Optimization Studies on the Extraction of Flavone Di-C-glycosides from *Premna fulva* Craib by Deep Eutectic Solvents

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Deep eutectic solvents (DESs) are emerging as new extraction solvents for the extraction of bioactive compounds from natural plants. In this work, a total of thirteen choline chloride-based DESs were synthesized and evaluated for the simultaneous extraction of five bioactive flavone di-C-glycosides from *Premna fulva* Craib. DES-F, which was composed of choline chloride and 1,3-propanediol, showed the best extraction efficiency for the flavone di-C-glycosides, exceeding that of the conventional industrial solvent (40% ethanol). Subsequently, the extraction parameters for ultrasonic-assisted extraction (liquid-solid ratio, water content (%) in the selected DES, and extraction time) were optimized using response surface methodology. Furthermore, the microstructural alterations in the samples after extraction were evaluated using scanning electron microscopy. In addition, we further verified the recovery of flavone di-C-glycosides from the DESs using macroporous resins and evaluated the reusability of the DESs as solvents. The results indicated that DES-F was an efficient and sustainable solvent for the extraction of bioactive flavone di-C-glycosides from *Premna fulva* Craib. Therefore, this novel strategy may represent a sustainable approach for the extraction of bioactive products and could make phytochemistry more attractive and environmentally friendly.

1. Introduction

*Premna fulva* Craib, which is also known as the Zhuang medicine “zhangu,” belongs to the Verbenaceae family and is mainly distributed in the Guangxi province in China [1]. Jian-Gu injection, a traditional Chinese medicine, is made from the roots and stems of *Premna fulva* Craib [2]. Clinical studies have shown that Jian-Gu injection has been widely used to treat cervical spondylosis, lumbar sprain, and osteoarthritis [3–7]. The main ingredients of Jian-Gu injection were reported to be flavone di-C-glycoside isomers in our previous research [8]. The structures of the five flavone di-C-glycoside congeners are shown in Figure 1. Moreover, the overwhelming majority of flavonoids in *Premna fulva* Craib are flavone C-glycosides [9]. Today, Jian-Gu injection is obtained from *Premna fulva* Craib using ultrasonic-assisted extraction, which has replaced conventional techniques such as maceration (40% ethanol) [10, 11], which have drawbacks such as inefficiency and the high vapor pressure of the solvent. Hence, there is a need to develop an efficient and environmentally friendly technique for the extraction of flavone di-C-glycosides from *Premna fulva* Craib.

Deep eutectic solvents (DESs) are rapidly emerging as a new class of green solvents to replace organic solvents and ionic liquids. DESs are eutectic systems obtained by the complexation of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). One of the most commonly used HBAs is choline chloride (ChCl), which is a cheap, biodegradable, and nontoxic salt. Publications dealing with the toxicity of ChCl-based DESs assessed them as being completely harmless towards the tested bacteria [12] and classified them as readily biodegradable with good prospects.
for wider use in the field of green technologies [13]. Because most HBAs and HBDs exist naturally, DESs present unique physicochemical properties such as low toxicity, low vapor pressure, nonflammability, and high biodegradability and can be prepared inexpensively. DESs have been widely applied for the extraction or separation of natural products from plants [14–18]. Recently, Duan et al. [19] synthesized DESs that were applied to extract three bioactive flavone mono-C-glycosides from Flos trollii. ChCl-ZnBr proved to be the most efficient solvent, but it is an ionic liquid that has more or less toxicity and is difficult to degrade. To the best of our knowledge, the efficiency of DESs in the extraction of bioactive flavone di-C-glycosides from Premna fulva Craib remains unknown. Hence, the aim of this study was to introduce a more efficient and environmentally friendly technique for the extraction of flavone di-C-glycosides using DESs. In this study, we evaluated the effect of DESs as green solvents in the extraction of flavone di-C-glycosides from Premna fulva Craib using ultrasonic-assisted extraction. Thirteen choline chloride-based DESs were prepared for the simultaneous extraction of five bioactive flavone di-C-glycosides. The most suitable DES with the highest extraction yield was determined by evaluating the extraction process. The independent variables of the extraction (liquid-solid ratio, water content (%)) in the selected DES, and extraction time) were optimized using the Box–Behnken design. Lastly, the microstructural alterations in the samples after extraction were observed using a scanning electron microscope. We further verified the recovery of flavone di-C-glycosides from the DESs using macroporous resins; the reusability of the DESs as solvents was also investigated.

