

Article

Supercritical CO₂ Extraction from Bacupari (*Garcinia brasiliensis*) and Leiteira (*Tabernaemontana catharinensis*) Seeds

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Abstract

This study evaluated the extraction of oils from the seeds of bacupari (*Garcinia brasiliensis* Mart.) and leiteira (*Tabernaemontana catharinensis*), using carbon dioxide (CO₂) in the supercritical state. The effects of temperature (40, 50, and 60 °C) and pressure (20, 24, and 28 MPa) on the yield and extraction kinetics were investigated. The results indicated that, within the studied limits, temperature had a negligible influence on the process, while pressure had a greater impact on the yields owing to its effect on the density of supercritical CO₂ and the solubility of the extracted compounds. The maximum yields obtained were 14.8% for bacupari and 15.2% for leiteira, with most of the oil extracted within the first 30 min, indicating initial rapid extraction. Chemical composition analysis revealed relevant bioactive compounds in bacupari, including oleic acid (35%) and delta-tocopherol (19.6%). In leiteira, the main compounds identified were hexanedioic acid (29.2%) and stigmast-5-ene (7.95%). These results suggest the potential application of these oils in the pharmaceutical, cosmetic, and food sectors, while also highlighting the feasibility of using supercritical CO₂ as an extraction method for these plant matrices.

Keywords: bacupari; leiteira; supercritical extraction; CO₂; bioactive compounds



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1. Introduction

Bioactive compounds present in plant matrices have garnered increasing interest because of their essential roles in promoting human health and in developing functional products. Secondary metabolites, such as flavonoids, saponins, fatty acids, and phenolic compounds, exhibit antioxidant, anti-inflammatory, antimicrobial, and hypoglycemic properties, making them valuable for applications in the pharmaceutical, food, and cosmetic industries [1]. Furthermore, the search for natural and sustainable alternatives to replace synthetic ingredients has driven the appreciation for plant-based sources rich in bioactive compounds. In this context, the efficient extraction of these compounds is a crucial

step to enable their application in high-value-added products, such as medicines, dietary supplements, and cosmetics [2,3].

Among the notable plant matrices, bacupari (*Garcinia brasiliensis*) and leiteira (*Tabernaemontana catharinensis*) exhibit rich and diverse chemical profiles, with significant potential to meet industrial demands.

Bacupari (*Garcinia brasiliensis*) is widely recognized for its rich content of bioactive compounds, including phenolic compounds, flavonoids, and xanthenes, which exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities [4,5]. These compounds play a crucial role in protecting against oxidative stress, a key factor in the development of chronic diseases such as cardiovascular disorders and neurodegenerative conditions [6]. Additionally, they contribute to the inhibition of inflammatory processes and provide antimicrobial effects, making bacupari a promising natural resource for the development of dietary supplements, pharmaceuticals, and cosmetics [4].

The oil extracted from bacupari seeds is particularly noteworthy because of its high content of essential fatty acids such as linoleic and oleic acids. Oleic acid, a monounsaturated fatty acid, is well known for its cardiovascular benefits, including the reduction in LDL cholesterol levels and improvement of overall heart health [7]. Linoleic acid, an essential polyunsaturated fatty acid, is vital for maintaining skin barrier function and preventing moisture loss, which enhances skin integrity and supports its use in cosmetic formulations [8]. Together, these fatty acids not only provide nutritional benefits, but also contribute to oil potential in functional food and skincare applications [9].

The combination of phenolic compounds and essential fatty acids in bacupari oil underscores its versatility and value as a bioactive compound. Phenolic compounds, such as xanthenes, are particularly effective in neutralizing free radicals, thereby preventing cellular damage and aging [4]. This antioxidant capacity, coupled with the fatty acid profile of the oil, makes it a multifunctional ingredient suitable for a wide range of applications, ranging from health-promoting supplements to anti-aging skincare products [6,9].

Leiteira (*Tabernaemontana catharinensis*), on the other hand, is a native South American plant that is often underutilized but has significant bioactive potential. Studies have indicated that leiteira is rich in compounds such as diterpenes, triterpenes, and flavonoids, which exhibit antioxidant, anti-inflammatory, and wound healing properties [10,11]. These secondary metabolites have been associated with tissue regeneration, the control of inflammatory processes, and protection against oxidative damage. Camponogara et al. [12] demonstrated that extracts from the leaves of leiteira possess topical anti-inflammatory activity, attributed to the presence of indole alkaloids, terpenes, and phenolic compounds.

Although the literature on leiteira (*T. catharinensis*) is limited, there is evidence that its extracts can be used in pharmaceutical and cosmetic products, reinforcing its potential as a source of high-value-added bioactive compounds [13]. Nicola et al. [14] highlighted that ethanolic extracts of the plant exhibit antioxidant and anticholinesterase activities, particularly because of the presence of alkaloids such as coronaridine and voacangine, which have neuroprotective properties.

The valorization of native species, such as leiteira, not only promotes sustainability but also offers opportunities for the development of innovative products in the pharmaceutical, food, and cosmetic industries. Furthermore, exploring the bioactive potential of underutilized plants contributes to biodiversity conservation and the generation of scientific knowledge regarding endemic species [6,15].

The extraction of bioactive compounds from these plant matrices presents challenges related to process efficiency and preservation of the chemical properties of the extracts. Supercritical CO₂ is widely used owing to its unique properties such as high density, low viscosity, and high diffusivity, which enable the selective extraction of target com-

pounds with high efficiency. Moreover, it is a non-toxic, inert, and environment-friendly solvent that eliminates the need for organic solvents and reduces the generation of toxic waste [16–18]. These characteristics make this technique ideal for applications in which extract purity is essential, such as in the production of medicines, dietary supplements, and cosmetics [19,20].

One of the main advantages of supercritical extraction is its high speed. Studies indicate that 70–80% of the oil or chemical constituents of plant matrices are extracted within the first 20 min of the process, significantly reducing the operation time compared to conventional methods [16–18]. Additionally, this technique allows for the extraction of thermosensitive compounds such as those present in bacupari and leiteira, while preserving their bioactive properties. The ability to adjust the temperature and pressure conditions of CO₂ also enables the selective extraction of different classes of compounds, such as lipids and phenolics, thereby increasing the versatility of the method [21,22].

