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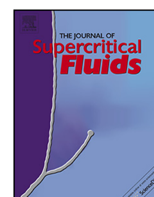

A bioactivity based comparison of *Echinacea purpurea* extracts obtained by various processes

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ABSTRACT

Echinacea species is provided as dietary supplements for various infectious and immune related disorders and has a potential role in cancer prevention. The aim of this study was to optimize the extraction of total flavonoids using different extraction methods and investigate the cytotoxic effects on various cancer cell lines (CaCo-2, MCF-7, A549, U87MG, and HeLa) and VERO (African green monkey) as a non-cancerous cell line. Box-Behnken statistical design was used to evaluate the effect of pressure (100–200 bar), temperature (40–80 °C) and ethanol as co-solvent (6–20 wt%) at a flow rate of 15 g/min for 60 min in supercritical CO_2 extraction and the effect of temperature (60–100 °C), time (5–15 min) and power (300–900 W) in microwave-assisted extraction. Optimum extraction conditions were elicited as 300 bar, 80 °C and 13% co-solvent yielding 0.472 mg rutin equivalent total flavonoids/g extract in SC-CO_2 extraction, whereas 60 °C, 10 min and 300 W yielded the highest (0.202 mg rutin equivalent) total flavonoids in microwave-assisted extraction. Additional trials with subcritical water (0.022 mg/g) and Soxhlet extraction with methanol (0.238 mg/g) yielded lower flavonoid contents. The exposures upto 50 μg/ml of extracts revealed no significant inhibition on the proliferation of both tested cancer cells and healthy VERO cells.

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1. Introduction

Preparations from *Echinacea purpurea* are widely used for herbal medicines where the emphasis is in immunological properties. *E. purpurea* is the best known of the dozen or so species of the genus *Echinacea*, perennial prairie wildflowers native to the central grasslands of North America and grouped within the Aster family [1,2]. The main active compounds are alkamides, polyacetylenes, caffeic acid derivatives, polysaccharides, glycoproteins and flavonoids such as chicoric and caffeic acid [3]. *E. purpurea* has potential for use as a phytochemical supplement due to its immunomodulatory, anti-inflammatory, antioxidant and anti-viral effects [4–6]. In addition, to stimulating the immune system, *Echinacea* has been reported to increase properdin levels in the body which could serve as an anticancer agent as well [7]. Glycerin extract of *E. purpurea* was reported to increase immunoglobulin *M* in mice [8]. *E. purpurea* has potential for increasing chemotoxicity in neutrophils and bactericidal activity against *Staphylococcus*, destroying tumor cells [9], stimulating macrophage and neutrophil serve [10]. However, these pharmacological activities vary based on the different parts of the plant material such as aerial parts and roots. Indeed, dry aerial parts were shown to include 20% higher flavonoid and 29% higher phenolic contents [11].

As for extraction processes, classical solvent extraction [12,13], Soxhlet [14], ultrasound [12,15], microwave-assisted extraction [11] and supercritical CO_2 extraction [15–17] methods have been applied but a detailed optimization study was not conducted.

The objective of this study was to carry out different extraction processes namely, supercritical CO_2, subcritical water, microwave-assisted and Soxhlet extractions by using aerial parts >of *E. purpurea* and determine optimum extraction conditions revealing the highest yields. In addition, the cytotoxic effects were investigated on various cancer cell lines such as CaCo-2, MCF-7, A549, U87MG, HeLa, and also a healthy cell line, VERO.

2. Materials and methods

2.1. Plant materials

Echinacea purpurea was supplied by Rasayana Organic (Antalya) which was harvested in 2012. The plant was harvested from Konya, Turkey and dried at room temperature. Prior to the extraction processes, the plant material was ground using a Waring laboratory scale blender and sifted using a 10 MESH-sieve (800 μm, average).

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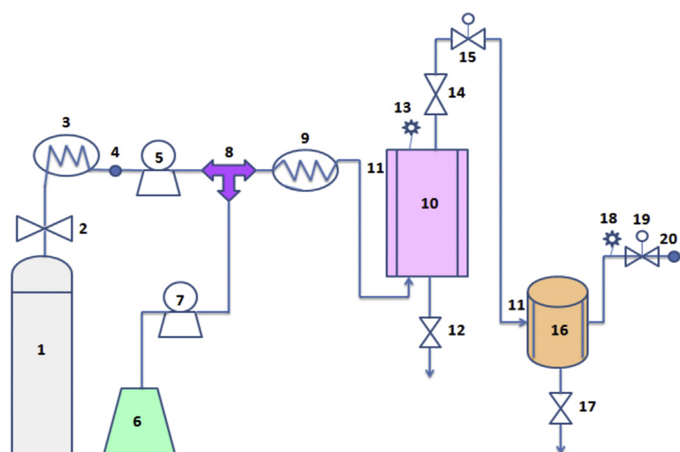

Fig. 1. A schematic diagram of a laboratory scale supercritical CO2 extraction system. 1 - CO2 supply in, 2 - CO2 on-off valve, 3 - cooling heat exchanger, 4 - flow meter, 5 - CO2 pump, 6 - co-solvent reservoir, 7 - co-solvent pump, 8 - mixer, 9 - heat exchanger, 10 - extraction vessel, 11 - heat jacket, 12 - bleed valve, 13 - gauge, 14 - on-off valve, 15 - automated back pressure regulator (ABPR), 16 - separator, 17 - drain valve, 18 - gauge, 19 - manual BPR, 20 - vent.

