



Processing tactics for low-cost production of pure nuciferine from lotus leaf

Yeqing Ruan, Jiahuan Xu, Jianbo Chu, Jing Shi, Qiyuan Shi*

School of Pharmacy, Hangzhou Medical College, Hangzhou, Zhejiang, China

ARTICLE INFO

Keywords:

Nuciferine
Lotus leaf
Low-cost production
Ultrasonic-assisted extraction-solid phase extraction
Liquid-liquid extraction
Crystallization

ABSTRACT

Nuciferine is an important drug candidate for the treatment of many diseases. However, there is no general method for its low - cost production. In this work, a feasible method for the production of nuciferine from lotus leaf, using ultrasonic-assisted extraction-solid phase extraction (UAE-SPE) as extraction and cleanup procedure, was developed. Petroleum ether and silica gel have been successfully used as extraction solvent and adsorbent to integrate UAE with SPE, respectively. Except for filtration, no treatment (e.g. concentration and redissolution, etc) was needed on UAE extract before SPE and the effluents obtained in the loading process of SPE could be used as UAE extraction solvent without purification. No obvious decline in the extraction efficiency of UAE and adsorption capacity of SPE was observed at least for 5 runs, which provides a feasible way for the continuous production of nuciferine in industry, i.e. Cyclic UAE-SPE. Moreover, SPE column could be conveniently regenerated and reused without significant decline in its adsorption capacity at least for 5 cycles, which can be used to reduce the cost of the whole system further. In comparison with other cleanup procedures, Cyclic UAE-SPE showed apparent advantages in energy conservation and emission reduction. LLE and crystallization were applied to separate nuciferine from other impurities further. Under optimum conditions, the total recovery rate of nuciferine with a purity over 90.0% from lotus leaf reached 50.1%. All in all, the developed method has advantages in convenient operation, low cost, and high efficiency, thus, is fitting for the production of high purity nuciferine.

1. Introduction

Nuciferine, one of the most important isoquinoline alkaloids in lotus leaf, is effective in managing obesity [1–2], stimulating insulin secretion [3], inhibiting the growth of non-small-cell lung cancer cells [4], and ameliorating the clinical symptoms of chronic ulcerative colitis [5], etc.

In order to further study the pharmacological activities of nuciferine, a large amount of pure nuciferine is urgently needed. So far as we know, there are few reports on the synthesis of nuciferine. Percim et al. (2020) have reported a method [6] for the total synthesis of (*S*)- and (*R*)-nuciferine through unprecedented approach involving diastereoselective reaction between chiral auxiliary-based isoquinoline compound and 2-(tri-methylsilyl) phenyl trifluoromethanesulfonate, but with a high cost. As a result, separation from lotus leaf is still the most appropriate choice (although this is hard too). The preparation of pure nuciferine usually needs special equipment, e.g. preparative liquid chromatography [7] and countercurrent chromatography [8–9], which hinders their practical use in industries.

Crystallization is a fundamental separation technology used for the

production of bio-actives [10–11]. Due to its excellent performance in promoting process design and operation, crystallization is very important for the preparation of organic chemicals in the field of fine chemicals, pharmaceuticals, and food additives in the chemical industry [12]. As the amounts of impurities could influence the rate of crystal formation and the overall growth rate [13], impurities existing in the extract of lotus leaf (such as pigments, poly-phenols and polysaccharides, etc.), especially extracted by ethanol aqueous solution [14], ionic liquid [15], or acidic aqueous solution [16], should be removed as much as possible before crystallization [13].

The separation of nuciferine and its analogues from other components is easy, which can be achieved by liquid–liquid extraction (LLE) [17] for their high solubility in acidic solution and low solubility in basic solution [18], but it is not convenient to separate nuciferine from other alkaloids for their similarities in structure and physical properties [19–20].

Solid phase extraction (SPE) has been extensively developed as a conventional technique in sample preparation processes from liquid or solid matrices [21]. Cation exchange resin PCX [22] and Fe₃O₄-

* Corresponding author.

E-mail address: shiqiyuan@hmc.edu.cn (Q. Shi).

<https://doi.org/10.1016/j.ultsonch.2022.106026>

Received 4 February 2022; Received in revised form 19 April 2022; Accepted 3 May 2022

Available online 6 May 2022

1350-4177/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

graphene oxide nanocomposite (MGO) [23] have been successfully used as adsorbents in SPE for the enrichment of alkaloids from lotus leaf before analysis. In our previous study (data is not shown in this work), we found that silica gel, a conventional and cheap adsorbent, also has a good performance on the selective separation of nuciferine, which is especially suitable for low-cost production.

Ultrasonic assisted extraction (UAE) is a modern extraction technology, which uses the cavitation effect, mechanical vibration, and thermal effect produced by ultrasound to destroy the plant cell walls, so as to promote the diffusion of solvent and accelerate the dissolution of compounds [24]. This method has the advantages of convenient operation and high efficiency.

In this work, a simple and efficient method was proposed to produce hyper pure nuciferine from lotus leaf. It was a modification of a crystallization process from literature [25], where the most critical extraction and cleanup steps were replaced with UAE-SPE and LLE. Petroleum ether was used as extraction solvent in UAE and silica gel was used as adsorbent in SPE to integrate UAE with SPE in order to eliminate concentration and re-dissolution procedures.

2. Experimental

2.1. Materials and reagents

Lotus leaf was provided by Hangzhou Tongjuntang Co., Ltd. (Hangzhou, Zhejiang, China). It was ground and sieved. The powder with a particle size of 0.150 and 0.420 mm was used for experiments. Silica gel with a particle size of 0.075–0.150 mm was purchased from Qingdao Haiyang Chemical Co., Ltd. (Qingdao, Shandong, China). Nuciferine was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). HPLC-grade acetonitrile was purchased from Tedia Company Inc (Fairfield, OH, USA). Acetic acid and triethylamine were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Analytical ammonia solution, petroleum ether, ethyl acetate, and methanol were purchased from Sinopharm chemical reagent Co., Ltd. (Shanghai, China). Deionized water was prepared by a Milli-Q water-purification system from Milipore (Bedford, MA, USA).

2.2. UAE-SPE

2.2.1. Effect of ultrasound power on the UAE of nuciferine from lotus leaf

UAE of nuciferine was performed by using a 53 kHz frequency ultrasonic equipment (SK8200HP, Shanghai Kedao Ultrasonic Instrument Co., LTD, Shanghai, China) according to the method [26] described by Gao et al. (2020) with modification. To evaluate the effect of ultrasound power on the UAE of nuciferine, 1 g of lotus leaf powder was firstly wetted with 1 mL of 5% ammonia solution for 5 min and then extracted with 50 mL of petroleum ether for 30 min under the assistance of ultrasound with different power (200, 250, 300, 350, 400, 450, and 500 W), respectively. All of the extracts were separated by filtration and placed in a 50 mL volumetric flask and diluted with petroleum ether before HPLC analysis, respectively.

2.2.2. Effect of concentration of ammonia solution on the UAE of nuciferine from lotus leaf

To evaluate the effect of concentration of ammonia solution on the UAE of nuciferine, 1 g of lotus leaf powder was firstly wetted with 1 mL ammonia solution with different concentration (1%, 2%, 5%, 10%, and 25%, w/v) for 5 min and then extracted with 50 mL of petroleum ether for 30 min under the assistance of ultrasound (53 kHz, 500 W). The extracts were treated using the method mentioned in Section 2.2.1.

2.2.3. Effect of volume of 5% ammonia solution on the UAE of nuciferine from lotus leaf

To optimize the volume of 5% ammonia solution, 1 g of lotus leaf powder was firstly moistened with different volume of 5% ammonia

solution (0.1, 0.5, 1, 2, and 5 mL) for 5 min and then then extracted with 50 mL of petroleum ether for 30 min under the assistance of ultrasound (53 kHz, 500 W). The extracts were treated using the method mentioned in section 2.2.1.

2.2.4. Effect of volume of petroleum ether on the UAE of nuciferine from lotus leaf

Lotus leaf powder (1 g) was firstly wetted with 1 mL of 5% ammonia solution for 5 min and then extracted with different volume of petroleum ether (10, 25, 50, 75, and 100 mL) for 30 min under the assistance of ultrasound (53 kHz, 500 W). The extracts were separated by filtration and placed in a 50 or 100 mL volumetric flask and diluted with petroleum ether before HPLC analysis.

2.2.5. Effect of extraction time on the UAE of nuciferine from lotus leaf

Lotus leaf powder (1 g) was firstly wetted with 1 mL of 5% ammonia solution for 5 min and then extracted with 75 mL of petroleum ether for 5, 15, 30, 45, and 60 min under the assistance of ultrasound, respectively. The extracts were separated by filtration and placed in a 100 mL volumetric flask and diluted with petroleum ether before HPLC analysis.

