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Research paper

Summer savory extracts prepared by novel extraction methods resulted in enhanced biological activity

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ABSTRACT

Satureja hortensis L. (summer savory) is herb from *Lamiaceae* plant family known by its usage in folk medicine and cooking. Despite these facts, this plant was not well studied regarding the application of nonconventional extraction techniques and utilization of prepared extracts. Thus, aim of this study was to prepare extract using conventional (maceration and Soxhlet) and non-conventional (ultrasound-assisted, microwave-assisted and subcritical water) approaches, to establish their chemical profile and biological activity using different assays and methods. Results confirmed the domination of subcritical water approach for isolation of natural compounds, following by microwave-assisted extraction. High performance liquid chromatography with photodiode array detection (HPLC-PDA) analysis confirmed the presence and domination of rosmarinic acid in conventionally prepared extracts, while rutin and quercetin dominated in non-conventionally prepared ones. Antioxidant and cytotoxic assays followed the trends of previous analysis, where the highest activity was exhibited by subcritical extract. Thus, results showed that extracts may be applied in food and pharmaceutical industries for utilization.

1. Introduction

Genus *Satureja* belongs to the *Lamiaceae* family of aromatic plants and comprises more than 30 species of herbs and shrubs, which are widely distributed over the Mediterranean region (Valizadeh et al., 2014). *Satureja hortensis* L. (summer savory) is one of the herb from this plant family known by its usage in folk medicine and cooking in several regions (Momtaz and Abdollahi, 2010). Aerial parts of the plant possesses a distinctive taste a may be used as a seasoning, while flowers and leaves found their application as a tea and in traditional medicine for treatment of cramps, muscle pain, nausea, indigestion, diarrhea, and infectious diseases (Dorman and Hiltunen, 2004; Güllüce et al., 2003). Beside these applications, plant exhibits antispasmodic, antidiarrheal, antioxidant, sedative, and antimicrobial activities (Deans and Svoboda, 1989; Güllüce et al., 2003; Hajhashemi et al., 2000; Madsen et al., 1996). It has been shown that plant leaves are rich in phenolic compounds, especially rosmarinic acid and flavonoids, which are one of the most potent antioxidants (Exarchou et al., 2002; Kemertelidze et al., 2004), while carvacrol and γ-terpinene have been reported to be the main compounds of essential oil (Góra et al., 1996).

Although, there are many studies regarding summer savory, they were usually dealing with the essential oil (Boyraz and Ozcan, 2006; Góra et al., 1996; Güllüce et al., 2003; Hajhashemi et al., 2002; Kemertelidze et al., 2004; Saeidi and Shahab-Ghayoor, 2015; Valizadeh et al., 2014) and application of conventional extraction approaches (Dorman and Hiltunen, 2004; Exarchou et al., 2002; Hajhashemi et al., 2002; Şahin et al., 2003). However, traditional extraction technique possess some drawbacks such as: thermal degradation of compounds of interest, occurrence of hydrolysis and residue of organic solvents in obtained extract (Zeković et al., 2016). Usually used organic solvents could express negative impact on environment or on human health, or can even be toxic (Wang and Weller, 2006; Veličković et al., 2017). This imply the necessity of further purification making the process of isolation of bioactive compounds more complicated, which can, in the end, increases the price of final products. Also, traditional techniques are very time-consuming and require relatively large quantities of solvents (Luque de Castro and Garcia-Ayuso, 1998).

On the other hand, during last years significant progress was made

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in the technology of isolation of bioactive compounds from plants and different non-conventional techniques have been developed. Assisted extraction techniques, such as ultrasound (UAE) and microwave extractions (MAE), became very popular due to the fact that these techniques considerably reduce the consumption of solvents, increase the speed and efficiency of the process, being considerate to the principles of the green chemistry (Švarc-Gajić et al., 2013). Furthermore, there is growing number of process which involve the use of solvents with altered physicochemical characteristics, such as sub- or supercritical fluids and among them special attention attracts subcritical water.

Subcritical water extraction (SWE) is extraction with water that remains in its liquid form in the temperature range between 100 and 374 °C under conditions of elevated pressure (Adachi, 2009). Such fluid has unique characteristics which helps it to efficiently solubilize compounds which are moderately or even sparingly soluble in water at room temperature. In contrast, the solubility of compounds which are well-soluble in water at room temperature (polar and moderately polar compounds) does not change significantly by bringing the water in its subcritical state (Cvetanović et al., 2017a,b).

To our knowledge there is a lack of reported data on the use of modern techniques in the extraction of the summer savory. Moreover, the composition of summer savory extracts obtained by modern approaches is not readily available.

Thus, the goal of this work was to characterize summer savory extracts obtained by ultrasound, microwave and subcritical water extraction in terms of their chemical composition and biological activity. Furthermore, used extraction technique was compared with conventional ones (maceration and Soxhlet extraction). Chemical screening of obtained extracts was performed by spectrophotometrically methods as well as by high performance liquid chromatography with photodiode array detection (HPLC-PDA). Extracts were further investigated regarding their biological activity (antioxidant, antibacterial and cytotoxic). Obtained results were compared in order to obtain valuable information regarding the further possible application of this plant in food and/or pharmaceutical industry.

