



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



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ABSTRACT

In this study, the extraction of sucupira fruits (*Pterodon* 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 CO₂ (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 polygalaeiflorus* 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 methanolic extract of the fruits of *P. polygalaeiflorus* 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 cytotoxicity 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 sesquiter-

penes, whose major components are β -caryophyllene, 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 (scCO₂) 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 scCO₂. 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 objective of this work was the study of the extraction of bioactive compounds of sucupira fruits using scCO₂ as solvent. The performance of the extraction with scCO₂ 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 CO₂ (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 scCO₂. 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 scCO₂ 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 CO₂ 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 CO₂ 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):

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

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 (CO₂, 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, CO₂ 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):

$$n_{CO_2} = \frac{\rho_{CO_2} \varepsilon V_{bed} M_{CO_2}}{\rho_{CO_2} \varepsilon V_{bed} + m_0} \quad (2)$$

$$n_{ext} = \frac{(1 - \rho_{CO_2} \varepsilon V_{bed}) M_{ext}}{\rho_{CO_2} \varepsilon V_{bed} + m_0} \quad (3)$$

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

2.6. Mathematical modelling

2.6.1. Phase equilibrium

The measurements of phase transitions were conducted by using the sucupira extract obtained at 333 K, 22 MPa, the CO₂ mass flow rate of 3 g min⁻¹ and extraction time of 80 min. The sucupira extract was considered as a pseudo-component. The extract composition was calculated by converting the percent in area of the components with values higher than 1% of the GC–MS analysis into mass percent. The thermophysical properties of the extracts were calculated by Kay rule [30]. The critical temperature and pressure of the pure components were calculated by Marrero-Gani method [31] and the acentric factor by the method of Constantinou et al. [32].

The thermodynamic modelling of the experimental data of vapor-liquid equilibrium was performed by using Peng-Robinson equation of state [33] combined with the Wong-Sandler mixing rule [34]. The parameters a and b are given by the Eqs. (5) and (6):

$$a = RT \frac{QD}{1-D} \quad (5)$$

$$b = \frac{Q}{1-D} \quad (6)$$

where R is the universal constant of the gases and:

$$Q = \sum_i \sum_j x_i x_j \left(b_{ij} - \frac{a_{ij}}{RT} \right) \quad (7)$$

$$D = \sum_i x_i \frac{a_i}{b_i RT} + \frac{G^E}{CRT} \quad (8)$$

where x is the molar fraction and:

$$C = -\frac{1}{\sqrt{2}} \ln(1 + \sqrt{2}) \quad (9)$$

As presented by Orbey and Sandler [35], the combining rule was used:

$$b_{ij} - \frac{a_{ij}}{RT} = \frac{(b_i + b_j)}{2} - \frac{\sqrt{a_i a_j} (1 - K_{ij})}{RT} \quad (10)$$

The calculation of Molar excess Gibbs energy, G^E , was made using the NRTL model [36]. In this work α_{ji} was considered equal to 0.2. The parameters of binary interaction, Δg_{12} , Δg_{21} and K_{12} were optimized by the minimization of the objective function given by Eq. (11):

$$OF = \sum_{i=1}^N (P_i^{exp} - P_i^{calc})^2 \quad (11)$$

where N is the number of experimental data and P_i^{exp} and P_i^{calc} are the experimental pressures and calculated, respectively. The stochastic simulated annealing method was used refining with the simplex method applied to the calculations of bubble point [37].