2.2.6. Conventional extraction methods

A comparison study was performed between the proposed optimal UAE method and the conventional extraction techniques in order to estimate and validate the efficiency of ultrasound on the extraction of nuciferine according to the method described by Christou et al. (2021) [27] with minor modification. Briefly, for agitation-assisted extraction, 1 g of louts leaf powder was mixed with 1 g of 5% ammonia solution and 75 mL of petroleum ether and agitated at a moderate speed at room temperature for 15 min using a magnetic stirrer. The extract was separated by filtration in order to remove the insoluble particles. And then it was placed in a 100 mL volumetric flask and diluted with petroleum ether before HPLC analysis. For Soxhlet extraction, 6 g of louts leaf powder was extracted with 6 g of 5% ammonia solution and 450 mL of petroleum ether, refluxing in a Soxhlet apparatus for 6 h. The obtained extract was cooled to room temperature and placed in a 500 mL volumetric flask and diluted with petroleum ether before HPLC analysis.

2.2.7. Effect of volume of UAE extract on the adsorption of nuciferine on silica gel column

The UAE extract was prepared by using the method mentioned in section 2.2.5 and loaded onto a pre-packed silica gel column (2 g, 25 mm × 15 mm I.D.) at different flow rate (3, 6, and 40 mL/min). The effluents obtained in the loading process of SPE were collected manually (70 mL for each, roughly equivalent to 1 g of lotus leaf powder) and analyzed by HPLC.

2.2.8. Effect of elution solvent on the desorption of nuciferine from silica gel column

The SPE column (2 g, 25 mm × 15 mm I.D.) loaded with 910 mL of UAE extract was eluted with different elution solvent (the upper layer of petroleum/ethyl acetate/25% ammonia solution, 0.5/1/0.1, 1/1/0.1, 2/1/0.1, 3/1/0.1, 4/1/0.1, 5/1/0.1, v/v/v). The effluents obtained in the elution process were collected manually (5 mL for each) and placed in a 50 mL volumetric flask and diluted with methanol before HPLC analysis.

Y_{SPE} (%) is the recovery rate of nuciferine in SPE,

$$Y_{SPE} = M_O/M_F \times 100\%$$

where, M_O (mg) is the obtained amount of nuciferine, M_F (mg) is the feed amount of nuciferine in UAE extract.

2.2.9. Cyclic UAE-SPE

A comparison study was performed between Cyclic UAE-SPE and UAE-SPE according to the method described by Shi et al. (2015) [28]

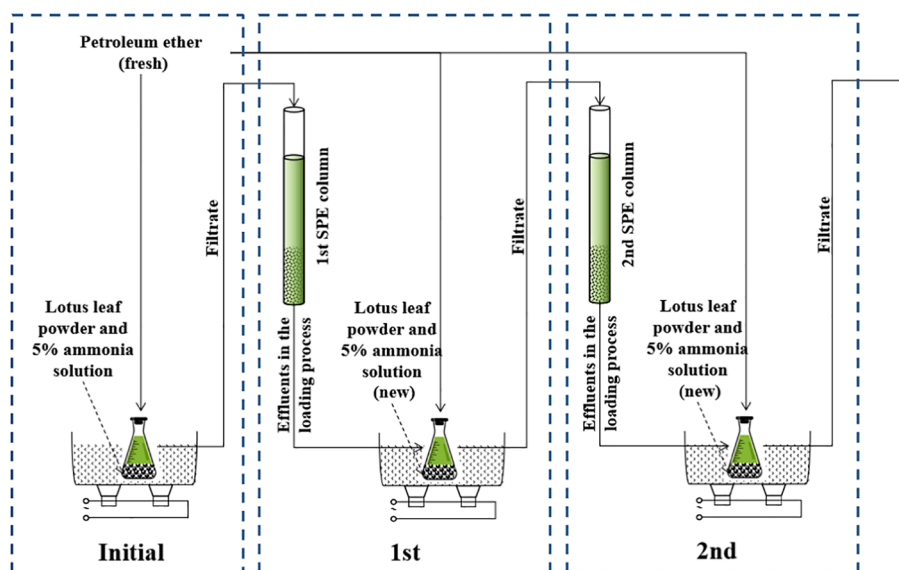


Fig. 1. Schematic picture of Cyclic UAE-SPE procedure.

with modification. Briefly, 910 mL of UAE extract was filtrated and loaded onto an SPE column pre-packed with 2 g of silica gel (25 mm × 15 mm I.D.) and the effluents obtained in the loading process of SPE were used as extraction solvent without any purification in the next UAE unit. Fresh petroleum ether was added in order to make up the loss. All of the above procedures were repeated for 5 times to validate the feasibility of Cyclic UAE-SPE. The contents of nuciferine in the UAE extract and effluents obtained in the loading process of SPE were monitored by HPLC method mentioned in Section 2.6. Fig. 1 shows the schematic picture of Cyclic UAE-SPE procedure.

In order to further reduce cost and protect environment, the regeneration of SPE column was also investigated. The silica gel column (2 g, 25 mm × 15 mm I.D.) was eluted with 50 mL of methanol and 50 mL of petroleum ether after loading with 910 mL of UAE extract, successively. It was then loaded with another 910 mL of UAE extract and the effluents obtained in the loading process of SPE were analyzed by HPLC method mentioned in Section 2.6. All of the above procedures were repeated for 5 times to validate the reusability of silica gel for adsorption.

2.3. LLE

325 g of Lotus leaf powder was treated using the method mentioned in Section 2.2.5 and the obtained UAE extract was loaded onto a pre-packed silica gel column (50 g, 80 mm × 48 mm I.D.) with a flow rate of 40 mL/min. The SPE column was then eluted with elution solvent (the upper layer of petroleum/ethyl acetate/25% ammonia solution, 1/1/0.1, v/v/v). The fractions were collected manually (75 mL for each), combined according to the results of HPLC analysis.

LLE of nuciferine from UAE-SPE extract was performed according to the method described by Ghang et al. (2016) [29] with modification. Briefly, the mixture was shaken on a vortex mixer (Vortex-2500MT, Shanghai Lichen-BX Instrument Technology, Co., Ltd, Shanghai, China) at a speed of 2000 rpm for 1 min and then centrifuged (TGL-16G-A, Shanghai Anting Scientific Instrument Factory, Shanghai, China) for 10 min at 10,000 rpm. Those parameters that could affect the LLE were optimized.

Y_{LLE} (%) is the recovery rate of nuciferine in LLE,

$$Y_{LLE}(\%) = M_O/M_F \times 100\%.$$

Where, M_O (mg) is the obtained amount of nuciferine in aqueous layer (in Sections 2.3.1 and 2.3.2) or organic layer (in Sections 2.3.3 and 2.3.4), M_F (mg) is the feed amount of nuciferine in UAE-SPE extract.

2.3.1. Effect of concentration of HCl solution

To investigate the effect of concentration of HCl solution on the extraction of nuciferine from organic layer into aqueous layer, 1 mL of the UAE-SPE extract was vortex-mixed with 1 mL HCl solution with different concentration (0.0001, 0.001, 0.010, 0.020, 0.050, 0.100, and 1.000 M). All of the aqueous layers were placed in a 10 mL volumetric flask and diluted with water before HPLC analysis, respectively.

2.3.2. Effect of ratio of 0.01 M HCl solution to UAE-SPE extract

1 mL of the UAE-SPE extract was vortex-mixed with different volume of 0.01 M HCl solution (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL). The aqueous layers were treated using the method mentioned in Section 2.3.1.

2.3.3. Effect of ratio of 0.1 M NaOH solution to UAE-SPE extract

1 mL of the UAE-SPE extract was vortex-mixed with 1 mL of 0.01 M HCl solution and the down layer was vortex-mixed with different volume of 0.1 M NaOH solution (0.025, 0.050, 0.100, 0.150, 0.200, 0.250, and 0.500 mL) and 1 mL of petroleum ether. The organic layers were collected and placed in a 10 mL volumetric flask and diluted with methanol before HPLC analysis, respectively.

2.3.4. Effect of ratio of petroleum ether to UAE-SPE extract

1 mL of the UAE-SPE extract was vortex-mixed with 1 mL of 0.01 M HCl solution and the aqueous layer was vortex-mixed with 0.2 mL of 0.1 M NaOH solution and different volume of petroleum ether (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL). The organic layers were treated using the method mentioned in Section 2.3.3.

2.4. Crystallization

The UAE-SPE extract was extracted with the same volume of 0.01 M HCl solution. And the aqueous layer was separated and mixed with 0.1 M NaOH solution (20% of the volume of UAE-SPE extract) and petroleum ether (2 times of UAE-SPE extract volume). And then the organic layer was collected and evaporated to dryness under reduced pressure (10.1 kPa) at 40 °C.

An appropriate amount of the obtained residue was dissolved in 50 mL of boiling solvent (petroleum ether, ethyl acetate, and methanol) to prepare a saturated solution. Then, the solution was filtered quickly and cooled to room temperature (20 °C). The crystal was filtered from mother liquor with a membrane of 0.45 μm after standing for 48 h, and then evaporated to dryness under reduced pressure (10.1 kPa) at 40 °C.

