

Research Article

Green and Efficient Extraction of Resveratrol from Peanut Roots Using Deep Eutectic Solvents

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Deep eutectic solvents (DESs), a new group of ecofriendly solvent combined with the ultrasonic-assisted extraction (UAE) technique, were first successfully used for extraction of resveratrol from peanut roots. Resveratrol in the extract was analyzed and quantified using a HPLC-UV method. A series of DESs consisting of choline chloride (ChCl) and 1,4-butanediol, citric acid, and ethylene glycol were formulated, finding ChCl/1,4-butanediol was a most proper extraction system. The optimal extraction parameters were obtained using a Box–Behnken design (BBD) test combined with response surface methodology as follows: 40% of water in ChCl/1,4-butanediol (1/3, g/g) at 55°C for 40 min and solid/liquid ratio of 1:30 g/mL. The total extraction content and extraction yield of resveratrol from peanut roots could reach 38.91 mg/kg and 88.19%, respectively, under such optimal conditions. The present study will provide a typical example for using DESs to extract natural bioactive compounds from plants.

1. Introduction

Peanut (*Arachis hypogaea* Linn.) belongs to the family of Rosales and is widely cultivated around the world as an important economical crop. This species is capable of producing stilbene derivatives, including resveratrol (3,5,4'-trihydroxy-stilbene) and other stilbenoids [1]. Resveratrol has attracted tremendous interest ascribing to its strong biological activity, such as antioxidant, anti-inflammatory, anticancer, cardiovascular protection, and cardioprotection functions [2–4]. In addition, it has been widely used as an active ingredient in cosmetic, medicine, and health products. Naturally, resveratrol is extracted from peanut [5], Japanese knotweed [6], grape [7], and other plants. Peanut roots are the waste products in the field after peanut harvesting, which are the cheapest sources of resveratrol [8].

Extraction of resveratrol from peanut roots should be based on green and sustainable technology. Conventionally, organic solvents, such as alcohols and acetone, are widely used in extraction of bioactive components from natural products resources in the fields of cosmetic, food, and pharmaceutical

industries [9, 10]. However, the organic solvents pollution is a serious environmental issue. Therefore, the green extraction by using the new and environmentally friendly solvents was needed urgently [11]. In this connection, the deep eutectic solvents (DESs) have emerged as a better alternative to conventional solvents [12]. DESs can be easily synthesized with quaternary ammonium salt and hydrogen bond donor (HBD) with a gentle heating temperature range (70°C–90°C), and no further purification is required [13]. Numerous DESs consisting of different components, such as choline chloride, urea, organic acids, polyols, and sugars, have been developed [14–16]. In addition to as a green extraction solvent, DESs have other advantages, including the simple synthesis, low cost, biodegradable, chemical inertness, and no toxic factors [17, 18]. In fact, DESs have been applied in the extraction of a wide variety of natural compounds including phenolic compounds [19, 20], flavonoids [21, 22], sugars [23], and proteins [24, 25].

With the aim of development of green extraction technology for separation bioactive compounds from oil processing by-products, the present study was to establish a

highly efficient and green extraction technology for the extraction of resveratrol from peanut roots using deep eutectic solvents assisted by ultrasonic extraction methods (DESs-UAE). First, a series of DESs were prepared by mixing the varying ratios of polyols, organic acid, and carbamide with ChCl. Second, various parameters of DESs-UAE in extracting resveratrol were optimized and systematically evaluated using a Box–Behnken design.

2. Materials and Methods

2.1. Chemicals and Reagents. 1,4-Butanediol (>98%) and citric acid (>99%) were obtained from Kwangfu Fine Chemical Industry Research Institute (Tianjin, China). Ethylene glycol (>98%) was purchased from Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Lactic acid (>95%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycerol (>99%), 1,2-propylene glycol (>99%), DL-malic acid (>99%), and carbamide (>99%) were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Choline chloride (>98%) was obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). Resveratrol (3,5,4'-trihydroxy stilbene, $\geq 98\%$) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol (the chromatographic grade) was purchased from VBS biologic Co. (USA). All samples and solutions prepared for HPLC analysis were filtered through the $0.45\ \mu\text{m}$ nylon membranes prior to use.

Standard stock solutions: resveratrol compound was directly prepared in methanol. The concentration of resveratrol in the standard stock solutions was all $500\ \mu\text{g}/\text{mL}$. Working standard solutions: resveratrol compound was obtained by diluting the stock solutions with methanol to a series of proper concentrations. The standard stock solutions and working standard solutions were all stored at 4°C in a dark place.

2.2. Plant Materials. Peanut roots were purchased from Hebei province in China. The plant material was cleaned and dried at 40°C . The dry plant material was ground into powder with a blender and passed through 50 mesh and then stored in a dry place at room temperature until used.

