Efficient Extraction and Purification of Isofraxidin from Acanthopanax Senticosus by DES

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Article

Efficient Extraction and Purification of Isofraxidin from *Acanthopanax senticosus* by DES

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Abstract: *Acanthopanax senticosus* (rupr. rt. Maxim.; AS) is a medicinal plant used in the clinical treatment of cerebrovascular diseases and central nervous system disorders, and significantly improves blood lipid levels and endothelial cell function in patients with acute cerebral infarction. Isofraxidin, one of the active ingredients of AS, is the core of the medical effects, and its extraction and purification depend on organic solvents. Deep eutectic solvents (DESs) are new green solvents, synthesized by intermolecular hydrogen bonding between hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA), which have non-toxic, high separation and purification efficiency, and environmentally friendly compared with traditional organic solvents. In this paper, DES was used for the extraction of isofraxidin from AS. The primary findings demonstrated that the DES had a viscosity higher than that of ethanol, and even added a tiny amount of water (about 10%) would trigger solvent redistribution, led to a considerable reduction in solvent viscosity. In comparison to ethanol, the extraction rate of isofraxidin by DES was 2-3 times higher. Thus, this work developed a new technique for using green extraction of isofraxidin that has some practical implications.

Keywords: *Acanthopanax senticosus*; deep eutectic solvents; isofraxidin; extraction

1. Introduction

A clonal plant species *Acanthopanax senticosus* (rupr. rt. Maxim.; AS) is capable of sexual seed reproduction in its native environment. Additionally, clone propagation is mostly practiced in northeast China; it is also present in Russia, South Korea, Korea, and Japan [1].

The active ingredients in AS are mainly polysaccharides, glycosides, terpenoids, lignans, flavonoids, and coumarins. The syringin, also known as Eleutheroside B, are terpenoids and are widely found in the leaves of AS; Eleutheroside E is a class of lignan monomers extracted from AS and is mainly found in the fruits of AS; quercetin is a flavonoid and is widely found in the fruits and leaves of AS[1]. Isofraxidin is a hydroxycoumarin monomeric substance that is widely found in the roots, leaves, and fruits of AS, and has a variety of physiological and pharmacological activities. Isofraxidin plays a wide range of pharmacological roles in various diseases such as osteoarthritis [2], cancer [3, 4], disorders of lipid metabolism [5], and Alzheimer's diseases [6]. Previous studies have shown that isofraxidin inhibits human hepatocellular carcinoma cell invasion by affecting MMP-7 expression. In hepatocellular carcinoma cells, isofraxidin inhibits ERK1/2 phosphorylation and reduces iNOS and COX-2 expression, and isofraxidin also inhibits the formation of TLR4/myeloid differentiation protein 2 (MD-2) complexes [7-8]. It can promote immune cells to regulate the immune system more efficiently (such as T cells, B cells, NK cells, and M cells), mediate interleukin and interferon, stimulate cytokines like tumor necrosis factor, enhance human immunity, and eventually execute its anti-tumor activity [9-10]. It also protected mice from acute lung injury by inhibiting cyclooxygenase-2 (COX-2) protein expression and decreasing inflammatory cell infiltration into lung tissues [11]. Pretreatment with isofraxidin prior to IL-1 could inhibit IL-1-stimulated expression of iNOS and COX-2, which in turn blocked the generation of nitric oxide (NO) and prostaglandin E2 in chondrocytes isolated from osteoarthritis patients (PGE2). Furthermore, matrix metalloproteinases'

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mRNA levels and secretion were considerably decreased by isofraxidin (MMPs). It was determined that isofraxidin controlled NF-B signaling to prevent IL-1-induced joint inflammation[12].

The varying distribution coefficients KD of solutes in various solvents are the basis for the separation and purification process known as organic solvent extraction. Numerous continuous extractions are necessary to get a greater recovery rate when the sample size is large or the KD value is too low [13]. This method's low extraction effective influence in a lack of selectivity for the extraction of the desired components, and the usage of numerous organic solvents significantly raises the cost of the procedure. In order to tackle these issues, Abbott et al. introduced the deep eutectic solvent (DESs) theory, which calls for the intermolecular synthesis of hydrogen bonds between hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA). The solvent is non-volatility, low toxicity, and eco-friendly [14].

