EFFECT OF MATRIX PRETREATMENT ON THE SUPERCritical CO2 EXTRACtion OF Satureja montana ESSENTIAL OIL

Article Highlights
- Supercritical CO2 extraction of Satureja montana was carried out with different pretreated matrices
- GC/FID analysis of extracts - dominant compound thymol was provided
- Gland physical disruption by fast decompression provides the highest content of thymol and carvacrol
- Extracts showed rather significant antimicrobial activity, similar to essential oil

Abstract
The effect of matrix pretreatment of winter savory (Satureja montana L.) on the supercritical CO2 (SC-CO2) extraction yield, composition and antimicrobial activity of extracts and essential oil (EO) was investigated. The herb matrix was submitted to conventional mechanical grinding, physical disruption by fast decompression of supercritical and subcritical CO2, and physical disruption by mechanical compression. The analyses of the essential oil obtained by SC-CO2 extraction and hydrodistillation were done by GC/FID method. The major compounds in winter savory EO obtained by SC-CO2 extraction and hydrodistillation were: thymol (30.4-35.4 and 35.3%), carvacrol (11.5-14.1 and 14.1%), γ-terpinene (10.2-11.4 and 9.1%) and p-cymene (8.3-10.1 and 8.6%), respectively. The attained results revealed that physical disruption of essential oils glands by fast CO2 decompression in supercritical region (FDS) achieved the highest essential oil yield as well as the highest content of thymol, carvacrol and thymoquinone. Antimicrobial activity of obtained winter savory SC-CO2 extracts was the same (FDS) or weaker compared to essential oil obtained by hydrodistillation.

Keywords: Satureja montana, supercritical CO2 extraction, herb matrix pretreatment, essential oil yield, essential oil composition.
ation of labile compounds, and avoiding toxic solvents residue in the product [9].

The need for a matrix pretreatment is related to the location of the EOs within the herbaceous matrices. In many aromatic herbs, EOs are largely located within glandular trichomes that develop on the surface of leaves and other organs of the plants. The so-called “peltate hairs” appear to contain most of the oil and will henceforth be called “the glands”. The glands are well described in literature and an extensive “library” of electronmicrographs and photomicrographs has been built up showing these glands and their location on the leaves of typical herbs [10]. Most of EOs of aromatic herbs from Lamiaceae family is found on the surface of the leaves in peltale gladular trichomes (peltate glands) [11,12]. The transport of material to or from the intact glands is highly restricted by the glandular walls which must be disrupted by mechanical means or other techniques to reach acceptable extraction rate of the oil using compressed CO2 as solvent [13].

Commonly, mechanical processes like flaking, grinding or pelletizing are applied [14]. However, during mechanical pretreatments, losses by degradation (oxidation and thermal degradation) and by evaporation of volatiles are observed, leading to a discrepancy between the EO composition of the herb and that of the extract [15]. In previously published investigations [16-18] a positive effect on extraction kinetics was observed after CO2 decompression of Lamiaceae species, Aloe vera as well as valerian and ginger roots. Also, it was found that carvacrol content in S. montana extracts can be significantly increased by application of ultrasound and high pressure pretreatments [19].

The aim of this work was to determine the effects of different S. montana matrix pretreatments on the supercritical CO2 extraction rate, yield and composition of extracts, as well as its antimicrobial activity.

EXPERIMENTAL

Matrix

Many factors can influence the amount of EO in aromatic herbs, such as climate and environmental conditions and age of plants. To minimize this influence in the present work, leaves of wild growing S. montana were collected manually from the same collection site from the central part of Montenegro (near Podgorica, 100 m a.s.l.) prior the flowering (in May). Collected leaves were air-dried at room temperature for 7 days, packed in double walled paper bags and stored at 5 °C before use. Voucher specimen was confirmed and deposited in Herbarium, Department of Biology, Faculty of Natural Sciences and Mathematics, University of Montenegro, voucher number: S809/08.

Matrix pretreatment procedure

The initial water inherent in the dried winter savory leaves was found to be 8.7 mass% using a Dean and Stark apparatus with n-heptane as the reflux solvent.