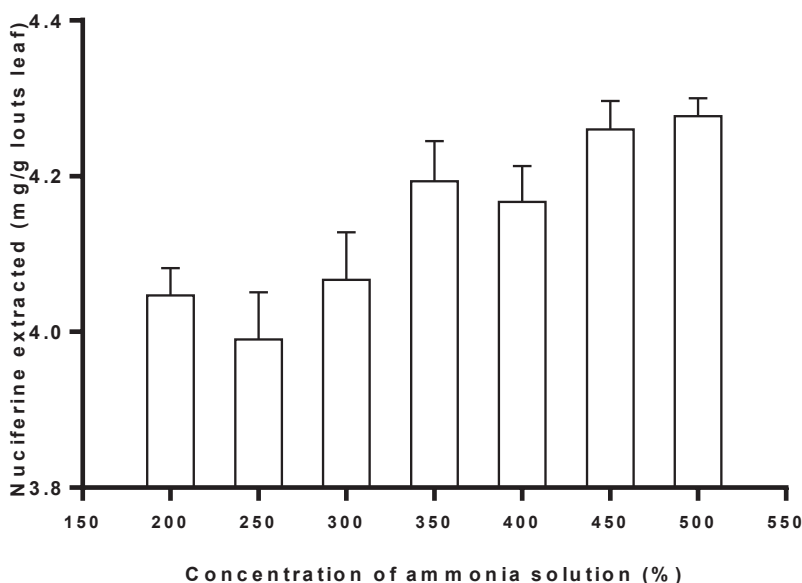


Fig. 2. Influence of ultrasound power on the extraction of nuciferine from lotus leaf.

to avoid unnecessary loss, as it was reported that nuciferine is unstable in the process of concentration and drying even at 60 °C [30]. The mother liquor was placed in a 500 mL volumetric flask and diluted with methanol before HPLC analysis. The purity of nuciferine was calculated by area normalization method.

Y_{CRY} (%) is the recovery rate of nuciferine in crystallization,

$$Y_{CRY}(\%) = M_O / (M_O + M_M) \times 100\%.$$

where, M_O (mg) is the amount of nuciferine in the obtained crystal, M_S (mg) is the amount of nuciferine in the mother liquor.

2.5. Characterization of purified nuciferine

The chemical structure of purified nuciferine was confirmed by mass spectrometry and NMR spectroscopy. Mass spectra was recorded on a UPLC-TOF MS/MS system (Waters Corporation, Milford, MA, USA). An ACQUITY UPLC BEH C_{18} column (50 mm \times 2.1 mm I.D., 1.7 μ m,

Waters Corporation, Milford, MA, USA) with a column temperature of 35 °C and an injection volume of 1.0 μ L was used. The mobile phase consisted of 2 mM ammonium acetate (A) and acetonitrile (B) was carried with linear elution gradient as follows: 0.00–0.50 min, 10% B; 0.50–2.50 min, 10%–90% B; 2.50–3.50 min, 90% B; 3.50–3.51 min, 90%–10% B; 3.51–4.50 min, 10% B, which was delivered at a flow rate of 0.3 mL/min. The mass detector was equipped with an ESI and operated in positive ionization mode. The conditions of TOF MS/MS were: Capillary, 3.0 kV; Sampling Cone, 40 V; Source Temp, 140 °C; Desolvation Temp, 500 °C; Cone Gas, 50 L/h; Desolvation Gas: 800 L/h; Collision Energy, 10–25 eV; scan range, 50–1200 m/z . The acquisition and analysis of data were controlled by Waters UNIFI software. 1H NMR and ^{13}C NMR spectra (in $CDCl_3$) were recorded at 500 and 125 MHz (Bruker Daltonic Inc., Karlsruhe, GER), respectively. Chemical shifts (δ) were reported in ppm relative to TMS and coupling constants (J) in Hz.

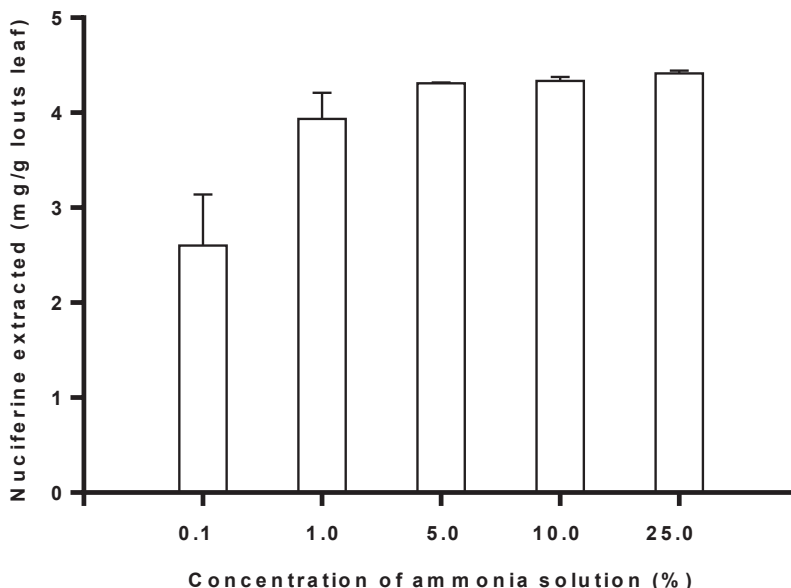


Fig. 3. Influence of concentration of ammonia solution on the extraction of nuciferine from lotus leaf.

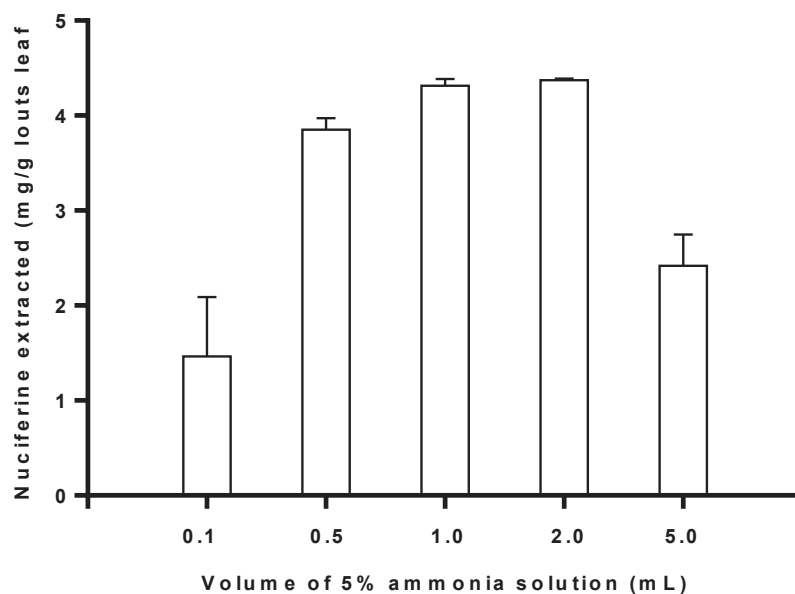


Fig. 4. Effect of volume of 5% ammonia solution on the extraction of nuciferine from lotus leaf.

2.6. Analytical HPLC

Samples were analyzed using an Agilent 1260 HPLC system. It was performed on an Agilent Poroshell 120 EC Xbridge C_{18} column (100 mm \times 4.6 mm I.D., 4 μ m, Agilent Technologies, Palo Alto, CA, USA). The mobile phase consisted of water – acetonitrile – triethylamine – acetic acid solution (70.6/27/1.6/0.78, v/v/v/v) (A) and water (B) using the following gradient elution program for separation: 0–10 min, 75% A; 10–15 min, 75%–100% A; 15–17 min, 100% A. The flow rate was kept at 1.0 mL/min. The column temperature was 35 $^{\circ}$ C and the injection volume was 5.0 μ L. The effluents were monitored by a UV–Vis detector at 270 nm. All of the samples were centrifuged at a speed of 13,000 rpm prior to injection. Quantitative analysis of nuciferine was performed using the peak area based on the standard curve.

2.7. Statistical analysis

All experiments were performed in triplicated unless otherwise indicated. Data is expressed as mean value \pm S.D.

