

Article

Kinetics of Supercritical CO₂ Extraction from Burrito (*Aloysia polystachya*) Leaves and Sucupira-Preta (*Bowdichia virgilioides*) Seeds

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Abstract: This study investigated the application of supercritical carbon dioxide (CO₂) for the extraction of essential oils from plant materials with anxiolytic potential, focusing on the leaves of burrito (*Aloysia polystachya*) and the seeds of sucupira-preta (*Bowdichia virgilioides*). The supercritical extraction technique was chosen for its ability to produce high-purity extracts without residual solvents and to reduce the environmental impact. This study evaluated the influence of temperature (40 °C, 50 °C, and 60 °C) and pressure (22 MPa, 25 MPa, and 28 MPa) on extraction efficiency using a 2² factorial design with triplicates at the central point. The maximum yields were 1.2% for burrito leaves and 4.2% for sucupira-preta seeds. Despite their relatively low yields, the extracts contained a diverse range of chemical compounds, including fatty acids (oleic, linoleic, and palmitic acids), squalene, β-carotene, vitamin E, and other bioactive molecules with antioxidant, anti-inflammatory, and immunomodulatory properties. Statistical analysis demonstrated that pressure was the most influential factor affecting yield, whereas temperature played a secondary role. The Sovová kinetic model provided a good fit for the extraction curves, with determination coefficients (R²) above 0.95, thus validating the efficiency of the method. These results highlight the pharmaceutical potential of these extracts, particularly for therapeutic and anxiolytic purposes.

Keywords: burrito; sucupira-preta; supercritical extraction; CO₂; Sovová; squalene

1. Introduction

Anxiety disorders are among the most prevalent mental health conditions worldwide and are characterized by excessive worry, fear, and physiological responses that can significantly impair daily functioning. While pharmacological treatments, such as benzodiazepines and antidepressants, are effective in managing anxiety symptoms, their use is often associated with adverse effects, including sedation, dependence, amnesia, sexual dysfunction, and gastrointestinal disturbances [1].

These limitations highlight the urgent need for alternative therapeutic approaches that are both effective and safe. Medicinal plants have emerged as promising sources of bioactive compounds with anxiolytic potential, offering a natural and potentially less harmful alternative to conventional drugs [2,3]. Therefore, there is a need to develop new anxiolytic drugs that do not produce these side effects. In this context, medicinal plants continue to demonstrate potential as anxiolytic agents [4].

The therapeutic potential of plants in the treatment of central nervous system disorders, including anxiety, has been well documented in traditional medicine. Many plant species synthesize secondary metabolites, such as terpenes, flavonoids, and alkaloids, which exhibit diverse pharmacological activities, including antioxidant, anti-inflammatory, and psychotropic effects [5]. These compounds are often produced as part of the plant defense mechanisms against environmental stressors, and their chemical diversity makes them valuable candidates for drug development. Essential oils and other bioactive constituents have shown particular promise in modulating the central nervous system, with several studies highlighting their anxiolytic properties [5–9].

Two plant species of particular interest are *Bowdichia virgilioides* (commonly known as sucupira-preta) and *Aloysia polystachya* (burrito). Both plants are widely used in traditional medicine and have been recognized for their pharmacological properties, including anti-inflammatory, antioxidant, and sedative effects [10].

Bowdichia virgilioides, a species native to South America, is traditionally used to treat rheumatism, arthritis, and skin diseases. Its seeds are rich in bioactive compounds, including fatty acids (e.g., oleic and linoleic acids), squalene, and flavonoids, which have been associated with anti-inflammatory, antioxidant, and neuroprotective effects [5,11]. In traditional medicine, its seeds are used to treat rheumatism, arthritis, and skin diseases, whereas its bark is used as an antidiabetic agent and for the topical treatment of inflammation [12]. Although there have been few scientific studies on the biological activity of this species, some reports have demonstrated its antimalarial properties [13], antinociceptive effects in peripheral analgesia models [14], and anti-inflammatory activities [14]. The specialized literature indicates the presence of various components of this species, including essential oils [15], alkaloids [16], flavonoids [17], triterpenoids, and resins [18]. Vieira et al. [11] reported that the secondary metabolites of sucupira-preta have significant potential as anxiolytics.

Aloysia polystachya is an aromatic plant native to the Verbenaceae family and is widely distributed in the subtropical regions of South America, particularly in Brazil, Paraguay, and northern Argentina. This species is commonly known as the burrito [19]. Its leaves and flowers are widely used in traditional medicine to treat gastrointestinal disorders, such as pain, nausea, vomiting, dyspepsia, and gastritis [20]. The therapeutic uses of *Aloysia* species include its use as a febrifuge, sedative, stomachic, diuretic, and antispasmodic [21]. Studies have indicated that the chemical composition of essential oils obtained from leaves includes monoterpenes, such as carvacrol, carvone, eucarvone, limonene, pinene, and sabinene [19,21]. Helli6n-Ibarrola et al. [21] and Mora et al. [19] have suggested that *Aloysia polystachya* is a potential anxiolytic agent.

