Ultrasonics Sonochemistry 19 (2012) 1150–1154

Contents lists available at SciVerse ScienceDirect

Ultrasonics Sonochemistry

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

Ultrasound-assisted extraction of three bufadienolides from Chinese medicine ChanSu

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ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form 16 February 2012
Accepted 1 March 2012
Available online 30 March 2012

Keywords:
Ultrasound-assisted extraction
Bufalin
Cinobufagin
Resibufogenin
ChanSu

ABSTRACT

In this study, the application of ultrasound-assisted extraction (UAE) method was shown to be more efficient in extracting anti-tumor bufadienolides (bufalin, cinobufagin and resibufogenin) from important animal medicine of ChanSu than the maceration extraction (ME) and soxhlet extraction (SE) method. The effects of ultrasonic variables including extraction solvent, solvent concentration, solvent to solid ratio, ultrasound power, temperature, extraction time and particle size on the yields of three bufadienolides were investigated. The optimum extraction conditions found were: 70% (v/v) methanol solution, solvent to solid ratio of 10 ml/g, ultrasound power of 125 W, temperature of 20 °C, extraction time of 20 min and particle size of 60–80 mesh. The extraction yields of bufalin, cinobufagin and resibufogenin were 43.17 ± 0.85, 52.58 ± 1.12, 137.70 ± 2.65 mg/g, respectively. In order to achieve a similar yield as UAE, soxhlet extraction required 6 h and maceration extraction required much longer time of 18 h. The results indicated that UAE is an alternative method for extracting bufadienolides from ChanSu.

1. Introduction

ChanSu, also called toad venom, is prepared from the skin secretions of giant toads of *Bufo bufo gargarizans* Cantor and *B. melanostictus* Schneider [1]. It is a widely used as a cardiotonic and local anesthetic agent in China and other countries. Many studies found that the major active constituents of ChanSu are bufadienolides, the characteristic structural feature of bufadienolides is a doubly unsaturated six membered lactone ring on position 17 of C-24 steroids [2–5]. Bufalin, cinobufagin and resibufogenin, the major bufadienolides in ChanSu, are usually believed to be responsible for the biological activity of anti-tumor [6]. In addition, they are often used as marker components in ChanSu [7].

Many different techniques have been employed for the extraction of bioactive compounds from natural products, such as maceration extraction (ME) [8], soxhlet extraction (SE) [9], ultrasound-assisted extraction (UAE) [10], microwave-assisted extraction (MAE) [11], supercritical fluid extraction (SFE) [12] and accelerated solvent extraction (ASE) [13]. Among these techniques, UAE has been proved to significantly decrease extraction time, reduce the consumption of organic solvents and increase extraction yields in many natural products [14,15]. Recently, UAE has been widely applied to extract active compounds, such as flavonoids [16,17], alkaloids [18], steroids [19] and anthraquinones [20,21] from plant materials. However, there have been no reports on application of UAE technique to extraction of bufadienolides from animal medicine of ChanSu. Thus, it is of interest to evaluate the influence of main extraction conditions including extraction solvent, solvent concentration, solvent to solid ratio, ultrasound power, temperature, extraction time and particle size on the yields of three bufadienolides from ChanSu. In addition, a comparison among the yields of bufadienolides from UAE, ME and SE was performed in order to evaluate the potential and feasibility of UAE for the rapid extraction of three bufadienolides from ChanSu.

2. Experimental

2.1. Materials and reagents

The ChanSu crude drug was purchased from Anguo, Hebei province, China, and authenticated by the Dr. Jianhua Wang (College of Agronomy, Shandong Agricultural University). The crude drug was pulverized into powder form by a disintegrator (Taisite Instrument Company, Tianjin, China), and then sieved with stainless steel sieves to classify the particle size. The powdered sample was kept in a dry and dark place until use.

Abbreviations: UAE, ultrasound-assisted extraction; ME, maceration extraction; SE, soxhlet extraction; BF, bufalin; CF, cinobufagin; RF, resibufogenin; TB, total bufadienolides.

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http://dx.doi.org/10.1016/j.ultsonch.2012.03.003

All organic solvents used for extraction were of analytical grade and purchased from Tianjin Chemical Factory, Tianjin, China. Acetonitrile used for HPLC was of chromatographic grade (Fisher Scientific, USA). Water used was redistilled water, and passed through a 0.22 μm filter prior to use in all the studies. Bufalin, cinobufagin and resibufogenin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (No. 2 Tiantan Xili, Chongwen District, Beijing) with purities of over 98%. The structures of the three bufadienolides were shown in Fig. 1.

2.2. Ultrasound-assisted extraction (UAE)

For the ultrasound-assisted extraction experiments, an ultrasonic bath was used as an ultrasound source. The bath (KQ-250DE, Kunshan Ultrasound Co. Ltd., China) was a rectangular container (300 × 240 × 150 mm), to which 40 kHz transducers were annealed at the bottom. The bath power rating was 250 W on the scale of 4–10. The extraction temperature was controlled and maintained at the desired value by circulating external water from a thermostatic water bath into the cleaning bath. The sample beakers were immersed into the ultrasonic cleaning bath for irradiation under different extraction conditions including solvents: chloroform, ethyl acetate, ethanol, methanol, acetonitrile and water; percentage of methanol in water of 50–90%; solvent to solid ratio of 5–50 ml/g; ultrasound power of 100–250 W; temperature of 20–60 °C; extraction time of 5–60 min; particle size of 20–100 mesh. Finally, extracts were filtered off through 0.22 μm membrane filter and the filtrate was collected for HPLC analyses. All samples were prepared and analyzed in triplicate.

