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Influence of pre-treatments on yield, chemical composition and antioxidant activity of *Satureja montana* extracts obtained by supercritical carbon dioxide

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ABSTRACT

Main objective of this work was to investigate the influence of pre-treatments of *Satureja montana* herbal material on supercritical extraction process, in terms: influence on extraction yield, composition and antioxidant activity of extracts. Four different pretreatments were investigated: water pre-treatment, ethanol pre-treatment, ultrasound and high pressure pre-treatment. Extraction yields were in the range from 1.68 to 2.35 g/100 g. Pre-treatments with water and ethanol increase the yield of extraction for 25% and 17%, respectively. According to GC/MS results the main compound of obtained extracts was carvacrol. Analyses confirmed that carvacrol content in extracts can be significantly increased by application of pre-treatments. Ultrasound pre-treatment and high pressure pre-treatment yielded highest concentration of carvacrol in extracts (around 66%). Using these two pre-treatments of herbal material, concentration of carvacrol, in extracts obtained by supercritical carbon dioxide extraction, could be increased for around 25%. Antioxidant activity of all extract was very high and in the range of well-known antioxidants.

1. Introduction

Herbs and spices have always been used as additives because of their ability to improve organoleptic properties of food. Today, they are valuated, not only for improvement of organoleptic properties, but also for their nutritive and medicinal benefits. One of such, valuated herbal species, is *Satureja montana* L. (winter savory), well-known aromatic plant, frequently used as spice and as a traditional medicinal herb. Beside traditional use in folk medicine, many scientific studies confirmed *S. montana* significant pharmacological activity (Table 1). *S. montana* contains various biologically active constituents including essential oil, triterpenes [22] and flavonoids [23]. Main components are aromatic monoterpens. The most dominant compound, carvacrol, is responsible for essential oil's characteristic taste.

Herbal plant and spices today are used not only in the native plant form, but also in the form of different extracts (produced by different extraction techniques) in which increase concentration of certain constituent of interest (with desirable biological activity or of some other desirable properties) could be achieved. Today, special attention is focused on essential oils and extracts contains essential oils, for which a significant pharmacological and antioxidative activity is confirmed and which can help to maintain nutritive and sensor quality of food [9]. According to Grosso et al. [24] many problems which occurs using standard method of extractions (thermal degradation, hydrolysis of individual components or the use of organic solvents) were exceeded by applying supercritical extraction. Several works have been published in order to report the use of SFE (supercritical fluid extraction) for isolation of different groups of plant metabolites, among them supercritical extraction of *S. montana* was also investigated [11,17,24]. The most frequent extraction fluid in supercritical extraction process is carbon dioxide. It is non-toxic and non-explosive extraction fluid, easy removed from the obtained products-extracts, thus allowing the preparation of solvent free extracts. Carbon dioxide possesses low critical temperature and pressure (T_c = 31.1 °C, P_c = 73.8 bar), therefore making it possible to avoid thermo-degradation of thermo-sensitive constituents. Regarding mild temperature extraction conditions and possibility of selective extraction (by tuning pressure and

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Table 1
Pharmacological activity of different *Satureja* extracts.

Pharmacological activity	*Satureja* extract	Reference
Diuretic activity	Infusion, ethanolic extract and essential oil	[1]
Antidiarrheal	Essential oil	[2,3]
Antispasmodic activity	Essential oil	[2]
Antiviral activity	Ethanolic extract, aqueous extract	[4,5]
Antibacterial and fungicidal activity	Essential oil, methanolic extracts, supercritical extract, soxhlet extract	[6,7,8,9,10,11]
Antioxidant activity	Ethanolic extract, methanolic extracts, supercritical extract, soxhlet extract, essential oil, powder extract obtained by spray drying of ethanolic extract	[12,13,14,9,10,15,16,17]
ACE inhibitory activity	Powder extract obtained by spray drying of ethanolic extract	[15]
Antiproliferative activity	Essential oil	[18]
Anticholinesterase	Supercritical extract, soxhlet extract, essential oil	[11]
Antinociceptive and antiinflammatory effects	Essential oil, ethanolic extract	[19]
Treatment of premature ejaculation	Ethanolic extract	[20,21]

temperature), supercritical carbon dioxide extraction is recognized as "method of choice" for extraction of essential oils and extracts containing constituents of essential oils.