2.6.2. Kinetic extraction

The modelling of the experimental data of sucupira extraction was performed by applying Sovová model [38]. This model takes three periods of extraction into consideration, constant extraction rate (CER), falling extraction rate (FER) and low extraction rate (LER) which are controlled by mechanisms of diffusion and convective mass transfer. The analytical solution is given by Eqs. (12)–(14):

For $t < t_{CER}$:

$$m(t) = \dot{m}_{CO_2} S_b t [1 - \exp(-Z)] \quad (12)$$

For $t_{CER} \leq t < t_{FER}$:

$$m(t) = \dot{m}_{CO_2} S_b \left\{ t - t_{CER} \exp \left[\frac{Z S_b}{W q_0} \ln \left[\frac{1}{1-r} \left(\exp \left(\frac{W \dot{m}_{CO_2}}{m_s} (t - t_{CER}) - r \right) \right) \right] \right] - Z \right\} \quad (13)$$

For $t \geq t_{FER}$:

$$m(t) = m_s \left\{ q_0 - \frac{S_b}{W} \ln \left[1 + \left(\exp \left(\frac{W q_0}{S_b} \right) - 1 \right) \exp \left(\frac{W \dot{m}_{CO_2}}{m_s} (t_{FER} - t) \right) \right] \right\} \quad (14)$$

where

$$Z = \frac{K_F a m_s \rho_{CO_2}}{\dot{m}_{CO_2} \rho_S} \quad (15)$$

$$W = \frac{m_s K_S a}{\dot{m}_{CO_2} (1 - \epsilon)} \quad (16)$$

where $m(t)$ is the extracted mass as function of time t , \dot{m}_{CO_2} is the CO₂ mass flow rate, S_b is the extract solubility in the solvent, Z and W are dimensionless model parameters, q_0 is the initial extract concentration in the solid matrix, m_s is the solid mass on an extract-free basis, ρ_{CO_2} and ρ_S are the fluid and solid densities respectively, $K_F a$ and $K_S a$ are the mass transfer coefficients of fluid and solid phases, respectively, ϵ the extraction bed porosity and r is the easily accessible oil fraction, an adjustable parameter of the model using the objective function given by Eq. (17):

$$OF = \sum_{i=1}^{n_{exp}} \sum_{j=1}^N (m_{i,j}^{calc} - m_{i,j}^{exp})^2 \quad (17)$$

The ends of the first and second period, t_{CER} and t_{FER} , respectively, are given by the Eqs. (18) and (19):

$$t_{CER} = \frac{(1-r)m_s q_0}{S_b Z \dot{m}_{CO_2}} \quad (18)$$

$$t_{FER} = t_{CER} + \frac{m_s}{W \dot{m}_{CO_2}} \ln \left[r + (1-r) \exp \left(\frac{W q_0}{S_b} \right) \right] \quad (19)$$

The parameters Z and W were calculated by minimization of the objective function given by Eq. (20) using the Downhill Simplex method:

$$OF = \sum_{j=1}^N (m_j^{calc} - m_j^{exp})^2 \quad (20)$$

where N is the number of experimental data and m_j^{calc} and m_j^{exp} are the mass calculated by Sovová method and experimental, respectively.

3. Results and discussion

3.1. Phase equilibrium

Table 1 shows the chemical components identified in the sucupira extract obtained by scCO₂ at 22 MPa, 333 K, the CO₂ mass flow rate of 3 g min⁻¹ and extraction time of 80 min used in the phase equilibrium experiments. Only the components identified by GC–MS with a percentage above 1% in area were considered. The mass and molar standard fractions, critical properties, acentric factor of the components identified and the pseudo-component are represented in Table 1.

Table 2 shows the experimental data of phase transition system CO₂ (1) + sucupira extract (2). The mass fractions of CO₂ measured ranged from 0.309 to 0.960 at temperatures 313, 323 and 333 K. Two kinds of transition of vapor-liquid phases were observed. For mass fractions until 0.700 bubble points were observed and for mass fractions over 0.900 dew points were observed. The values of the pressures are

Table 1

Chemical composition of the components, critical properties and acentric factor of the sucupira extract used in the measurements of phase transition.