DESs are excellent for electrochemical and phytochemical extraction and have good medicinal qualities. With high extraction rates for flavonoids, lignin, and phenolic glycosides, DESs have demonstrated tremendous potential for the extraction and recovery of phytochemical residents that are activating [15]. To extract polyphenols, vanillin, and flavonoids, natural deep eutectic solvents (NADES) and therapeutic deep eutectic solvents (THEDES) were developed in accordance with this principle. NADES are frequently used to improve the biological activity of medications because they are made of primary metabolites including amino acids, carbohydrates, or organic acids through the complexation of HBA and HBD [16-17]. The extract can be used directly in products for human or animal consumption due to the food-grade qualities of its constituent molecules, avoiding the requirement for expensive purifying processes. To focus on the current challenges of improving drug bioavailability and high-efficiency and high-selectivity phytochemical extraction. The purpose of this research was to develop extraction and purification of the active components from AS that used a new green solvent.

This study used the heating and stirring procedure to create one type of DES. The DES was used to extract the isofraxidin, then it was quantitatively evaluated through UHPLC-MS/MS. Finally, the extract's component was purified that used the macroporous resin procedure. All in all, this study created a novel method for the use of DES in the field of green extraction and purification of chemical components of plants and traditional Chinese medicine, provided a new technical path for the optimal utilization of active pharmaceuticals in AS, and has the potential industrial application prospects. The above advancements followed the development of the concept of green chemistry.

2. Material and Methods

2.1. Plants Materials

The rhizomes of AS were collected from the Sifeng Mountain wild plant area of Jiamusi and identified by Professor Ximing Zong. The samples were dried at 60°C until constant weight, then crushed in a high-speed pulverizer (FW100, Tianjin Teste) and sieved through an 80-mesh drug sieve before being vacuum-sealed and stored in vacuum-sealed bags until use.

2.2. Chemicals

Formic acid, methanol, and acetonitrile (>99.99%, chromatographic grade) were obtained from Fisher Chemical, USA; choline chloride, and citric acid and purchased from Aladin; a Master Touch-S15 ultrapure water system were used to purify water (HHitech, Shanghai, China); the standard isofraxidin(110837-202009, >=99.8%) was purchased from National Institute for Food and Drug Control of China; AB-8, HPD100C macroporous resin, purchased from Zhengzhou Ainuo Chemical Technology Co., Ltd.

2.3. Synthesis of DES

A precise amount of HBA (choline chloride) and HBD (citric acid) were weighed into a conical bottle, and a mixer was added. Water was then added to wet the two phases, and the bottle top was sealed with tin Paper. The bottle was then placed on a hot plate magnetic stirrer. The clarity and

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transparency of DES (choline chloride and citric acid, 1:1) were obtained by constantly stirring for 2 hours at a rate of 500 rpm in an 80 °C constant temperature water bath.

2.4. Viscosity test

Using a digital viscometer(Shanghai Precision Instruments Co, NDG-8S), determine the solution's viscosity. Equipment that controls temperature has a 0.01K precision. At a temperature of 23 °C, the viscosity of DES was measured with an accuracy of 0.001 mPa·s.

2.5. UHPLC-MS/MS

Dionex Ultimate 3000 high-performance liquid chromatography was used to separate the samples' chemical components (Thermo Fisher Scientific, USA). Using a Thermo Scientific Q-Exactive Series ultra-high-resolution mass spectrometer, the phytochemicals in the samples were identified.

The vacuum exhaust system, pump system, sample tray with the temperature control system, automatic sampler, chromatographic column, column temperature control system, and DAD detector were all parts of the UHPLC system. The instrument was used in conjunction with a Thermo Scientific Hypersil GOLD aQ (2.1 mm × 100 mm,1.9 μm) column (C18, 4.6 × 150 mm, 5 μm). The chromatographic conditions were as follows: formic acid water (0.1%, v/v) and formic acid acetonitrile (0.1%, v/v as mobile phases A and B, respectively). Elution gradient procedure: 0–2 min, 5% B; 2–20 min, 5–45% B; 20–21 min, 45-100% B; 21-25 min, 100% B; 25–26min, 100%-5% B; 26-30min 5%B. The gradient flow rate was maintained at 0.3 mL/min, column temperature 40 °C, and the injection volume was 5 μL.

