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Supercritical fluid extraction of coumarins from *Pterocaulon balansae*: mathematical modeling and evaluation of antifungal properties

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ABSTRACT

The composition of the *Pterocaulon* extracts obtained by supercritical extraction is well defined and cumarins are their major components. Three of these compounds contain epoxy groups, and one of them, 7,7-((3,3-dimethyloxiran-2-yl) methoxy)-5,6-dimethoxy-2H-chromen-2-one, was identified as the main compound in these extracts. These compounds are considered privileged structures due to their wide range of biological activities such as antibacterial, antifungal and cytotoxic. Aerial parts of *Pterocaulon balansae* were collect in the Rio Grande do Sul state, Brazil. The dry and powdered plant material was subjected to extraction with supercritical carbon dioxide in three pressure conditions (90, 120, and 150 bar) at 40 °C. Yield curves were generated for each extraction condition. Aiming to promote the improvement of the knowledge about the extraction process, the mathematical simulation of the experimental data was performed. This procedure is a fundamental step in the project of an efficient industrial process avoiding experimental costs related to the use of solvent, energy and raw material. The chosen model considers the solvent density and CO_2 flow rate are constant throughout the bed. The extract is assumed to be one component due to the lack of experimental phase equilibrium data. The chosen model considers the solvent flow constant and is established from the material balance for the solid material and for the fluid phase generating two differential equations coupled by an equilibrium relation between the phases. The method for quantification of coumarins was validated and antifungal tests were carried out aiming to control chromoblastomycosis, a fungal infection of the skin and the subcutaneous tissue caused by traumatic inoculation of a specific group of dematiaceous fungi. The disease has been described worldwide but the prevalence is higher in countries with a tropical or subtropical climate. The global yields obtained were 1.2%, 2.3% and 1.6% in gram of extract per gram of plant, for pressure condition: 90, 120, and 150 bar, respectively. Good results were observed with respect to the simulated extraction curves.

Keywords: *Pterocaulon balansae*, SFE, Mathematical modeling, antifungal activity

INTRODUCTION

Plants from the genus *Pterocaulon* (Asteraceae) are used in Brazilian traditional medicine to treat several skin diseases caused by bacteria or fungi. In the last decade, pharmacological studies carried out with the extracts of these plants have supported their ethnomedical use and the biological properties are attributed to coumarins, the most abundant compounds found in these species [1]. Furthermore, plants from the genus *Pterocaulon* (Asteraceae) are used in south Brazil to treat mycoses in both domestic animals and human skin[2].

Coumarins are a large class of compounds with a common scaffold formed by the fusion of benzene and α-pyrone rings. They display a wide range of biological properties [3], including anticoagulant [4], antioxidant [5,6], anticancer [7] and antimicrobial activities [8]. Among the species previously studied for the coumarins it can be highlighted *P. balansae*. This species shows a distinct chemical profile, mainly characterized by 6,7-dioxygenated, 5,6,7-trioxygenated and 6,7-methylenedioxy coumarins [3] for which we have a particular interest due to their activities against cancer cells and pathogenic fungi [1].

Generally, the authors reported in the literature the use of processes of CO_2 supercritical extraction to obtain natural products as an alternative to processes using organic solvents [9,10]. Characterizing this technology as a green extraction technique, it uses a non-toxic solvent and generates a smaller amount of waste when compared to techniques that employ organic solvents. Moreover, supercritical fluid extraction using carbon dioxide is a useful tool to increase yields and the selectivity profile in the extraction of organic compounds from plants [11,12].

The design of engineering processes requires the knowledge of quantities relative to the species involved. In this sense, the adjustment of mathematical models associated with extraction processes has been intensively addressed in the engineering literature. Specifically, for supercritical extraction there are several models available. Mathematical modeling for SFE can be divided into three main categories: empirical models, models based on the heat transfer analogy and models based on differential mass balance equations [9]. Mathematical models of the extraction process are important for generating predictions about process efficiency to simulate the process on different scales and to evaluate the influence of the operating parameters on the technical and economic feasibility of an industrial process [13]. The SFE process has also been successfully modelled by integrating the differential mass balances written on a section of a fixed bed composed of vegetable particles [14]. A dynamic model proposed by Reverchon [15] was used for the mathematical representation of the extraction process. This model consists of a one-dimensional mass balance for the extract, considering the hypothesis of a linear behavior for the solid-fluid phase equilibrium.

