Original article

High-efficient extraction of principal medicinal components from fresh Phellodendron bark (cortex phellodendri)

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Abstract

There are three key medicinal components (phellodendrine, berberine and palmatine) in the extracts of Phellodendron bark, as one of the fundamental herbs of traditional Chinese medicine. Different extraction methods and solvent combinations were investigated to obtain the optimal technologies for high-efficient extraction of these medicinal components. Results: The results showed that combined solvents have higher extracting effect of phellodendrine, berberine and palmatine than single solvent, and the effect of ultrasonic extraction is distinctly better than those of distillation and soxhlet extraction. Conclusion: The hydrochloric acid/methanol-ultrasonic extraction has the best effect for three medicinal components of fresh Phellodendron bark, providing an extraction yield of 103.12 mg/g berberine, 24.41 mg/g phellodendrine, 1.25 mg/g palmatine.

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1. Introduction

Phellodendron bark has been widely known as one of the fundamental herbs of traditional Chinese medicine. Phellodendron bark is also called cortex phellodendri, which is the bark of two Phellodendron trees: P. amurense and P. chinense. Phellodendron bark is characterized by humorism, as bitter and cold, affecting the kidney, urinary bladder and large intestine meridians in a traditional Chinese medicine counterpart (Zhang et al., 2016). Phellodendron bark is also medically used to clear heat, reduce fire, dry dampness and release toxins (Li et al., 2014). Modern pharmacological researches indicated that Phellodendron bark has the functions of anti-pathogenic microorganism, anti-ulcer, antihypertensive and anti-arrhythmic, etc. Phellodendron bark can reduced blood uric acid levels in mice with hyperuricemia by inhibiting xanthine oxidase activity (Yang et al., 2015).

Many scientific findings had revealed the biomedical activities of extracts from Phellodendron bark. The further researches indicated that there are three key bioactive components (phellodendrine, berberine and palmatine) in the extracts of Phellodendron bark. Ethanol extracts of Phellodendron bark displayed antidiarrheal activity by attenuating ion transport by intestinal epithelium (Tsai et al., 2004; Guo et al., 2017). Medicinal extracts of Phellodendron bark reduced the rate of growth of Candida, which has been ascribed to berberine and palmatine content (Park et al., 1999). Different extracts of Phellodendron bark showed multiple functions, especially including; protect against airway inflammation in response to lipopolysaccharide treatment of mice (Mao et al., 2010; Gao et al., 2017), reduce blood glucose levels and slow the development of diabetic nephropathy in mice treated with streptozocin to induce diabetes (Kim et al., 2008; Muhammad et al., 2017a), reduce contractions of smooth muscle of isolated rat prostate glands (Xu and Ventura, 2010), and reduce cell replication of tumors in mice (Park et al., 2004).

Therefore, the efficient extraction of medicinal components from Phellodendron bark was focused by researches (Boost et al., 2016). The widely used extraction methods were soxhlet extraction, distillation and ultrasonic extraction, and the extraction solvents were water, organic solvents and their combinations (Wang et al., 2015; Chen et al., 2014). To obtain the optimal technologies for high-efficient extraction of principal medicinal components (phellodendrine, berberine and palmatine) from fresh Phellodendron bark, the three extraction methods and several solvent combinations were systematically analyzed.
2. Methodology

2.1. Preparation of Phellodendron barks

The fresh Phellodendron barks were collected from the average 15a trees in Phellodendron plantation in Cili country, Hunan province, China (Choi et al., 2014; Xian et al., 2011). The barks were treated by drying at 50 °C for 10 h, then meshed sieved by 60 meshes. The bark powders obtained were stored 4 °C.

2.2. Preparation of standard samples

Three standard samples (phellodendrine hydrochloride, berberine and palmatine hydrochloride) were dissolved in solution of acetonitrile/0.1% phosphoric acid to form sample solutions of gradient concentration (Hu et al., 2010; Garazd et al., 2017).

2.3. Distillation extraction

Accurately weigh 5.0 g powders of Phellodendron barks and put them into the round bottom flask, then add 40 times of ultrapure water (200 mL), followed by refluxing extraction in 100 °C. The extracts were collected finally by centrifugation (1000r/min) for 1 min (Jung et al., 2009).

2.4. Ultrasonic extraction

Weigh 0.50 g powders (by 20 mesh sieve) for 3 copies, then put them in the conical flask of 25 mL and add different extraction solvents: hydrochloric acid/methanol (1:100 v/v), hydrochloric acid/ethanol (1:100 v/v) and hydrochloric acid/water (1:100 v/v) in 50 mL (Kim et al., 2008). Powders were extracted with ultrasonic 20 min under the condition of 40 kHz and 250 W. The extracts were collected by centrifugation (1000 r/min) 1 min.

2.5. Soxhlet extraction

Ethanol extraction. Weigh 10.0 g powders, wrapped with filter paper and bounded with string, then put them into the drawer tube, then add 20 times amount of 70% ethanol (Chan et al., 2007). The extraction condition: extraction temperature 80 °C, 3 siphoning treatment (each for 1 h). Finally, combine the 3 extracting solutions to form extracts.

