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Free-radical scavenging activity and antibacterial impact of Greek oregano isolates obtained by SFE

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ABSTRACT

The antioxidant and antibacterial properties of Greek oregano extracts obtained by fractional supercritical fluid extraction (SFE) with carbon dioxide were investigated and compared with the properties of essential oil obtained by hydrodistillation. According to DPPH·, hydroxyl radical and superoxide anion radical scavenging activity assays, the supercritical extracts expressed stronger antioxidant activity comparing to the essential oil. The most effective was the supercritical extract obtained by fractional extraction at 30 MPa and 100 °C after the volatile fraction had been extracted at lower pressure. At the same time this extract showed strong antibacterial activity against staphylococci, including MRSA strain, but did not affect *Escherichia coli* of normal intestinal flora. The essential oil obtained by hydrodistillation showed stronger antibacterial activity against *E. coli*, *Salmonella* and *Klebsiella pneumoniae*, comparing to the supercritical extracts but at the same affected the normal gut flora.

1. Introduction

Greek oregano (*Origanum heracleoticum* or *Origanum vulgare* L. ssp. *hirtum*) belongs to *Origanum* genus of the Lamiaceae family. It is widely used as a culinary spice due to its flavouring properties originating from its essential oil. The amount of essential oil in Greek oregano is considered to be high, up to 8% (Kokkini & Vokou, 1989) and rich mainly in four components: carvacrol, thymol, *p*-cymene and γ-terpinene. However, numerous studies showed that the amounts of these four components present in the essential oil, as well as the amount of the oil, can significantly vary depending on factors such as geographical location of the crops and/or harvest season (Bonfanti et al., 2012; De Martino, De Feo, Formisano, Mignola, & Senatore, 2009; Esen, Azaz, Kurkcuoglu, Can Baser, & Tinmaz, 2007; Gavalas, Kalburtji, Kokkini, Mamolos, & Veresoglou, 2011; Jerkovic, Mastelic, & Milos, 2001; Kokkini, Karousou, Dardioti, Krigas, & Lanaras, 1997; Kokkini, Karousou, & Vokou, 1994; Russo, Galletti, Bocchini, & Carnacini, 1998; Tibaldi, Fontana, & Nicola, 2011; Vokou, Kokkini, & Bessiere, 1993).

Since species of *Origanum* genus had found their place in traditional medicine from ancient times, attempts have been made to investigate the antioxidant as well as antimicrobial potentials of Greek oregano. Sivropoulou et al. (1996) investigated antibacterial effect of carvacrol rich Greek oregano essential oil obtained by hydrodistillation, as well as the same effect of main individual components against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Rhizobium leguminosarum* and *Bacillus subtilis*. It was found that the essential oil, carvacrol and thymol had strong antibacterial effect (except on *P. aeruginosa*), unlike *p*-cimene and γ-terpinene. Adam, Sivropoulou, Kokkini, Lanaras, and Arsenakis (1998) presented results on antifungal activity of thymol and carvacrol rich Greek oregano essential oil. It was shown that the Greek oregano oil was superior compared to those of mint, lavender and sage against the human pathogens *Malassezia furfur*, *Trichophyton rubrum*, and *Trichosporon beigelii*. Thymol rich oil of *O. vulgare* L. ssp. *hirtum* from Dalmatia was also found to have good antioxidant activity which was fairly better than the activity of individual components of the oil (Milos, Mastelic, & Jerkovic, 2000). Zheng and Wang (2001) examined the phenolic compounds content and antioxidant activities of almost thirty culinary and medicinal herbs. It was found that the content of phenolic components was the highest and the antioxidant effect

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of essential oil was the strongest in the case of Greek oregano. Esen et al. (2007) investigated antibacterial and antifungal effect of essential oils from numerous samples of wild and cultivated *O. vulgare* L. ssp. *hirtum* collected from 20 locations that greatly differed in composition. Fairly good results were obtained, but without a definitive conclusion regarding favourable composition, since the variations were very large in the examined samples. Dorman and Deans (2000) as well as Sarac and Ugur (2008) found that Greek oregano essential oil had strong antibacterial effect on both gram-positive and gram-negative bacteria. Fasseas, Mountzouris, Tarantilis, Polissiou, and Zervas (2007) examined the antioxidant activity of Greek oregano essential oil in meat. The results showed that the essential oil treatments significantly reduced the oxidation process. De Martino et al. (2009) examined antibacterial effect of Greek oregano essential oil against numerous strains of bacteria. Positive effect was found for gram-positive species, while only *E. coli*, amongst the Gram-negative ones, was successfully inhibited by the essential oil.

