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The extraction efficiency enhancement of polyphenols from *Ulmus pumila* L. barks by trienzyme-assisted extraction

Zhihao Zhou, Hongjun Shao*, Xiao Han, Kaijie Wang, Cuiping Gong, Xingbin Yang*

Shaanxi Engineering Laboratory for Food Green Processing and Security Control, College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an 710062, China

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ABSTRACT

This study aimed to optimize trienzyme-assisted extraction (EAE) conditions for total polyphenols (TP) from *Ulmus pumila* barks (UPB). Response surface methodology (RSM) was used to optimize EAE conditions including pH, temperature, and time. The extraction efficiency of three extraction procedures on the TP yield, antioxidant activities and chemical composition of UPB extracts was also compared and characterized. Our results showed that the maximum extraction yield of TP was 16.04 ± 0.38 mg gallic acid equivalents/g dry weight (GAE/g DW) under the optimum EAE conditions (pH = 4.63, 52.6 °C and 62 min). Meanwhile, the EAE gave a higher extraction yield of TP and then a greater *in vitro* antioxidant capacity compared with those obtained from both ultrasound-assisted extraction (UAE) and conventional heat extraction (CHE). In addition, seven polyphenolic compounds were validated by high-performance liquid chromatography analysis in the extracts at the optimized conditions. The results of this study further confirmed that EAE could be explored as a state-of-the-art environmentally friendly technology for recovering optimum amounts of antioxidant polyphenols from plant sources.

1. Introduction

Polyphenolic compounds, which are non-nutritive chemicals occurring widely in plants and food, have recently emerged as antioxidants exerting proven health-prompting effects (Pandey and Rizvi, 2012; Quideau et al., 2011; Rodrigo et al., 2014; Valavanidis and Vlachogianni, 2013). It is thus not surprising that their extraction, availability and bioactivity from different matrices have increasingly been a topic of intensive investigation in food- and health-related research.

In general, the recovery of biological compounds including polyphenols from samples of interest is traditionally achieved by using the Soxhlet, heated reflux solvent extraction. Due to the time- and solvent-consuming drawbacks associated with the conventional extraction methods, several cost-effective and environmentally friendly alternatives for the extraction of polyphenols, such as ultrasound-assisted extraction (UAE), microwave- assisted extraction (MAE), supercritical fluid extraction (SFE), and sub-critical water extraction (SCWE), have been developed recently. The applications as well as detailed features of these techniques were available in some comprehensive reviews (Dai and Mumper, 2010; Khoddami et al., 2013; Santos-Buelga et al., 2012; Shi et al., 2005).

Furthermore, enzyme-assisted extraction (EAE) of polyphenolic substance has recently attracted special attention for the enhanced release and recovery of polyphenols covalently or non-covalently bonded to the plant cell wall components (Fu et al., 2008; Tomaz et al., 2016; Weinberg et al., 1999; Y. Zhu et al., 2016). Plant cell walls, mainly composed of highly complex polysaccharides such as cellulose, hemicellulose, lignin and pectin, limit the polyphenolic accessibility and then the efficiency of polyphenolic extraction using the aforementioned extraction procedures. Therefore, enzymatic pretreatment of plant samples favoring the release of bioactive compounds, has been increasingly applied either alone or in combination with other extraction approaches such as UAE, MAE and SFE in more recent years. The successful use of this technique to facilitate the recovery of polyphenols from various plant sources, including grape skin (Gomez-Garcia et al., 2012; Tomaz et al., 2016), seaweed (Rodrigues et al., 2015), tomato waste (Strati et al., 2015), pomegranate peel (Mushtaq et al., 2015a), cauliflower

Abbreviations: UPB, *Ulmus pumila* barks; TP, total polyphenol; TS, total sugar; EAE, enzyme-assisted extraction; UAE, ultrasound-assisted extraction; CHE, conventional heat extraction; MAE, microwave-assisted extraction; SFE, supercritical fluid extraction; SCWE, sub-critical water extraction; RSM, response surface methodology; BBD, Box-Behnken design.

* Corresponding authors.
E-mail addresses: shjxue@163.com, shaohj@snnu.edu.cn (H. Shao), xbyang@snnu.edu.cn (X. Yang).

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(Nguyen Thai et al., 2014), peanut shells (Zhang et al., 2013), and ginger (Nagendra chari et al., 2013), has been well studied.

Ulmus pumila L., known as the Chinese elm, Asiatic elm and dwarf elm, is native to East Asia, and now has been widely cultivated throughout the many parts of the world such as Americas, Asia and southern Europe (Zalapa et al., 2009). Traditionally, the stem and root barks of this plant are frequently used in the treatment of edema, mastitis, gastric cancer, and inflammation in traditional Chinese medicine (Ghosh et al., 2012; Wang et al., 2004a), and the powder of inner stem barks is also acting as a thickening agent in bread making. Phytochemical investigations on *U. pumila* L. resulted in the isolation of two new cytotoxic sesquiterpenoids mansonone E and mansonone F and nine known triterpenoids (Wang et al., 2006). In addition, polyphenolic compounds such as naringenin, catechin and catechin-7-*O*-β-apiofuranoside were identified in the stem bark of *U. pumila* L. (Cho et al., 2016; Jin et al., 2013). A recent study indicated that the methanolic extracts of *U. pumila* L. powder inhibited adipogensis through regulation of cell cycle progression in 3T3-L1 cells (Wang et al., 2004b). Kim et al. (2010) also reported that the plant was the most potent antioxidant among ten Korean medicinal plants. Although *U. pumila* L. represents a valuable source of bioactive molecules and has numerous uses in traditional medicine, to the best of our knowledge, EAE of polyphenols from this plant has not yet been reported.

