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Ultrasound-assisted extraction of natural antioxidants from the flower of *Limonium sinuatum*: Optimization and comparison with conventional methods

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ABSTRACT

Natural antioxidants are widely used as dietary supplements or food additives. An optimized method of ultrasound-assisted extraction (UAE) was proposed for the effective extraction of antioxidants from the flowers of *Limonium sinuatum* and evaluated by response surface methodology. In this study, ethanol concentration, ratio of solvent to solid, ultrasonication time and temperature were investigated and optimized using a central composite rotatable design. The optimum extraction conditions were as follows: ethanol concentration, 60%; ratio of solvent to solid, 56.9:1 mL/g; ultrasonication time, 9.8 min; and temperature, 40 °C. Under the optimal UAE conditions, the experimental values (483.01 ± 15.39 μmol Trolox/g DW) matched with those predicted (494.13 μmol Trolox/g DW) within a 95% confidence level. In addition, the antioxidant activities of UAE were compared with those of conventional maceration and Soxhlet extraction methods, and the ultrasound-assisted extraction could give higher yield of antioxidants and markedly reduce the extraction time.

1. Introduction

Excessive oxidative stress is implicated in the development of several chronic diseases, such as diabetes, cardiovascular diseases, cancer and ageing diseases (Kaur & Kapoor, 2001; Kumar & Singh, 2015; Young & Woodside, 2001). Many risk factors, such as cigarette smoking, irradiation and environmental pollutants, may induce excessive oxidative stress in human body, and the intake of antioxidants is regarded as a preventative method to reduce the negative effects induced by oxidative stress (Boots, Haenen, & Bast, 2008; Li et al., 2015; Roleira et al., 2015). Antioxidants could delay or inhibit the oxidation of biological macromolecules (such as DNA, protein and lipid) in cells and tissues by inhibiting the initiation or propagation of oxidative chain reactions (Das, Das, Miyaji & Deka, 2016; Young & Woodside, 2001). In addition to the capacity of scavenging free radicals in the body, the natural antioxidants could have multiple activities in food, including reducing hydroperoxides into stable hydroxyl derivatives, inactivating metal catalysts by chelation, and interacting synergistically with other reducing compounds (Frankel & Finley, 2008; Shahidi & Ambigaipalan, 2015). Fruits, vegetables, cereals, spices and herbs are the main sources of natural antioxidants and many of them have displayed strong free radical scavenging abilities and anti-inflammatory activities. (Deng et al., 2012; Embuscado, 2015; Hung, 2016; Kaur & Kapoor, 2001; Li et al., 2013; Shahidi & Ambigaipalan, 2015). With the increased application of natural antioxidants in medicine and food processing, the effective extraction of natural antioxidants from plant sources is required.

Limonium sinuatum belongs to the family Plumbaginaceae, and has a worldwide distribution (Chen, Funnell, & Morgan, 2010; Igawa, Hoshino, & Mii, 2002). Historically, the flower of *Limonium sinuatum* was often used as a form of tea for the prevention of ageing. In previous studies, the flower of *Limonium sinuatum* has been found to have strong antioxidant capacity in comparison with 51 edible flowers, and therefore could potentially be a rich resource of natural antioxidants (Li et al., 2014). The antioxidants from the flower of *Limonium sinuatum* could be used as dietary supplement or food additive. Thus, maximum extraction of natural antioxidants from the flower of *Limonium sinuatum* was investigated.

Extraction of antioxidant compounds from plants mainly utilise conventional solvent and Soxhlet methods, which have long extraction times, high usage of organic solvents and poor extraction efficiency. Recently, more efficient techniques have been

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developed to extract antioxidant compounds from plant materials, including microwave-assisted extraction (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015), ultrasound-assisted extraction (UAE) (Liu et al., 2015; Zou et al., 2014), pressurized liquid extraction (Povilaitis, Sulniute, Venskutonis, & Kraujaliene, 2015), and supercritical fluid extraction (Diaz-Reinoso, Moure, Dominguez, & Parajo, 2006). Among them, several UAE methods have been developed for the extraction of natural antioxidants from plant materials (Bimakr et al., 2012; Liu et al., 2008; Peng et al., 2013; Shirsath, Sonawane, & Gogate, 2012; Xu et al., 2016). UAE has the capacity to improve the extraction efficiency by promoting the mass transfer and possible rupture of cell wall due to its effect of acoustic cavitation (Shirsath et al., 2012). The efficiency of an extraction method is affected by many process parameters, e.g. solvent composition, the ratio of solvent to solid, ultrasonication time and temperature (Esclapez, Garcia-Perez, Mulet, & Carcel, 2011). Therefore, the optimization of the extraction parameters is required to obtain the maximum amount of antioxidants from plant materials. As a mathematical and statistical technique, response surface methodology (RSM) is an effective tool for optimizing the process parameters (Bimakr et al., 2012). The application of RSM may enable reduction of the number of experimental trials and quantify the interactions between multiple parameters.