Despite the promising pharmacological potential of these plants, studies focusing on the optimization of extraction methods to maximize the yield and bioactivity of their compounds are lacking. Supercritical fluid extraction (SFE) using carbon dioxide (CO₂) has emerged as a green and efficient technique for obtaining high-purity extracts. This method offers several advantages over conventional extraction techniques, including the absence of residual solvents, reduced environmental impact, and the ability to operate under controlled temperature and pressure conditions to selectively extract desired compounds. Moreover, SFE is particularly suitable for preserving the integrity of thermolabile compounds, which are often present in essential oils and other bioactive plant metabolites [22–25].

This study aimed to investigate the application of supercritical CO₂ extraction for the recovery of bioactive compounds from sucupira-preta seeds and burrito leaves, focusing on their potential anxiolytic properties. By evaluating the influence of temperature and pressure on extraction efficiency, this study sought to optimize the process conditions to enhance the yield and quality of the extracts. Additionally, this study employed the Sovová kinetic model to characterize the extraction process and provide insights into the mass transfer mechanisms involved. These findings are expected to contribute to the growing body of knowledge on the use of supercritical CO₂ extraction for medicinal plants and support the development of phytotherapeutic products for anxiety management.

2. Experimental Section

2.1. Sample Preparation

Plant materials, comprising burrito leaves (*Aloysia polystachya*) and sucupira-preta seeds (*Bowdichia virgilioides*), were obtained from the Pontal do Paranapanema region. These materials were dried in a ventilated oven at 105 °C for 24 h until a stable mass was reached [26,27]. After drying, the materials were ground using a domestic blender and sieved with W.S. Tyler sieves (Mentor, OH, USA). A particle size of 0.85 mm (20 mesh) was selected for sample preparation. The processed samples were vacuum-sealed in polyethylene bags and stored under freezing conditions. Particle density (ρ_t) was measured using a helium gas pycnometer (Ultracyc 1200e, Quantachrome Instruments, Boynton Beach, FL, USA). Carbon dioxide with a purity of 99.9% in the liquid phase was supplied by Air Liquide S.A (Presidente Prudente, SP, Brazil).

2.2. Supercritical CO₂ Extraction

Experimental procedures were carried out using a bench-scale extraction system comprising a CO₂ cylinder, a thermostatically regulated bath, a syringe pump (ISCO 260D, Lincoln, NE, USA), and a jacketed extraction vessel made of 304 stainless steel (Figure 1). Additional details regarding the equipment and experimental methodologies can be found in previous studies [28,29].

The extractor was loaded with 0.01 kg of material, and the remaining void space in the extraction cell was filled with glass beads to form an inert bed. The CO₂ introduced into the system initially passed through the inert bed before reaching the ground plant material. Once the desired extraction temperature was reached, the pump and extractor were pressurized simultaneously. After the operational pressure was established, the system was left to rest for 30 min to ensure equilibrium and confirm that the solvent was fully saturated at the start of the extraction process.

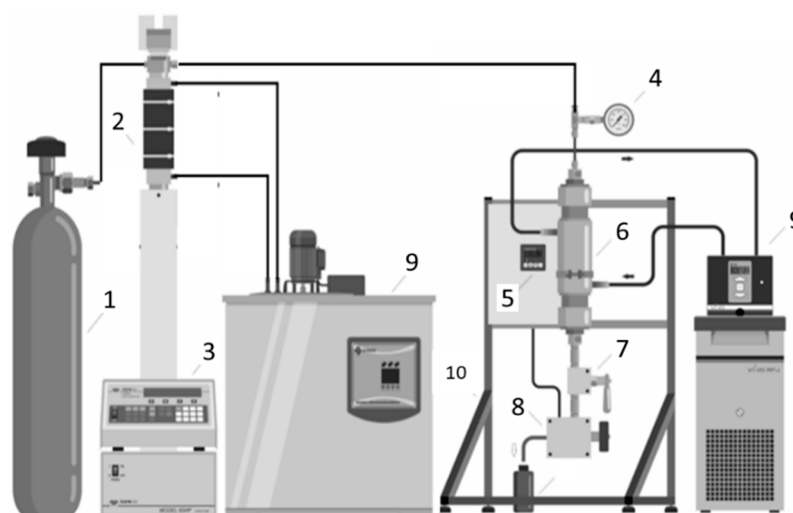


Figure 1. A schematic of the experimental supercritical extraction unit: 1—CO₂ cylinder; 2—syringe pump; 3—thermostatic bath; 4—pressure indicator; 5—temperature controller/indicator; 6—extractor; 7—valve; 8—needle-type valve attached to an aluminum jacket for heating; 9—thermostatic bath; 10—aluminum structure.

Extractions were carried out at pressures of 22, 25, and 28 MPa; temperatures of 40 °C, 50 °C, and 60 °C; and a CO₂ flow rate of 2.0 mL min^{−1}, following a 2² factorial design with triplicates at the central point (Table 1). The solvent flow rate of 2.0 mL min^{−1} was controlled using a micrometric valve (Parker Autoclave Engineers, Erie, PA, USA) and maintained at 100 °C. The total extract was collected in glass vials and weighed over six initial cycles of 5 min, followed by six to nine additional cycles of 10 min.

Table 1. Two-level factorial design.

Factors	Symbols	Units	Levels		
			−1	0	+1
Temperature	T	°C	40	50	60
Pressure	P	MPa	22	25	28

2.3. Oil Characterization

The extract samples were weighed using an analytical balance, diluted in dichloromethane ($n = 3$) to achieve a concentration of 1000 µg mL^{−1}, and injected into the pre-defined chromatographic system.