Thus, the aim of this study was to extract by supercritical CO2 and identify the coumarins from *P. balansae*, to develop mathematical models and to determine the antifungal activity against chromoblastomycosis agents

MATERIALS AND METHODS

Supercritical fluid extraction

Aerial parts of *Pterocaulon balansae* were collect in the Rio Grande do Sul state, Brazil, dried at room temperature and powdered in a cutting mill. The supercritical fluid extractions (SFE) were carried out on a pilot-scale equipment located in the LOPE. The pilot unit consists of two cylinders of carbon dioxide (99.9% purity - AirProducts), a high-pressure pump responsible for raising the pressure above the critical condition, a condenser located before the pump to ensure that CO_2 is in the liquid state, avoiding cavitation. In addition, a heat exchanger raises the temperature above the critical condition, an extraction vessel where the vegetable material is placed; a micrometric valve used for solute/solvent separation through system expansion, an extract collection system and a mass flowmeter. The supercritical fluid extractions were performed in an extraction vessel (Waters) with 500 mL capacity, 6.3 cm diameter and 19 cm height.

The investigated conditions were determined according to previous works [3], in order to obtain extracts rich in coumarins using the SFE methodology; four different pressures conditions (90, 120, and 150 bar) at 40 °C, with a 1000 $g.h^{-1}$ CO_2 flow rate. Aiming to promote the improvement of the knowledge about the extraction process, the mathematical simulation of the experimental data was performed once the experimental data was retrieved. The experimental mass *versus* time curve was built in triplicate for further mathematical modeling. Samples were collected with time interval of 10 min in order to evaluate the extract mass until the plant exhaustion (extract mass constant after three consecutive measurements).

Mathematical modeling

In order to fit the experimental data and obtain important mass transfer parameters, the mathematical model used in this work was based in the model developed by Reverchon [15]. It consists of one-dimensional mass balance for the extract, assuming the hypothesis of a linear behavior for the solid-fluid phase equilibrium among other assumptions. The mass balance model was created considering axial dispersion negligible; density and solvent flow rate are constants throughout the bed and assumed the extract as a single pseudo-component. The mass balance is given below (equation 1 and 2).
Fluid phase mass balance:

$$\frac{\partial C(z,t)}{\partial t} = -\upsilon \frac{\partial C(z,t)}{\partial z} - \frac{1-\varepsilon}{\varepsilon} \rho_s \frac{\partial q(z,t)}{\partial t} \tag{1}$$

Mass balance in the solid phase:

$$\frac{\partial q(z,t)}{\partial t} = -k_{TM}[q(z,t) - K \cdot C(z,t)] \tag{2}$$

The concentration of the extract in the vapor phase is given by the function $C(z,t)$ and the concentration in the plant is described by the $q(z,t)$ function. Where υ is the interstitial vapor velocity; ε is the porosity of the bed; k_{TM} is the internal mass transfer coefficient; ρ_s is the specific mass of the plant and K is the equilibrium constant between the phases. The model also considers some initial and boundary conditions: $q(z,0) = q_0$ and $C(z,0) = 0$, q_0 is defined by the total amount of extract contained in the solid phase and the $C(z,0) = 0$ as a boundary condition. The linear comportment for solid-fluid phase equilibrium is expressed by $q^*(z,t) = K \cdot C(z,t)$.

HPLC analyses

The samples were analyzed by high performance liquid chromatography (HPLC), in an apparatus equipped with a UV detector. Phenomenex-C18 Synergi column (150 mm × 4.6 mm, 4 μm) coupled to a refillable pre-column filled with C18 silica was used. The mobile phase was composed of a gradient of acetic acid 2% (A) and acetonitrile (B). The gradient elution was 17% B in 0.01 min, 17%–20% B in 10 min, 20% B in 15 min, 20%–25% B in 20 min, 25%–27% B in 22 min, 27%–30% B in 25 min, 30%–35% B in 30 min, 35% B in 35 min, 35%–17% B in 40 min. The system operated at flow rate of 1 mL/min, the time for 45 min at 30°C, injection volume of 20 μL and the wavelength adjusted to 327 nm.