Therefore, this study aimed to evaluate the supercritical extraction kinetics of oils from the plant matrices bacupari (*Garcinia brasiliensis*) and leiteira (*Tabernaemontana catharinensis*) using supercritical carbon dioxide (CO₂) as a solvent. This study sought to determine the extraction yields and analyze the effects of process variables, such as temperature and pressure, on the efficiency of the method. Additionally, the constituents present in the obtained oils and extracts will be characterized to identify bioactive compounds and explore their potential for applications in high-value-added products, particularly in the pharmaceutical, food, and cosmetic industries.

2. Materials and Methods

2.1. Sample Preparation

Bacupari and leiteira seeds were collected in June 2024 in Rosana, São Paulo, Brazil (22°34'47" S, 53°03'33" W). A total of 200 g of bacupari and leiteira seeds was dried in an oven at 60 °C for 72 h until a constant weight was achieved. Subsequently, the material was ground using a household blender (Britânia, model BLQ1350, Curitiba, PR, Brazil) and sieved through meshes of 16–20 (MyLabor, São Paulo, SP, Brazil) to achieve particle size standardization. Particle size, a critical parameter that influences extraction yield and kinetics by affecting surface area and porosity [2,18], was restricted to values above 24 mesh (710 µm) due to the technical limitations of the supercritical extraction equipment [23,24]. The processed samples were stored in polyethylene bags and frozen until extraction.

2.2. Supercritical CO₂ Extraction

The extraction experiments were conducted using a bench-scale system designed for supercritical extraction, consisting of a CO₂ cylinder (Linde Gass, 99.9%), high-pressure pump (ISCO 260 D, Lincoln, NE, USA), thermostatic bath, and extractor made of 304 stainless steel (Figure 1). Additional information on the equipment and experimental procedures can be found in previous studies [23,24].

For each experiment, 10 g of plant material was placed in the extractor, and the remaining void space was filled with glass beads to form an inert bed. The experimental conditions were defined based on a 2² factorial design, including temperatures of 40, 50, and 60 °C and pressures of 20, 24, and 28 MPa, with triplicate measurements at the central point (Table 1). The CO₂ flow rate was maintained constant at 2.5 mL·min^{−1}. After reaching the desired temperature, the system was pressurized and allowed to equilibrate for 30 min before starting extraction. The solvent flow was controlled using a micrometering valve (Parker Autoclave Engineers, Erie, PA, USA) and was maintained at 100 °C. The extracts were collected at regular intervals and stored in glass vials for subsequent analysis. The extraction yield was determined gravimetrically using an analytical balance and calculated

as the ratio of the total mass extracted to the initial mass of the leaves in the extractor (on a dry basis).

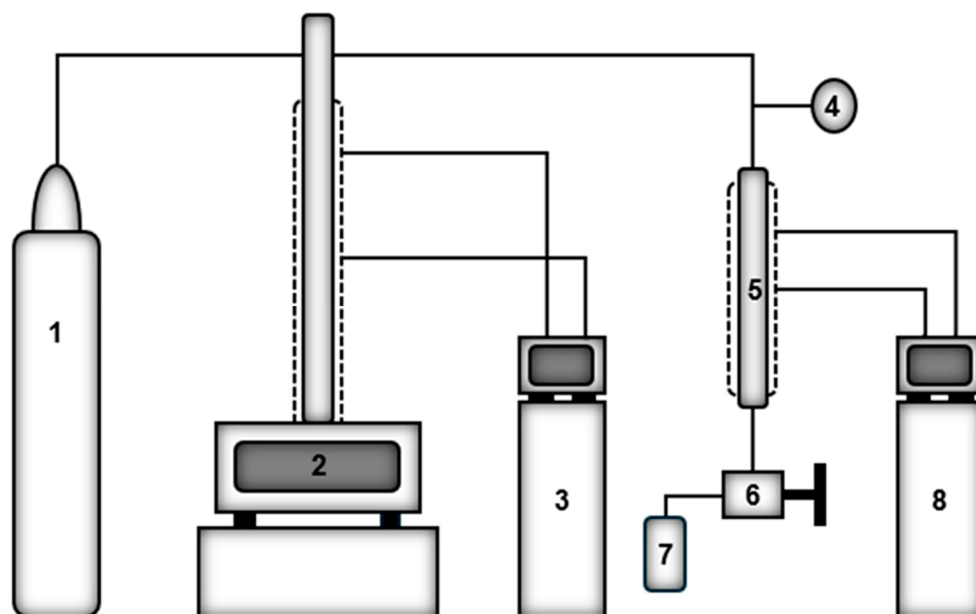


Figure 1. Schematic representation of the supercritical extraction system: 1—CO₂ cylinder, 2—syringe pump, 3—thermostatic bath, 4—pressure gauge, 5—extractor, 6—needle valve with aluminum heating jacket; 7—Collecting container; and 8—thermostatic bath.

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. Soxhlet Extraction

Soxhlet extraction was performed using ethanol (Êxodo Científica, 98.8%) as solvent. This procedure introduced 10 g of material crushed into the Soxhlet extractor and subjected it to an 8 h reflux process. After the extraction phase, the samples were subjected to solvent removal under reduced pressure, using a rotary evaporator. The samples were placed in a forced air circulation oven to ensure complete solvent evaporation. After drying at room temperature (25 °C), samples were transferred to a silica gel desiccator. The lipid extraction yield was determined using an accurate digital analytical balance, and the results were expressed in grams per 100 g of sample, according to the AOAC method.

2.4. Oil Characterization

The obtained extracts were weighed using an analytical balance and diluted in dichloromethane ($n = 3$) to a concentration of 1000 µg mL^{−1}. The diluted samples were analyzed using a previously configured chromatographic system.

The analysis was performed by Gas Chromatography coupled with Mass Spectrometry (GC-MS) using a gas chromatograph coupled to a mass spectrometer (model GCMS-QP2010 Plus, Shimadzu, Kyoto, Japan). The system was equipped with an AOC-20i autosampler (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 was set to start at 40 °C, with a heating rate of 2 °C min^{−1} up to 200 °C, followed

by an increase of $10\text{ }^{\circ}\text{C min}^{-1}$ up to $300\text{ }^{\circ}\text{C}$, where it was held for 15 min. The injector, interface, and ion source temperatures were set to 250, 300, and $280\text{ }^{\circ}\text{C}$, respectively. Helium gas (99.999% purity) was used as carrier gas at a flow rate of 0.8 mL min^{-1} . Injections ($1\text{ }\mu\text{L}$, $1000\text{ }\mu\text{g mL}^{-1}$) were performed in the split mode with a split ratio of 1:50. Data acquisition and processing were carried out using the GCMS Postrun Analysis software (Ver. 2.53, Shimadzu, Kyoto, Japan), which included the NIST14.lb and NIST14.lbs spectral libraries. Additional details of the analytical methods used can be found in previous studies [24,25].

