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Determination of process parameters, chemical composition and antioxidant activity of *Calycolpus goetheanus* (O. Berg) extract obtained by supercritical CO_2

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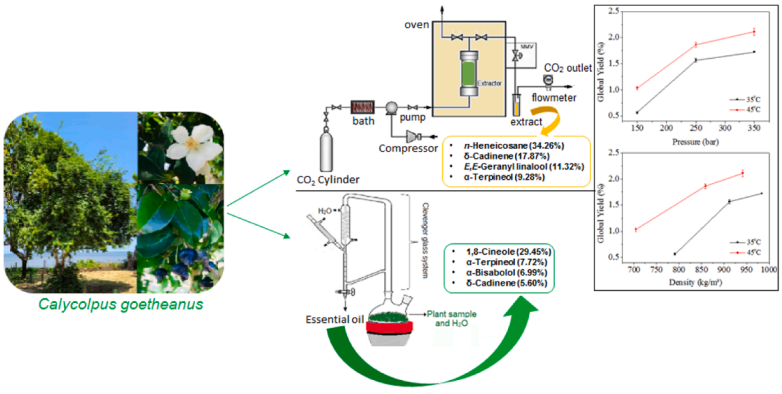
HIGHLIGHTS

- First research to report extraction of *Calycolpus goetheanus* using $scCO_2$.
- Plant extracts were produced by hydrodistillation and supercritical extraction.
- Extraction with different operating conditions of pressure and temperature.
- $scCO_2$ extraction produced a unique chemical profile with n-Heneicosane.
- Extracts obtained by $scCO_2$ demonstrated superior antioxidant activity compared to HD.

GRAPHICAL ABSTRACT



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ABSTRACT

This pioneering study is the first to explore the extraction of Calycolpus goetheanus using supercritical carbon dioxide ($scCO_2$), novel approach to harnessing the bioactive potential of this Amazonian species. By examining extractions under varying temperatures (35 and 45 °C) and pressures (150, 250, and 350 bar), the research not only evaluates the yields but also uncovers significant differences in chemical composition and antioxidant activity compared to traditional hydrodistillation. Notably, the highest yield (2.11 ± 0.07 %) was achieved under 45 °C and 350 bar conditions, where *n*-heneicosane (34.26 %) was the dominant compound, alongside δ-cadinene, α-terpineol, and other important terpenes and fatty alcohols. The $scCO_2$ extracts demonstrated superior antioxidant activity compared to hydrodistillation, highlighting the method's ability to preserve and concentrate

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bioactive compounds. This research advances the field by showcasing $scCO_2$ as a greener and more efficient extraction technique, with potential applications in pharmaceuticals, cosmetics, and nutraceuticals, opening new pathways for the sustainable exploitation of Amazonian biodiversity.

1. Introduction

Calycolpus goetheanus (O. Berg), a relatively unexplored shrub and fruit species native to the Brazilian Amazon, is commonly known as "Araçá or suim," "beach plum," "Murta," and "goiabinha do mato." It belongs to the *Myrtaceae* family, which encompasses numerous species recognized for their rich chemical profiles and bioactive properties [1–4]. Plants in this family produce essential oils rich in phenolics, terpenes, and flavonoids with a broad spectrum of pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory properties [5,6]. Despite the potential of *C. goetheanus*, limited research has been conducted on its phytochemical composition and biological activities, with most studies focusing on its essential oils obtained by traditional extraction methods [7–9].

The antioxidant potential of plant extracts and essential oils has garnered considerable attention due to their ability to neutralize free radicals and protect against oxidative stress-related diseases [10]. Antioxidant activity is primarily attributed to the presence of phenolic compounds and terpenes, which are known for their free radical scavenging, metal chelating, and lipid peroxidation inhibitory [11,12]. In a previous study by Franco et al. [7], *C. goetheanus* essential oil demonstrated a high capacity for eliminating free radicals, with activity mainly attributed to sesquiterpene hydrocarbons such as (*E*)-caryophyllene, germacrene D, and δ-cadinene, along with oxygenated sesquiterpenes like γ-eudesmol, α-cadinol, and epi-α-muurolol.

The chemical composition and bioactivity of essential oils and plant extracts can be significantly influenced by the extraction method employed. Conventional methods, such as hydrodistillation (HD), often involve high temperatures and prolonged processing times, which can result in the degradation or transformation of thermolabile constituents [13,14]. In contrast, supercritical fluid extraction using carbon dioxide ($scCO_2$) has emerged as a green and efficient alternative for obtaining high-quality plant extracts. $scCO_2$ extraction operates at relatively low temperatures, preserving heat-sensitive bioactive compounds while eliminating the need for organic solvents, thus offering a more sustainable approach [15–17].

Supercritical CO_2 ($scCO_2$) extraction is particularly advantageous due to the tunable nature of its parameters, such as pressure, temperature, and solvent flow rate, which can be optimized to selectively target specific compounds in the matrix [18,19]. Several studies have successfully applied $scCO_2$ extraction to species within the *Myrtaceae* family, obtaining extracts with high yields and enhanced antioxidant activities. For instance, Frohlich et al. [20] optimized $scCO_2$ extraction conditions for *Eugenia uniflora*, showing that increased pressure facilitated the extraction of terpenes, while lower temperatures preserved phenolic content. Similarly, Haro-González et al. [21] demonstrated that varying the pressure and temperature during $scCO_2$ extraction of *Psidium guajava* altered the concentration of antioxidant-rich flavonoids in the extracts. Barzotto et al. [22] also highlighted the impact of $scCO_2$ parameters on the extraction efficiency and antioxidant activity of *Myrciaria dubia*, suggesting that temperature and pressure play a crucial role in balancing yield and bioactivity.

