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Chemical composition and antioxidant properties of *Ocimum basilicum* L. extracts obtained by supercritical carbon dioxide extraction: Drug exhausting method

Snežana Filip[a,*], Senka Vidović[a], Jelena Vladić[a], Branimir Pavlić[a], Dušan Adamović[b], Zoran Zeković[a]

[a] Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[b] Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

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ABSTRACT

Supercritical fluid extraction of thermolabile volatile compounds from *Ocimum basilicum* was applied. The highest extraction yield has been achieved in the case of extract obtained using carbon dioxide at pressure of 150 bar and temperature of 60 °C on drug previously exhausted by carbon dioxide at temperature of 60 °C and pressure of 100 bar. Obtained extracts were analyzed by gas chromatography–mass spectrometry. A few compounds were dominant in analyzed extracts: linalool, eugenol, α-bergamotene, germacrene D, γ-cadinene, δ-cadinene and β-selinene. Linalool was major compound in extracts present in range from 12.2 to 141.5 g/kg. Antioxidant activity of extract obtained using carbon dioxide at 100 bar and at temperature of 60 °C was approximately three times higher than antioxidant activity of basil essential oil. Obtained results indicated that supercritical extraction of basil, with the principles of drug exhausting, can be considered as more effective method for volatile non-polar components extraction and preparation of extracts with high antioxidant activity, in comparison to classical Soxhlet and hydro-distillation procedures.

1. Introduction

In recent years herbal extracts and essential oils (EOs) have attracted a great deal of scientific interest as they formed the basis of applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [1–3]. The main investigated and explored characteristics of herbal extracts and essential oils are antioxidant activity. Antioxidant activity of natural products, such as herbal extracts, are linked to the reduced risk of many diseases, especially cancer and cardiovascular. Furthermore, in the field of food production carcinogenicity of some synthetic antioxidants led to the substitution of such antioxidants with safe additives/extracts obtained from natural sources with proven antioxidant capacity. *Ocimum basilicum* L. (sweet basil) is an important herbal representative that can be used in mentioned applications. It is aromatic herb belonging to the *Lamiaceae* family, and is the most cultivated variety in the world, especially in Mediterranean countries. Among more than 150 species of the genus *Ocimum*, basil is the major essential oil crop [4]. The basil leaves can be used fresh or dry, or as a spice to enhance the food flavor. In folk medicine basil leaves and flowering tops are usually used as carminative, stomachic and antispasmodic [5].

The antioxidant properties of basil have been investigated intensively and mainly these properties have been linked to the phenolic constituents of basil and its extracts [6,7]. Composition and antioxidant characteristics of basil essential oils, as extracted mixture of volatile compounds, were investigated by Hussain et al. [8]. In the basil essential oil, linalool and methylchavicol (estragole) were marked as a two major components [9,10]. These components, together with various sesquiterpenes, are being responsible for fresh, minty and sweet flavor of plant [11]. Eugenol has been also marked as one of the major compounds in basil essential oil [9,12–14]. The presence of such specific compounds makes basil oil economically important [15]. The value of basil oil can be explained with its major constituents—linalool. Linalool and linalool-rich essential oils are known to exhibit various biological activities such as antimicrobial, anti-inflammatory, anticancer and antioxidant [16]. Linalool is also a key compound for industrial production of fragrance chemicals such as geraniol, nerol, citral

* Correspondence to: Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia. Tel.: +381 638571488.
E-mail address: filipsneza@gmail.com (S. Filip).

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and its derivates, as well as a major compound in the synthesis of vitamins A and E [17]. All above mentioned compounds are extremely sensitive to heat and due to that they are subject to chemical changes. Therefore, during the extraction process some losses of such volatile compounds, in higher or lower concentrations, depending on the applied method of extraction, may occur [18].

Several methods can be used for extraction of mentioned complex volatile mixtures and compounds. Hydro-distillation is classical method for volatile constituents/essential oils extraction. The main disadvantage of this process is high process temperature. This disadvantage can be overcome by employment of supercritical fluid extraction (SFE), which can be consider as a successful technique for extraction of complex components mixtures including essential oils, fatty acids, tocopheroles, etc. [19–21]. The most commonly used extraction fluid in SFE is carbon dioxide. It is non-toxic, non-explosive and easy removable from the product, allowing preparation of solvent free extracts. Carbon dioxide possesses low critical temperature and pressure (T_c = 31.1 °C, P_c = 73.8 bar), therefore allowing the preservation of thermolabile compounds, such are essential oils components.

The present work was undertaken with main objectives to investigate the composition and antioxidant activity of supercritical basil extracts. Supercritical extraction process has been applied regarding its efficiency for extraction of temperature unstable volatile compounds. Beside supercritical extraction technique for extraction of basil volatile compounds hydro-distillation, as comparable classical method of extraction, was used. During the investigation, the influence of applied extraction techniques on preparation and characteristics of supercritical extracts was also explored.