For one-dimensional Gas Chromatography–Mass Spectrometry (GC–MS) analysis, a Shimadzu gas chromatograph coupled with a mass spectrometer (model GCMS–QP2010 Plus, Kyoto, Japan) was employed. The system was equipped with an AOC–20i auto-sampler/auto-injector (Shimadzu, Kyoto, Japan) and a DB–5MS capillary column (50 m length, 0.25 mm internal diameter, and 0.25 µm stationary phase thickness). The temperature program began at 313.15 K, with a heating rate of 2 K min^{−1} up to 473.15 K, followed by a ramp of 10 K min^{−1} up to 573.15 K, where it was held for 15 min. The injector, interface, and ionization source temperatures were set to 523.15 K, 573.15 K, and 553.15 K, respectively. Helium gas (purity 99.999%) was used as the carrier at a flow rate of 0.8 mL min^{−1}. A 1 µL injection (1000 µg mL^{−1}) was performed in split mode with a split ratio of 1:50. Data acquisition and processing were conducted using the GCMS Postrun Analysis software (Ver.2.53, Shimadzu, Kyoto, Japan), which incorporates the NIST14.lb and NIST14.lbs spectral library databases. Additional details regarding the equipment and experimental methodologies can be found in previous studies [29].

2.4. Statistical Analysis

The dataset underwent an analysis of variance (ANOVA) at a 5% significance level, followed by Tukey test, which was performed using Statistica Software version 8.0 [30]. The main effects and interactions were examined using Design Expert software version 12 [31], which evaluates the primary effects, interactions, and influence of the independent variables on the response outcomes.

2.5. The Kinetics of the Extraction—Sovová Model

The Sovová model [32] explains the two stages of mass transfer and three distinct extraction phases. Phase 1: Extraction of easily accessible oil. This phase is primarily determined by the solubility of oil in the fluid phase and is represented by a linear curve, where the slope closely approximates the solubility of oil in the solvent. Phase 2: A reduction in the extraction rate occurred, followed by oil extraction, which is more difficult to access. This phase was controlled by an internal diffusion mechanism. Phase 3: The extraction curve became nearly linear again, but the extraction rate was considerably lower than that observed in the first phase.

The model establishes a comprehensive framework for analyzing the supercritical CO₂ extraction process and supports the refinement of the extraction parameters for a wide range of plant materials. Additional details regarding the equipment and experimental methodologies can be found in previous studies [29].

3. Results and Discussion

3.1. Extraction Yield

The extraction yields obtained for burrito leaves (*Aloysia polystachya*) and sucupira-preta seeds (*Bowdichia virgilioides*) using supercritical CO₂ are presented in Table 2. The maximum yield was 1.2% for burrito leaves and 4.2% for sucupira-preta seeds, which was achieved at 60 °C and 28 MPa. These results indicate that the sucupira-preta seed matrix exhibited a higher concentration of extractable compounds than the burrito leaves, which was expected because of the higher lipid content of the seeds.

Table 2. Experimental conditions and extraction yield data for the recovery of metabolites from burrito leaves and sucupira-preta seeds using supercritical CO₂ at a constant flow rate of 2.0 mL min^{−1}.

Run	Temperature (K)	Pressure (MPa)	Yield (wt%)
Burrito Leaves			
1	40	22	0.78
2	60	22	0.85
3	40	28	1.11
4	60	28	1.20
5	50	25	0.87
6	50	25	0.91
7	50	25	0.87
Sucupira-preta Seeds			
1	40	22	3.91
2	60	22	3.97
3	40	28	4.11
4	60	28	4.23
5	50	25	3.99
6	50	25	3.96
7	50	25	3.92

Most of the extracts were recovered within the first 20 min of extraction, which was followed by a significant reduction in the extraction rate. This behavior is consistent with the Sovová kinetic model, which describes the transition from a convection-controlled regime to a diffusion-controlled regime. Statistical analysis identified pressure as the most significant factor influencing the extraction yield, whereas temperature had a secondary effect. This can be attributed to the increased density of CO₂ at higher pressures, which enhances its solvating capacity and, consequently, the efficiency of the extraction process.

The extraction kinetic curves for burrito leaves and sucupira-preta seeds are presented in Figures 2 and 3, respectively. These curves illustrate the cumulative yield as a function of extraction time adjusted according to the Sovová kinetic model. The extraction process was characterized by three distinct phases: an initial constant-rate phase, an intermediate falling-rate phase, and a final residual-rate phase. In the constant-rate phase, extraction was predominantly governed by convective mass transfer mechanisms in the fluid phase. As the process progressed, the falling-rate phase began, where the extraction rate decreased owing to the transition to diffusive mechanisms. Finally, in the residual-rate phase, the yield plateaued as the less accessible compounds were gradually extracted.