2.5. Statistical Analysis

The experimental data were analyzed using analysis of variance (ANOVA) at a 5% significance level. Statistical tests were performed to compare the means, and the interactions and main effects of the independent variables on the responses were evaluated using the Design Expert software, version 12 [26].

3. Results

3.1. Extraction Yield

The extraction yields of bacupari and leiteira seeds using supercritical CO_2 are listed in Table 2. For bacupari, the yield ranged from 11.4% to 14.8%, whereas for leiteira, it varied between 9.5% and 15.2%. The highest bacupari yield was achieved at $40\text{ }^{\circ}\text{C}$ and 28 MPa, whereas the best results were obtained for leiteira at $60\text{ }^{\circ}\text{C}$ and 28 MPa. Soxhlet extraction with ethanol, which was used as a comparative method, resulted in lower yields for leiteira (8.2%), but higher yields for bacupari (17.5%).

Table 2. Experimental conditions and extraction yield data for the recovery of compounds from bacupari and leiteira seeds using supercritical CO_2 at a constant flow rate of 2.5 mL min^{-1} .

Run	Temperature ($^{\circ}\text{C}$)	Pressure (MPa)	Yield (wt%)
Bacupari Seeds			
1	40	20	11.4
2	60	20	12.2
3	40	28	14.2
4	60	28	14.8
5–7	50	24	13.1 ± 0.7 *
Ethanol Soxhlet (360 min)		Atmospheric	17.4 ± 1.1 *
Leiteira Seeds			
1	40	20	9.5
2	60	20	12.2
3	40	28	13.6
4	60	28	15.2
5–7	50	24	13.4 ± 0.4 *
Ethanol Soxhlet (360 min)		Atmospheric	8.2 ± 0.6 *

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

The kinetic extraction curves for bacupari and leiteira seeds (Figures 2 and 3) provide valuable insights into extraction dynamics. For bacupari, the majority of the extractable compounds were recovered within the first 40 min of extraction, with approximately 97% of the oil extracted at $40\text{ }^{\circ}\text{C}$ and 28 MPa. Similarly, for leiteira, 95% of the oil was extracted within the first 25 min at $60\text{ }^{\circ}\text{C}$ and 28 MPa.

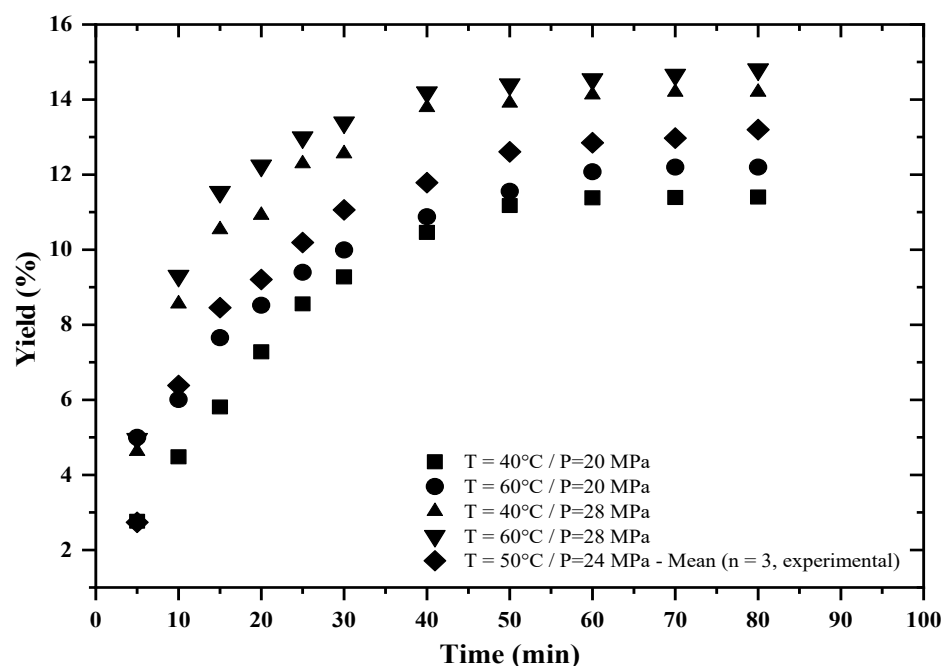


Figure 2. Experimental kinetic extraction curves with supercritical CO₂ of bacupari seeds: 40 °C (■, 20 MPa; ▲, 28 MPa); 50 °C (◆, 24 MPa); 60 °C (●, 20 MPa; ▼, 28 MPa) with a constant flow rate of 2.5 mL min^{−1}.