In order to determine effects of winter savory matrix pretreatment on SC-CO2 extraction yield, following pretreatments were applied:

- Intact herb matrix.
- Conventional mechanical grinding of herb matrix with different exposure periods: typically, 100 g of material was milled in a domestic blender (Multi Moulinex, 260 W) during 20, 40 and 60 s and, after sieving (laboratory Erweka sieves, mesh from 0.1 to 2 mm), mean particle size was determined (d20 = 1.1 mm, d40 = 0.9 mm and d60 = 0.6 mm, respectively). Herb batch was used immediately in extraction experiments in order to minimize losses of volatile compounds.
- Physical disruption of EOs glands by fast decompression of supercritical and subcritical CO2 (FDS and FDL) respectively, as alternative physical pre-treatment:
  - The physical disruption of EOs glands with compressed CO2 was studied at 40 (FDS) and 26 °C (FDL). 80 g intact herb leaves was enclosed in an extraction vessel and compressed CO2 was fed from the bottom of extractor. When the desired pre-expansion pressure was achieved (90 bar), inlet valve was closed and the bed was exposed to CO2 at this pressure for a designed period (60 min) before fast decompression of the bed was carried out (∼1.20 s). FDS and FDL pretreatments were immediately followed by CO2 extraction in the same apparatus.
  - Physical disruption of EOs glands by mechanical compression (pellet): 30 g of intact herb leaves was charged in a 25 mm diameter die, and exposed 5 min to 15 t m−2 mechanical pressure in a Graseby Specac 25.011 hydraulic press to make a pellet. The obtained pellets were smashed using a pestle and mortar, and used immediately in extraction experiments.

Supercritical carbon dioxide extraction procedure

The supercritical carbon dioxide extraction was carried out on extraction apparatus in the Laboratory of Supercritical Fluid Technology Group, University of Birmingham (Figure 1).
Extraction procedure was previously described in detail elsewhere [15]. Shortly, commercial CO₂, from storage cylinders at bottle pressure, was fed through valves, V1 and NRV1, and a filter, F1. In order to reduce the temperature of the CO₂, the gas passes through a coil immersed in a refrigeration bath, HE1. The cooled CO₂ was then pressurized in the liquid compressor pump and passed through a 50 µm filter, F2 and NRV2. The CO₂ then passes through a heat exchanger (HE2), where now the solvent is a supercritical fluid and enters the external insulated air bath housing. The gas was transported through another heat exchange before entering the pressure vessel.

For each experiment, 80 g of pre-treated herb was weighed and, before loading, some glass wool was inserted, after which the herb was transferred in the pressure vessel. Finally, two steel mesh filters of different types were inserted to stop particles leaving the vessel.

Obtained extract was collected in first collector, after depressurized CO₂. Additionally, EO was attached to the "cold collector", which was immersed in a mixture of acetone/dry-ice (−82 °C). Its purpose was to collect the volatile components (solute) from the CO₂ that may have escaped from first collector.

The extraction conditions for all experiments were: temperature 40 °C and pressure 100 bar, extraction time 360 min and CO₂ flow rate 0.3 kg CO₂/h. Commercial carbon dioxide (99.995% purity, BOC grade N4.5-CP) as well as acetone (capillary GC grade, ≥99.9%, Sigma-Aldrich, UK) and dry ice (−82 °C) were used for extractions.

**Hydrodistillation procedure**

80 g of herb material (60 s milled in a domestic blender, d = 0.6 mm) was submitted to hydrodistillation in a Clevenger-type apparatus for 2 h according to Yugoslav Pharmacopoeia IV. The obtained essential oil was dried over anhydrous sodium sulphate, measured, poured in hermetically sealed dark-glass containers and stored in a freezer at −4 °C until analyzed by GC.

**Gas chromatography**

The yield of the gained essential oil was evaluated by GC-FID using a VARIAN 3400 gas chromatograph with a DB-5 capillary column (30 m×0.32 mm, film thickness 25 µm). The analysis conditions were: oven temperature was programmed at 50 °C for 5 min, then increased to 140 °C at a rate of 8 °C/min and then increased to 250 °C at a rate of 13 °C/min; the injector and detector temperature were 120 and 270 °C, respectively. Helium as carrier gas was adjusted to flow rate of 1.4 ml/min, and the injection mode was splitless.

The constituents of extracts were identified by comparing their retention times with authentic standards (Fluka, Great Britain) and their mass was calculated from a predetermined peak area response factor.
Microbial strains

In order to evaluate the activity of the essential oil of *Satureja montana*, the following microorganisms were used: as reference strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853 and *Candida albicans* ATCC 10231 (Torlak, Belgrade, Serbia); further, the clinically isolated *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*.