In this paper, effects of extraction process parameters (ethanol concentration, the ratio of solvent to solid, ultrasonication time and temperature) were evaluated in order to optimise extraction of antioxidants from *Limonium sinuatum* flower and analyzed using RSM with a five-level, three-variable central composite rotatable design (CCRD). Furthermore, the antioxidant activities of the flower extracts using UAE were compared with conventional maceration and Soxhlet extraction methods. Besides, the polyphenolic compounds of extracts obtained under optimized conditions were quantified using high-performance liquid chromatography-photodiode array detector (HPLC-PAD).

2. Materials and methods

2.1. Plant materials

The flowers of *Limonium sinuatum* were bought from different markets in Guangzhou, China. The flowers were dried to 1.8% residual moisture utilizing freeze-drying technology and reduced to a fine particle (<150 μm) in an electric grinder (RHP-100; Ronghao Insustry & Trade Co., Ltd, Yongkang, China) for the subsequent experiments.

2.2. Reagents

The standards of ABTS (2,2′-azinobis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and polyphenolic standards (epigallocatechin, cyanidin, chlorogenic acid, coffeic acid, p-coumaric acid, rutin, resveratrol, 2-hydrocinnamic acid, quercetin, gallic acid, ferulic acid) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Potassium persulfate was purchased from Tianjin Chemical Factory (Tianjin, China). Deionized water was used. Ethanol of analytical grade were used in the part of extraction, and solvents (formic acid and methanol) of HPLC grade were used in the part of HPLC analysis.

2.3. Extraction of antioxidant compounds

2.3.1. Ultrasound-assisted extraction

Ultrasound-assisted extraction was carried out in a domestic ultrasonic bath device (Kj1012B; Guangzhou Kejin Ultrasonic Instrument Factory, Guangzhou, China) with a constant power of 400 W, at a frequency of 40 kHz. The apparatus was equipped with a digital control system for sonication time and temperature.

A ground particle sample (0.1 g) was placed in a capped plastic tube (15 mL, round bottom) and mixed with an appropriate volume of ethanol solution. The tube with suspension was sonicated at different time periods and temperatures in the ultrasonic device containing 7 L water. All the samples in each extraction were placed in the centre of ultrasonic device with the depth of six centimeters and subsequent each batch of samples to be sonicated was kept the same position. Besides, all the samples of each experimental design were sonicated simultaneously in the ultrasonic device. Each sample was done in triplicate. According to the different levels of each experimental design, namely each extraction parameter in single factor experiments and response surface experiment, the number of tubes used in each extraction varied from 18 to 60. After UAE, the mixtures were centrifuged and the supernatants were obtained for the subsequent antioxidant activity determination.

2.3.2. Maceration extraction

A ground particle sample (0.1 g) was mixed with 60% ethanol solution (5.69 mL). The extraction was performed in a shaking water bath at 25 °C for 24 h according to the method displayed by Xu et al. (2016). Then, the extract was centrifuged and the supernatants were obtained for the subsequent antioxidant activity determination.

2.3.3. Soxhlet extraction

A ground particle sample (2.0 g) was weighed, and filtered using Whatman filter paper. The extraction was carried out with 400 mL of 60% ethanol at 95 °C for 4 h, refluxing in a Soxhlet apparatus according to the method displayed by Xu et al. (2016). Then, the extract was collected for the subsequent assays.

2.4. Determination of antioxidant capacity

The assay of ABTS radical-scavenging capacity was performed according to the method displayed by Jing, Dong, and Tong (2015) previously. 7 mM ABTS solution was prepared and added to the same volume of 2.45 mM potassium persulfate solution (v/v), then, incubated for 16 h at room temperature in the dark. The stock solution was diluted to make the absorbance of $ABTS^{\cdot+}$ working solution be 0.710 ± 0.05 at 734 nm before usage. For the spectrophotometric assay, 3.8 mL of the $ABTS^{\cdot+}$ working solution and 100 μL of the diluted sample were mixed and the absorbance was measured at 734 nm after 6 min of incubation. The results of ABTS radical-scavenging assay were expressed as μmol Trolox/g dry weight (DW).

2.5. HPLC analysis

Polyphenolics of extracts obtained under optimized conditions were analyzed on the basis of the method described previously with minor modification (Cai, Luo, Sun, & Corke, 2004). HPLC-PAD equipped with a Waters (Milford, MA, USA) 1525 binary HPLC pump and a Waters 2996 photodiode array detector was applied. Separation was carried out using an Agilent Zorbax Extend-C18 column (250 × 4.6 mm, 5 μm) at 40 °C with a gradient elution at a flow rate of 0.8 mL/min. The mobile phase composed of solution A (0.1% formic acid–water solution) and solution B (methanol), which were delivered as follows: 0 min, 95% (A); 15 min, 80% (A); 20 min, 70% (A); 25 min, 63% (A); 40 min, 60% (A); and 60 min, 50% (A). The UV spectra were monitored between 200 and 600 nm. In this part, 11 polyphenolic standards (epigallocatechin, cyanidin, chlorogenic acid, coffeic acid, p-coumaric acid, rutin, resveratrol, 2-hydrocinnamic acid, quercetin, gallic acid,