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COMPARISON OF EXTRACTION METHODS FOR THE HYPOTENSIVE DRUGS FROM *EUCOMMIA ULMOIDES*

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Abstract - Extraction methods using Soxhlet extraction (SE), enzyme-assisted aqueous extraction (EE), semi-bionic extraction (SBE) and supercritical fluid extraction (SFE) were evaluated for the yields of geniposidic acid (GPA) and geniposide (GPS) from the bark of *Eucommia ulmoides*. The results showed that the yields of GPA and GPS attained by EE and SBE were highest, respectively. Compared with various extraction methods for the extraction of GPA and GPS, the SBE was more efficient than other methods. There was no organic solvent used in SBE. The pH values of semi-bionic extraction were the same as in the human body. The results indicate that SBE can be used for large-scale and efficient extraction of GPA and GPS from plant materials. The time taken by SFE was longer than other methods, but the yields of GPA and GPS were the lowest.

Keywords: Geniposidic acid, geniposide, *Eucommia ulmoides,* enzyme-assisted aqueous extraction, supercritical fluid extraction, Soxhlet extraction, semi-bionic extraction.

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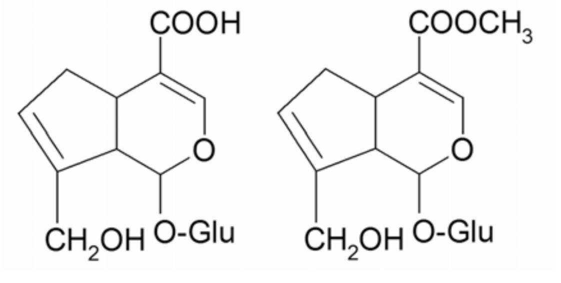
INTRODUCTION

The tree *Eucommia ulmoides* Oliver, whose bark is called Du-Zhong in Chinese, is a traditional medicine used as a tonic for reducing blood pressure (Huang et al., 2002). The bark of *E. ulmoides* is sweet in taste and warm in nature, active in nourishing the liver and kidney, strengthening the bone and muscle, and preventing miscarriage, etc (Deyama and Nishibe, 2001). Previous studies have found that many bioactive components are present in the bark of *E. ulmoides,* including derivatives of iridoids, flavonoids and phenolic compounds (Deyama et al., 1086; Deyama, 1983). Geniposidic acid (GPA, Fig. 1a) and geniposide (GPS, Fig. 1b) are two important hypotensive components in the bark of *E. ulmoides* (Deyama and Nishibe, 2001). The former may promote collagen synthesis (Li et al., 1998), and the latter has anti-inflammatory activity (Nishizawa et al., 1988).



a. Geniposidic acid b. Geniposide

Fig.1. Formula of geniposidic acid and geniposide

The extraction of essential oil components using solvent at high pressure, or supercritical fluids (SCF), has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative for conventional processes such as organic solvent extraction and steam distillation (Zougagh et al., 2006). The semi-bionic extraction method comes from the concept of combining holistic medicine and molecular medicine to simulate the progress and circumstances in which oral medicines are taken and absorbed in the gastrointestinal tract (A G L et al, 2006; Li et al., 2006). Enzyme-assisted aqueous extraction is for the elimination of the useless starch, pectin, protein, etc., effectively with the relevant enzyme digestion. The enzymes used in the extraction process and most frequently referred to in the literature are protease, amylase, cellulase and pectinase Thakur and Gupta, 2006; Li et al, 2004).

A microwave-assisted solvent extraction method was reported for leaching geniposidic and chlorogenic acids from the bark of *E. ulmoides* (Li et al., 2004). However, there is little data concerning the extraction of the hypotensive drugs geniposidic acid and geniposide from *E. ulmoides* using the Soxhlet extraction, enzyme-assisted aqueous extraction, semi-bionic extraction and supercritical fluid extraction. The aim of this work was to evaluate the extraction efficiencies of the hypotensive drugs geniposidic acid and geniposide from the bark of *E. ulmoides* using different extraction techniques.

MATERIALS AND METHODS

SFE grade carbon dioxide (99.998% pure) supplied in cylinders with a dip tube was purchased from Central South University (Hunan, PR China). Acetic acid and methanol were chromatographic grade. The solvents used were filtered through a 0.45 μm nylon membrane filter (Schleicher & Schnell, Keene, NH, USA) before use. Ethanol and HCl were of analytical grade. Standards of GPA (>98% pure) and GPS (>98% pure) were generously provided by the Japanese professor, Dr Deyama. The dried barks of *E. ulmoides* were obtained from the Traditional Chinese Medicine Co., Ltd. (Hunan, PR China).