The quadrupole ultra-high-resolution mass trap (Q Exactive hybrid quadrupole-Orbitrap) was connected in series with the UHPLC system and equipped with a HESI-II type ESI ion source. The ESI-QE-Orbitrap mass spectrum parameters were set as follows: sheath gas (N_2) pressure, 40 psi; auxiliary gas (N_2) pressure, 20 psi; sweep gas (N_2) pressure, 10 psi; capillary voltage, 3 KV; ion transfer tube temperature, 320 °C; AUG gas heating temperature, 350 °C; collision gas, N_2; normalized collision energy, 20, 40, or 60 eV; and RF lens amplitude (s-lens), 60. The primary mass spectrometry full scan was selected in combination with auto-trigger secondary mass spectrometry scan mode (Fullms-ddms^2). Resolution: primary and secondary high-resolution mass spectrometry (MS^1) (70 000 FWHM), scan range, m/z 100-1000; (MS^2) (17500 FWHM), scan range, m/z 50–750; loop count, 3; quadrupole isolation window (isolation window), 1.5 m/z; dynamic exclusion time, 5s.

2.6. Extraction of specific components from AS

In this experiment, isofraxidin was extracted from AS by ethanol, 70%, 50%, and 30% ethanol aqueous solution (v/v) water, and DES. The same ratio of extracting medium for different solvents was used for all sample preparation processes to determine the isofraxidin concentration of different solvent extracts. The 80 mesh AS powder that had been filtered through was dried at 80 °C. 20 mL of solvent (1:20 w/v) was used to extract 1.0 g of the powder. Prior to UHPLC/MS analysis, the water and ethanol extract was diluted 10 times, DES extract was diluted 20 times with methanol and filtered using an organic membrane syringe filter with a 0.22 μm pore size.

2.7. Analysis methodology validation

2.7.1. Specific test

5 μL of DES solution, isofraxidin solution (0.01 mg/mL), 70% alcohol, and blank control aqueous solution were measured precisely and analyzed by UHPLC-MS/MS conditions for specificity test.

2.7.2. Linear regression test

Add 2mg of the standard sample of isofraxidin into a 200 mL volumetric flask, dilute, and fix the volume with methanol to obtain 0.01mg/mL of mixed standard stock solution. The solutions were diluted and fixed with methanol to obtain 0.007mg/mL, 0.005mg/mL, 0.003mg/mL, 0.002mg/mL, and

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0.001mg/mL of the above five concentrations of standard solutions. The concentrations of the standard solutions of isofraxidin were measured according to the UHPLC-MS/MS conditions, and the linear range and regression equation were obtained by the least squares method.

2.7.3. Quantitative limit

Isofraxidin was assayed several times according to the UHPLC-MS/MS conditions in order to establish a standard curve and calculate the standard deviation S of the peak area. The Quantitation limit was calculated after obtaining the deviation of the response value S and the slope of the standard curve K, respectively.

Quantitation limit=10S/K (1)

2.7.4. Precision test

Six standard sample solutions of 0.002, 0.01, and 0.1 mg/mL were measured precisely and operated according to the above assay method, and the above concentrations were calculated using the standard curve established on the same day, and the data were analyzed to calculate the RSD%.

2.7.5. Accuracy test

The precision of the method was investigated by adding the results of the standard sample recovery experiment. A volume of 10 mL of DES was added to 0.5 mL of isofraxidin (0.2 μg/mL) standard solution. The peak areas were measured by UHPLC. Six simultaneous treatments were performed for each sample. The established regression equation was used to determine the sample concentration of isofraxidin and the recovery of the DES blank matrix. In addition, to accurately quantify the concentration of isofraxidin, 10 mL of each AS-DES extract was measured, and the sample extraction recovery was calculated by adding the standard solution operating as described above.

3. Results

3.1. Chemical and physical characteristics of DES

3.1.1. Solvent stability of DES

The DES that was synthesized in this research is shown in Extended Data Figure 1 A and B. No component precipitation was detected in the DES, indicating that the solvents' hydrogen bonds with other solvent systems were not broken and that they remained stable for three months at 4°C.

