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Extraction and characterization of Burdock extracts (leaves, seeds and roots) with compressed solvents technologies

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In this study, an advanced green extraction technique applying supercritical CO_2 ($scCO_2$) and $scCO_2$ with co-solvent (esters, alcohols or hydroalcoholic solutions) is used to obtain value added compounds from the aerial (leaves and seeds) and underground (roots) parts of *Arctium lappa*, commonly known as burdock. In order to increase the yields multi-step $scCO_2$ extraction was also tested. Thus, the highest yield of 12.78 wt % for *A. lappa* leaves was achieved by a six-step sequential $scCO_2$ extraction with ethanol as a co-solvent, for *A. lappa* roots - 32.82 wt % by a three-step sequential $scCO_2$ extraction with hydroalcoholic solution (methanol-water), while for the *A. lappa* seeds - 19.02 wt % by using $scCO_2$ with ethanol as co-solvent. Finally, the effectiveness of the above techniques with that of a conventional Soxhlet extraction with regard to yield was compared.

Keywords: *Arctium lappa*; supercritical CO_2 extraction; co-solvent; multi-step extraction.

INTRODUCTION

Presently, more and more people turn away from modern medicine and look for solutions to their health problems in traditional medicine and natural products [1]. One such example is the *Arctium lappa* plant, more commonly known as burdock. It is native to the Eurasian region but due to its rapid growth it has spread to other parts of the world such as South America where it is considered an invasive species [2]. Though not a very common dish in most countries it is cultivated and regularly used in East Asian cuisine [3-5], while in the UK, Dandelion and burdock is a popular and widely consumed beverage.

The roots are the edible part of the plant, however, it has been demonstrated that the seeds and leaves contain compounds with antioxidant [6-10], antibacterial [11,12] and anti-inflammatory biological activities [13,14]. Hence, recently some considerable effort has been invested into their obtainment, characterization and applications [6,7,9-17].

The aim of the present study is to examine the capabilities of mild and green techniques that apply a supercritical solvent without and with co-solvents to obtain high value compounds from the different parts of the burdock plant, which nowadays is treated as a waste, with the view of its complete valorization and in accordance to the principles of the circular economy.

Fig. 1. *Arctium lappa* [18]

MATERIALS AND METHODS

Sample preparation

The burdock seeds were supplied by a producer from Ivaiporã city, state of Paraná, Brazil (GPS location: 24°14'47.4"S 51°40'32.8" W), in 2018. The material was dried, milled and separated using

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Tyler series sieves of different mesh size in a mechanical shaker. The plant material used for all experiments had particle size of (0.41 – 0.71) mm.

Soxhlet extraction

Soxhlet extraction was performed on all three parts of the burdock plant – the seeds, leaves and roots. All extractions were done in triplets and the yields given below are the average yields. These results served as references in our further investigations. The yield of each extraction was calculated according to:

$$Yield(\%) = \frac{mass\ of\ extract\ (g)}{mass\ of\ sample} * 100 \qquad (1)$$

Supercritical fluid extraction

All supercritical fluid extractions (SFE) were performed in two identical, home-made laboratory extraction units at LACTA laboratory at UFPR – Brazil (Fig. 2.).

The specifics of the SFE units are extensively described in previous works of the research group [19-25]. In summary, the extraction equipment used consists of an extraction vessel equipped with temperature-regulation jacked with internal volume of $62.4 \cdot 10^{-6}$ m^3 ($\varnothing = 1.9 \cdot 10^{-3}$ m and L = $22.0 \cdot 10^{-3}$ m). The pressure is controlled by a syringe pump and monitored by a manometer, while the actual flow rate of the dynamic extraction was controlled by a needle valve (V5, Fig. 2.).

The extraction procedure involved: Firstly, the sample was loaded within the vessel. In the experiments with a co-solvent, the latter was mixed with the sample before loading it into the extraction vessel. Then, after each step of the sequential extractions, the top of the extraction vessel was opened and fresh co-solvent was introduced.

When the desired pressure of the syringe pump was reached, the gas was introduced into the extraction vessel. When pressure equilibrium was attained, the static extraction started (60 minutes for all experiments), after which the dynamic extraction was performed by opening Valves 4 and 5 at a flow rate of 2 ml·min^{-1}. The extract was collected in glass vials and weighed to determine the extraction yield. In the cases when a co-solvent was used it was evaporated before weighing the yield.