2.3. Preparation of DESs. All of the chemicals used in DESs preparations were dried at 60°C for 24h. The DESs were prepared at specific ratios of choline chloride to the hydrogen donor (i.e., 1,4-butanediol, glycerol, and lactic acid; Table 1). The varying ratios of choline chloride with the hydrogen donor were stirred in a flask at 80°C for 5–10 min, until a homogeneous transparent colourless liquid was formed. These DESs samples were treated by vacuum drying prior to use.

2.4. Deep Eutectic Solvents Ultrasonic-Assisted Extraction of Resveratrol. About 0.50 g peanut roots powder was weighed into an 50 mL centrifuge tube and followed by addition of 10 mL of extraction solvent. The mixture was then ultrasonically treated. Ultrasound-assisted extraction (UAE) was

TABLE 1: Different systems of natural deep eutectic solvents (DESs).

Abbreviation	Component 1	Component 2	Molar ratio
DES-1	Choline chloride	1,4-Butanediol	1 : 2
DES-2	Choline chloride	1,2-Propylene glycol	1 : 2
DES-3	Choline chloride	Glycerol	1 : 2
DES-4	Choline chloride	Ethylene glycol	1 : 2
DES-5	Choline chloride	DL-Malic acid	1 : 2
DES-6	Choline chloride	Lactic acid	1 : 2
DES-7	Choline chloride	Carbamide	1 : 2
DES-8	Choline chloride	Citric acid	1 : 2

performed using an ultrasonicator under 40 kHz and 400 W (SCQ-7201B, Shengyan Ultrasonic Instrument Co., Ltd., Shanghai, China). The extraction was carried out under different conditions. After extraction, the mixture was centrifuged (10 min, 2500 rpm) with a bench-scale centrifuge. The supernate was separated and filtered through a $0.45\ \mu\text{m}$ membrane prior to HPLC analysis.

The extraction content was calculated according to the following equation:

$$\text{extraction content (mg/kg)} = \frac{\text{mass of resveratrol (mg)}}{\text{mass of weighed peanut roots powder (kg)}} \quad (1)$$

The extraction yield was defined as follows [26]:

$$\text{extraction yield (\%)} = \frac{\text{mass of the resveratrol in extraction solution}}{\text{sum of the mass of resveratrol in sample}} \times 100\% \quad (2)$$

The mass of resveratrol in the extraction solution (one-step extraction) was determined by HPLC-UV. The sum mass of resveratrol in the sample was calculated by analysis of the total mass of resveratrol in the combined extraction solutions afforded by continuously extracting three times with methanol according to the standard method.

2.5. Experimental Design and Statistical Analysis. Firstly, the mole ratio of hydrogen bond donors and ChCl (1,4-butanediol:ChCl = 1:1, 2:1, 3:1, 4:1, and 5:1), the percentage of water (10%, 20%, 30%, 40%, 50%, and 100%), solid-liquid ratio (1:10, 1:20, 1:30, 1:40, and 1:50), extraction temperature (20, 30, 40, 50, 60°C , and 70°C), and extraction time (20, 30, 40, 50, and 60 min) were optimized by single-factor experiments, respectively. Furthermore, Box–Behnken experimental design (BBD) with response surface methodology (RSM) was used to estimate the most effective combination of extraction parameters according to the single-factors experiments. A three-level (–1, 0, and +1) four-factor Box–Behnken design (BBD) was applied to evaluate the interaction effect of the factors: the percentage of water (A), solid/liquid ratio (B), extraction temperature

(C), and extraction time (D) on the extraction content of resveratrol (Y). 29 experiments running with different combinations of four factors were carried out totally. The second-order polynomial given in the equation was applied to correlate the interaction of each factor to the response. For the four factors, this equation is

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1}^4 \sum_{j=i+1}^4 \beta_{ij} X_i X_j, \quad (3)$$

where X_i and X_j are the independent coded variables which influence the response Y . Y is the predicted response; β_i is the parameter estimated for the variable; β_{ii} and β_{ij} are the parameters estimated for the interaction between variables i and i and i and j ; and β_0 , β_i , β_{ii} , and β_{ij} are the regression coefficients for intercept, linearity, square, and interaction, respectively. The variables of each factor were changed in the range of between -1 and 1 for the appraisals, while the dependent variable was the extraction content of resveratrol.

All above experimental statistical analyses were analyzed using the software Design-Expert 8.0.6 (Stat-Ease, Minneapolis, MN, USA). Analysis of variance (ANOVA) was carried out to evaluate the optimal conditions for the resveratrol extraction from peanut roots using the DES-UAE method. A significance level of $p < 0.05$ was performed for each influential factor.