2. Materials and Methods

2.1. Materials and Reagents. Premna fulva Craib was purchased from a local traditional Chinese medicine market (Yulin, Guangxi, China) and ground into a fine powder. The reference compounds apigenin 6,8-di-C-β-D-glucopyranoside (1), apigenin 6-C-β-D-xlyopyranosyl-8-C-β-D-glucopyranoside (2), apigenin 6-C-β-D-xlyopyranosyl-8-C-β-D-galactopyranoside (3), apigenin 6-C-β-D-glucopyranosyl-8-C-β-D-xlyopyranoside (4), and apigenin 6-C-β-D-galactopyranosyl-8-C-β-D-xlyopyranoside (5) were isolated and prepared from Jian-Gu injection (Premna fulva Craib) as described in our previous report [8].

Compounds for DES preparation, including choline chloride, mannose, D-fructose, glucose, xylose, glycerol, levulinic acid, 1,3-propanediol, glycolic acid, D-lactic acid, malonic acid, tartaric acid, citric acid, and urea, were obtained from Aladdin Reagent Company (Shanghai, China). HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Water was prepared using a Milli-Q water purification system (Millipore, MA, USA). Other reagents and chemicals were of analytical grade.

2.2. HPLC Analysis for Quantification of Flavone Di-C-glycosides. Quantitative analysis was performed using a Shimadzu LC-20AT HPLC unit equipped with LabSolutions software for data acquisition and analysis. An Agilent Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm) was used to separate the compounds. The mobile phase was 0.1% aqueous acetic acid (solvent A) and methanol (solvent B) using the following gradient: 0–15 min, 5–20% B; 15–50 min, 20–40% B; 50–60 min, 40–50% B; 60–65 min, 50–65% B; 65–66 min, 5% B. UV detection over the wavelength range 190–400 nm was used. The flow rate was 1.0 mL/min, and the column temperature was set at 35°C. The peak areas of the samples were monitored at 280 nm. Typical HPLC chromatograms of the Premna fulva Craib sample and the five flavone di-C-glycoside reference compounds are presented in Figure 2. The mass of flavone di-C-glycosides was determined by means of a calibration curve established using a regression equation in which y was the peak area and x was the mass of the flavone di-C-glycosides standard (μg). Each sample was analyzed using HPLC with duplicate measurements. During HPLC analysis, it was observed that the presence of DESs in the samples did not affect the chromatograms obtained.

2.3. Preparation of DESs. The DESs were prepared according to previous studies [20]. In brief, the DESs were formulated with different molar ratios of the HBA to the HBD. The eutectic mixtures were prepared by rotary evaporation of the two components at 80°C–90°C in a water bath until a homogeneous transparent liquid was formed. Table 1 presents the compositions of the studied DESs, along with the abbreviations used for them this study.

2.4. Extraction Procedure and Selection of DESs. The DES-based ultrasonic-assisted extraction procedure was as follows: an accurately weighed 100 mg sample of powdered Premna fulva Craib (screened through 200 mesh sieves) was added to 1.4 mL (DES: water = 1:0.4 v/v, equivalent to 28.6% water content) of one of the 13 different DESs in a 5 mL Erlenmeyer flask. The mixture was sonicated (KQ5200B, 200 W, 40 kHz, Kunshan, China) at 50°C in a water bath for 30 min and then centrifuged at 12000 rpm for 10 min. The suspension was diluted three times with water and analyzed to determine the extractant using HPLC. The extraction was performed in triplicate. The same procedure...
was repeated to screen the extraction performance of the reference solvent (40% ethanol) [10].

Based on the standard curve equation (Eq.), the mass of flavone di-C-glycosides in the extract was calculated, and the extraction efficiency (EE) was calculated from this value using the following equation:

\[
EE (\text{mg/g}) = \frac{\text{mass of flavone di-C-glycoside based on standard curve equation}}{\text{mass of Premna fulva Craib power}}
\]  

(1)

The extraction efficiencies of the flavone di-C-glycosides for different DESs were used to select the most appropriate DES for use as the extraction solvent in subsequent experiments.

2.5. Optimization of the DES Extraction Parameters. The optimal extraction parameters for the total flavone di-C-glycosides were determined using the software package Design-Expert Ver. 8.0.6. Box–Behnken design (BBD) in combination with response surface methodology (RSM) was used to find the optimal values for the three independent variables: liquid-solid ratio \((X_1)\), water content \((X_2)\) in the selected DESs, and extraction time \((X_3)\) at three levels \((-1, 0, 1)\). The total amount of the five flavone di-C-glycosides extracted was used as the response in the design experiments. Regression analysis was performed using the experimental data. Subsequently, additional confirmation experiments were conducted to confirm the validity of the statistically determined experimental strategies.