2. Materials and methods

2.1. Chemicals

Commercial carbon dioxide (Messer, Novi Sad, Serbia) purity >99.98% was used for laboratory supercritical fluid extraction. The standard compounds for GC analysis (GC purity) were purchased by Ehrenstorfen, Germany and Carl Roth, Germany. All other chemicals were of analytical reagent grade.

2.2. Plant material

Basil was cultivated at the Institute of Biodiversity, Bački Petrovac, Serbia, in 2011. The collected plant material (leaves and flowering tops) were air dried and stored at room temperature. Moisture content of basil was analyzed using standard procedure, i.e., by drying the plant sample at 105 °C until constant weight. This analysis was performed in three replicates. The dried basil was grounded in a domestic blender and the particle size of ground material was determined using sieve sets (Erweka, Heusenstamm, Germany).

2.3. Hydrodistillation

The content of essential oil in grounded plant material was determined by hydrodistillation according to the standard *Ph. Jug.* IV procedure [22].

2.4. Soxhlet extraction

Sample of basil (20.0 g) was extracted by methylene chloride using Soxhlet apparatus. After 6 h of extraction the extraction solvent was evaporated under vacuum and extraction yield was determined.

2.5. Supercritical carbon dioxide extraction

The extraction process was carried out on laboratory scale high pressure extraction plant (HPEP, NOVA, Swiss, Efferikon, Switzerland; Fig. 1) [23]. The main plant parts and properties, by manufacturer specification were: gas cylinder with CO_2, the diaphragm type compressor with pressure range up to 1000 bar, extractor with heating jacket for heating medium with internal volume 200 ml, maximum operating pressure of 700 bar and temperature 100 °C, separator with heating jacket for heating medium (with internal volume 200 ml, maximum operating pressure of 250 bar), pressure control valve, temperature regulation system and regulation valves.

Extracts were prepared according to the following procedure: the ground basil (50.0 g) was placed in the extractor vessel and first extract (E1) was obtained using carbon dioxide at the pressure of 100 bar and temperature of 60 °C. The exhausted herbal material (after extraction of E1) was again extracted by carbon dioxide, but at the pressure of 150 bar and temperature of 60 °C, for preparation of extract E2. Further, exhausted material (after extraction of E1 and E2) was extracted using carbon dioxide at 200 bar and 60 °C for isolation of extract E3, and the same procedure was applied for preparation of E4 extract using carbon dioxide at 300 bar and 60 °C (Fig. 2). The extraction time and flow rate was the same in all cases of extraction, 4 h and 3.225 g CO_2/min.

The separator conditions were 15 bar and 23 °C. After extraction obtained extracts were placed in the glass bottles, sealed and stored at 4 °C to prevent any possible degradation.

2.6. Antioxidant assay—DPPH test

The free radical scavenging activity of supercritical basil extracts was determined using simple and fast spectrophotometric method as described by Espin et al. [24]. Briefly, prepared extracts were mixed with methanol (96%) and 90 μM 2,2-diphenyl-1-picrylhydrazyl (DPPH) to give different final concentrations. After 60 min at room temperature, the absorbance of samples was measured at 517 nm. Radical scavenging capacity (%RSC) was calculated by the following equation:

$$\%RSC = 100 - (A_{sample} \times 100)/A_{blank} \quad (1)$$

where, A_{sample} is the absorbance of sample solution and A_{blank} is the absorbance of control. Antioxidant activity was also expressed as IC_{50} which represent the concentration of test (extract solution) required for obtaining the 50% of radical scavenging capacity.

2.7. Chromatographic procedure

GC/MS analysis was run on Agilent GC6890N system coupled to mass spectrometer model Agilent MS 5795. An HP-5MS column (30 m length, 0.25 mm inner diameter and 0.25 μm film thicknesses) was used. Injected volume of sample solution in methanol was 5 μl with split ratio 30:1. The compounds were identified using the NIST 05 and Wiley 7*n* mass data base and by comparing their retention times to those in mass spectral libraries. Quantifications of aromatic compound linalool was performed by FID detector and calibration curve for compound. The percentage composition (relative amount) was calculated from the peak area. The GC/MS operating conditions were as follows: injector temperature 250 °C, temperature program was: from 60 °C to 150 °C (4 °C/min), carrier gas He with flow rate 2 ml/min. The GC/FID operating conditions were: injector temperature 250 °C, temperature program from 60 °C to 150 °C (4 °C/min), detector temperature 300 °C.

All data are presented as the means ± standard deviation of at least three replicates.