

Article

An Extraction Process Based on the Collaborative Extraction of *Coptis chinensis* Franch. Phytoconstituents Using a Deep Eutectic Solvent and an Organic Solvent

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Abstract: A low-cost method for the simultaneous extraction of alkaloids and water-insoluble flavonoids and esters from *Coptis chinensis* Franch. (Abbrev. *C. chinensis*) was explored to provide a reference for the production of green plant-based preparations and traditional Chinese medicine formula granules. A combined extraction method with the deep eutectic solvents (DESs) of choline chloride and urea (molar mass ratio of 1:2) and organic solvent ethanol was used, supplemented by ultrasonic-assisted extraction (ultrasonic power: 150 W; ultrasonic temperature: 60 °C; treatment time: 15 min). The extraction efficiency of the 50% DES (choline chloride and urea) aqueous solution for berberine, palmatine, jatrorrhizine, and magnoflorine was found to be the highest and was superior to traditional ultrasonic extraction and water bath reflux extraction methods. Furthermore, the flavonoids and esters from *C. chinensis* residue were extracted using ethanol. The results from high-performance liquid chromatography and gas chromatography–mass spectrometry indicated a high extraction efficiency overall.

Keywords: *Coptis chinensis* Franch.; deep eutectic solvent; organic solvent; extraction process



Citation: Liu, C.; Gong, F.; Xiong, Z.; Wang, C.; Ran, X.; Ran, J.; Li, R.; Ou, Y.; Xia, Q.; Wei, P.; et al. An Extraction Process Based on the Collaborative Extraction of *Coptis chinensis* Franch. Phytoconstituents Using a Deep Eutectic Solvent and an Organic Solvent. *Separations* **2024**, *11*, 249. <https://doi.org/10.3390/separations11080249>

Academic Editor: Aleksandra Mišan

Received: 12 July 2024

Revised: 15 August 2024

Accepted: 15 August 2024

Published: 16 August 2024



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1. Introduction

Coptis chinensis Franch. (Abbrev. *C. chinensis*) belongs to the genus *Coptis* Salisb. from the Ranunculaceae family and is widely used as a traditional Chinese medicine. The medicinal value of *C. chinensis* was first recorded in *Shen Nong's Herbal*. It is bitter-cold in nature and has the effect of clearing heat, drying dampness, clearing fire, and detoxifying toxins, and it has been used as a medicine in China for more than 2000 years [1–4]. Chinese medicine tablets are the main form of medicine used in Chinese medicine clinics. They are typically slices of Chinese herbal medicine that can be directly used in clinics after processing and concocting. The Chinese Pharmacopoeia lists different methods for processing *C. chinensis* such as stir-frying with wine, stir-frying with vinegar, stir-frying with ginger, and a preparation with *Evodia rutaecarpa* [5–8]. The purpose is to promote the dissolution of berberine, epiberberine, palmatine, jatrorrhizine, coptisine, magnoflorine, columbamine, evodiamine, rutaecarpine, chlorogenic acid, evodine, and other active ingredients in *C. chinensis* via the combined effect of alcohol, vinegar, ginger juice, or other plants [9–11]. The structures of the main compounds are illustrated in Figure 1. In addition to alkaloids, *C. chinensis* also contains sugars, volatile oils, organic acids, flavonoids, and terpenes, as well as trace elements such as copper and zinc [4]. Modern pharmacological studies have shown that *C.*

chinensis has anti-inflammatory [12], bactericidal [13], antidiarrheal [14], hypoglycemic [15], hypolipidemic [16], immunity-enhancing [17], anticancer [18], antitumor [19], and other effects. If the active components of *C. chinensis* can be extracted and prepared into Chinese patent medicine products such as formula particles, tablets, capsules, etc., it would be convenient for patients to consume and carry such preparations, and the safety and efficacy would be more guaranteed. Active phytochemicals are generally less expensive, safer, and more reliable.

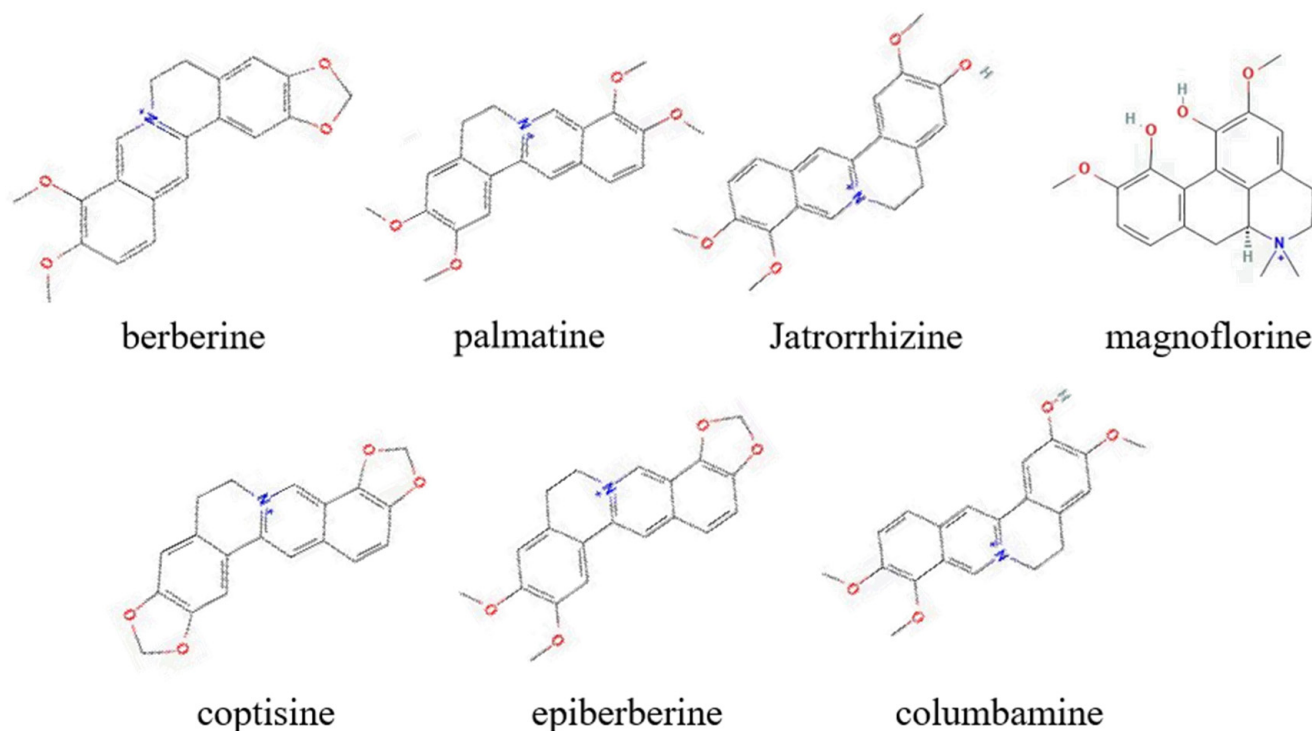


Figure 1. The structures of the main compounds of *C. chinensis*.

The extraction of active ingredients is an important step in the preparation of traditional Chinese medicine. The extraction quality is the key factor that affects the curative effect of traditional Chinese medicine preparations, and advanced and effective extraction processes are directly related to the extraction quality of their active components. The traditional methods of extraction of *C. chinensis* include ultrasonication, reflux, cold immersion, auxiliary enzyme extraction, and supercritical fluid extraction, all of which are generally associated with disadvantages such as low extraction efficiency, long extraction time, and environmental harm [20–22]. Over the years, studies on the extraction of active ingredients from *C. chinensis* have focused solely on changes in the chemical components or pharmacological effects to clarify the processing principle [12,23–25]. However, the therapeutic effect of traditional Chinese medicine results from the collective action of various chemical components. The material basis of its medicinal properties is an ordered whole that is composed of inorganic ions, organic macromolecules, and small organic molecules, and there is compatibility and a matching relationship among the monomers in the composition [26]. Furthermore, simple boiling or other traditional methods of extraction cannot completely dissolve all the medicinal components of *C. chinensis*. Therefore, there is an urgent need to identify a green and efficient extraction technology to improve the extraction efficiency and purity of the active components of *C. chinensis* and provide safer and more effective drugs for clinical use.