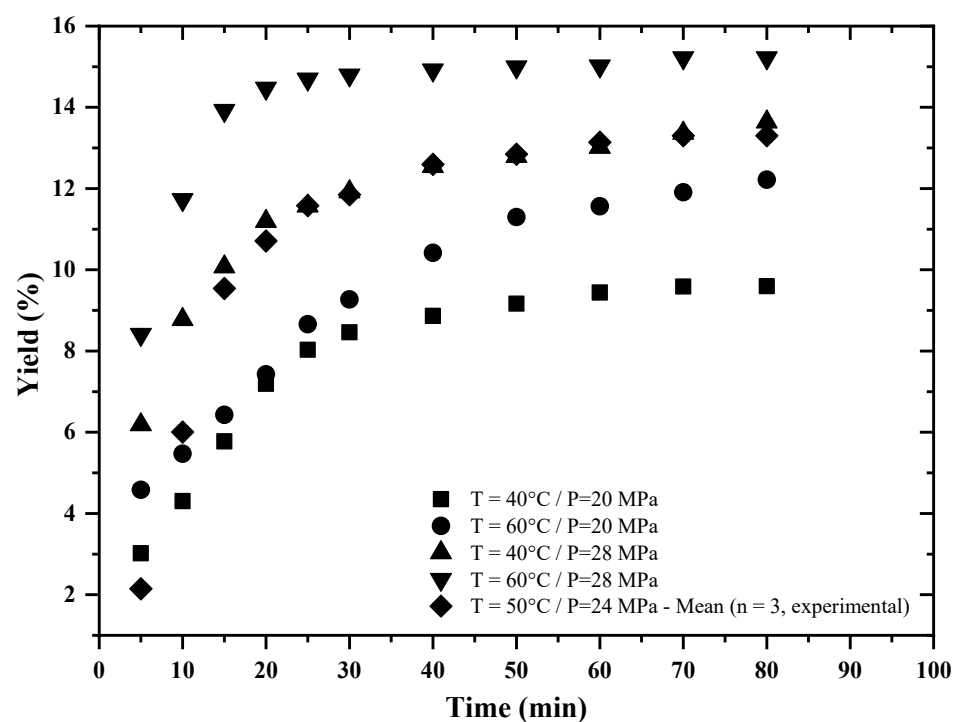


Figure 3. Experimental kinetic extraction curves with supercritical CO₂ of leiteira seeds: 40 °C (■, 20 MPa; ▲, 28 MPa); 50 °C (◆, 20 MPa); 60 °C (●, 20 MPa; ▼, 28 MPa) with a constant flow rate of 2.5 mL min^{−1}.

3.2. Statistical Analysis Results

The response surface plots (Figures 4 and 5) illustrate the combined influence of temperature and pressure on the extraction yields of bacupari and leiteira. For bacupari, the highest yields were obtained at 40–50 °C and 28 MPa, whereas for leiteira, optimal conditions were 60 °C and 28 MPa.

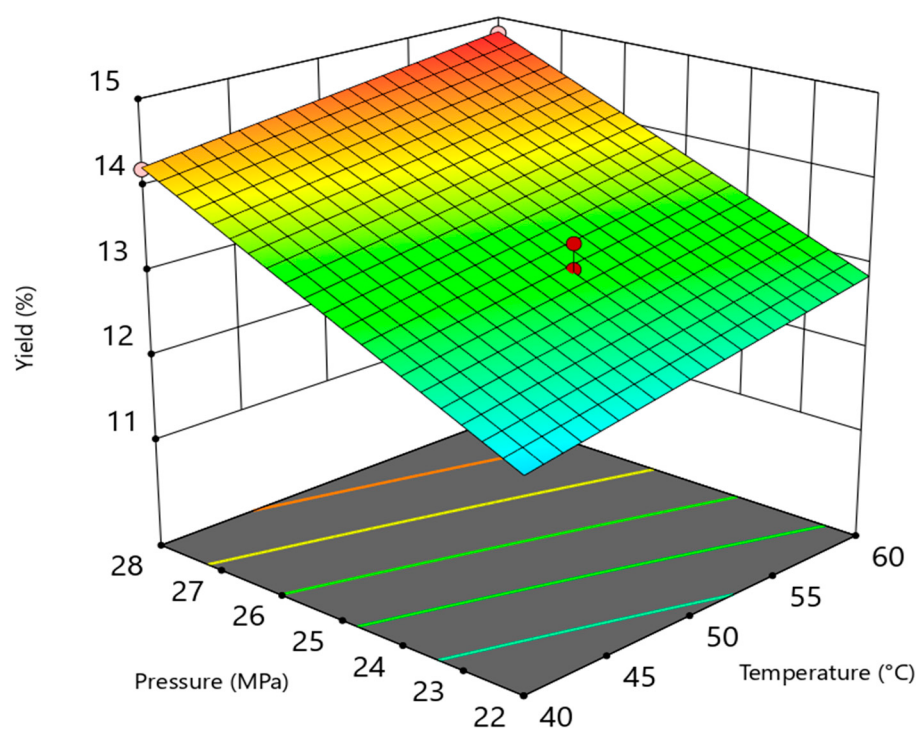


Figure 4. Response surface plot illustrating the extraction yield of oil from bacupari seeds as a function of temperature and pressure at a constant flow rate of 2.5 mL min^{-1} .

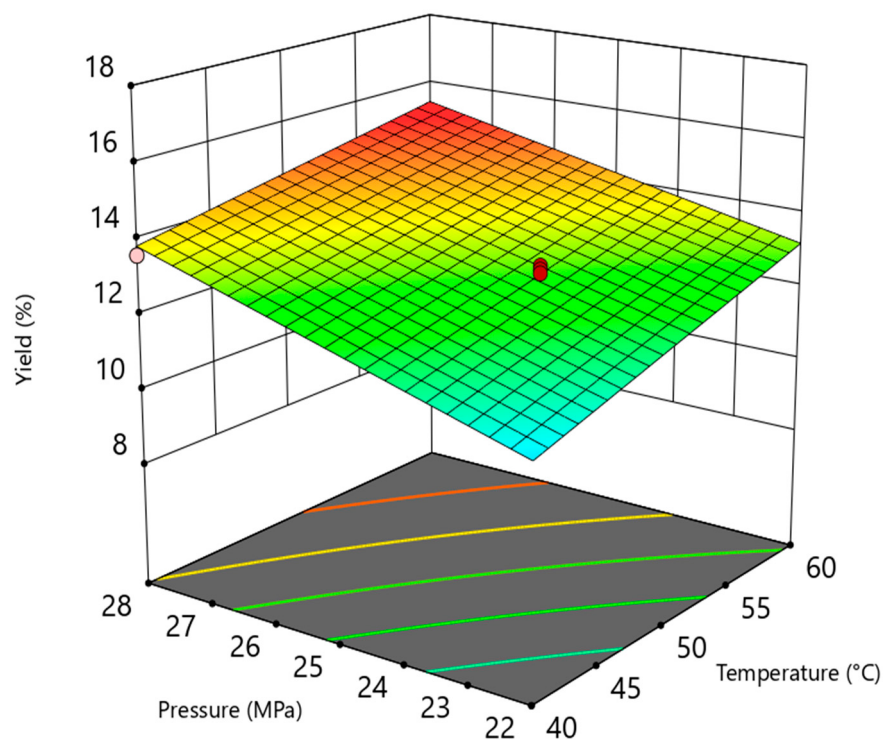


Figure 5. Response surface plot showing the oil extraction yield from leiteira seeds as influenced by temperature and pressure at a fixed flow rate of 2.5 mL min^{-1} .

