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Extraction of *Arctium Lappa* leaves using supercritical CO_2 + ethanol: Kinetics, chemical composition, and bioactivity assessments

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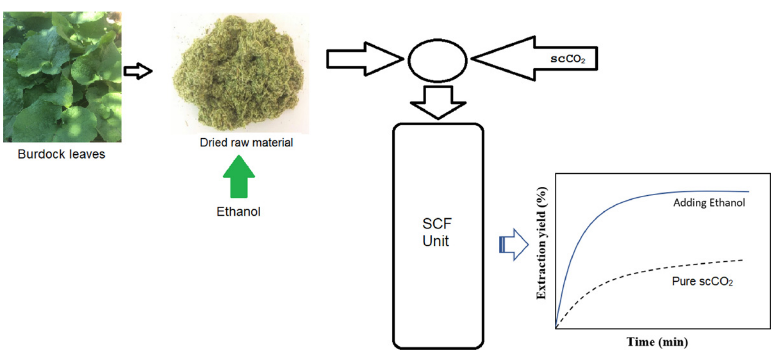
GRAPHICAL ABSTRACT



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ABSTRACT

This study reports the extraction of *Arctium lappa* leaves using $scCO_2$ with ethanol as solvent ($scCO_2$ + EtOH) aiming the recovery of bioactive components. Experiments were performed varying the temperature (313.15–353.15 K), pressure (15–25 MPa), and ethanol to CO_2 mass ratio in the static extraction step, where extraction yield, chemical composition, phenolic total content (TPC), antibacterial and antioxidant activity were determined. GC analysis showed diisooctyl phthalate, amyrin acetate, lupeol acetate and phytol as major compounds. It was observed the best relation between maximum yield (6.09%) and biological activities for the extract obtained at 353.15 K and 15 MPa. High values of antioxidant activity were found for the extracts obtained at 353.15 K and 15 MPa determined by the DPPH method and the phosphomolybdenum reduction method. The same extract presented the high phenolic content (35.51 mgGAE/gextract). High antibacterial activity was observed for all the extracts obtained with $scCO_2$ + EtOH against *Staphylococcus aureus*.

1. Introduction

Arctium lappa, commonly known as burdock or bardana (in Brazil), is a plant from Asteraceae family that has been considered a good source of bioactive compounds [1–4]. Extracts obtained from different parts of the *A. lappa* have been reported to present biological activities and pharmacological functions, including antioxidant activity [5–7], antibacterial activity [8,9], effect neuroprotective [10], anti-inflammatory [11,12], anti-diabetic and anti-cancer [4]. Thus, *A. lappa* is considered a promising plant for the treatment of chronic diseases, such

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as cancer, diabetes, and AIDS and due to the increasing evidence of functional compounds contributions over a variety of health beneficial properties the *A. lappa* has received increasing scientific interest.

In previous studies reported in the literature, the extracts from different parts of *A. lappa* have been assessed by distinct extraction methods, such as solvent extraction, Soxhlet extraction with ethanol, methanol and hexane solvents [13], ultrasonic and microwave extraction [14]. However, the conventional extraction methods, such as Soxhlet extraction, can be environmentally unfriendly due to the large amounts of organic solvents used, which are often expensive and potentially harmful [15]. Other disadvantages of conventional methods include long processing times, low selectivity (i.e., low-quality extracts) and in some cases thermally sensitive components are degraded by the heating process, resulting in low extraction yields and poor quality of the extracts [16]. These drawbacks can be overcome with the application of non-traditional technologies. Among these technologies, supercritical carbon dioxide extraction is an environment-friendly technology, which offers several advantages over conventional extraction techniques. Carbon dioxide (critical temperature of 304 K and critical pressure of 7.4 MPa) is the most commonly used supercritical fluid ($scCO_2$) solvent because it is inert, non-toxic, and allows extraction at low temperatures and at relatively low pressures [17]. Nevertheless, it is known that the solubility of supercritical carbon dioxide is more limited to the non-polar, non-volatile organic materials and also to some molecules with small and medium-sized [18], but the addition of a small amount of an organic solvent, such as ethanol, can increase the solubility of polar compounds in a phenomenon known as the entrainer effect [19]. Recently, some authors [20–25] have shown that the addition of a polar solvent in the $scCO_2$ can improve both the selectivity and extraction yield of compounds from vegetal matrixes. Ethanol improves the solubility of a variety of compounds due to the increase in the chemical forces, mainly the hydrogen bonds, or physical interactions of the solutes with the polar solvent. The addition of this solvent can also cause a swelling of the solid matrix, facilitating the solute transport from the interstitial pores to the surface of the matrix [26].