Determination of GPA and GPS

GPA and GPS were determined according to the Li et al. (2004). HPLC analysis was performed on a Shimadzu 2010A apparatus equipped with a SPD-M10A VP detector. GPA and GPS were analyzed using an ODS RP-C_{18} column (150 × 4.6 mm i.d, 5 μm, Hanbon Science &Technology Co., Ltd). The mobile phase consisted of a mixture, methanol-acetic acid-H_2O= 20:0.5:79.5 (v/v). The detecting wavelength was 237 nm. The flow was 1.0 ml/min. The yield of extraction was calculated by the equation: yield (%) = $(m_1/m_2)\times100\%$ (1), where m_1 is mass of extract and m_2 is mass of raw material.

Soxhlet extraction (SE)

For Soxhlet extraction, 5 g of dried plant material was placed in a 200 ml Soxhlet thimble. The apparatus was fitted with a 250 ml round bottom flask. The extraction temperature was controlled between 50-80°C. Because these two components are easily dissolved in water and ethanol, ethanol aqueous solutions (50%-80%) were used to extract. The flask was heated for 0.5-2.0 h and the solvent was refluxed until a given time was up.

Supercritical fluid extraction method (SFE)

The SFE experiments were performed on a HA121-50-01-C SFE module. 100 g of the sample was weighed in a filter paper and packed into a 1-L thimble. Ethanol (95%, v/v) was added directly into the sample inside the thimble. The thimble was closed with hand screw caps and the system pressurized with pressures 30 MPa at temperatures from 40 to 60°C. The extraction time was between 2 and 4 h. The CO_2 flow rate was 15 –25 l/h and the modifier volume was 200-400 ml.

Enzyme-assisted aqueous extraction (EE)

10 g of the sample was weighed in a filter paper and packed into a 250-ml thimble. The cellulase, pectase and glucanase were chosen for experiment and cellulase was selected for its highest efficient. The optimum extraction conditions were determined by the orthogonal design method L_9 (3^4). The parameters, extraction temperature, pH of solvent, extraction time and solid-enzyme ratio optimum, were studied.

Semi-bionic extraction method (SB)

Ten g of the sample were packed into a 250-ml thimble which was extracted first by acidic water (100 ml) and then by alkaline water (100 ml) with appropriate pH values. The optimal pH values and other progressing parameters were obtained from the yields of GPA and GPS.

RESULTS AND DISCUSSION

Soxhlet method

During the Soxhlet extraction of GPA and GPS from *Eucommia ulmoides*, there are many variables affecting the yield. However, studying all parameters is time-consuming and unnecessary. Hence, in this work, only five main factors, i.e. solvent concentration, extraction temperature, extraction time, solvent volume and extraction times, were tested. For each variable factor, the influence on yields was considered on four levels. Table 1 lists the factors and levels used in the tests. An L_{16} (4^5) orthogonal design graph was used (Fig. 2).

To analyze the influence of each variable on extraction results, an intuitionistic plot was shown in Fig. 2. It can be seen from Fig. 2a that for GPA the effects of the four factors on extraction efficiency decreased in the order of extraction time (C), extraction temperature (A), solvent volume (E), extraction time (B) and ethanol concentration in water (D). When the extraction time was increased from 1 to 4 s, the yield of GPA was increased by 0.28%. When the extraction time was increased from 0.5 to 2.0 h, the yield of GPA increased at first, followed by a decrease, before increasing again. However, a different situation occurred with the factors: the higher the extraction temperature, the lower the yield of GPA. Unlike GPA, the influence of these factors on the extraction efficiency of GPS decreased in the order of extraction time (C), ethanol concentration in water (D), extraction time (B), extraction temperature (A) and solvent volume (E). (Fig. 2b).

