Content of phenolic compounds in herbs used in the Czech Republic

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Abstract

Herbs are recognized as sources of natural antioxidants and thus play a significant role in the chemoprevention of diseases resulting from lipid peroxidation. The Trolox equivalent antioxidant capacities (TEAC) and phenolic contents of the aqueous extracts of 17 spices from 6 botanical families grown in the Czech Republic were investigated. The herbs were extracted by means of different extraction techniques. Classical solvent extraction (Randall extraction), ultrasonication, maceration and shaking were used and compared. The antioxidant capacity (AC) was estimated by method based on ABTS free radical scavenging abilities. Qualitative and quantitative analyses of major phenolics were conducted by reverse-phase ultrahigh-performance liquid chromatography (RP-UHPLC). The phenolic compounds identified in the analyzed species were caffeic acid, ferulic acid, p-coumaric acid, protocatechuic acid and cinnamic acid as the major constituent obtained. From all extraction methods, Randall extraction and maceration were considered the best, because the extracts obtained revealed the highest phenolic contents and the best antioxidant properties measured by chemical assays. The highest contents of phenolic compounds were determined in the Lamiaceae family – in lavender (319.75 ± 1.77 mg/100 g dry weight) and lemon balm (249.00 ± 0.35 mg/100 g dry weight) – by means of Randall extraction.

Keywords

Phenolic compounds
Ultrahigh-performance liquid chromatography
Herbs
Antioxidant capacity

Introduction

Natural antioxidants are in high demand because of their potential to promote health and to help prevent disease, and also because of their relative safety and acceptability to consumers. Herbs are widely known for their numerous applications in the food industry; however, there is increasing interest in their pharmaceutical properties. Their antioxidant and antibacterial properties have been studied in many papers (Czapecka et al., 2005; Pérez-Tortosa et al., 2012; Chiang Chan et al., 2012; Berdowska et al., 2013).

Phenolic acids are usually present in leaves, flowering tissues, and woody parts such as stems and bark. They generally exist as glycosylated derivates in plants, although conjugation with inorganic sulfate or organic acids is also known. The structure–activity relationships of phenolic compounds present in spices require further investigation (Czapecka et al., 2005; Ivanova et al., 2005; Terpinc et al., 2009).

The most commonly used methods for estimating antioxidant capacity are ABTS•⁺ and DPPH•: Both of them are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their responses to antioxidants. The DPPH free radical (di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium) does not require any special preparation, while the ABTS radical cation must be generated by enzymes or chemical reactions (Arnao, 2000). Another important difference is that ABTS•⁺ can be dissolved in aqueous and organic media, in which the antioxidant capacity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples. In contrast, DPPH• can only be dissolved in organic media, especially in ethanol. This is an important limitation when interpreting the role of hydrophilic antioxidants.

The technique of phenolic compound extraction from plant material depends generally on the method, the type of extracting solvent, and the type of phenolic compound, as well as the presence of interfering substances (Goli et al., 2004). The results of previous studies showed that the extraction yield of phenolic content is greatly dependent on the solvent polarity (Lapornik et al., 2005; Turkmen et al., 2006). The polarities of polyphenols range from polar to non polar and thus a wide range of extraction solvents (water, acetone, methanol or ethanol, and aqueous solutions of the mentioned organic solvents) have been studied.

Kivilompolo et al. (2009) developed a dynamic sonication-assisted extraction method for the determination of phenolic acids in basil, oregano, rosemary, sage, spearmint and thyme. In a later study, Kivilompolo et al. (2012) performed a dynamic sonication-assisted ethanol extraction method for isolating phenolic acids from oregano,
rosemery, sage, spearmint, thyme and basil. The results were compared with conventional solvent extraction techniques (Kivilompolo et al., 2012). Conventional, microwave-assisted and ultrasound-assisted extraction methods using ethanol and water as solvents for the isolation of antioxidants from rosemary were developed in a study by Rodriguez-Rojo et al. (2012). The extraction efficiency was more significant for aqueous extracts. Borrás Linares et al. (2007) conducted a comparison of different extraction procedures for the characterization of phenolic compounds in rosemary. In a study by Proestos et al. (2013), both the conventional (reflux) and microwave-assisted extraction of phenolic compounds from aromatic plants using different solvents were examined. Water, 60% methanol, 60% acetone, and ethyl acetate/water (2:1, v/v) were used as the extraction solvents. The results demonstrated similar solvent effects for the two extraction methods. It was also shown that microwave-assisted extraction is a more effective method compared to the conventional method. Barros et al. (2010) optimized the extraction conditions (water and ethanol/water 50% v/v; 30 min at 25°C and boiling temperature; ethanol and methanol, 24 h at 25°C) to isolate phenolics from mastic thyme. The best method (25°C, 50 ml of methanol, 24 h) was further applied to the other Lamiaceae. The plant species in our study belonged to several botanical families, such as Lamiaceae, Apiaceae, Amaryllidaceae, Hypericaceae, Lauraceae and Rosaceae. For the purposes of this study, it was possible to use only water as the extraction solvent and during the antioxidant procedure. This was because the herbal extracts used were intended for whey modification; organic solvents or strong bases would cause coagulation.