All the investigations mentioned so far were conducted with essential oils obtained by hydrodistillation. Besides steam distillation, which is generally used for production of essential oils for commercial purposes, supercritical fluid extraction (SFE) has received particular attention in the field of natural compounds isolation. Carbon dioxide is the most used supercritical fluid due to its favourable critical parameters (31.1 °C; 7.38 MPa), low cost and availability. Extraction with supercritical carbon dioxide provides isolation of essential oil rich fractions from plant material under mild temperature conditions (40–60 °C), thus avoiding thermal degradation of active compounds. SFE also offers the advantage of fractional extraction or separation (extraction or separation at different pressure and temperature conditions) whereby extracts rich in compounds with antioxidant properties but free of the lower molecular weight aromatic compounds can be obtained. Applying fractional SFE, natural antioxidants that are soluble in SC CO_2 and free of aromatic compounds that could have an impact on organoleptic properties of food, can be isolated. In the open literature, data on the SFE from Greek oregano are scarce. However, a considerable amount of data can be found on SFE from *O. vulgare* (Cavero et al., 2006a; Díaz-Maroto, Pérez-Coello, & Cabezudo, 2002; Fornari et al., 2012; Omar, Alonso, Garaikoetxea, & Etxebarria, 2013; Rodrigues et al., 2004; Simandi et al., 1998).

Mohácsi-Farkas, Tulok, and Balogh (2003) found that isolates of Greek oregano and winter savory obtained by hydrodistillation as well as by SFE exhibited significant growth-inhibitory effect against selected food-borne bacteria and fungi. Greek oregano extracts showed higher activity than those of winter savory, and extracts obtained by hydrodistillation showed higher activity than those obtained by the SFE. Both essential oil and supercritical extract of oregano had a significant growth-inhibitory effect against *E. coli*, *Pseudomonas fluorescens*, *Bacillus cereus* and *Aspergillus niger* strains in 0.05% and 0.1% (v/v) concentrations. The supercritical extract of oregano in 0.1% concentration was proved to be able to inhibit the growth of *Saccharomyces cerevisiae*. In the mentioned study, the supercritical extract was obtained with SC CO_2 at 40 °C and 10 MPa, which were the common conditions for isolation of essential oil rich fractions. At higher pressures, besides essential oils, co-extraction of higher molecular weight compounds takes place as well. Karakaya, Nehir El, Karagözlü, and Sahin (2011) investigated antioxidant and antibacterial effect of Greek oregano isolates obtained by hydrodistillation, solvent free microwave extraction (SFME) and SFE at 40 °C and 10 MPa. Good results were achieved with essential oil obtained by hydrodistillation and SFME extracts regarding both antioxidant and antibacterial effect with the exception of *S. aureus*. On the other hand, the supercritical extracts exhibited neither antioxidant nor antibacterial effect. It was concluded that the supercritical extract composition was low in oxygenated compounds that are responsible for antioxidant and antibacterial effect. Data on the antibacterial effects of supercritical extracts obtained from *O. vulgare* can be found as well (Cavero, Senoráns, Jaime, Reglero, & Ibañez, 2006b; Munoz et al., 2009). However, to the best of our knowledge there are no data in the open literature on antibacterial not antioxidant properties of Greek oregano extracts obtained by SFE at pressures higher than 10 MPa.

The main objective of this study was the investigation of the antioxidant and antibacterial properties of Greek oregano extracts obtained by fractional SFE with carbon dioxide and comparison with essential oil obtained by hydrodistillation. The importance of the impact of essential oil and supercritical extracts on the normal intestinal flora was also evaluated.

2. Materials and methods

2.1. Plant materials and chemicals

O. heracleoticum was collected in 2011 in Southern Serbia (Vlasina) and identified in the Institute for Medicinal Plant Research, Belgrade, Serbia. The plant material was collected just before the phase of full flowering, and dried in the shadow, at the place protected from direct sunlight. The material was ground and sieved. The fraction with an average particle diameter of 0.4 mm (caught between the 0.3 mm and 0.5 mm sieves) was used for further studies.

2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH·), *N*,*N*-dimethylformamide (DMF), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) and dimethylsulphoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Carvacrol and thymol of analytical reagent grade were purchased from Sigma Aldrich, Germany. Butylated hydroxyanisole (BHA), synthetic antioxidant, was purchased from ICN Biomedicals, Inc. Aurora, Ohio, United States. All other chemicals and solvents were of the highest analytical grade and obtained from "Zorka" Šabac, Serbia. Commercial carbon dioxide (99% purity, Messer-Tehnogas, Belgrade, Serbia) was used for the SFE.

2.2. Supercritical fluid extraction and hydrodistillation

Essential oil rich fractions were obtained by SFE with carbon dioxide at pressure of 10 MPa and temperature of 40 °C. Extraction of the higher molecular weight fractions followed at a pressure of 30 MPa and temperatures of 40 °C and 100 °C. Extractions with supercritical carbon dioxide were performed in an Autoclave Engineers Screening System previously described (Zizovic, Stamenic, Orlovic, & Skala, 2007). The flow rate of supercritical carbon dioxide was 0.5 kg/h for the experiments at 10 MPa and 0.3 kg/h for the experiments at 30 MPa. The initially used mass of the plant samples was 45 g.

Isolation of essential oil by hydrodistillation was performed in a Clevenger-type apparatus for 4 h, up to the point at which the oil contained in the herbaceous matrix was exhausted.

2.3. Chemical analyses

2.3.1. Determination of total phenolic content

The total phenolic content was determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). 100 μL of MeOH solution of the investigated extracts were mixed with 0.75 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distiled water) and allowed to stand at 22 °C for 5 min; 0.75 mL of sodium bicarbonate (60 g/L) solution was added to mixture. After 90 min at 22 °C, the absorbance was measured at 725 nm. Gallic acid (0–100 mg/L) was used for calibration of a standard curve. The