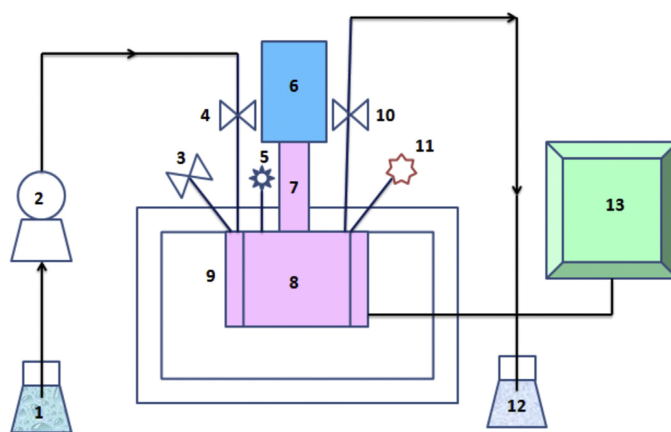

Fig. 2. A schematic diagram of a laboratory scale supercritical water extraction system. 1 - solvent system, 2 - HPLC pump, 3 - sampling valve, 4 - input valve, 5 - pressure gauge, 6 - motor, 7 - magnetic drive, 8 - extraction vessel, 9 - heating jacket, 10 - output valve, 11 - rupture disk, 12 - collecting flask, 13 - electrical control panel.

Powdered plant material was then packed in plastic bags and stored at +4 °C until use.

2.2. Materials and reagents

CO_2 (99%) was taken from Habas, Izmir, Turkey. Folin-Ciocalteu's reagent, 2,2-diphenyl-1 picrylhydrazyl hydrate (DPPH), sodium carbonate (Na_2CO_3) and aluminium chloride ($AlCl_3$) were obtained from Sigma. Acetic acid (100%), pyridine, methanol and ethanol were purchased from Merck. All other chemicals were of analytical grade purity. Nanopure water used in the analysis was prepared by using in-house nanopure water system (Sartorius Arium 611, Sartorius-Stedim, Gottingen, Germany).

2.3. Extraction processes

2.3.1. Supercritical CO_2 extraction

Supercritical CO_2 extraction was carried out using SFE 100 System (Thar Instruments, Inc., UK, 2006). The extractor volume was 100 ml, thus it was filled with about 10 g of ground *E. purpurea*. The independent variables were temperature (40, 60, 80 °C), pressure (100, 200, 300 bar) and co-solvent (ethanol) ratio (6%, 13%, 20%). These variables were set according to the experimental design (Box-Behnken) which consists of a group of mathematical approaches for the modelling and analysis of the response influenced by the independent variables. When all the desired parameters were reached, the extraction was started at a flow rate of 15 g/min and continued for 60 min under each condition. At the end of the extraction process, valve of the CO_2 tube was sealed off for depressurization. Extracts were collected from the separator outlet after releasing CO_2 from the system (Fig. 1) and concentrated to dryness at 55 °C under vacuum by Hahnvapor RS2005V-N rotary evaporator and subsequently lyophilized.

2.3.2. Subcritical water extraction

Subcritical water extraction (SCW) was carried out using a continuous system (Amar Equipments, India) comprised a Shimadzu HPLC pump (LC-20AT, Japan), an electrical control panel and an extraction vessel (Amar Equipments, India) with a volume of 100 ml (Fig. 2). About 10 g of aerial parts of *E. purpurea* was loaded and the system was operated at a flow rate of 5 ml/min at 60 °C and 250 bar for duration of 60 min. The obtained extract was concentrated to dryness at 55 °C in vacuum by Hahnvapor RS2005V-N rotary evaporator and subsequently lyophilized.

2.3.3. Microwave-assisted extraction

About 1 g of dried aerial parts of *E. purpurea* was transferred into a vessel containing 20 ml ethanol. The independent variables, temperature (60, 80, 100 °C), time (5, 10, 15 min) and power (300, 600, 900 W), which were set according to the experimental design (Box-Behnken) and automatically adjusted throughout the system. After extraction, the system was allowed to cool to room temperature for an additional 5 min prior to opening the vessels. The extract obtained in each vessel was then filtered through a 0.20 mm membrane filter. The obtained extract was concentrated to dryness at 55 °C in vacuum by Hahnvapor RS2005V-N rotary evaporator and subsequently lyophilized.

2.3.4. Soxhlet extraction

Aerial parts of *E. purpurea* (50 g) were extracted with 1000 ml of methanol for four cycles (about 4.5 h) using a Soxhlet (1000 ml) apparatus [18]. The extract was concentrated to dryness at 55 °C in vacuum by Hahnvapor RS2005V-N rotary evaporator and subsequently lyophilized.

2.4. Total phenol content

The total phenols in the extracts were determined by Folin–Ciocalteu method as described in Akay et al. [19]. Briefly, 100 μl aliquot of the extract was made up to 10 ml with distilled water. Then 500 μl of Folin–Ciocalteu's reagent was added, stirred by vortex and left to stand for 5 min. Finally, 1.5 ml of saturated sodium carbonate solution was added, stirred for the last time and left at room temperature for an hour. The absorbance was determined at 760 nm using a Shimadzu UV-2401 spectrophotometer. Gallic acid was used as a standard. The total phenols were determined in duplicates; the results were expressed as mean values and were given as gallic acid equivalent (GAE) per gram of extract.

2.5. Free radical scavenging activity

The free radical scavenging activities of the extracts were determined as described in Yesil-Celiktas [20]. The extracts were dissolved in 4 ml of methanol and then added to 0.5 ml of 1 mM methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazil hydrate, Sigma). The contents were stirred vigorously for 15 s and then left to stand at room temperature for 30 min. The decrease in

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