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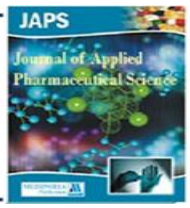

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Investigation on ultrasound-assisted extraction of three dibenzylbutyrolactone lignans from *Hemistepta lyrata*

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ABSTRACT

Ultrasound-assisted extraction is evaluated as a simpler and more effective alternative to conventional extraction methods for the extraction of bioactive compounds from natural product. This study investigated the use of ultrasound-assisted extraction to extract three dibenzylbutyrolactone lignans, including tracheloside, hemislienoside, and arctiin from *Hemistepta lyrata*. Factors such as extraction solvent, solvent concentration, solvent to material ratio, and extraction time were examined. High-performance liquid chromatography with photodiode array detection was used for simultaneous determination of the target compounds in the corresponding extracts. Results showed that the optimal parameters to extract the target compounds from *H.* lyrata were as follows: extraction solvent: 70% aqueous ethanol; solvent to material ratio: 20:1 (v/w, ml/g); extraction time: 20 min under the conditions: ultrasonic frequency: 40 Hz; extraction temperature: 30 ˚C. With all these merits, ultrasound-assisted extraction should be considered for wider application in the extraction of tracheloside, hemislienoside, and arctiin from other medicinal plants.

Keywords: *Hemistepta lyrata*, ultrasound-assisted extraction, tracheloside, hemislienoside, arctiin.

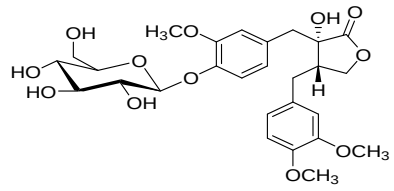
INTRODUCTION

Hemistepta lyrata Bunge (Compositae), commonly known as "NiHuCai" in China, is an herbaceous plant growing on mountain slopes, wasteland, and along roadsides in eastern and southern areas of Asia and Australia (Lin and Shi, 1987). It is used as folklore medicine for reducing fever and detoxification, eliminating stagnated blood, and dispersing swelling (Jiangsu New Medical College, 1977). H. lyrata reportedly harbors lignans (Ren and Yang 2001; Zou *et al.*, 2006a), flavonoids (Dong et al, 2010; Huang *et al.*, 1991; Zou *et al.*, 2006b; Zou *et al.*, 2006c), sesquiterpene lactones (Ha *et al.*,2003; Jiang *et al.*,1998; Jiang *et al.*,1999), and so on. Our phytochemistry study showed the whole plant of H. lyrata to be a rich source of dibenzylbutyrolactone lignans including tracheloside, hemislienoside, and arctiin (Figure 1). Among them, hemislienoside is a new component obtained from H. lyrata (Ren and Yang, 2001). Naturally occurring dibenzylbutyrolactone lignans exhibit a variety of biological activity. Tracheloside displays anti-estrogenic effects (Yoo *et al.*, 2006). Arctiin has been reported to show a variety of biological activities and a number of important pharmacological properties, such as antiproliferative (Csapi *et al.*, 2010), anti-tumor-promoting (Takasaki *et al.*, 2000), ameliorative (Wu *et al.*, 2009), neuroprotective (Jiang *et al.*, 2001) activities.

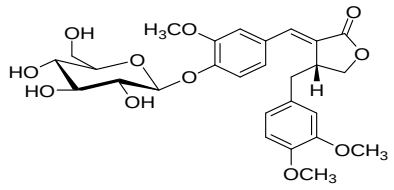
Furthermore, arctiin was found to attenuate lipopolysaccharide-induced inducible nitric oxide synthetase and cyclooxygenase-2 expressions through activation of nuclear factor-κB and mitogen-activated protein kinase in RAW264.7 cells (Li *et al*. 2010).

Reflux extraction of bioactive compounds from plant materials is a traditional technique applied in many industrial processes, particularly the pharmaceutical industry. The conventional reflux extraction requires long time and has low efficiency. Moreover, many natural products are thermally unstable and may degrade during thermal extraction. The ideal extraction methods could obtain the maximum of the bioactive constituents in a shortest processing time with a low cost. Ultrasound-assisted extraction allows the solvent to penetrate cell walls, and the bubbles produced by acoustic cavitation aid in the disruption of the cell wall, which then releases active ingredients. This method has been applied in the extraction of many chemical constituents from different plant materials (Albu *et al*. 2004; Dong *et al*. 2010; Li *et al*. 2005; Ma *et al*. 2008; Wu *et al*. 2001; Zhao *et al*. 2007), indicating that it is a highly efficient, low energy required and reduced solvent- and time-consuming method.