2.6. HPLC Analysis of Resveratrol. The determination of resveratrol content was carried out on an HPLC system (Waters e2695, USA). The chromatographic separation of resveratrol was performed on Waters Symmetry C18 reversed-phase column (250 mm \times 5 mm \times 4.6 mm, 5 μ m). The mobile phase consisting of 0.5% formic acid aqueous solution (A) and methanol (B) was filtered through a 0.45 μ m membrane filter prior to use. The gradient elution was performed as follows: 0–6 min, 28% B; 6–15 min, 28–60% B; 15–20 min, 60–100% B; and 20–25 min, 100% B. The flow rate and injection volume were 1.0 mL/min and 10 μ L, respectively, and the column temperature was set at 30°C. The resveratrol was identified by comparing the retention time with the standard, and the quantification of resveratrol was carried out at 306 nm. The HPLC analysis of the resveratrol standard and peanut roots sample was shown in Figure 1.

2.7. Statistical Analysis. Experimental results were obtained as the mean value \pm standard deviation (SD) ($n = 3$). The significance of difference was assessed using ANOVA. Differences were considered significant when the p value was < 0.05 .

3. Results and Discussion

3.1. Effect of DESs on Resveratrol Extraction. The components of DESs have significant influence on their physicochemical properties, such as polarity, viscosity, and dissolving capacity, which will directly influence their extraction efficiency. In the present study, ChCl-based DESs were

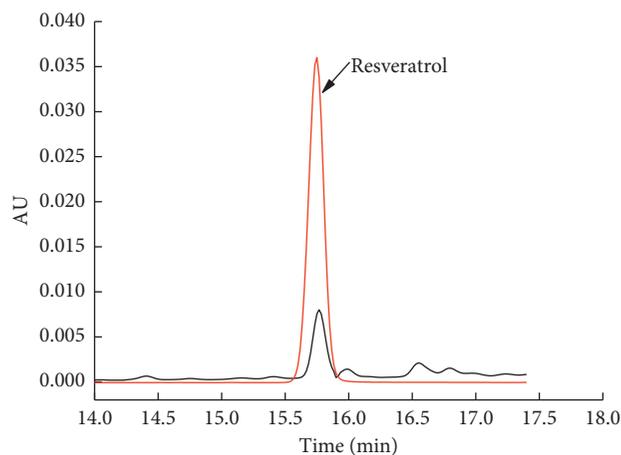


FIGURE 1: HPLC chromatogram of resveratrol extracted from peanut roots (the red and black lines mean the standard of resveratrol and sample, respectively).

synthesized by ChCl combining with different hydrogen bond donors (HBDs) including 1,4-butanediol, citric acid, ethylene glycol, lactic acid, glycerol, 1,2-propylene glycol, DL-malic acid and carbamide. The obtained eight DESs with different physicochemical properties were used for extracting resveratrol from peanut roots. The extraction contents were shown in Figure 2. The results indicated that the DESs type indeed strongly influenced the resveratrol extraction efficiency. The sequence of DESs for the extraction contents of resveratrol was as follows: DES-1 (ChCl/1,4-Buta) > DES-2 (ChCl/1,2-PG) > DES-4 (ChCl/EG) > DES-6 (ChCl/LA) > DES-7 (ChCl/Ca) > DES-3 ((ChCl/Gly) > DES-5 (ChCl/MA) > DES-8 (ChCl/CA). The optimal DES which provided the highest extraction content (26.44 ± 0.06 mg/kg) of resveratrol was DES-1 (composed of ChCl/1,4-butanediol). These data indicated that the polyalcohol-based DESs had a better extraction efficiency than organic acid-based DESs except for DES-3 ((ChCl/glycerol). This was because the strength of H-bonding interactions of the organic acid-based DESs would be the most efficient or the enthalpy of hole formation of polyalcohol-based DESs would be better for the resveratrol extraction [27]. Moreover, resveratrol belong to polyhydroxyphenols, and the polyalcohol-based DESs had a more suitable polarity for the resveratrol extraction. In addition, the much higher viscosity of glycerol-based DES also limited its extraction efficiency of resveratrol. Therefore, the following experiments were aimed at optimizing the extraction processing of resveratrol using choline chloride-1,4-butanediol (ChCl/1,4-Buta) (DES-1) as an extraction solvent.

3.2. Effect of Choline Chloride/1,4-Butanediol Molar Ratio on Resveratrol Extraction. The effect of the choline chloride/1,4-butanediol molar ratio (1:1, 1:2, 1:3, 1:4, and 1:5) on the resveratrol extraction was examined (Figure 3). The results showed that the maximum resveratrol content (28.79 ± 0.12 mg/kg) could be achieved at choline