The mathematical models adjusted to the yields are expressed in Equations (1) and (2) (where T and P are temperature and pressure, respectively). The results of the analysis of variance (ANOVA) are presented in Table 3

$$\text{Yield}_{\text{Bacupari}} = 13.51 + 0.3375 T + 1.01 P - 0.0375 T \times P \quad (1)$$

$$Yield_Leiteira = 13.32 + 1.01 T + 1.33 P - 0.2026 T \times P \quad (2)$$

Table 3. 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	p-Value	R ²
Bacupari Seeds						
Model	7.79	3	2.60	42.27	0.0059	0.977
T	0.4288	1	0.4288	6.98	0.0775	
P	7.29	1	7.29	118.67	0.0017	
T.P	0.0100	1	0.0100	0.1628	0.7136	
Residual	0.1843	3	0.0614			
Cor Total	7.97	6				
Leiteira Seeds						
Model	17.53	3	5.84	29.87	0.0098	0.968
T	3.81	1	3.81	19.49	0.0216	
P	12.60	1	12.60	64.43	0.0040	
T.P	0.3025	1	0.3025	1.55	0.3020	
Residual	0.5868	3	0.1956			
Cor Total	18.11	6				

T = temperature; P = pressure.

3.3. Chemical Profile of Extracts

The oil extracted from bacupari seeds at 60 °C and 28 MPa contained a complex mixture of bioactive molecules (Table 4; Figure 6). Among the identified compounds, oleic acid (35.05%), palmitic acid (25.84%), and delta-tocopherol (19.66%) were the most abundant, whereas stearic acid (5.25%) and 1-monooleoylglycerol (4.30%) were the most abundant.

Table 4. Chemical profiles of oils extracted from bacupari seeds.

ID	Retention Time	Math with the Library (%)	Compound	Chemical Class	Peak Area (%)
1	9.760	100	Palmitelaidic acid	Lipid—Unsaturated fatty acid	1.16
2	10.118	100	Palmitic Acid	Lipid—Saturated fatty acid	25.84
4	11.386	99	Heptadecanoic acid	Lipid—Saturated fatty acid	0.19
5	12.534	96	Oleic Acid	Lipid—Monounsaturated fatty acid	35.05
6	12.779	100	Stearic acid	Lipid—Saturated fatty acid	5.25
8	15.752	99	Hexadecanoic acid	Lipid—Saturated fatty acid	0.35
9	15.922	98	1-Monopalmitin	Lipid—Monoglyceride	1.94
10	16.902	99	2-linoleoylglycerol	Lipid—Monoglyceride	0.55
11	17.058	98	2-Oleoylglycerol,	Lipid—Monoglyceride	0.54
12	17.207	99	1-Monooleoylglycerol	Lipid—Monoglyceride	4.30
13	17.371	97	Glycerol monostearate	Lipid—Monoglyceride	1.19
14	17.794	100	Hexanedioic acid	Carboxylic acid—Dicarboxylic acid	0.69
15	18.372	99	Cholestane	Hydrocarbon—Steroid hydrocarbon	0.79
17	20.074	100	delta-Tocopherol	Phenolic compound—Vitamin E	19.60
18	20.569	99	Farnesol	Isoprenoid alcohol	0.51
19	21.432	99	beta-Tocopherol	Phenolic compound—Vitamin E	1.62
20	24.679	98	Stigmasterol	Steroid—Phytosterol (plant sterol)	0.43

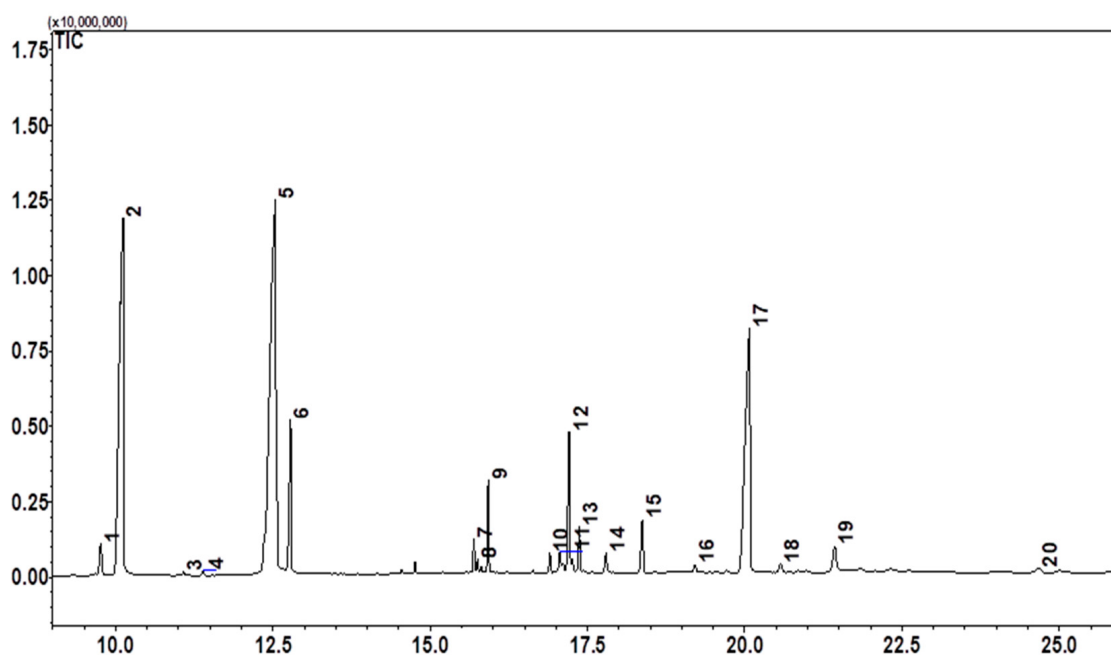


Figure 6. Gas chromatography of bacupari essential oil, intensity (eV) vs. time (min).

The oil obtained from leiteira seeds under identical extraction parameters (60 °C and 28 MPa) had markedly different compositions (Table 5; Figure 7). The profile was dominated by hexanedioic acid (29.17%) and stigmast-5-ene (7.95%), followed by considerable levels of palmitic acid (14.00%), oleic acid (8.91%), and stigmasterol (4.99%).

Table 5. Chemical profile of the oil extracted from leiteira seeds.

