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Research Article

Optimization of microwave and ultrasound-assisted extraction of sinomenine from *Sinomenium acutum* using a natural deep eutectic solvent

Lin-Xin Chen†, Hui Cao†, Long Chen, Bin Liu, Bao-Zhong Zhu, Wei-Zhuo Tang (✉)

College of Biological and Chemical Engineering, Changsha University, Changsha 410022, China
† *Lin-Xin Chen and Hui Cao contributed equally to this work.*

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Abstract: Natural deep eutectic solvents (NADEs) have emerged as green and sustainable options for the efficient extraction of bioactive components from natural plants. This study aimed to evaluate the effectiveness of NADE-based microwave and ultrasound extraction techniques for sinomenine from *Sinomenium acutum*, screening nine different NADEs in the process. To optimize the extraction processes, a single-factor experiment was conducted alongside response surface methodology, utilizing a Box-Behnken design. An optimized high-performance liquid chromatography (HPLC) method was also developed to quantify the sinomenine content in various samples. The results revealed that chloride-lactic acid (ChCl-LA) was the most effective NADE for sinomenine extraction, with microwave extraction demonstrating superior efficiency compared to ultrasound extraction. For ultrasound extraction, the optimal parameters included a ChCl-LA ratio of 1:2, 30% of water content, a liquid-to-solid ratio of 1:20, an extraction time of 20 min, and an ultrasonic power of 240 W. In contrast, for microwave extraction, the optimal conditions were a ChCl-LA ratio of 2:1 with 57% of water content, an extraction time of 20 min, and a power level of 423.33 W. Under these conditions, the sinomenine content was found to be 16.03 mg/g for ultrasound extraction and 20.08 mg/g for microwave extraction, respectively. These findings provide a foundation for the further utilization of sinomenine from *S. acutum* .

Keywords: natural deep eutectic solvents; *Sinomenium acutum*; response surface methodology; ultrasonic extraction; microwave extraction.

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1 Introduction

Deep eutectic solvents (DESs) represent a new class of green solvents composed of two or more components that typically include hydrogen bond donors and acceptors in specific stoichiometric ratios. DESs were first proposed by Abbott *et al.*[1] in 2003 and can be formed from natural substances such as glucose, mannitol, and amino acids. The strong intermolecular interactions (including hydrogen bonds and electrostatic forces) among the components result in a melting point lower than that of each individual component[2]. Compared to traditional organic solvents, DESs are more environmentally friendly due to their primary constituents being derived from natural products or biodegradable compounds, which generally exhibit low toxicity and high biodegradability[3]. Additionally, DESs offer higher selectivity and efficiency during the extraction process, demonstrating a stronger affinity for target compounds, improved extraction efficiency and reduced resource wastage[4-6]. In recent years, DESs have found widespread application in extracting bioactive ingredients from Chinese herbal medicines, including flavonoids[7], lignin[8], phenols[9], alkaloids[10], and polysaccharides[11].

Sinomenium acutum, the dried vine stem of *S. acutum* (Thunb.), was first recorded in the "Illustrated Classic of Materia Medica" from the Song Dynasty. It contains a variety of components, including sterols, alkaloids, and esters, with alkaloids being the primary active ingredients[12]. *S. acutum* is known for its effects in dispelling wind and dampness, unblocking meridians, and promoting urination[13]. Clinically, it is used to treat conditions such as rheumatic joint pain and scabies, showcasing its pharmacological properties of anti-inflammatory and analgesic effects. Sinomenine is a natural alkaloid extracted from the traditional Chinese medicine *S. acutum*. It has many biological activities such as anti-inflammatory, anti-oxidation, anti-tumor and immune regulation[14]. Luo *et al.*[15] analyzed the chemical constituents of *S. acutum*, focusing on various alkaloids (including sinomenine, magnoflorine, dauricine, l-stepholidine, cepharanthine, higenamine, and acumenine), sterols, volatile oils, and phenols.

Sinomenine is the principal alkaloid compound isolated from

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Address correspondence to Wei-Zhuo Tang, tweizhuo@126.com

S. acutum and has been reported to exhibit various pharmacological effects, including anti-inflammatory, immunosuppressive, anti-tumor, and other pharmacological effects[16, 17]. Liu *et al.*[18] examined the protective effects of sinomenine against oxidative stress injury and pulmonary fibrosis, investigating its relationship with the Kelch-like ECH-associated protein 1 (Keap1)/nuclear factor E2-related factor 2 (Nrf2) signaling pathway. They found that sinomenine inhibited oxidative stress and pulmonary fibrosis by activating the Keap1/Nrf2 signaling pathway. Moreover, Wang[19] studied the effects of sinomenine on the proliferation and apoptosis of U266 myeloma cells, in which sinomenine was found to influence the proliferation and apoptosis of multiple myeloma U266 cells by modulating the signal transducer and activator of transcription 3 (STAT3) and nuclear factor-κ-gene binding (NF-κB) signaling pathways.

In recent years, ultrasonic extraction technology has become a mature and efficient method for extracting active ingredients from traditional Chinese medicines. This technique utilizes the strong vibrations, high acceleration, thermal effects, and stirring generated by ultrasonic waves to facilitate the rapid transfer of effective components into the solvent, thereby improving extraction efficiency, shortening extraction times, and minimizing heat-related damage to the extracted compounds[20]. Similarly, microwave-assisted extraction technology has gained popularity in the extraction of plant samples. This method uses microwave radiation to heat water molecules within the sample, enabling the rapid and efficient extraction of target components[21]. Sun *et al.*[22] used ultrasound-assisted deep eutectic solvent to extract polysaccharides from licorice. The optimum conditions for the extraction of licorice polysaccharide were determined by single factor test and response surface test. The results showed that the process conditions were mild, the extraction rate of polysaccharide was high, the loss rate of hydrolysis and biological decomposition was low, and the application prospect was good. Fu *et al.*[23] optimized the extraction process of proanthocyanidins from grape pomace by microwave-assisted DESs through orthogonal experiments, and investigated the stability of the extracted proanthocyanidins. Cao *et al.*[24] employed ethanol heating reflux extraction and utilized central composite design-response surface methodology to assess the influence of ethanol concentration, material-liquid ratio, and extraction time. The optimal parameters for extracting total alkaloids from *S. acutum* were identified as 75% ethanol, a material-liquid ratio of 1:10, and three cycles of heating reflux extraction for 1.8 h each. Chen optimized the ultrasonic extraction process via an orthogonal test, achieving optimal conditions of 70% ethanol concentration, 15 mL of ethanol, 30 min of ultrasonic time, and a frequency of 30 Hz[25]. Li *et al.*[26] further refined the microwave extraction process for sinomenine in *S. acutum* investigating the effects of extraction time, ethanol volume fraction, and microwave power on extraction efficiency. They established the optimal parameters using response surface methodology: a material-liquid ratio of 1:20, 75% ethanol, 450 W microwave power for two cycles of 8.30 min each, resulting in a yield of 0.803% for sinomenine. Given the advantages of DESs, utilizing ultrasound and microwave-assisted DESs for extracting active ingredients may yield favorable results. However, to date, there have been no reports on the extraction of sinomenine from *S. acutum* using DESs.

In this study, the content of sinomenine was used as the indicator to identify the optimal types and ratios of DESs for extracting sinomenine using both ultrasound and microwave methods with optimizing the process by single-factor experiment and response surface methodology.

2 Material and methods

2.1 Experimental reagents and instruments

The *S. acutum* medicinal material was purchased online in March 2023 from Hangzhou Jiawei Technology Co., Ltd. (China). It originated from Henan Province and was identified by Professor Chen Jianrong from the Biological and Chemical Engineering College of Changsha University as the root of *S. acutum* and hairy ivy. The material is located at the Small Molecule Natural Products Research Center of Changsha University.