3. Results and discussion

3.1. UAE-SPE

3.1.1. Effect of ultrasound power on the UAE of nuciferine from lotus leaf

The optimization of ultrasound power was achieved by comparing the extraction efficiency at the condition of 200, 250, 300, 350, 400, 450, and 500 W. The experimental results show that the extraction yield increases with the increase of ultrasonic power (Fig. 2). This is in agreement with the report of Shirsath et al. (2013) [31], which also showed a higher recovery rate with increasing acoustic power. As a result, 500 W was recommended for the UAE of nuciferine from lotus

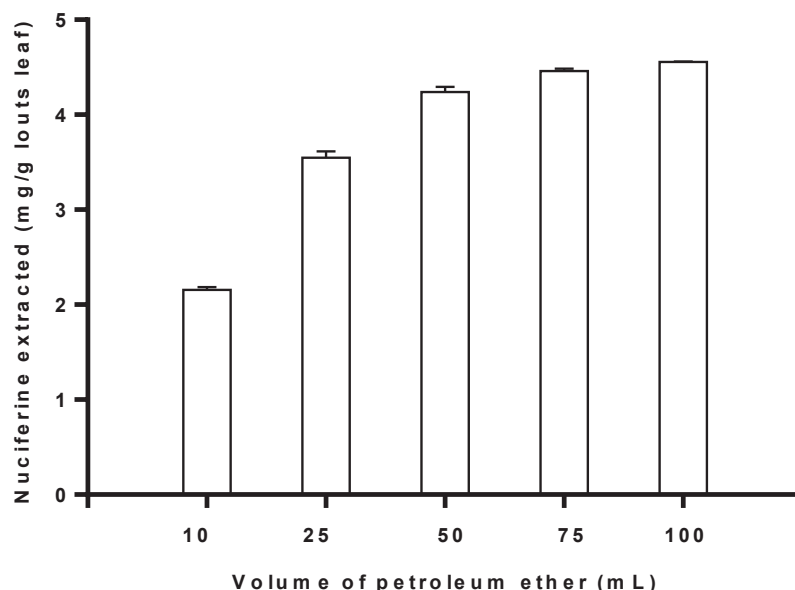


Fig. 5. Effect of volume of petroleum ether on the extraction of nuciferine from lotus leaf.

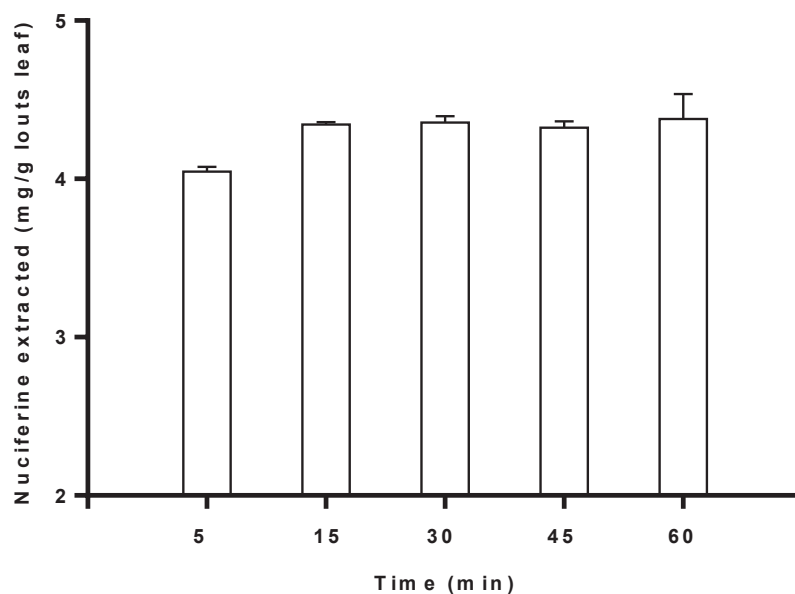


Fig. 6. Effect of extraction time on the extraction of nuciferine from lotus leaf.

leaf.

3.1.2. Effect of concentration of ammonia solution on the UAE of nuciferine from lotus leaf

Since nuciferine contains a basic group, the extent of ionization of nuciferine could be influenced by the pH value of the solvent, subsequently their solubility in solvent could also be affected. Wetting with ammonia solution is a commonly used procedure to inhibit ionization in the extraction of alkaloids [32]. As the alkaloid of interest is a lipophilic compound, petroleum ether, a non-polar solvent, was used as extraction solvent [33]. As shown in Fig. 3, the concentration of ammonia solution plays an important role in the extraction of nuciferine. The yield of nuciferine increased when the concentration of ammonia solution increased from 0.1% to 5% and maintained at a stable level when the concentration increased from 5% to 25%. In order to reduce the composition of ammonia, 5% ammonia solution was used in the following experiments.

3.1.3. Effect of volume of ammonia solution on the UAE of nuciferine from lotus leaf

To evaluate the consumption of 5% ammonia solution on the extraction of nuciferine, different volume of 5% ammonia solution was added to lotus leaf powder. As shown in Fig. 4, the yield of nuciferine increases with the increase of volume of 5% ammonia solution. But, it does not mean the more the better. If the volume of 5% ammonia solution was too large, it would have a negative effect on the extraction of nuciferine. The traditional theory, “like dissolves like”, can not explain this abnormal phenomenon [34]. It has been reported that a gradient of water-rich transition layers could be formed between the polar bonded phase and the poorly polar bulk mobile phase in hydrophilic interaction liquid chromatography (HILIC) acting as HILIC stationary phases to retain polar solutes [35]. Inspired by this, it was proposed that the thickness of aqueous liquid film formed on the surface of raw materials played an important role in the diffusion of nuciferine from raw materials or aqueous phase to petroleum ether. This hypothesis was confirmed by stationary extraction for another 16 h after UAE. There was no obvious difference between 1, 2, and 5 mL groups (data is not shown in this work). In order to reduce the composition of ammonia as much as possible, 1 mL of 5% ammonia solution was used in the following experiments.

3.1.4. Effect of volume of petroleum ether on the UAE of nuciferine from lotus leaf

Except for the concentration and volume of ammonia solution, UAE process could be influenced by liquid-to-solid ratio, extraction time [36], etc. The consumption of petroleum ether is one of the most crucial factors that should be investigated. As shown in Fig. 5, with the increase of petroleum ether, the extraction yield increased to 4.46 ± 0.03 mg/g with a consumption of 75 mL of petroleum ether and maintained at a stable level. This liquid-to-solid ratio is much higher than that reported in literature [37] using ethanol solution as extraction solvent. Traditionally, so much solvent consumption may cause inconveniences, leading to more energy consumption and solvent loss in the following concentration process. But, as concentration step was not needed before SPE in our method and the effluents obtained in the loading step of SPE could be reused as extraction solvent without any purification (data is shown in Section 3.1.9), such solvent consumption was still acceptable.

3.1.5. Effect of extraction time on the UAE of nuciferine from lotus leaf

To optimize extraction time, experiments were carried out using 75 mL of petroleum ether as extraction solvent. As shown in Fig. 6, when the extraction time increased from 5 to 15 min, the extraction efficiency increased dramatically. When the variable was changed from 15 to 60 min, slight improvements were observed. Therefore, 15 min was selected for further experiments.

3.1.6. Comparison of the optimized UAE extraction procedure with conventional ones

The effect of ultrasound was investigated by comparing the extraction yield of nuciferine from lotus leaf powder using optimized UAE method (4.37 ± 0.06 mg/g) with the one obtained by using agitation-assisted extraction method (3.59 ± 0.29 mg/g) performed under the same experimental conditions, but without the presence of ultrasound. The extraction yield of nuciferine was greatly enhanced by the use of ultrasonic waves (more than 20%).

Recovery obtained by the Soxhlet approach (2.38 ± 0.36 mg/g), demonstrated the inadequacy of the Soxhlet extraction method. Taking into consideration of the duration of the process and the extremely low levels of nuciferine obtained, it was concluded that Soxhlet extraction is not an appropriate method for nuciferine extraction.

The results of the experiment are consistent with other studies [27,38]. Increased mass transfer and significant disruption of cell walls

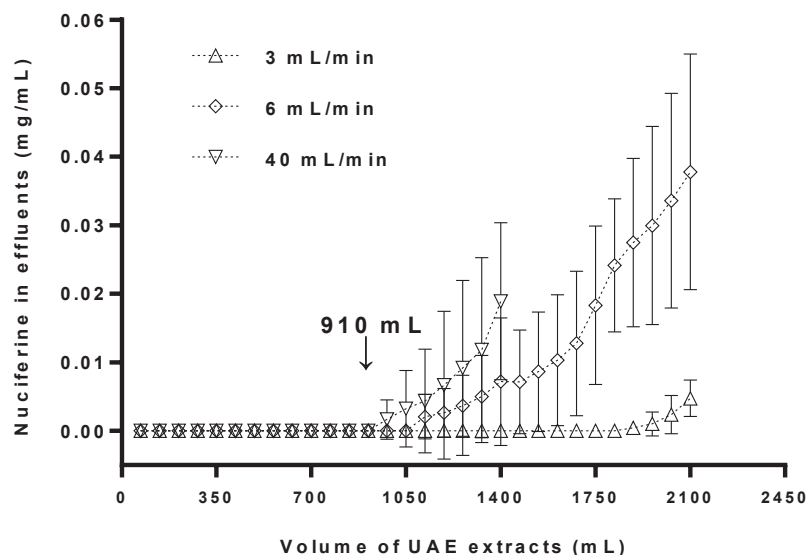


Fig. 7. Effect of volume of UAE extract on the adsorption of nuciferine on SPE column.