The microorganisms were isolated from clinically treated patients of the Clinical Centre of Montenegro (Podgorica, Montenegro).

Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A broth microdilution method was used to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) [20]. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5 vol.%. Briefly, serial doubling dilutions of the extracts were prepared in a 96-well microtiter plate ranged from 0.09 to 25.00 mg/ml. To each well 10 μL of resazurin indicator solution (prepared by dissolving a 270-mg tablet in 40 mL of sterile distilled water) and 30 μL of Mueller-Hinton broth were added. Finally, 10 μL of bacterial suspension (10^6 CFU/mL) was added to each well to achieve a concentration of 10^4 CFU/mL.

Two columns in each plate were used as controls: one column with a broad-spectrum antibiotic as a positive control (amykacine) and one column containing the methanol as negative controls. Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and prepared in triplicate, and then they were placed in an incubator at 37 °C for 18-24 h. Color change was then assessed visually. The lowest concentration at which color change occurred was taken as the MIC value. The average of 3 values was calculated and that were the MIC and the MBC for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by the turbidity. The MBC was defined as the lowest concentration of the extract at which incubated microorganism was completely killed.

Assay of in vitro antifungal activity

Broth microdilution assays were performed in accordance with the guidelines [21]. Briefly, stock solutions were prepared in water for nystatin and in methanol for oil. The final dilution was prepared in RPMI 1640 medium, adjusted to pH 7.0 with 0.165 M morpholinenepropansulfonic acid buffer, in the range from 0.09 to 25 mg/ml and inoculum size of 103 CFU/ml. The growth (drug free) and sterility controls were also included. Microdilution trays were incubated in ambient air at 35 °C. MICs were determined visually after 48 h of incubation, as the lowest concentration of the drug that caused no detectable growth. The average of 3 values was calculated giving the MIC and the MBC for the tested oil.

Statistical analysis

Extraction experiments were carried out in triplicate. Means and standard deviation (SD) were calculated using Origin Pro 8 (OriginLab, USA). Duncan’s test was conducted to analyze the difference between various pre-treatments. A value of *P* < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The main volatile components and average yield of *Satureja montana* essential oil, after 6 h supercritical CO₂ extraction from differently treated matrix and hydrodistillation are shown in Table 1.

Major compounds in winter savory EO obtained by SC-CO₂ extraction and HD were: thymol (30.4-35.4 and 35.3%), carvacrol (11.5-14.1 and 14.1%), γ-terpinene (10.2-11.4 and 9.1%) and p-cymene (8.3-10.1 and 8.6%), respectively.

The extraction yield value of *S. montana* EO was similar to that previously found [5,22-24] but much higher than the yield reported by other researchers [1,25]. The phytochemical profile of the winter savory EO in this study was in agreement with the results of several authors who have also evaluated this vegetal species [1,5,26,27]. In contrast, the savory EO evaluated by Cavar et al. [25] was characterized by a high content of alcohols, such as geraniol and terpinen-4-ol. It was found that the final chemical depends on: each organ and its stage of development; the climatic conditions of the plant collection site; the degree of terrain hydration; macro- and micronutrient levels; and the plant material’s drying conditions [9,28].

The percentage of sesquiterpene hydrocarbons was rather high in all samples (45.1-54.5%), due to high content of major compounds thymol and carvacrol (Figure 2).

The percentage of oxygenated monoterpenes was highest in essential oil obtained by hydrodistillation (16.3%) while in other extract varied from 14.5% (20 s grind matrix, d₂₀ = 1.1 mm) to 7.1% (FDS). The content of monoterpenic monomers was quite similar in all samples (27.0-28.4%) except...
Table 1. Major components (%) of S. montana essential oil obtained by SC-CO₂ extraction (after 6 h) from different pretreated herb matrices and hydrodistillation

