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Experimental and chemometric study of antioxidant capacity of basil (*Ocimum basilicum*) extracts

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ABSTRACT

The interest in a natural and healthy lifestyle has moved the plant crops under the spotlight. The aim of the work was to test the antioxidant activity of basil (*Ocimum basilicum* L.) extracts obtained by extraction with water (in presence and absence of light), methanol (95%), ethanol (30, 40, 50, 60, 96%), chloroform, dichloromethane and hexane. Fragmentation of plant material was 0.3 and 2 mm and the extraction was performed during 10 and 30 min. The total phenolic content ranged from (5.17 ± 0.15 to 65.25 ± 2.19) mg of gallic acid equivalents per gram of a dry weight of extract, and the content of the total flavonoids from (0.11 ± 0.01 to 40.63 ± 2.14) mg of quercetin per gram of a dry weight of extract. All the extracts showed an antioxidant activity with an IC_{50} values of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition in the range from (0.22 ± 0.01 to 20.49 ± 1.54) μg/ml. The evaluation of experimental data for 44 basil extracts was performed by applying hierarchical cluster analysis (HCA) and principal component analysis (PCA). It was found that the increased time of extraction, solvent polarity and plant fragmentation increase the quality of the extracts in terms of the content of phenolic components and antioxidant effects. Extracts with the strongest antioxidant capacity were obtained by concentrated ethanol and methanol maceration. The chemometric analysis showed good correlation between the yield and total phenolic composition, and between the flavonoid content and antioxidant activity, predicting thus, basil extract quality.

1. Introduction

Looking for a new approach to nutrition, more and more people believe that plant crops contribute directly to their health. Functional food plays an important role considering the fact that it can offer an excellent benefit (Urala and Lahteenmaki, 2007). The healthy lifestyle has a great impact on the well-being by preventing nutrition-related diseases, scavenging of free radicals and blocking chain reactions (Menrad, 2003). Free radicals are the normal product of human body metabolic reactions (Salla et al., 2016). They are usually defined as any molecular species with unpaired electron in an atomic orbital capable of independent existence. The presence of an unpaired electron is responsible for many common properties shared by most radicals. Many radicals are unstable and highly reactive and can behave as oxidants or reductants due to ability to donate or to accept an electron from other molecules. The most important oxygen-containing free radicals in many diseases are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical (Lobo et al., 2010). They can be products of tobacco smoke, pollutants, radiation, organic solvents, pesticides, etc. Free radicals are highly reactive oxygen species capable of attacking unsaturated fatty acids of the membrane system and cause lipid peroxidation, which is one of the reactions that lead to oxidative stress (Kaurinovic et al., 2011). Overproduction of these free radicals can cause the oxidative damage of biomolecules (lipids, proteins, DNA) and finally induce the occurrence of many chronic diseases such as atherosclerosis, cancer, diabetes, stroke, heart diseases, gastric ulcer, aging and other degenerative diseases in humans (Masuoka et al., 2012; Cai et al., 2004; Gülçin et al., 2007).

Abbreviations: DPPH, 2,2–diphenyl–1–picrylhydrazyl radical; HCA, hierarchical cluster analysis; PCA, principal component analysis; FC, Folin-Ciocalteu; GAE, gallic acid equivalents; DE, dried extract; QE, quercetin equivalents; RSC, radical scavenger capacity; IC_{50}, inhibitory concentration; TPC, total phenolic compound; FLV, flavonoid compound.

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Antioxidants are substances which can scavenge free radicals and prevent those disorders. They have redox properties due to their ability to reduce agents, hydrogen donors and singlet oxygen structure (Hakkim et al., 2007). The most commonly used synthetic antioxidants in food are butyl-hydroxytoluene (BHT) and butyl-hydroxyanisole (BHA) which are very effective as antioxidants. However, their usage in nutrition is avoided due to their structural instability and potential carcinogenic effect. Nowadays, the tendency in pharmaceutical and food industries is to replace synthetic antioxidants with the natural ones. For these reasons there is a growing interest in analyzing natural, healthy and non-toxic additives as potential antioxidants (Politeo et al., 2007).

Some plants which contain many phenolic compounds are increasingly of interest in food industry due to their ability to scavenge free radicals (Gülçin et al., 2007). Basil (*Ocimum basilicum* L.) is one of the most important industrial and pharmaceutical crop species from *Lamiaceae* family having a major application in the food, pharmaceutical and cosmetic industries. It contains many antioxidant substances which contribute to its intense antiradical activity (Kwee and Niemeyer, 2011) and could have potential human health benefits (Flanigan and Niemeyer, 2014; Lee and Scagel, 2009). The main antioxidant compounds in basil extracts are chlorogenic, *p*-hydroxybenzoic, caffeic, vanillic and rosmarinic acids, as well as apigenin, quercetin and rutin. Basil is an ornamental, medicinal and aromatic plant native to Asia, Africa and India, but is widely cultivated in many countries under variety conditions (Makri and Kintzios, 2007). It is used for pharmaceutical and cosmetic preparations because of the rich phenolic and flavonoid content. Due to the strong antioxidant activity, basil acts as a protector to prevent heart diseases, reduce inflammation, lower the incidence of cancers and diabetes (Mastaneh et al., 2014). Antioxidant compounds from natural plants can be obtained by different procedures under different process conditions (time, lightening) and using various solvents. Maceration with organic solvents provides a great amount of phenolic acids and flavonoids which are holders of an antioxidant activity (Vidović et al., 2012).