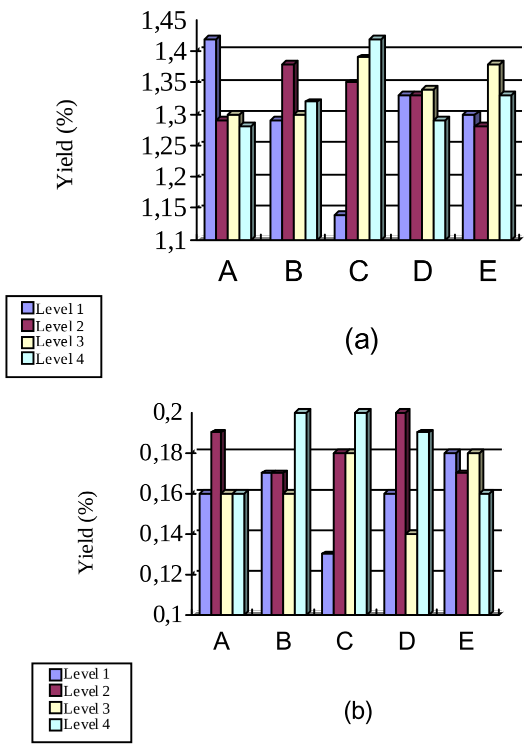


Fig. 2. The yields of GPA (a) and GPS (b) by SE under orthogonal conditions (A, extraction temperature (50, 60, 70, 80 °C) ; B, extraction time (0.5, 1.0, 1.5, 2.0 h); C, extraction times (1, 2, 3, 4); D, ethanol concentration (50%, 60%, 70%, 80%), and E, Solvent volume (10, 12, 14, 16 ml/g).

Supercritical fluid extraction

During supercritical fluid extraction of GPA and GPS from *Eucommia ulmoides*, there are many

Table 1. Results of different enzymes extracting GPA and GPS

	Yield of GPA (%)				Yield of GPS (%)			
Enzyme	1	2	3	Average	1	2	3	Average
Cellulase	1.26	1.21	1.23	1.23	0.20	0.18	0.21	0.20
Pectase	1.12	1.10	1.16	1.13	0.15	0.14	0.18	0.17
Glucanase	0.78	0.71	0.74	0.74	0.09	0.07	0.10	0.09

variables affecting the yields. However, studying all parameters is time-consuming and unnecessary. Hence, in this work, only 4 main factors, i.e. extraction temperature, extraction time, CO_2 flow rate and modifier volume, were tested. The factors and levels used in the tests and an L_9 (3^4) orthogonal design graph was used (Fig. 3).

To analyze the influence of each variable factor on the yields, an intuitionistic plot was shown in Fig. 3. It can be seen from Fig. 3a that for GPA the effect of the four factors on extraction efficiency decreased in the order of extraction temperature (A), CO_2 flow rate (C), extraction time (B) and modifier volume (D). Unlike the GPA, the influence of these factors on the yield of GPS decreased in the order of modifier volume (D), extraction temperature (A), extraction time (B) and CO_2 flow rate (C) (Fig. 3b).

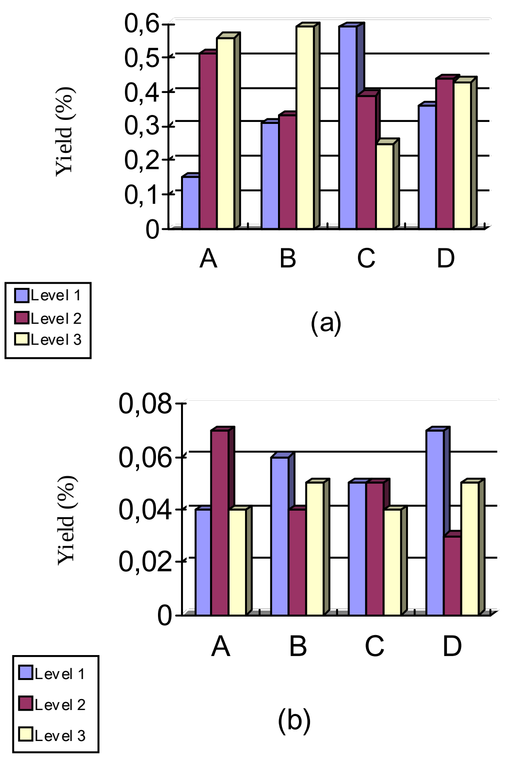


Fig. 3. The yields of GPA (a) and GPS (b) by SFE under orthogonal conditions (for designations of factors A, Temperature (40, 50, 60 °C); B, time (2.0, 3.0, 4.0 h); C, flow rate (15, 20, 25 L/h), and D, Modifier volume (200, 300, 400 ml).

Enzyme-assisted aqueous extraction

Cellulase, pectase and glucanase were chosen to carry out the experiment. The results are shown in Table 1. From Table 1, the yields of GPA and GPS extracted by cellulase were higher than by the other enzymes. Thus, cellulase was selected to carry out further experiments to find the best extraction condition.