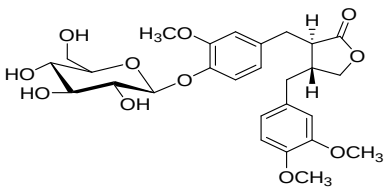
So far, for the extraction of tracheloside, hemislienoside, and arctiin from natural materials using ultrasound-assisted extraction, no such work has been reported in the literature. In this study, we investigated the factors that influence the ultrasound-assisted extraction, such as extraction solvent, solvent concentration, solvent to material ratio, and extraction time, to define optimal technological parameters for the extraction of tracheloside, hemislienoside and arctiin from *H. lyrata*.



Tracheloside



Hemoslienoside



Arctiin

Fig 1: Chemical structures of the three investigated compounds.

MATERIALS AND METHODS

Ultrasonic instrument

The ultrasound-assisted extraction was carried out in an ultrasonic cleaning bath (Kunshan Ultrasound Co. Ltd., Kushan, China; inner dimension: 300 mm × 150 mm × 100 mm) with an ultrasound power of 100 W, heating power of 400 W, and frequencies of 40 kHz, equipped with digital timer and temperature controller.

High-performance liquid chromatography (HPLC) system

The HPLC equipment used is Shimadzu 2010A HT series liquid chromatographic system (Shimadzu, Kyoto, Japan) equipped with a quaternary solvent delivery system, an on-line degasser, an auto-sampler, a column temperature controller and SPD-M10A photodiode-array detector (PAD) coupled with an analytical workstation.

Reagents

Solvents used in the extraction, such as methanol, ethanol, and *n*-butanol were of analytical grade and purchased from Beijing Chemical Factory (Beijing, China). Acetonitrile used in the analysis of HPLC was of chromatographic grade and purchased from TEDIA Company (Fairdield, USA). HPLC-grade water was purified by use of Milli-Q system (Millipore, Bedford, USA). Tracheloside, hemislienoside, and arctiin were isolated by the authors from the whole plant of *H.lyrata*, and their structures were fully characterized by spectroscopic methods (mass spectrometry (MS) and nuclear magnetic resonance (NMR))(Kim *et al*. 2007; Ren and Yang 2001; Zou *et al.*, 2006a). The purities of the compounds were determined on the basis of the peak area of each compound, in comparison with other peaks detected, in HPLC-PAD analysis with wavelength of detection at 202, 230, and 280 nm. This showed purity to be better than 98 % for all the compounds.

Plant material

The whole plants of *H. lyrata* were collected from Changbai mountain, Jilin Province of China, in September 2008, and authenticated by Prof. Zhongkai Yan, Jilin Academy of Chinese Medicine Sciences, China. A voucher specimen (HLNHC20080902) is deposited at the Institute of Phytochemistry, Jilin Academy of Chinese Medicine Sciences, China. Samples were dried in vacuum oven (35 ˚C), then pulverized into powder by a disintegrator and sieved with stainless steel sieves to classify the particle size. The powdered samples were kept in a dry and dark place until use.

Ultrasound-assisted extraction

A 10.0 g aliquot of accurately weighted powder of *H. lyrata* was loaded into a 500 ml conical beaker. The selected solvent was added and the breaker sealed. The extraction was conducted under certain conditions in the ultrasonic cleaning bath. After the extraction was finished, the extracted solution was clarified by filtration on a water pump, and was then filtered

through a 0.45 μm Nylon membrane filter. The filtrate was then analyzed by HPLC.

The parameters investigated include extraction solvent, such as water, 50 % methanol, methanol, 50 % ethanol, ethanol, and butanol, among them ethanol (diluted with water) was further examined with concentrations of 0 %, 10 %, 30 %, 50 %, 70 %, 90 %, and 100 %; the ratios of solvent to material with the proportions of 10, 20, 30, and 40 (v/w, ml/g); extraction time with designed periods of 5, 10, 15, 20, 25, and 30 min. All experiments were prepared in triplicate.