These previous studies help establish a foundation for determining optimal extraction conditions for *C. goetheanus*. In particular, research on related *Myrtaceae* species shows that moderate pressure and low temperatures are often ideal for preserving the integrity of phenolic compounds while maximizing the extraction of bioactive terpenes [19–21].

Despite the successful application of $scCO_2$ extraction in various *Myrtaceae* species, no studies to date have investigated the extraction of bioactive compounds from *C. goetheanus* using $scCO_2$. This study will focus on the distinct phytochemical profile of *C. goetheanus*, which remains largely unexplored. Unlike previous investigations that primarily evaluated the extraction of essential oils or single compound groups, this study aims to assess the impact of $scCO_2$ extraction variables on a broader range of bioactive compounds, including terpenes.

Given the lack of research on *C. goetheanus* extracted by $scCO_2$, the present study aims to evaluate the influence of process variables (temperature and pressure) on the yield, chemical composition, and antioxidant activity of *C. goetheanus* extracts obtained by $scCO_2$. Additionally, the extracts will be compared to essential oils obtained by HD to better understand the impact of extraction methods on the bioactive profile and antioxidant properties of this promising species.

2. Material and methods

2.1. Plant material

The aerial parts of *Calycolpus goetheanus*, containing leaves, branches, and, in smaller quantities, fruits, were collected from a wild specimen in the city of Salvaterra, Marajó Archipelago, Pará, Brazil (0°45'18.7"S 48°30'41.8"W). The taxonomic identification of the specimen was carried out in collaboration with a specialist in the family and subsequently deposited in the IAN herbarium of Embrapa Amazônia Oriental, located in the city of Belém, in Pará, Brazil, under the voucher Mota, RV 01.

2.2. Preparation and characterization of the raw material

The collected sample was dried by freeze-drying. For this, the material with the aerial parts of the plant (leaves, branches and fruits) were reduced into smaller parts, mixed and previously frozen at −18 °C for 24 h and then subjected to freeze-drying (Terroni, model LH/E). Subsequently, the dried material containing leaves, branches and fruits were ground together in a knife mill (Marcone, model MA1340) for approximately 10 s. The samples containing the aerial parts of the plant, used in the extraction experiments, were selected using stainless steel TYLER sieves in the 20–35 mesh range. Moisture content was measured on an infrared moisture analyzer (Marte científica, model V1.8). The actual density of the particles was determined using an automatic helium gas pycnometer from the Chemistry Institute of the State University of Campinas (Unicamp). The apparent density was calculated by relating the mass of the sample used in the extraction to the volume of the extraction cell. The porosity of the bed was calculated using the mathematical relationship between the apparent density and the real density. All experiments were performed in triplicate.

2.3. Hydrodistillation (HD)

C. goetheanus extraction by hydrodistillation (HD) was carried out in a Clevenger glass system, using 100 g of sample, containing the aerial part of the plant. The extraction period was 10,800 s at a temperature of 100 °C, with the device coupled to a refrigeration system to keep the condensation water close to 12 °C. After the extraction time, the extract obtained was centrifuged for 300 s at 2500 rpm, dehydrated with anhydrous sodium sulfate and centrifuged again for another 300 s. The mass yield of the extract was calculated on a dry basis (db) by relating the mass of oil obtained in HD with the dry mass used in the extraction process.

2.4. Soxhlet extraction (SE)

To compare the mass yields, an extraction with n-hexane (95 %, Vetec, Brazil) was performed in a Soxhlet apparatus, according to AOAC method 920.39 C [23]. The ground mass containing the aerial parts of *C. goetheanus* used in the extraction was 5 g. A volume of 0.150 L of solvent was used for the extraction process. The solute/solvent mixture was maintained under reflux at the boiling temperature of the solvent in a Soxhlet apparatus for 18,000 s. The extracts obtained were rotoevaporated (Laborota 4000, Heidolph, Germany) under vacuum of 400 mmHg and rotation of 30 rpm, until complete elimination of *n*-hexane. The yield was determined from the ratio between the mass of the extract and the mass of the sample and calculated on a dry basis.

2.5. Supercritical CO_2 extraction ($scCO_2$)

Extractions to obtain the *C. goetheanus* extract occurred using supercritical carbon dioxide ($scCO_2$) through the Spe-edTM SFE system (model 7071, Applied Separations, USA). The volumetric capacity of the extractor vessel used was 100 mL (with an internal diameter of 0.0317 m and a height of 0.1244 m). Isotherms were determined using approximately 8 ± 0.1 g of sample, containing the aerial part of the plant. Extractions took place at temperatures of 35 and 45 °C and pressures of 150, 250 and 350 bar. The extraction period was divided into two stages: a static period of 1800 s and a dynamic period of 1800 s. During the second period, the CO_2 output was kept constant at 8.85 × 10−5 kg/s, with the extract and CO_2 released continuously into an amber penicillin vial closed with a rubber cap and metal seal, placed in an ice bath to avoid volatilization of the compounds. The $scCO_2$ density was calculated using software developed by the National Institute of Standards and Technology, which uses the equation of state developed by Span and Wagner [24]. The overall yield was calculated as the ratio between the mass of oil obtained and the initial mass value of the raw material (on a dry basis) used to obtain the *C. goetheanus* extract.