For cellulase extraction, 4 main factors, i.e. extraction temperature, pH value of solvent, extraction time and solid-enzyme ratio, were tested. For each variable, the influence on the yield was considered on four levels. Table 6 lists the factors and levels used in the tests. In general, a full evaluation of the effect of the four factors from three levels on the yield requires 81 (3^4) experiments. In order to reduce the number of experiments, an L_9 (3^4) orthogonal design graph was used (Fig. 4). In this way, only nine experiments were needed. The yields of GPA and GPS obtained under orthogonal conditions are also shown in Fig. 4.

To analyze the influence of each variable on extraction results, an intuitionistic plot was shown in Fig. 4. It can be seen from Fig. 4a, for GPA the effects of the four factors on yield decreased in the order of extraction temperature (A), pH value of solvent (B), extraction time (D) and solid-enzyme ratio (C). The influences of these factors on the yield of GPS decreased in the order of solid-enzyme ratio (C), extraction time (D), extraction temperature (A), pH value of solvent (B) (Fig. 4b).

Semi-bionic extraction method - pH value of water

The semi-bionic extraction method comes from the concept of combining holistic and molecular medicine to simulate the progress and circumstances in which oral medicines are taken and absorbed in the gastrointestinal tract. The pH values of gastric juice, small intestinal juice and large intestinal juice are 0.9-1.5, 7.6 and 8.3-8.4, respectively. In this experiment, the samples were extracted firstly by water (pH=1.5, 100 ml), then by water (pH=7.5, 100 ml), and at last by water (pH=8.5, 100 ml) .

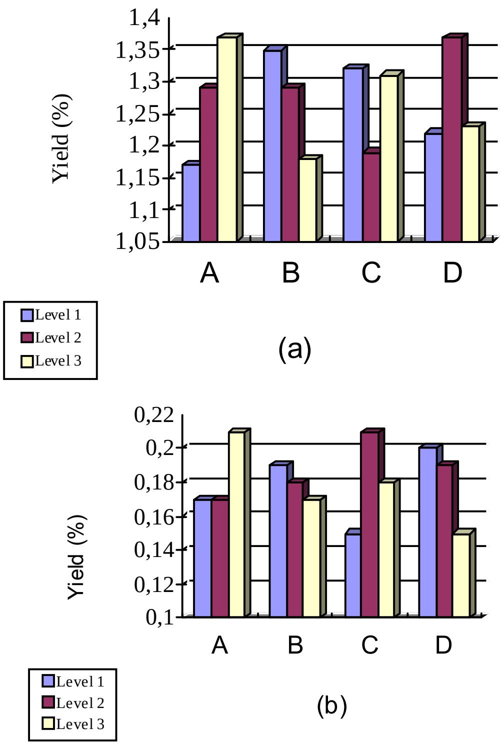


Fig. 4. The yields of GPA (a) and GPS (b) by EE under orthogonal conditions (for designations of factors A, Extraction temperature (35, 40, 45 °C); B, pH value of solvent (5.0, 6.0, 7.0); C, Solid-enzyme ratio (1000:1, 1000:2, 1000:3), and D, Extraction time (0.5, 1.0, 1.5 h).

Influence of the extraction time on the yields of GPA and GPS

Fig. 5 shows the influence of the extraction time on the yields of GPA and GPS. When the extraction time was increased from 0.5 to 2.5 h, the yields of GPA and GPS at first increased and then decreased (after 2 h).

Influence of extraction temperature on the yields of GPA and GPS

Fig. 6 shows the influence of extraction temperature on the yields of GPA and GPS. When the extraction temperature was increased from 40 to 60°C, the yield of GPA increased followed by a decrease after 60°C. For GPS, when the extraction temperature was below 70°C, the yield increased, but after 70°C the yield decreased.

Results of semi-bionic extraction

Analyzed as above, the following conditions for the extraction of GPA were selected: sample, 10 g; H_2O, 100 ml; pH, 1.5; temperature 60°C; time 0.5 h. After filtration, the residue was extracted with water with pH values at 7.5 and 8.5 two times. The yield of GPA was 1.50%. The best extraction condition of GPS was the same as for GPA except for the extraction temperature (70°C). The yield of GPS was 0.51%.