Deep eutectic solvent (DES) extraction is an extraction technique that utilizes a deep eutectic solvent mixture combining a certain stoichiometric ratio of hydrogen-bonded acceptors and hydrogen-bonded donors [27]. The hydroxyl, carboxyl, or amino functional

groups in the DES can form intermolecular hydrogen bonds and result in a high solubility of the components in the extract, thereby improving the extraction rate of the medicinal plant. The combination of choline chloride and urea can form various types of hydrogen bonds, including OH...O, NH...O, OH...Cl, NH...Cl, etc. These hydrogen bonds exhibit significant flexibility in both quantity and strength [28]. Although both Choline chloride and urea have high melting points, their eutectic point of a 1:2 mixture is significantly reduced. This characteristic makes the mixture liquid or solid with a low melting point at room temperature, which helps to form DESs [28]. The structure and properties of the mixture can also be adjusted through the interaction between choline chloride and urea. In particular, the formation of double ionic hydrogen bonds, like the CH...Cl hydrogen bond mode, provides comparable or greater covalency compared to neutral or ionic hydrogen bonds [29]. DES extraction is a low-cost, environmentally friendly method that is easy to implement. Owing to its good biodegradability, it has been widely used for the extraction of natural products [30,31]. Currently, there are only a few studies reporting the use of DESs in the extraction of Chinese medicinal materials, and the main focus is on the extraction of phenolic acids, flavonoids, polysaccharides, and other active ingredients [32–37]. Compared with traditional extraction solvents, DESs have better extraction effects on bioactive natural products. For example, Tang et al. extracted quercetin and myricetin from Ginkgo biloba leaves using DESs of choline chloride–oxalic acid–ethylene glycol, with final extraction rates of 1.40 and 1.11 mg·g^{−1}, respectively, which were 104.7% and 90.0% higher than that of traditional solvent ethanol [38]. Duan et al. investigated the effects of DES combinations of choline chloride, betaine, and L-proline on the extraction efficiency of phenolic acids such as salvianolic acid B, rosmarinic acid, and shikonin in Salvia miltiorrhiza. The results showed that most DES combinations exhibited higher phenolic acid extraction rates compared to methanol, with the highest extraction rate being achieved by using choline chloride/acetamide at a ratio of 1:1 [39]. Zhang et al. extracted yam polysaccharides using DESs of choline chloride-1,4-butanediol, with an average extraction rate of 15.98 ± 0.15%, which was higher than hot water extraction and water–ultrasound-assisted extraction methods [36].

In this study, a novel method to extract the active components from *C. chinensis* is reported. The alkaloids, flavonoids, and esters in *C. chinensis* can be extracted more effectively by using DESs/organic solvents. Furthermore, the influence of various extraction methods and technological parameters on extraction was compared, and the optimal extraction process was determined. Our findings are of great significance for the transition of traditional Chinese herbs for use in a clinical setting, as well as for the extraction and preparation of phytochemicals. Additionally, this method can be useful in studying the changes in chemical components before and after the preparation of herbal concoctions and serve as an important guide for revealing the mechanisms of action of these herbal preparations.

2. Materials and Methods

2.1. Materials

Standard products and reagents: choline chloride (CAS: 67-48-1, purity ≥ 99%, MACKLIN/Macklin Reagent Co., Ltd., Shanghai, China); urea (CAS No.: 57-13-6, purity ≥ 99%, J&K Bailingwei Technology Co., Ltd., Beijing, China); berberine (CAS: 2086-83-1, purity ≥ 99%, ALADDIN/Aladdin, Jebel Ali, United Arab Emirates); palmitine (CAS No.: 3486-67-7, purity ≥ 98%, MACKLIN/Macklin, Shanghai, China); jatrorrhizine (CAS No.: 3621-38-3, purity ≥ 98%, MACKLIN/Macklin, Shanghai, China); magnoflorine (CAS number: 6859-66-1, purity ≥ 98.0%, Stende, Shanghai, China); chromatographic-grade ethanol and acetonitrile (Thermo Fisher/Thermo Fisher Technology Co., Ltd., Waltham, MA, USA).

The morphology of the *C. chinensis* powder was determined using scanning electron microscopy (SEM; Quanta 650FEG, Manufacturer: FEI, Hillsboro, OR, USA). The composition of the secondary extract was determined using gas chromatography–mass spectrometry (GC-MS; 7890B-5977B, Agilent Corporation, Santa Clara, CA, USA). Infrared spectroscopy was performed using a Nicolet 6700 infrared spectrum tester (FTIR, Thermo

Feld, Waltham, MA, USA). Deionized water was prepared in the laboratory, and all other reagents were of analytical grade.

2.2. Preparation of *C. chinensis* Powder

The impurities, fibrous roots, and gathered sediments from *C. chinensis* roots were removed, and the mildewy, oily, moth-eaten, and nonmedicinal parts were discarded. After washing with water and drying in the oven at 50 °C for 5–6 h, the powder was obtained by pulverizing in a grinder for 5 min. The mixture was sifted through a second screen to remove larger particles and finally obtain the powder.

2.3. Synthesis of DES

Choline chloride (hydrogen bond acceptor) and urea (hydrogen bond donor) were added to a sealed reactor in a 1:2 molar ratio, mixed with a magnetic stirrer at 80 °C, and continuously stirred for 8–10 h until a clear liquid was formed, i.e., the DES.

2.4. Preparation of Aqueous DES

Using a beaker and measuring cylinder, the target DES was dissolved in water according to the volume fraction, and aqueous DESs with a certain water content (30%, 50%, 80%) were configured. A homogeneous and clear aqueous solution was finally obtained after shaking and allowing to stand.

2.5. Extraction of the Effective Components of *C. chinensis* Using DES

Crude extraction of the effective components of *C. chinensis* using DES: First, 2.0 g of the crude powder was accurately weighed in a flask. Next, 50 mL of different DESs were added and the samples were ultrasonicated. The ultrasonic power was 150 W, the temperature was 60 °C, and the ultrasonic time was 15 min. After extraction, the supernatant was collected and filtered through a 0.45 µm filter membrane and stored until future use.

Secondary extraction of the residue after DES extraction: The residue after extraction with the DES was transferred to a new flask. Next, 50 mL of organic solvent ethanol was added, and the samples were ultrasonicated. The ultrasonic power was 150 W, the temperature was 60 °C, and the ultrasonic time was 15 min. After extraction, the system was cooled to room temperature, and the loss in weight was made up. The supernatant was collected and filtered into a small bottle, and the filtrate was collected using a needle tube and refiltered through a 0.45 µm membrane.

Control experiments: The effective components of *C. chinensis* were extracted using water as a solvent. Briefly, 2.0 g of the powder was accurately weighed into a flask; 50 mL of water was added, and the samples were ultrasonicated. The ultrasonic power was 150 W, the temperature was 60 °C, and the ultrasonic time was 15 min. After completion, the samples were cooled slightly and weighed, and the weight lost during the process was made up. The samples were shaken and filtered through a 0.45 µm filter membrane for reserve use. Extraction in a water bath by reflux was also performed, wherein the water bath temperature was set at 80 °C and the extraction time was 3 h. After completion, the supernatant was absorbed and filtered through a 0.45 µm filter membrane for reserve use.