There are many studies about the antioxidant activity of the basil extract, but there is no evidence how different extraction conditions affect yield and total phenolic compounds of obtained extracts. Factors which have an impact on the phenolic composition are important because an antioxidant capacity, bioavailability and bioefficacy strongly depend on phenolic compounds (Nguyen et al., 2010; Manach et al., 2005). Thus, the main objectives of this study were: analysis of the influence of operational conditions and extraction methods on antioxidant activity, correlation between antioxidant activity and total phenolic/flavonoid content and optimization of its application in the industry.

Although basil is popular and widely consumed, there is no data of optimization of extraction procedure performed by different chemometric techniques in order to obtain extracts with the highest amount of bioactive compounds. In this work large amounts of obtained experimental data were analyzed by applying different chemometric tools – hierarchical cluster analysis (HCA) and principal component analysis (PCA). The HCA and PCA procedures were applied in order to find similarities between the analyzed data and they have been already described elsewhere (Miller and Miller, 2004). HCA searches for objects which are close together in the variable spaces and puts them into the same cluster. Successive stages of clustering can be shown on a dendrogram where the vertical axis is a measure of similarity between two objects in obtained clusters. Principal component analysis is an effective method which is used for reducing the amount of data without much loss of information finding new variables – principal components (PCs) which are linear combinations of the original variables. The principal components are formed in the way that, unlike the original variables, they are not correlated with each other. However, the principal components are also chosen so that the first principal component (PC1) accounts for most of the variation in the data set, while the second (PC2) accounts for the next largest variation and so on. Hence, when the significant correlation occurs, the number of useful PCs is much smaller than the number of the original variables. Usually, two PCs are adequate to describe the most of the data variations in the specified data analysis (Vastag et al., 2014).

2. Material and methods

2.1. Chemicals

Gallic acid, aluminium chloride, dichloromethane, hexane and chloroform were obtained from Sigma Aldrich (St. Louis, MO, USA), methanol and sodium-carbonate from POCH (Gliwice, Poland) and quercetin from Extra synthese (Genay Cedex, France). Ethanol was purchased from J.T. Baker (Nederland) and 2, 2-diphenyl-1-picrilhydrazil (DPPH) reagent from Alfa Aesar (Karlsruhe, Germany). The Folin-Ciocalteu's reagent was obtained from Merck (Darmstadt, Germany). Ultra-pure water was used for the preparation of all solutions. All solvents and reagents were of an analytical grade unless indicated otherwise.

2.2. Plant material and preparation

Voucher specimens (*Ocimum basilicum* L. 1753, Institute for Medicinal Plant Research "*Dr Josif Pancic*", Belgrade, Serbia, 2-1518) were confirmed and deposited at the Herbarium BUNS of the University of Novi Sad, at the Department of Biology and Ecology, Faculty of Sciences. Ground parts of basil (*Ocimum basilicum* L.) were run through different sieves and standardized on 0.3 and 2 mm. The extraction was performed with ethanol-water mixtures (30, 40, 50, 60, 96%, v/v), concentrated methanol (95%, v/v), water (in presence and absence of light), dichloromethane, chloroform and hexane during different periods of time (10 and 30 min). Different concentrations of ethanol and presence/absence of light have been applied in order to find the most appropriate solvent and condition for the extraction that can be applied in any of the industrial processes. For that purpose, 1 g of dry plant was overflowed with 5 ml of solvent and shaken on magnetic stirrer. After 10 or 30 min samples were filtered and rinsed with another 5 ml of specific solvent and evaporated on rotary evaporator. Dry extracts were used for further analysis. Total extraction yield, total phenolic content, flavonoides and the inhibition of DPPH radical were determined in 44 obtained dry extracts and were performed in triplicate.

2.3. Analysis of total phenolic compounds

The amount of total phenolic compounds in the extracts was determined colorimetrically with the Folin-Ciocalteu (FC) reagent (Božin et al., 2008). The reaction mixture contained 0.1% dilution of a dry extract (0.1 ml), a freshly prepared 0.2 M FC reagent (0.5 ml) and a 7.5% sodium carbonate solution (0.4 ml) and it was kept in the dark under ambient conditions for 30 min to complete the reaction. The absorbance of the resulting solution was measured at 760 nm in a UV–vis spectrophotometer (model 8453 Hewlett Packard, Agilent Technologies, USA). The concentration of the total phenolic compounds was expressed as a milligram of gallic acid equivalents (GAE) per gram of a dried extract (d.e.), using the standard curve of gallic acid. All the measurements were carried out in three replicates.

2.4. Estimation of total flavonoid content

The measurement of total flavonoid content in the investigated extracts was determined spectrophotometrically (Božin et al.,