2.1.1 *Experimental materials*

The sinomenine reference substance, with a purity of ≥ 90%, was obtained from Shanghai Yuanye Biotechnology Co., Ltd. (China). Methanol, choline chloride, urea, and glucose were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Additionally, lactic acid and betaine were bought from Shanghai Aladdin Biochemical Technology Co., Ltd. (China). Sucrose and glycerol were obtained from Shanghai McLean Biochemical Technology Co., Ltd. (China). Acetonitrile and phosphoric acid were acquired from Anhui Tiandi High-Purity Solvent Co., Ltd. (China). Lastly, Yibao drinking water was purchased from China Resources Yibao Beverage Co., Ltd. (China).

2.1.2 *Experimental instruments*

The following instruments were utilized in the experiments: an electronic balance ME204E from Mettler toledo Instruments Co., Ltd. (Switzerland); a pure water apparatus ZVV4 u72-05 from Zhongwo Water Affairs Environmental Protection Technology Co., Ltd. (China); a constant temperature magnetic stirrer 85-2A from Jintan District Xicheng Xinrui Instrument Factory (China); a high-speed grinder DFY-600D from Wenling Lindao Machinery Co., Ltd. (China); and a water bath SB-1300 from Elan Technology International Trade Co., Ltd. (China). Additionally, a circulating water vacuum pump SHZ-(Ⅲ) from Yuhua Instrument Co., Ltd. (China); a refrigerator and freezer BCD 146AD from Ningbo Haidian Electric Appliance Co., Ltd. (China); a PDA detector 2998 from Waters Company (USA); a high-performance liquid chromatograph Waters e2695 from Waters Company; a CNC ultrasonic cleaner KQ 400DE from Kunshan Ultrasonic Instrument Co., Ltd. (China); and a pipette TopPette from Dalong Xingchuang Laboratory Instrument Co., Ltd. (China) were also used.

2.2 Ultrasonic experimental design

2.2.1 *Pre-processing of materials*

Choline chloride and betaine were selected as hydrogen bond donors, while lactate, glucose, sucrose, urea, and glycerol served as hydrogen bond acceptors. The specific preparation steps are as follows: the hydrogen bond donors were mixed with the hydrogen bond acceptors in specific molar ratios. This process resulted in the preparation of nine types of DESs, which were then heated in a water bath until they melted into a clear solution. The roots of *S. acutum* were dried, ground, and sieved to obtain powdered samples, which were stored in sealed bags for further use.

2.2.2 The optimization of DESs for sinomenine extraction

A precision balance was used to weigh 1.0 g of the powdered sample, which was placed in a 50 mL centrifuge tube. Different types of DESs were utilized as extraction solvents under fixed conditions: ultrasonic time was set at 20 min, ultrasonic power at 240 W, water content at 40%, and a material-to-liquid ratio of 1:20 (g/mL), all at room temperature. After the extraction, 1 mL of the filtrate was accurately measured and transferred into a 10 mL volumetric flask, where pure water was added to reach the fixed volume. Next, the diluent was passed through a 0.45 μm microporous filter membrane using a 1 mL syringe, and the filtered solution was collected in a 0.5 mL vial. The concentration of sinomenine in the test solution was then determined using HPLC to identify the DES solution with the best extraction efficacy.

2.2.3 Optimize the ultrasound extraction process

2.2.3.1 Single-factor experiments

The extraction rate of sinomenine from *S. acutum* was investigated by weighing 1.0 g of the powdered sample. The study focused on the influence of three key factors: ultrasonic power, ultrasonic time, and solvent water content. To comprehensively assess the impact of each condition, single-factor experiments were conducted by varying the ultrasonic power (160, 200, 240, 280, and 320 W), ultrasonic time (10, 15, 20 and 30 min), and water content (20%, 25%, 30%, and 40%). The effects of these variables on the extraction of sinomenine were evaluated to determine their significance.

2.2.3.2 Response surface optimization experiment

Based on the selection of the best DESs and the results from the single-factor experiments, 1.0 g of the powdered sample was accurately weighed and placed in a 50 mL centrifuge tube. The three levels of each optimal single-factor condition were employed as variable factors during extraction. Following extraction, 1 mL of the filtrate was measured and transferred into a 10 mL volumetric flask, where pure water was added to achieve the desired volume. The resulting diluent was then passed through a 0.45 μm microporous filter membrane using a syringe, and the filtered solution was collected in a 0.5 mL vial. The concentration of sinomenine in the test solution was determined using HPLC. The content of sinomenine extracted from the samples served as the evaluation index. Through this response surface experiment, the comprehensive effects of each factor on the extraction of sinomenine were further investigated, ultimately leading to the optimization of the ultrasonic extraction process.

2.3 Microwave experiment design

2.3.1 Pre-processing of materials

Choline chloride and betaine were selected as hydrogen bond donors, while lactate, glucose, sucrose, urea and glycerol were chosen as hydrogen bond acceptors. To prepare the DESs, the hydrogen bond donors were mixed with the hydrogen bond acceptors in specific molar ratios. The mixture was then heated in a water bath until it melted into a clear solution. The roots of *S. acutum* were dried, ground, and sieved to obtain drug powder samples, which were subsequently stored in sealed bags.

2.3.2 Selection of the optimal DES for sinomenine extraction

A precision balance was used to weigh 1.0 g of the powder sample, which was placed in a 250 mL four-neck flask. The extraction was conducted under fixed conditions, specifically an extraction time of 15 min, microwave power of 300 W, a solvent-to-sample ratio of 1:20 (g/mL), and a solvent moisture content of 40%. Different types of DESs were employed as extraction solvents. Following extraction, 1 mL of the filtrate was transferred to a 10 mL volumetric flask and diluted with pure water to achieve a fixed volume. The diluted solution was then filtered through a 0.45 μm microporous membrane using a 1 mL syringe and placed in a 0.5 mL vial. The content of sinomenine was determined through HPLC to identify the most effective DES for subsequent experiments.

2.3.3 Optimization of the microwave extraction process

2.3.3.1 Single-factor experiments

The extraction rate of sinomenine from *S. acutum* was investigated by accurately weighing 1.0 g of sample powder. The study focused on the influence of microwave power, microwave time, and solvent water content as essential factors affecting the extraction process. To comprehensively evaluate the impact of each variable, single-factor experiments were conducted by varying microwave power (200, 240, 280, 320, 360 W), microwave time (10, 20, 30, 40, 50 min), and water content (10%, 20%, 30%, 40%, 50%). This approach allowed for a more thorough understanding of how each factor influenced the extraction of sinomenine.

2.3.3.2 Response surface optimization experiment

Based on the selection of optimal DESs and the findings from the single-factor experiments, 1.0 g of the powdered sample was weighed and transferred to a four-neck flask. The three levels of each identified optimal single-factor condition were used as variable factors for extraction. Following extraction, 1 mL of the filtrate was pipetted into a 10 mL volumetric flask, and purified water was added to the mark. The resulting solution was then drawn into a 1 mL syringe and passed through a 0.45 μm microporous filter membrane into a 0.5 mL vial, preparing it for testing. The content of sinomenine in the test solution was determined using HPLC. The extracted sinomenine content was used as the evaluation index, allowing for a comprehensive investigation of the effects of each variable on sinomenine extraction through the response surface methodology and ultimately facilitating the optimization of the microwave extraction process.

2.4 Determination of sinomenine content in *S. acutum*

2.4.1 HPLC conditions for sinomenine determination

The mobile phase for HPLC analysis comprised acetonitrile (A) and 0.2% phosphoric acid in water (B), using a linear gradient elution. The elution procedure was performed as follows: from 0 min to 8 min, the mobile phase consisted of 10% acetonitrile (A); from 8 min to 15 min, a gradient was applied to increase the concentration of A from 10% to 15%; and from 25 min to 27 min, the concentration of A was further increased from 15% to 25%. The flow rate was set at 1.0 mL/min, with UV detection wavelengths of 265 nm for the ultrasonic method and 224 nm for the microwave method. The column was maintained at a temperature of 25 °C, and an injection volume of 10 μL was utilized. The column used for the analysis was a Medium Spectrum Red RD-C_{18} (4.6 mm × 250 mm, 5 μm).