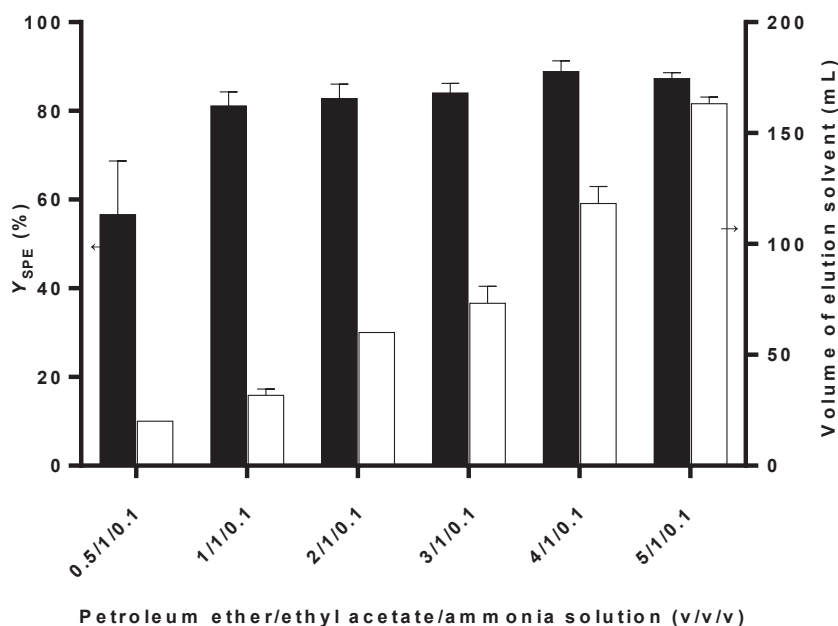


Fig. 8. Effect of solution solvent on the desorption of nuciferine from SPE column.

come as a result of these combined effects, offering higher extraction yields and significantly reduced processing times in comparison to the conventional techniques [39]. In addition, when ultrasound is applied to our solid – liquid – liquid extraction system, an emulsion of petroleum ether in 5% ammonia solution is created. This formation of emulsion leads to increased interfacial areas between the two immiscible phases resulting in an enhanced mass transfer between the phases [40].

3.1.7. Effect of volume of UAE extract on the adsorption of nuciferine on silica gel column

UAE extract was continuously loaded onto the silica gel column. Fig. 7 indicated that the flow rate played an important role in the adsorption of nuciferine. More time is needed for nuciferine to enter into silica gel to interact with internal silanol groups when external ones were saturated in the final stage of loading process [41]. Low flow rate (3 mL/min) is more favorable for adsorption. But it may also take more time to complete the whole loading process. In order to ensure

adsorption efficiency [42] and reduce irreversible adsorption as much as possible, 910 mL of extract (equivalent to 13 g of lotus leaf) loaded at a flow rate of 40 mL/min was chosen as the optimal one.

3.1.8. Effect of elution solvent on the desorption of nuciferine from silica gel column

The aim of the elution process is to reduce the occurrence of specific interactions among the adsorbents and targeted molecules, which depends on the pH value [43] and the polarity of elution solvent, and concurrently, to eliminate the contaminants that may interfere the crystallization of nuciferine. As a result, ammonia solution was added to surmount the strong interactions between silica gel and alkaloids. And the polarity of elution solvent was changed by adjusting the ratio of petroleum ether to ethyl acetate. The fractions were combined according to the results of HPLC analysis. With the increase of the ratio of petroleum ether to ethyl acetate from 0.5/1 to 5/1, not only the yield of nuciferine, but also the consumption of elution solvent increased

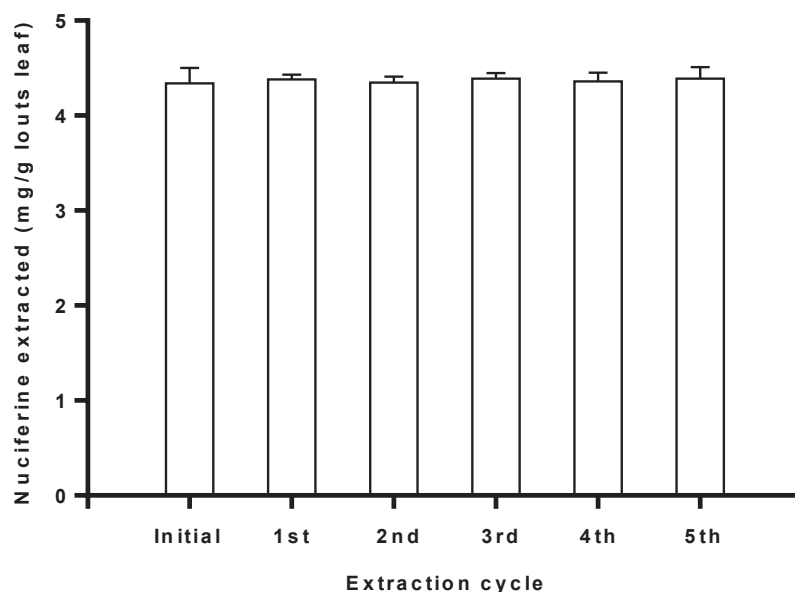


Fig. 9. Effect of cycle times on the extraction efficiency of UAE.

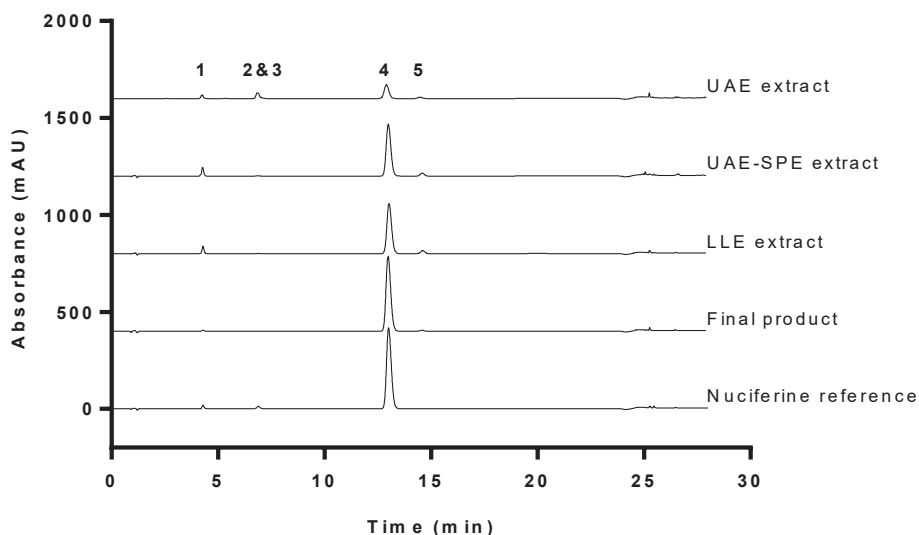


Fig. 10. Chromatogram of the extracts of lotus leaf and the reference solution of nuciferine. Peaks 1–5 in the profile were O-nornuciferine, N-methylnuciferine, N-nornuciferine, nuciferine, and unknown alkaloid, respectively.

(Fig. 8). In order to reduce the solvent consumption, the upper layer of petroleum ether/ethyl acetate/ammonia solution (1/1/0/1, v/v/v) was chosen as the optimal one.

3.1.9. Cyclic UAE-SPE

The results in Fig. 9 showed that the extraction yield of nuciferine from lotus leaf powder using effluents obtained in the loading process of SPE as extraction solvent remained about 100% of its initial after 5 cycles, needing no extra purification. No nuciferine was detected in the above effluents, which demonstrated that the impurities existed in those effluents almost have no effect on the adsorption of nuciferine on silica gel.

In addition, silica gel could be easily regenerated by washing with methanol and petroleum ether successively, which demonstrated that silica gel presented good reusability over several adsorption–desorption cycles.

To summarise, no treatment (e.g. concentration and redissolution, etc) on UAE extract was needed before SPE except for filtration and no

obvious decline in the extraction and adsorption efficiency was observed in Cyclic UAE-SPE (5 runs). That is, Cyclic UAE-SPE showed apparent advantages in energy conservation and emission reduction in comparison with other cleanup procedures, which provides a feasible way for the continuous production of nuciferine in industry. Moreover, SPE column could be conveniently regenerated and reused, which can further reduce the system cost. In comparison with other cleanup procedures, Cyclic UAE-SPE showed apparent advantages in energy conservation and emission reduction.

3.2. LLE

Although no apparent difference was observed among the chromatograms of UAE-SPE and LLE extracts (shown in Fig. 10), the cleanup effect of LLE on pigment was quite obvious. The color of the extract changed from dark green to light yellowish-green. As a result, the influence of factors on the LLE of nuciferine from UAE-SPE extract were investigated in this section.

