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A sustainable process for (−)-α-bisabolol extraction from *Eremanthus erythropappus* using supercritical CO_2 and ethanol as co-solvent

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Supercritical carbon dioxide (sc-CO_2) extraction of Candeia (*Eremanthus erythropappus*) wood was studied using ethanol as a co-solvent. This tree has a great potential for producing (−)-α-bisabolol, an interesting compound for the cosmetic and pharmaceutical industries because of its properties. Using a Box–Behnken experimental design, the effects of pressure, temperature, extraction time and co-solvent percentage on the global yield were systematically evaluated. Static and dynamic extraction modes were compared. The use of ethanol as co-solvent proved itself fundamental for raising the extracted percentage of the desired compound. Both static and dynamic modes provided extracts with (−)-α-bisabolol as the major compound, although dynamic system provided the best extraction yields. In the best experimental condition, an extraction yield equal to 0.74% was achieved, which is much higher than the yields obtained using the standard steam distillation reported in the literature. Pressure showed greater influence in the obtained (−)-α-bisabolol percentage by the dynamic mode, while temperature was very important in the static system.

1. Introduction

Essential oils are lipophilic liquids obtained from plants, usually by steam distillation [1]. They are responsible for the flavor and perform many of the functions involved in specific ecological interactions between species [2]. Most of the essential oils consist of mixtures of terpene and sesquiterpene hydrocarbons and oxygenated derivatives of phenylpropanoid [3].

Sesquiterpenes have been identified as the active compounds in various plants widely used in traditional medicine and have anti-infectious, anti-oxidant, anti-inflammatory, and anti-carcinogenic properties [4]. The compound (−)-α-bisabolol is a monocyclic sesquiterpene alcohol, whose structure is showed in Fig. 1, found in the essential oils of many plants, such as chamomile (*Chamommila maricaria*) [5], arnica (*Arnica amplexicaulis* and *Arnica chamissonis*) [6], and sage (*Salvia stenophylla*) [7].

Due to its properties, it has been widely used in cosmetic formulations. It is also an efficient enhancer that helps other compounds to be absorbed by the skin [8].

Candeia (*Eremanthus erythropappus*) is a Brazilian tree that is characteristic of biomes such as the Atlantic Forest and the Cerrado. These trees grow to form dense forest regions with low diversity, which makes them viable for sustainable forest management [9]. Commercial interest in this tree has grown substantially in recent years due to its great potential for producing (−)-α-bisabolol. Data found in the literature show that the oil content obtained via steam distillation of candeia wood varies from 0.3% to 0.5%, with the (−)-α-bisabolol content ranging from 60% to 95% [10–12].

Although traditional and widely used, the steam distillation process has some drawbacks, such as the high temperatures in which the biomass is placed; this results in thermal degradation or hydrolysis and altered flavor of the extracts [13], long processing times, low selectivity, and low extraction yields [14]. Moreover, according to chromatographic results showed in the supplementary material, when used at the industrial level, this technique causes a considerable waste of water and leads to the extraction of high levels of isovaleric acid, about 8.4%,[1] which requires an auxiliary treatment process for removal because of its unpleasant odor.

In recent years, there has been increasing interest in the use of green solvents to reduce the impact on health and the environment. In this context, supercritical carbon dioxide (sc-CO_2) has attracted much attention because it is environmentally friendly.

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[1] Information provided by the company ATINA Ativos Naturais, located in Pouso Alegre, Brazil.

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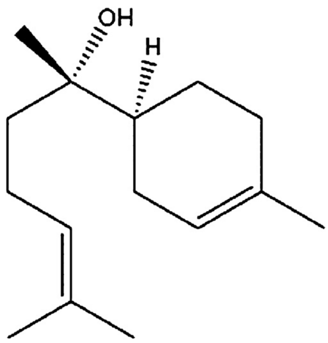

Fig. 1. (−)-α-Bisabolol structure.

Supercritical fluids typically have very desirable characteristics for use in extraction processes, such as high diffusivity, low viscosity, surface tension, and their density changes are strongly dependent on pressure and temperature [15–17]. CO_2 in particular has relatively low critical temperature and pressure; it is also inert and non-toxic and is the most widely used solvent in supercritical fluid extraction processes [18]. CO_2 has been used under supercritical conditions for obtaining the essential oil *E. erythropappus*, with the yield being investigated as a function of temperature and pressure [19,20]. However, the amount of (−)-α-bisabolol in these extracts is limited because of its low solubility in sc-CO_2. Sc-CO_2 has a dielectric constant equal to 1.5 (200 bar, 40 °C) and is a highly non-polar solvent appropriate for dissolving non-polar substances [21]. It is known that the addition of a small amount of a volatile co-solvent to a supercritical fluid can increase the solubility of a solute, a phenomenon known as the entrainer effect [22]. Recently, many authors [19,23–28] have shown that the addition of a polar co-solvent can improve both the selectivity and extraction yield of compounds from vegetal matrixes. Ethanol is used as a co-solvent for the extraction of natural products because it is not overly toxic to humans or the environment [29] and is easily recovered and reused.