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<tr>
<th>Component</th>
<th>Pretreatment procedure</th>
<th>HD</th>
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<tr>
<td></td>
<td>Pellet 1st 20 s grind</td>
<td>40 s grind</td>
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<tr>
<td>α-Pinene</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Sabinene</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>β-Pinene</td>
<td>0.3</td>
<td>1.1</td>
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<tr>
<td>β-Myrcene</td>
<td>1.9</td>
<td>2.9</td>
</tr>
<tr>
<td>α-Terpinene</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>β-Cymene</td>
<td>8.3</td>
<td>9.1</td>
</tr>
<tr>
<td>Limonene</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>γ-Terpine</td>
<td>10.9</td>
<td>10.2</td>
</tr>
<tr>
<td>Linalool</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Terpinene-4-ol</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Thymol</td>
<td>30.7</td>
<td>30.4</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>12.5</td>
<td>11.5</td>
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<tr>
<td>β-Caryophyllene</td>
<td>3.6</td>
<td>4.9</td>
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<tr>
<td>Germacrene D</td>
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<tr>
<td>β-Farnesene</td>
<td>3.4</td>
<td>4.5</td>
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<tr>
<td>β-Cadinene</td>
<td>3.8</td>
<td>4.8</td>
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<tr>
<td>Caryophyllene oxide</td>
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<td>0.1</td>
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<tr>
<td>Total identified</td>
<td>85.9</td>
<td>87.3</td>
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<td>Yield, mass%</td>
<td>1.62</td>
<td>1.28</td>
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Figure 2. Yield (mass%) of S. montana essential oil isolated by SC-CO₂ extraction and hydrodistillation (HD) with respect to grouped components, expressed as mean ± standard deviation; a,b,c,d means for each pre-treatment, without a common letter, are significantly different from each other (P < 0.05).

for the essential oil obtained by HD (24.1%). This result is probably due to an uncontrolled loss of some volatile components from the Clevenger apparatus [24]. The major difference between these two techniques (SC-CO₂ and HD) is the occurrence of thymoquinone, an oxygen-containing monoterpane in all SC-CO₂ extracts. This compound is of great importance to the pharmaceutical industry, due to its anticancer, antioxidant and anti-inflammatory properties, as well as the neuroprotective effect against forebrain ischemia and Alzheimer disease [23].

From the general phytochemical knowledge and also from the obtained results it is evident that the preprocessing of plant material plays rather significant role in chemical composition of the essential oil. Thus, highest content of thymol, carvacrol and γ-ter-
pinene (35.4, 14.1 and 11.4%, respectively) as well as limonene (3.33%) was recorded in the EO obtained from winter savory matrix treated by FDS. The highest content of p-cymene and linalool (10.1 and 3.8%, respectively) was found in EO gained savory matrix treated by FDL. The smallest content of very important compounds as thymol and carvacrol (30.4 and 11.5%, respectively) was recorded in EO obtained from 20 s grind herb matrix ($d_{20} = 1.1$ mm). For mechanically treated samples, it is noticeable that content of phenolic compound thymol, carvacrol and thymoquinone rise as particle diameter size decreases (Table 1). Grosso et al. [23] observed similar trend during SC-CO$_2$ extraction (at 40 °C, 90 bar and particle size 0.4, 0.6 and 0.8 mm) of $S$. montana volatile oil for carvacrol and thymol content.

High antimicrobial activity of savory EO is probably due to the presence of phenolic components, such as thymol and its isomer carvacrol as well as its precursors, $\gamma$-terpinene and p-cymene which activities have been confirmed. Moreover, a number of researchers had shown that components present in lower amount in savory EO, such as terpinen-4-ol, linalool and limonene, could also contribute to the antimicrobial activity of the oil [7,29].

To test the effect of the winter savory leaves pretreatment on the CO$_2$ extraction of EOs, the extraction curves from differently treated matrices were compared to that of the untreated matrix (Figure 3). The yields are expressed as the percentage ratio between the mass of EO extracted and the initial mass of dry herb sample.

As can be seen, the extraction yields are considerably enhanced when pretreated matrices are extracted, compared to intact herb. Relatively limited extraction rate was observed from the intact herb matrix, probably because of intraparticle resistance due to the location of the EO in the herb. It has been shown in the literature that the ability of CO$_2$ to penetrate intact glands is restricted by the low solubility of both cuticle and cell wall components [30].