2.4.2 Construction of the standard curve

Standard solutions were prepared in a series according to a defined concentration gradient, and the content of sinomenine was evaluated by HPLC. The concentration of sinomenine in the standard solution was denoted as *X*, while the corresponding chromatographic peak area was denoted as *Y*. The regression curve equation was calculated to evaluate the sinomenine content in subsequent samples. The specific procedure involved dissolving 10 mg of sinomenine standard in a 10 mL volumetric flask, followed by dilution with methanol to achieve a concentration of 1 mg/mL. Subsequently, aliquots of this solution (0.1, 0.5, 2.0, 3.0 mL) were transferred to a 10 mL volumetric flask and diluted with methanol to the mark. Filtered solutions of sinomenine at concentrations of 0.01, 0.05, 0.2, 0.3, and 0.4 mg/mL were obtained using a 0.45 μm microporous filter. These standard solutions were analyzed using the HPLC system at a wavelength of 265 nm. The chromatographic peak areas were calculated, allowing for the construction of the standard curve for sinomenine.

3 Results and discussion

3.1 Screening results of nine types of DESs for ultrasound extraction of *S. acutum*

The properties of nine types of DESs are summarized in Table 1. HPLC analysis was conducted to identify the peak corresponding to sinomenine in the samples by comparing the retention times with those of standard sinomenine. The peak areas of the chromatograms were utilized to evaluate the effectiveness of each DES. The results of are presented in Fig. 1. The evaluation of the extraction solvents indicated that the DES prepared from a molar ratio of choline chloride to lactic acid (1:2) exhibited the highest extraction efficiency for sinomenine. Therefore, choline chloride lactate was selected as the optimal DES and was employed as the extraction solvent for subsequent experiments.

Table 1 List of DESs initially tested using ultrasound

No.	Abbreviation	HEBA	HBD	Mole ratio
DES-1	ChCl-Ur	Choline chloride	Urea	1:2
DES-2	ChCl-Suc-Water		Sucrose	4:1:5
DES-3	ChCl-Glu-Water		Glucose	1:2:4
DES-4	ChCl-Lac		Lactic acid	1:2
DES-5	ChCl-Gly		Glycerol	1:2
DES-6	Bet-Ur-Water	Betaine	Urea	1:1:2
DES-7	Bet-Suc-Water		Sucrose	2:1:5
DES-8	Bet-Glu-Water		Glucose	1:1:2.5
DES-9	Bet-Lac		Lactic acid	1:2

3.1.1 Single factor experiment results

A single-factor ultrasonic extraction experiment was conducted to investigate the effects of varying ultrasound duration, ultrasound power, and solvent moisture content on the extraction of sinomenine. Figure 2A illustrates the influence of different ultrasound durations on sinomenine extraction. The results indicate that, within the range of 10–20 min, the concentration of sinomenine generally increases, reaching a peak. However, between 20 and 30 min, a decline in sinomenine concentration is observed, particularly a rapid decrease between 20 and 25 min. Beyond 25 min, the concentration begins to rise again, although the extraction rate remains lower than that observed during the 10- to 20-min interval. Therefore, the optimal ultrasound duration was determined to be 20 min.

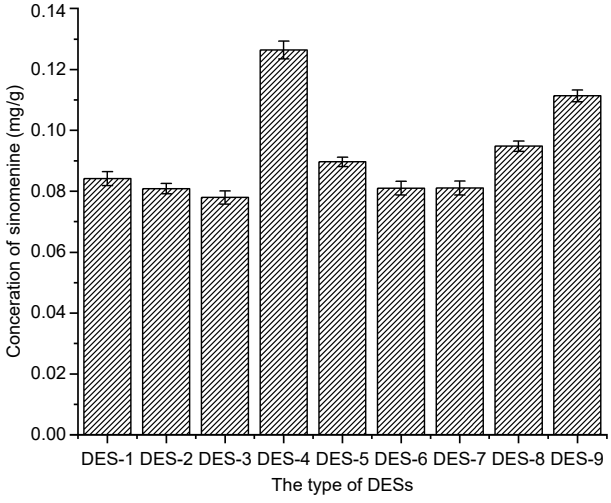


Figure 1 Effect of nine DESs on the extraction of sinomenine.

The effect of different ultrasound powers on the extraction rate of sinomenine was also assessed. Figure 2B shows the relationship between ultrasound power and sinomenine extraction, indicating that as the power increases from 160 W to 240 W, the concentration of sinomenine rises. However, as the power continues to increase to 320 W, a decline in sinomenine concentration is observed, particularly between 240 and 280 W. After reaching 280 W, although the concentration increases, the extraction rate during this power segment remains lower than that at 240 W. Consequently, the optimal ultrasound power was identified as 240 W.

Next, we assessed the effects of different moisture contents on the extraction rate of sinomenine. As shown in Fig. 2C, when the moisture content is between 20% and 30%, the concentration of sinomenine rises. However, as the moisture content increases beyond 30% to 40%, a decreasing trend is observed. Based on these findings, the optimal moisture content was determined to be 30%.

3.1.2 Response surface optimization experiment results

Using the content of *S. acutum* as an indicator, a response surface experiment was conducted based on the results of the single-factor experiment. The design of the response surface optimization experiment followed the scheme is outlined in Table 2, and the results are presented in Table 3. The regression coefficients were calculated from the data, resulting in a quadratic multiple regression model that describes the relationship between the ultrasound parameters and the extraction of sinomenine. The equations are as follows:

Coded equation:

$$Y = 15.92 - 0.41 \times A - 0.095 \times B + 0.58 \times C - 1.19 \times AB - 0.20 \times AC + 0.067 \times BC - 2.33 \times A^2 - 2.37 \times B^2 - 1.06 \times C^2$$

Actual equation:

$$Y = -175.555 + 5.316,5 \times A + 0.816,625 \times B + 2.644,5 \times C - 0.005,962 \times AB - 0.007,900 \times AC + 0.000,337 \times BC - 0.093,25 \times A^2 - 0.001,479 \times B^2 - 0.040,85 \times C^2$$

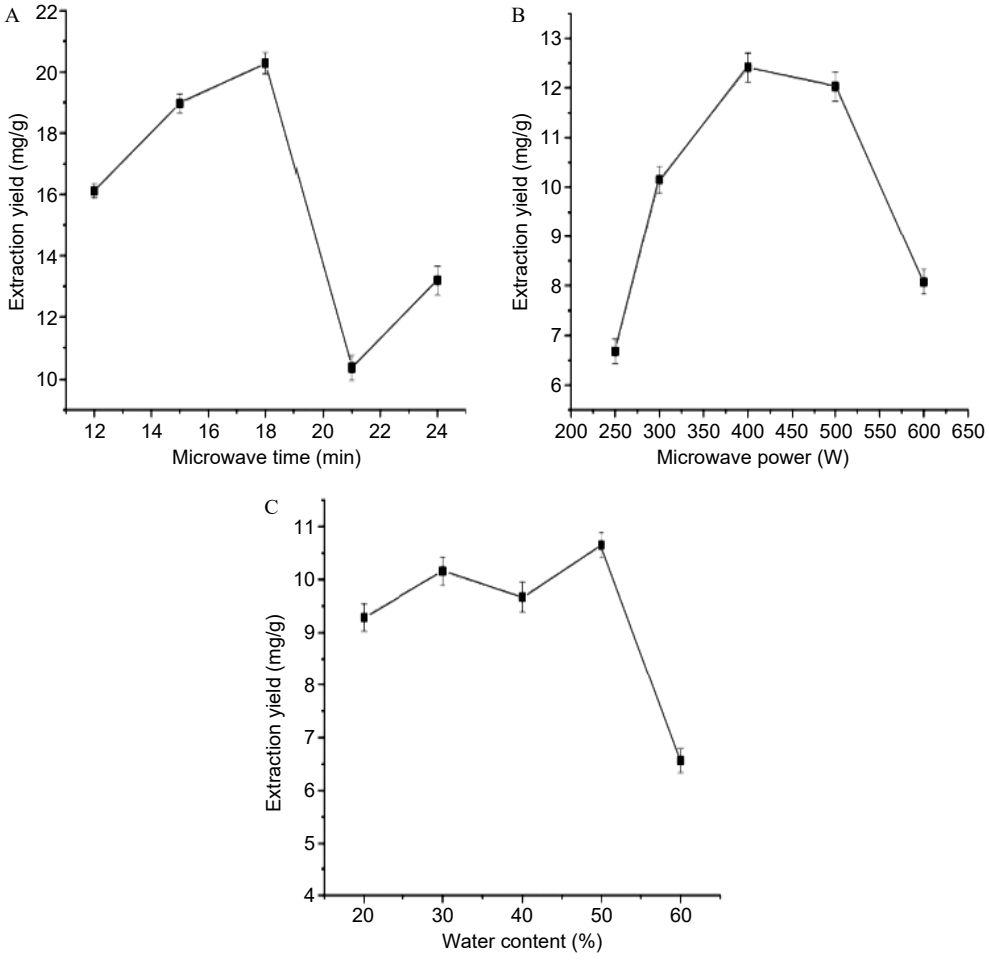


Figure 2 Effects of varying ultrasonic time (A), ultrasonic power (B), and water content (C) on the extraction yield of sinomenine.