HPLC analysis

The chromatographic separation was carried out on a ZORBAX Extend-C18 (5 μm, 250 mm × 4.6 mm, Agilent Technologies, USA). The mobile phase consisted of acetonitrile and water (84:16, v/v). All solvents were filtered through a 0.45 μm Nylon membrane filter prior to use. The sample injection volume was 20 μl. A flow rate of 1.0 mL/min and a column temperature of 40 ˚C were used throughout the study. The PDA acquisition wavelength was set at 202 nm. Under the above conditions, the chromatograms of standards and ultrasonically extracted *H. lyrata* are shown in Figure 2. The chromatographic peaks of tracheloside, hemislienoside, and arctiin were confirm by comparing their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak area using external standard method.

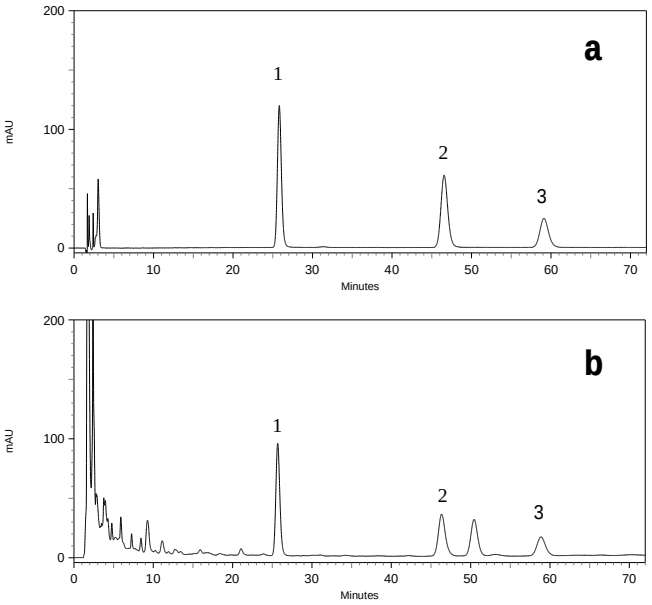


Fig 2: HPLC chromatograms of standards and sample. (a) standards; (b) ultrasonically extracted *H. lyrata*. Peaks 1, 2, and 3 correspond to tracheloside, hemislienoside, and arctiin, respectively.

RESULTS AND DISCUSSION

Effect of solvent

The selection of the most appropriate solvent for extracting the compounds of interest from the sample is an essential step for developing any extraction method. In this study, water, methanol, ethanol, and butanol were tested to extract tracheloside, hemislienoside, and arctiin from *H. lyrata*. Figure 3 shows the effects of different solvents on the extraction yield of the target compounds. The extraction time was 30 min, extraction temperature was 30 ˚C, ultrasound frequency was 40 kHz, and the ratio of solvent to material was 30:1. Four different solvents exhibited different effects on the extraction yield under same extraction conditions. Water is not suitable solvent for extracting the target compounds. Because tracheloside, hemislienoside, and arctiin are polar compounds, solvents with high polarities such as methanol and ethanol are better for the extraction. Butanol, with lower polarity than methanol and ethanol, exhibited a lower extraction yield. This result indicates that solvents show different extraction results on the objective constituents due to polarity and viscosity differences between them.

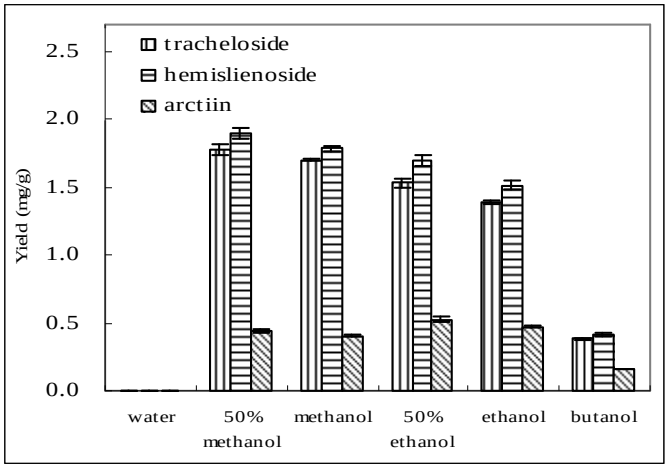


Fig 3: Effects of different solvents on the extraction yields of tracheloside, hemislienoside, and arctiin. Other conditions were fixed at: sonication time duration, 30 min; extraction temperature, 30 ˚C; ultrasound frequency, 40 kHz; the ratio of solvent volume to sample mass, 30:1.