RESULTS AND DISCUSSIONS

On all samples firstly a conventional Soxhlet extraction was applied. The results obtained served as a reference for the consecutive yields comparison. All experiments were performed in triplets and at a fixed extraction time (360 minutes) with the following solvents:

- leaves – water;
- seeds – methanol, ethanol, ethyl acetate and hexane;
- roots – methanol, ethanol, ethyl acetate, hexane and water.

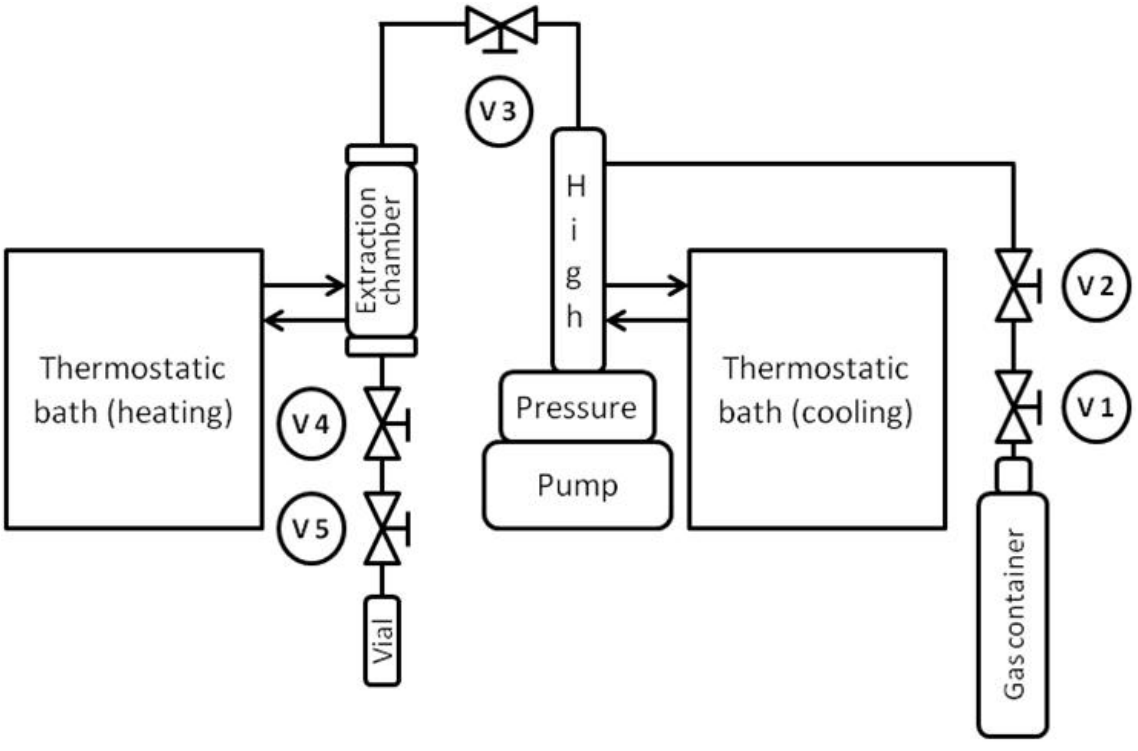

Fig. 2. Schematic representation of the supercritical fluid extraction equipment used

The extraction yield for the leaves was 33.58 wt %, while the highest yields for the seeds and roots were 40.88 wt % with ethanol and 35.44 wt % with water, respectively. The second highest yield for the roots was achieved applying methanol (21.76 wt % – over 3 times higher than that with the third solvent, ethanol). This result was used as a basis for further $scCO_2$ extraction with a hydroalcoholic co-solvent (methanol-water).

Extractions with $scCO_2$ followed and it was demonstrated that for the seeds the average yield was 6.45 wt %. At the conditions applied, and because of the low oil content in the leaves and roots, supercritical CO_2 extraction seems to be unsuitable for those materials, so $scCO_2$ extractions with ester, alcoholic and hydroalcoholic co-solvents were carried out, increasing thus the yields significantly.