Acid-water extraction. Weigh 5.0 g powders, rapped with filter paper and bounded with string, then soaked in 2% hydrochloric acid for 1 h. Put them in the drawer tube and add 10 times amount of water (Li et al., 2006). The extraction condition: extraction temperature 100 °C, 3 siphoning treatment (each for 1 h). Finally, combine the 3 extracting solutions to form extracts (Muhammad et al., 2017b).

Double solvents extraction. Weigh 5.0 g powders of Phellodendron barks for 3 copies, wrapped with filter paper and bounded with string, then put them into the drawer tube (Liu et al., 1993; Wu et al., 2017). Then add 200 ml double solvents: hydrochloric acid/methanol (1:100 v/v), hydrochloric acid/ethanol (1:100 v/v) and hydrochloric acid/water (1:100 v/v). The extraction condition: extraction temperature 90 °C, 3 siphoning treatment (each for 1 h). Finally, combine the 3 extracting solutions to form extracts.

2.6. HPLC condition

Phellodendrine and berberine were gradient eluted with Kromasil RP-C18 chromatographic column (4.6 mm RP-C18), and palmatine is gradient eluted with Waters symmetry C18 chromatographic column (4.6 × 250 mm, 5 μm).

Mobile phase for berberine: Acetonitrile/0.1% phosphoric acid (50:50 v/v) (plus 0.1 g sodium dodecyl sulfonate per 100 mL), and the detection wavelength is 265 nm.

Mobile phase for Phellodendrine: Acetonitrile/0.1% phosphoric acid (36:64 v/v) (plus 0.2 g sodium dodecyl sulfonate per 100 mL), and the detection wavelength is 284 nm.

Mobile phase for Palmatine gradient eluting: 0.05% TFA ddH2O (A)/0.05% TFA acetonitrile (B), and the detection wavelength is 270 nm.

3. Results & discussion

3.1. Component identification of Phellodendron barks extracts by HPLC

According to the peak area integral (mAU) and concentrations (μg/mL) of three standard samples (phellodendrine hydrochloride, berberine and palmatine hydrochloride), their regression equations were established (Fig. 1).

Nine combinations of three extraction methods and solvent combinations were used to extract Phellodendron barks, and their principal medicinal components were identified by HPLC (High Performance Liquid Chromatography) and verified by three different standard samples (Fig. 2).

3.2. Extraction yield of medicinal components from Phellodendron barks

The extraction effects of principal medicinal components in fresh Phellodendron barks were investigated using five extracting...
solvents (distilled water, methanol, ethanol, hydrochloric acid/methanol, hydrochloric acid/ethanol, hydrochloric acid/distilled water) and three extracting methods (soxhlet extraction, distillation and ultrasonic extraction).

The analytical result showed that the extraction effects of hydrochloric acid/methanol, hydrochloric acid/ethanol, hydrochloric acid/distilled water are better than those of hydrochloric acid, methanol, ethanol and distilled water, suggesting that combined solvents have higher extracting effect of phellodendrine, berberine and palmatine than single solvent (Mustafa et al., 2017). Further, the hydrochloric acid/methanol (1:100 v/v) display the highest extracting efficiency. The three-good extraction yield of phellodendrine were achieved by hydrochloric acid/methanol-ultrasonic, hydrochloric acid/distilled water-ultrasonic and hydrochloric acid/ethanol-ultrasonic. The three-good extraction yield of berberine were achieved by hydrochloric acid/methanol-ultrasonic, hydrochloric acid/ethanol-ultrasonic. The three-good extraction yield of palmatine were achieved by hydrochloric acid/methanol-ultrasonic, hydrochloric acid/methanol-ultrasonic and hydrochloric acid/methanol-soxhlet (Nawaz et al., 2017).

Among the three extraction methods, the effect of ultrasonic extraction is distinctly better than those of distillation and soxhlet extraction. According to the analytical result, it is confirmed that the hydrochloric acid/methanol-ultrasonic extraction has the best effect, providing an extraction yield of 103.12 mg/g berberine, 24.41 mg/g phellodendrine, 1.25 mg/g palmatine (see Fig. 3).

It is reported in the literatures that the results of the experiment are different because of raw material origin, variety, pretreatment methods and data analysis methods. The findings here focused on the exploration of the best extraction technology of medicinal components from fresh Phellodendron barks, which maybe have high quality than those of long-term stored barks (Sarfraz et al., 2017).

Fig. 2. HPLC chromatograms of Palmatine in nine extracts of Phellodendron barks.
to use the ultrasonic extraction as the best extraction method for medicinal components from fresh *Phellodendron* barks.

4. Conclusion

Combined solvents have higher extracting effect of than single solvent for the extraction of medicinal components from fresh *Phellodendron* barks, and the effect of ultrasonic extraction is distinctly better than those of distillation and soxhlet extraction. The hydrochloric acid/methanol-ultrasonic extraction has the best effect for three medicinal components of fresh Phellodendron bark, and the extraction yield reaches to 103.12 mg/g berberine, 24.41 mg/g phellodendrine, 1.25 mg/g palmatine.

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