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Natural antioxidant fine particles recovery from *Eucalyptus globulus* leaves using supercritical carbon dioxide assisted processes

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A B S T R A C T

Supercritical antisolvent (SAS) processes were carried out to obtain precipitates from eucalyptus leaves with potent antioxidant activity. For that, an extract was previously obtained by supercritical fluid extraction (SFE) from these leaves using CO_2 and ethanol as a solvent and co-solvent, respectively. Around fifty compounds were identified by gas chromatography-mass spectrometry (GC–MS) in the supercritical fluid extract and most of them were in the precipitates. The effects of different parameters on the outcome of the SAS process were analyzed such as temperature (35 and 50 °C), pressure (100 to 200 bar), CO_2 flow rate (2 to 5 g/min), liquid flow rate (10 to 30 mL/min) and washing time (30 and 60 min). The antioxidant activities of both the supercritical fluid extract of eucalyptus and SAS precipitates were evaluated by DPPH assay. Most of the SAS precipitates of eucalyptus micro particles showed stronger antioxidant activity compared to the eucalyptus supercritical fluid extract.

1. Introduction

In recent years, the development of nanostructures of natural products are of special interest in cosmetic, nutraceutical and pharmaceutical industries for the reason of environmental, health and economic concern [1,2]. Nano and microparticles can be obtained from a great variety of traditional methods such as physical and chemical vapor deposition, colloidal chemistry approach, mechanical alloying techniques, mechanical milling, micro emulsion based techniques and sol–gel techniques [3–5].

Nevertheless, these methods, with the exclusion of microemulsions, should not be applied to the production of organic micro- or nanoparticles, because of the (extremely) high temperature which is essential for processing. In the case of micro emulsions, the major drawback is the aging time of several hours which is required for the complete processing of particles. Furthermore, dry particles recovery and handling are also very difficult [6–8].

The use of supercritical fluids (SCFs) technology has emerged as an important alternative to conventional process for the generation of natural product based micro- and nanoparticles [9–11]. SCFs technique offers several advantages such as higher product quality in terms of purity, more uniform dimensional characteristics, a variety of compounds to process and a substantial improvement in terms of environmental benign. Furthermore, supercritical conditions are sufficiently mild to permit the micronization of thermolabile solutes. Of all possible SCFs, carbon dioxide (CO_2) at supercritical conditions are mainly used due to its relatively low critical pressure (73.8 bar) and critical temperature (31.1 °C), natural abundance, low cost, non-flammable, non-corrosive nature to the apparatus and environmental safety [12–15].

The SCFs properties (solvent power and selectivity) can also be adjusted continuously by changing the experimental conditions (pressure and temperature). These properties make CO_2 an excellent medium to solve the problems associated with the processing of organic pharmaceutical compounds using the SCF as antisolvent process (SAS) when the active substances is not soluble in CO_2 [9,13–15].

The use of harmless extraction method is essential to apply with SAS process. Conventional extraction methods suffer from several drawbacks such as lack of selectivity, the need for large volume of organic solvents and several hours is required for extraction. Supercritical fluid extraction (SFE) is an important alternative as it has

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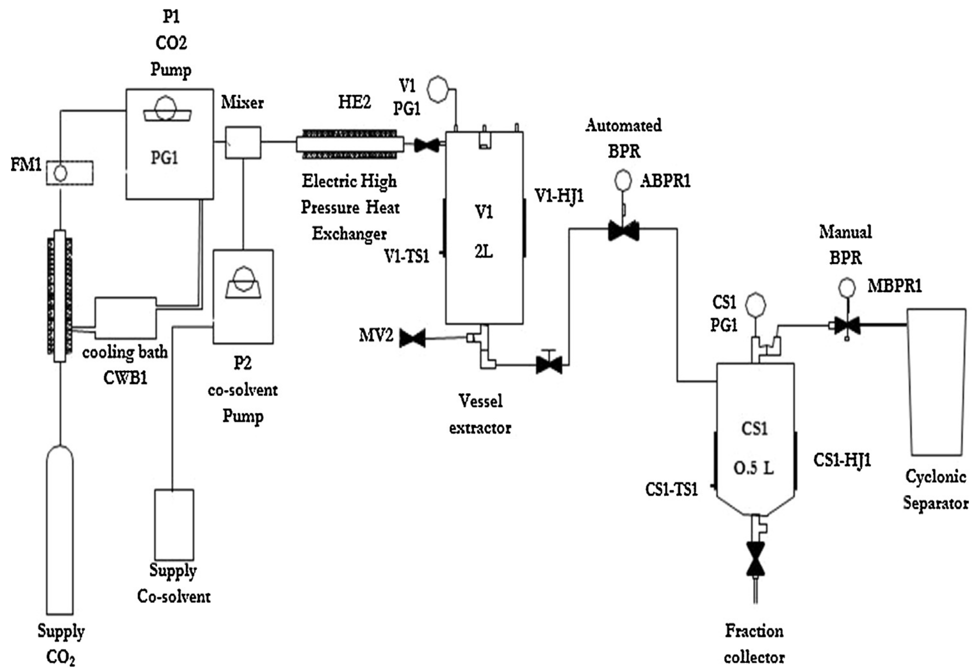