Also, as can be seen from Figure 3, two different shapes of extraction curves can be observed among pretreated herb matrices. Mechanically prepared matrices revealed high extraction rates in first 120 min of extraction, which was followed by rather low extraction rates in the later stages. This trend is especially noticeable during extraction from mechanically pretreated herb matrix with diameter particle size $d_{20} = 1.1$ mm and $d_{40} = 0.9$ mm. On the other hand, physically treated and mechanically pressured matrices as well as mechanically pretreated matrices (with diameter particle size $d_{60} = 0.6$ mm) show a smoother curve through extraction.

The disruption of glands during the mechanical grinding of the leaves occurred either by direct contact with the blender blades or by particle collision helped by the turbulence within the blender. This turbulence probably resulted in higher dislocation of the EO from disrupted glands, so this oil is accessible to the extraction solvent. This fraction was easily extracted in the earlier phase, whereas the remaining EO was extracted very slowly in the later phase.

![Figure 3. Essential oil extraction curves from different pretreated $S$. montana matrices isolated by SC-CO$_2$ extraction, expressed as mean ± standard deviation.](image-url)
In physically treated herb matrices by fast CO$_2$ decomposition and mechanical pressure, the lack of turbulence during the preparation process results in lower displacement of the EO from glands; consequently, extraction curves are more gradual. When exposed to compressed CO$_2$, the gas slowly penetrates the glands and dissolves in the intraglandular oil until the solubility limit is reached. During the fast decompression of the bed, the dissolved gas is desorbed from the oil phase and discharged to the bulk solvent. The inability of the glands to discharge the gas, at a rate dictated by the loss of solubility in the oil with the decompression of the bed, generates a pressure gradient across the glands that may leads to its rupture [30].

Particle size plays an important role in SC extraction processes, when internal mass transfer resistance is reduced and the extraction is controlled by equilibrium conditions. Furthermore, the extraction rate increases because of a shortening in the diffusion path. In our work, the yields of EO from herb leaves treated by mechanical grinding in 20 and 40 s period (particle size $d_{20} = 1.1$ mm and $d_{40} = 0.9$ mm) were 1.28 and 1.35%, respectively. The smaller yield could be explained with low efficiency of grinding treatment in liberating intraglandular EO.

It was previously found [31-34] that reduced substrate particle size positively affects essential oils yield and SC-CO$_2$ extraction rate. Smaller particles caused increases in the specific surface area as well as a disruption of the cell walls and other inner barriers, thus leaving the essential oil more accessible to the SC-CO$_2$. In our study, at the smallest diameter particle size ($d_{60} = 0.6$ mm), the SC-CO$_2$ extraction yield was higher (1.48%), compared to mechanically treated samples, but not as expected. We assume there is some loss of essential oil during extended grinding period (60 s) due to higher temperature through this pretreatment process. Temperature could rise to the extent of 42 to 93 °C during spice grinding, causing loss of volatile oil and flavour constituents [35]. The temperature rise can be minimized to some extent by circulating cold air or water around the grinder, but this technique is not sufficient. It was previously found that black pepper volatile oil content was 26% higher after the cryogenic grinding in comparison with grinding at room temperature [36].

After 6 h of extraction, rather similar extract yields were obtained from FDL extraction and mechanically compressed matrices (1.64 and 1.62%, respectively), while the extraction yield from FDS pretreated herb matrix was the highest in this set of experiments (1.75%) with further increase trend. It should be noted that, in our study, the essential oil yield obtained by hydrodistillation is 1.82%, which is comparable to the estimate content within the FDS-treated matrix, i.e., 1.75%. In previously published investigation, Stamenić et al. [18] also observed higher yields for extraction of several materials - among them hyssop, thyme and mint during extraction with CO$_2$ with subsequent decompression to atmospheric conditions.

The results of the bioassays (Table 2) show that tested S. montana extracts obtained by SC-CO$_2$ extraction from different pretreated herb matrices exhibited the same (FDS) or weaker antimicrobial activity in comparison with essential oil obtained by hydrodistillation.

