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ORIGINAL ARTICLE

The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts

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Reducing power

Abstract The objectives of this study were to determine best conditions for the extraction of phenolic compounds from fresh, frozen and lyophilized basil leaves. The acetone mixtures with the highest addition of acetic acid extracted most of the phenolic compounds when fresh and freeze-dried material have been used. The three times procedure was more effective than once shaking procedure in most of the extracts obtained from fresh basil leaves – unlike the extracts derived from frozen material. Surprisingly, there were not any significant differences in the content of phenolics between the two used procedures in the case of lyophilized basil leaves used for extraction. Additionally, the positive correlation between the phenolic compounds content and antioxidant activity of the studied extracts has been noted. It is concluded that the acetone mixtures were more effective than the methanol ones for polyphenol extraction. The number of extraction steps in most of the cases was also a statistically significant factor affecting the yield of phenolic extraction as well as antioxidant potential of basil leaf extracts.

1. Introduction

Plant foods provide abundant natural bioactive compounds which have many proved health-promoting activities like antioxidant, antibacterial, antihypertensive, anti-inflammatory etc. Phenolic compounds are one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants. Additionally, there are many reports that this group of phytochemicals possesses biological activity. The antioxidant

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activity of polyphenols is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations (Amarowicz et al., 2004; Balasundram et al., 2006). The presence of electron-donating and electron-withdrawing substituents in the ring structure of phenolics as well as the number and arrangement of the hydroxyl groups determines their antioxidant potential (Zhang et al., 2003). Additionally, a huge number of phenolic compounds which have been reported in the literature, show differences in possible biochemical modification (glycosylation, acetylation, manolynation, esterification to organic acids etc.) (Dai and Mumper, 2010; Balasundram et al., 2006).

Differences in the structure of phenolic compounds also determine their solubility in solvents of different polarity. Therefore type of extraction solvent as well as the isolation procedures may have a significant impact on the yield of extraction polyphenols from plants material. There are some reports concerning optimization of extraction conditions of phenolic compound content and antioxidant activities of some plant foods but as some researches indicated optimal procedure is usually different for different plant matrices (Rababah et al., 2010; Pellegrini et al., 2007).

In the present study basil (*Ocimum basilicum* L.) was selected as model food matrices as it is one of the most common herbs consumed as spice and is a rich source of phenolic compounds especially phenolic acids (like rosmarinic acid, chicoric acid, vanillic acid, *p*-coumaric acid, benzoic acid, hydroxybenzoic acid, syringic acid, ferulic acid, protocatechuic acid, caffeic acid and gentisic acid), flavonol-glycosides and anthocyanins (Lee and Scagel, 2010; Tarchoune et al., 2012).

The aim of the present work was to determine best conditions for extraction of phenolic compounds from fresh, frozen and lyophilized basil leaves.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteau reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid) were purchased from Sigma–Aldrich Company, USA. Any other chemicals were of analytical grade.

2.2. Materials

Basil plants (*O. basilicum* L.) were purchased at commercial maturity from a local store. One proportion of the samples after weighing was frozen and the other one was freeze-dried.

2.2.1. Sample preparation

Two extraction ways were used for sample preparation:

(a) Fresh material (2 g) (or an appropriate amount of frozen or lyophilized material) was ground in a mortar and pestle with 20 mL of an appropriate solvent mixture (see Table 1) and the polyphenols were extracted for 1 h at 4 °C, then centrifuged at 9000*g* for 30 min. and adjusted to 50 mL of final volume with used solvent – this was an extract of polyphenols labeled as once shaking.

(b) 2 g of fresh material or optional respectively to the weight of the quantity of frozen or lyophilized material was ground in a mortar and pestle with 15 mL of an appropriate solvent mixture (see Table 1) and the polyphenols were extracted for 20 min at 4 °C, then centrifuged at 9000*g* for 30 min. – this procedure was repeated three times and the supernatants were pooled and adjusted to 50 mL of final volume with used solvent – this was an extract of polyphenols labeled as three times shaking.

Table 1 Composition of solvents used for phenolic compound extraction.

Number of solvent mixture	Solvent composition
I	Acetone/water/acetic acid (70/28/2, v/v/v)
II	Acetone/water/acetic acid (70/29.5/0.5, v/v/v)
III	Acetone/water/acetic acid (70/29.8/0.2, v/v/v)
IV	Methanol/water (50/50, v/v)
V	Methanol/water/acetic acid (50/49.5/0.5, v/v/v)
VI	Methanol/acetic acid (99.5/0.5, v/v)

2.3. Determination of total phenolic compounds (TPC)

The amount of total phenolics was determined using Folin–Ciocalteau reagent (Singleton et al., 1974). To 0.5 mL of the sample, 0.5 mL H_2O, 2 mL Folin–Ciocalteau reagent (1:5 H_2O) was added, after 3 min, 10 mL of 10% (w/v) Na_2CO_3 and the contents were mixed and allowed to stand for 30 min. Absorbance at 725 nm was measured in a UV–Vis spectrophotometer. The amount of total phenolics was calculated as gallic acid equivalent (GAE) in mg per g of fresh weight (FW).

2.4. Determination of free radical scavenging activity

The free radical scavenging activity was measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) – according to Brand-Williams et al. (1995) as the source of the free radicals. For the DPPH assay, the 80 μL of methanolic extracts was mixed with 1.92 mL 6×10^{-5} M solution of DPPH$^{\cdot}$ in methanol. Absorbance at 515 nm was measured immediately and after 2.5 mins of incubation. The affinity of the test material to quench DPPH free radicals was evaluated according to the equation:

$$\text{scavenging}\% = [(AC - AA)/AC)] \times 100, \text{where :}$$

AC – absorbance of control at 0 min, AA – absorbance of sample after 2.5 min.

The antiradical activity was related to Trolox (an analog of vitamin E) and expressed as mM of Trolox per gram of fresh weight (FW) (TEAC, Trolox equivalent antioxidant activity).