2.6. Chemical composition of extracts

The chemical compositions of *C. goetheanus* extracts obtained by HD and under different $scCO_2$ conditions were evaluated according to the methodology described by Oliveira et al. [25], using gas chromatography/mass spectrometry (QP-2010 plus system, Shimadzu, Japan), under the following conditions: silica capillary column Rtx-5MS (30 m×0.25 mm, 0.25 μm film thickness), program temperature of 60–240 °C (3 °C/min), injector temperature of 250 °C, as carrier gas helium (linear velocity of 32 cm/s, measured at 100 °C), and splitless injection (1 μl of a 2:1000 hexane solution). Ionization was obtained by the electronic impact technique at 70 eV, and the temperature of the ion source and other parts was 200 °C. The quantification of volatile substances was determined by gas chromatography with a flame ionization detector (FID) (Shimadzu, QP 2010 system), under the same conditions as gas chromatography coupled to mass spectrometry (GC–MS), with hydrogen used as the carrier gas. The retention index was calculated for all volatile substances using a homologous series of n-alkanes (C8 – C20), and they were identified by comparison of their mass spectra and retention indices to those in the literature [26].

2.7. Antioxidant activity

2.7.1. DPPH radical assay

The evaluation of antioxidant activity was carried out by capturing the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH), according to the methodology described by Brand-Williams et al. [27], with adaptations. The *C goetheanus* extracts obtained by HD and under the different $scCO_2$ conditions were diluted in 99.5 % methanol (Merck, Brazil) at concentrations of 100, 70, 60, 50 and 30 μg/mL. Then, 100 μL of solution of each concentration were added to 3.9 mL of solution containing the DPPH radical (10 mg/L). After 1800 s protected from light and room temperature, a reading was taken at 515 nm on an ultraviolet-visible spectrophotometer (model Evolution 60S, Thermo Scientific). The construction of the analytical curve was carried out at concentrations of 10–60 μM of the DPPH radical. Analyzes were performed in triplicate and the calculation of the percentage of DPPH inhibition was evaluated as described by Sánchez-Moreno et al. [28].

2.7.2. ABTS radical assay

The antioxidant capacity of *C. goetheanus* extracts obtained by HD and under different SC-CO_2 conditions was determined using the Trolox Equivalent Antioxidant Capacity (TEAC) method, as proposed by Re et al. [29], with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich Co., St. Louis, USA) as the reference antioxidant. The ABTS radical was dissolved in distilled water to a concentration of 7.0 mM and reacted with 2.45 mM potassium persulfate to form the radical in solution at a 1:1 ratio. The solution was stored in the dark prior to use. The ABTS radical solution was diluted in ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. Different dilutions of the extracts were prepared, and 30 μL aliquots of each dilution were transferred to test tubes, followed by the addition of 3 mL of the ABTS radical solution, and kept protected from light for 360 s. Readings were taken using a spectrophotometer at 734 nm absorbance. As a reference, an analytical curve was prepared with Trolox at concentrations of 0.01–0.20 mg/mL, and the results were calculated and expressed as mg of Trolox/mL [25].

2.8. Statistical analysis

The experimental tests to obtain the extracts were carried out in duplicate. To verify the existence of a significant difference between the $scCO_2$ conditions, the mean results were subjected to analysis of variance and, when significantly different, compared using the Tukey test with a 95 % confidence level, using the STATISTIC®7.1 software. (Statsof, Inc., Tulsa, USA).

3. Results and discussion

3.1. Raw material characterization

The step prior to obtaining the *C. goetheanus* extracts by HD and $scCO_2$ consisted of freeze-drying the raw material containing the aerial parts of the plant, with the aim of avoiding possible losses of volatile compounds during the conventional drying process, followed by grinding. The moisture content of the fresh raw material (leaves, branches and mixed fruits) was 65.54 ± 0.20 %, and after freeze-drying, it was reduced to 10.88 ± 0.10 %, on a dry basis. According to Brunner [30], reducing the moisture content of the raw material can contribute to enhancing contact between the supercritical solvent and the solid surface of the plant matrix, generating benefits for mass transfer throughout the extraction process. Another important operational aspect calculated was the bed porosity (ε), which presented a value of 0.8112 from a feed mass of 8 ± 0.1 g of dry and crushed raw material. This parameter can vary between values that range from 0.0 to 1.0, however, the appropriate value will depend on the research objectives.

3.2. Global yields of HD, SE and $scCO_2$

Table 1 presents the statistical differences ($p < 0.05$) between the extraction yields obtained by the different methods (HD, SE and $scCO_2$), as well as in the different operational conditions of the $scCO_2$ process (temperature, pressure and density). The only extraction conditions that did not present significant differences between them were $scCO_2$ at 35 °C/350 bar (1.72 ± 0.01 %) and 45 °C/250 bar (1.86 ± 0.04 %); all the others presented statistically significant differences between them ($n = 8$; $p < 0.05$). The HD extraction presented the lowest yield

Table 1
Means of the mass yields of extracts from *C. goetheanus* obtained by hydrodistillation (HD), soxhlet extraction (SE) and different conditions of supercritical carbon dioxide extraction ($scCO_2$).

