yield and the kinetic extraction. The chemical profiles of the extracts obtained were determined by applying gas chromatography coupled to mass spectrometry (GC–MS). The antioxidant activities of the extracts were evaluated by DPPH method. The conditions of the supercritical extractions were defined by experimental data of phase transition system CO_2 (1) + sucupira extract (2). In addition, sucupira fruit oil was obtained by hydrodistillation and the results of mass percent yield, chemical profile and antioxidant activities were compared to the extract obtained from the extraction with $scCO_2$. Finally, the experimental data of equilibrium phases were modeled by Peng-Robinson equation of state combined with the Wong-Sandler quadratic mixing rule. The kinetics of the extractions with $scCO_2$ were represented by Sovová model.

2. Material and methods

2.1. Sample preparation

The sucupira fruits were acquired in a local Market in Maringá, Brazil. The fruits were kiln-dried with closed circulation (Nova Ética 400/4ND) at a temperature of 323 K until constant mass is reached. The fruits were crushed with an average size of 2 mm and stored at temperature 277 K. The moisture content of the fruits determined by the gravimetric method was 3%.

2.2. Phase equilibrium measurements

The phase equilibrium measurements of CO_2 system (1) + sucupira extract (2) were performed by applying the synthetic-static method [20–22]. The experimental apparatus consists basically of a high pressure cell with a variable volume with two sapphire windows. This cell is connected to a syringe pump (Isco 260D) and attached to a heating jacket, in which the pressure and the temperature are monitored by a pressure transmitter (Smar LD301) and a thermocouple (PT-100), respectively.

Initially, a certain quantity of mass of the extract is introduced in the cell with variable volume via a glass syringe. The mass of extract is determined using an analytical balance (Denver Instrument APX-200). After that, a pre-established amount of CO_2 is added by using the syringe pump. Since the composition is known and the temperature is fixed, the system pressure is increased through the piston inside the cell with variable volume until a single phase is reached. After a stabilization period, about 30 min, depressurization starts (0.1–0.3 MPa min^{-1}) until a new phase is formed. With a visual observation of the phase transition formed, through the sapphire window, it is possible to classify it as bubble point (BP) or dew point (DP). The experiments were conducted at temperatures 313, 323 and 333 K. The pressures of phase transitions were calculated in triplicate.

2.3. Extraction methods

2.3.1. Hydrodistillation (HDE)

The essential oil of sucupira fruits was obtained by hydrodistillation by using the Clevenger apparatus. Forty grams of sucupira crushed fruits are added in 500 mL of distilled water at normal boiling temperature. The process of extraction of hydrodistillation lasted 2 h [23]. The experiments of extraction were made in triplicate and the samples of the essential oil were dried with anhydrous sodium sulfate and stored at 255 K. The mass percent yield was expressed as a mass percent of the extracted oil in relation to the initial mass of the fruits used for extraction according to Eq. (1):

$$Y(\%) = \left(\frac{massextracted}{initialsolidmass}\right) \times 100 \quad (1)$$

2.3.2. Extraction with supercritical fluid (SFE)

The experiments of supercritical extraction were conducted in a laboratory scale unit reported in previous works [24–26]. Briefly, the unit consists in a solvent reservoir (CO_2, White Martins S.A. with purity of 99.9%), a syringe pump (Isco 500D), two thermostatic baths (Julabo F25-ME and Quimis Q214M2) and a stainless steel extractor with 28 cm length and 2.85 cm internal diameter. The experimental procedure starts with the introduction of 20 g of sucupira fruits in the extractor. Then, CO_2 is added and the expected conditions of temperature and pressure are adjusted for one hour. The experiments were conducted at a constant mass flow of 3 g min^{-1}. The samples of extracts were collected in five minute intervals on the first 30 min, and ten minutes intervals until the extraction process finished.

The extraction temperature range (303–333 K) was defined, initially, through values used in the literature for the extraction of bioactive compounds [27]. The molar fractions inside the extractor were calculated according to Eqs. (2) and (3):

$$n_{CO2} = \frac{\rho_{CO2}\varepsilon V_{bed} M_{CO2}}{\rho_{CO2}\varepsilon V_{bed} + m_0} \quad (2)$$

$$n_{ext} = \frac{(1 - \rho_{CO2}\varepsilon V_{bed}) M_{ext}}{\rho_{CO2}\varepsilon V_{bed} + m_0} \quad (3)$$

where n_{CO2} and n_{ext} are the number of mols of CO_2 and extract, respectively, ε is the extraction bed porosity, V_{bed} is the extraction bed, M_{CO2} and M_{ext} are the molar mass of CO_2 and extract, respectively, and m_0 is the initial extract mass.

The conditions of extraction pressure were defined through the knowledge of phase diagram of CO_2 (1) + sucupira extract (2).

2.4. Antioxidant activity

The analyses of the antioxidant activities were performed through the free radical method DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) described by Mensor et al. [28]. The results were expressed in inhibition percentage of free radical based on the decrease of the absorbance measured at 516 nm according to Eq. (4):

$$AA(\%) = \left(\frac{A_{DPPH} - A_{sample}}{A_{DPPH}}\right) \times 100 \quad (4)$$

where A_{DPPH} is the absorbance of the DPPH solution and A_{sample} is the absorbance of the sample in solution. The analyses were performed in triplicate.

2.5. Gas chromatography

The chemical profiles of the extracts were performed by GC–MS in a chromatograph (Agilent Technologies 7890A) coupled to a mass detector (Agilent Technologies 5975C) using a column DB-5 (30 m × 0.25 mm × 0.25 mm) with helium as carrier gas (1 mL min^{-1}). The temperature of the injector was 493.15 K and of the detector was 553.15 K. The *Split* ratio was 1:5. The temperature of the oven increased from 343.15 to 553.13 K at a rate of 3 K min^{-1} and maintained constantly for 5 min. The injected volume was 1 μL in triplicate. The identification of the components was made by comparing the mass spectra of the databank of the equipment, by the Kovat index [29] and by comparing the chromatograms with the results obtained from the oilseed extract [9] and ethanolic fruit extract [10] of *Pterodon pubescens* Benth.