Table 2 Design of ultrasonic factors and levels for the Box-Behnken method

Factor level	Ultrasonic time (min)	Ultrasonic power (W)	Water content (%)	DES
−1	15	200	25	/
0	20	240	30	ChCl-Lac
1	25	280	35	/

In these equations, *A* represents ultrasound time, *B* represents ultrasound power, and *C* represents water content. The correlation coefficient R^2 for the model is 0.975,8. Further, variance analysis of the data was performed, and the results are summarized in Table 4. The *F* value and *P* value are important indicators for assessing the significance of the model. A higher *F* value and a lower *P* value indicate a more significant model. The results of the variance analysis indicated that the model's *F* value is 31.43, suggesting a high significance. The *P* values for the quadratic terms *AB*, A^2, B^2, and C^2 are all less than 0.01, demonstrating a significant difference and indicating that the extraction efficacy of sinomenine is significantly influenced by ultrasound time and ultrasound power. The *P* values for the linear terms *A* and *C* are both less than 0.05, confirming that ultrasound time and water content significantly affect the extraction process. The *P* value for the residual term is 0.581,6, indicating that the fit between the theoretical data and actual results is high, suggesting that the model accurately reflects the experimental outcomes. Furthermore, the fitted R^2 value of 0.837,9 is close to the actual R^2 value of 0.944,8, indicating a strong alignment between expected and observed results.

Table 3 Box-Behnken design and corresponding experimental results

Runs	Ultrasonic time (*A*, min)	Ultrasonic power (*B*, W)	Water content (*C*, %)	Extraction yield (mg/g)
C-1	−1 (15)	−1 (200)	0 (30)	10.44
C-2	1 (25)	−1 (200)	0 (30)	12.06
C-3	−1 (15)	1 (280)	0 (30)	12.77
C-4	1 (25)	1 (280)	0 (30)	9.62
C-5	−1 (15)	0 (240)	−1 (25)	11.96
C-6	1 (25)	0 (240)	−1 (25)	11.49
C-7	−1 (15)	0 (240)	1 (35)	14.04
C-8	1 (25)	0 (240)	1 (35)	12.78
C-9	0 (20)	−1 (200)	−1 (25)	12.44
C-10	0 (20)	1 (280)	−1 (25)	11.98
C-11	0 (20)	−1 (200)	1 (35)	12.95
C-12	0 (20)	1 (280)	1 (35)	12.76
C-13	0 (20)	0 (240)	0 (30)	15.60
C-14	0 (20)	0 (240)	0 (30)	16.41
C-15	0 (20)	0 (240)	0 (30)	16.40
C-16	0 (20)	0 (240)	0 (30)	15.96
C-17	0 (20)	0 (240)	0 (30)	15.23

The response surface equations were further analyzed through 3D surface plots, as illustrated in Fig. 3. In comparison to the center surface of the response surface, the center point of the 3D

Table 4 Variance analysis for the fitted regression model

Source	Sum of squares	Degrees of freedom	Mean square	*F*-value	*P*-value	Significance
Model	65.98	9	7.33	31.43	<0.000,1	Significant
A-Ultrasonic time	1.33	1	1.33	5.70	0.048,4	
B-Ultrasonic power	0.072,2	1	0.072,2	0.309,5	0.595,3	
C-Water contend	2.71	1	2.71	11.64	0.011,3	
AB	5.69	1	5.69	24.39	0.001,7	
AC	0.156,0	1	0.156,0	0.668,9	0.440,4	
BC	0.018,2	1	0.018,2	0.078,1	0.787,9	
A^2	22.88	1	22.88	98.10	<0.000,1	
B^2	23.58	1	23.58	101.07	<0.000,1	
C^2	4.39	1	4.39	18.83	0.003,4	
Residual	1.63	7	0.232,3			
Lack of fit	0.582,3	3	0.194,1	0.738,9	0.581,6	Not significant
Pure error	1.05	4	0.262,7			
Cor total	67.61	16				

diagram depicting the interaction between ultrasonic time and ultrasonic power stands out prominently, with the steepest response surface observed. This observation indicates a significant effect of both parameters, which aligns with the differences noted in the variance analysis for the interaction term *A*, *B*, and *AB*. Additionally, the gradient of the response surface with respect to ultrasonic time is greater, suggesting that ultrasonic time exerts a more substantial influence on the extraction of *S. acutum* than ultrasonic power does. Analysis of the 3D diagram illustrating the interaction between ultrasonic time and water content reveals that the effect of water content on the extraction process is greater than that of ultrasonic time, suggesting that water content plays a more significant role in the extraction of *S. acutum*. A similar conclusion was reached when examining the interaction between ultrasonic power and water content.

In summary, the hierarchy of impact on the extraction of sinomenine is as follows: water content > ultrasonic time > ultrasonic power. Furthermore, simulations using Box-Behnken software indicate that the optimal ultrasonic extraction process consists of DESs with a molar ratio of choline chloride to lactic acid of 1:2, a solvent-to-sample ratio of 1:20 (g/mL), an ultrasonic time of 19.49 min, an ultrasonic power of 240 W, and a water content of 31.47%. Under these conditions, the theoretical extraction yield of sinomenine can reach 16.03 mg/g.

3.1.3 *Verification experiment results*

To assess the reliability of the simulated optimal extraction process generated by the software, the experimental conditions were verified in accordance with the material-to-liquid ratio of 1:20 (g/mL), ultrasonic time of 20 min, ultrasonic power of 240 W, and water content of 30%. The best ultrasonic extraction experiment was repeated three times, and the content of sinomenine in the samples was determined to yield an average value of 15.6 mg/g. The experimental data closely approximates the theoretical extraction value, indicating that the experimental model developed from the response surface analysis and the proposed optimal ultrasonic extraction process are highly reliable.

3.2 Analysis of sinomenine from *S. acutum* by ultrasonic extraction

3.2.1 *Precision experiment results*

Samples obtained as described in Section 2.2 were analyzed according to the content evaluation and determination method outlined in Section 2.4. The control solution of *S. acutum* was sampled five times continuously to calculate the relative standard deviation (RSD) of the chromatographic peak area. The RSD value was found to be less than 2.6%, indicating that the analytical method exhibits good precision.

3.2.2 *Repetitive experiment results*

According to the procedures in Section 2.2 and the content evaluation and determination method in Section 2.4, five aliquots of the same sample solution were measured consecutively, and the RSD of the chromatographic peak area was calculated. The RSD value was determined to be less than 4.1%, demonstrating that the content determination method has excellent repeatability.

3.2.3 *Stability experiment results*

The samples were prepared as described in Section 2.2, and the same sample solution was analyzed at 0, 4, 6, 12, and 24 h using the evaluation method from Section 2.4. The RSD value of the chromatographic peak area was calculated, yielding a value of less than 3.8%, which indicates that the sample solution is stable over time.