Order	Retention Time	Math with the Library (%)	Compound	Chemical Class	Peak Area (%)
1	10.018	100	Palmitic Acid	Lipid—Saturated fatty acid	14.00
2	12.409	98	Oleic Acid	Lipid—Monounsaturated fatty acid	8.91
3	12.491	98	13-Octadecenoic acid	Lipid—Unsaturated fatty acid	1.08
4	12.734	100	Stearic acid	Lipid—Saturated fatty acid	3.62
6	15.636	99	Octacosane	Hydrocarbon—Alkane	4.96
8	15.917	99	Hexacosane	Hydrocarbon—Alkane	3.64
10	17.183	96	1-Monooleoylglycerol	Lipid—Monoacylglycerol	0.89
11	17.433	99	Heneicosane	Hydrocarbon—Alkane	3.76
12	17.814	100	Hexanedioic acid	Carboxylic acid—Dicarboxylic acid	29.17
13	18.372	99	Cholestane	Hydrocarbon—Steroid hydrocarbon	10.93
15	19.701	91	gamma-Tocopherol	Phenolic compound—Vitamin E	0.61
16	19.948	98	delta-Tocopherol	Phenolic compound—Vitamin E	1.48
17	21.767	92	alpha-Tocopherol	Phenolic compound—Vitamin E	1.84
18	24.074	100	Campesterol	Steroid—Phytosterol (plant sterol)	2.16
19	24.688	99	Stigmasterol	Steroid—Phytosterol (plant sterol)	4.99
20	26.018	100	Stigmast-5-ene	Steroid—Phytosterol (plant sterol)	7.95

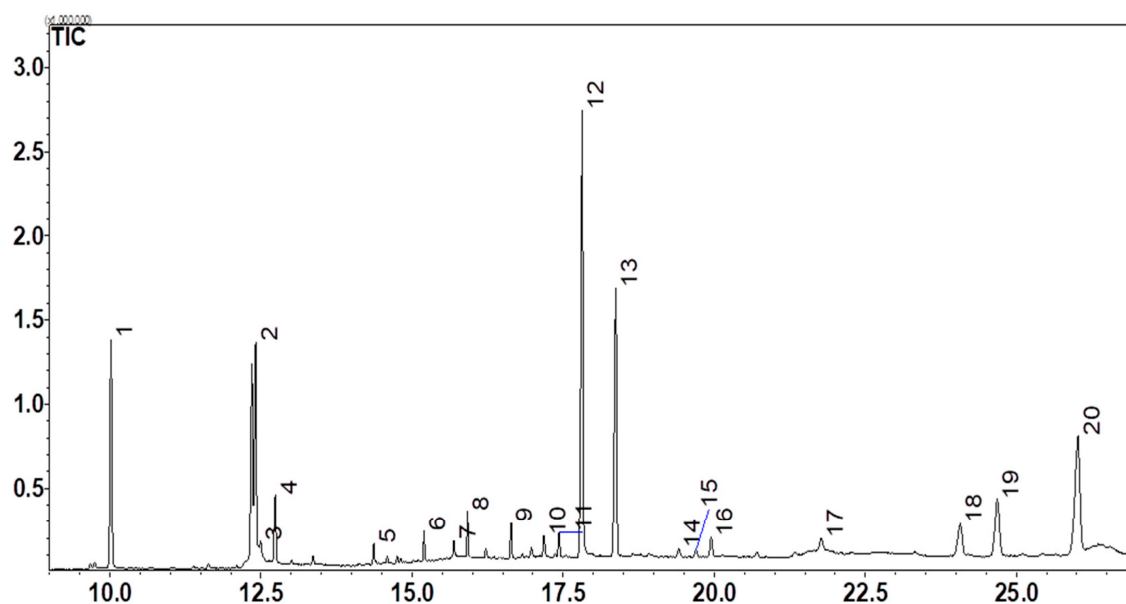


Figure 7. Gas chromatography of leiteira essential oil, intensity (eV) versus time (min).

4. Discussion

The extraction yields highlight the efficiency of supercritical CO₂ for the rapid extraction of bioactive compounds from bacupari and leiteira seeds. Approximately 97% of bacupari oil was extracted within the first 40 min at 40 °C and 28 MPa, whereas 95% of leiteira oil was extracted within 25 min at 60 °C and 28 MPa, demonstrating the rapid and efficient performance of the process.

The extraction curves exhibit the characteristic three-phase behavior commonly observed in supercritical fluid extraction: an initial rapid extraction phase dominated by convection, a slower intermediate phase controlled by diffusion, and a final depletion phase, where the extraction rate stabilizes. For bacupari (Figure 2), the rapid extraction phase lasted approximately 20 min, after which the extraction rate decreased significantly, indicating that most easily accessible compounds were removed. In contrast, for leiteira (Figure 3), the rapid extraction phase was shorter, lasting approximately 15 min, suggesting that the compounds in this matrix were more readily accessible to supercritical CO₂. This difference may be attributed to the structural characteristics of the matrices, with leiteira seeds potentially having a more porous structure or a higher concentration of surface-accessible compounds. Studies by McHugh and Krukonis [18] and Martín and Cocero [16] describe these phases as intrinsic to the process, with the initial phase dominated by convection, enabling the rapid extraction of easily accessible compounds, whereas the subsequent phases are controlled by diffusion.

Despite the differences in the rapid phase, the overall extraction times were short, with most of the oil being recovered within 40–50 min. This efficiency minimizes the processing time and energy consumption compared to conventional methods, such as Soxhlet extraction.

However, the differences in yield across the tested conditions were relatively small, with variations of less than 3% for bacupari and approximately 5% for leiteira. These small differences suggest that under the tested conditions, the majority of the oil was already extracted at lower temperatures and pressures, and the observed variations may fall within the range of the experimental error. This indicates that the extraction process for both matrices is efficient, even under milder conditions, which could reduce energy consumption and operational costs in industrial applications.

The duration of this rapid phase is consistent with previous studies that relate the structure of the plant matrix to the accessibility of its compounds. For instance, Corrêa et al. [23] observed similar behavior in *Tropaeolum majus* seeds, highlighting that the porosity and chemical composition of the matrix directly influence the extraction efficiency. Additionally, the presence of short-chain lipids or low-polarity compounds may facilitate the initial solubilization, as discussed by Azmir et al. [2].

The maximum extraction yields were 14.8% for bacupari and 15.2% for leiteira, both at 60 °C and 28 MPa. In comparison, the Soxhlet method showed a higher yield for bacupari (17.4%) but lower yield for leiteira (8.2%), highlighting that the polarity of the ethanol solvent favors the extraction of specific compounds from bacupari, whereas supercritical CO₂ is more efficient for leiteira. This demonstrates the role of solvent polarity and selectivity in determining the yield behavior between matrices.