The objectives of this study were to: (1) identify and quantify the major phenolic compounds present in the tested species by RP-UHPLC; (2) compare extraction techniques; and (3) determine the relationship between antioxidant capacity and the phenolic compounds of 17 species extracts in order to confirm that phenolic constituents are responsible for the antioxidant capacity of the plants. The examined phenolic acids included hydroxybenzoic acids (e.g. gallic acid, 4-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, vanillic acid), hydroxycinnamic acids (e.g. ferulic acid, caffeic acid, p-coumaric acid, cinnamic acid, sinapic acid), and aldehydes (4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, vanillin).

**Materials and Methods**

**Chemicals and reagents**

Acetonitrile was from Biosolve BV (Valkenswaard, Netherlands). Methanol, potassium persulfate, phosphate buffer (sodium monohydrogen phosphate, sodium dihydrogen phosphate, sodium chloride), hydrochloric acid, and acetic acid were from Penta (Praga, Czech Republic). Gallic acid, benzoic acid, salicylic acid and caffeic acid were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Protocatechuic acid was purchased from Chromadex (Irvine, CA, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), trans-cinnamic acid, 3,4-dihydroxybenzaldehyde and vanillic acid were purchased from Fluka (Sigma-Aldrich, St. Luis, MO, USA). ABTS⁺, syringic acid, p-coumaric acid, vanillin, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, ferulic acid and sinapic acid were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Luis, MO, USA).

**Plant materials**


**Phenolics extraction**

A total of 20 ml of MilliQ water was added to 2 g of herbs. Four samples of each herb were prepared for four different types of extraction. Each sample was either (1) extracted by sonication in a Bandelin Sonorex ultrasonic cleaning instrument (BANDELIN electronic GmbH, Berlin, Germany) for 15 min at ambient temperature, (2) extracted by
Results and Discussion

Determination of free radical-scavenging ability by the use of a stable ABTS radical cation

Free radical-scavenging activity was determined by ABTS radical cation decolorization assay as described by Re et al. (1999). ABTS was dissolved in MilliQ water to a 7.50 μM concentration. ABTS radical cation (ABTS⁺) was produced by reacting ABTS stock solution with 4.95 μM potassium persulfate and kept in the dark at room temperature for 12–16 h before use. ABTS⁺ was dissolved 1:18 in phosphate buffer prior to analysis. A calibration curve for Trolox was prepared in the concentration range 0 – 50 μmol/L. Samples of herbs were diluted if necessary. The absorbance of the resulting blue color was measured at 734 nm with a Genesys 100 UV-VIS spectrophotometer exactly 20 min after the initial mixing. Quantification was performed with respect to the standard curve for Trolox. The results were corrected for dilution and expressed in mM Trolox per 100 g dry weight (DW) as Trolox equivalents (TEAC). All determinations were performed in triplicate.

UHPLC-PDA instrumentation and conditions

UHPLC analyses were performed on a Knauer Platin Blue (Knauer, Berlin, Germany) equipped with two pumps (PLATINblue P-1 and PLATINblue P-2), a PLATINblue AS-1 autosampler, a PLATINblue T-1 column thermostat, and a PLATINblue PDA-1 photodiode array detector. The sample was separated using a Phenomenex Kinetex C18 column (I.D. 4.6 mm × 100 mm, 2.6 μm particle size; Phenomenex) and the column temperature was 30°C. Gradient elution was applied with 0.8% acetic acid and acetonitrile using the following settings (A - acetic acid/ B - acetonitrile): 0.0 min – 90/10; 1.0 min – 92/8; 1.1-4.0 min – 97/3; 5.0-8.9 min – 92/8; 9.0-13.0 min – 89/11; 13.6-15.5 min – 65/35; 16.0-18.0 min – 90/10. The flow rate was 2.0 mL/min, and the injection volume was 5 μL. The detector wavelengths were set to 270, 290, 310, and 324 nm.

Antioxidant capacity of herbs provided by ABTS⁺ radical scavenging

The antioxidant capacity of herbs determined with respect to the ABTS⁺ radical is shown in Figure 3. The measured values indicate an extremely large variation in antioxidant capacity. Differences in
lemon balm, 2013; Farhat, 2005; Surveswaran, 2005), suggesting that cinnamon, lavender, rosemary, lavender, thyme, sage, and thyme, from the Rosaceae family. A quite low antioxidant capacity was determined in the case of caraway (11.62 mmol/100 g DW). These findings were in agreement with the orders of decreasing antioxidant capacities are probably due to (1) genotypic and environmental differences within species, (2) the choice of parts tested, (3) the time at which samples were taken, and (4) the methods of determination. The antioxidant capacity of the aqueous extracts ranged from 0.88 to 85.51 mmol Trolox equivalents per 100 g dry weight (mmol TEAC/100 g DW), and the average TEAC value was 27.42 mmol/100 g DW.