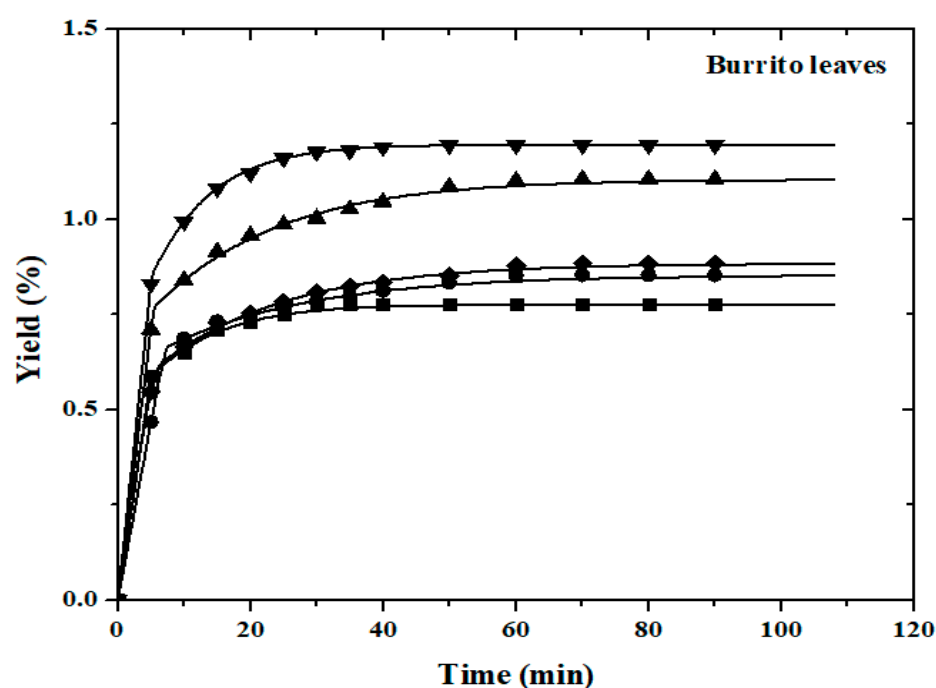


Figure 2. Experimental kinetic extraction curves with supercritical CO₂ fitted using the Sovová model (—) of burrito leaves: 40 °C (■, 22 MPa; ▲, 28 MPa); 50 °C (◆, 25 MPa); 60 °C (●, 22 MPa; ▼, 28 MPa) with a constant flow rate of 2.0 mL min^{−1}.

The Sovová model demonstrated a strong fit to the experimental data, with determination coefficients (R^2) exceeding 0.95 for both matrices. The adjusted parameters, including the mass transfer coefficients and apparent solubility, are summarized in Table 3.

The changes in the slopes of the extraction kinetic curves reflect the transitions between convective and diffusive mass transfer mechanisms. Initially, the convective mechanism dominated, facilitating the rapid removal of easily accessible compounds. However, as extraction progressed, a discontinuity effect was observed on the surface layer, leading to a decline in the extraction rate. At this stage, the process becomes diffusion-controlled, and less accessible oils are extracted. This shift highlights the increasing challenges associated with the solubilization of these compounds.

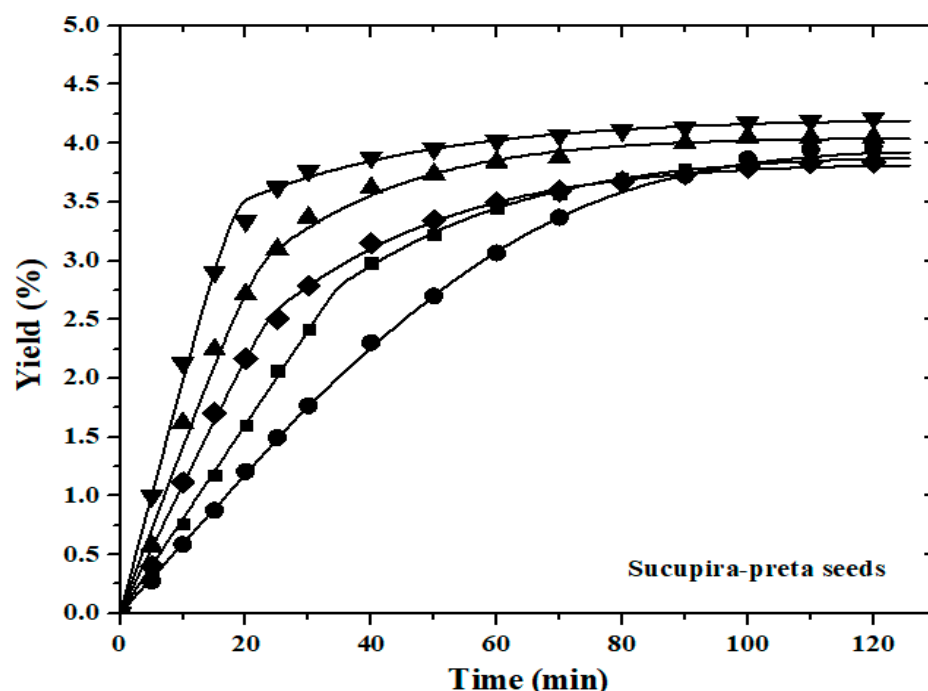


Figure 3. Experimental kinetic extraction curves with supercritical CO₂ fitted using Sovová model (—) of sucupira-preta seeds: 40 °C (■, 22 MPa; ▲, 28 MPa); 50 °C (◆, 25 MPa); 60 °C (●, 22 MPa; ▼, 28 MPa) with a constant flow rate of 2.0 mL min^{−1}.

Table 3. Experimental conditions, extraction yield outcomes, and mathematical model adjustment parameters for supercritical CO₂ extraction.