2.6. Content Determination

Alkaloids are the main effective components of *C. chinensis*, with berberine being the primary component with the highest content (up to 10%). In addition, *C. chinensis* also contains palmatine, coptisine, jatrorrhizine, and magnoflorine, which account for 70–80% of the total alkaloid content [40,41]. However, due to the poor water solubility of coptisine, the 4 alkaloids berberine, palmatine, jatrorrhizine, and magnoflorine were selected as the targets to study the extraction effect of DESs. Appropriate amounts of the control products berberine, palmatine, jatrorrhizine, and magnoflorine were accurately weighed and dissolved in methanol to prepare control solutions with a concentration of 1 mg/mL. An appropriate amount of the control solution was diluted with water to make a

mixed control solution with appropriate concentration, shaken well, and passed through a 0.45 µm filter membrane as a spare. The mixed control solution was filled in a 10 mL volumetric flask and diluted with methanol to yield different concentrations of berberine (5, 10, 20, 50, 100 µg/mL), palmatine (5, 10, 20, 50, 100 µg/mL), jatrorrhizine (5, 10, 20, 50, 100 µg/mL), and magnoflorine (5, 10, 20, 50, 100 µg/mL) solutions and stored in a refrigerator at 4 °C until subsequent use.

HPLC conditions: Chromatography was performed using a Waters Xbridge C18 (4.6 × 150 mm, 5 µm) column with acetonitrile as mobile phase A and water as mobile phase B (containing 0.10% formic acid). The gradient elution method was used (0–2 min, 0% A → 5% A; 2–4 min, 5% A → 95% A; 4–30 min, 95% A). Prebalancing with mobile phase A-B (50:50) was performed for 5 min before each injection. The flow rate was 1.2 mL/min, the column temperature was 40 °C, and the injection volume was 5 µL.

2.7. GC-MS

A DB-5MS chromatographic column (30 mm × 250 mm, 0.25 µm) was used: 5% phenyl methyl siloxane; inlet temperature: 260 °C; injection mode: no shutter; flow rate: 1 mL/min; column pressure: 9.08 psi. Mass spectrum conditions: ion source temperature: 250 °C; transmission temperature: 280 °C; solvent switching time: 2 min; ion source: electron bombardment source; scanning mode: Scan.

2.8. SEM

The sample was mounted to a conductive adhesive surface to avoid contamination during the process, and then, the sample surface was sputter-coated with gold. SEM conditions were high-vacuum mode, and the acceleration voltage was 15 keV.

2.9. Infrared Spectroscopy

Infrared spectroscopy was performed using the KBr compression method or KBr wafer direct measurement method. Infrared spectroscopy conditions: wave-number range 850–4000 cm^{−1}, resolution ratio 4–8 cm^{−1}, and scan number 32.

3. Results and Discussion

3.1. Method Validation

In this study, a rapid, sensitive, and specific HPLC method was developed for the determination of berberine, palmatine, jatrorrhizine, and magnoflorine in *C. chinensis* powder. The results show that the method is accurate and convenient to perform.

3.1.1. Linear Relationship

The control solutions of berberine, palmatine, jatrorrhizine, and magnoflorine were each analyzed using HPLC as described in Section 2.5 to determine the retention time of each analyte. An appropriate amount of the control solution was taken in the sample for determination, and the mass concentration of the control solution was taken as the abscissa and the peak integral area as the ordinate for regression. The mass concentration at S/N = 10 was used as the detection limit, and the mass concentration at S/N = 3 was used as the quantitation limit (Table 1). The linear relationship of each component was good in the range of 5.0–100.0 µg/mL.

Table 1. Linear relationships of four target components.

Component	Linear Equation	r ²	Concentration Range/µg·mL ^{−1}	Detection Limit/µg·mL ^{−1}	Quantification Limit/µg·mL ^{−1}
Berberine	Y = 44536 + 11.87 × X	0.9992	5.0~100.0	1.0 × 10 ^{−2}	5 × 10 ^{−3}
Palmatine	Y = 65567 + 12.36 × X	0.9997	5.0~100.0	1.0 × 10 ^{−2}	5 × 10 ^{−3}
Jatrorrhizine	Y = 42643 + 8.63 × X	0.9996	5.0~100.0	1.0 × 10 ^{−2}	5 × 10 ^{−3}
Magnoflorine	Y = 61237 + 12.66 × X	0.9997	5.0~100.0	1.0 × 10 ^{−2}	5 × 10 ^{−3}

3.1.2. Sample Recovery

A sample solution of 0.5 mL of each component was precisely measured, and the control product was precisely added to prepare and analyze the test solution. The recoveries of berberine, palmatine, jatrorrhizine, and magnoflorine were in the range of 92.84–113.72%, indicating that the recoveries using this method were good (Table 2).

Table 2. Recovery rates of four target components.

Component	Original Concentration/ $\mu\text{g}\cdot\text{mL}^{-1}$	Concentration after Addition/ $\mu\text{g}\cdot\text{mL}^{-1}$	Monitoring Concentration/ $\mu\text{g}\cdot\text{mL}^{-1}$	Recovery Rate/%
Berberine	50.00	75.00	78.43	113.72
Palmatine	50.00	75.00	76.86	107.44
Jatrorrhizine	50.00	75.00	75.63	102.52
Magnoflorine	50.00	75.00	73.21	92.84

3.2. Effect of DES with Different Water Content Levels on the Extraction Rate of *C. chinensis*

DES was dissolved in deionized water according to the volume fraction, and aqueous DESs with different water content levels (30%, 50%, 80%) were configured. The contents of berberine, palmatine, jatrorrhizine, and magnoflorine in the solutions were determined after DES extraction with choline chloride/urea with different water content levels, ultrasonic extraction, and water bath reflux extraction (Table 3).

Table 3. Extraction effects of four target components using different methods.

Component	DES-30% / $\mu\text{g}\cdot\text{mL}^{-1}$	DES-50% / $\mu\text{g}\cdot\text{mL}^{-1}$	DES-80% / $\mu\text{g}\cdot\text{mL}^{-1}$	Ultrasonic Extraction/ $\mu\text{g}\cdot\text{mL}^{-1}$	Water Bath Reflux Extraction/ $\mu\text{g}\cdot\text{mL}^{-1}$
Berberine	75.87	79.23	57.97	67.89	74.21
Palmatine	12.44	15.63	10.87	7.86	12.57
Jatrorrhizine	17.45	21.85	15.87	12.34	14.56
Magnoflorine	20.68	26.79	16.87	11.34	18.64

The test results indicated that the extraction efficiency of aqueous DESs was higher than that of traditional ultrasonic extraction and water bath reflux extraction in terms of the individual contents of berberine, palmatine, jatrorrhizine, and magnoflorine, as well as the total contents of the four alkaloids (see Figure 2). It is speculated that in the mixed DES system, these four alkaloids are more likely to form strong intermolecular hydrogen bonds with the DESs, resulting in their better solubility in DESs [38,42,43] and, thus, higher extraction efficiency.

The results also showed that the 50% aqueous DES had the best extraction efficiency for berberine, palmatine, jatrorrhizine, and magnoflorine. It has been reported that different eutectic solvents have different effects on the extraction rate. A 1:2 molar ratio of urea as a hydrogen bond donor in deep eutectic solvents and choline chloride as a hydrogen bond acceptor is the ideal ratio reported in the literature. Under these conditions, the hydrogen bond force is the strongest [44–46]. Aqueous DESs with different contents exhibit different properties in terms of polarity, solubility, viscosity, surface tension, and other parameters, and these differences affect the equilibrium and solubility of berberine, palmatine, jatrorrhizine, and magnoflorine [47,48], resulting in a difference in the extraction rate of the alkaloids in *C. chinensis* powder extracted with DES with different water content levels.