The purpose of the study reported here was to optimize the EAE of polyphenolics from *U. pumila* L. barks (UPB). EAE parameters such as pH, temperature, and time were firstly optimized using response surface methodology (RSM), and total sugar (TS) was measured to analyze the relevance between the extent of cell wall degradation and the extraction yield of TP. The composition and extraction efficiency of polyphenolic compounds among EAE, UAE and CHE were then systematically compared. In addition, the *in vitro* antioxidant capacity of the polyphenolic extracts from *U. pumila* L. was also evaluated.

2. Materials and methods

2.1. Materials

The stem barks of *U. pumila* L. were collected from Tianshui, China in October 2015 and were identified by Prof. Xunjun Dong, College of Life Science, Shaanxi Normal University. The air-dried samples were well powdered (40 mesh) and stored into an air tight bottle in a freezer (about 4 °C) until further analysis.

2.2. Enzymes and chemicals

Three different enzymes, including cellulase (≥40 U/mg, E.C. 3.2.1.4), pectinase (≥20 U/mg, E.C. 3.2.1.15) and β-glucosidase (≥10 U/mg, E.C. 3.2.1.21) were selected on the basis of the structural composition of plant barks, and purchased from Sigma (China).

Gallic acid, Folin-Ciocalteu reagent, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were all purchased from Sigma (China). $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, muriatic acid, sodium acetate, acetic acid, and ethanol were from Tianli Biotech Co. Ltd (Xi'an, China). Deionized water used in the experiments was prepared using a Millipore Milli Q-Plussystem (Millipore, Bedford, MA, USA). Acetonitrile and methyl alcohol were chromatographic grade reagent (Honeywell, U.S.A.). All other chemicals were of analytical grade if not specified otherwise.

2.3. Enzyme assisted extraction

The Box-Behnken design (BBD) was used to optimize enzyme-assisted extraction of polyphenols from UPB. The ranges of preliminarily selected independent variables including pH, extraction temperature, and incubation time were designed in Table 1.

Table 1
Experimental values of the independent variables used Box-Behnken design (BBD).

Factor levels	Independent variables		
	A (−)	B (°C)	C (min)
−1	4	40	30
0	5	50	60
1	6	60	90

A–C represent pH, extraction temperature (°C), incubation time (min), respectively.

Table 2
Box-Behnken design (BBD) experimental design with the independent variables and experimental data for the responses.

Run	Extraction conditions			Results	
	A (−)	B (°C)	C (min)	TP (mg/g DW)	TS (mg/g DW)
1	5	50	60	15.45 ± 0.11	103.10 ± 2.17
2	5	50	60	15.61 ± 0.19	103.48 ± 1.57
3	6	50	90	13.49 ± 0.14	109.82 ± 2.96
4	4	50	30	14.19 ± 0.35	95.66 ± 1.82
5	6	50	30	11.79 ± 0.27	82.74 ± 2.74
6	5	40	30	13.59 ± 0.18	90.45 ± 1.69
7	5	50	60	15.60 ± 0.10	103.47 ± 3.01
8	4	50	90	14.03 ± 0.11	123.68 ± 3.96
9	5	60	30	13.78 ± 0.13	95.78 ± 2.13
10	5	40	90	13.75 ± 0.31	119.41 ± 2.26
11	6	40	60	13.22 ± 0.19	84.49 ± 1.63
12	4	40	60	14.50 ± 0.33	98.87 ± 2.23
13	5	50	60	15.56 ± 0.15	105.49 ± 3.16
14	5	60	90	14.45 ± 0.23	116.52 ± 2.19
15	6	60	60	13.30 ± 0.12	86.89 ± 1.85
16	4	60	60	15.16 ± 0.14	102.41 ± 2.46
17	5	50	60	15.67 ± 0.18	108.23 ± 3.05

A–C represent pH, extraction temperature (°C), incubation time (min), respectively. TP—means total polyphenol; TS—means total sugar.

The ground powder of stem barks of *U. pumila* L. (0.50 g) was mixed with 10 ml buffer solution including three enzymes mixture, and extracted following the conditions in Table 2. The enzyme mixtures including cellulase 200 U/g sample, pectinase 20 U/g sample and β-glucosidase 16 U/g sample were placed in a conical flask (100 ml). The optimal concentration of each enzyme was selected in preliminary experiments by testing the impact of different concentrations of the enzymes on the yield of total polyphenols at pH 5.0 and 50 °C for 60 min (data not shown). After the enzymatic hydrolysis, additional 10 ml of ethanol was added to the conical flask for polyphenol extraction (50% ethanol final). Then the extract was centrifuged at 5000 r/min for 5 min at room temperature. The collected supernatant was brought to a final volume of 20 ml with 50% ethanol, and stored at 4 °C for further study.

2.4. Ultrasound assisted extraction

To compare extraction efficiency of TP between EAE and UAE, UAE extraction was performed according to the optimal conditions for EAE in Section 3.2 and to He et al. (2016). In detail, plant samples (0.50 g) were extracted at 52.6 °C for a dynamic time (10–90 min), using 20 ml of 50% ethanol. Ultrasonic power (200 W) was utilized to assist extraction by using an ultrasonic generator (Model KQ-300DE, 40 KHz, China). At the end of the process, the extracted solution was centrifuged and separated in the same way as shown in the EAE.

2.5. Conventional heat extraction

Conventional heat extractions (CHE) were performed similarly to UAE (see Section 2.4) and compared with EAE. At the end of the