2.6. Scanning Electron Microscopy. Scanning electron microscopy (SEM) was conducted as described previously [21]. In brief, the samples were mounted on aluminum stubs and sputtered with a thin gold layer. The prepared samples were imaged using an EVO18 ZEISS scanning electron microscope (Zeiss, Germany).

2.7. Recovery of Flavone Di-C-glycosides from DESs and Reusability of the DESs. The recoveries of the flavone di-C-glycosides from the DES extracts were determined using macroporous resin column chromatography [10, 11]. A 1 mL aliquot of the extract was loaded onto a glass column filled with 10 g of D101 macroporous resin. The polar DES components were removed using 100 mL deionized water,

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**Table 1:** List of the choline chloride-based DESs synthesized in this work.

| No. | Abbreviation | HBA       | HBD         | Molar ratio | Appearance               |
|-----|--------------|-----------|-------------|-------------|--------------------------|
| 1   | DES-A        | Mannose   | 1:1         | Colorless viscous liquid |
| 2   | DES-B        | D-Fructose| 1:1         | Colorless viscous liquid |
| 3   | DES-C        | Glucose   | 1:1         | Light white viscous liquid |
| 4   | DES-D        | Xylose    | 1:1         | Colorless viscous liquid |
| 5   | DES-E        | Glycerol  | 1:2         | Colorless liquid         |
| 6   | DES-F        | 1,3-Propanediol | 1:2 | Colorless liquid         |
| 7   | DES-G        | Choline chloride (ChCl) | 1:2 | Colorless liquid         |
| 8   | DES-H        | Leulinic acid | 1:2 | Light yellow liquid     |
| 9   | DES-I        | Glycolic acid | 1:2 | Colorless liquid         |
| 10  | DES-J        | D-Lactic acid | 1:2 | Colorless liquid         |
| 11  | DES-K        | Malonic acid | 1:2 | Colorless liquid         |
| 12  | DES-L        | Tartaric acid | 1:2 | Colorless liquid         |
| 13  | DES-M        | Citric acid | 1:2 | Colorless viscous liquid |
| 14  | DES-N        | Urea      | 1:2         | Colorless liquid         |

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and the sample was then eluted with 100 mL of ethanol. The ethanol-eluted fractions were dried with a vacuum evaporator and redissolved in 10 mL of methanol for HPLC analysis. The DES solutions were recovered by removing the residual water at 90°C under a vacuum pump. The recovered DEs could be used for additional extractions of flavone di-C-glycosides from Premna fulva Craib.

3. Results and Discussion

3.1. Method Validation. After determining the optimum HPLC conditions, the linearity, limit of detection (LOD), and limit of quantification (LOQ) were assessed. For the linearity tests, standard solutions of the analytes with ten different concentration levels were injected in triplicate. The calibration curves were constructed by plotting the integration areas of the chromatographic peaks (Y) versus the corresponding mass of the analyte in the injected standard solution (X, μg).

Good linearity was obtained for each analyte over the concentration range tested (r² > 0.99) (Table 2). The LOQ and LOD were determined by gradually diluting the individual standard solution with methanol and were calculated for signal-to-noise ratios (S/N) of 10 and 3, respectively. The LOD and LOQ values in the present study were less than 0.06 μg and 0.18 μg, respectively (Table 2), which indicated that the developed method had excellent sensitivity and allowed the direct determination of flavone di-C-glycosides in Premna fulva Craib without any previous enrichment step.

3.2. Evaluation of Various DESs in the Extraction of Flavone Di-C-glycosides. The composition of DESs determines their physicochemical properties and consequently influences the EEs of natural compounds. In this study, thirteen different ChCl-based DESs (Table 1) were selected to test their EEs for flavone di-C-glycosides from Premna fulva Craib.

However, the high viscosity of the DESs restricted their application due to slow mass transfer. The addition of water to DESs has been reported to change their viscosity and surface tension, which may affect their extraction efficiency toward target compounds [22]. Thus, a 28.6% (v/v) water content in the DESs was used as the standard condition in the preliminary screening. The extraction temperature and time were set at 50°C and 30 min, respectively. To compare the EE of the DESs and the reference solvent (40% ethanol), the extraction was performed in triplicate under the same operational conditions. The EEs of the five flavone di-C-glycosides achieved using the different DESs are shown in Figure 3.