The HPLC spectra of the aqueous DES extracts under optimal conditions were obtained (Figure 3). Based on the peak positions of the standard solutions of the four substances measured using HPLC, a peak time of 13.38 min corresponded to jatrorrhizine, a peak time of 13.74 min corresponded to magnoflorine, a peak time of 10.97 min corresponded to palmatine, and a peak time of 10.15 min corresponded to berberine. The peaks of the four alkaloids are obvious in the 50% aqueous DES, further indicating the higher extraction

efficiency. It is worth noting that in addition to the four substances mentioned above, there are peaks that are attributable to other substances in the extract prepared using 50% aqueous DES, which are the other compounds extracted from the *C. chinensis* powder.

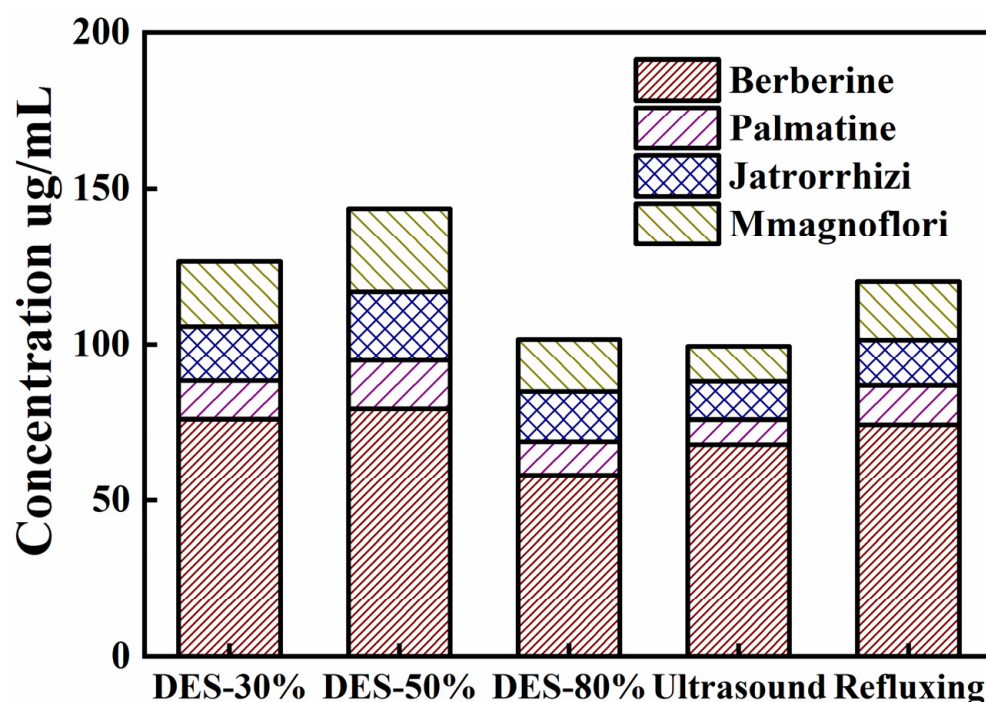


Figure 2. Comparison of extraction effects of four target components.

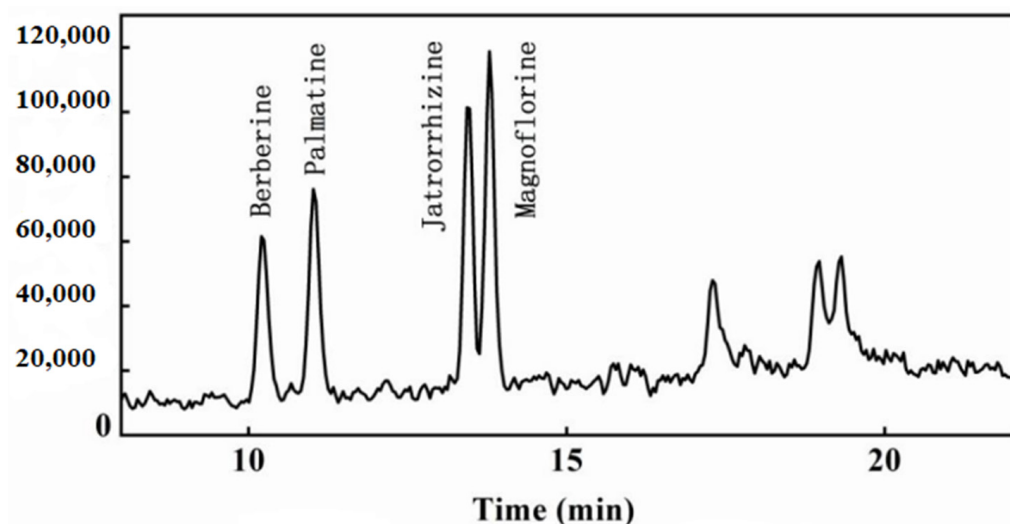


Figure 3. HPLC chromatogram of the extraction solution using a 50% DES aqueous solution.

3.3. Optimization of DES Extraction

During the extraction of the functional components of *C. chinensis* powder using DESs, the main influencing factors include the role of ultrasonication (auxiliary power, temperature, and treatment time). Ultrasound can produce high-speed eddies and strong cavitation and stirring effects, thereby destroying the cell walls of plant materials and promoting the diffusion of target products to the solvent. This, in turn, accelerates and enhances the dissolution and extraction of alkaloids [12]. Therefore, a single-factor test (Table 4) was used in this study. Taking the extraction rates of berberine, palmatine, jatrorrhizine, and magnoflorine as indicators, the effects of the ultrasonic auxiliary power

(80, 150, and 200 W), reaction temperature (40, 50, and 60 °C), and treatment time (5, 15, and 25 min) on the extraction efficiency of alkaloids were investigated using a 50% aqueous DES.

Table 4. Design table for single-factor experiment.

Variables		Level		
Power (W)	80	150	200	
Temperature (°C)	40	50	60	
Time (min)	5	15	25	

The effect of the ultrasonic auxiliary power (80, 150, and 200 W) on the extraction of the four alkaloids based on a 50% aqueous DES to extract the active components of *C. chinensis* powder is shown in Figure 4. At high powers (150 and 200 W), the extraction efficiency for berberine, palmatine, jatrorrhizine, and magnoflorine improved significantly, likely because a higher ultrasonic power can provide sufficient energy for the rupture of cell walls, whereas a lower ultrasonic power is not adequate to destroy the cell walls and completely dissolve the components [49]. When the power was 150 W and 200 W, the extraction rate did not differ much. A very high ultrasonic power may make it challenging to control the extraction temperature, resulting in the degradation of some active ingredients. Therefore, 150 W was selected as the ultrasonic extraction power. At 150 W, the extraction yields of berberine, palmatine, jatrorrhizine, and magnoflorine were 75.87, 12.87, 17.98, and 27.97 µg/mL, respectively.