Fig. 1. A schematic diagram of SFE 1000 equipment.

several advantages such as reducing the use of organic solvents, increasing the selectivity, low degradation of chemical compounds and environmental safety [16,17]. SFE of eucalyptus leaves have been investigated and volatile and high molecular weight compounds were obtained with demonstrated bioactivity, which raises the importance of this biomass [17–19].

Eucalyptus leaves enjoy an ever-increasing recognition not only by the scientific community but also, and most remarkably, by the general public because of their health promoting benefits. Eucalyptus leaves contain various pharmacological active compounds such as 1,8-cineole, gallic acid, ellagic acid, hydrolyzable tannins, leucoanthocyanins, hydroxyl cinnamaic acids, quercetin acid, pedunculagin, tellimagrandin, flavonol glycosides, monoterpenes, sesquiterpenes, polyphenols and valoneic acid isomers. Eucalyptus chemical composition have exhibited wide range of biological activities such as antioxidant activity, antimicrobial activity, antibacterial activity, antifungal, anti-inflammatory and anti-proliferative properties and long history of use against the effects of cold, influenza and respiratory infections [20,16,21–23].

Many antioxidant particles and extracts have been precipitated by supercritical technology mostly with supercritical antisolvent process in order to obtain precipitates and encapsulates with high value added [24–32]. To the best of our knowledge, the micronization of eucalyptus compounds with potent antioxidant activity by SAS process has not been investigated so far. The aim of the present study was to establish highly sensitive and viable method for production of eucalyptus micro particles with potent antioxidant activity by SAS process due to their potential in effective production of eucalyptus antioxidants.

2. Materials and methods

2.1. Plant materials

Eucalyptus leaves were collected in 2013 in the region of Puerto Real (Spain), dried in a well ventilated shady place and afterwards stored. Before the extraction, leaves were grinded using Bosch 6000 W grinder with a sieve of approximately 5 mm.

2.2. Solvents and reagents

Carbon dioxide (CO_2) with a minimum purity of 99.8% was provided by Linde (Spain). Ethanol HPLC gradient grade was obtained from Panreac (Barcelona, Spain). The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) reagent was obtained from Sigma-Aldrich (Steinheim, Germany).

2.3. *Supercritical fluid extraction of* Eucalyptus leaves *(SFE)*

The extraction was carried out in a high pressure apparatus supplied by Thar Technology (Pittsburgh, PA, USA, model SF 1000). A schematic diagram of the equipment used in this work is shown in Fig. 1. This set-up included an extraction vessel (capacity of 1000 mL) with a thermostatic jacket to control the extraction temperature, two pumps with a maximum flow rate of 150 g/min CO_2 and 50 g/min of co-solvent. The cyclonic separator allowed periodical discharge of the extracted material during the SFE process. For the extraction, the sample vessel was loaded with approximately 150 g of eucalyptus leaves. The extraction process was performed on the following operating conditions: pressure of 100 bar, temperature of 55 °C, CO_2 flow rate of 16 g/min, ethanol flow rate of 4 mL/min as co-solvent and an extraction time of 3 h. These extraction conditions were chosen on the basis of previous studies on SFE of several leaves [9,33,34].

All the operating conditions apart from the flow of CO_2 were optimized and when the system was stabilized the extractor was pressurized with CO_2. When the pressure in the extractor was near to the desired extraction pressure valve MV-2 was opened to start the flow of solvent, which led to a balanced state. BPR1 was opened automatically and the process began. The extract were recovered in a cyclonic separator and then collected in brown glass bottles and stored. One part of the extract was evaporated to dryness using a rotary evaporator (IKA® RV 10) at 40 °C and subsequently submitted to scanning electron microscope to analyze the morphology of the eucalyptus extract and the other part of this liquid extract was used to SAS process.