Separation and Purification Technology 133 (2014) 375–379

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Optimization and comparison of ultrasound-assisted extraction and microwave-assisted extraction of shikimic acid from Chinese star anise

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ARTICLE INFO

Article history:
Received 1 September 2013
Received in revised form 28 May 2014
Accepted 27 June 2014
Available online 18 July 2014

Keywords:
Shikimic acid
Chinese star anise
Ultrasound-assisted extraction
Microwave-assisted extraction

ABSTRACT

Shikimic acid is a main raw intermediate for manufacturing oseltamivir phosphate (Tamiflu), which is the only antiviral treatment for Influenzavirus A (H5N1 and H2N1), recently. Chinese star anise is a natural resource of shikimic acid which can be extracted by some typical technologies. Two effective technologies, ultrasound-assisted extraction (USE) and microwave-assisted extraction (MWE), were introduced to extract shikimic acid from Chinese star anise in this study. Effects of various variables, such as applied power, ratio of solvent to material, and extraction time on the yield by two different technologies were investigated. Results showed that ratio was the most significant factors in both methods, followed by applied power and time. In USE process, the best yield of shikimic acid, 1.367%, were obtained at an optimum condition of 480 W, 15 mL/g and 20 min using orthogonal design; while in MWE, 2.75% of yield was achieved at 500 W, 15 mL/g and 16 min by response surface methodology. USE and MWE are two promising technologies for shikimic acid extraction; however, it is still necessary to develop some integrated methods to improve the extraction yield of shikimic acid from Chinese star anise.

1. Introduction

Chinese star anise, the fruit of *Illicium Verum Hook.f.*, is an important traditional Chinese medicine for treating stomachaches, skin inflammation, vomiting, etc., as well as a popular spice used in food. Chinese star anise contains many active chemicals, such as essential oils, preylated C6-C3 compounds, lignans, sequiterpenes and flavonoids [1]. It is a main resource of natural shikimic acid, as shown in Fig. 1, which has activities of antioxidant, antibacterial, antiinflammatory, anticoagulant and antithrombotic [2]. Moreover, shikimic acid has an important role in the synthesis of some compounds, especially as an intermediate to manufacture the oseltamivir phosphate (Tamiflu), regarding as an oral antiviral treatment for Influenzavirus A (H5N1 and H2N1). In recent decades, influenza pandemic happened frequently, and it may continue to emerge in the future [3]. Therefore, it is necessary to develop some effective medicines such as Tamiflu or other medicines for this disease. Thus, how to abundant supply the intermediate of shikimic acid is a crucial problem at the moment.

In Abrecht's review [4], it reports that it is more advantage to begin the manufacture of oseltamivir phosphate from shikimic acid than developing shikimic acid independent approaches. Recently, there are three main technologies to obtain shikimic acid used for producing oseltamivir phosphate, the chemical synthesis, microbial fermentation and extraction from natural plants [5]. Microorganisms such as *Escherichia coli* or their modifications are fed by glucose to produce shikimic acid in the fermentation process [6]. In the extraction process, shikimic acid is extracted from some natural plants, such as Chinese star anise. The extraction of shikimic acid from natural plant is very difficult because of the low yield of shikimic acid. Many efforts have been carried out for improving the efficiency of extraction technologies. Water is widely used as the solvent in extraction of shikimic acid, as well as ethanol, methanol and n-butanol, because of its solubility in water of 204 g/kg H_2O at 25 °C and practically zero solubility in chloroform, benzene or petroleum ether [7]. Though many researchers investigated the technologies for extraction of shikimic acid from some natural plants [8,9], few study focused on the Chinese star anise in the world. Ohira et al. [7] found shikimic acid can be rapidly separation from Chinese star anise with hot water extraction at temperatures of 120 °C or higher to obtain recoveries of 100%. Some researchers in China have investigated the extraction technologies of shikimic acid from Chinese star anise. Ultrasound-assisted extraction (USE) and microwave-assisted extraction (MWE) have been admitted as two effective technologies in some primary studies [10,11].

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http://dx.doi.org/10.1016/j.seppur.2014.06.064
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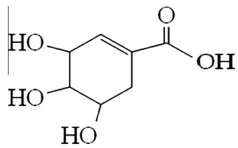

Fig. 1. Structure of shikimic acid.

However, these studies used methanol as the extraction solvent which is not safe and acceptable in food and pharmaceutical industries. Therefore, it is necessary to confirm and optimize these effective technologies applying in extraction of shikimic acid from Chinese star anise with water.

In this work, technologies of USE and MWE will be introduced to extract shikimic acid from Chinese star anise with water. Various factors in these two processes are evaluated by single factor experiments, and optimized by orthogonal experiments and response surface methodology, respectively. Results obtained by USE and MWE will be compared.