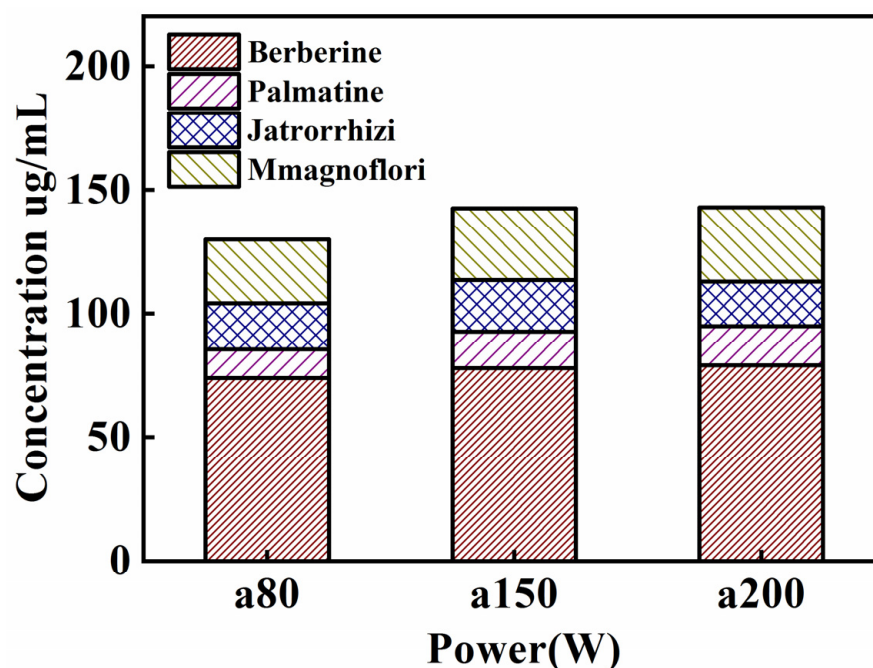


Figure 4. Comparison of extraction efficiency under different ultrasonic powers.

The extraction temperature is an important factor affecting the extraction rate, and the extraction yield will differ at different temperatures. The effect of the ultrasonic treatment temperature (40, 50, and 60 °C) on the yields of the four alkaloids from *C. chinensis* powder extracted using 50% aqueous DES is shown in Figure 5. The yields of berberine, palmatine, jatrorrhizine, and magnoflorine gradually increased with an increase in extraction temperature. Therefore, we chose 60 °C as the processing temperature for ultrasonic extraction. As most active components in plants are destroyed when the treatment temperature exceeds 60 °C [7], higher processing temperatures were not evaluated in this study.

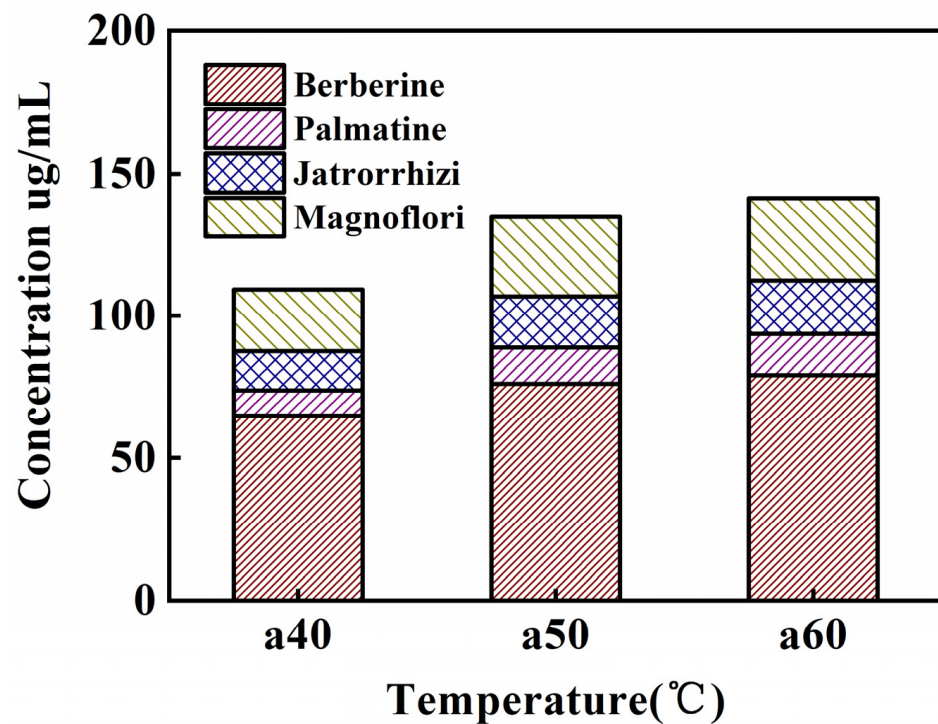


Figure 5. Comparison of extraction efficiencies at different treatment temperatures.

The extraction time is another important factor affecting extraction. A very short extraction time leads to incomplete extraction, whereas a prolonged extraction time may destroy the active components and cause losses. The effect of the ultrasonic treatment time (5, 15, and 20 min) on the yields of the four alkaloids from *C. chinensis* powder using a 50% aqueous DES is shown in Figure 6. When the extraction time was 5–25 min, the yield gradually increased with an increase in time. After 15 min, as the extraction time continued to increase, the yield increased gradually. Considering energy consumption and other factors, the optimal extraction time was determined to be approximately 15 min.

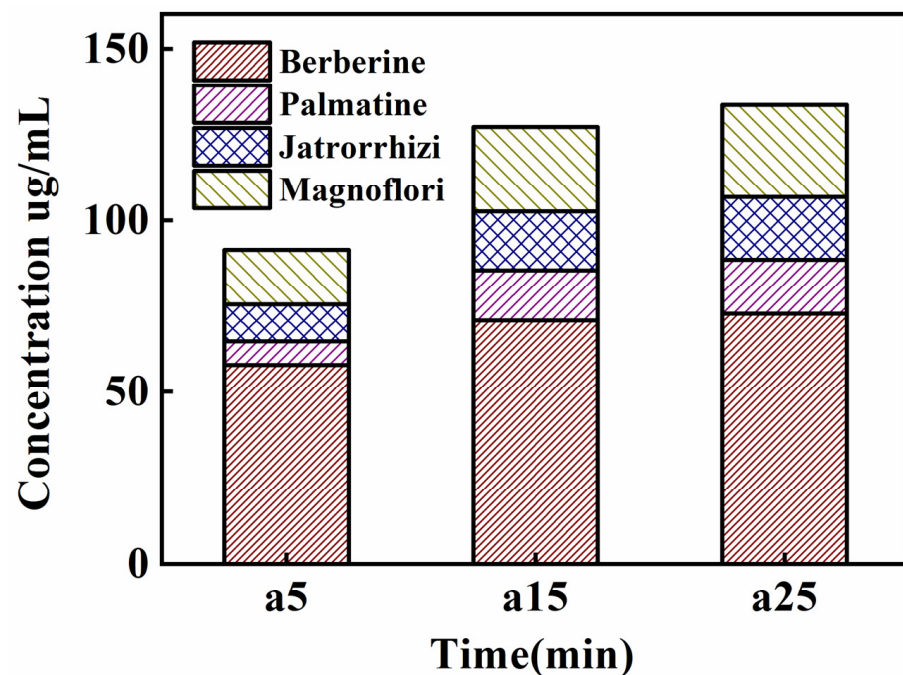


Figure 6. Comparison of extraction efficiencies at different treatment times.

Therefore, the optimal parameters were confirmed based on a 50% aqueous DES in combination with ultrasonic-assisted extraction (ultrasound power: 150 W; ultrasound temperature: 60 °C; processing time: 15 min). Under these conditions, the yields of berberine, palmatine, jatrorrhizine, and magnoflorine were 79.23, 15.63, 21.85, and 26.79 µg/mL, respectively.

3.4. Secondary Extraction of *C. chinensis* Residue after Extraction with DES

After extraction with the DES, the residue was filtered and dried, and the extraction was continued with ethanol. The extracted organic solution was analyzed using GC-MS (Figure 7). As shown in the total ion flow diagram, no component was detected until 15 min, as the low-boiling and volatile substances in the residue were already extracted or consumed during the previous extraction step.