Run	Z	W	r	S (g _{oil} ·g _{solvent} ^{−1})	<i>t</i> _{CER} (min)	<i>t</i> _{FER} (min)	<i>K</i> _{Fa} (min ^{−1})	<i>K</i> _{Sa} (min ^{−1})	ADD (%)	R ²
Burrito Leaves										
1	87.59	0.23	0.30	0.0028	0.05	5.05	9.36	0.014	0.57	0.987
2	13.57	0.12	0.25	0.0025	0.50	7.64	1.45	0.007	0.61	0.995
3	36.79	0.12	0.35	0.0032	0.13	5.54	3.93	0.008	0.70	0.994
4	82.33	0.29	0.37	0.0041	0.05	5.21	8.80	0.017	0.12	0.999
5–7 *	1.03	0.12	0.32	0.0044	3.22	6.68	0.11	0.008	0.51	0.996
Sucupira-Preta Seeds										
1	43.58	0.097	0.510	0.002	0.51	35.47	4.66	0.006	1.67	0.999
2	82.46	0.142	0.898	0.002	0.082	29.43	8.82	0.008	1.68	0.999
3	6.28	0.073	0.215	0.004	2.639	20.32	0.67	0.005	1.12	0.995
4	6.00	0.117	0.378	0.003	2.941	24.54	0.64	0.007	3.39	0.994
5–7 *	10.27	0.097	0.486	0.003	1.754	25.69	1.09	0.006	3.30	0.997

Note: Extraction was carried out in triplicate *.

In the Sovová model, the dimensionless parameters Z and W represent specific system characteristics, while S denotes solubility, and r refers to the mass of easily accessible oil. The mass transfer coefficients in the solvent phase (*K*_{Fa}) were higher than those in the solid phase (*K*_{Sa}), indicating that the easily accessible oil contributed more significantly to the extraction yield. The lower *K*_{Sa} values, compared to *K*_{Fa}, underscore the greater difficulty associated with the diffusion process and the extraction of less accessible oils, as corroborated by previous studies [33–36]. The parameters *t*_{CER} and *t*_{FER} represent, respectively, the point at which oil extraction from within the particles begins and the point at which the easily accessible crude compounds are extracted. The coefficient of

determination (R^2) serves as a statistical measure of the model accuracy in fitting the experimental data.

The response surfaces shown in Figures 4 and 5 illustrate the combined influence of temperature and pressure on extraction yields. Statistical analysis confirmed that pressure was the most significant factor affecting the extraction yield, whereas temperature had a secondary but still relevant effect. The adjusted equations for the extraction yields are presented in following equations, which demonstrate that increasing the pressure has a more pronounced positive impact on the yields for both plant matrices.

$$\text{Yield_Burrito} = 0.9414 + 0.0400 T + 0.1700 P + 0.0050 T * P$$

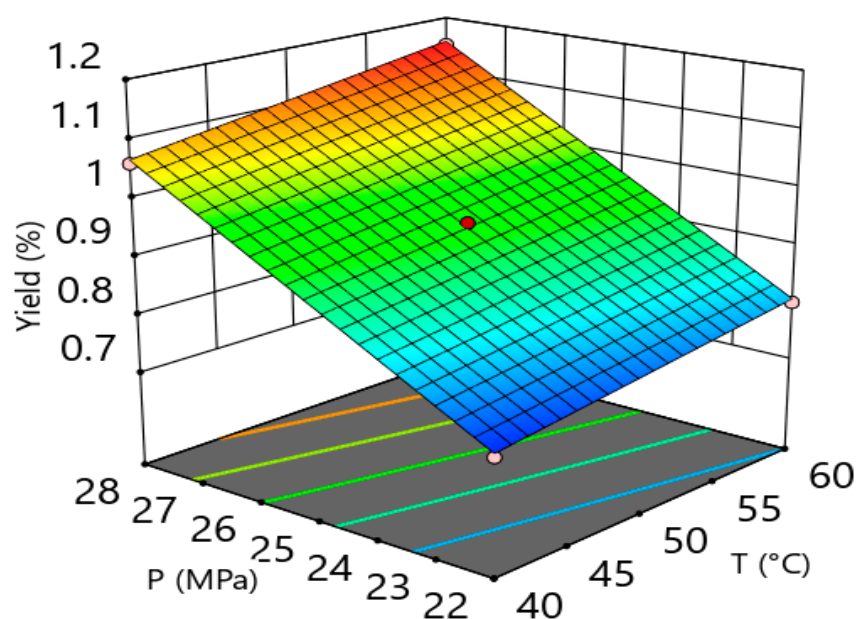


Figure 4. A response surface plot illustrating the extraction yield of oil from burrito leaves as a function of temperature and pressure, with a constant flow rate of 2.0 mL min^{-1} .