2.3. Maceration extraction (ME)

ME method was performed with 0.4 g (60–80 mesh) of dried samples and 100 ml of 70% methanol extracted three times, each for 6 h, and then mixed them at room temperature. The extracts were combined and concentrated by a rotary vacuum evaporator. All solutions were filtered through 0.22 μm membrane filter before direct injection into the HPLC system. All samples were prepared and analyzed in triplicate.

2.4. Soxhlet extraction (SE)

Soxhlet extraction was performed in a soxhlet apparatus. The powders of 0.4 g (60–80 mesh) and 100 ml methanol were placed into the soxhlet apparatus. The exhaustive extraction was performed for 6 h at 85 °C. All solutions were filtered with 0.22 μm membrane filter before the HPLC analysis. All samples were prepared and analyzed in triplicate.

2.5. High-performance liquid chromatography (HPLC) analysis

The HPLC equipment used is Waters 600E (USA) HPLC system including a 4-Solvent delivery system 600E start-up kit, a 600 pump, 0–20 ml/min, a 2996 photodiode array detector, an Empower Add-on Single System, China, a Degasser in-line 4-chamber, and a 600E controller. Analysis was achieved on a Hypersil C_{18} column (250 mm × 4.6 mm, 5 μm) from Dalian Elite Analytical Instruments Co. Ltd.

For HPLC analysis, the mobile phase was CH_3CN-H_2O (85:15, v/v) at a flow rate of 1.0 ml/min, the column temperature was 30 °C and sample volume injected was 20 μl. The absorbance was measured at a wavelength of 300 nm for the detection of bufalin, cinobufagin and resibufogenin. The chromatograms of standards and ultrasonically extracted ChanSu are shown in Fig. 2. The chromatographic peaks of bufalin, cinobufagin and resibufogenin were confirmed by comparing their retention time with those of the reference standards. Quantification was carried out by the integration of the peak using external standard method by means of a six-point calibration curve. The regression equations, correlation coefficient and linear range are listed in Table 1.

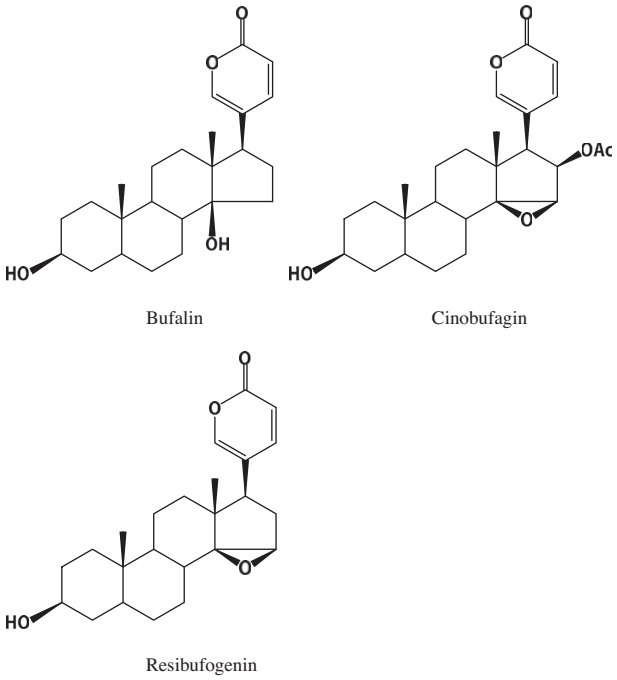

Fig. 1. Chemical structures of bufalin, cinobufagin and resibufogenin.

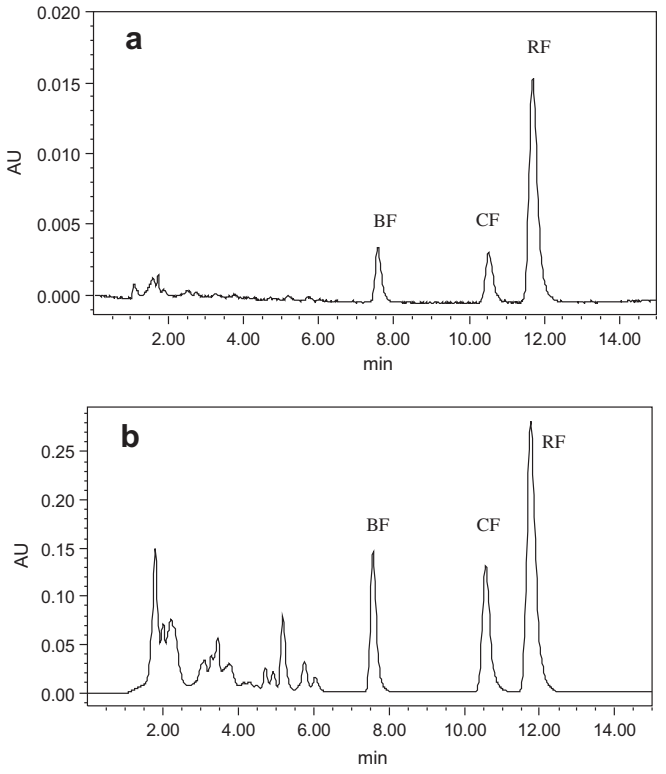

Fig. 2. The HPLC chromatograms of the standard mixture solutions and samples. (a) standard substances; (b) crude extract by UAE from ChanSu. Peaks BF, CF and RF correspond to bufalin, cinobufagin and resibufogenin, respectively.