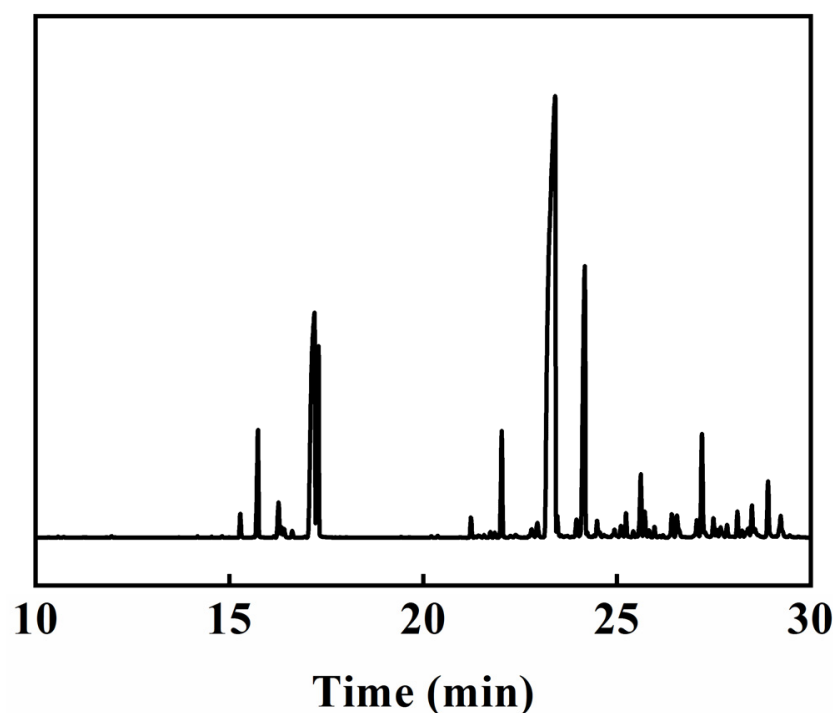


Figure 7. TIC diagram of GCMS test for ethanol extraction solvent.

The GC-MS revealed up to 20 kinds of alkenes, in addition to ketones, esters, and other substances, in the secondary ethanol extract. The results are summarized in Table 5.

Table 5. Matching results of GC-MS test.

Compound Number (#)	Retention Time (min)	Name	CAS Number	Relative Content
1	14.186	(+)-3-Carene	498-15-7	0.0296%
2	14.81	Fenchyl acetate	13851-11-1	0.0365%
3	16.267	D-Limonene	5989-27-5	0.9304%
4	16.413	endo-Borneol	507-70-0	0.2203%
5	16.623	gamma.-Terpinene	99-85-4	0.1439%
6	17.299	2-Carene	554-61-0	5.8446%
7	21.239	alpha.-Cubebene	17699-14-8	0.4279%
8	21.431	Eugenol	97-53-0	0.0892%
9	21.577	1,3-Diethyl-4-methylbenzene	1758-85-6	0.0676%
10	22.032	Copaene	3856-25-5	2.5769%
11	22.253	beta.-Bourbonene	5208-59-3	0.0714%
12	22.725	Methyleugenol	93-15-2	0.0301%
13	23.407	Caryophyllene	87-44-5	44.6313%
14	23.466	Aromandendrene	489-39-4	0.4429%

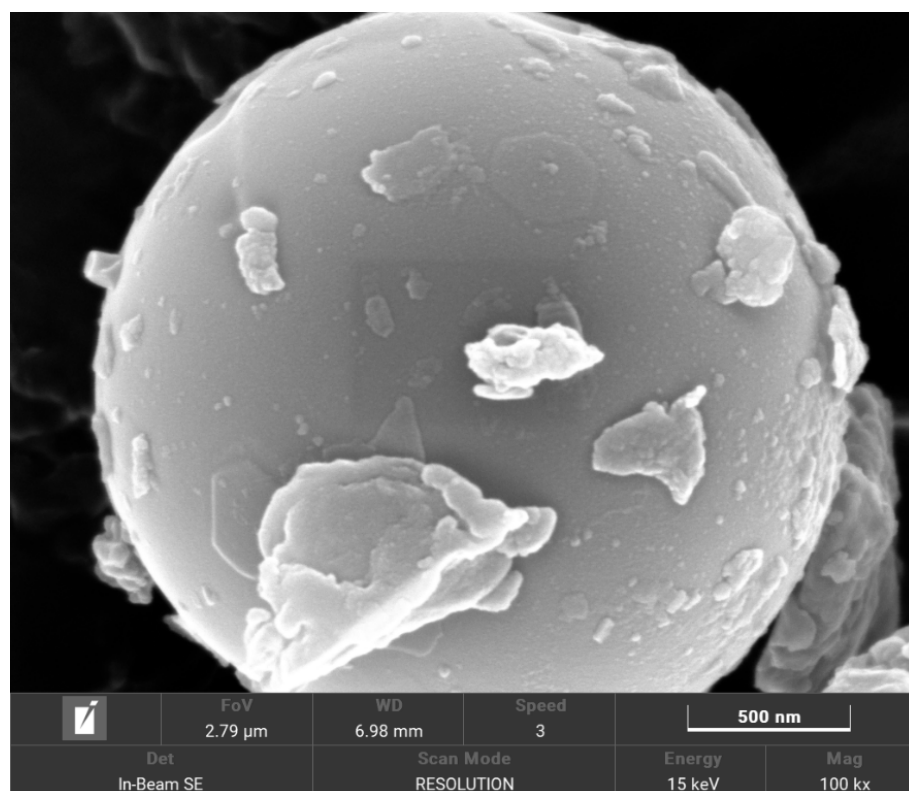
Table 5. Cont.

Compound Number (#)	Retention Time (min)	Name	CAS Number	Relative Content
15	24.177	Humulene	6753-98-6	10.4788%
16	24.235	Alloaromadendrene	25246-27-9	0.1454%
17	24.561	gamma.-Muurolene	30021-74-0	0.1399%
18	25.721	trans-Calamenene	73209-42-4	0.7079%
19	25.832	alpha.-Guaiene	3691/12/1	0.1815%
20	25.972	Cubenene	29837-12-5	0.2501%
21	26.193	alpha.-Calacorene	21391-99-1	0.0562%
22	27.196	Longifolene	475-20-7	2.8377%
23	27.277	beta.-Guaiene	88-84-6	0.1401%
24	27.581	cis.-alpha.-Bisabolene	29837-07-8	0.1668%
25	27.68	beta.-Longipinene	41432-70-6	0.2350%
26	27.843	alpha.-Farnesene	502-61-4	0.2725%
27	28.391	Neoisolongifolene,8,9-dehydro	67517-14-0	0.3519%
28	28.566	Germacrene D	23986-74-5	0.2943%
29	29.463	aR-Himachalene	19419-67-1	0.0692%
30	44.542	trans-Isoeugenol	5932-68-3	0.6854%
31	45.888	Spiro[5.5]undec-8-en-1-one	7353-75-5	0.0323%
32	49.246	Eugenol	97-53-0	0.2447%