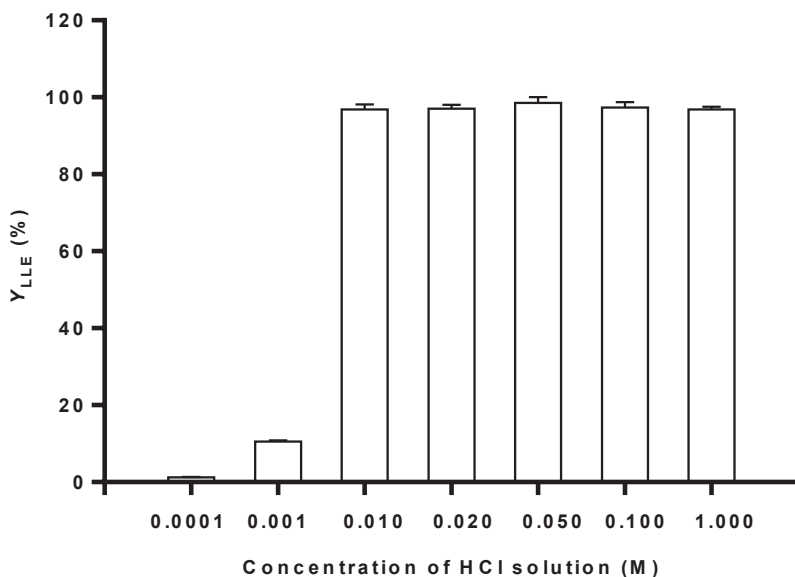


Fig. 11. Effect of concentration of HCl solution on the LLE of nuciferine.

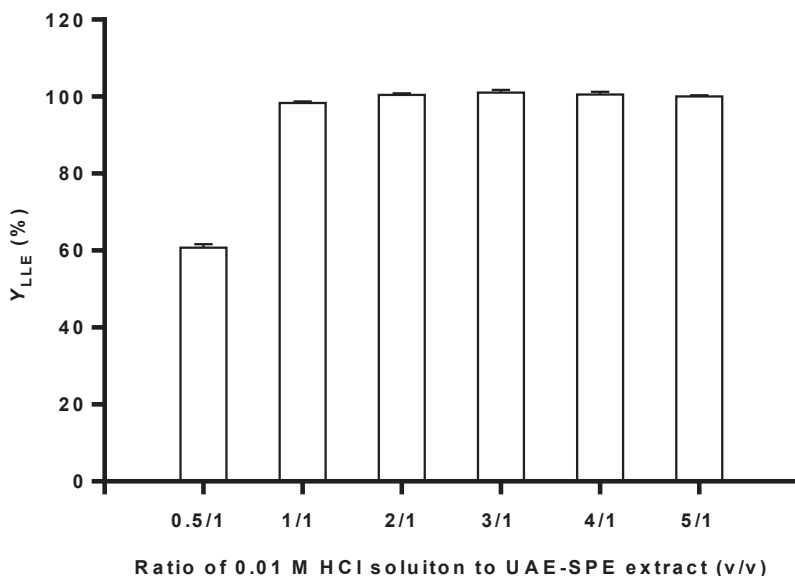


Fig. 12. Effect of ratio of 0.01 M HCl solution to UAE-SPE extract on the LLE of nuciferine.

3.2.1. Effect of concentration of HCl solution

As shown in Fig. 11, the concentration of HCl solution played an important role in the recovery of nuciferine. The recovery rate increased when the concentration increased from 0.0001 to 0.01 M and maintained at a stable level when the concentration increased from 0.01 to 1.00 M. In order to reduce the composition of HCl, 0.01 M HCl solution was used in the following experiments.

3.2.2. Effect of ratio of 0.01 M HCl solution to UAE-SPE extract

To evaluate the effect of ratio of 0.01 M HCl solution to UAE-SPE extract on the LLE of nuciferine, different volume of 0.01 M HCl solution was added to 1 mL of UAE-SPE extract. As shown in Fig. 12, with the increase of volume of 0.01 M HCl solution from 0.5 to 1 mL, the recovery rate increased to $98.3\% \pm 0.3\%$ and maintained at a stable level. As a result, a ratio of 1/1 between 0.01 M HCl solution to UAE-SPE extract was used in the following experiments with a pH value of 2.26 in aqueous layer.

3.2.3. Effect of ratio of 0.1 M NaOH solution to UAE-SPE extract

To investigate the effect of the ratio of NaOH solution (to adjust pH value) to UAE-SPE extract on the LLE of nuciferine, different volume of 0.1 M NaOH solution was added to the aqueous layer prepared by the method mentioned in Section 3.2.2. With the addition of NaOH solution, the color of aqueous layer changed from light yellowish-green to milky white. Fig. 13 shows that the recovery of nuciferine increases with the increase of the volume of 0.1 M NaOH from 0.025 to 0.2 mL. However, further increase leads to a decrease in extraction efficiency. We found that there was still some white flocculent precipitate existing in the aqueous layer after liquid-liquid extraction by 1 mL of petroleum ether if too much NaOH solution was added. It was proposed that tiny intact grains of nuciferine might be preserved within the precipitate formed by impurities which is hard to extract. According to the results, a ratio of 0.2/1 between 0.1 M NaOH solution and UAE-SPE extract was chosen as the optimal one. It is worth mentioning that in the presence of 0.2 mL of 0.1 M NaOH, the pH value of aqueous layer was 6.96.

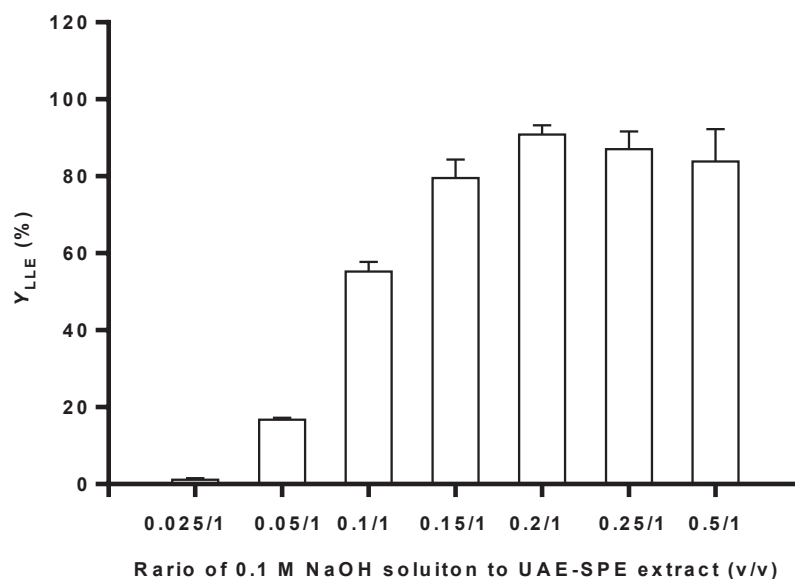


Fig. 13. Effect of ratio of 0.1 M NaOH solution to UAE-SPE extract on the LLE of nuciferine.

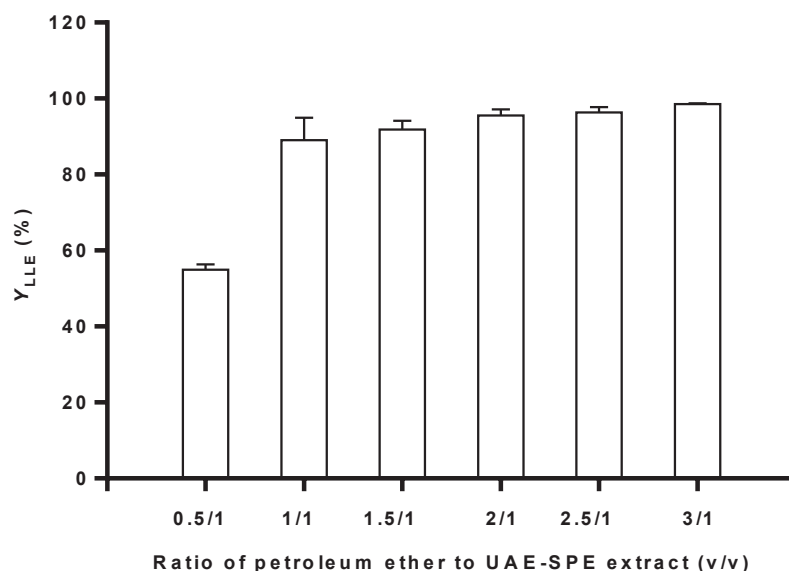


Fig. 14. Effect of ratio of petroleum ether to UAE-SPE extract on the LLE of nuciferine.

3.2.4. Effect of ratio of petroleum ether to UAE-SPE extract

In order to study the effect of the ratio of petroleum ether to UAE-SPE extract on the LLE of nuciferine, different volume of petroleum ether was tested. According to Fig. 14 and considering the experimental errors on the data points, the extraction recovery of nuciferine was found to increase to $95.5\% \pm 1.7\%$ with a ratio of 2/1 between petroleum ether and UAE-SPE extract and maintained at a stable level. Thus, 2/1 was considered as the optimal one.

3.3. Crystallization

Solvent cooling crystallization method is a common and useful method for separation and purification [44]. It has been used by Wei and Xiao [25] in the refining of nuciferine. To our knowledge, there is no report on the evaluation of the influence of different solvents on the crystallization of nuciferine. In this work, the effect of different solvents on the cooling crystallization of nuciferine was investigated. It was found that the solubility of nuciferine increased with the increase of

Table 1

Effect of different solvents on the crystallization of nuciferine.