Compound	Molar fraction	Molar mass	T _c ^a (K)	P _c ^a (MPa)	ω ^b
α-copaene	0.027	204.35	722.19	2.04	0.29
β-cubebene	0.018	204.35	724.69	2.04	0.22
β-elemene	0.066	204.35	707.46	1.85	0.34
β-caryophyllane	0.281	204.35	753.19	2.05	0.48
α-humulene	0.034	204.35	747.52	2.04	0.58
Alloaromadendrene	0.013	204.35	744.39	2.03	0.33
γ-murolene	0.204	204.35	748.13	2.06	0.38
bicyclogermacrene	0.102	204.35	681.22	2.05	0.51
Sphatulenol	0.036	220.35	760.00	2.20	0.76
6α-acetoxycouacapan	0.053	344.49	826.35	1.54	0.41
6α,7β-dimethoxycouacapan-17-ene	0.116	344.49	838.29	1.56	0.35
6α-acetoxy-7β-hidroxiyouacapan	0.019	360.49	858.61	1.56	0.79
6α,7β-diacetoxycouacapan	0.030	402.52	857.80	1.39	0.34
Sucupira extract		237.51	759.23	1.93	0.44

^a Calculated by Marrero-Gani method [31].

^b Calculated by Constantinou et al. method [32].

average values of triplicate, and ranged from 8.26 to 28.72 MPa.

Table 3 shows the adjusted values of the parameters of binary interaction of Peng-Robinson equation with Wong-Sandler mixing rule (PR-WS) using Eq. (11). Only BP experimental data were considered for the parameter estimation and the dew points were used to check the thermodynamic model performance. The value of the root mean square deviation calculated represents a satisfactory adjust of the experimental data by the proposed model.

The composition-diagram of the system CO₂ (1) + sucupira extract (2) is represented in Fig. 1. The symbols which are not filled represent the data of phase transition. The rise in temperature results in a higher pressure of phase transition classifying the system as type I according to the classification of Scott and Konynenburg [39].

3.2. Yield and kinetic extraction

The temperatures of 313, 323 and 333 K are recommended by the literature [27] for the extraction of bioactive compounds. The range of pressure of extraction (10–22 MPa) was defined to evaluate the performance of mass percent yield and the kinetic extraction in the regions of different phases. According to Fig. 1, at pressure 10 MPa the system is found on the biphasic region, at 22 MPa on monophasic region and at 16 MPa next to vapor-liquid transition region. The symbols filled in Fig. 1 represent the extraction conditions.

Table 4 shows the mass percent yield and antioxidant activities of the supercritical extractions and by hydrodistillation of the sucupira fruits. The values of the scCO₂ densities as a function of temperature and pressure were obtained from Angus et al. [40]. The yields obtained by supercritical extraction, particularly, for the Exp. 2 (21.2%) and 4

Table 2

Experimental values for the phase equilibrium of the system CO₂ (1) + sucupira extract (2).

w ₁	x ₁ , y ₁	Equilibrium type	P (MPa) and T (K)		
			313 K	323 K	333 K
0.309	0.707	BP	8.26 ± 0.04	9.70 ± 0.01	11.26 ± 0.04
0.400	0.783	BP	13.42 ± 0.03	15.06 ± 0.12	17.38 ± 0.05
0.500	0.844	BP	18.92 ± 0.04	21.71 ± 0.08	23.1 ± 0.07
0.600	0.890	BP	23.19 ± 0.19	24.78 ± 0.06	26.59 ± 0.02
0.700	0.926	BP	26.33 ± 0.05	27.51 ± 0.10	28.72 ± 0.06
0.900	0.980	DP	23.98 ± 0.15	25.97 ± 0.18	28.52 ± 0.06
0.950	0.990	DP	14.33 ± 0.01	17.05 ± 0.09	19.50 ± 0.07
0.960	0.992	DP	12.97 ± 0.04	16.06 ± 0.06	18.48 ± 0.09

Table 3

Parameters of interaction adjusted of PR-WS model of phase equilibrium CO₂ (1) + sucupira extract (2).