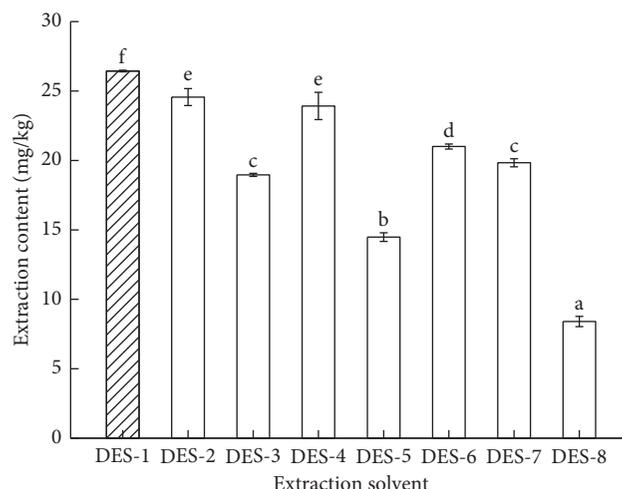


FIGURE 2: Effect of DESs on the extraction content of resveratrol from peanut roots. UAE conditions: solid/liquid ratio of 1: 20 (g/mL), 25°C, 30 min, ultrasonic power of 40 kHz, and choline chloride/1,4-butanediol molar ratio of 1 : 2, 20% of water (v/v) (DES-1: ChCl/1,4-Buta; DES-2: ChCl/1,2-PG; DES-3: ChCl/Gly; DES-4: ChCl/EG; DES-5: ChCl/MA; DES-6: ChCl/LA; DES-7: ChCl/Ca; and DES-8: ChCl/CA) (data are expressed as the mean \pm SD; means in the group with different letters differ significantly at $p < 0.05$).

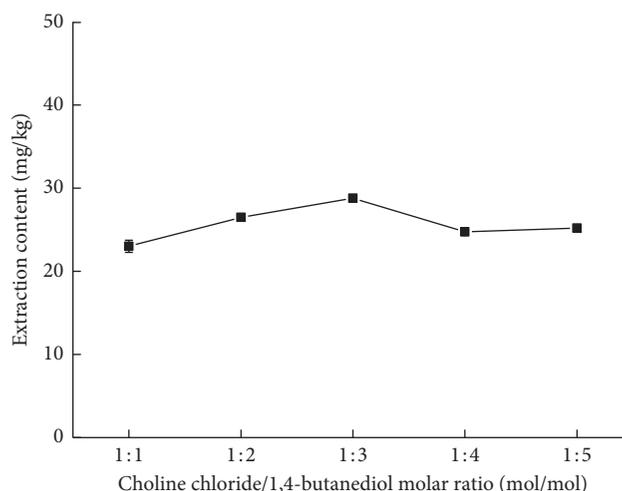


FIGURE 3: Effect of choline chloride/1,4-butanediol molar ratio on the extraction content of resveratrol from peanut roots. UAE conditions: solid/liquid ratio of 1: 20 (g/mL), 25°C, 30 min, ultrasonic power of 40 kHz, extraction solvent of choline chloride/1,4-butanediol (DES-1), and 20% of water (v/v).

chloride/1,4-butanediol molar ratio of 1 : 3. Further increasing of the proportion of 1,4-butanediol (>1 : 3) would cause the decline of resveratrol content (1 : 4, 24.76 ± 0.47 mg/kg, and 1 : 5, 25.21 ± 0.30 mg/kg). The increase of the 1,4-butanediol molar ratio in DES would result in the viscosity of DES decreasing and the polarity increasing, which might affect the effectiveness of mass transport and diffusion of resveratrol from peanut roots [28–30]. Therefore, the ChCl/1,4-butanediol molar ratio of 1 : 3 (mol/mol) was selected for the next experiments.

3.3. Effect of Water Content in DESs on Resveratrol Extraction.

The high viscosity of DESs not only hinders the mass transport from plant matrices to solution but also leads to handling difficulties. Polarity is another important property

of DESs since it affects the solubilizing ability of DESs. The addition of water to DES can decrease the viscosity of the DESs, adjust the polarity, and increase the solubility of the target compounds. In this study, ChCl/1,4-Buta- (DES-1-) water mixture with water fraction ranging from 10% to 50% (v/v) was evaluated for the extraction of resveratrol from peanut roots (Figure 4).

As shown in Figure 4, the extraction power of the resveratrol was significantly improved with the increasing proportion of water, up to maximum (28.26 ± 0.77 mg/kg) at 30% (v/v). This was because that the addition of water led to a decrease in the viscosity of the reaction media, improving the mass transfer from peanut roots to solution, therefore, enhancing the extraction efficiency. However, higher concentration of water in ChCl/1,4-Buta (DES-1) (40%–100%) led to the decrease in the extraction amount of resveratrol