3.1.2. Viscosity test

An essential solvent property parameter is viscosity. Excessively high solvent viscosity for nonionic common organic solvents increases the mass transfer impedance during extraction, which lowers extraction efficiency [14]. Although the influence of low-eutectic solvent viscosity on extraction efficiency is less than that of non-ionic solvent, high viscosity still has some effect.

This part investigated the viscosities of a single type of DES findings demonstrated that the initial viscosity of DES tested under the tical circumstances was much higher than that of conventional organic chemical solvents. The stability of the hydrogen bond system and the viscosity of DES are both significantly impacted by water. As shown in Figure 2, the water concentration had a significant relationship with the DES viscosity, and the viscosity of DES is dramatically reduced by the addition of water.

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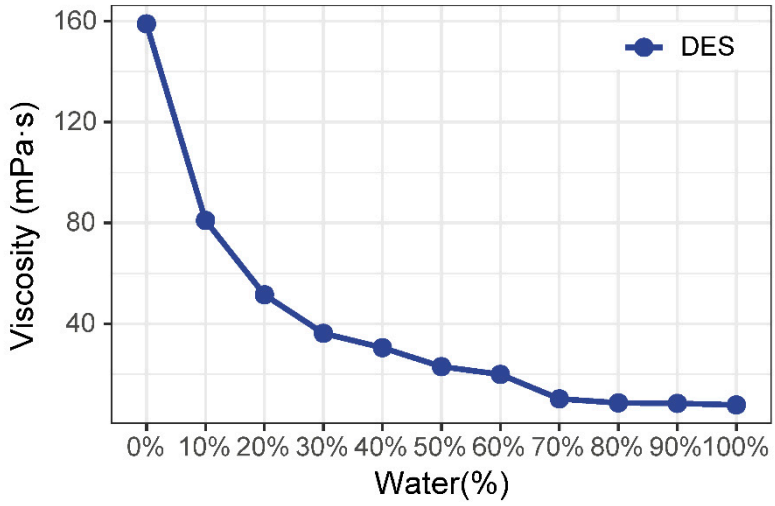

Figure 2. The viscosity of DES was related to the water content.

Adding water will significantly reduce the DES viscosity, with DES containing 10% water, the viscosity is reduced by 49.06%, and DES viscosity decreased by 68.03% when DES contains 20% water. After containing 50% water, the viscosity decreases slowly, when the hydrogen bonding network of the DES system is destroyed, and the properties are seriously affected by water. Therefore, the water content of DES is typically less than 30% in multiple applications, considering the effect of viscosity on the performance of natural product extraction, the effect of solvent on the chromatographic system, and the stability of the solvent system [13-14].

The DES exhibited a higher viscosity than traditional solvents, and this viscosity improved as the quantity of active neutral hydroxyl groups from hydrogen-bonded donors increased. Water plays a crucial role in determining the solvent's viscosity. Small amounts of water can significantly reduce the solvent viscosity after DES production. In the extraction test of natural plant chemical components, the amount of water used should not exceed 20% to ensure the physical and chemical qualities of the DES.

3.2. DES extraction of pharmacologically active compounds from AS

3.2.3. Identification of isofraxidin from AS

Isofraxidin had the chemical formula $C_{11}H_{10}O_5$ and the excimer ion $[M+H]^+$ (m/z^+ 223.05977).Figure 3 shows the chromatograms of isofraxidin extracted using different solvents. In Figure 3, A, B, and C are the chromatograms of water, ethanol, and DES as blank solvents, respectively. The peaks in the chromatograms are spurious peaks at the same mass-to-charge ratio (m/z+ 223.05977) as isofraxidin, and the signal response intensity is relatively low (10^4) and can be ignored. Besides, this indicates that the blank solvent chromatogram has a stable baseline with no interfering peaks from other components in 0-20 min. The retention time of the isofraxidin reference standard, as shown in Figure 3D, is 11.17 minutes, and the chromatographic peaks of 70% ethanol (Figure 3E) and DES (Figure 3F) extract have the same retention time. This demonstrated that isofraxidin could be extracted from AS with 70% ethanol and DES.