T (K)	Δg ₁₂ (K)	Δg ₂₁ (K)	K ₁₂	rmsd (MPa) ^a
313–333	1899.75	−663.19	0.2593	0.72

$$^a \text{root mean square deviation: } rmsd = \sqrt{\frac{\sum_{i=1}^N (p_i^{exp} - p_i^{calc})^2}{N}}$$

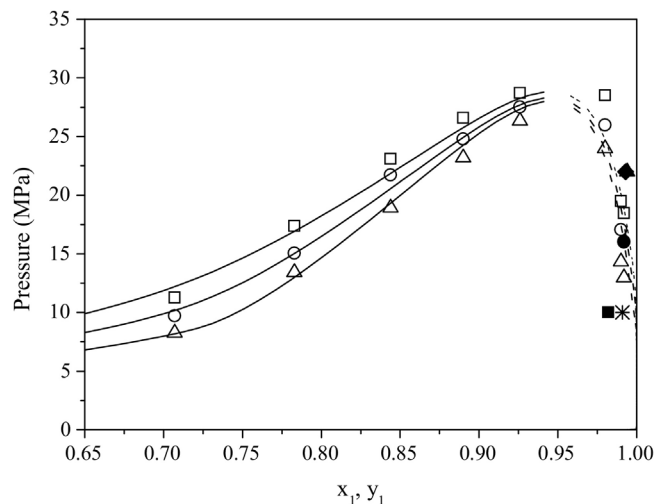


Fig. 1. Pressure-composition diagram for CO₂ (1) + sucupira extract (2) at 313 K (Δ), 323 K (○), 333 K (□). Solid and dashed lines denote BP and DP, respectively, fitted by using by PR-WS model. Extraction conditions: Exp 1 (*); Exp 2 (▲); Exp 3 (■); Exp 4 (◆); Exp 5 (○).

Table 4

Mass percent yield and antioxidant activity of the supercritical extractions and by hydrodistillation of sucupira fruits.

Exp.	P (MPa)	T (K)	ρ _{CO2} (g cm ⁻³)	Y(%)	AA (%)
1 ^{br}	10	313	0.630	11.0	74.03
2 ^{mtr}	22	313	0.858	21.2	77.13
3 ^{br}	10	333	0.290	1.0	75.71
4 ^{mtr}	22	333	0.754	20.4	77.39
5 ^{tr}	16	323	0.721	17.6 ± 0.7	77.59 ± 0.12
HDE	0.1	373		2.6 ± 0.8	50.26 ± 0.44

^{br}biphasic region; ^{mtr}monophasic region; ^{tr}VL transition region; Y(%): mass percent yield; AA(%):antioxidant activity.

(20.4%) were higher than the hydrodistillation (2.6%). These results demonstrate the adaptation of the supercritical technology on the extraction of biocompounds in relation to the conventional technologies.

The values of mass percent yield were higher in the extraction conditions of higher density of carbon dioxide and in the monophasic

Table 5
Adjusted parameters of Sovová model of the supercritical extractions of sucupira fruits.

Exp.	Z	W	r	t_{CER} (min)	t_{FER} (min)	K_{Fa} (min^{-1})	K_{Sa} (min^{-1})
1 ^{br}	2.21 ± 0.17	0.03 ± 0.002	0.59 ± 0.01	17.7	63.4	0.097	1.1 × 10 ⁻³
2 ^{mr}	2.80 ± 0.71	0.73 ± 0.204	0.59 ± 0.01	4.3	21.2	0.092	2.6 × 10 ⁻²
3 ^{br}	0.68 ± 0.04	0.19 ± 0.020	0.59 ± 0.01	497.7	1520.0	0.064	6.9 × 10 ⁻³
4 ^{mr}	2.80 ± 0.46	0.29 ± 0.029	0.59 ± 0.01	5.8	32.4	0.107	1.1 × 10 ⁻²
5 ^{tr}	1.04 ± 0.03	0.13 ± 0.003	0.59 ± 0.01	13.9	32.2	0.041	4.6 × 10 ⁻³

^{br}biphasic region; ^{mr}monophasic region; ^{tr}VL transition region.