Antifungal susceptibility testing

The extracts were evaluated by the broth macrodilution technique against some chromoblastomycosis agents, as preconized by the Clinical and Laboratory Standards Institute for filamentous fungi.

RESULTS AND DISCUSSION

The supercritical extraction was carried out using the milled aerial parts of the plant. In the experiments 0.1 kg of material was used. The experiments were finalized when the mass of extract did not change after three successive measurements for each experimental condition. The experimental data of mass extracted by supercritical fluid extraction is showed in Table 1.

Table 1. Experimental mass data at each extraction condition.

	Yield ($g_{extract}/g_{plant}$)		
Time (min)	**90 bar**	**120 bar**	**150 bar**
0	0	0	0
10	0.0016	0.0053	0.0061
20	0.0035	0.0099	0.0092
30	0.0056	0.0132	0.0119
40	0.0070	0.0161	0.0133
50	0.0084	0.0178	0.0142
60	0.0099	0.0187	0.0150
70	0.0109	0.0194	0.0152
80	0.0113	0.0201	0.0155
90	0.0117	0.0208	0.0157
100	0.0119	0.0214	0.0161
110	0.0122	0.0220	0.0161
120	0.0123	0.0226	0.0162
130	0.0124	0.0232	0.0162
140	0.0124	0.0233	-
150	0.0125	0.0235	-
160	0.0125	0.0235	-

According to the proposed methodology, the mathematical modeling was performed and the Figure 1 presents the mass of extracts obtained from the extractions of *P. balansae* and the curves associated to each pressure. The required parameters to perform the fitting of the experimental data are shown in Table 2. The estimated parameters values together with the determination coefficient (R^2) for each method are presented in Table 3.

Table 2. Required input data for the mathematical model.

Model input data		
ρ_s =	1240	kg/m³
ρ_f (90 bar) =	485.6	kg/m³
ρ_f (120 bar) =	689.4	kg/m³
ρ_f (150 bar) =	780.3	kg/m³
d =	0.063	m
h =	0.19	m
Q =	1000	g/h