3.2.4 *HPLC determination of sinomenine*

According to the chromatographic conditions specified in Section 2.4.1, both the control product and the sinomenine sample were analyzed. By comparing the retention time and ultraviolet absorption characteristics of the chromatographic peaks (Fig. 4A), it was confirmed that peak 1 in the *S. acutum* rattan sample (Fig. 4B) corresponds to the sinomenine peak.

3.3 Screening results of nine types of DESs for microwave extraction of *S. acutum*

Solution preparation was conducted according to the methodology outlined in Section 2.3.1, yielding 10 sets of DESs. The preparation results are detailed in Table 5 and illustrated in Fig. 5. After preparation and screening of DESs as described in Sections 2.3.1 and 2.3.2, a single-factor experiment was performed using these ten low co-melting DESs, followed by HPLC to determine the peaks in the samples. The evaluation of the DESs was based on the peak areas of the 10 solvents, with results depicted in Fig. 6. From the comparison of peak areas across the

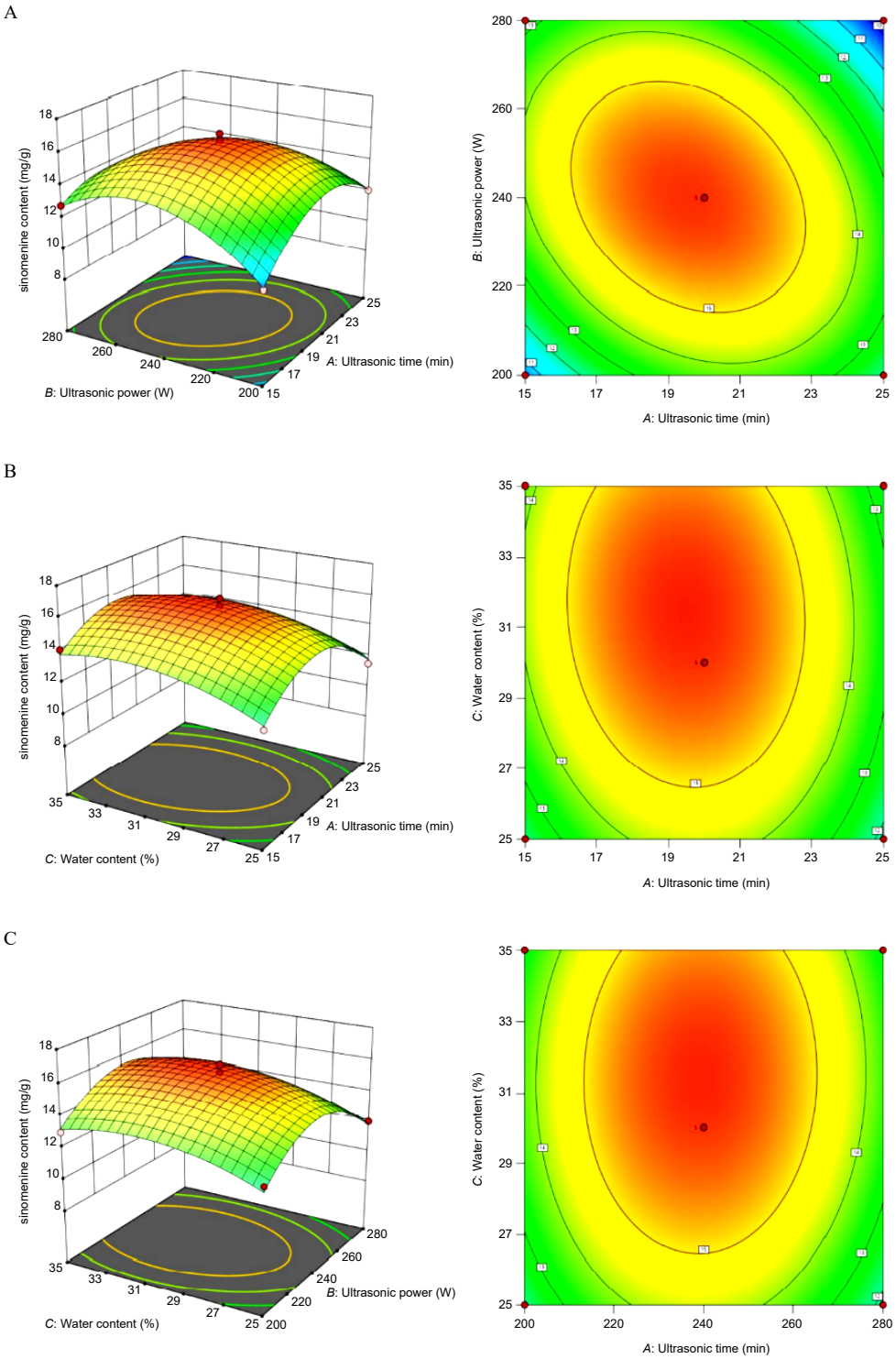


Figure 3 Response surface analysis demonstrating the effects of ultrasonic time (A), ultrasonic power (B), and water content (C) on the extraction rate of sinomenine.

ten solvents, it was concluded that lactic acid was the optimal extraction solvent. Therefore, choline chloride-lactate was identified as the best DES for subsequent extraction experiments.

3.3.1 *Single factor experiment results of microwave extraction*

To determine the optimal microwave extraction time, Fig. 7A illustrates the significant effect of extraction time on the yield of sinomenine. During the extraction period of 12–18 min, the content of sinomenine exhibited an upward trend across all samples. The maximum content was achieved at 18 min; however, from 18 min to 24 min, a downward trend was observed. Therefore, 18 min was established as the optimal extraction time for microwave-assisted extraction. To determine the optimum microwave power, Fig. 7B illustrates the trend in the effect of microwave power on the extraction of sinomenine. It is evident from Fig. 7B that microwave power significantly influences sinomenine extraction. Specifically, as the microwave power increased from 250 W to 400 W, the content of sinomenine extracted also exhibited an upward trend across all samples. The highest yield of sinomenine was observed at 400 W; however,

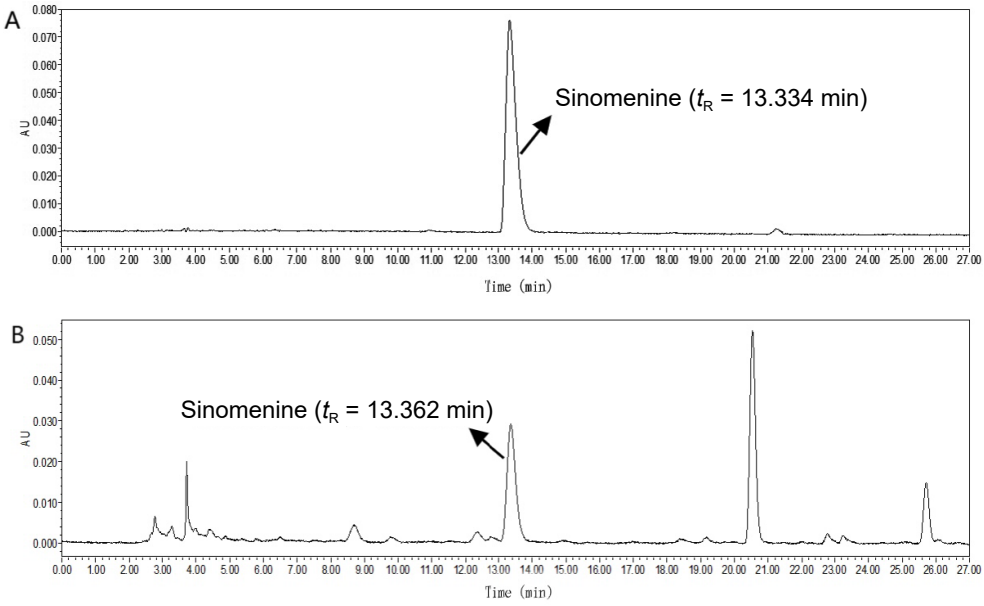


Figure 4 HPLC chromatograms of sinomenine under ultrasonic conditions. (A) Chromatogram of the sinomenine standard, (B) chromatogram of the sinomenine sample.