Comparison of various extraction methods

Fig. 7 shows the yields of GPA and GPS that were attained by EE, SBE, SE and SFE. The results show that the yield of GPA by EE (1.58%, w/w) was higher than the yields obtained with SBE (1.50%,

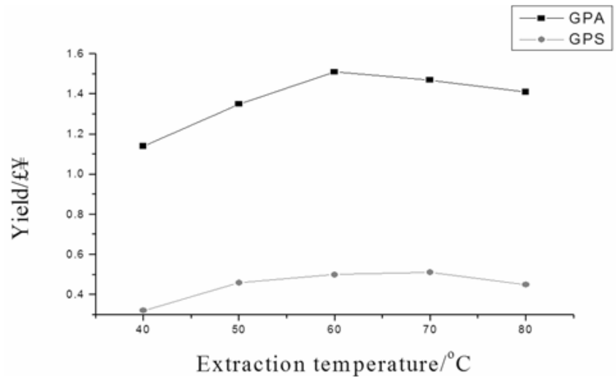


Fig. 5. Influence of extraction time on yields of GPA and GPS by SBE.

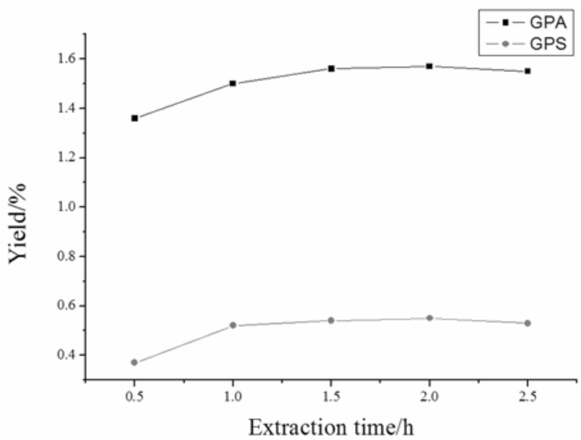


Fig. 6. Influence of extraction temperature on yields of GPA and GPS by SBE.

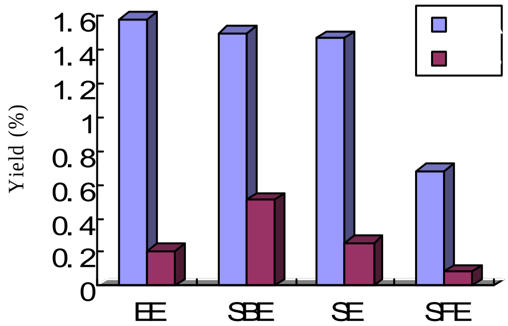


Fig. 7. Comparison of percentage extraction of GPA and GPS from the plant material of *E. ulmoides* by different extraction methods. Values expressed as means±S.D. (n = 3). The optimum condition for GPA was obtained by EE: extraction temperature, 45 °C; pH, 5.0; extraction time, 1h; solid-enzyme ratio, 100:0.3(w/w). The optimum condition for GPS was obtained by SBE: sample, 10g; H_2O, 100ml; pH, 1.5; temperature 60 °C; time, 1.5 h. After filtration, the residue was extracted with water whose pH was 7.5 to 8.5 for two times.

w/w), SE (1.47%, w/w) and SFE methods (0.68%, w/w) in that order. The results also showed that the yield of GPS by SBE (0.51%, w/w) was higher than as obtained by SE (0.25%, w/w), EE (0.21%, w/w) and SFE methods (0.08%, w/w), in that order.

The yield of GPA extracted by EE was highest, but was lower for GPS. The yield of GPS extracted by EE was only half of that obtained by SBE extraction. By comparing the various extraction methods for GPA and GPS, the SBE was more efficient, providing high yields of GPA and GPS. No organic solvents were used in SBE. The pH values of the SBE were the same as in the human body. The results indicate that SBE is suitable for large-scale and efficient extraction of GPA and GPS from plant materials.

SFE appears to be a cost-effective technique on a laboratory scale, but an accurate economic evaluation for large-scale use requires additional experiments. The advantages of SFE-CO2 extraction over the solvent extraction include: low operating temperature, hence no thermal degradation of most of the labile compounds; a shorter extraction period; high selectivity in the extraction of compounds; no solvent residue with negative effects on the oil quality. An essential drawback in the use of supercritical CO_2 is its low polarity, making the extraction of polar analytes difficult. GPA and GPS are highly-polar compounds. Even when a modifier is added, the yields of GPA and GPS are low. So, the yields of GPA and GPS attained by SFE are lowest of the four extraction methods.

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