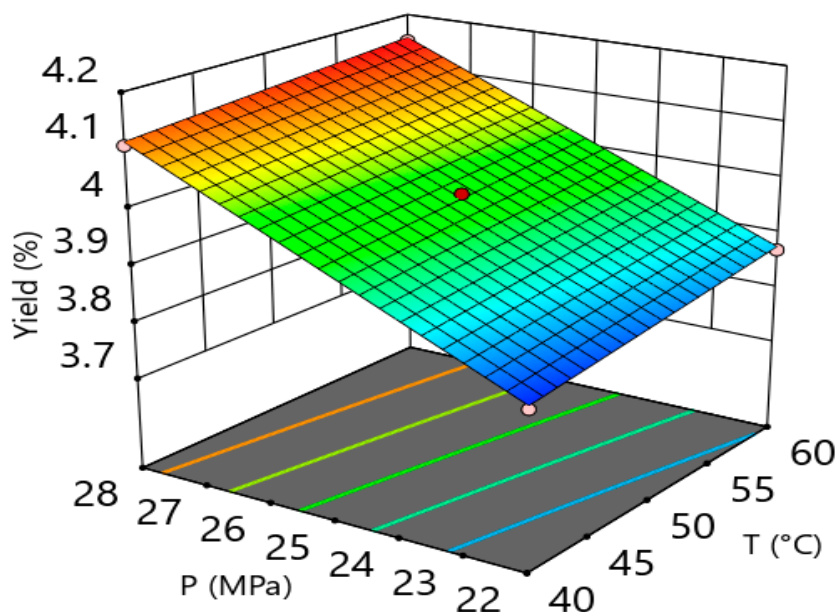


Figure 5. A response surface plot showing the oil extraction yield from sucupira-preta seeds as influenced by temperature and pressure at a fixed flow rate of 2.0 mL min^{-1} .

$$Yield_Sucupira-preta = 3.99 + 0.0250 T + 0.1450 P - 0.0050 T * P$$

These equations indicate that increasing the pressure had a more pronounced positive impact on the extraction yields for both matrices.

The statistical analysis results, summarized in Table 4, further validated the influence of temperature (*T*) and pressure (*P*) on the extraction yields. A significant linear model was observed for both variables across the studied plant matrices, highlighting their critical roles in determining the outcomes of the extraction process. The analysis of variance (ANOVA) confirmed the statistical significance of the models, with *R*² values exceeding 0.99, indicating an excellent fit to the experimental data and the reliability of the proposed models.

Table 4. Variance analysis results for the extracts obtained using a 2² factorial design for the extraction of plant matrices with carbon dioxide.

Terms	Sum of Squares	Degrees of Freedom	Mean Squares	F-Value	<i>p</i> -Value	<i>R</i> ²
Burrito Leaves						
Model	0.1221	3	0.0407	316.56	0.0003	0.997
T	0.0064	1	0.0064	49.78	0.0059	
P	0.1156	1	0.1156	899.11	0.0001	
T.P	0.0001	1	0.0001	0.7778	0.4428	
Pure Error	0.0000	2	0.0000			
Cor Total	0.1225	6				
Sucupira-Preta Seeds						
Model	0.0867	3	0.0289	224.78	0.0005	0.996
T	0.0025	1	0.0025	19.44	0.0216	
P	0.0841	1	0.0841	654.11	0.0001	
T.P	0.0001	1	0.0001	0.7778	0.4428	
Pure Error	0.0000	2	0.0000			
Cor Total	0.0871	6				

T = temperature; P = pressure.

The ANOVA results, as depicted in the Pareto chart in Figure 6A,B, further emphasized the significant effects of temperature (*T*) and pressure (*P*) on the extraction yield (*Y*), with *p*-values below 0.05. Among these variables, pressure was identified as having a substantial impact on the extraction yields of both burrito leaves and sucupira-preta seeds, reinforcing its critical role in optimizing the process.

3.2. Extract Components

3.2.1. Burrito Seeds

The chemical profile of the burrito leaf extract was analyzed under the experimental conditions that yielded the highest extraction efficiency (*T* = 60 °C and *P* = 28 MPa), as detailed in Table 5. The results revealed diverse compositions of bioactive compounds, including squalene (9.23%), nonacosane (17.98%), vitamin E (9.39%), hexadecanoic acid (9.51%), hexadecanal (8.58%), β-sitosterol acetate (8.27%), oleic acid (4.62%), and β-carotene (2.65%). Additionally, minor compounds, such as alkanes (e.g., tetracosane, pentacosane, hexacosane, heptacosane, and octacosane), and unidentified components accounted for a significant portion of the extract, reflecting the chemical complexity of the burrito leaves.