The choice of the co-solvents was based on the yields achieved applying the Soxhlet extractions and were different for the different plant matrices. Additionally for the leaves and the roots, sequential $scCO_2$ extractions with a co-solvent were carried out to determine the effects of a single-step and multi-step extractions on the yield.

The co-solvents for the seeds were ethanol and ethyl acetate at a co-solvent to sample mass ratio of 2:1 and yielded 19.02 wt % and 13.40 wt %, respectively.

Methanol was used as a co-solvent for the experiments with roots and the yield was 4.13 wt % for the single-step extraction, and 5.65 wt % for the three-step one, respectively.

For the leaves ethanol was used as a co-solvent and the yield for the single-step extraction was 6.10 wt %, while that for the six-step sequential extraction was 12.78 wt % (Fig. 3.).

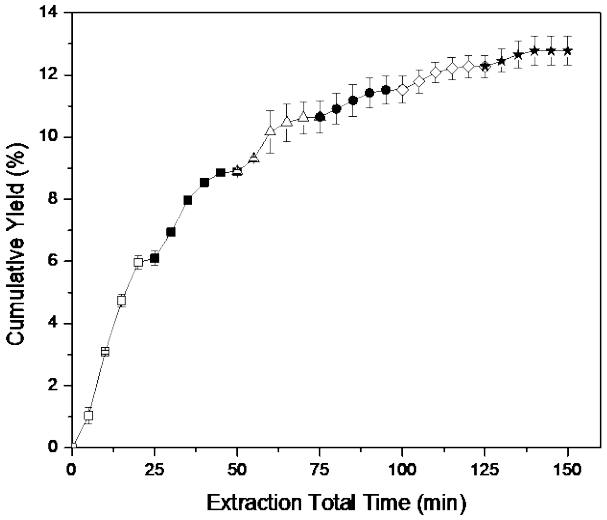

Fig. 3. Kinetic curves of a six-step sequential $scCO_2$ extraction with ethanol as a co-solvent: step 1 - □; step 2 - ■; step 3 - Δ; step 4 - ●; step 5 - ◇; step 6 - ★.

Additional extractions with a hydroalcoholic solution were carried out for the roots and the leaves. The yields obtained were 2.40 wt % for the roots with methanol-water solution and 9.12 wt % for the leaves with ethanol-water solution.

Due to the unexpected low yield for the roots, a three-step sequential extraction was carried out with the same solvent. The results obtained showed a considerable increase in the yield - 1.37 wt % for the first step, 20.22 wt % for the second step and 11.23 wt % for the third one, with a 32.82 wt % cumulative yield.

We attribute this extremely low yield of the initial step to difficulties for the solvent to penetrate and break the cellular walls of the samples.

GC–MS analyses were performed for all burdock roots extracts [23]. The major compounds found were diisooctyl phthalate (DIOP) and 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), glycerol, methyl oleate, butanoic acid and pentadecanal.

For the *A. lappa* leaves, the phytochemical compounds profile obtained from thin layer chromatography revealed the presence of lactones, terpenoids, and esters in extracts [7]. Furthermore, the DPPH and the phosphomolybdenum reduction methods found high values of antioxidant activity in the extracts, and a number of important phenolic compounds like lupeol acetate, amyrin acetate, diisooctyl phthalate and phytol were identified by GC analysis [7].

CONCLUSIONS

The present work investigates the potential of green techniques for extraction of value added compounds from *A. lappa* leaves, seeds and roots as alternatives to conventional extractions using large quantities of organic solvent.

The highest yield obtained for the leaves – 12.78 wt % was achieved by a sequential $scCO_2$ extraction with ethanol as co-solvent. For the seeds the highest yield was obtained by $scCO_2$ with ethanol as a co-solvent – 19.02 wt %, while for the roots a yield of 32.82 wt % was achieved applying $scCO_2$ with a hydroalcoholic solution (methanol-water) in a three-step sequential extraction.

These results prove that extraction with $scCO_2$ plus co-solvents is a viable green alternative to conventional extraction techniques, which allows obtainment of high value substances with a wide spectrum of applications. Furthermore, the application of supercritical extractions processes within a biorefinery will pave the way to valorization of burdock – a plant that is considered an invasive

weed in the majority of countries and is highly underused at present.

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