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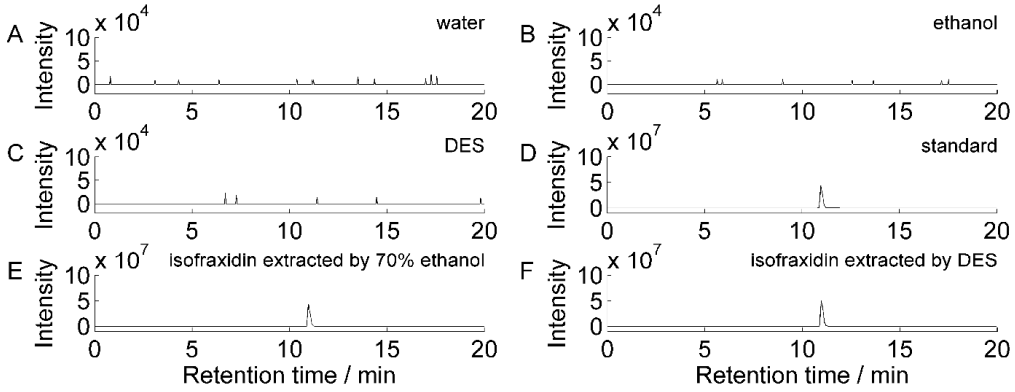

Figure 3. The extraction ion(m/z$^+$: 223.05977) chromatograms of isofraxidin. (A: chromatogram of aqueous; B: chromatogram of ethanol; C: chromatogram of DES; D: chromatogram of isofraxidin reference standard; E: chromatogram of isofraxidin extracted by 70% ethanol; F: chromatogram of isofraxidin extracted by DES).

3.2.4. The capacity to extract isofraxidin from different solvents

Water and ethanol are the most commonly used solvents for extracting natural compounds. The excimer ion (m/z$^+$ 223.05977) chromatograms of isofraxidin extracted by different solvents are shown in Figure 4 to compare the frequently used solvents' ability to extract isofraxidin from AS. This result demonstrates that 70% ethanol has the best extraction performance compared to water and other ethanol concentrations. At the same time, DES has a greater ability than 70% ethanol.

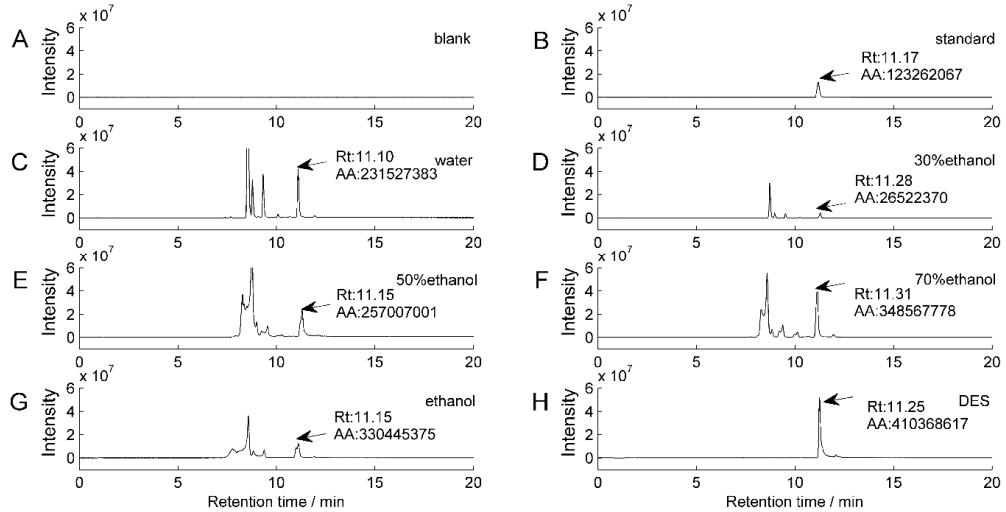

Figure 4. The extraction ion(m/z$^+$: 223.05977) chromatograms of isofraxidin extracted by different solvents. (A: chromatogram of aqueous; B: chromatogram of isofraxidin reference standard; C: chromatogram of isofraxidin extracted by aqueous; D: chromatogram of isofraxidin extracted by 30% ethanol; E: chromatogram of isofraxidin extracted by 50% ethanol; F: chromatogram of isofraxidin extracted by 70% ethanol; G: chromatogram of isofraxidin extracted by anhydrous ethanol; H: chromatogram of isofraxidin extracted by DES).