3.5. Structural Characterization of *C. chinensis* Powder Extracted Using DES at Different Stages

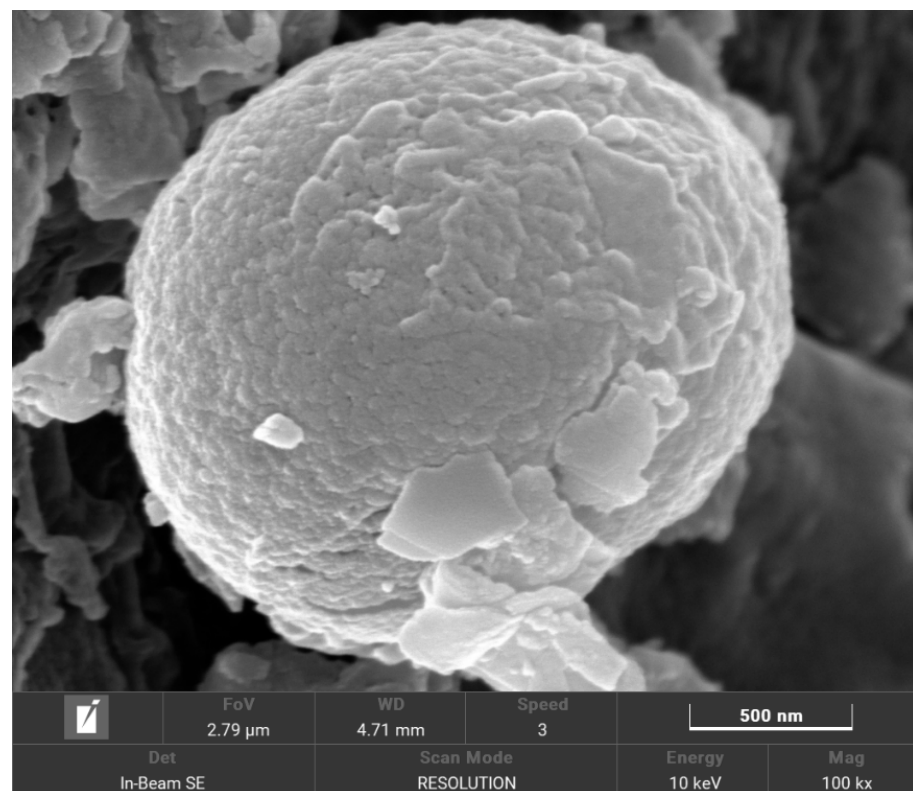
3.5.1. The Effect of Different Extraction Stages on the Morphology of *C. chinensis* Powder

SEM was used to observe the microstructures of *C. chinensis* powder before extraction, after extraction with DES, and after ethanol extraction of the dried powder after the first extraction to compare the microstructural changes in the samples at different extraction stages. Under the same microscopic magnification, the microstructure showed considerable differences (Figure 8a–c).

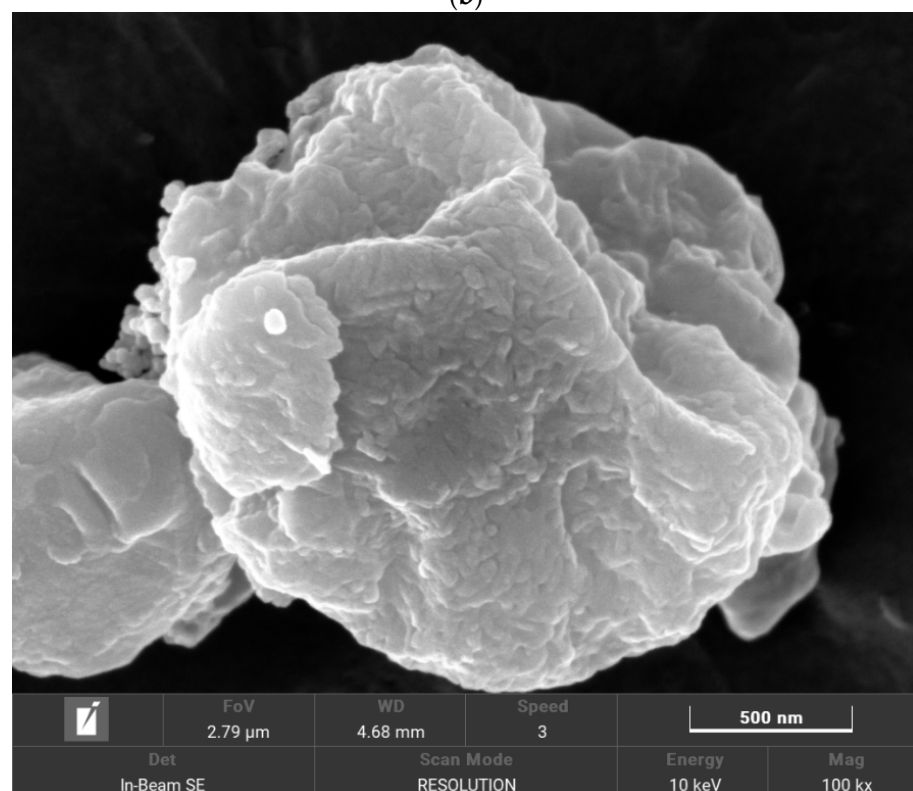


(a)

Figure 8. Cont.



(b)



(c)

Figure 8. (a) SEM image of unextracted powder particles of *Coptis chinensis*; (b) SEM image of *Coptis chinensis* powder particles after DES extraction; (c) SEM image of *Coptis chinensis* powder particles after ethanol extraction.

Before sample extraction, the particle surface was smooth and predominantly intact, indicating the characteristics of complete powder particles (Figure 8a). Figure 8b shows the morphology of *C. chinensis* powder extracted using DESs. The surface shows collapse and obvious damage, which may be caused by the dissolution of some components. This finding indirectly verified that the DES had a strong dissolution ability for the alkaloids of *C. chinensis* including jatrorrhizine, magnoflorine, berberine, and palmatine [34,50,51]. The microstructure of the dried residue extracted with ethanol is shown in Figure 8c. The particles appear more “dry”, indicating further extraction of the additional components in the powder and further verifying the complementarity of the extraction ability of the DES and the organic solvent ethanol.

3.5.2. Comparison of the Infrared Spectra of *C. chinensis* Powder at Different Extraction Stages

To compare the changes in the overall composition of the *C. chinensis* powder samples at different extraction stages, the infrared spectrum of the powder before extraction, after extraction using DES, and after extraction of the residue using ethanol were compared (Figure 9).

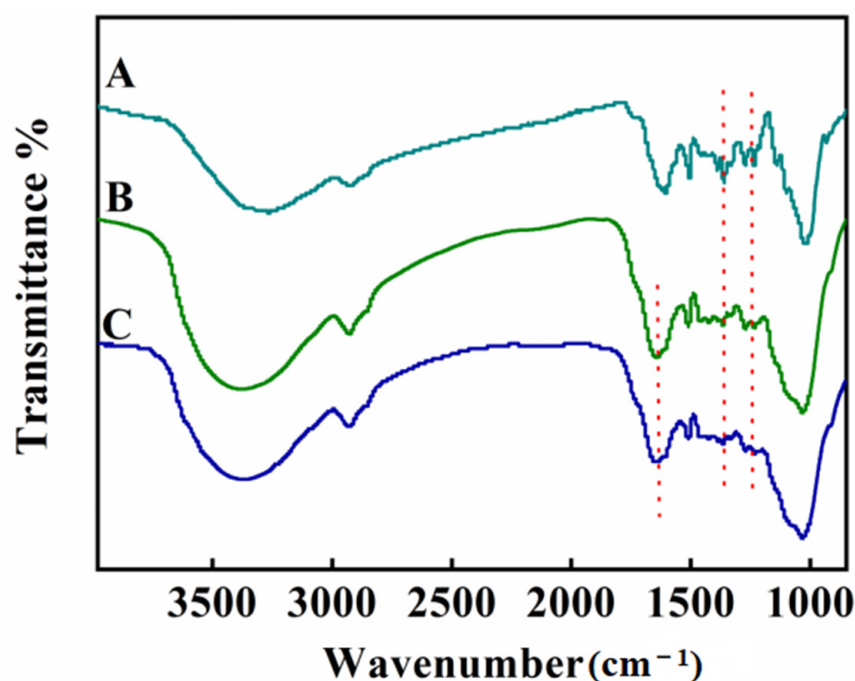


Figure 9. Comparison of FTIR spectra at different extraction stages (Curve A represents the infrared spectrum of *Coptis chinensis* powder before extraction; Curve B represents the infrared spectrum of *Coptis chinensis* powder after DES extraction; Curve C represents the infrared spectrum of the remaining residue after ethanol extraction).