Table 5 List of DESs for initial microwave testing

No.	Abbreviation	HEBA	HBD	Mole ratio
DES-1	Bet-Ur-Water	Betaine	Urea	1:1:2
DES-2	Bet-Suc-Water		Sucrose	2:1.5
DES-3	Bet-Glu-Water		Glucose	1:1:2.5
DES-4	Bet-Lac		Lactic acid	1:2
DES-5	Bet-Gly		Glyerol	1:2
DES-6	ChCl-Ur	Choline chloride		2:1
DES-7	ChCl-Suc-Water		Surose	4:1:5
DES-8	ChCl-Glu-Water		Glucose	1:2:8
DES-9	ChCl-Lac		Lactic acid	2:1
DES-10	ChCl-Gly		Glycerol	1:2



Figure 5 Visual appearance of different natural deep eutectic solvents (NADES) prepared in this study.

extraction at power levels between 400 and 600 W resulted in a decline in sinomenine content. Consequently, 400 W was established as the optimal microwave extraction power.

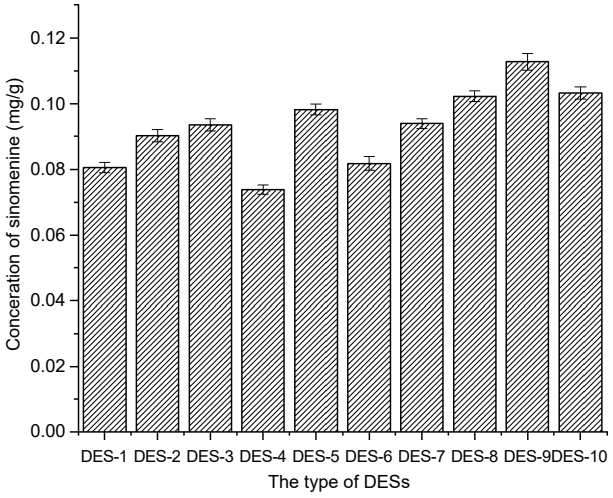


Figure 6 Effect of 10 DESs on the extraction of sinomenine.

Next, the determination of the optimum solvent water content is depicted in Fig. 7C, which shows the impact of water content in the DESs on sinomenine extraction. The results indicate that water content significantly affects the extraction process. Specifically, the content of sinomenine demonstrated an increasing trend within the 30%–50% water content range for each solvent. At 50% water content, the extraction of sinomenine reached its highest value. Conversely, when the water content was increased from 50% to 60%, a downward trend in sinomenine content was observed. Therefore, 50% water content was determined to be the optimal condition for solvent use in the extraction process.

3.3.2 Response surface experimental results of microwave extraction

The response surface optimization experiment was conducted according to the experimental scheme outlined in Table 6 and the results presented in Table 7. Data from Table 7 were analyzed using Design-Expert 8.0.6 software, which facilitated the calculation of regression coefficients. A quadratic multiple

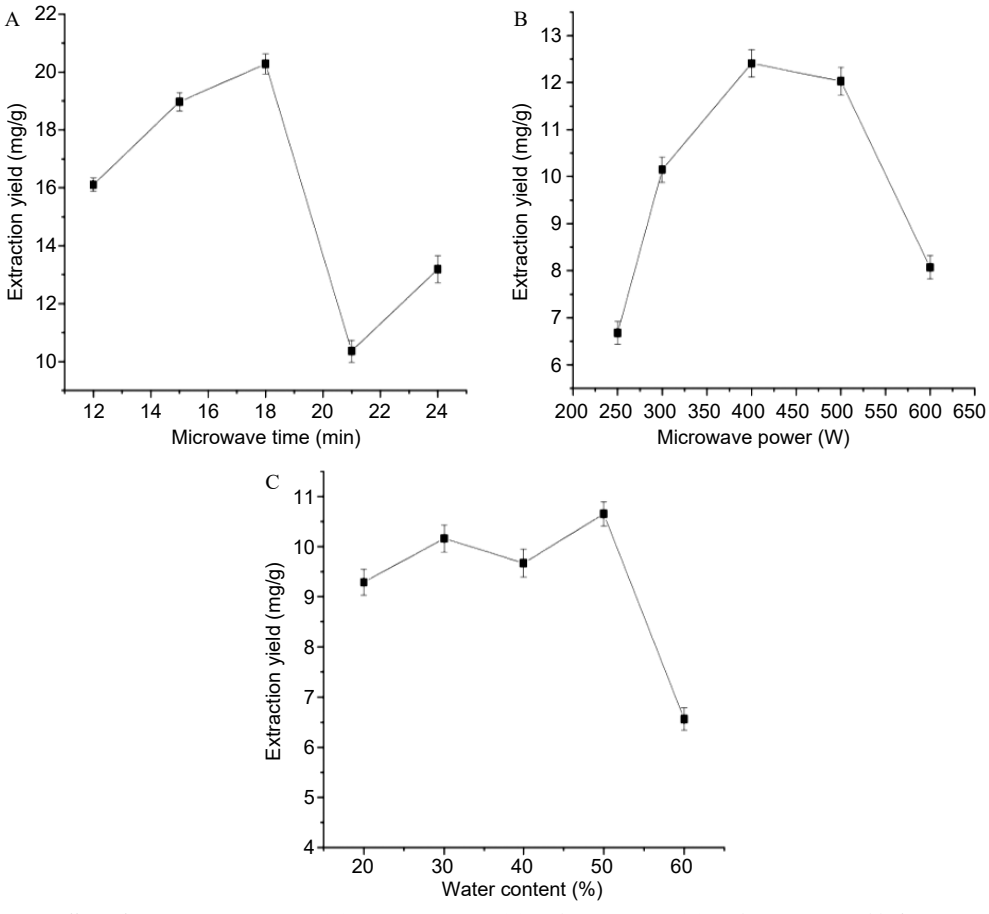


Figure 7 Effects of varying microwave time (A), microwave power (B), and water content (C) on the extraction yield of sinomenine.

regression model for the sinomenine content in microwave-assisted extraction products of *S. acutum* was obtained:

Coded equation:

$$Y = 19.68 - 0.25 \times A + 0.70 \times B + 1.55 \times C - 0.10 \times AB + 0.60 \times AC + 0.50 \times BC + 0.36 \times A^2 - 1.94 \times B^2 - 2.24 \times C^2$$

Actual equation:

$$Y = -37.85 - 2.39 \times A + 0.143,2 \times B + 1.835 \times C - 0.000,333 \times AB + 0.2 \times AC + 0.000,5 \times BC + 0.4 \times A^2 - 0.000,194 \times B^2 - 0.022,4 \times C^2$$

Table 6 Factors and levels for the Box-Behnken method

Factor level	Microwave time (min)	Microwave power (W)	Water content (%)
−1	15	300	40
0	18	400	50
1	21	500	60

In the equations, A represents microwave extraction time, B denotes microwave power, and C indicates water content. The correlation coefficient R^2 for the quadratic multiple regression equation is 0.979,9. The above response surface experimental data were analyzed by variance analysis, and the results are shown in Table 8. The P value and F value serve as parameters to evaluate the goodness of fit between the hypothesis test results and the overall model, with a smaller P value indicating greater significance, while a larger F value showing a better model fit. As shown in Table 8, the regression model exhibits a $P < 0.000,1$, indicating high significance. The loss of fit term yielded $F = 2.96$ with $P > 0.093,0$, suggesting this term is not significant and confirming the model's feasibility. Furthermore, P values less than 0.01 indicate extremely significant differences, while those less than 0.05 indicate significant differences.