3.3. Methodology validation

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3.3.1. Specific test

The extraction ion chromatograms in the blank solvents of water, ethanol, and DES are shown in Figures 3A-3C, respectively. No extra component interference peaks have a negative impact on these findings within 0-20 minutes, as indicated in the figures, and the baseline of the blank solvent chromatogram is stable.

3.3.2. Linear regression test

Isofraxidin in AS had standard concentration curves. The findings demonstrated that isofraxidin strongly correlated with 0.1-20 μg/mL. The linear regression equation is

$$A=1.006\times10^{11}\times C+1.998\times10^{7} \quad (2)$$

correlation coefficient R^2 is 0.9812.

3.3.3. Quantitative limit

The limit of quantification for isofraxidin was determined to be 0.27 μg/mL by establishing the equation for the UHPLC-MS/MS data analysis.

3.3.4. Precision test

The repeatability test was conducted to determine the method's precision. The test result data was shown in Table 1. According to this data, the RSD% of the isofraxidin is less than 2.11%, and the system error had no impact on the results.

Table 1. Precision test results (n = 6).

Concentration	Isofraxidin	
(mg/mL)	Mean± SD	RSD%
0.002	0.0019±0.00004	2.11%
0.01	0.0115±0.00017	1.47%
0.1	0.1083±0.0021	1.93%

3.3.5. Accuracy test

The recovery test results were used to evaluate the method's accuracy. The recovery was 93.1% (low concentration: 1μg/mL, RSD: 1.93%); 82.1% (Intermediate concentration: 100 μg/mL, RSD: 1.47%); and 80.7%(high concentration: 200 μg/mL, RSD: 2.11%), respectively.

3.3.6. The highest content of DES solvent extraction isofraxidin

Table 2 shows the quantity of isofraxidin in AS that was extracted by different solvents. As shown in the table, the extraction efficiency of isofraxidin in AS varied significantly among the different solvents. DES displayed the highest isofraxidin extraction efficiency among all the test solvents as shown in Figure 5. The quantity was 1.56 mg/g, which was 2-3 times more than 70% ethanol.

Table 2. The content of isofraxidin in AS extracted by different solvents.

Compound	Formula	$[M+H]^+$	$[m/z]^+$	solvent	Area of peak×10^8			Content
					1	2	3	mg/g
Isofraxidin	$C_{11}H_{10}O_5$	$C_{11}H_{11}O_5$	223.05977	DES	4.104	4.115	4.124	1.56
				70%ethanol	3.486	3.357	3.412	0.64

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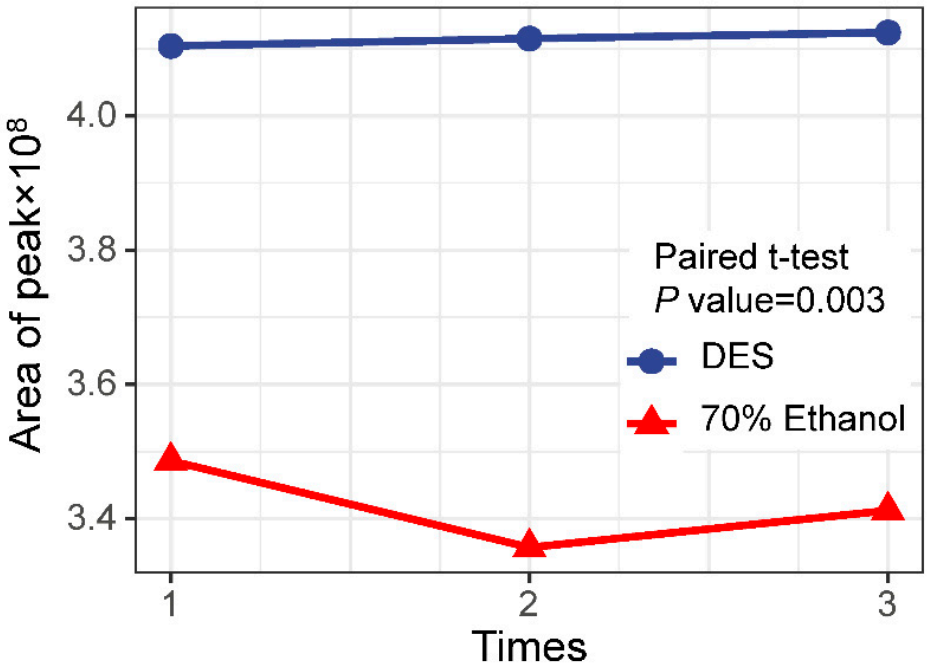

Figure 5. The efficiency of different solvents for the extraction of isofraxidin.