Solvents	Recovery rate (%)	Purity (%)
Methanol	76.7 ± 1.7	98.5 ± 0.2
Ethyl acetate	67.9 ± 1.0	99.1 ± 0.1
Petroleum ether	80.2 ± 0.7	98.5 ± 0.1

temperature as expected [44]. Petroleum ether had the highest recovery rate, while ethyl acetate had the highest purity (see Table 1). Methanol's performance was less than stellar. This, coupled with its toxicity, made us not considering using methanol. We prefer to use ethyl acetate for its lower solvent consumption (accounts for about 14% of the volume of solvent used in crystallization using petroleum ether as solvent) and higher solvent selectivity [45].

Under optimum conditions, the total recovery rate of pure nuciferine (purity over 99.0%) from lotus leaf reached 50.1%. The developed method has advantages in convenient operation, low cost, and high

efficiency, thus, is fitting for the production of high purity nuciferine.

3.4. Characterization of purified nuciferine

Nuciferine (C₁₉H₂₁NO₂) showed [M + H]⁺ ion at m/z 296.1621 (calculated 296.1651). ¹H NMR (500 MHz, CDCl₃): δ 8.36 (d, J = 7.8 Hz, 1H), 7.33–7.20 (m, 3H), 6.63 (s, 1H), 3.88 (s, 3H), 3.65 (s, 3H), 3.20–2.98 (m, 4H), 2.54 (s, 3H), 2.72–2.46 (m, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 152.0, 145.2, 136.5, 132.2, 128.7, 128.3, 127.9, 127.3, 127.0, 126.9, 111.3, 62.4, 60.2, 55.9, 53.3, 44.0, 35.2, 29.3. The mass and NMR spectrum Data is listed in the [supplementary data](#). All of the spectral data is consistent with those reported in literature [46].

4. Conclusions

In this study, an efficient method was developed to extract and purify nuciferine (over 99.0%) from lotus leaf (with a total recovery of 50.1%), which is particularly proper for low - cost production of nuciferine. Integrated extraction and purification procedure, UAE-SPE, was applied to reduce the cost. Except for filtration, no treatment (e.g. concentration and redissolution, etc) was needed on UAE extract before SPE. No obvious decline in efficiency was observed in the use of effluents obtained in the loading process of SPE directly as UAE extraction solvent. As a result, UAE-SPE could be integrated with other UAE-SPE units to form a cyclic UAE-SPE to minimize the production cost of the whole system. Silica gel was considered as a very suitable SPE adsorbent not only for its excellent performance in the enrichment of nuciferine but also for its reusability.

CRedit authorship contribution statement

Yeqing Ruan: Investigation, Writing – original draft. **Jiahuan Xu:** Investigation. **Jianbo Chu:** Validation, Data curation. **Jing Shi:** Funding acquisition, Writing – review & editing. **Qiyuan Shi:** Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project was supported by the Natural Science Foundation of Zhejiang Province (LY20H280002), “13th Five-Year” Chinese Medicine Key Discipline in Zhejiang Province-Chinese Medicine Quality and functional evaluation (2017-XK-A43) and Provincial first-class (B class) discipline project-Pharmacy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ultsonch.2022.106026>.

References

- [1] F. Guo, Y. Xue, X. Li, R. Feng, C. Guan, Y. Wang, Y. Li, C. Sun, M.A. Avila, Nuciferine prevents hepatic steatosis and injury induced by a high-fat diet in hamsters, *PLoS One* 8 (2013), e63770, <https://doi.org/10.1371/journal.pone.006377>.
- [2] Y. Wan, J. Xia, J.-F. Xu, L.u. Chen, Y.u. Yang, J.-J. Wu, F. Tang, H. Ao, C. Peng, Nuciferine, an active ingredient derived from lotus leaf, lights up the way for the potential treatment of obesity and obesity-related diseases, *Pharmacol. Res.* 175 (2022) 106002, <https://doi.org/10.1016/j.phrs.2021.106002>.
- [3] K.H. Nguyen, T.N. Ta, T.H.M. Pham, Q.T. Nguyen, H.D. Pham, S. Mishra, B.L. G. Nyomba, Nuciferine stimulates insulin secretion from beta cells-An in vitro comparison with glibenclamide, *J. Ethnopharmacol.* 142 (2) (2012) 488–495, <https://doi.org/10.1016/j.jep.2012.05.024>.
- [4] W. Liu, D.D. Yi, J.L. Guo, Z.X. Xiang, L.F. Deng, L. He, Nuciferine extracted from *Nelumbo nucifera* Gaertn inhibits tumor-promoting effect of nicotine involving Wnt/β-catenin signaling in non-small cell lung cancer, *J. Ethnopharmacol.* 165 (2015) 83–93, <https://doi.org/10.1016/j.jep.2015.02.015>.
- [5] C. Li, J. Wang, R. Ma, L. Li, W. Wu, D. Cai, Q. Lu, Natural-derived alkaloids exhibit great potential in the treatment of ulcerative colitis, *Pharmacol. Res.* 175 (2022) 105972, <https://doi.org/10.1016/j.phrs.2021.105972>.
- [6] G.P. Perecim, V.M. Deflon, G.R. Martins, L.M.C. Pinto, G.A. Casagrande, D. Oliveira-Silva, C. Raminelli, Stereoselective total synthesis of (S)- and (R)-nuciferine using benzyne chemistry, *Tetrahedron* 76 (38) (2020) 131461, <https://doi.org/10.1016/j.tet.2020.131461>.
- [7] J.J. Liu, B.X. Luo, B. Chen, S.Z. Yao, Isolation and purification of nuciferine by preparative HPLC, *Chin. Tradit. Herbal Drugs* 37 (2006) 55–57.
- [8] Z.J. Zheng, M.L. Wang, D.J. Wang, W.J. Duan, X. Wang, C.C. Zheng, Preparative separation of alkaloids from *Nelumbo nucifera* leaf by conventional and pH-zone-refining counter-current chromatography, *J. Chromatogr. B* 878 (2010) 1647–1651, <https://doi.org/10.1016/j.jchromb.2010.04.020>.
- [9] Y.T. Fang, Q. Li, Q. Shao, B.H. Wang, Y. Wei, A general ionic liquid pH-zone-refining countercurrent chromatography method for separation of alkaloids from *Nelumbo nucifera* Gaertn, *J. Chromatogr. A* 1507 (2017) 63–71, <https://doi.org/10.1016/j.chroma.2017.05.048>.
- [10] X.B. Jiang, Y.C. Niu, S.F. Du, G.H. He, Membrane crystallization: Engineering the crystallization via microscale interfacial technology, *Chem. Eng. Res. Des.* 178 (2022) 454–465, <https://doi.org/10.1016/j.cherd.2021.12.042>.
- [11] X. Jiang, Y. Shao, L. Sheng, P. Li, G. He, Membrane crystallization for process intensification and control: A review, *Engineering-PRC* 7 (1) (2021) 50–62, <https://doi.org/10.1016/j.eng.2020.06.024>.
- [12] X. Zhao, H. Gao, Y. Hou, L. Gbolagoh, X. Zeng, Y. Wang, Analysis of crystallization and deposition process using electrochemical-quartz crystal microbalance: A review, *J. Electroanal. Chem.* 904 (2022) 115936, <https://doi.org/10.1016/j.jelechem.2021.115936>.
- [13] A. Markande, A. Nezzal, J. Fitzpatrick, L. Aerts, A. Redl, Influence of impurities on the crystallization of dextrose monohydrate, *J. Cryst. Growth* 353 (1) (2012) 145–151, <https://doi.org/10.1016/j.jcrysgro.2012.04.021>.
- [14] Y.X. Wang, B. Liu, Y.Y. Jiang, Extraction and purification processes of alkaloids in Heye (*Folium Nelumbinis*), *J. Beijing Univ. Tradit. Chin. Med.* 9 (2013) 622–626.
- [15] W. Ma, Y. Lu, R. Hu, J. Chen, Z. Zhang, Y. Pan, Application of ionic liquids based microwave-assisted extraction of three alkaloids N-nornuciferine, O-nornuciferine, and nuciferine from lotus leaf, *Talanta* 80 (3) (2010) 1292–1297, <https://doi.org/10.1016/j.talanta.2009.09.027>.
- [16] X.L. Zhang, Y. Zhou, X.F. Xiong, Y.J. Wu, L.J. Luan, Process optimization for extraction of procyandinids and nuciferine from the lotus leaf, *Sci. Technol. Food Indus.* 23 (2012) 295–298.
- [17] M.X. Lin, G.R. Lin, J.F. Chen, Y.H. Guo, Preparation of high purity nuciferine from lotus leaf and study on its pharmaceutical efficiency, *J. Fuzhou Univ. (natural science edition)* 36 (2008) 303–307.
- [18] J.R. Zhao, X.L. Ren, M. Wang, Biopharmaceuticals classification system (BCS) of nuciferin in *Nelumbinis Folium*, *Zhongguo Zhong Yao Za Zhi* 43 (2018) 511–519.
- [19] P. Wang, X.B. Luo, B. Chen, S.Z. Yao, Isolation and purification of aporphine alkaloids with macroporous adsorption resin, *Chin. Tradit. Herbal Drugs* 3 (2006) 355–358.
- [20] Y. Li, J. Wu, Z.M. Zhu, X. Liu, Y.S. Yu, C.B. Li, Isolation and purification of nuciferine and HPLC detection, *J. Dalian Minzu Univ.* 1 (2020) 21–25.
- [21] G.J. Maranata, N.O. Surya, A.N. Hasanah, Optimising factors affecting solid phase extraction performances of molecular imprinted polymer as recent sample preparation technique, *Heliyon* 7 (1) (2021) e05934, <https://doi.org/10.1016/j.heliyon.2021.e05934>.
- [22] S. Zou, C. Wang, J. Li, J. He, X. Gao, Y.-x. Chang, Microwave assisted solid phase microextraction for extraction and selective enrichment of four alkaloids in lotus leaf, *Sustain. Chem. Pharm.* 18 (2020) 100345, <https://doi.org/10.1016/j.scp.2020.100345>.
- [23] J.P. Fan, B. Zheng, Y. Qin, D. Yang, D.D. Liao, X.K. Xu, X.H. Zhang, J.H. Zhu, A superparamagnetic Fe₃O₄-graphene oxide nanocomposite for enrichment of nuciferine in the extract of *Nelumbinis Folium* (Lotus leaf), *Appl. Surf. Sci.* 364 (2016) 332–339, <https://doi.org/10.1016/j.apsusc.2015.12.160>.
- [24] J. Nie, D. Chen, J. Ye, Y. Lu, Z. Dai, Optimization and kinetic modeling of ultrasonic-assisted extraction of fucoxanthin from edible brown algae *Sargassum fusiforme* using green solvents, *Ultrason. Sonochem.* 77 (2021) 105671, <https://doi.org/10.1016/j.ultsonch.2021.105671>.
- [25] Y.H. Wei, Y.C. Xiao, Alkali precipitation agent for nuciferine extraction and nuciferine extraction method, CN201310099422.4.
- [26] J. Gao, J.Y. You, J.H. Kang, F.H. Nie, H.W. Ji, S.C. Liu, Recovery of astaxanthin from shrimp (*Penaeus vannamei*) waste by ultrasonic-assisted extraction using ionic liquid-in-water microemulsions, *Food Chem.* 325 (2020) 126850, <https://doi.org/10.1016/j.foodchem.2020.126850>.
- [27] A. Christou, I.J. Stavrou, C.P. Kapnissi-Christodoulou, Continuous and pulsed ultrasound-assisted extraction of carob's antioxidants: Processing parameters optimization and identification of polyphenolic composition, *Ultrason. Sonochem.* 76 (2021) 105630, <https://doi.org/10.1016/j.ultsonch.2021.105630>.
- [28] Q.Y. Shi, S. Geng, J.L. Chen, Q.F. Zhou, Y. Jin, H.L. Lei, L.J. Luan, X.S. Liu, Y.J. Wu, An efficient procedure for preparing main acylated pentasaccharides from *Polygalae Radix* using integrated extraction-adsorption method followed by semi-preparative high performance liquid chromatography, *Sep. Purif. Technol.* 153 (2015) 84–90, <https://doi.org/10.1016/j.seppur.2015.08.037>.

