J. of Supercritical Fluids 50 (2009) 29–32

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Extraction of rosavin from *Rhodiola rosea* root using supercritical carbon dioxide with water

Pamela Iheozor-Ejiofor, Estera Szwajcer Dey*

Lund University, Pure and Applied Biochemistry, Box 124, 221 00 Lund, Sweden

ARTICLE INFO

Article history:
Received 24 September 2008
Received in revised form 18 April 2009
Accepted 19 April 2009

Keywords:
Rhodiola rosea
Rosavin
Bioactive substances
Supercritical carbon dioxide ($scCO_2$) with water
Solvents
Extraction
HPLC

ABSTRACT

In this study, a new extraction method for the isolation of rosavin from dried crushed roots of *Rhodiola rosea* is being developed using supercritical CO_2 and water. Rosavin extracts quantitatively and qualitatively were compared to commonly used solvents such as methanol, ethanol and ethyl acetate. By HPLC analysis rosavin was found to be the dominant compound in extracts obtained by both extraction methods. Quantitative differences were observed between the two extraction methods. Among the solvents, methanol yielded 3.3% while ethanol only 1.2% of rosavin. Supercritical CO_2 and water at extraction temperature 80 °C and 5 h yielded 4.5% of rosavin.

1. Introduction

A variety of high value added products present in plant material can be successfully processed in supercritical fluids. Roots are a typical matrix from which bioactive compounds are extracted. Some of the natural resources with very high value bioactive substances are the roots and rhizomes of *Rhodiola rosea*, an herbaceous plant of the Crassulaceae family. They were traditionally used in Asia and Europe, especially in the Artic region for medicinal purposes [1,2], but with limited research. Several studies have been carried out on the plant, of its adaptogenic properties, and bioactivity components. These properties include inhibition of acetyl cholinesterase [3], anti-stress and anti-cardiac damage effect [4], in cancer therapy [5], as an antidepressant [6] or antioxidant [7], reduction of mental fatigue [8] chemo-preventive and/or therapeutic agent in the treatment of type II diabetes and hypertension [9]. Hence this plant is a favorite product for the development of wholesome foods, nutraceuticals, pharmaceutical and cosmetics.

The effects mentioned are attributed to the phenylpropanoids, organic acids and flavonoids of the plant such as; rosavin (cinnamyl-6(6′-0-α-L-arabinopyranoside)-0-β-D-glucopyranoside), salidroside(2-(4-hydroxyphenyl)ethyl-0-β-D-glucopyranoside) and its aglycon tyrosol, rosarine(cinnamyl-β′-0-α-L-arabinofuranosyl)-0-β-D-glucopyranoside); rosin (cinnamyl-0-β-D-glucopyranoside), gallic acid and rhodioflavanoside. Several clinical research works have made use of commercial standardized extract of 3–5% rosavin and 1% salidroside [10–12]. All the bioactive compounds are amphiphilic.

The increasing demand of bioactive substances from rose-root leads to its destructive exploitation. The whole plant must be destroyed in each harvest after 5-years of growth. Consequently, an efficient and environmentally friendly isolation method is called for. Supercritical CO_2 ($scCO_2$) method is the most attractive and environmentally friendly method of choice. Overall, our and others results demonstrate comparable and often with high recoveries of bioactive compounds from the $scCO_2$. The extracts generated by $scCO_2$ contains lower quantities and number of interfering compounds [13–15]. This method is already accepted in industrial scale in food and pharmaceutical applications. Its qualities are: low cost, low toxicity, solvent-free product, low reactivity and non-flammability with attraction to hydrophobic compounds. Therefore, for the isolation of amphiphilic compounds, CO_2 has to be combined with a polar co-solvent [16]. The most commonly used co-solvents are: ethanol and methanol.

Water has been very successfully used for the isolation of lipopolysaccharides [17].

The study focuses on the extraction of rosavin using $scCO_2$ by changing process factors such as extraction temperature, time, and co-solvent type and concentration. The results from $scCO_2$ based method were compared to conventional solvent extraction. The yield of rosavin was monitored by HPLC and compared with a commercial standard.

* Corresponding author. Tel.: +46 46 222 8258; fax: +46 46 222 4611.
E-mail address: estera.dey@tbiokem.lth.se (E.S. Dey).

0896-8446/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.supflu.2009.04.011

2. Material and methods

2.1. Chemicals

The rosavin standard was from ChromaDex. HPLC grade acetonitrile (99.9%) and methanol (99.9%), LAB-SCAN Dublin, Ireland. Ethanol (99.5%) Kemetyl (Haninge, Sweden); analytical grade sulphuric acid, ethyl acetate (99.7%) Sigma–Aldrich (Steinheim, Germany); chloroform and ortho-phosphoric acid (analytical grade) provided by Merck (Darmstadt, Germany) and HPLC grade diethyl ether Fluka. Milli-pore water (18.2 MΩ) Maxima Ultra pure water (Abino Lab.)

2.2. Samples

Dried crushed root of *R. rosea* were provided by Gösta Lilius courtesy of SWEPHARM AB, Sweden.

2.3. Solvent extraction

The crushed root was sieved to obtain a particle fraction of 0.5–1.0 mm in size. 2 g of dried root samples were extracted with 16 ml of solvent (ethanol, methanol or ethyl acetate) in a parafilm sealed beaker with magnetic stirring (50% of the max scale rpm) for 3 h. Samples were later carefully decanted and the spent roots rinsed with the same volume of fresh solvent. The extracts were pooled and filtered, centrifuged at 6000 rpm (4960 × *g*) for 10 min and the supernatant carefully decanted and collected. The organic solvents were removed on rotary evaporator at 55 °C (Bůchner rotarvapor R-200). The dry masses of crude extracts were weighed and reconstituted with ethanol. All extractions were done in triplicates.