Process conditions	Yield (%)	Density (kg/m^3) $scCO_2$
HD	0.40 ± 0.03^g	
SE	7,74 ± 0.03^a	
35 °C/ 150 bar	0.56 ± 0.01^f	790.95
35 °C/ 250 bar	1.57 ± 0.04^d	912.86
35 °C/ 350 bar	1.72 ± 0.01^c	984.48
45 °C/ 150 bar	1.03 ± 0.03^e	704.10
45 °C/ 250 bar	1.86 ± 0.04^c	858.73
45 °C/ 350 bar	2.11 ± 0.07^b	941.86

Mean ± standard deviation, different letters indicate significant difference between values ($p < 0.05$) by Tukey test.

(0.40 ± 0.03 %), while the extraction with n-hexane (7.74 ± 0.03 %) presented the highest yield. The $scCO_2$ extraction yields varied significantly between the different process conditions, obtaining the lowest (0.56 ± 0.01 %) and highest (2.11 ± 0.07 %) yields under the extreme conditions of the $scCO_2$ process, respectively at 35 °C/150 bar and 45 °C/350 bar. Similar behavior was reported by Danh et al. [31] and Oliveira et al. [25].

In the literature, there are only three reports of essential oil extraction yields from *C. goetheanus*, all obtained by hydrodistillation. Pereira et al. [5] obtained a yield of 1.0 % in the extraction of essential oil from *C. goetheanus* from the northeastern region of the state of Pará, Brazil. Santos et al. [6], when studying the seasonal and circadian influences on the essential oil yield of *C. goetheanus* from Marajó Island (Amazon), observed yields ranging from 1.2 % to 2.4 %. Franco et al. [7], when evaluating the essential oil yield of three specimens of *C. goetheanus* from the Amazon region, obtained yields of 1.10 %, 0.69 %, and 0.20 %. This variation in extraction yields may be related to soil and climate conditions, as well as the period and time of collection of plant material, as these are factors that can significantly influence the yield of plant extracts from aromatic plants, in addition to their chemical composition, both qualitatively and quantitatively [6,32].

In general, HD may present some disadvantages, such as low yield, caused by the boiling point of the mixture being lower than that of water and essential oil, in addition to the possibility of chemical changes and loss of volatile compounds due to thermal degradation, and the long time required for extraction [13–15]. Although extraction with organic solvents usually presents high yields compared to HD and $scCO_2$, it also has some disadvantages, such as the extraction of non-lipid compounds of greater polarity, such as chlorophyll, long extraction times, and obtaining extracts with organic solvent residues, which reduces the quality of the final product. This is different from what is observed in extracts obtained by green technologies, such as supercritical extraction [15–17]. $scCO_2$ extraction can produce extracts with higher amounts of bioactive compounds compared to HD. Etudies have shown that $scCO_2$ extraction can selectively extract fatty acids, carotenoids, and phenolic compounds that are not typically found in essential oils produced by conventional methods such as HD. In comparison, HD is more efficient at extracting compounds such as 1,8-cineole, which are found in high concentrations in many aromatic plants, but may not efficiently recover other bioactive components present in the plant matrix [14,17].

The overall yields of extracts containing the aerial parts of *C. goetheanus* obtained by $scCO_2$ are shown in Fig. 1, through the extraction isotherms. It can be observed that the density of the solvent did not favor the extraction process, because although the density was higher at all points in the 35 °C isotherm, it was where it presented the lowest yields. On the other hand, in the 45 °C isotherm, it was observed that as the pressure increased, the density of the solvent increased and consequently, the solubility of CO_2 increased. Therefore, the extraction yield was significantly influenced by temperature, which, according to Brunner [30], is a variable that can be controlled in order to enhance the

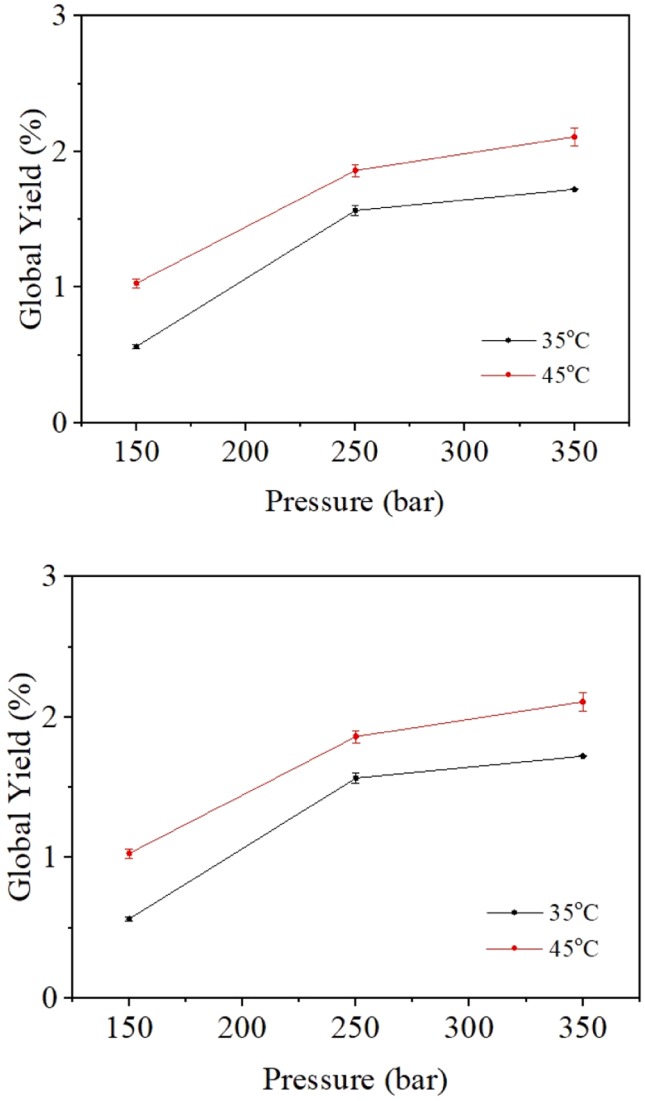

Fig. 1. Curves isotherms for the global yield of *C. goetheanus* leaves extracts obtained by $scCO_2$.