The results indicated that the EEs of the flavone di-C-glycosides were influenced by the type of DES used. Obviously, viscosity was an important property in the solid-liquid extraction process and affected the flavonoid extraction. Compared to 40% ethanol, the four DESs (DES-A/B/C/D) exhibited lower EE values for the total flavone di-C-glycosides. However, DES-J and L resulted in even lower EEs than these DESs (DES-A/B/C/D); this may have been due to the chemical reaction between flavone di-C-glycosides and the malonic acid and citric acid-base DESs, which changed the solution from light yellow to dark green during the extraction process.

Under the designed parameters, four of the synthesized DESs (DES-F/G/H/I) provided higher EEs for the total flavone di-C-glycosides. DES-F (choline chloride/1,3-propanediol) had a slightly higher EE than DES-G/H/I. The experimental results can be ascribed to the hydrogen bonding interactions between the DESs and the target solutes (flavone di-C-glycosides). In addition, similar results have been reported in which choline chloride/1,2-propanediol, (1:2 mol ratio) was selected as the most effective solvent for extraction of flavonoids in pollen typhae [23]. However, the experimental results are not in agreement with those reported by Duan et al. [19]. A possible explanation is that the flavone C-glycosides species contained in each plant are different and therefore have different physicochemical properties that result in different extraction results for similar DESs. Consequently, DES-F was selected and applied in further tests as the best DES for the extraction of flavone di-C-glycosides in Premna fulva Craib.

3.3. Optimization of the DES Extraction Parameters for Total Flavone Di-C-glycosides. As described above, DES-F (choline chloride/1,3-propanediol) provided the highest EE of flavone di-C-glycosides from Premna fulva Craib. Subsequently, eutectic mixtures of choline chloride/1,3-propanediol were prepared at mole ratios of 1:1, 1:2, 1:3, and 1:4 and used to extract flavone di-C-glycosides from Premna fulva Craib. The results demonstrated that DES-F, which had a 1:2 mole ratio, provided the highest EE. To obtain the optimal EE for bioactive flavone di-C-glycosides, three factors (liquid-solid ratio (X₁), 10–50 mL/g), water content (X₂, 10%–50%), and extraction time (X₃, 20–60 min)) were considered using a Box–Behnken design (BBD) through the preliminary single-factor test. Response surface methodology (RSM), a valuable statistical method in multivariate statistical techniques, was employed to simultaneously optimize the levels of the investigated factors to attain the highest EE in this study. The experimental orders, levels of variables, and response values are presented in Table 3. The EE of total flavone di-C-glycosides was taken as the response of the design experiments.

By fitting the experimental data obtained using these variables and responses, a quadratic polynomial model for the total flavone-C-glycosides was established as follows:

\[ Y = 17.58 + 0.35X₁ + 0.68X₂ + 0.53X₃ + 0.20X₁X₂ - 0.81X₁X₃ - 0.96X₂X₃ - 2.17X₁^2 - 2.00X₂^2 - 1.12X₃^2, \]

(2)

where Y represents the EE for total flavone-C-glycosides (mg/g) and X₁, X₂, and X₃ are the coded variables for the liquid-solid ratio (mL/g), water content (%), and extraction time (min), respectively.

Statistical analysis (Table 4) indicated the significant variables affecting the EE of flavone di-C-glycosides and the
interaction effects between the variables. The high F value (68.09) and low p values (<0.0001) indicated that the regression model was significant and thus accurately represented the experimental data. The p value for lack of fit (p = 0.4215 > 0.05) implied that it was not significant compared with the pure error, which indicates that the established predictive mathematical model explained all the data well. The determination coefficient ($R^2 = 0.9887$), which is an index of the fitting degree and applicability of the model, adjusted coefficient (Adj. $R$-squared = 0.9742), and Adeq. precision (22.075) of the regression model showed that the model was highly precise and reliable. Therefore, the statistical results revealed that the model adequately represented the real relationship between the EE of the flavonoids and the independent variables.

To describe the relationship between the extraction parameters and the EE of the flavonoids, three-dimensional (3D) response surface plots were studied by RSM, as shown in Figure 4. All the 3D response surfaces were arched, which indicated that the ranges of the variables were within the experimental ranges and were appropriately optimized.