Effect of solvent concentration

Figure 3 shows that the extraction of the target compounds in 50 % aqueous methanol and 50 % aqueous ethanol are better than in pure methanol and ethanol, respectively. Although the use of 50 % aqueous methanol as the extracting solvent produced higher yields of tracheloside and hemislienoside than 50 % aqueous ethanol, its disadvantage over 50 % aqueous ethanol is a toxic solvent, which makes it more harmful to health. It has been observed that sometimes the addition of small percentage of water to the extraction solvent helps to increase the extraction yield of the target compounds from the sample (Barbero et al 2008; Martino *et al.* 2006). The effects of different concentrations of aqueous ethanol on the extraction were further examined. Figure 4 shows the effects of different concentrations of aqueous ethanol on the extraction yield of tracheloside, hemislienoside, and arctiin from *H. lyrata.* Other extraction conditions were fixed at: extraction time: 30 min, extraction temperature: 30 ˚C, ultrasonic frequency: 40 kHz, and ratio of solvent to material 30:1. A 70 % aqueous ethanol solution showed the highest extraction efficiency and was chosen as the optimal solvent for the following extraction experiments.

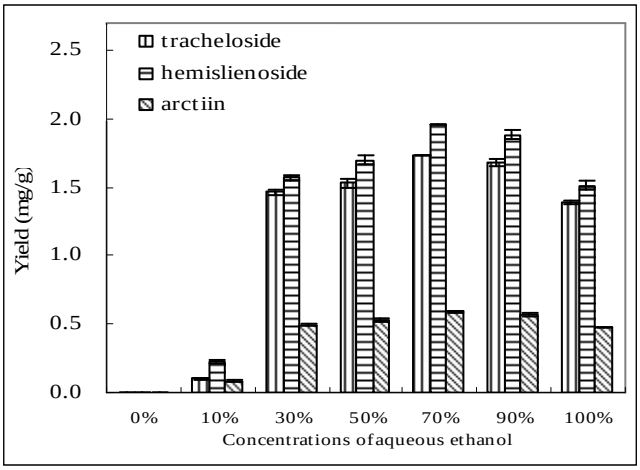


Fig 4: Effects of concentrations of aqueous ethanol on the extraction yields of tracheloside, hemislienoside, and arctiin. Other conditions were fixed at: sonication time duration, 30 min; extraction temperature, 30 ˚C; ultrasound frequency, 40 kHz; the ratio of solvent volume to sample mass, 30:1.

Effect of solvent to material ratio

The influence of the ratio of solvent to material on the extraction yields of tracheloside, hemislienoside, and arctiin from *H. lyrata* was evaluated. The extractions were performed with 70% aqueous ethanol at four different solvent to material ratio of 10, 20, 30, and 40 ml/g, respectively. The extraction time was 30 min, extraction temperature was 30˚C, and the frequency of ultrasound was 40 kHz. The data shown in Figure 5 indicated no significant differences of extraction yields of the objective constituents when the solvent to solid ratio was increased from 10 to 40 mg/g. A higher ratio of solvent to material will lead to excess work in the concentration process, causing the waste of solvent. For commercial application, a solvent to material ratio of 20 ml/g should be optimum to avoid waste of solvent and bulky handing in the subsequent processes.