In our study, the ranking of AC values for the herbs was as follows: rosemary (39.81 ± 2.56 mmol/100 g DW) > skullcap (26.35 ± 5.70 mmol/100 g DW) > marjoram (31.21 ± 2.41 mmol/100 g DW) > thyme (29.75 ± 6.92 mmol/100 g DW) > oregano (27.50 ± 1.26 mmol/100 g DW) > wild thyme (20.10 ± 2.06 mmol/100 g DW) > lavender (20.19 ± 2.55 mmol/100 g DW). The ability to scavenge the ABTS+ free radical was also very high in the Randall extract of agrimony (32.33 ± 1.12 mmol/100 g DW) from the Rosaceae family. A quite low antioxidant capacity was determined in the case of caraway (11.62 mmol/100 g DW). These findings were in agreement with

Shan et al. (2005). The lowest AC values (5.28 and 5.06 mmol/100 g DW) were obtained for extracts of fennel and anise. Similar results were reported by Przygodzka et al. (in press), who also determined the lowest AC values for extracts of fennel and anise, despite the fact that they used ethanol/water (1:1, v/v) extracts of herbs.

In the presented experiments, the relationship between antioxidant capacity and the content of phenolic compounds was determined. This was observed mainly for rosemary and lavender, and further also for wild thyme, lemon balm, thyme, and sage from the Lamiaceae family and for wild garlic (Amaryllidaceae) and cinnamon (Laureaceae) (Table 2). The correlation between total phenolic content and antioxidative capacity was previously reported in several studies (Chun et al., 2005; Surveswaran et al., 2007; Kamdem et al., 2013; Farhat et al., 2013). With respect to the other herbs investigated in our study, no direct correlation between phenolic content and antioxidative capacity was demonstrated. Ambiguous relationships between the content of phenolic substances and antioxidant capacity are difficult to explain on the basis of quantitative analyses only (Czapecka et al., 2005), suggesting that not only the level of antioxidants but also a synergy occurring between them and other plant elements might influence apparent differences in the antioxidant capacities of plant extracts.

Conclusions

It is well known that phenolic compounds are constituents of many plants and herbs, and they have attracted a great deal of public and scientific interest because of their health-promoting effects as antioxidants. The results of this systematic
investigation of various herbs are important for determining their chemical constituents, and are a contribution to the search for natural sources of potent antioxidants. They confirm the existence of unique antioxidant properties with respect to all the examined species of herbs.

All the results are supported by findings from literature. Randall extraction and maceration achieved better results than the other methods. This indicates that hot water might be considered as an appropriate solvent for extracting phenolic compounds from spices, with a reduced level of pungency. The contents of phenolics in hot water extracts were found to be lower than those reported previously in organic solvent extracts, which might be attributed to differences in the polarities of solvents, to differences in the extraction techniques used, as well as to differences in other environmental factors such as climate, sun exposure, and soil composition, which may alter the phenolic metabolism of spice plants. Using a higher heating temperature might accelerate polyphenol extraction in comparison to other techniques. Antioxidant capacity, expressed as the ability to scavenge ABTS free radicals, was high in the case of all the examined herbs, especially in the Lamiaceae family. Lower ABTS values were measured in the extracts of the Apiaceae family. It may be noted that all the examined herbs of the six families are rich sources of antioxidants, in particular those from the group of derivates of cinnamic acids. The contents in herb material differed significantly depending on the species tested.

The slight differences in results may likely be due to genotypic and environmental differences within species, the choice of parts tested, the time of year when the herbs were collected, extraction times, and differences in the methods of determination. For the purposes of this study, it was possible to use only water as the extraction solvent and during the antioxidant procedure as well. This was because the herbal extracts used were intended for whey modification; organic solvents or strong basics would cause coagulation. Herb extracts, especially from lavender and lemon balm, can be considered as a powerful and effective additive in numerous applications in the food industry. It should be also investigated which extractives used were intended for whey modification; this was because the herbal extracts were found to be lower than those reported previously in organic solvent extracts, which might be attributed to differences in the polarities of solvents, to differences in the extraction techniques used, as well as to differences in other environmental factors such as climate, sun exposure, and soil composition, which may alter the phenolic metabolism of spice plants. Using a higher heating temperature might accelerate polyphenol extraction in comparison to other techniques. Antioxidant capacity, expressed as the ability to scavenge ABTS free radicals, was high in the case of all the examined herbs, especially in the Lamiaceae family. Lower ABTS values were measured in the extracts of the Apiaceae family. It may be noted that all the examined herbs of the six families are rich sources of antioxidants, in particular those from the group of derivates of cinnamic acids. The contents in herb material differed significantly depending on the species tested.

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