2.4. $ScCO_2$ extraction

The rosavin from crushed root with particle size fraction of 0.5–1.0 mm (Section 2.3) was extracted by supercritical CO_2, using the Thar SFE 100X2F system (Thar technology Inc., Pittsburgh, PA, U.S.A.). The schematic diagram of the original system is shown in Fig. 1.

In the present studies only the extraction vessel 2 (V2), and both cyclone collectors (CS1 and CS2) were used. For each extraction, 5 g of sample was placed and covered with a small piece of glass wool, to prevent loss/bumping of samples and subsequent blocking of the extraction pipes, before closing the extraction vessel. The carbon dioxide pressure was adjusted to 20 MPa. This fixed pressure was used in all trials. The temperatures were kept; at 70 and 80 °C and the extraction time was 3 and 5 h. In all trials carbon dioxide flow rate was kept constant at 9 g/min, and co-solvents concentration as carbon dioxide percentage, at 10%. Co-solvents details are given in the text of the corresponding tables. All co-solvents used were degassed.

The extracts collected from cyclone/separator were filtered, and centrifuged at 6000 rpm (4960 × *g*) for 10 min. The organic co-solvents were removed on rotary evaporator at 55 °C (Bůchner rotarvapor R-200), and the samples extracted with water as co-solvent were freeze dried (Ab Nino lab. Sweden). The extracts were reconstituted in ethanol. Each extraction was performed in triplicates and the samples were stored at 4 °C before analysis. The standard deviation of *n* measurements was calculated by applying "Statistics of repeated measurement" [18].

2.5. High performance liquid chromatography (HPLC)

All samples were analyzed using Agilent HP 1050HPLC equipped with a UV detector and an auto sampler. A reversed phase Kromasil C18 (250 mm × 4.0 mm), 5 μm particle size column (Chromatech AB) was used and controlled by ChemStation software (Agilent Technologies).

ChromaDex protocol (2005) was used with a slight modification due to differences in HPLC equipments and minor problems encountered during analysis. Prior to use, all mobile phase were vacuum degassed and samples were passed through 0.2 μm Acrodisc syringe filter from Pall (Ann Arbor, MI, U.S.A.). The UV wavelength was kept at 254 nm. Five microliters of extract (0.1 mg/ml) was injected in triplicates with an auto sampler and elution was conducted at ambient temperature. 0.2% (v/v) phosphoric acid/acetonitrile (96:4, v/v) was used as the mobile phase at a flow rate of 0.6 ml/min for 20 min.

After 20 min, elution continued for 30 min with 0.2% (v/v) phosphoric acid/acetonitrile (70:30, v/v) at a constant flow rate. Rosavin standard in methanol (0.5, 1, 1.5, 2.0 and 2.5 μg) was used. To determine the peak identity, the extracted samples were also spiked with 12 μl of the standard rosavin sample.

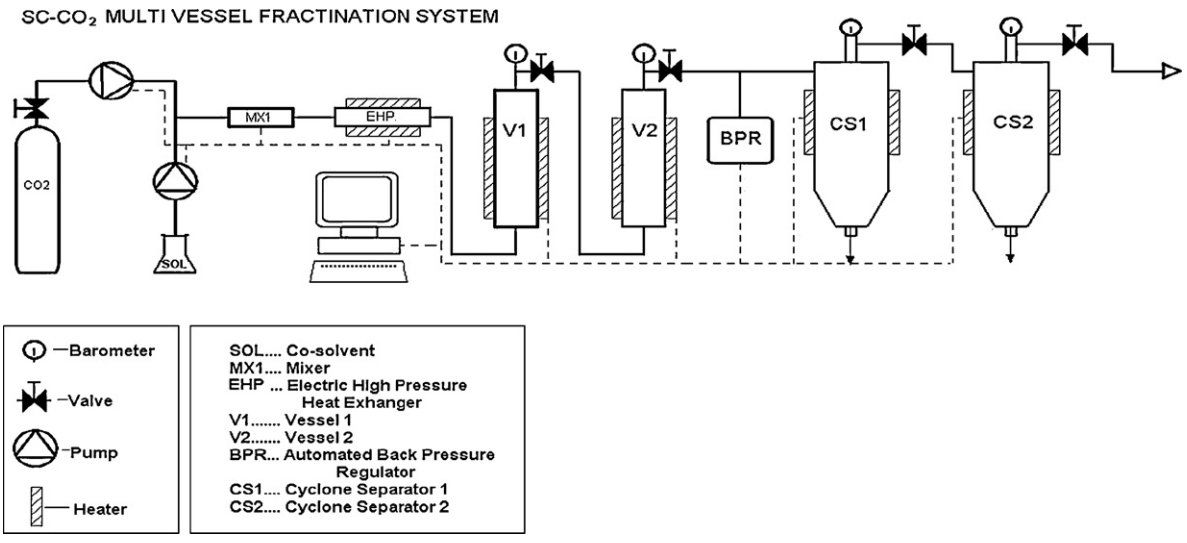

Fig. 1. Schematic diagram of the supercritical fluid extractor Thar SFE 100X2F.