2. Materials and methods

2.1. Materials

Chinese star anise (*Illicium Verum Hook.f.*) were purchased from LBX Pharmacy (Hangzhou, China), and pulverized by an ultra mill (Shanghai Dianjiu Chinese Machinery Manufacturing Co. Ltd., Shanghai, China). The crude powder was sifted using a 0.35 mm mesh.

2.2. Ultrasound-assisted extraction

10 g pulverized powder of Chinese star anise were put into a beaker of 250 mL with a specific volume of water. Ultrasonic transducer probe at 28 Hz (FS-1200, Shanghai Shengxi Ultrasonic instrument Co. Ltd., Shanghai, China) was immersed in the water to enhance the extraction process. Optimization experiments were carried out according to the L_{16} (4^5) orthogonal design, and each run was operated for three times.

2.3. Microwave-assisted extraction

In MWE, 10 g pulverized powder of Chinese star anise were put into a 1000 mL flask with a specific volume of water. It was carried out in a homebred microwave extraction apparatus (MAS II Microwave extraction apparatus, Sineo Microwave Chemical Technology Co. Ltd., Shanghai, China). Various parameters are shown detailed in Table 1. All the factors selected in our studies were according to our primary studies and some other reported researches.

2.4. Analytical method

The method to determine the concentration of shikimic acid was according to Tian's [12] with some modifications. 0.02 g standard shikimic acid was dissolved into a 100 mL volumetric flask with methanol to be stock solution. 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mL stock solution were moved to another 100 mL volumetric flask and diluted with methanol. The adsorption of different samples were measured by UV spectrophotometer (752 Model, Shanghai Optical instrument Co. Ltd.) at 213 nm. Standard curve of shikimic acid can be obtained, as shown in Fig. 2. The extracted solution of shikimic acid was dissolved in 250 mL volumetric flask, and 0.5 mL of the sample was used for analyzing by UV at 213 nm.

Table 1
Factors and levels of experiments.

Factors	USE				MWE		
	1	2	3	4	−1	0	1
Extraction time (min)	10	20	30	40	10	16	22
Power (W)	240	360	480	600	400	500	600
Ratio (mL/g)	5	10	15	20	10	15	20

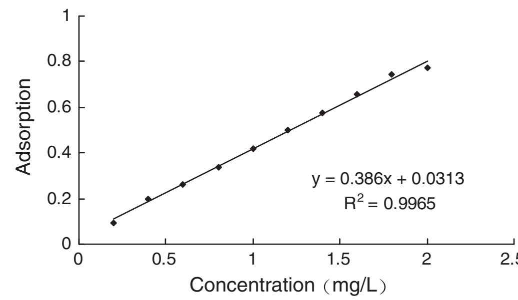

Fig. 2. Standard curve of shikimic acid.

2.5. Statistical analysis

All the tests were carried out for three times. Orthogonal experimental design was conducted by the orthogonal design assistant II, and response surface methodology was performed using the software of Design-Expert 7.1 (Stat-Ease, Inc., Minneapolis, MN, USA).

3. Results and discussion

3.1. Ultrasound-assisted extraction

Effects of three parameters, ratio of solvent to material, extraction time and US power, on the extraction yields of shikimic acid from Chinese star anise by USE were investigated, as shown in Fig. 3. It shows that all the yields of shikimic acid gradually increase when the factors increase during the processes. When the ratio increased from 5 to 15 mL/g, the yield rose almost linearly, and after that point it almost kept steady. This demonstrates that the diffusion speed of shikimic acid from the fruit inside to outside became faster when the star anise immersed into a huger volume of solvent because of the great concentration gradient. It took only 20 min to achieve at an equilibrium status in the extraction, as shown in Fig. 3B. The yield of shikimic acid increased sharply when the US power changed from 240 to 480 W, as shown in Fig. 3C. It indicates that US power can significantly affect the recovery which maybe caused by the vibration and cavitation mechanisms of US [13]. The yield changed slightly when the power increased from 480 W, which indicates that any excess power is useless. These one factor at time experiments shows that it is necessary to do an optimization to obtain the optimum conditions.

L_{16} (4^5) orthogonal experimental design was employed to optimize the USE process of shikimic acid, and results are shown in Table 2. It shows that ratio is the most significant factor while the extraction time is the insignificant, in an order of A > C > B. According to this experimental design, the optimum conditions for USE of shikimic acid from Chinese star anise are ratio of 15 mL/g, extraction time of 20 min and US power of 600 W. Considering the actual operation and energy consumption, the best conditions can be at ratio of 15 mL/g, extraction time of 20 min and US power of 480 W. Confirmation experiments were carried