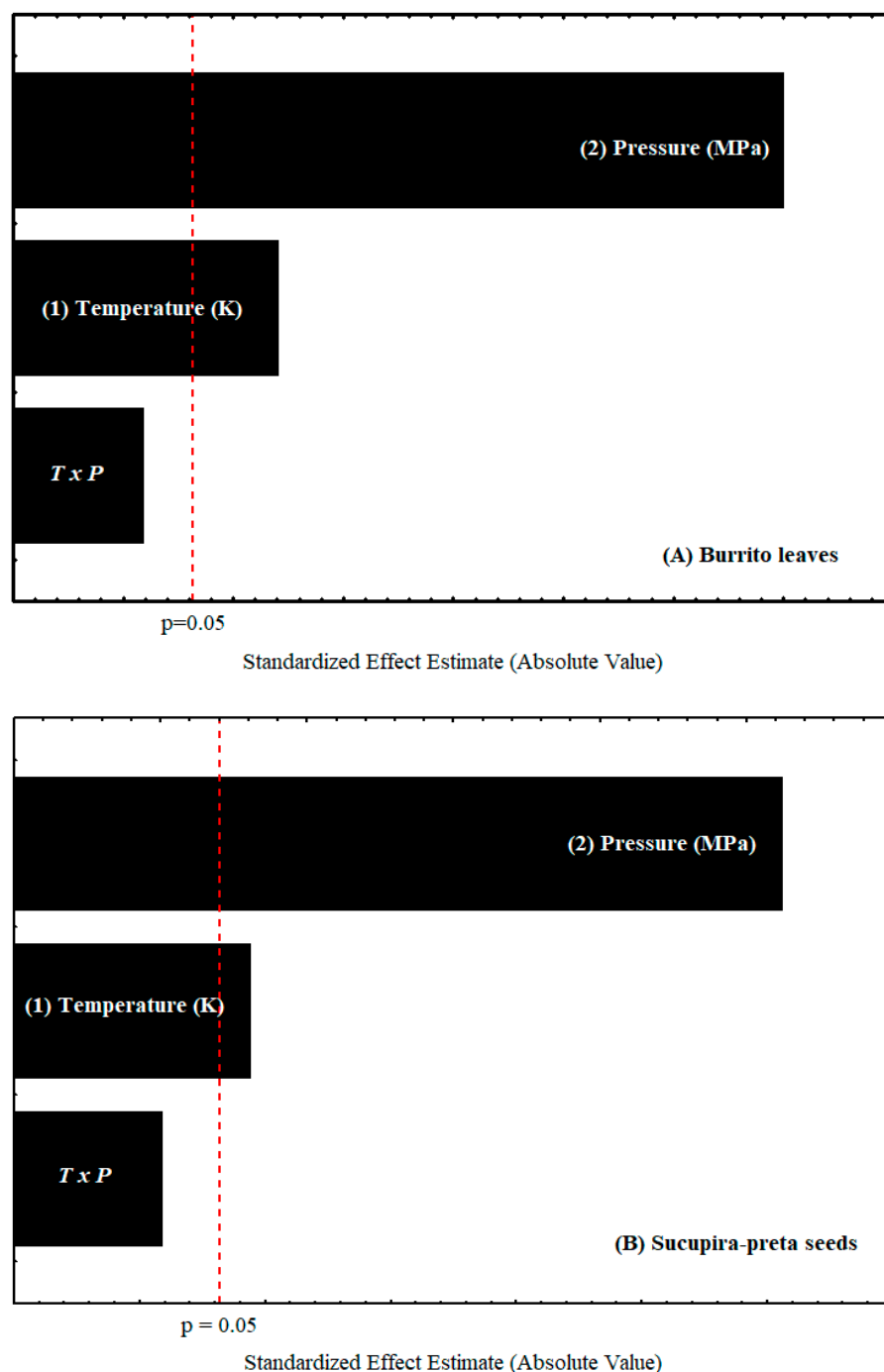


Figure 6. Pareto chart: analysis of linear effects of variables. (A) Burrito leaves and (B) sucupira-preta seeds.

Among the identified compounds, nonacosane had the highest concentration (17.98%). Its elevated concentration in burrito leaves may be attributed to the adaptation of plants to environmental stressors, such as high temperatures or arid conditions, which necessitates enhanced protective mechanisms. Beyond its ecological role, nonacosane has been associated with potential pharmacological benefits, including antimicrobial, anti-inflammatory, and neuroprotective properties. These characteristics suggest that the high nonacosane content in burrito leaves could contribute to their therapeutic potential, particularly in formulations that target skin protection or inflammation-related conditions [37].

Other major compounds also exhibit significant pharmacological properties. Squalene, a sterol precursor, is known for its antioxidant, anti-inflammatory, and immunomodulatory activities. It has been widely used as a vaccine adjuvant and has demonstrated antitumor

potential by inducing apoptosis in cancer cells [38,39]. Vitamin E and β -carotene are potent antioxidants that protect cells from oxidative damage, support immune function, and contribute to skin and eye health. These compounds are also associated with the prevention of chronic diseases such as cardiovascular and neurodegenerative disorders [38,40].

Table 5. Chemical profile of oil extracted from burrito leaves.

Compound	Chemical Class	Peak Area (%) *
Nonacosane	Alkane	17.98
Hexadecanoic acid	Fatty acid	9.51
Vitamin E	Alcohol	9.39
Squalene	Alkane	9.23
Hexadecanal	Aldehyde	8.58
β -sitosterol acetate	Ester	8.27
Unknown	-	3.53
Farnesyl acetone <(5E,9Z)->	Cetone	2.06
Unknown	-	1.12
Pentacosane	Alkane	0.42
Hexacosane	Alkane	0.40
Tetracosane	Alkane	0.33
unknown	-	11.88
Untriacontane	Alkane	1.81
Unknown	Aldehyde	5.80
β -Carotene	Carotenoid	2.65
Oleic acid	Fatty acid	4.62
Octacosane	Alkane	0.65
Heptacosane	Alkane	0.81
Farnesyl acetone <(Z,Z)->	Cetone	0.96

Mean $\pm$ standard deviation (n = 3) *.

Fatty acids such as hexadecanoic acid (palmitic acid) and oleic acid play essential roles in maintaining cellular membrane stability and promoting cardiovascular health. Oleic acid is recognized for its ability to reduce LDL cholesterol levels, thereby improving circulatory health [41]. Hexadecanal, an aldehyde, exhibits antimicrobial properties and may act as a plant defense compound [42]. β -sitosterol acetate, an ester, is widely acknowledged for its anti-inflammatory effects, cholesterol-lowering properties, and anticancer potential [43]. Additionally, farnesyl acetone, a ketone, may contribute to plant defense mechanisms against environmental stressors [44].