4. Discussion

AS is a medicinal plant in the family of Araliaceae. Previous studies have shown that the active ingredients of AS can significantly improve blood lipid levels and endothelial cell function in patients with acute cerebral infarction, promote the proliferation of neural stem cells in ischemic brain tissue and have therapeutic effects on cerebrovascular diseases such as hypertension and cerebral infarction [18-20]. In addition, AS has a sedative effect on the central nervous system, which can improve anxiety, irritability, insomnia, and other symptoms, and is commonly used clinically for the treatment of abnormal affective disorders and neurological disorders [21-22]. Therefore, it is particularly important to efficiently extract the active ingredients in AS. The extraction of active ingredients from plants with traditional organic solvents has many disadvantages, including the higher cost of organic solvents used in traditional extraction processes, difficulty in controlling the extraction parameters such as temperature and pH, the potential for toxicity due to the use of hazardous organic solvents and inability to extract polar compounds due to the non-polar nature of the organic solvents. Under the concept of upholding safety and environmental protection, more and more green extraction solvents have emerged. As early as 2003, a researcher discovered that by mixing choline chloride and urea under certain conditions, it can form a transparent liquid through hydrogen bonding, and called it DES, which is highly efficient, degradable, and non-polluting and can dissolve a variety of substances, including polysaccharides, alkaloids, flavonoids, etc., thus receiving the attention of many researchers to apply it in the extraction of active ingredients of traditional Chinese medicine. Compared with previous studies, DES extracts isofraxidin from AS with great advantage. Low melting points: The melting point of the deep eutectic solvents produced from choline chloride and citric acid is much lower than that of traditional solvents, making them easier to use and handle; High solubility: The deep eutectic solvents produced from choline chloride and citric acid have high solubility for a wide range of substances, making them ideal for a variety of applications; Low toxicity: The deep eutectic solvents produced from choline chloride and citric acid are much less toxic than traditional solvents, making them safer to use; Cost-effective: Choline chloride and citric acid are much cheaper than traditional solvents, making them a cost-effective choice for synthesizing deep eutectic solvents.

Previous studies have found that using liquid-liquid extraction methods purified 90 mg of isofraxidin from 80 g dried ethyl acetate extract of Chloranthus japonicus root, and the extraction rate is 1.12 mg/g [23]. However, in this study, we used DES synthesized by choline chloride and citric acid for the first time as an extraction solvent for the extraction of isofraxidin from AS. In addition, the extraction rate of DES was 2-3 times higher than that using conventional solvents.

At the initiation of isofraxidin extraction, it is crucial to choose a stable and efficient DES. In this study we described only one DES synthesized from choline chloride and citric acid, but in my pre-experiments, we synthesized 5 DES including DES synthesized from choline chloride and citric acid, DES synthesized from choline chloride and L-ascorbic acid, betaine and proline, betaine and maleic acid, and betaine and xylitol.

Indeed, previous studies have used the above-mentioned DES to extract other active ingredients, but it is worth noting that almost all previous studies have synthesized DES and used them directly to extract active ingredients or for other purposes. For example, Tsvetov et al[24] used choline chloride and L-ascorbic acid to synthesize DES to extract bioactive components from *chamaenerion angustifolium*, Luo et al[25] used choline chloride and L-ascorbic acid to synthesize DES to extract secondary metabolites from eucommia ulmoides leaves, and Zhang et al[26] used glycerol and levulinic acid to synthesize DES extracting flavonoids from Acanthopanax senticosus, other functions include the synthesis of DES removal of copper corrosion products using choline chloride and L-ascorbic acid by Akiko Tsurumaki et al[27]. However, there are a few studies that have performed short-term storage of newly synthesized DES, for example, Andrew J Maneffa et al[28] used choline chloride and other compounds to synthesize DES. After 7 days of storage, it was found that some DES had crystallized, indicating that the eutectic state of these DES had changed.