This study used a Box–Behnken design to systematically investigate the effect of pressure, temperature, extraction time, and the entrainer effect of ethanol on the yield of (−)-α-bisabolol extracted from Candeia (*E. erythropappus*) under static and dynamic conditions.

2. Materials and methods

2.1. Samples

The Candeia raw material after grinding process, the standard (−)-α-bisabolol was provided by Atina Indústria e Comércio de Ativos Naturais S/A, located in Pouso Alegre, Minas Gerais, Brazil. The raw material, without any drying process, was stored at room temperature. The plant material had a humidity of 11.35 wt.% (mass of water/mass of raw material). Data regarding the particle size distribution are showed in supplementary material.

2.2. Soxhlet extraction

Candeia sawdust (6 g) was continuously extracted with 150 mL ethanol for 6 h in a conventional Soxhlet apparatus.

2.3. Experimental apparatus for supercritical CO_2 extraction

The equipment consists of a CO_2 reservoir (R-1) connected to a thermostatically controlled bath at −5 °C to liquefy the gas. A hydropneumatic pump, a Maximator M-111 Model (P-1), was used to inject liquid CO_2 until the desired working pressure was reached in the extraction vessel (EV-1) with 1 L of internal volume (a reduction measuring 3.4 cm in diameter and 23.2 cm in height, corresponding to an internal volume of 210.5 mL was also used). The vessel containing the material for extraction was then heated to the necessary temperature to perform the procedure. The temperature inside the vessel, as well as the inlet and outlet temperatures were continuously monitored. A Waters pump, model 515 (P-2), was used to inject the co-solvent from a reservoir (R-2) if necessary. Next, a block valve (V-4) was heated to prevent clogging of the pipe due to the viscosity of the extracted oil. Finally, the extracted material was collected in a container (C-1), and the CO_2 flow can be optionally directed to another container holding a NaOH solution in order to capture part of the released CO_2 and reduce emissions resulting from the extraction process. A schematic model of the apparatus is shown in Fig. 2.

2.4. Operational modes

Two types of supercritical fluid extraction were used. In the static method, the extraction cell was filled with the material for extraction in addition to the necessary volume of ethanol for each condition, and CO_2 was injected until the desired pressure was reached, keeping the V-3 valve closed. The extractor vessel was maintained at the chosen temperature for the corresponding time for each investigated condition. In the dynamic method, the cell containing the material for extraction was heated to the required temperature. Subsequently, CO_2 was injected until the desired working pressure was reached. Next, the material was continuously washed with CO_2 and ethanol at a flow rate previously chosen for the time corresponding to each condition.

2.5. Experimental design and statistical analysis

An experimental design was used to systematically evaluate, with minimal number of experiments, the influence of the independent variables (pressure, temperature, extraction time, and ethanol amount) and the interactions between them and extracted α-bisabolol content (Y, %, Eq. (2)). The influence of pressure (P, bar, coded variable X_1, Eq. (3)), temperature (T, °C, coded variable X_2, Eq. (4)), extraction time (t, h, coded variable X_3, Eq. (5)), and volume of ethanol (V, mL, coded variable X_4, Eq. (6a)) on the static mode/ethanol flow rate (E, mL/min, coded variable X_4, Eq. (6b)) and the dynamic mode was analyzed.

To do this, we used an experimental Box–Behnken design, which belongs to a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs [30]. A total of 27 experiments were carried out for each operational mode, with 24 experimental points with 4 levels and 3 replicates of the central point. In the static mode, an extra central point was made.

Statistical analysis was performed using the program MATLAB®. After performing the experimental design, the coefficients of the model that describes the experimental results for each operating mode were estimated by a least-squares regression. The model generated is generally described as:

$$\begin{aligned} Y = {} & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14} \\ & + b_{23}X_{23} + b_{24}X_{24} + b_{34}X_{34} + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2, \end{aligned} \quad (1)$$

where b_{ij} represents the coefficients related to each one of the variables and their interactions. The Eqs. (2)–(6b) relate the coded variables to the experimental data used.

$$\text{(-)-}\alpha\text{-bisabolol yield extraction} = Y\,(\%\,\text{wt.}) = \frac{\text{(-)-}\alpha\text{-bisabolol weight}}{\text{raw material weight}} \times 100 \quad (2)$$