Curve A shows the infrared spectrum of *C. chinensis* powder before extraction, with the characteristic peaks mainly being distributed in the regions of 1500–1700 cm^{-1} and 1000–1200 cm^{-1} . The characteristic peaks in the region of 1500–1700 cm^{-1} are mainly attributable to the C=C stretching vibration and C=O stretching vibration of the benzene ring structure, whereas the characteristic peaks in the region of 1000–1200 cm^{-1} are mainly due to the C-O stretching vibration and C-H bending vibration of the phenylpropyl structure. The lignin in *C. chinensis* Franch. powder mainly contributes to these signals. In addition, there are signals generated by the C-N bond vibration in the 1250–1350 cm^{-1} region, and the peaks generated by carbonyl group vibration at around 1600 cm^{-1} . After extraction with an aqueous DES, the signal in the region of 1250–1350 cm^{-1} reduced significantly. This was likely due to the numerous alkaloidal components such as berberine, palmatine, jatrorrhizine, and magnoflorine, which were extracted from the sample, thereby weakening

the vibration signal of the C-N bond. The infrared spectrum (Curve C, Figure 9) of the residue extracted using ethanol continued to show a decrease at about 1600 cm^{-1} compared with that of *C. chinensis* powder extracted using DES (Curve B, Figure 9), indicating that after ethanol extraction, the powder continued to lose organic components such as alenes and esters.

Curve A is the infrared spectrum of *C. chinensis* powder before extraction, Curve B is the infrared spectrum of the powder after extraction with DES, and Curve C is the infrared spectrum of the residue after ethanol extraction.

3.6. Methodological Summary

Aqueous DES could extract the active ingredients of *C. chinensis* more efficiently, especially the alkaloids berberine, palmatine, jatrorrhizine, and magnoflorine. However, aqueous DES could not effectively extract the water-insoluble alkaloids such as coptisine and other components such as flavonoids and esters. Therefore, based on the organic/inorganic composite extraction theory, we continued to extract the powder residue with ethanol after DES extraction to obtain the active ingredients from *C. chinensis*. The extraction process is shown in Figure 10.

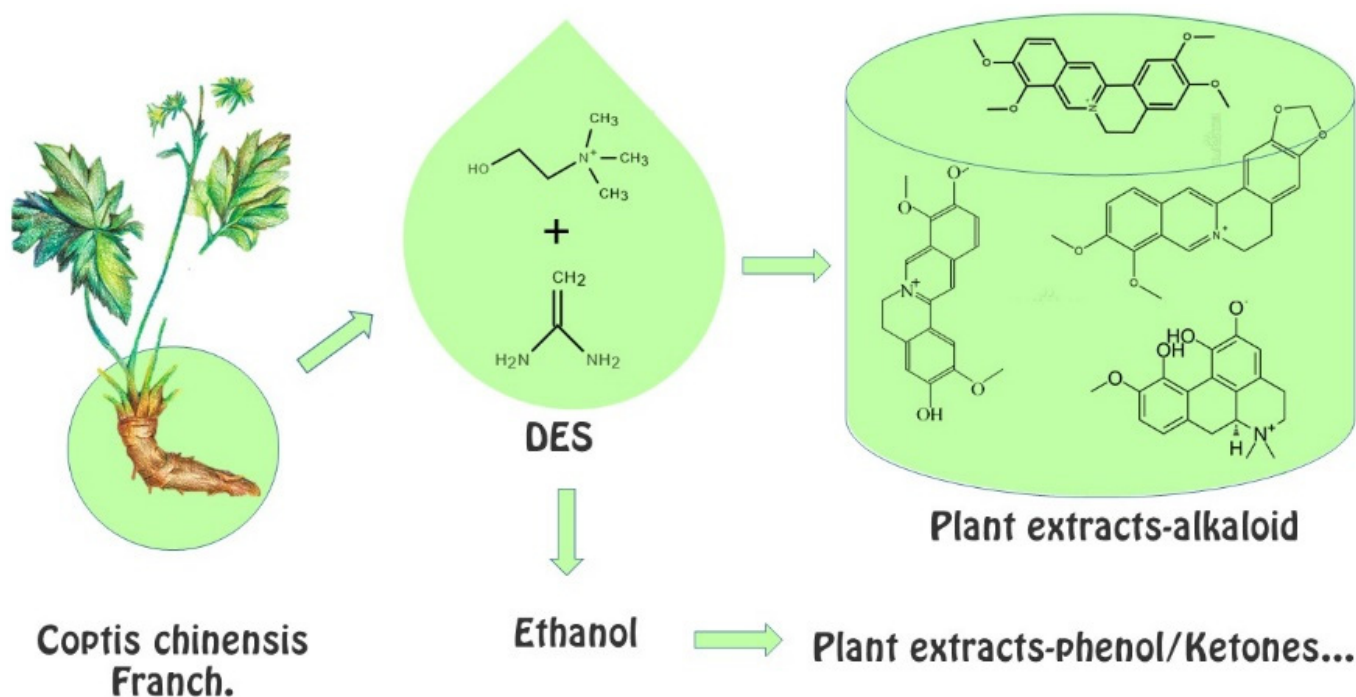


Figure 10. Schematic diagram of synergistic extraction of active components from *Coptis chinensis* using deep eutectic solvent (DES) and organic solvent.

4. Conclusions

In this study, a low-cost method for the simultaneous extraction of alkaloids and water-insoluble flavonoids and esters from *C. chinensis* is reported, which could serve as a reference for the production of green plant-based preparations and finished products of traditional Chinese medicine formulations such as granules, tablets, and capsules. The method is specified as follows: (1) *C. chinensis* was cleaned and dried at $50\text{ }^{\circ}\text{C}$ for 5–6 h and crushed (for 5 min) into a powder; (2) a DES composed of choline chloride and urea in a 1:2 molar mass ratio was used as the extraction solvent, and ultrasonication was used to aid extraction; (3) the residue was extracted with a combination of ethanol and ultrasonication, and the collaborative extraction with DES and organic solvents was thus accomplished. The results demonstrated that 50% DES (choline chloride–urea) had the highest extraction efficiency of the active ingredients of *C. chinensis* and was superior to that

achieved using traditional ultrasonic extraction and water bath reflux extraction methods. The optimal parameters for ultrasound-assisted extraction were an ultrasound power of 150 W, ultrasound temperature of 60 °C, and processing time of 15 min. The results of the secondary extraction of the residue using ethanol showed that the alkenes, ketones, and esters in *C. chinensis* continued to be extracted. Furthermore, the results from both HPLC and GC-MS showed an overall high extraction efficiency of the multiple constituents of *C. chinensis*. SEM observations of the *C. chinensis* powder before and after extraction also proved this aspect based on the appearance.

Author Contributions: Conceptualization, C.L. and Z.X.; methodology, F.G.; software, C.W.; validation, X.R.; formal analysis, J.R.; investigation, R.L.; resources, R.L.; data curation, Y.O.; writing—original draft preparation, C.L. and F.G.; writing—review and editing, Z.X.; supervision, Q.X.; project administration, P.W.; funding acquisition, J.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Scientific and Technological Research Program of Chongqing Municipal Education Commission (Grant No. KJQN202101626 and KJQN202201606), Chongqing Special Project for Technological Innovation and Application Development (grant number: CSTB2023TIAD-LDX0012), Chongqing University of Education High-level Talents Scientific Research Startup Project (grant number: 2023BSRC014), and School-level Research Program of Chongqing University of Education (grant number: KY202301A). This study was supported by the National Natural Science Foundation of China (22204010), the Natural Science Foundation Project of CQ CSTC (Grant No. cstc2021jcyj-msxmX0156), and the Science and Technology Research Program of Chongqing Municipal Education Commission (Grant No. KJQN202201627).

Data Availability Statement: All data in this manuscript are available from the corresponding author by e-mail.

Conflicts of Interest: The authors declare no conflicts of interest.

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