2. Material and methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, aluminium chloride, gallic acid, 2,2-difenyl-1-picrylhydrazyl (DPPH), rutin and potassium iodate (Sigma Chemical Company, St. Louis, SAD). All standard for HPLC analysis were of analytical grade and were purchased from Sigma chemicals Co (St Louis, MQ, USA) and Alfa aesar (Karlsruhe, Germany). Acetonitrile and phosphoric acid were of HPLC grade (Tedia Company, USA). Ethanol and methanol was of analytical grade (Aldrich Chemical Co, Steinheim, Germany).

2.2. Plant material

Satureja hortnesis L. (summer savory) was collected in Ovčarsko-Kablarska klisura (western region of Serbia) at an altitude of 278 m, in August 2015. Botanical identification of the plant was done by Dr. Milan Stankovic (Institute of Biology, Faculty of the Science and Mathematics, Kragujevac, Serbia). The voucher number is 128/017. Plant material was stacked in a crate with perforated bottom, in order to ensure air flow. Drying was performed naturally in the draft and dark until moisture content of 10%. Before extraction material was crushed and homogenized into small 3–5 mm pieces by a cylinder crusher ground. The fraction of the same particle size was used in all extraction runs.

2.3. Extraction procedures

Soxhlet extraction was conducted in the following manner: plant material (75.0 g) was placed in the Soxhlet apparatus. Extraction process was carried out for eight hours using 96% ethanol as a solvent (600 mL).

Maceration was conducted using the following procedure: plant samples (10.0 g) were extracted using 96% ethanol (300 mL) as a solvent. The extraction process was carried out under the laboratory conditions at a temperature of 22 °C in a sheltered, dry place for seven days, with occasional shaking to improve the maceration process.

Ultrasound-assisted extraction (UAE) was performed in ultrasonic water bath (EUP540A, Euinstruments, France). A sample (5 g) was placed in volumetric flask and 100 mL of solvent (96% ethanol) was added. The mixture was sonificated for thirty minutes at frequency of 40 kHz and ultrasound power of 90% (216 W).

Microwave-assisted extraction (MAE) was performed in domestic microwave oven, which was previously modified for this purpose. Extraction was conducted using the same sample weight, solvent volume and extraction time. The extraction procedure program was as follows: one min pre-heating at 160 W; one min pre-heating at 320 W and thirty min extraction at 600 W.

Subcritical water extraction (SWE) was performed in previously described home-made extractor system (Cvetanović et al., 2017a,b). In all experimental runs, 5.0 g of plant sample was mixed with 100 mL of double-distilled water. Extraction was performed at pressure of 40 bar and temperature of 140 °C. Agitation was assured by vibrational movements of vessel platform at the frequency of 3 Hz. Extraction duration in all experiments was 30 min. After the extraction, the process vessel was immediately cooled in flow-through water-bath at 20 °C.

All extracts were filtered through filter paper (Whatman, No.1) and concentrated to dry mass by a rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenia) under vacuum and dried at 60 °C to the constant weight. The dried extracts were stored in a dark glass bottle at 4 °C to prevent oxidative damage.

2.4. Chemical composition of extracts

Total phenolics (TPC) and flavonoids (TFC) contents were established using previously described procedures (Brighente et al., 2007; Singleton and Rossi, 1965). Results were expressed as mg of gallic acid equivalents (mg GAE) and mg of rutin equivalents (mg RU) per g of dry extracts. Condensed tannins (CT) and gallotannins (GA) were determined using described potassium iodate assay (Vermerris and Nicholson, 2006). Both results were expressed as mg GAE/g. Total anthocyanins content (TAC) was determined applying previously described procedure (Cheng and Breen, 1991; Vulic et al., 2011) using pH single and differential methods. Result was expressed as cyanidin-3-glucoside equivalents per g of dry extract (mg CGE/g).

2.5. HPLC-DAD analysis

Quantification of individual phenolic compounds was performed using reversed phase HPLC analysis. The equipment used was an HPLC Agilent-1200 series with UV–vis DAD detector for multi wavelength detection. After injecting 5 μL of sample, the separation was performed in an Agilent-Eclipse XDB C-18 column (4.6 × 150 mm), which was thermostated at 25 °C. Two solvents were used for the gradient elution: A (H_2O + 2%HCOOH) and B (80%ACN + 2%HCOOH + H_2O). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increased 0–25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increased 25–50% B, from 35 to 40 min gradually increased 50–80% B, and finally for the last 5 min gradually decreased 80-0% B. Phenolic compounds in the samples were identified by comparing their retention times and spectra with retention time and spectra of standards for each component. Quantitative data were calculated from the calibration curves. Calibration curve, coefficient of correlation (R^2), limit of detection (LOD) and limit of quantification (LOQ) are shown in Table 1. Content of phenolic compound were