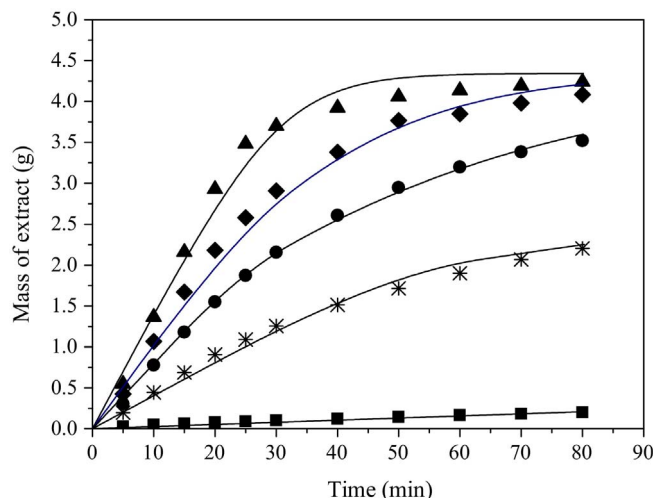


Fig. 2. Kinetic curves of experimental and fitted extractions of sucupira: (—) Sovová model; (*) Exp 1; (▲) Exp 2; (■) Exp 3; (◆) Exp 4; (●) Exp 5).

region. The solvation of scCO₂ is favored by the increase of the density [41] and decrease of the resistance of the mass transfer due to the presence of a single phase [19]. The values of the mass percent yield of the extractions performed in the biphasic are compatible to Santos et al.

Table 6
Chemical composition of extracts obtained by supercritical extractions and by hydrodistillation.

Compound	RI	Experiments (mass fraction)					
		1 ^{br}	2 ^{mr}	3 ^{br}	4 ^{mr}	5 ^{tr}	HD
γ-elemene	1335	0.51	0.30	0.60	0.45	0.32	
α-copaene	1373	2.77	2.07	3.70	2.16	2.14	5.77
β-cubebene	1388	1.75	1.33	2.25	1.39	1.34	4.32
β-elemene	1389	5.18	3.45	8.48	5.27	4.50	5.73
β-caryophyllane	1417	35.71	25.18	35.49	22.34	26.93	40.24
α-humulene	1451	3.82	2.73	4.36	2.72	2.83	5.45
alloaromadendrene	1458	1.41	1.02	1.64	1.03	1.08	1.92
γ-murolene	1479	22.24	15.73	24.17	16.24	16.96	15.61
bicyclgermacrene	1494	10.70	7.85	11.13	8.13	8.33	9.31
δ-cadinene	1522	0.37	0.26	0.38	0.32	0.29	0.61
sphatulenol	1573	3.62	2.85	3.03	3.10	3.15	8.85
1-hexadecanol	1874	0.34	0.36		0.40	0.36	
vouacapane	2196	0.30	0.61	0.14	0.68	0.52	
6α-hydroxyvouacapane-7,17β-lactone	2308		0.52		0.49	0.43	
18α-hydroxyvouacapane-7,17β-lactone	2432		0.48		0.39	0.35	
6α-acetoxylvouacapane	2457	1.99	7.45	0.72	7.05	5.35	
6α,7β-dimethoxyvouacapane-17-ene	2512	5.59	19.78	1.56	15.54	15.86	
6α-acetoxyl-7β-hydroxyvouacapane	2516	0.22	1.42		2.60	1.43	
6α,7β-diacetoxylvouacapane	2545	1.02	5.39	0.19	4.77	4.32	
sesquiterpene hydrocarbons		84.47	59.92	92.19	60.05	64.73	88.96
Oxygenated sesquiterpenes		3.62	2.85	3.03	3.10	3.15	8.85
vouacapane diterpenes		9.12	35.66	2.62	31.52	28.27	0
Other		0.34	0.36	0.00	0.40	0.36	0
Total identified		97.54	98.79	97.84	95.06	96.50	97.81

^{br}biphasic region; ^{mr}monophasic region; ^{tr}VL transition region.

work [17].