Table 7 Box-Behnken design and corresponding experimental results

Runs	Microwave time (A, min)	Microwave power (B, W)	Water content (C, %)	Extraction yield (mg/g)
W-1	−1 (15)	−1 (300)	0 (50)	17.6
W-2	1 (21)	−1 (300)	0 (50)	17.6
W-3	−1 (15)	1 (500)	0 (50)	18.8
W-4	1 (21)	1 (500)	0 (50)	18.4
W-5	−1 (15)	0 (400)	−1 (40)	17.6
W-6	1 (21)	0 (400)	−1 (40)	15.6
W-7	−1 (15)	0 (400)	1 (60)	18.8
W-8	1 (21)	0 (400)	1 (60)	19.2
W-9	0 (18)	−1 (300)	−1 (40)	13.2
W-10	0 (18)	1 (500)	−1 (40)	14
W-11	0 (18)	−1 (300)	1 (60)	16
W-12	0 (18)	1 (500)	1 (60)	18.8
W-13	0 (18)	1 (500)	1 (60)	20
W-14	0 (18)	1 (500)	1 (60)	19.6
W-15	0 (18)	0 (400)	0 (50)	20
W-16	0 (18)	0 (400)	0 (50)	19.2
W-17	0 (18)	0 (400)	0 (50)	19.6

Table 8 Variance analysis for the fitted regression model

Source	Sum of squares	Degrees of freedom	Mean square	*F*-value	*P*-value	Significance
Model	65.20	9	7.24	26.30	0.000,1	Significant
A-Ultrasonic time	0.500,0	1	0.500,0	1.82	0.219,8	
B-Ultrasonic power	3.92	1	3.92	14.23	0.007,0	
C-Water contend	19.22	1	19.22	69.78	<0.000,1	
AB	0.040,0	1	0.040,0	0.145,2	0.714,4	
AC	1.44	1	1.44	5.23	0.056,1	
BC	1.000,0	1	1.000,0	3.63	0.098,4	
A^2	0.545,7	1	0.545,7	1.98	0.202,1	
B^2	15.85	1	15.85	57.53	0.000,1	
C^2	21.13	1	21.13	76.70	<0.000,1	
Residual	1.93	7	0.275,4			
Lack of fit	1.48	3	0.493,3	4.40	0.093,0	Not significant
Pure error	0.448,0	4	0.112,0			
Cor total	67.12	16				

Herein, the analysis reveals that the first-term microwave power (*B*), water content (*C*), and the second-term B^2 and C^2 were significantly different, indicating the substantial impact of microwave power and water content on sinomenine extraction. Additionally, the significant differences observed in *A*, *AC*, *BC*, and A^2 highlight the considerable influence of microwave extraction time on sinomenine extraction. The model fitting index R^2 = 0.971,3 and adjusted R^2 value of 0.934,3 are both close to 1, with the difference between them falling within an acceptable range, indicating good model stability. The predicted values align closely with the experimentally measured values, validating the model's utility for analyzing and predicting sinomenine extraction rates. Overall, the data fitting and variance analysis indicate that the experimental factors significantly influence the extraction rate of sinomenine from *S. acutum*, with statistically significant differences observed in the material-liquid ratio and extraction time. Analysis of the *F* values for each test factor revealed that the factors influencing the extraction rate, ranked from most to least significant, are water content, microwave power, and microwave extraction time.

The Design-Expert 8.0.6 software was employed to generate a 3D surface diagram based on the regression equation mentioned above, and the results are shown in Fig. 8. It can be found that microwave power, water content, and microwave extraction time demonstrate interactive effects. The response surface appears convex, indicating that, as microwave power, water content and extraction time increase, the content of sinomenine first rises and then declines, suggesting a strong correlation between the extraction rate and these three factors. The variations in contour line colors and the slopes of the response surface highlight that microwave power and water content are the primary influencing factors, with microwave extraction time following. Furthermore, the interaction between water content and microwave power is marked by significant changes in high-line color and a steep response surface slope, signifying statistically significant effects on the extraction rate of sinomenine, aligning with the *F* values reported in the variance analysis. Overall, the relative influence of various factors on sinomenine extraction from *S. acutum* can be ranked as follows: water content > microwave power > microwave extraction time. Based on the Box-Behnken response surface method, the optimal extraction process was determined to be as follows: DESs (choline chloride:lactic acid = 2:1), microwave extraction time of 20.70 min, microwave power of 423.33 W, and water content of 57.67%. Under these conditions, the content of sinomenine in *S. acutum* could reach 20.08 mg/g.

3.3.3 *Validation test*

To verify the accuracy and reliability of the process optimization experiment, a verification test was conducted based on the conditions outlined by the response surface. To enhance operational feasibility and minimize error, the microwave extraction time was adjusted to 21 min, with a microwave power of 425 W and water content of 58%. This test was repeated three times, yielding an average value of 18.08 mg/g. The minor difference between the actual and theoretical values indicates a degree of reliability in the results.

3.4 Analysis of sinomenine from *S. acutum* by microwave extraction

3.4.1 *Precision experiment results*

An HPLC experiment was conducted on a sample of *S. acutum* extracted via microwave. The sample was injected six times, and the peak area of each of the six chromatograms was recorded. The RSD of the calculated peak areas was then determined. The RSD value was found to be less than 3%, indicating that the error associated with the HPLC instrument was minimal. This low RSD suggests that the methodology employed is reliable and consistent for the analysis of sinomenine content in the extracted samples.

3.4.2 *Repetitive experiment results*

To assess the method's repeatability, the chromatographic peak areas of six samples extracted under identical extraction conditions were obtained through continuous injection. The RSD of the calculated peak areas was determined to be less than 3%, indicating that the method exhibits good repeatability.

3.4.3 *Stability experiment results*

The stability of the extracted samples was evaluated by measuring the chromatographic peak areas at 0, 4, 6, 12, and 24 h post-extraction. The RSD value of the peak areas obtained from