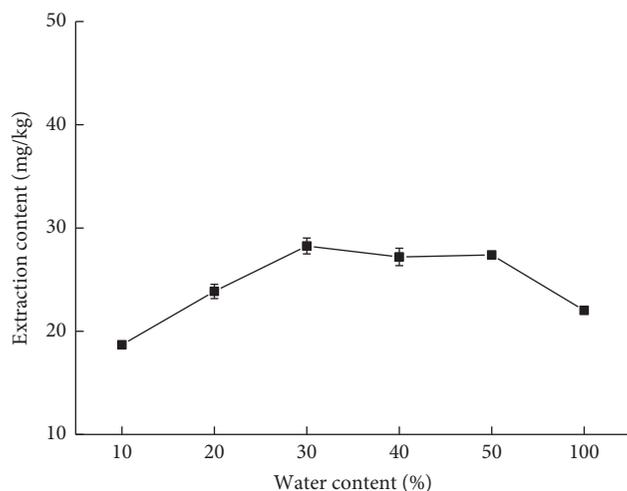


FIGURE 4: Effect of water content on the extraction content of resveratrol from peanut roots. UAE conditions: solid/liquid ratio of 1: 20 (g/mL), 25°C, 30 min, ultrasonic power of 40 kHz, extraction solvent of choline chloride/1,4-butanediol (DES-1), and choline chloride/1,4-butanediol molar ratio of 1:3.

(40%, 27.20 ± 0.84 ; 50%, 27.39 ± 0.14 ; and 100%, 22.02 ± 0.21) (mg/kg). This was probably because higher concentration of water weakened the interactions between resveratrol and ChCl/1,4-Buta (DES-1) and also increased the polarity of extraction solution. Furthermore, the excess water made the ChCl/1,4-Buta (DES-1) diluted, which might result in the disruption of hydrogen bonds of DESs consequently [31]. Hence, water content of 30% in the ChCl/1,4-Buta was considered as the optimal ratio.

3.4. Effect of Solid/Liquid Ratio on Resveratrol Extraction.

The solid/liquid ratio was evaluated (Figure 5). From the results, we could find that the extraction efficiency of resveratrol increased from 13.17 ± 0.32 to 38.34 ± 0.54 mg/kg with the increase of the solid/liquid ratio from 1:10 to 1:30 (g/mL). But further increase of the solid/liquid ratio had no obvious effect on the extraction content of resveratrol (1:40, 36.88 ± 0.18 , and 1:50, 38.04 ± 0.21) (mg/kg), indicating that the target compound could be fully extracted at 1:30 g/mL of the solid/liquid ratio. Therefore, 1:30 g/mL of solid/liquid ratio was selected for the further experiments.

3.5. Effect of Extraction Temperature on Resveratrol Extraction. The temperature affects the viscosity and solubility of solvents and therefore affects the extraction efficiency of resveratrol. As shown in Figure 6, the extraction content of the resveratrol increased continually with the increasing extraction temperatures from 20°C to 60°C (20°C, 35.66 ± 0.15 , and 60°C, 40.53 ± 0.67) (mg/kg). The elevated temperature might decrease the viscosity of the DESs, inducing the full contact of the material with the extraction solvent. The extraction efficiency at 70°C (40.52 ± 0.67 mg/kg) had no change compared to 60°C. However, with further increase of

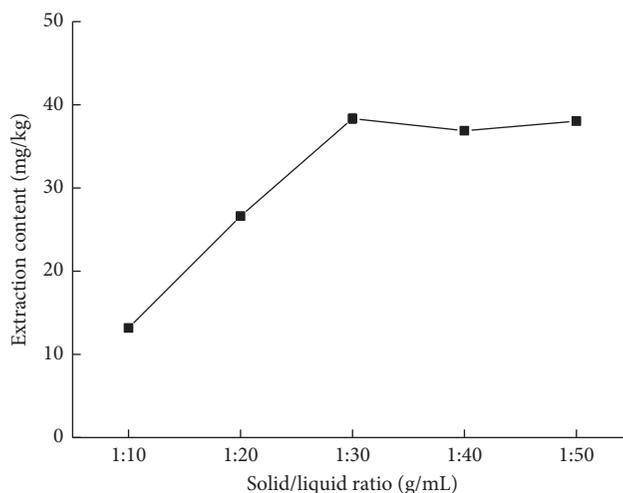


FIGURE 5: Effect of solid/liquid ratio on the extraction content of resveratrol from peanut roots. UAE conditions: 25°C, 30 min, ultrasonic power of 40 kHz, extraction solvent of choline chloride/1,4-butanediol (DES-1), choline chloride/1,4-butanediol molar ratio of 1:3, and 30% of water (v/v).

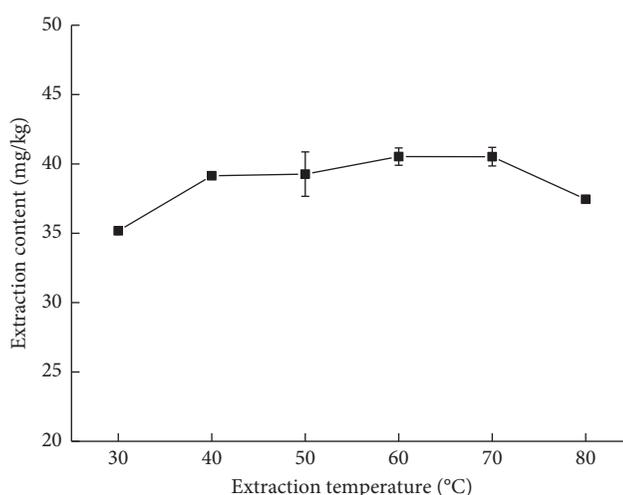


FIGURE 6: Effect of extraction temperatures on the extraction content of resveratrol from peanut roots. UAE conditions: solid/liquid ratio of 1:30 (g/mL), 30 min, ultrasonic power of 40 kHz, extraction solvent of choline chloride/1,4-butanediol (DES-1), choline chloride/1,4-butanediol molar ratio of 1:3, and 30% of water (v/v).