The presence of minor compounds and unidentified constituents further highlights the chemical diversity of the burrito leaves. This complexity reflects the adaptive capacity of plants to produce a wide range of bioactive molecules, some of which may have unexplored pharmacological or ecological roles. The detection of uncharacterized compounds underscores the need for further research to uncover their potential applications.

In summary, the chemical composition of burrito leaves, including major, minor, and unidentified compounds, demonstrated the potential of the plant as a source of bioactive molecules. The high concentration of nonacosane along with other pharmacologically relevant compounds reinforces the importance of burrito leaves in biotechnological and medicinal research. Furthermore, several of these constituents, such as β -sitosterol acetate, vitamin E, and squalene, have been associated with anxiolytic properties, suggesting that burrito leaves hold promise for the development of natural therapies targeting anxiety and related disorders.

3.2.2. Sucupira-Preta Seeds

The chemical composition of sucupira-preta seed oil was analyzed under the experimental conditions that produced the highest yield ($T = 60\text{ }^{\circ}\text{C}$ and $P = 28\text{ MPa}$). The results revealed a composition consisting predominantly of linoleic acid (28.05%), oleic acid (26.94%), and palmitic acid (24.83%), along with a significant concentration of squalene (20.18%) (Table 6).

Table 6. Chemical profile of oil extracted from sucupira-preta seeds.

Compound	Chemical Class	Peak Area (%) *
Linoleic acid	Fatty acid	28.05 ± 0.09
Oleic acid	Fatty acid	26.94 ± 0.05
Palmitic acid	Fatty acid	24.83 ± 0.01
Squalene	Alkene	20.18 ± 0.13

Mean $\pm$ standard deviation ($n = 3$) *.

Fatty acids identified in sucupira-preta seeds are essential for human health and nutrition. Linoleic acid, an omega-6 polyunsaturated fatty acid, plays a critical role in maintaining healthy skin, regulating inflammatory responses, and preserving the structural integrity of the cellular membranes [45]. Oleic acid, an omega-9 monounsaturated fatty acid, is known for its cardiovascular benefits [46]. Palmitic acid, a saturated fatty acid, is a natural constituent of many oils and fats and is indispensable for cellular membrane stability [47].

The presence of 20.18% squalene further enhanced the pharmacological value of sucupira-preta seed oil. Squalene is a bioactive compound with diverse biological properties including antioxidant, anti-inflammatory, and immunomodulatory effects. It is extensively used in the pharmaceutical industry as a vaccine adjuvant and has shown potential for preventing cardiovascular and neurodegenerative diseases. In addition, squalene exhibits antitumor activity by inducing apoptosis in cancer cells, making it a compound of significant importance for human health [38,39,48].

The chemical profile of the sucupira-preta seeds highlights their nutritional and therapeutic potential. The high concentrations of linoleic, oleic, and palmitic acids underscore their value as sources of essential fatty acids, while the substantial presence of squalene reflects their pharmacological richness. Furthermore, squalene and oleic acid have been associated with anxiolytic effects, suggesting that sucupira-preta seed oil may have applications in the development of natural treatments for anxiety- and stress-related disorders [49].

Both burrito leaves and sucupira-preta seeds contain bioactive compounds with anxiolytic properties. In burrito leaves, β -sitosterol acetate, squalene, and vitamin E have been linked to the modulation of stress responses and reduction in anxiety-like behaviors in preclinical studies. Similarly, in sucupira-preta seeds, high concentrations of squalene and oleic acid are noteworthy, as these compounds have demonstrated anxiolytic effects through their ability to regulate oxidative stress and inflammation, which are often associated with anxiety disorders. The presence of these compounds in both plant matrices suggested that they may serve as valuable natural sources for the development of anxiolytic therapies.

Burrito leaves and sucupira-preta seeds contain bioactive compounds with therapeutic properties, such as antioxidants and anxiolytics, making them promising candidates for the development of pharmaceuticals, cosmetics, and supplements. Future research should focus on validating their effects, identifying unknown compounds, and optimizing extraction processes, thereby expanding their application in natural and sustainable products.

4. Conclusions

This study demonstrated that supercritical CO₂ extraction is a highly efficient technique for obtaining high-purity plant extracts, even when the yields are relatively modest. Burrito leaves achieved a maximum yield of 1.2%, while sucupira-preta seeds reached 4.2%, confirming the higher concentration of essential oils in the seeds. Despite the limited yields, the extracts displayed a complex chemical composition, including bioactive compounds such as fatty acids (oleic, linoleic, and palmitic acids), squalene, β -carotene, and vitamin E, all of which are recognized for their antioxidant, anti-inflammatory, and anxiolytic properties.

Statistical analysis revealed that pressure was the most critical factor influencing the yield, whereas temperature had a comparatively smaller effect. The Sovová kinetic model exhibited a strong fit to the extraction curves, thus validating the robustness of the methodology. These findings emphasize the pharmaceutical potential of the extracts, particularly for the development of therapeutic and anxiolytic products, and reinforce the significance of supercritical extraction as a sustainable and effective method for isolating bioactive compounds.

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