The highest antibacterial efficiency was shown by the SC-CO$_2$ extract after FDS pretreated herb matrix where MIC ranged between 0.09 mg/ml and 25.0 mg/ml. The lowest activity of FDS extract was observed for P. aeruginosa (25.0 mg/ml), while the highest activity was observed against S. aureus and E. coli (0.09 mg/ml). This efficiency could be attributed to the high content of compounds with known antimicrobial activity, such as phenolic components thymol and its isomer carvacrol as well as its pre-

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<th>Microorganism</th>
<th>Pretreatment procedure</th>
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<td>Pellet 15 t</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>0.36/0.36</td>
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<tr>
<td>S. aureus ATCC 25923</td>
<td>0.18/0.36</td>
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<tr>
<td>Escherichia coli</td>
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</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&gt;25.0</td>
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<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>12.5/12.5</td>
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<tr>
<td>Candida albicans</td>
<td>0.36/0.36</td>
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<tr>
<td>C. albicans ATCC 10231</td>
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cursors, γ-terpinene and p-cymene [29]. Thymol disintegrates the outer membrane and increases the permeability of the cytoplasmic membrane to ATP of E. coli [37]. Carvacrol is an isomer of thymol and has been shown to cause damage in B. subtilis cells [38]. The presence of the hydroxyl group seems to be more important for the antimicrobial activities of these compounds than the ability to expand and consequently to destabilize the bacterial membrane.

The weakest antimicrobial efficiency was shown by the SC-CO₂ extract after 20 s grinding (MIC ranged between 0.72 and 25.0 mg/ml), probably due to the smallest content of very important compounds such as thymol and carvacrol.

The mentioned results suggest the significance of individual oil components ratio in the antimicrobial mixture. Antimicrobial action is often determined by more than one component; each of them contributes to the beneficial or adverse effects [9]. The obtained results showed that SC-CO₂ extracts as well as essential oil obtained by hydrodistillation were more effective against ATCC strains than against clinically isolated strains. The data indicated that Gram-positive S. aureus was the most sensitive strain tested to the savory extracts while P. aeruginosa was the most resistant. Gram-negative P. aeruginosas known to have a high level of intrinsic resistance to virtually all known antimicrobials and antibiotics, due to a very restrictive outer membrane barrier [39]. Pseudomonas species are known to have the ability to metabolise a wide range of organic compounds and for this fact is used extensively in bioremediation; this may explain their high level of resistance. They may simply metabolise the compounds in extracts that are inhibitory to many of the other bacteria [40]. Previous reports [28,30,44] showed that Gram-positive bacteria are generally more sensitive to the effects of the savory essential oil, which was confirmed in this study. S. montana extracts and essential oil generally exhibited relatively high antifungal activity, whether as clinically isolated or as ATCC strain, regardless of the individual components’ percentage values, what confirms previous results on this subject [5,7,40].

CONCLUSION

In this study, it was found that different matrix pretreatment of S. montana leaves, namely mechanical grinding, FDS, FDL treatment and physical disruption of EOs glands by mechanical compression are effective for the supercritical CO₂ extraction of EOs. However, FDS (fast decompression of supercritical CO₂) matrix pretreatment results in the highest content of EO which is believed to be of better quality than those obtained from mechanically treated matrix, due to higher content of oxygenated compounds thymol, carvacrol and thymoquinone.

The supercritical CO₂ extracts, as well as the essential oil obtained by hydrodistillation, were the most effective against S. aureus and E. coli. Any microbiological activity depends on the chemical composition of oil and the investigated strain sensitivity. Therefore, it can be concluded that the similar antimicrobial activities of essential oil and FDS extract are probably caused by phenolic components, thymol and carvacrol which are the main components in extract as well as in essential oil. Microbial susceptibility tests confirm potential use of S. montana supercritical CO₂ extracts in the food and pharmaceutical industry.

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REFERENCES

UTICAJ PREDTRETMANA BILJKE *Satureja montana* NA EKSTRAKCiju ETARSKOG ULJAla NATKRITIČNIM CO₂

Ispitan je uticaj različitih predtretmana vrjaška (*Satureja montana L.*) na ekstrakciju etarskог ulja natkritičnom CO₂ - prinosa, hemijski sastav i antimikrobne aktivnosti dobijenih ekstraktara i etarskог ulja. Matriks biljke je, prije ekstrakcije, podvrgnut konvencionalnom mijenjanju s hemijskim razaranjem uljnih komponenti. Antimikrobna aktivnost ekstraktara dobijenih natkritičnom CO₂ ekstrakcijom bila je ista (FDS) ili slabija u poređenju sa etarskim uljem dobijenim hidrodestilacijom.

**Ključne reči:** *Satureja montana*, natkritično CO₂ ekstrakcija, predtretman biljnjeg matriksa, prinosa etarskог ulja, hemijski sastav etarskог ulja.