temperature, the extraction content of resveratrol decreased slightly (80°C, 37.46 ± 0.002 mg/kg), probably because the higher extraction temperature would make the resveratrol oxidized or decomposed. Comprehensively considering the extraction efficiency and energy saving, 60°C was selected as the optimal extraction temperature.

3.6. Effect of Extraction Time on Resveratrol Extraction.

The extraction time was also investigated, and the results were shown in Figure 7. The highest extraction content of the resveratrol was obtained at 30 min (39.15 ± 0.07 mg/kg).

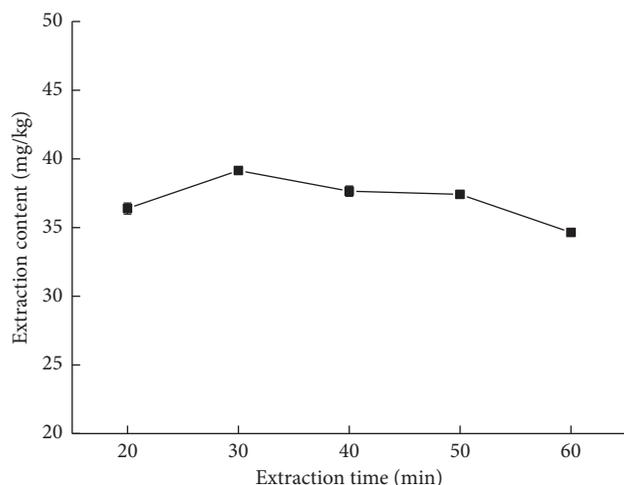


FIGURE 7: Effect of extraction time on the extraction content of resveratrol from peanut roots. UAE conditions: solid/liquid ratio of 1 : 30 (g/mL), 60°C, ultrasonic power of 40 kHz, extraction solvent of choline chloride/1,4-butanediol (DES-1), choline chloride/1,4-butanediol molar ratio of 1 : 3, and 30% of water (v/v).

With the prolonging of extraction time from 30 to 60 min, the resveratrol content decreased slightly (40 min, 37.65 ± 0.37 ; 50 min, 37.41 ± 0.05 ; and 60 min, 34.64 ± 0.06) (mg/kg). This trend might be because resveratrol took place oxidation or decomposition during the long time extraction process. Thus, 30 min was chosen as the optimal extraction time.

3.7. Optimization of the Extraction Process by the BBD Assay. Further optimization of DESs-UAE resveratrol extraction conditions (water content, liquid/solid ratio, extraction temperature, and extraction time) was carried out by a Box-Behnken design (BBD) method. The data were analyzed using Design-Expert 8.0.6 software for statistical analysis of variance (ANOVA) and regression analysis (Table 2). The regression equation model for resveratrol extraction was obtained and shown in the following equation:

$$\begin{aligned}
 Y = & 37.58 + 2.52A + 4.81B + 1.47C + 1.74D + 0.85AB \\
 & - 1.55AC - 0.8AD - 1.53BC + 0.57BD - 0.93CD \\
 & - 6.21A^2 - 9.48B^2 - 1.82C^2 - 3.27D^2,
 \end{aligned}
 \tag{4}$$

where Y is the extraction content of resveratrol (mg/kg) and A , B , C , and D represented water content, solid/liquid ratio, extraction temperature, and extraction time, respectively.

The analysis of variance (ANOVA) was performed to evaluate the optimal extraction conditions of resveratrol (Table 3). The F -value of the model was 31.03 ($p < 0.0001$), indicating that the afforded model was significant. "Lack of fit F -value" was 1.74 ($p = 0.3117$), demonstrating that the lack of fit of the quadratic models was not significant, and the experiment data fitted well to the model. The regression analysis of the data showed the coefficient of the

TABLE 2: Experimental data and the obtained response values with different combinations of water content (A), solid/liquid ratio (B , g/mL), extraction temperature (C , °C), and extraction time (D , min) used in BBD.