In the practical application in industry, the stability of DES is also a concern, therefore, in this study, I stored all five newly synthesized DES at 4° dark for 100 days and found that choline chloride and L-ascorbic acid synthesized DES(Extended Data Figure 1E), betaine and proline synthesized DES(Extended Data Figure 1F), betaine and maleic acid DES(Extended Data Figure 1G) have shown very obvious crystallization, similarly, betaine and xylitol DES(Extended Data Figure 1H) have shown partial crystallization, however, DES from choline chloride and citric acid was still stable(Extended Data Figure 1C and 1D). As such, we may infer that this composition should be the closest to the eutectic composition of the choline chloride and citric acid mixtures, which prompted me to use synthetic DES. Moreover, the DES molecular formula can be accurately detected based on the advantages of high sensitivity and high resolution of mass spectrometry. The molar ratio of hydrogen bond donor and acceptor may not be a critical factor in the molecular composition of DES, and most DES are synthesized in a 1:1 molar ratio of hydrogen bond donor and hydrogen bond acceptor. Then, we chose a molar ratio of 1:1. In addition, the viscosity of DES is a key factor in the extraction rate.

The viscosity of DES is mainly determined by van der Waals forces and hydrogen bonding, and the solvent viscosity can influence the extraction efficiency of chemical components. The viscosity of DES is usually larger at room temperature, which may be caused by van der Waals forces or electrostatic interactions between ions [29]. Some research results found that temperature also has an effect on the viscosity of DES, and the higher the temperature the lower the viscosity, therefore, the viscosity of the solvent can be changed by heating. There is a strong hydrogen bonding interaction between the two constituents of DES, and appropriate water content can reduce the viscosity of the solvent, thus increasing its solubility to the extract, and can lead to an increase in the conductivity of some DES [30]. However, the interaction between hydrogen bonds gradually decreases when the water content reaches 50%, so the effect of the water content of DES should be considered when extracting chemical components.

The high-resolution Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer is a new type of mass spectrometry analyzer developed in recent years, which has the advantages of faster speed and higher resolution compared with time-of-flight mass spectrometry. Especially, the combination of UHPLC can greatly shorten the sample analysis time and improve the efficiency of chemical composition identification, which has become a powerful tool for the analysis of complex

samples in recent years and has been widely used in the basic research of the pharmacodynamic components of various Chinese medicines. This technique was used to establish a rapid method for the analysis of isofraxidin in AS. An in-depth study of its chemical composition and further clarification of its material basis is of great significance for the elucidation of the mechanism of the medicinal effect of AS.

5. Conclusion

In this study, we developed a new green extraction method of isofraxidin with some practical significance. By heating and stirring, DES was synthesized at 80°C using citric acid and choline chloride. the initial polarity of DES was like that of 60-80% ethanol, and the hydrogen bond donor was the main factor affecting the polarity of DES. the viscosity of DES was higher than that of ethanol, and even the addition of a small amount of water (10%) resulted in a significant decrease in the viscosity of the solvent, which eventually led to the destruction of the hydrogen bond. the DES was left for three months at 4°C under light-proof conditions solvent It is still stable. The extraction efficiency of isofraxidin with different solvents was different, and the extraction rate of DES was 1.56 mg/g which was 2-3 times higher than that of ethanol. The results of UHPLC-MS/MS showed that the limit of quantification of isofraxidin was 0.27 μg/mL, and the standard curve of the concentration of isofraxidin in AS was measured. The results of the methodological validation analysis showed that the regression equation correlated well within the range of 1-200 μg/mL, the RSD values of the experimental method were within the limits, and the systematic errors did not affect the experimental determination results, thus indicating that the method has good precision. The recoveries were within 80%~100%, indicating that the method is accurate. In conclusion, the new green DES has an important role in the study of the separation and extraction of phytochemical components of AS, with the advantages of high extraction efficiency, safety, and economy, and can be used in the future as a green solvent instead of organic reagents such as ethanol for the separation and extraction of active components of AS. In this study, following the development concept of green chemistry, a new method for DES application in the field of green extraction and purification of medicinal phytochemicals was developed, which provides a new technical pathway for the optimal utilization of active medicinal components of AS, and has potential industrial application prospects.

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Conflicts of Interest: The authors have declared no conflict of interest.

Data Availability Statement: Not applicable.

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