Based on the quadratic model and 3D response surfaces, the optimum conditions for the extraction of flavone di-C-glycosides from *Premna fulva* Craib were calculated to be the following (Table 5): a liquid-solid ratio of 31.18 mL/g, water content of 32.74%, extraction time of 43.10 min, which gave the highest EE of total flavone di-C-glycosides (17.68 mg/g). Taking into account the accuracy of the instrument, the optimal conditions were modified to a liquid-solid ratio of 31.00 mL/g, water content of 33.00%, and extraction time of 43.00 min. Then, the verification experiments were carried out in triplicate. The EE of total flavone di-C-glycosides was 17.37 mg/g, which was close to the predicted value of 17.68 mg/g. The total EE was greater than that of the

### Table 2: Calibration curve, LOD, and LOQ of the five investigated compounds.

| Analyte | Calibration curve | $r^2$ | LOD (μg) | LOQ (μg) |
|---------|-------------------|-------|----------|----------|
| Apigenin 6,8-di-C-β-D-glucopyranoside (1) | $Y = 1000000X + 81351$ | 0.9992 | 0.18 | 0.54 |
| Apigenin 6-C-β-D-xylopyranosyl-8-C-β-D-glucopyranoside (2) | $Y = 446642X - 6968.9$ | 0.9977 | 0.14 | 0.43 |
| Apigenin 6-C-β-D-xylopyranosyl-8-C-β-D-galactopyranoside (3) | $Y = 723977X - 154149$ | 0.9936 | 0.24 | 0.72 |
| Apigenin 6-C-β-D-glucopyranosyl-8-C-β-D-xylopyranoside (4) | $Y = 309159X - 5670.3$ | 0.9918 | 0.19 | 0.57 |
| Apigenin 6-C-β-D-galactopyranosyl-8-C-β-D-xylopyranoside (5) | $Y = 817494X + 15891$ | 0.9998 | 0.06 | 0.18 |
Table 3: Experimental data and obtained response values for different combinations of three factors.

| Run | Liquid-solid ratio ($X_1$, mL/g) | Water content ($X_2$, %) | Extraction time ($X_3$, min) | Total flavone di-C-glycosides (mg/g) |
|-----|----------------------------------|--------------------------|-----------------------------|--------------------------------------|
| 1   | 10                               | 50                       | 40                          | 13.638                               |
| 2   | 30                               | 30                       | 40                          | 17.9165                              |
| 3   | 30                               | 50                       | 20                          | 15.475                               |
| 4   | 30                               | 30                       | 40                          | 17.8521                              |
| 5   | 10                               | 10                       | 40                          | 12.7462                              |
| 6   | 50                               | 10                       | 40                          | 12.7954                              |
| 7   | 50                               | 30                       | 20                          | 15.2052                              |
| 8   | 30                               | 10                       | 60                          | 15.3776                              |
| 9   | 10                               | 30                       | 20                          | 12.617                               |
| 10  | 30                               | 30                       | 40                          | 17.2204                              |
| 11  | 50                               | 50                       | 40                          | 14.4825                              |
| 12  | 50                               | 30                       | 60                          | 14.3368                              |
| 13  | 30                               | 10                       | 60                          | 12.1016                              |
| 14  | 30                               | 30                       | 40                          | 17.568                                |
| 15  | 30                               | 50                       | 60                          | 14.9027                              |
| 16  | 10                               | 30                       | 60                          | 15.0012                              |
| 17  | 30                               | 30                       | 40                          | 17.3653                              |

Table 4: ANOVA results of the quadratic multiple regression model for flavone di-C-glycosides.