- [29] C.-Y. Chang, W.-H. Chung, W.-H. Ding, Vortex-assisted liquid-liquid microextraction for the rapid screening of short-chain chlorinated paraffins in water, *J. Sep. Sci.* 39 (2) (2016) 427–432, <https://doi.org/10.1002/jssc.201500991>.
- [30] H. Liu, X.L. Ren, H.J. Zhang, L.L. Sun, M. Wang, Degradation kinetics of four active ingredients in compound of Xuezhining, *J. Tianjin Univ. Trad. Chin. Med.* 5 (2017) 363–367.
- [31] C. Da Porto, E. Porretto, D. Decorti, Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds, *Ultrason. Sonochem.* 20 (4) (2013) 1076–1080, <https://doi.org/10.1016/j.ultsonch.2012.12.002>.
- [32] S. Singh, N. Pathak, E. Fatima, A.S. Negi, Plant isoquinoline alkaloids: Advances in the chemistry and biology of berberine, *Eur. J. Med. Chem.* 226 (2021) 113839, <https://doi.org/10.1016/j.ejmech.2021.113839>.
- [33] A.A. Salamatin, A.S. Khaliullina, R.S. Khaziev, Extraction of aromatic abietane diterpenoids from *Salvia officinalis* leaves by petroleum ether: Data resolution analysis, *Ind. Crop. Prod.* 143 (2020) 111909, <https://doi.org/10.1016/j.indcrop.2019.111909>.
- [34] W. Yang, X. Wang, S. Ni, X. Liu, R. Liu, C. Hu, H. Dai, Effective extraction of aromatic monomers from lignin oil using a binary petroleum ether/dichloromethane solvent, *Sep. Purif. Technol.* 267 (2021) 118599, <https://doi.org/10.1016/j.seppur.2021.118599>.
- [35] L. Redón, X. Subirats, M. Rosés, Volume and composition of semi-adsorbed stationary phases in hydrophilic interaction liquid chromatography. Comparison of water adsorption in common stationary phases and eluents, *J. Chromatogr. A* 1656 (2021) 462543, <https://doi.org/10.1016/j.chroma.2021.462543>.
- [36] F. Chemat, N. Rombaut, A. Meullemestre, M. Turk, S. Perino, A.S. Fabiano-Tixier, M. Abert-Vian, Review of green food processing techniques. Preservation, transformation, and extraction, *Innov. Food Sci. Emerg.* 41 (2017) 357–377, <https://doi.org/10.1016/j.ifset.2017.04.016>.
- [37] W. Xiong, X. Chen, G. Lv, D. Hu, J. Zhao, S. Li, Optimization of microwave-assisted extraction of bioactive alkaloids from lotus plumule using response surface methodology, *J. Pharm. Anal.* 6 (6) (2016) 382–388, <https://doi.org/10.1016/j.jpha.2016.05.007>.
- [38] X. Luo, J. Cui, H. Zhang, Y. Duan, D. Zhang, M. Cai, G. Chen, Ultrasound assisted extraction of polyphenolic compounds from red sorghum (*Sorghum bicolor* L.) bran and their biological activities and polyphenolic compositions, *Ind. Crop. Prod.* 112 (2018) 296–304, <https://doi.org/10.1016/j.indcrop.2017.12.019>.
- [39] M. Ramić, S. Vidović, Z. Zeković, J. Vladić, A. Cvejin, B. Pavlič, Modeling and optimization of ultrasound-assisted extraction of polyphenolic compounds from *Aronia melanocarpa* by-products from filter-tea factory, *Ultrason. Sonochem.* 23 (2015) 360–368, <https://doi.org/10.1016/j.ultsonch.2014.10.002>.
- [40] J.J. John, S. Kuhn, L. Braeken, T. Van Gerven, Ultrasound assisted liquid-liquid extraction with a novel interval-contact reactor, *Chem. Eng. Process.* 113 (2017) 35–41, <https://doi.org/10.1016/j.cep.2016.09.008>.
- [41] J.L. Urraca, M. Castellari, C.A. Barrios, M.C. Moreno-Bondi, Multiresidue analysis of fluoroquinolone antimicrobials in chicken meat by molecularly imprinted solid-phase extraction and high performance liquid chromatography, *J. Chromatogr. A* 1343 (2014) 1–9, <https://doi.org/10.1016/j.chroma.2014.03.045>.
- [42] S. Sadeghi, M. Jahani, Selective solid-phase extraction using molecular imprinted polymer sorbent for the analysis of Florfenicol in food samples, *Food Chem.* 141 (2) (2013) 1242–1251, <https://doi.org/10.1016/j.foodchem.2013.04.027>.
- [43] M. Tabandeh, S. Ghassamipour, H. Aqababa, M. Tabatabaei, M. Hasheminejad, Computational design and synthesis of molecular imprinted polymers for selective extraction of allopurinol from human plasma, *J. Chromatogr. B* 898 (2012) 24–31, <https://doi.org/10.1016/j.jchromb.2012.04.009>.
- [44] M. Jiang, X.-W. Ni, Effects of solvents and impurity on crystallization kinetics and crystal properties in a reactive crystallization of paracetamol, *J. Cryst. Growth.* 523 (2019) 125150, <https://doi.org/10.1016/j.jcrysgro.2019.125150>.
- [45] C.P. Ye, Y.F. Qiao, R.N. Wang, W.Y. Li, Purification of carbazole by solvent crystallization under two forced cooling modes, *Chin. J. Chem. Eng.* 35 (2021) 173–179, <https://doi.org/10.1016/j.cjche.2020.12.018>.
- [46] A.F.C. Rossini, A.C.A. Muraca, G.A. Casagrande, C. Raminelli, Total syntheses of aporphine alkaloids via benzyne chemistry: an approach to the formation of aporphine cores, *J. Org. Chem.* 80 (20) (2015) 10033–10040, <https://doi.org/10.1021/acs.joc.5b01634>.