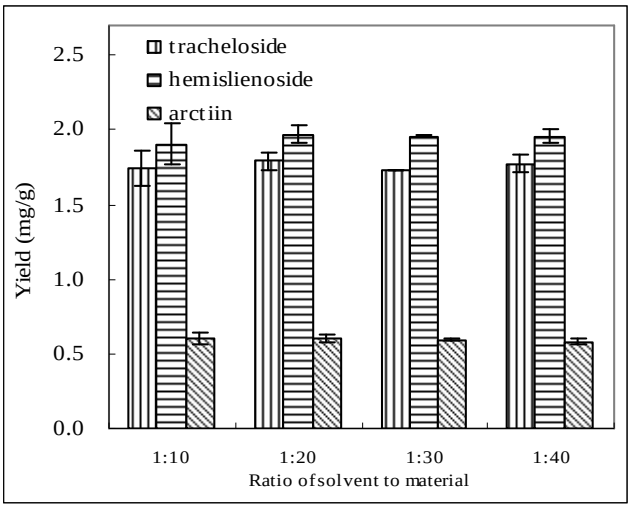


Fig 5: Effects of the ratio of solvent volume to sample mass on the extraction yields of tracheloside, hemislienoside, and arctiin. Other conditions were fixed at: extraction solvent, 70% aqueous ethanol; sonication time duration, 30 min; extraction temperature, 30 ˚C; ultrasound frequency, 40 kHz.

Effects of extraction time

Figure 6 shows the extraction results carried out under different time durations with fixed conditions of other factors, such as extraction solvent (70 % aqueous ethanol), extraction temperature (30 ˚C), ultrasound frequency (40 kHz). The results indicated that when extraction time increased from 5 to 20 min, the extraction yields of tracheloside, hemislienoside, and arctiin increased from 1.48 to 1.79, 1.56 to 1.97, 0.48 to 0.60 mg/g, respectively. After 20 min, the extraction yields decreased slowly. These results can be explained as the effects of acoustic cavitation and rupture of plant cells, which caused the intensification of mass transfer and thus closed interaction between the solvent and the plant tissues. Along with the increase of extraction time, all the plant cells will be completely cracked by acoustic cavitation effect, and the extraction yield will increase within a certain time duration. As the plant cells rupture, impurities such as insoluble substances, as well as cytosol and lipids suspend in the extraction liquid, resulting in the lower permeability of the solvent. Dissolved constituents will also re-adsorb on the smashed plant particles due to their relatively large specific surface areas lowering yields of recovered compounds. Hence, 20 min is suitable time duration for the extraction of tracheloside, hemislienoside, and arctiin from *H. lyrata.*

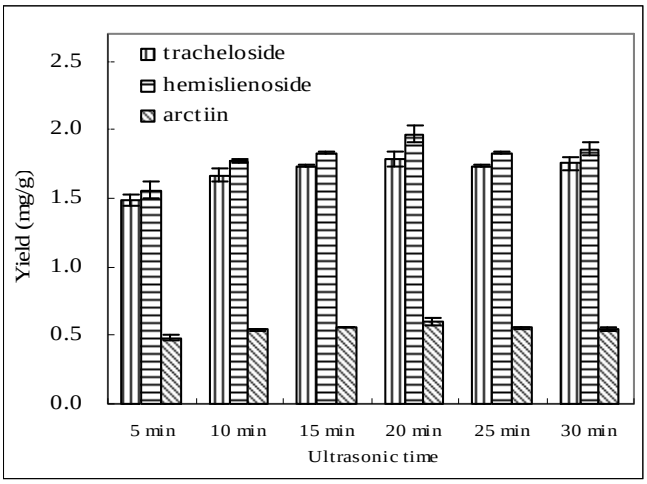


Fig 6: Effects of extraction time on the extraction yield of tracheloside, hemislienoside, and arctiin. Other conditions were fixed at: extraction solvent, 70% aqueous ethanol; extraction temperature, 30 ˚C; ultrasound frequency, 40 kHz; the ratio of solvent volume to sample mass, 20:1.

CONCLUSION

This study investigated the use of ultrasound-assisted extraction to extract three dibenzylbutyrolactone lignans, including tracheloside, hemislienoside, and arctiin from *Hemistepta lyrata*. The experiments suggest that the optimal parameters to extract the target compounds are as follows: extraction solvent: 70 % aqueous ethanol; solvent to material ratio: 20:1 (v/w, ml/g); extraction time: 20 min under the conditions: ultrasonic frequency: 40 Hz; extraction temperature: 30 ˚C. Higher yield of extraction can be achieved under lower temperature with shorter period of time when

ultrasound-assisted extraction method is adopted in the extraction of the target compounds from *H. lyrata*. The applicability of ultrasound-assisted extraction to the extraction of tracheloside, hemislienoside, and arctiin from other medicinal plants is also expected.

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