Run	Factor				Extraction content Y
	A	B	C	D	
1	70	30	30	40	32.40
2	40	30	55	40	38.33
3	40	10	30	40	18.17
4	10	30	55	10	22.44
5	70	50	55	40	29.08
6	40	50	55	70	30.66
7	10	30	30	40	23.54
8	40	30	30	10	26.86
9	40	30	55	40	38.12
10	40	50	55	10	27.88
11	40	30	55	40	38.19
12	10	30	55	70	28.26
13	10	50	55	40	2.93
14	70	30	55	10	28.95
15	10	30	80	40	30.76
16	70	30	55	70	31.56
17	70	30	80	40	33.41
18	40	10	55	10	21.07
19	40	30	55	40	38.03
20	40	30	80	10	33.14
21	40	10	55	70	21.57
22	40	30	30	70	33.34
23	70	10	55	40	18.77
24	40	30	55	40	35.22
25	40	10	80	40	21.49
26	40	30	80	70	35.88
27	40	50	80	40	30.73
28	10	10	55	40	16.03
29	40	50	30	40	33.53

determination ($R^2=0.9688$) value for resveratrol was significant, implying that this quadratic model was suitable to describe the response of the experiment regarding to the resveratrol.

The effect of these factors affecting the resveratrol extraction was in an order of B (liquid/solid ratio) $> A$ (water content) $> D$ (extraction time) $> C$ (extraction temperature), which was determined by the absolute value of the liner term coefficient of the regression equation. The p value of the quadratic term of A^2 and B^2 was both < 0.0001 , respectively, implying that water content (A) and solid/liquid (B) ratio both had significant effects on the extraction content of resveratrol.

The effect and interaction of four factors on the extraction yields of resveratrol were examined by the three-dimensional response surface (Figure 8). Figure 8(a) showed the effects of water content, solid/liquid ratio, and their interaction on the extraction content of resveratrol. It was observed that the highest extraction content was afforded with the water content range of 15%–55% and solid/liquid ratio of 25–38 mL/g. When solid/liquid ratio was a certain value, the extraction content of resveratrol had the trend of increasing first and then decreasing with the increase of water content. When the water content was fixed, the yield of

TABLE 3: ANOVA statistics analysis of the model for the extraction of resveratrol.

Source	Sum of squares	df	Mean square	F value	p value probability > F	Significance
Model	1168.03	14	83.43	31.03	<0.0001	**
A (water content; %)	76.10	1	76.10	28.31	0.0001	**
B (solid/liquid ratio; g/mL)	277.52	1	277.52	103.22	<0.0001	**
C (extraction temperature; °C)	25.78	1	25.78	9.59	0.0079	*
D (extraction time; min)	36.45	1	36.45	13.56	0.0025	*
AB	2.9	1	2.9	1.08	0.3164	
AC	9.64	1	9.64	3.58	0.0792	
AD	2.56	1	2.56	0.95	0.3458	
BC	9.38	1	9.38	3.49	0.0829	
BD	1.31	1	1.31	0.49	0.4969	
CD	3.49	1	3.49	1.30	0.2738	
A ²	249.87	1	249.87	92.24	<0.0001	**
B ²	583.45	1	583.45	217.01	<0.0001	**
C ²	21.44	1	21.44	7.97	0.0135	
D ²	69.44	1	69.44	25.83	0.0002	
Residual	37.64	14	2.69			
Lack of fit	30.62	10	3.06	1.74	0.3117	Not significant
Pure error	7.02	4	1.76			
Correlation total	1205.67	28				

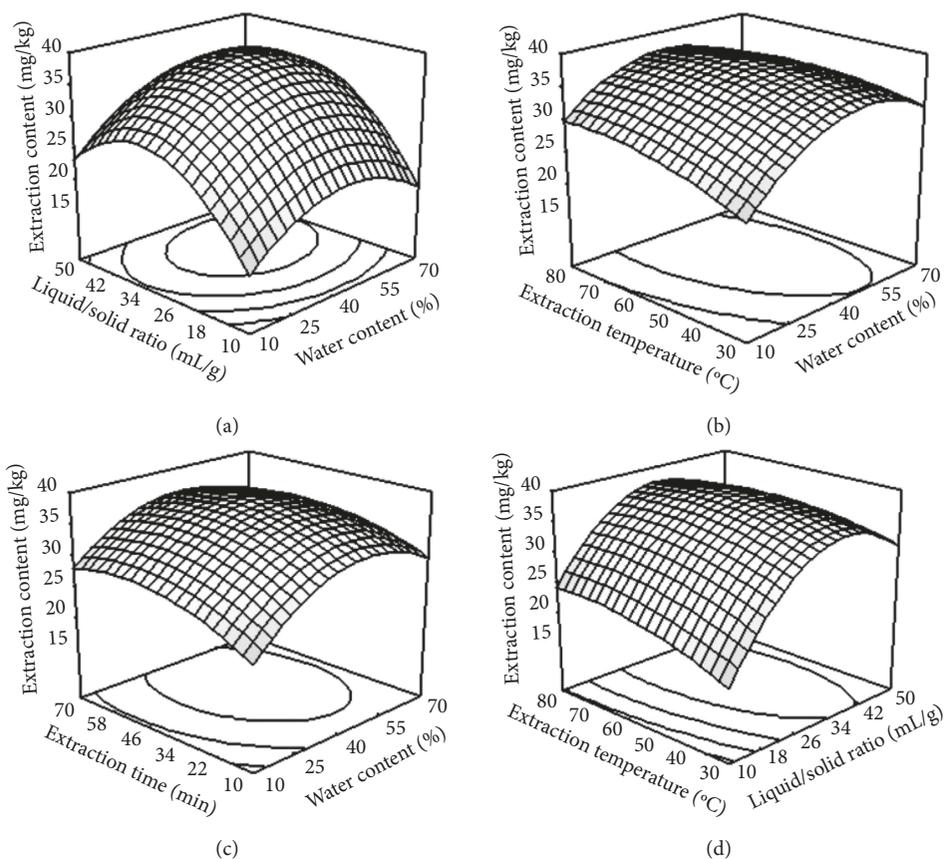
* $p < 0.01$; ** $p < 0.001$.