solvation capacity of the solvent in the plant matrix, contributing to obtaining higher extraction yields. Thus, our findings highlight that temperature, together with pressure, significantly impacts the solvation power of supercritical CO_2, with higher temperatures promoting better yields due to increased solubility. This emphasizes the importance of carefully selecting temperature and pressure conditions to maximize the extraction efficiency of bioactive compounds from the plant matrix under study.

As can be seen in Table 1 and Fig. 1, CO_2 density decreases with increasing temperature when pressure is constant, indicating higher extraction yields at lower densities for the same pressure condition. As for the effect of pressure on the process, it was observed that in the 45 °C isotherm, there was an approximately two-fold increase in the extraction yield was observed when increasing the pressure from 150 to 350 bar, highlighting the dominant effect of solvent density, as previously described. At the 35 °C isotherm, an even greater increase is observed, with an increase of more than three times in the extraction yield when increasing the pressure from 150 to 350 bar.

3.3. Chemical composition of extracts

The chemical compositions of the extracts of the dried aerial parts of

C. goetheanus, obtained by HD and $scCO_2$ under different operating conditions, are shown in Table 2. In total, 87 compounds were identified and quantified by gas chromatography coupled to GC-MS mass spectrometry. The compounds obtained by HD and $scCO_2$ were distributed, respectively, in the following groups: monoterpene hydrocarbons (HD: 0.21 %), oxygenated monoterpenes (HD: 38.58 %; $scCO_2$: 8.11–16.79 %), sesquiterpene hydrocarbons (HD: 28.72 %; $scCO_2$: 36.82–56. 83 %), oxygenated sesquiterpenes (HD: 27.03 %; $scCO_2$: 7.95–18.12 %), oxygenated diterpenes (HD: 0.85 %; $scCO_2$: 4.00–14.61 %), primary fatty alcohol (HD: 0.75 %; $scCO_2$: 2.78–7.58 %), alkane ($scCO_2$: 34.26 %) and others (HD: 1.37 %; $scCO_2$: 0.26–1.03 %). Thus, it was observed that extraction by HD shows a higher concentration of compounds distributed in oxygenated monoterpenes and oxygenated sesquiterpenes compared to extraction by $scCO_2$. The latter shows a higher concentration of compounds distributed in sesquiterpene hydrocarbons, oxygenated diterpenes, primary fatty alcohol and alkane.

As observed in Table 2, the chemical composition of essential oils obtained by HD and plant extracts obtained by $scCO_2$ extraction differed significantly due to the distinct extraction mechanisms and the specific selectivity of each method. The main constituents identified, which had average concentrations (% area) of more than 3 %, for HD extraction were 1,8-cineole (29.45 %), α-terpineol (7.72 %), α-bisabolol (6.99 %) and δ-cadinene (5.60 %), and for $scCO_2$ extraction were *n*-heneicosane (34.26 %), obtained exclusively in the extreme process condition (45 °C/ 350 bar), followed by δ-cadinene (11.91–17.87 %), *E,E*-geranyl linalool (4.00–11.32 %), α-terpineol (6.22–9.28 %), α-bisabolol (4.77–8.14 %), *n*-hexadecanol (2.78–7.58 %), 1,8-cineole (1.62–7.51 %), α-copaene (4.62–7.00 %), β-selinene (4.14–6.65 %) and α-selinene (2.95–4.66 %), these with significant variations in concentration between the different operating conditions.

This is the first work in the literature that describes the chemical composition of the *C. goetheanus* extract obtained by $scCO_2$. Other studies that described the chemical composition of the essential oil of *C. goetheanus*, but obtained by HD, were by Franco et al. [7], when evaluating three species of C. goetheanus collected in the municipality of Magalhães Barata, Pará, Brazil, Santos et al. [6] who studied the seasonal and circadian influences on the composition of the essential oil of *C. goetheanus* collected on Marajó Island, Pará, Brazil and by Pereira et al. [5], when evaluating the chemical composition of the essential oil of *C. goetheanus* collected in the municipality of Maracanã, Pará, Brazil.

Thus, it is observed that, although extraction by $scCO_2$ indicates a higher concentration of sesquiterpene hydrocarbon compounds (36.82–56.83 %), close to the results in which the extracts were obtained by HD, described by Franco et al. [7] (43.6–60.17 %) and Santos et al. [6] (16.8–46.6 %), extraction by $scCO_2$ shows greater selectivity to obtain more non-polar compounds, such as *n*-heneicosane and *n*-hexadecanol. This may be related to the solubility characteristics of CO_2 and the pressure used in the process [15].

The differences in the chemical composition of *C. goetheanus* extracts obtained by HD and by $scCO_2$ can be attributed to the nature of the extraction methods, which target different compounds based on their polarity, molecular weight and volatility [16]. HD typically captures volatile compounds such as monoterpenes and sesquiterpenes. This technique tends to extract compounds with lower boiling points and higher water solubility. As a result, essential oils obtained by this method are often dominated by lighter aromatic molecules such as 1, 8-cineole, as observed in the present study [14,16,25].