An interesting way of combining a polar solvent with supercritical CO_2 is using it as a gas-expanded liquid (GXL) procedure, in which a mixed solvent composed of a compressible gas dissolved in an organic solvent is obtained. The use of expanded liquids combines the beneficial properties of compressed gases such as CO_2 and organic solvents, leading to a new class of tunable solvents called gas expanded liquids (GXLs). Because of the safety and economic advantages of CO_2, CO_2-expanded liquids (CXL) are the most commonly used class of GXL. A large amount of CO_2 favors mass transfer and, in many cases, gas solubility, and the presence of polar organic solvents enhances the solubility of solid and liquid solutes [27].

Studies regarding the extraction of *A. lappa* matrix using supercritical fluids and the evaluation of biological activities of its extracts have been little investigated in the literature. In the previous study presented by Rodriguez et al. [7], the extraction of *A. lappa* roots with $scCO_2$ was investigated. The results demonstrated that the addition of methanol was necessary to reach suitable extraction yields (around 8%), and extracts with high antioxidant activity and total phenolic content were obtained.

In this study the main subject was to evaluate the potential of *A. lappa* leaves extracts obtained using CO_2-expande ethanol solvent (a CXL-type process), in terms of antioxidant activity, antibacterial activity, and total phenolic content. The variables temperature and pressure were also evaluated in order to assess the extraction yield and process information on the chemical composition of the volatile fraction and the bioactivity of the extracts. In addition, a first-order kinetic model, previously reported by Guedes et al. [25] was also applied in order to evaluate and better compare the extraction curves and process parameters.

2. Materials and methods

2.1. Materials

Whole aerial parts of burdock (*Arctium lappa*), including leaves and stems, were used as raw material in the present study. The plants were collected in July 2016 in a local property in Ivaiporã (State of Paraná, Brazil). All samples used in this study belong to a single lot harvested.

All reactants and solvents used in this work were of analytic grade. The carbon dioxide (> 99% purity in the liquid phase) was acquired from White Martins (Curitiba, Brazil). Ethanol (99.8% purity, Sigma–Aldrich), Folin–Ciocalteu phenol reagent (2N, Sigma–Aldrich), DPPH (99.9% purity, Sigma–Aldrich) and gallic acid (99.9% of purity, Sigma–Aldrich). All chemicals were used without further purification.

2.2. Sample preparation

A. lappa leaves were harvested, sanitized and dried in an air circulation oven at 323.15 ± 2.0 K for 24 h. The dried samples were ground in a domestic food processor. After this, the raw material was separated using different Tyler series sieves coupled to a mechanical stirrer (Produtest, São Paulo State, Brazil). The average particle diameter was estimated using the method described by Gomide [28], considering the mass fractions of milled material retained on the following sieves: 8 mesh (28.8 wt%), 12 mesh (14.93 wt%), 20 mesh (6.33 wt%), 24 mesh (13.45 wt%), 32 mesh (6.80 wt%), 48 mesh (9.03 wt%) and bottom (20.63 wt%). All fractions were blended, and the material was packaged in low-density polyethylene bags and sealed under vacuum and stored in a freezer at 268.15 ± 2.0 K until use.

2.3. Determination of moisture content and the particle density

Moisture content (*H*) of *A. lappa* leaves samples was measured by toluene distillation method according to AOCS method [29], which can distinguish water from the volatile material. The values were calculated as follows:

$$H = \left(\frac{V_{H_2O}\, x\, \rho_{H_2O}}{w_{sample}}\right) \times 100 \tag{1}$$

where V_{H_2O} is the measured volume of aqueous phase, ρ_{H_2O} is the water density and w_{sample} is the sample mass. The real density of the milled leaves was measured using a helium pycnometer at the Institute of Chemistry / Unicamp, Campinas, Brazil.

2.4. Soxhlet extraction

Soxhlet extractions were performed with ethanol as solvent, as a reference extraction procedure aiming the comparison of the extraction using compressed solvents (CLX). The extractions were carried out according to a method adapted from AOAC [30], using 5 g sample for 200 mL of solvent during six hours. All experiments were performed in triplicate to better assess the results. After the extractions, the solvent was rotary evaporated and then placed into an air circulation oven at 333.15 ± 2.0 K, until constant weight.

2.5. Supercritical fluid extraction (SFE) with ethanol

Experiments of extraction were performed using a bench-scale supercritical extraction unit (8.0×10^{-5} m³ inner volume, length L = 0.16 m, diameter $\Phi = 2.52 \times 10^{-2}$ m). Experimental apparatus and procedure used in this study were the same described in previous studies [7,31–34]. Briefly, the experimental system consists of a jacketed-vessel (extractor) with an ultrathermostatized bath, a needle valve to control the solvent flow rate inside the extractor, a syringe-type pump (ISCO, model 500D), and pressure and temperature sensors and