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Application of ultrasonic technique for extracting chlorogenic acid from *Eucommia ulmodies* Oliv. (*E. ulmodies*)

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Abstract

An ultrasonic method for the extraction of chlorogenic acid from fresh leaves of *Eucommia ulmodies* Oliv. was investigated and optimized. The influence of four extraction variables on extraction efficiency of chlorogenic acid was investigated. The optimum extraction conditions found were: 70% aqueous methanol; solvent: sample ratio = 20:1 (v/w); extraction time 3×30 min. The recovery of chlorogenic acid was studied (HPLC) and the reproducibility of the extraction method was determined. The optimized ultrasonic extraction conditions were applied to extract chlorogenic acid from fresh leaves, fresh bark and dried bark of *E. ulmodies* and four traditional Chinese medicines. The application of sonication method was shown to be highly efficient in the extraction of chlorogenic acid from *E. ulmodies* and other Chinese medicines compared with classical methods.

Keywords: Ultrasound; Chlorogenic acid; *Eucommia ulmodies* Oliv.; Extraction

1. Introduction

Eucommia ulmodies Oliv. is an important Chinese materia medica. Its therapeutic action is mainly related to the liver and kidney. It is efficacious in nourishing the liver and kidney, but also as bone and muscle strengthening and preventing abortion. Recently, it was reported [1] that the leaves of *E. ulmodies* contained the same chemical components as the bark, resulting in identical therapeutic potency.

In conventional methods, the extraction of chlorogenic acid is accomplished by heating, boiling, or refluxing. One disadvantage of this procedure is the loss of chlorogenic acid due to ionization, hydrolysis and oxidation during extraction [2–4]. The other is the consumption of a large amount of solvent and the long extraction time [5–7]. To get more effective constituents from the herbal materials so that the Chinese medical materials could be utilized more efficiently and economically is an important research task for pharmacists and chemists. In this respect, a procedure that could obtain the most of the effective constituents in a shortest processing time with low cost will be an ideal technology. Modern techniques, such as ultrasonically assisted extraction, could be used to get bioactive components from plants [8–10]. Ultrasounds produce cell disruption, particle size reduction and ultrasonic jet towards solid's surfaces leading to a greater contact area between solid and liquid phase, better access of solvent to valuable components, compared with traditional methods [11,12]. This type of extraction has been applied to biological matrices such as plant materials [13–15] and even animal tissues [16]. In some cases, yields similar to those obtained by microwave-assisted extraction have been found [17]. The application of supersonic technique to plants has produced satisfactory results [18], and industrial processing has been proposed for obtaining compounds with pharmacological properties [19,20]. In many of these cited studies, the optimum extraction conditions were established

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using experimental design techniques to optimize extraction variables [21].

So far, for the extraction of chlorogenic acid from fresh leaves and bark of *E. ulmodies* using ultrasound, no such work has been reported in the literature. Hence, it is of interest to investigate the effect of ultrasound on the extraction of chlorogenic acid from these materials. In this paper, the extraction conditions for extracting chlorogenic acid from fresh leaves of *E. ulmodies* and other herbal medicines under supersonic have been studied and optimum condition for ultrasonic assisted extraction established.

2. Experimental

2.1. Apparatus

A cleaning bath (Shanghai Branson Ultrasound Co. Ltd., China) working at 50 kHz frequency and 160 W input power was employed as ultrasonic device. High performance liquid chromatographer was remodeled from HIC-6A ion chromatographer made by Shimadzu Co. Ltd. (Japan) with a SCL 6B system controller and a SIL-6B auto-injector connected to an auto-injection pump. LC-10uv UV detector, chromatographic work station with a data auto-collection card, and C_{18} column (250 mm × 4.6 mm, ID 5 μm) were purchased from Dalin Chromatographic Instrument Co. Ltd. (China).

2.2. Reagents and materials

Methanol, glacial acetic acid and redistilled water were filtrated through a 0.45 μm membrane before use. Standards of chlorogenic acid, caffeic acid, geniposidic acid, and geniposide were provided by Chemical Research Institute, Hunan Normal University. All chemical reagents were of analytical-reagent grade.

Fresh leaves and bark of *E. ulmodies* were collected from the same tree in Cili County, Hunan Province, China, in the middle of April. Dried bark of *E. ulmodies* was purchased from the Traditional Chinese Medicine Market (Hunan). *Yinhuang* Deliquation Dose was produced by 999 Nankai Pharmaceutical Co. Ltd., China, with an approval number of 011051 (1994). *Yinqiao* Detoxication Pill came from Hunan Jiu Zhi Tang Co. Ltd., China, with an approval number of 002119 (1992). *Yinhuang* Oral Liquid was prepared by Hubei Newland Pharmaceutical Co. Ltd., China, with an approval number of 000991 (1990). *Tianma Duzhong* Capsule was produced by Guizhou Assun Pharmaceutical Co. Ltd., China, with an approval number of 100191 (1996). These four traditional Chinese medicines were made from medicinal plants abundantly containing chlorogenic acid, and were of antibacterial, antiphlogistic, detoxicative, and other pharmacological activities. The content of chlorogenic acid was regarded as a major evaluation index of these Chinese medicines in the Chinese Pharmacopoeia [22]. Conventional determination method, like heat refluxing, was prone to destroy the active constituents in medicines.

2.3. Extraction method

Before experiment, fresh leaves and bark of *E. ulmodies* were cut into pieces (5 mm × 5 mm) and dried bark of *E. ulmodies* was ground into powder (particle diameter: 0.2–0.9 mm). Samples of 0.2 g were mixed with the extracting solvent (solvent/sample ratio 20/1 (v/w)), placed in ultrasonic cleaning bath having as coupling liquid water, sonicated for 30 min, and filtered off through 0.45 μm microporous membrane. The filtrate was collected and the solid was extracted again (two times) with the same volume of fresh solvent.

2.4. Chromatographic analysis

For HPLC analysis, CH_3OH–H_2O–CH_3COOH (19:81:1.5, v/v) was used as the mobile phase, flow rate was 1 ml/min, the column temperature was room temperature and sample volume injected was 10 μl. Detector: LC-10uv UV at 240 nm. Calibration curve method was used for the HPLC analysis of chlorogenic acid in *E. ulmodies* and from traditional Chinese medicines.

2.5. Determination of the chlorogenic acid content in dried material

The sample (dried in air for 15 days, 1.0 g) was ground in powder and then treated by refluxing method [23] for 5 × 60 min with 20 ml freshly prepared 70% aqueous methanol at 60 °C. The collected filtrate was concentrated under vacuum and then analyzed by HPLC. The content of chlorogenic acid in dried material was calculated.

2.6. Determination of the recovery

The determination of the recovery of chlorogenic acid was carried out by standard addition method with sonication under the same optimized extraction conditions for the same material.

2.7. Stability test of the sonication method

Five samples with the same weight (0.2 g) were individually processed under sonication and the same optimized extraction conditions for the same material. The filtrate was analyzed by HPLC and the extraction efficiencies of chlorogenic acid were calculated. By comparing these results, the stability of the sonication method was evaluated.