In contrast, $scCO_2$ extraction is known for its tunable solubility properties, which depend on variations in temperature and pressure [18, 19]. This allows $scCO_2$ extraction to target a broader range of bioactive compounds, including non-volatile and thermally sensitive compounds such as fatty acids, waxes, and high molecular weight terpenoids, which are generally not present in oils obtained by hydrodistillation [14,15, 17]. Furthermore, $scCO_2$ extraction at higher pressures and moderate temperatures, as used in the present study, helps to retain thermolabile compounds, which may degrade at the higher temperatures used in HD. This results in a wider range of bioactive compounds, including primary fatty alcohols such as *n*-hexadecanol, which were found in higher concentrations in $scCO_2$ extracts. As observed in the present study, where $scCO_2$ extraction resulted in a higher concentration of sesquiterpene hydrocarbons (36.82–56.83 %) and oxygenated diterpenes (4.00–14.61 %), which are less volatile and not easily extracted by HD. Similar results were described by Bezerra et al. [15], Oliveira et al. [25], Danh et al. [31] and Silva et al. [33].

Based on the chemical composition of the essential oil of *C. goetheanus* obtained by HD, whose main compound is 1,8-cineole, there is a possibility of application in different industrial sectors, since studies with other species that also have 1,8-cineole have demonstrated its antimicrobial, analgesic, mucolytic, antiasthmatic, anti-inflammatory and antihypertensive activities [34–36]. The plant extract of *C. goetheanus* obtained by $scCO_2$, which presented *n*-Heneicosane as the main compound, can have wide application in the industrial market, being used as an emollient, emulsifier and thickening agent in skin creams, or as a new emulsifier in biofertilizers [37]. Another activity of *n*-heneicosane is its antimicrobial action against *Streptococcus pneumoniae* and *Aspergillus fumigatus*. These antimicrobial and anti-inflammatory properties may make the extract valuable in the development of pharmaceutical products, particularly for treating infections or inflammatory conditions [38].

Although *n*-heneicosane and *n*-hexadecanol are compounds rarely found in extracts obtained by hydrodistillation, due to their high molecular weight, the literature still reports the presence of *n*-heneicosane in essential oil extracted by HD from the plant species *Trembleya phlogiformis*, this being the main compound identified in the study [39].

3.4. Antioxidant activity

The free radical scavenging effect of the extracts of the aerial parts of *C. goetheanus* obtained by HD and $scCO_2$ was evaluated (Table 3). The antioxidant capacities of the extracts obtained under the different operating conditions were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS (TEAC) radical scavenging tests. DPPH scavenging activity is generally presented by the IC_{50} value and is defined as the amount of sample required to reduce the initial DPPH concentration by 50 %. A lower IC_{50} value represents better antioxidant activity. Thus, the extract obtained by $scCO_2$ at 35 °C/150 bar had the lowest IC_{50} (120.54 ± 6.81 μg/mL) and the highest inhibition value (66.82 ± 0.81 %), with no significant difference ($p < 0.05$) for the extract obtained at 45 °C/150 bar (125.35 ± 6.63 μg/mL). This same behavior was observed for the other $scCO_2$ operating conditions. In the present study, it was observed that the extracts with the highest antioxidant activities were those obtained under the mildest temperature and pressure conditions. Similar behavior was described by Cunha et al. [40] and Bezerra et al. [15].

The extract obtained by HD shows the highest IC_{50} value (334.87 ± 7.51 μg/mL) and the lowest inhibition percentage (25.04 ± 0.81 %). According to the results obtained, extracts obtained by HD generally present lower antioxidant activity compared to $scCO_2$ extracts, which can be attributed to the low content of active components. During the extraction of essential oils by HD, the antioxidant activity is partially lost. These essential oils contain volatile compounds, which generally have low antioxidant activity and can be applied to a limited extent in the general industry as natural antioxidants [41]. Studies have shown that the antioxidant activity of extracts obtained from the same plant, but by different methods, can significantly influence the antioxidant capacity of the extracts [15,41]. This can be explained by the moderate volatility and partial water solubility of the phenolic components, which are partially lost during HD [11].

The antioxidant capacity of *C. goetheanus* extracts by TEAC shows that the fractions obtained by HD and $scCO_2$ were also efficient in scavenging the ABTS radical. A significant difference ($p < 0.05$) was

Table 2
Chemical composition of *C. goetheanus* extracts obtained by hydrodistillation (HD) and different conditions of supercritical carbon dioxide extraction ($scCO_2$).