| Source                  | Sum of squares | df | Mean square | F value | p value prob. > F | Remarks       |
|-------------------------|----------------|----|-------------|---------|-------------------|---------------|
| Model                   | 59.93          | 9  | 6.66        | 68.09   | <0.0001           | Significant   |
| $X_1$: liquid-solid ratio | 0.99           | 1  | 0.99        | 10.15   | 0.0154            | Significant   |
| $X_2$: water content    | 3.75           | 1  | 3.75        | 38.35   | 0.0004            | Significant   |
| $X_3$: extraction time  | 2.23           | 1  | 2.23        | 22.76   | 0.002             | Significant   |
| $X_1 X_2$               | 0.16           | 1  | 0.16        | 1.62    | 0.2441            |              |
| $X_1 X_3$               | 2.64           | 1  | 2.64        | 27.05   | 0.0013            | Significant   |
| $X_2 X_3$               | 3.7            | 1  | 3.7         | 37.86   | 0.0005            | Significant   |
| $X_1^2$                 | 19.86          | 1  | 19.86       | 203.04  | <0.0001           | Significant   |
| $X_2^2$                 | 16.8           | 1  | 16.8        | 171.78  | <0.0001           | Significant   |
| $X_3^2$                 | 5.31           | 1  | 5.31        | 54.29   | 0.0002            | Significant   |
| Residual                | 0.68           | 7  | 0.098       |         |                   |               |
| Lack of fit             | 0.32           | 3  | 0.11        | 1.18    | 0.4215            | Not significant|
| Pure error              | 0.36           | 4  | 0.091       |         |                   |               |
| Cor. total              | 60.61          | 16 |             |         |                   |               |
| R-squared               | 0.9887         |    |             |         |                   |               |
| Adj. R-squared          | 0.9742         |    |             |         |                   |               |
| Pred. R-squared         | 0.9057         |    |             |         |                   |               |
| Adeq. precision         | 22.075         |    |             |         |                   |               |

Figure 4: 3D response surface plots of the model for the EE of total flavone di-C-glycosides.
conventional extraction system (40% ethanol, 13.70 mg/g; Table 5). The results obtained through the confirmation experiments indicated that the model was adequate for predicting the optimized parameters.

3.4. Microstructure Characterization Using a Scanning Electron Microscope. To better understand why DES-F showed the best EE, the microstructure of Premna fulva Craib before and after extraction with the DES solvents was characterized (Figure 5). The structure of Premna fulva Craib root is formed of sclereids (stone cell group). Prior to extraction, the cell wall was smooth and invisible, and the cell cavity had a large crevice (Figure 5(a)). After treatment with 40% ethanol, the cell wall was obviously swollen (Figure 5(b)). After treatment with DES-F, the cell wall was significantly swollen, and the crevice of the cell cavity was diminished, indicating that intercellular matter had been exposed and come into contact with the extraction solvent. This agrees with a previous study [24] in which a DES (ChCl: 1,2-butandiol) formed porous openings on Salvia miltiorrhiza Bunge due to the dissolution of cellulose after extraction, thereby releasing the natural products into the surroundings.

3.5. Recovery of Flavone Di-C-glycosides from DES and DES Reusability. DESs have low vapor pressures, making it fairly challenging to recover the extracted compounds from the extracts. However, several approaches, such as liquid-liquid extraction, solid-liquid extraction, supercritical carbon dioxide, and recrystallization, can be used to recover the targeted extracts from DESs [25]. In this work, the recovery of total flavone di-C-glycosides was selected as the evaluation index, and the solid-liquid extraction method showed an acceptable recovery yield of 81.59%. The results indicated that D101 resin could also effectively adsorb flavone di-C-glycosides in DES-F. However, the recovery of and reusability of the DES remained to be addressed. After eluting the DES from the D101 resin with deionized water, the water was evaporated from DES-F by heating at 90°C using a vacuum pump. The results for the recovery and reusability of the DES in terms of the EE of flavone di-C-glycosides are shown in Figure 6. After three cycles, the EE of flavone di-C-glycosides was the same as that of the CON. However, in subsequent cycles, the EE decreased rapidly, which could potentially be attributed to changes in its performance parameters due to the polar impurities in the plants. Therefore, DES-F can be effectively recovered by simple evaporation.

4. Conclusions

In summary, a simple, green, and efficient extraction method using a DES as an extraction solvent was established for the
extraction of bioactive flavone di-C-glycosides from *Premna fulva* Craib. A total of thirteen different ChCl-based DESs were successfully synthesized and applied to extract five bioactive flavone di-C-glycosides. The results indicated that DES-F (choline chloride/1,3-propanediol) was the most efficient solvent. Moreover, the optimal extraction conditions for flavone di-C-glycosides were obtained using RSM. In addition, the flavone di-C-glycosides can be recovered from the DES extracts using a D101 macroporous resin, and the recovered DES can be reused for additional extractions. Based on the beneficial effect of DESs on the extraction efficiency of target compounds, the developed DESs may represent a sustainable approach for the extraction of bioactive products and thereby make phytochemistry more attractive and environmentally friendly.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author or the first author upon request.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

**Authors’ Contributions**

Yueyuan Chen and Jiaoyang Dang contributed equally to this work.

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