The kinetics curves of supercritical extractions of sucupira were modeled by Sovová. To calculate the parameters of Sovová model, showed in Table 5, the following parameters were used: $\dot{m}_{CO_2} = 3 \text{ g min}^{-1}$, $q_0 = 0.27 \text{ g}_{\text{ext}} \text{ g}_{\text{sólid}}^{-1}$ was calculated as the maximum extract mass obtained in relation to the solid matrix mass, $m_s = 15.76 \text{ g}$ and $\epsilon = 0.813$. The solubility was determined by the dynamic method from the adjustment of the equation in the linear part of the extraction curve (mass of oil extracted as function of the solvent mass). The solid density was calculated based on the added solids mass, extractor volume and porosity.

The mass transfer coefficients of solid-liquid phase, K_{Fa} and K_{Sa} , were calculated based on the adjustable non-dimensional parameters Z and W, respectively. The values of mass transfer coefficients, K_{Fa} and K_{Sa} , indicate predominance of the convective stage compared to the diffusive stage.

In the monophasic region the values of K_{Sa} and the extraction periods (t_{CER} , and t_{FER}) were higher and lower, respectively, in relation to the values of biphasic region. In the monophasic region the resistance of mass transfer is lower than the biphasic region.

Fig. 2 shows the experimental and calculated data of kinetic extraction curves are found. The kinetic extraction curves were represented satisfactorily by Sovová model.

3.3. Gas chromatography

Table 6 shows the chemical composition of sucupira extracts obtained by supercritical extraction and by hydrodistillation. A total of 19 components were identified, divided in three classes of terpenes: sesquiterpene hydrocarbons, oxygen sesquiterpenes and vouacapan diterpenes.

The sesquiterpene hydrocarbons were predominant on the chemical composition of the extracts regardless the extraction technique used. The vouacapan diterpenes, related to the biological activities [15,16] of the extracts, are present only on the extracts obtained by scCO₂. The values of concentration of diterpenes obtained from the extractions conducted on the biphasic region are compatible to Santos et al. work [17]. The major components (β -caryophyllene and the γ -muurolene) of the essential oil obtained from hydrodistillation were compatible to Alves et al. work [13].

3.4. Antioxidant activity

Table 4 shows the antioxidant activity values of the extracts obtained by scCO₂ and by hydrodistillation of sucupira fruits. The extracts which have higher concentrations of vouacapan diterpenes show higher values of antioxidant activities. The hydroxyl groups present in the vouacapan diterpenes favor the capacity of free-radical inhibition [42]. In the monophasic region and next to the phase transition the extracts obtained show higher concentrations of vouacapan diterpenes favoring the antioxidant activity.

4. Conclusion

The extraction of chemical components from the sucupira fruit of highest biological activities using scCO₂ were higher than hydrodistillation, shown higher effectiveness on the extraction of bioactive compounds. The knowledge of the phase diagram enabled a better definition of the supercritical extraction conditions resulting in higher mass percent yield compared to the available data in the literature. The supercritical extractions conducted in the monophasic region resulted in higher vouacapan diterpenes concentration, and, consequently more antioxidant activities. The values of mass transfer coefficients calculated confirm the information found in the phase diagram generated. The experimental data of phase transition were only bubble and dew point types. The equation of state of Peng-Robinson with the Wong-Sandler quadratic mixing rule and the Sovová model represented satisfactorily the experimental data.

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