RI	Constituents	Concentrations (% Area)*						
		HD	scCO2					
			35 °C			45 °C		
			150 bar	250 bar	350 bar	150 bar	250 bar	350 bar
1032	**1,8-Cineole**	**29.45**	**1.62**	**1.37**	**4.71**	**7.51**	**7.35**	**5.90**
1055	γ-Terpinene	0.10						
1083	Terpinolene	0.11						
1100	Linalool	1.03		0.28	0.47			
1166	Isoborneol	0.01						
1170	Borneol	0.35						
1180	Terpinen−4-ol	0.63		0.24	0.38			
1196	**α-Terpineol**	**7.72**	**7.05**	**6.22**	**9.25**	**9.28**	**9.03**	**8.03**
1199	γ-Terpineol	0.02						
1290	Safrole				0.44			
1165	Cyclosativene	0.61						
1368	α-Ylangene		0.76	0.68	0.71	0.85	0.80	0.53
1374	**α-Copaene**	**2.32**	**5.76**	**4.62**	**4.93**	**7.00**	**6.89**	**4.76**
1394	Sativene	0.12						
1401	β-Longipinene	0.05						
1405	α-Gurjunene	0.16	0.20	0.22	0.19			
1411	**α-**Santalene	0.08						
1417	*E*-Caryophyllene	2.13	4.18	3.63	3.74	4.58	4.37	2.94
1430	*trans*-α-Bergamotene	0.04						
1439	6,9-Guaiadiene	0.62	0.66	0.90	0.86	0.67	0.61	0.40
1444	*cis*-muurola−3,5-diene	0.24						
1447	Isogermacrene D	0.32	0.29	0.41	0.38	0.30	0.30	0.20
1452	*E*-β-Farnesene			0.18	0.14			
1453	α-Humulene	0.73	0.58	0.70	0.65	0.63	0.57	0.39
1458	*allo*-Aromadendrene	0.16		0.21	0.15			
1464	Isobornyl *n*-butanoate	0.62		0.11	0.11			
1471	Selina−4,11-diene	1.10	0.58	0.58	0.48			
1473	Germacrene D		0.32	0.24	0.25			
1477	α-Amorphene	0.49	0.41	0.41	0.38			
1480	α-Curcumene	0.26	1.16	0.82	0.83	0.99	0.87	0.65
1482	γ-Muurolene	0.16						
1487	**β-Selinene**	**2.57**	**6.15**	**4.64**	**4.66**	**6.08**	**5.97**	**4.14**
1491	γ-Amorphene	0.14		0.17				
1494	α-Selinene	1.71	4.66	3.56	3.66	4.43	4.34	2.95
1496	α-Muurolene	0.68	1.38	1.09	1.13	1.42	1.3	0.97
1501	δ-Amorphene	0.94		0.82	0.77			
1506	β-Bisabolene	0.69	3.16	2.42	2.38	3.17	3.01	2.16
1511	γ-Cadinene	0.85	2.31	1.79	1.72	2.18	1.97	1.47
1516	**δ-Cadinene**	**5.60**	**17.87**	**14.93**	**14.12**	**17.39**	**16.89**	**11.91**
1520	Zonarene	0.77	1.39	1.12	1.13	1.39	1.30	0.97
1526	(-)−7-*epi*-α-selinene	0.07						
1529	*trans*-Cadina−1,4-diene	0.20						
1535	α-Cadinene	0.27	0.30	0.30	0.24			0.16
1539	α-Calacorene	1.93	2.29	2.26	2.02	2.06	1.97	1.37
1559	*E*-Nerolidol	0.47						
1560	β-Calacorene		0.35	0.52	0.36	0.21	0.52	
1564	Longipinanol	0.08						
1567	Palustrol	0.19						
1574	Caryolan−8-ol	1.70		0.24	0.35			
1580	Caryophyllene oxide		0.28	0.22	0.22			
1582	α-Thujopsan−2-ol	0.77						
1584	Globulol	0.27		0.23	0.19			
1594	Viridiflorol	0.79	0.33	0.75	0.79			
1600	Sesquithuriferol	1.21						
1603	Ledol			0.34	0.40			
1607	Copaborneol	2.26	0.92	0.96	1.15	0.81	0.78	0.46
1616	Cedrol	0.72			0.26			
1620	Junenol	1.10	1.31	1.44	0.53	1.35	1.21	0.72
1622	Eremoligenol	0.25		0.46	0.28			
1626	1,10-*di*-*epi*-cubenol	1.10		0.83	0.90	0.60	0.90	
1633	1-*epi*-Cubenol	1.58	0.64	0.13	0.20		0.93	0.41
1635	γ-Eudesmol	1.48						
1638	*epi*-α-Cadinol	0.92						
1641	*epi*-α-Muurolol	0.81	0.42					
1643	*cis*-Cadin−4-en−7-ol				0.67			
1646	α-Muurolol	1.19	0.31	1.04	0.49			
1650	α-Eudesmol	2.00						
1653	Himachalol		1.07	1.27	1.35	1.05	1.45	0.55
1655	α-Cadinol	0.70						
1657	Selin−11-en−4-α-ol	1.67	1.85	2.07	2.61		2.25	1.04

(*continued on next page*)

Table 2 (*continued*)