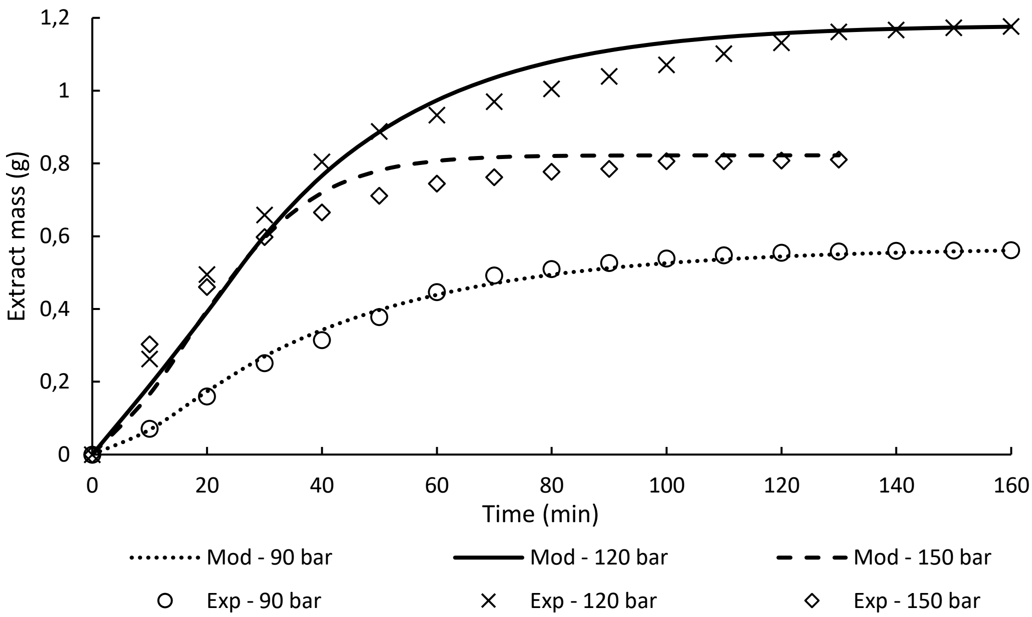


Figure 1. *Pterocaulon balansae* supercritical fluid extraction curves (mass extract *vs.* time) at three different pressure conditions.

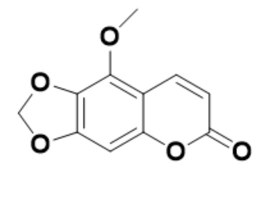
The coefficient of determination represents the adhesion of the model to the experimental data. In relation to this statistical parameter, the simulations are good and representative of the experimental procedures, which means that the mathematical model proposed to represent the extraction process fitted well to the experimental data.

The different orders of magnitude found in the parameters related to mass transfer and chemical equilibrium are in line with the dominant regime of the process at each pressure. The first extraction step is dominated by phase equilibrium and is a faster step characterized by higher values of *K*. Internal mass transfer is a slower step but may also dominate the extraction process, characterized by a value higher k_{TM} and usually associated with heavier compounds (higher-pressure conditions).

Table 3. Parameters obtained through the modeling of supercritical fluid extraction (SFE) data.

Pressure	**Global yield** ($g_{extract}/100g_{plant}$)	**Parameters**		
		k_{TM} *(s^{-1})*	*K (m^3/kg)*	R^2
90	1.249	4.79E-04	5.16E-05	0.9726
120	2.354	8.74E-04	9.26E-03	0.9868
150	1.622	3.24E-03	6.60E-03	0.9956

The extracts obtained from the aerial parts of *P. balansae* were analyzed by High Performance Liquid Chromatography showing the presence of four main compounds, presented in Figure 2, which were identified as 5-methoxy-6,7-methylenedioxycoumarin (**1**), 7-(2′,3′-epoxy-3′-methyl-3′-butyloxy)-6-methoxycoumarin (**2**), 5-(2′,3′-epoxy-3′-methylbutyloxy)-6,7-methylenedioxycoumarin (**3**), and 5,6-dimethoxy-7-(2′,3′-epoxy-3′-methylbutyloxy)coumarin (**4**), being the last compound the most abundant in all the fractions obtained. The compounds were identified by comparison of HPLC retention time with a standard sample previously isolated from the same plant.



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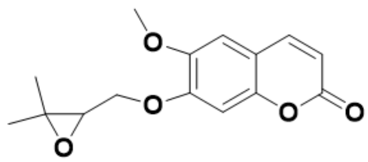

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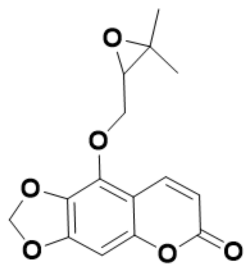

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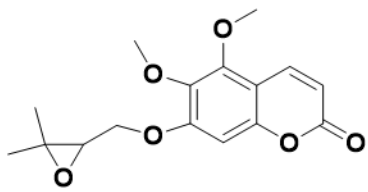

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Figure 2. Structures of coumarins from *P. balansae*.

The antifungal activity of the extracts was evaluated in vitro against chromoblastomycosis agents. The results were inconclusive since high standard deviations were obtained. Thus, the experiments will be further repeated.

CONCLUSION

The method demonstrated to be efficient in extracting the main coumarins from *P. balansae.* Determination of the values for the adjusted parameters agreed with data in the available literature in terms of order of magnitude. Good results were observed with respect to the simulated extraction curves obtained from the mathematical model when compared with the experimental data. Finally, the evaluation of model parameters in this work could be useful during the scale-up of the extraction process and/or during pilot or industrial operation to evaluate the extraction time required to obtain a given yield at a different temperature.

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