Few studies have reported the extraction of bioactive compounds from selected plant matrices, particularly their seeds. For bacupari, Silva et al. [9] reported a global lipid yield of approximately 3.7% using the Bligh and Dyer method, significantly lower than the 14.8% obtained in this study using supercritical CO₂. This highlights the superior efficiency of the supercritical method for the recovery of lipophilic compounds. For leiteira, most studies have focused on other parts of the plant, such as the leaves. Pereira et al. [9] reported a maximum yield of 1.29% for leaves extracts using supercritical CO₂ at 25 MPa and 45 °C, whereas a subsequent study by the same authors [27] achieved a slightly higher yield of 2.4% with the addition of a cosolvent. However, it is important to note that these values cannot be directly compared to the 15.2% yield obtained for leiteira seeds in this study, as the data from the literature refer to leaf extractions, whereas this study focuses on seed extraction.

The results of the statistical analysis, summarized in Table 3, validate the influence of temperature and pressure on the extraction yields for both bacupari and leiteira. The ANOVA results (Table 3) indicated significant models for both matrices, with R² values of 0.977 for bacupari and 0.967 for leiteira. The F and *p*-values confirmed the statistical significance of the models, with bacupari showing an F-value of 42.27 (*p* = 0.0059) and leiteira showing an F-value of 17.53 (*p* = 0.0098). Pressure (P) was the most influential factor for both matrices, contributing 91.4% to bacupari and 69.5% to leiteira. Temperature (T) played a secondary role, contributing 5.3% and 21.0%, respectively. The interaction term (T × P) made a negligible contribution to both matrices (0.13% for bacupari and 1.77% for leiteira). These values indicated that the combined effect of temperature and pressure had a minimal influence on the extraction yields for both matrices.

The response surface plots (Figures 4 and 5) and statistical analysis confirmed that pressure was the most significant factor influencing the extraction yield, accounting for 91.4% in the case of bacupari and 69.5% for leiteira. Temperature played a secondary role, with a smaller but still relevant effect. The predominance of pressure as the most significant variable is related to the increased density of CO₂ under supercritical conditions, which enhanced the solubility of the compounds. Azmir et al. [2] and Martín and Cocero [16] confirmed that pressure had a more pronounced impact on yield, whereas temperature played a secondary role. These findings suggest that increasing the pressure has a more pronounced positive impact on the extraction yield, whereas the interaction between the temperature and pressure is minimal.

The Pareto charts (Figure 8A,B) (Design Expert software, version 12 [26]) further corroborate the relative importance of pressure and temperature during the extraction process. Pressure emerged as the most influential factor, with a *t*-value exceeding the Bonferroni limit, underscoring its critical role in enhancing extraction yields. Temperature also demonstrated statistical significance, albeit with a less pronounced effect than that

of pressure. However, the interaction term ($T \times P$) was not statistically significant, as its t-value fell below the significance threshold, suggesting that the effects of temperature and pressure on the extraction process are largely independent.

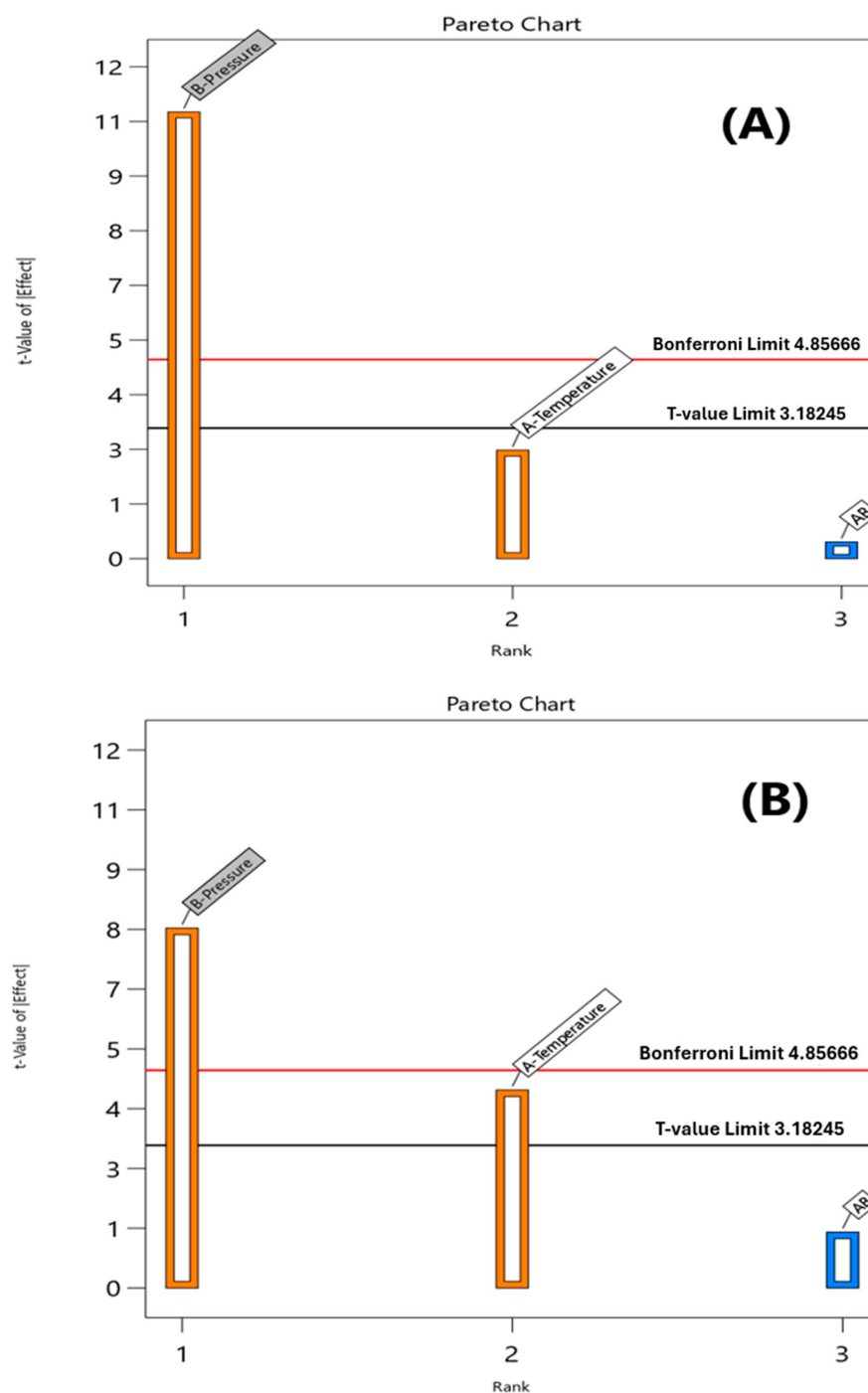


Figure 8. Pareto chart: Analysis of the linear effects of variables. (A) bacupari and (B) leiteira seeds.