The pre-treatment of herbal material to be extracted can change the "direction of extraction process", leading to the higher extraction yields, to the production of extracts with increase content of certain constituents, and production of extracts of desirable properties, sensory or biological. Several pre-treatments have been investigated in the case of supercritical extraction with carbon dioxide (SECD): mechanical pre-treatment, rapid gas decompression, different drying pre-treatments, ultrasound pre-treatment etc. Most of them are used for disruption of plant material structures and cells. Ivanović et al. [25] investigated influence of two different pre-treatments (mechanical pre-treatments, using different types of mills, and rapid gas decompression) on extraction of *Usnea barbata* by supercritical carbon dioxide. They found out that recovery of usnic acid from *U. barbata* using supercritical carbon dioxide was dependent on the pre-treatment and extraction conditions [25]. Crampon et al. [26] also focused their research on the influence of pre-treatments (two drying methods and one mechanical pre-treatment-grinding) upon extraction kinetics and yields in the case of *Nannochloropsis oculata* supercritical extraction. They found out that drying under air flow leads to faster extraction kinetics. The extraction curve obtained in this research illustrated that the smaller particle size, the higher the extraction kinetics [26]. A positive effect on extraction kinetics was observed after CO_2 decompression for materials of the *Lamiaceae* species, as well as valerian and ginger roots [27,28]. The rapid gas decompression, in the case of St. John's wort by SECD, led to increase of extraction yield [29]. During the exposure to compressed CO_2 prior to extraction – beside static extraction of compounds to the surrounding dense gas atmosphere – the gas sorbs into plant material. In the case of St. John's wort extractables are stored within various secretory structures. The rapid gas decompression leads to disruption of cell therefore certain amount of extractables being stored in the discrete secretory structures becomes accessible for the extraction [29]. Several studies confirmed the positive influence of the ultrasound pre-treatment on the efficacy of supercritical extraction. Riera et al. [30] showed that the presence of ultrasound can increase the yield of almond oil by 20% compared to traditional SECD. Luo et al. [31] also showed that SC-CO_2 reverse microemulsion extraction of ginsenosides from ginseng was significantly accelerated by applying ultrasound. Gao et al. [32] reported that the using of ultrasound can significantly increase the yield of lutein esters.

The present study was undertaken to investigate the influence of different pre-treatments on *S. montana* on further supercritical extraction process, composition and antioxidant activity of obtained extracts. SECD has been applied regarding its efficiency for extraction of aromatic and thermolabile volatile compounds, which are the main constituents of *S. montana* essential oil. Influence of four different pre-treatments was observed and analyzed: water pre-treatment, ethanol pre-treatment, ultrasound and pre-treatment with high pressure.

Table 2
Methods of pretreatment applied prior *S. montana* SECD, and obtained extracts.

Extract	Method of pre-treatment and extraction
E0	Extraction of herbal material without pre-treatment
E1	Extraction of herbal material with water pre-treatment
E2	Extraction of herbal material with high pressure pre-treatment
E3	Extraction of herbal material with ultrasound pre-treatment
E4	Extraction of herbal material with ethanol pre-treatment

2. Material and methods

2.1. Chemicals

Commercial carbon dioxide (Messer, Novi Sad, Serbia) was used for laboratory supercritical fluid extraction. All other chemicals were of analytical reagent grade.

2.2. Plant material

S. montana was cultivated at Institute of Field and Vegetable Crops, Bački Petrovac, Serbia, in year 2012. The collected plant material was air dried and stored in a paper bags, at a room temperature. The dried *S. montana* was grounded in a domestic blender prior extraction, and the particle size of grounded material were determined using sieve sets (Erweka, Germany).

2.3. Pretreatment of S. montana material

Before SECD, *S. montana* herbal material was the subject of different pre-treatments. All experiments were performed in three replicates.

In pre-treatment with water 60 g of investigated herbal material was moisturized by addition of 36 g of water in extraction vessel. This mixture was vigorously stirred for 1 h. In the same extraction vessel obtained wet sample was extracted by high pressure carbon dioxide. This way extract E1 was obtained (Table 2).

In a pre-treatment with high pressure 60 g of investigated herbal material was placed in extractor vessel and exposed to carbon dioxide at pressure of 100 bar and temperature of 40 °C for 1 h. After pre-treatment with high pressure such herbal material was extracted by high pressure carbon dioxide. This way extract E2 was obtained (Table 2).

In pre-treatment with ultrasound 60 g of investigated herbal material was placed in glass flask, without addition of any solvent,