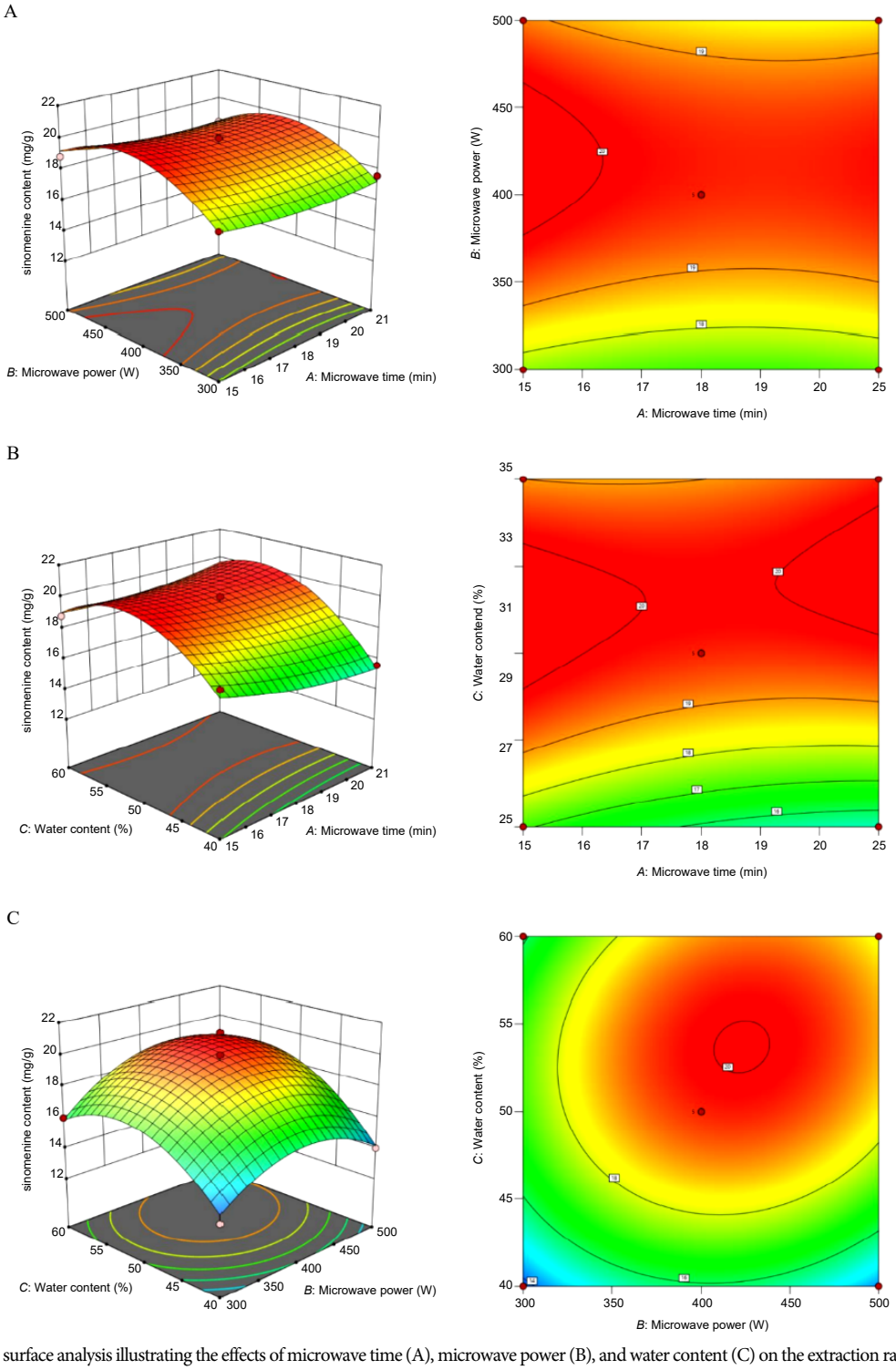


Figure 8 Response surface analysis illustrating the effects of microwave time (A), microwave power (B), and water content (C) on the extraction rate of sinomenine.

these measurements was found to be less than 3%, further demonstrating that the method has good repeatability.

3.4.4 HPLC analysis results

According to the chromatographic conditions specified in Section 2.2.4.1, both the sinomenine standard and the sinomenine sample were injected into the HPLC system. By comparing the retention times and ultraviolet absorption characteristics of the sinomenine standard with those of the sample, the chromatographic peak corresponding to sinomenine in the extracted sample was identified, as illustrated in Fig. 9.

3.5 Comparison of sinomenine content extracted by traditional solvent and DESs

To demonstrate the efficiency of natural DESs in extracting sinomenine from *S. acutum*, a fixed HPLC method and chromatographic column were utilized. The extraction solvent was altered, using traditional solvents such as ethanol for comparison. The content of sinomenine in samples obtained through ultrasonic and microwave extraction using traditional solvents was compared with that of samples extracted using DESs via ultrasonic and microwave methods. The chromatograms

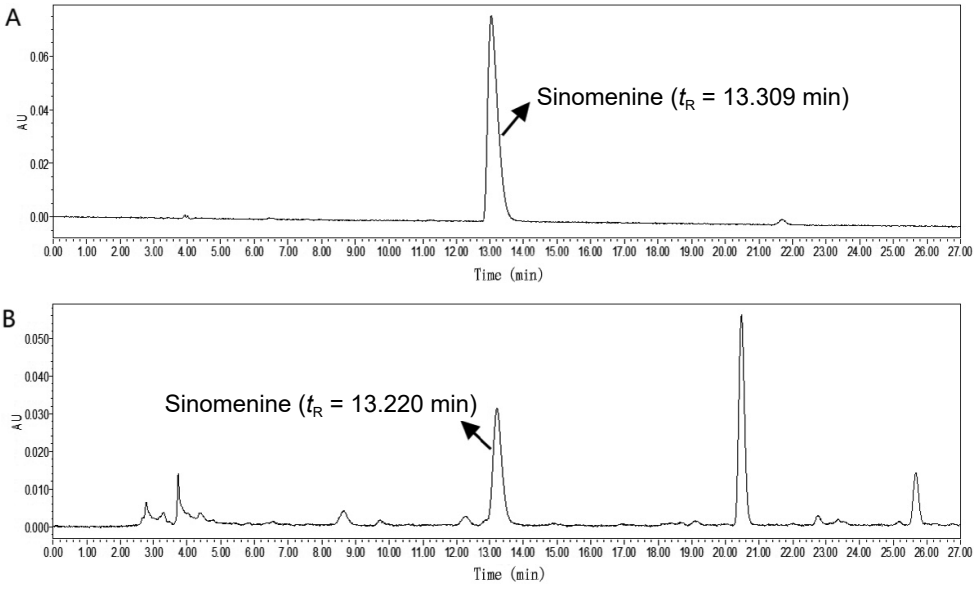


Figure 9 HPLC chromatograms of sinomenine under microwave conditions. (A) Chromatogram of the sinomenine standard, (B) chromatogram of the sinomenine sample.

illustrating the different extraction methods are presented in Fig. 10.

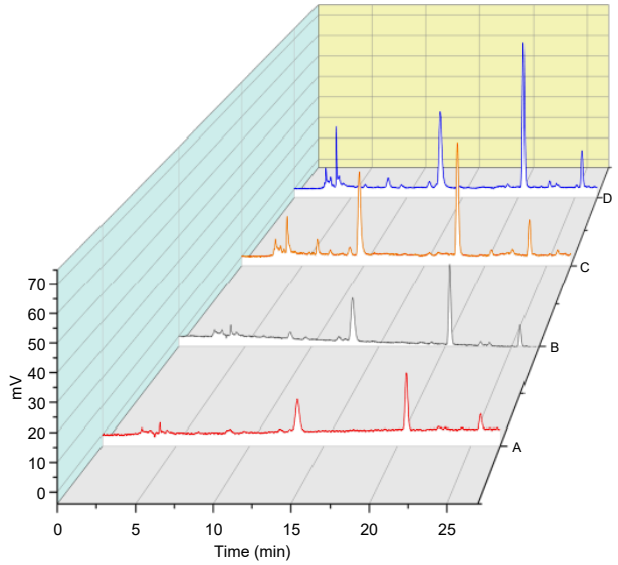


Figure 10 Chromatograms of different extraction methods. A, Ethanol-ultrasonic, B, Ethanol-microwave, C, DES-ultrasonic, D, DES-microwave.

3.5.1 *Analysis of standard curve drawing results*

According to the standard curve drawing method outlined in Section 2.4.2, a series of standard solutions with gradient mass concentrations was prepared. The concentration of the standard substances was plotted on the *X*-axis, and the corresponding chromatographic peak areas were plotted on the *Y*-axis. The resulting regression equation was $Y = 8 \times 10^6 X - 7{,}146.7$, with an R^2 value of 0.999,9, indicating excellent linearity within the concentration range of 0.01–0.4 mg/g.

3.5.2 *Calculation of the content of sinomenine*

The content of sinomenine in the extracted sample can be calculated using the following formula:

$$\text{Content (mg/g)} = \frac{(y + 7146.7) \times D \times V}{8 \times 10^6 \times M}$$

In this formula, y represents the chromatographic peak area of the sample, the variable D denotes the dilution factor of the liquid phase sample, V indicates the volume of the extraction solvent (mL), and M refers to the mass of *S. acutum* (g).

4 Conclusions

This paper presented a comprehensive study of the ultrasonic and microwave-assisted extraction of sinomenine from *S. acutum* by using DESs. Through single-factor and response surface experiments, the process of the above two extraction methods was systematically investigated. By screening nine of different DESs, and it was ultimately determined that the suitable extraction solvent for sinomenine was a combination of choline chloride and lactic acid. During the extraction, the parameters of extraction time, extraction power, and water content significantly influence the extraction efficiency, with water content exerting the greatest impact on the extraction process. For ultrasound extraction, the optimal parameters included a chloride-lactic acid (ChCl-LA) ratio of 1:2, 30% water content, a liquid-to-solid ratio of 1:20, an extraction time of 20 min, and an ultrasonic power of 240 W. In contrast, for microwave extraction, the optimal conditions were a ChCl-LA ratio of 2:1 with 57% water content, an extraction time of 20 min, and a power level of 423.33 W. Under these conditions, the sinomenine content was found to be 16.03 mg/g for ultrasound extraction and 20.08 mg/g for microwave extraction.

In summary, this study successfully investigated and optimized the ultrasonic and microwave-assisted extraction process of sinomenine based on DESs, providing a valuable reference for the comprehensive utilization of *S. acutum* resources.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

Acknowledgments

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