The small increases in yields observed under more severe temperature and pressure conditions further reinforce the efficiency of supercritical extraction under moderate conditions. Studies by McHugh et al. [18] and Dixon and Johnston [17] emphasized that, after the initial convection-dominated phase, additional yield gains are minimal because of the reduced availability of easily accessible compounds. Furthermore, Wrona et al. [21] indicated that extending the extraction time beyond the rapid phase may not be energetically viable, because energy consumption increases disproportionately relative to the additional yield

obtained. However, the small differences in yields observed across the tested conditions suggest that the majority of the oil was extracted under milder conditions, and the observed variations may fall within the range of the experimental error. This indicates that extending the extraction time beyond the rapid phase may not be energetically or economically viable as the additional yield is minimal. This is particularly relevant in industrial applications, where cost and process efficiency are critical. The data suggest that operating under milder conditions can be sufficient to achieve high yields while minimizing energy demand, which increases process viability.

The oils extracted from bacupari and leiteira exhibit diverse chemical profiles, highlighting their potential for functional, industrial, and pharmaceutical applications. Both oils contain bioactive compounds and fatty acids, which underscores their relevance in various sectors while presenting distinct characteristics.

Bacupari oil is rich in oleic acid (35.05%), a fatty acid that is widely recognized for its cardiovascular benefits. This concentration is comparable to that of avocado oil (40–70%) [7], higher than that of soybean (22–30%) [28], sunflower oil (14–39%) [29], and sunflower oil (14–39%) [29], and lower than that of olive oil (55–83%) [8]. Palmitic acid, the second most abundant fatty acid in bacupari oil, was present at 25.84%, which is lower than the 36.2% reported by Silva et al. [9]. These differences may be attributed to extraction conditions, which influence the solubility and recovery of specific compounds.

The fatty acid profile of bacupari seeds was reported by Silva et al. [9] highlights a palmitic acid content of 36.2%, which is significantly higher than that obtained in this study (25.84%). This discrepancy may be due to differences in the extraction methods, as supercritical CO₂ extraction is known for its selectivity and ability to preserve bioactive compounds under milder conditions. Additionally, oleic acid was reported in 47.2% of the seeds by Silva et al. [9], which was higher than the 35.05% observed in this study. This variation could reflect differences in the plant growth conditions, genetic factors, or specific parameters of the extraction process. Furthermore, bacupari oil contains 19.66% delta-tocopherol, a bioactive compound with antioxidant properties, which makes it particularly suitable for cosmetic and preservative applications. This concentration is significantly higher than that in soybean (1–3%) and sunflower oils (traces) [6], and comparable to wheat germ oil, which is known for its high antioxidant content [15]. Thus, the chemical profile of bacupari oil supports its strong nutritional, cosmetic, and preservative potential.

In contrast, leiteira oil contained 8.91% oleic acid and 14.00% palmitic acid, along with notable amounts of hexanedioic acid (29.17%) and stigmast-5-ene (7.95%). Hexanedioic acid is widely used in the production of polymers and resins [30], whereas stigmast-5-ene, a phytosterol, has anti-inflammatory and cholesterol-lowering properties, enhancing the value of oil for nutraceutical and pharmaceutical applications [31]. Although leiteira oil contains delta-tocopherol at a lower concentration (1.47%) than bacupari oil, it remains relevant when compared to other vegetable oils. The presence of 1.35% octacosane, a long-chain alkane, may reflect the adaptation of plants to adverse environmental conditions, such as high temperatures or arid environments [15].

For leiteira (*Tabernaemontana catharinensis*), no studies have specifically addressed the extraction of oils from its seeds. Previous studies, such as those by Boligon et al. [11] and Pereira et al. (2004, 2005) [27,28], have focused on the extraction of bioactive compounds from other parts of the plant, including the leaves, stems, and flowers, using different methods. Boligon et al. [11] reported the presence of phenolics, flavonoids, and tannins in extracts from stem bark, while Pereira et al. [27,28] highlighted the extraction of indole alkaloids, such as coronaridine and voacangine, using supercritical CO₂ with ethanol as a co-solvent. These pharmacologically significant compounds differed substantially from the lipophilic compounds extracted from seeds in this study. The lack of studies on seed oil

extraction limits direct comparison with the literature. However, this study provides an initial contribution to the characterization of seed oil from leiteira.

Chemical diversity of oils reflects the adaptation of plants to environmental conditions. In leiteira oil, the presence of 1.35% octacosane, a long-chain alkane, may be associated with plant adaptation to adverse conditions such as high temperatures or arid environments [15]. Bacupari oil stands out for its high concentration of antioxidant compounds, such as delta-tocopherol, which enhances its potential for cosmetic and preservative formulations. These findings suggest that supercritical CO₂ extraction is an efficient method for obtaining bioactive compounds under mild operational conditions, offering significant potential for optimization without compromising the performance.

5. Conclusions

Supercritical CO₂ extraction proved to be efficient for obtaining oils from bacupari and leiteira seeds, with most compounds extracted in less than 30 min, highlighting its reduced processing time compared to the Soxhlet method. Variations in temperature and pressure resulted in small differences in the yields, allowing the process to be conducted under milder conditions. Bacupari oil contains 35.05% oleic acid and 19.66% delta-tocopherol, offering antioxidant potential and applications in cosmetics and functional foods. Leiteira oil, on the other hand, is notable for its 29.17% hexanedioic acid and 7.95% stigmast-5-ene contents, with potential applications in the industrial and pharmaceutical sectors. Both plant matrices, native to the Pontal do Paranapanema region of the Atlantic Forest biome (São Paulo, Brazil), underscore the importance of exploring Brazilian biodiversity for the development of innovative products.

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