FIGURE 8: Continued.

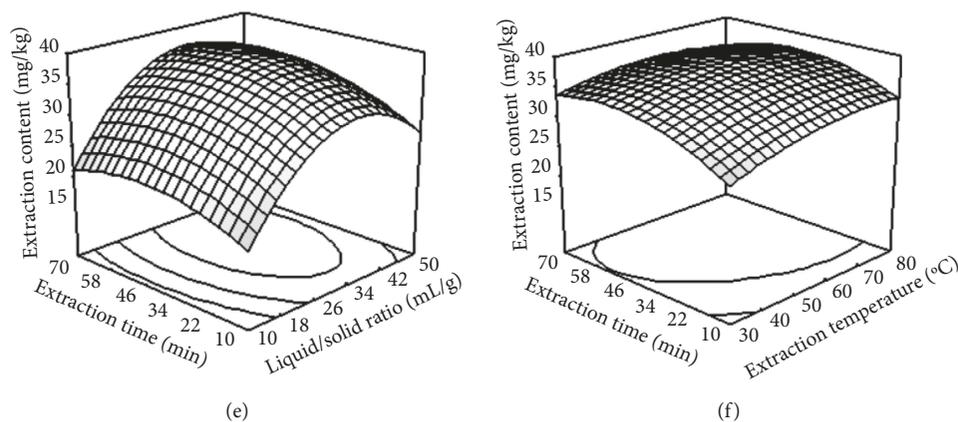


FIGURE 8: (a–f) refers to the target compounds response values under the two variables. Response surfaces representations for resveratrol from peanut roots: (a) varying water content and liquid/solid ratio; (b) varying water content and temperature; (c) varying water content and time; (d) varying temperature and liquid/solid ratio; (e) varying liquid/solid ratio and time; (f) varying temperature and time.

resveratrol was increased and then kept at the stable level with the increase of solid/liquid ratio. The interaction effect of water content and solid/liquid ratio on the resveratrol content was not significant. The same trend was observed in Figures 8(b)–8(f) (the interactive effects of water content, extraction time, and solid/liquid ratio on the extraction content of resveratrol). In summary, the interactions in water content, extraction time, extraction temperature, and solid/liquid ratio on the resveratrol extraction from peanuts roots were no significant, which was consistent with the analysis result of the regression model.

The optimal conditions for the extraction of resveratrol from peanut roots were obtained based on the established model, which were as follows: water content of 40% (v/v), solid/liquid ratio of 1:30 g/mL, extraction temperature of 55°C, and extraction time of 40 min. Under these optimum conditions, the predicted extraction content of resveratrol from peanut roots was 38.39 mg/kg. The verification experiment was also performed, and the obtained extraction content of resveratrol was up to 38.91 mg/kg, which indicated that the established model was considered to be reliable and reasonable.

4. Conclusion

In this study, a new type of green and efficient solvent DESs coupled with ultrasonic-assisted extraction (UAE) and HPLC-UV was developed to extract resveratrol from peanut roots. The optimal DES-UAE conditions were obtained using a BBD test combined with a response surface methodology as follows: extraction solvent 40% of water in ChCl/1,4-butanediol (1/3, g/g), extraction temperature 55°C, solid/liquid ratio 1:30 g/mL, and extraction time 40 min. Under the above optimum conditions, the total extraction content of resveratrol from peanut roots was up to 38.91 mg/kg, and the extraction yield was 89.46%. It was concluded that this DES-UAE-HPLC method was a fast, safe, and efficient extraction method for the preparation and determination of resveratrol from peanut roots.

Abbreviations

DESs: Deep eutectic solvents
 UAE: Ultrasonic-assisted extraction
 BBD: Box–Behnken design
 HBD: Hydrogen bond donor.

Data Availability

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

Conflicts of Interest

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

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