RI	Constituents	Concentrations (% Area)*						
		HD	scCO2					
			35 °C			45 °C		
			150 bar	250 bar	350 bar	150 bar	250 bar	350 bar
1663	Bulnesol	0.11						
1671	Cadalene	1.62	1.57	1.94	1.54	1.36	1.24	0.85
1677	4(15),5,10(14)-Germacratrien−1-ol	0.10						
1685	**α-Bisabolol**	**6.99**	**7.10**	**8.14**	**6.71**	**7.75**	**7.55**	**4.77**
1696	Eudesm−7(11)-en−4-ol	0.09						
1699	10-*nor*-calamenen−10-one	0.33		0.14	0.14			
1711	5-*cis*-Hydroxy calamenene	0.03						
1712	(*E,Z*)-farnesol	0.11						
1801	β-Eudesmol acetate				0.18			
1816	Cryptomeridiol				0.17			
1836	Neophytadiene		0.31	0.64	0.49	0.47	0.45	0.26
1842	Phytone	0.04		0.18	0.13			
1882	***n*-Hexadecanol**	**0.75**	**6.12**	**7.58**	**5.57**	**5.13**	**4.97**	**2.78**
1911	5*E*,9*E*-Farnesyl acetone			0.23	0.09			
2022	***E,E*-Geranyl linalool**	**0.74**	**8.95**	**11.32**	**7.82**	**7.89**	**7.56**	**4.00**
2104	Phytol	0.11	2.79	3.29	2.50	1.69	2.79	
2111	***n*-Heneicosane**							**34.26**
Monoterpenes hydrocarbons		0.21						
Oxygenated monoterpenes		38.58	8.67	8.11	15.25	16.79	16.48	13.93
Sesquiterpene hydrocarbons		28.72	56.83	49.51	47.53	54.71	53.51	36.82
Oxygenated sesquiterpenes		27.03	14.23	18.12	16.74	13.32	14.24	7.95
Oxygenated diterpenoid		0.85	11.74	14.61	10.32	9.58	10.35	4.00
Primary fatty alcohol		0.75	6.12	7.58	5,57	5.13	4.97	2.78
***n*-Alkane**								34.26
Other Compounds		1.37	0.31	0.95	1.03	0.47	0.45	0.26
No identified		2.49	2.10	1.12	3.56			
Total		100	100	100	100	100	100	100

RI: Retention index (DB-5ms column). *Concentrations expressed in percentages relative to the área. Bold highlights the main constituents (average above 4 %)

Table 3
Antioxidant activity of *C. goetheanus* extracts obtained by hydrodistillation (HD) and different conditions of supercritical carbon dioxide extraction ($scCO_2$).

Process conditions	DPPH inhibition (%)	IC_{50} (μg/mL)	ABTS (mg Trolox/mL)
HD	25.04 ± 0.81 ^{f}	334.87 ± 7.51^{a}	195.56 ± 6.44 ^{f}
35 °C/ 150 bar	66.82 ± 0.81^{a}	120.54 ± 6.81^{d}	405.10 ± 9.31^{a}
35 °C/ 250 bar	58.08 ± 1.64^{c}	157.83 ± 8.64^{c}	386.13 ± 7.40^{c}
35 °C/ 350 bar	55.36 ± 0.79^{c}	209.35 ± 4.59^{b}	327.30 ± 10.34^{d}
45 °C/ 150 bar	63.35 ± 0.63^{b}	125.35 ± 6.63^{d}	396.11 ± 9.40^{b}
45 °C/ 250 bar	53.78± 0.94^{d}	147.31± 6.44^{c}	316.50 ± 11.22
45 °C/ 350 bar	51.54 ± 0.73^{e}	198.32 ± 7.73^{b}	294.68 ± 8.75^{e}

Mean ± standard deviation, different letters indicate significant difference between values ($p < 0.05$) by Tukey test.

observed between all results, with variations from 195.56 ± 6.44 mg Trolox/mL (extract obtained by HD) to 405.10 ± 9.31 mg Trolox/mL (extract obtained by $scCO_2$ at 35 °C/150 bar). According to Oliveira et al. [25], this difference may be related to variations in the chemical composition of the extract fractions, as shown in Table 2, indicating that the antioxidant activities presented may not be directly associated with the isolated action of secondary metabolites present in higher concentrations such as 1,8-cineole present in higher concentration in the extract obtained by HD, but may rather be related to the synergistic effect between the chemical constituents of the extracts. Thus, each compound can contribute in a different way to the results of antioxidant activity. In addition, similar behaviors for greater antioxidant capacity of extracts obtained by $scCO_2$ compared to HD were reported by Danh et al. [31], Sodeifian and Sajadian [41], Bezerra et al. [15], Oliveira et al. [25] and Reyes-Solano et al. [42].

4. Conclusions

This pioneering research demonstrates the effectiveness of extracting *C. goetheanus* using $scCO_2$, revealing that temperature and pressure conditions have a significant impact on both the overall yields and the chemical composition of the extracts. Extraction by $scCO_2$ shows good selectivity compared to extraction by HD, especially for the classes of sesquiterpene hydrocarbons and oxygenated diterpenoids. The results highlight the condition of 45 °C and 350 bar as the most efficient, providing the highest mass yield and the identification of *n*-Heneicosane as the predominant compound. In addition, the extracts obtained by $scCO_2$ exhibited higher antioxidant activity than the essential oil extracted by HD, suggesting the potential of this technique for obtaining bioactive compounds with industrial and pharmaceutical applications. These findings open up new perspectives for the use of $scCO_2$ in the extraction of Amazonian natural substances, contributing to the advancement of extraction technologies and the valorization of plant resources.

CRediT authorship contribution statement

Rafael Vitti Mota: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Nayara Janaina Neves da Silva:** Investigation, Methodology, Validation, Writing - original draft. **Eduardo Gama Ortiz Menezes:** Investigation, Methodology, Validation, Writing - review & editing. **Maria Eduarda Ferraz de Carvalho:** Writing - original draft. **Jean Maurício Leão Pinheiro:** Investigation, Methodology, Validation. **Diego Aires da Silva:** Investigation, Methodology. **Eloisa Helena de Aguiar Andrade:** Investigation, Methodology. **Raul Nunes de Carvalho Júnior:** Conceptualization, Validation, Resources, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declared that they have no conflicts of interest to this work. We declared that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Data availability

The data that has been used is confidential.

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