Research Article

Response Surface Optimization of Rotenone Using Natural Alcohol-Based Deep Eutectic Solvent as Additive in the Extraction Medium Cocktail

Zetty Shafiqah Othman, Nur Hasyareeda Hassan, and Saiful Irwan Zubairi

School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

Correspondence should be addressed to Saiful Irwan Zubairi; saiful-z@ukm.edu.my

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Rotenone is a biopesticide with an amazing effect on aquatic life and insect pests. In Asia, it can be isolated from *Derris* species roots (*Derris elliptica* and *Derris malaccensis*). The previous study revealed the comparable efficiency of alcohol-based deep eutectic solvent (DES) in extracting a high yield of rotenone (isoflavonoid) to binary ionic liquid solvent system ([BMIM]OTf) and organic solvent (acetone). Therefore, this study intends to analyze the optimum parameters (solvent ratio, extraction time, and agitation rate) in extracting the highest yield of rotenone extract at a much lower cost and in a more environmental friendly method by using response surface methodology (RSM) based on central composite rotatable design (CCRD). By using RSM, linear polynomial equations were obtained for predicting the concentration and yield of rotenone extracted. The verification experiment confirmed the validity of both of the predicted models. The results revealed that the optimum conditions for solvent ratio, extraction time, and agitation rate were 2 : 8 (DES : acetonitrile), 19.34 hours, and 199.32 rpm, respectively. At the optimum condition of the rotenone extraction process using DES binary solvent system, this resulted in a 3.5-fold increase in a rotenone concentration of 0.49 ± 0.07 mg/ml and yield of 0.35 ± 0.06 (% w/w) as compared to the control extract (acetonitrile only). In fact, the rotenone concentration and yield were significantly influenced by binary solvent ratio and extraction time ($P < 0.05$) but not by means of agitation rate. For that reason, the optimal extraction condition using alcohol-based deep eutectic solvent (DES) as a green additive in the extraction medium cocktail has increased the potential of enhancing the rotenone concentration and yield extracted.

1. Introduction

Rotenone extract has a great impact as an insecticidal product and can be isolated naturally from the roots of *Derris* sp. in Asia, *Lonchocarpus* in South America, and several other legumes in the tropics region [1]. *Derris elliptica* is widely available as a local plant and it contains approximately 4% to 5% (w/w) of rotenone in dried roots [2, 3]. However, the rotenone content is still low as compared to the commercial grade rotenoids resin [4] and the organic solvents as its extraction medium has a high risk toward humans and the environment. Thus, alternative green solvent with high extraction efficiency is crucial in enhancing the quality and quantity of rotenone extract. In the case of rotenone (Figure 1(a)) extraction, the previous study showed the potential

of environmental friendly ionic liquid binary solvent system to increase the yield of rotenone extracted compared to organic solvent (acetone) [5]. However, in terms of costing and convenience, ionic liquid is expensive, hygroscopic, and hard to handle, limiting its usage as an extraction medium. Thus, this leads to the introduction of deep eutectic solvents (DESs) as an additive in extraction medium. Deep eutectic solvents (DESs) are emerging green solvents well known for their biodegradability, nontoxicity, and low cost which overcome the drawbacks of conventional organic solvents and ionic liquids (ILs). Previous study has shown that DESs which are comprised of choline chloride and 1,4-butanediol with a ratio of 1 : 5 produced the highest yield of extracted flavonoids (myricetin and amentoflavone) [6]. In addition, a preliminary study on comparing the efficiency between

FIGURE 1: Molecular structure of (a) rotenone and (b) alcohol-based deep eutectic solvent (DES).

FIGURE 2: (a) *Derris elliptica* roots and (b) ground fine roots.

ionic liquid (IL), acetone, and alcohol-based deep eutectic binary solvent system in extracting rotenone compound also showed a remarkable potential of alcohol-based deep eutectic binary solvent system (Figure 1(b)) to be used as a green additive in extraction of rotenone (isoflavonoid) [5]. A study conducted by Zubairi et al. [7, 8] on the optimal condition for rotenone extraction which was comprised of solvent types, solvent-to-solid ratio, and particle size recorded acetone as the best solvent for extraction with optimal solvent-to-solid ratio of 10 ml/g and 0.84 mm particle size. Therefore, this study aims to enhance the optimal extraction conditions with additional parameters such as solvent ratio, extraction time, and agitation rate for *Derris elliptica* roots by means of response surface methodology (RSM) to obtain the optimal levels of the rotenone yield and concentration by introducing a new approach of a green extraction process.

2. Material and Methods

2.1. Sample Collection and Preparation. *Derris elliptica* roots were first collected from Ladang 2, Faculty of Agriculture, Universiti Putra Malaysia, UPM, Malaysia. The collected roots (Figure 2(a)) were cleaned and cut into smaller parts prior to the rapid drying. The cleaned parts of the roots were placed in the freezer to maintain their freshness and were dried using vacuum oven at temperature ($28 \pm 2^\circ\text{C}$) for 24

hours. Once dried, the roots were ground into smaller particles of the size of approximately 0.86 ± 0.20 mm (Figure 2(b)). The selected sieved ground samples were weighed prior to the normal soaking extraction process (NSE).

2.2. Preparation of Alcohol-Based Deep Eutectic Solvent. Deep eutectic solvent (DES) was prepared by mixing choline chloride, ChCl (98% in purity, Sigma Aldrich), with 1,4-butanediol (99% in purity, Sigma Aldrich) at mol ratio of 1/5 according to Bi et al. [6]. The mixture was continuously stirred at 80°C until a homogenous mixture was obtained. The solution was then kept in Scott bottle once cooled down. The density of DES was recorded and the structure elucidation of DES was analyzed using FTIR, ^1H NMR, and ^{13}C NMR.

2.3. Preparation of Several Alcohol-Based Deep Eutectic Binary Solvent Systems. The binary solvent systems were prepared according to design run order (Table 1) using Design Expert software (DX 6) with five different solvent ratios of DES to acetonitrile (95% in purity) (1:0, 8:2, 5:5, 2:8, and 0:1). The mixtures were stirred by using magnetic stirrer for 5 to 6 hours to homogenize the combined solvents.

2.4. Design of Experiment (DOE). Response surface methodology (RSM) is a collection of mathematical and statistical techniques for empirical model building. The RSM was

TABLE 1: Response surface optimization run order actual and coded value.

Run order	Actual value			Coded value		
	X_1 : Solvent ratio (DES : acetonitrile)	X_2 : Extraction time (hour)	X_3 : Agitation rate (rpm)	X_1 : Solvent ratio	X_2 : Extraction time (hour)	X_3 : Agitation rate (rpm)
1	8:2	19.34	50.67	1.00	1.00	-1.00
2 (CP)	5:5	12.50	125.00	0.00	0.00	0.00
3	8:2	19.34	199.33	1.00	1.00	1.00
4 (CP)	5:5	12.50	125.00	0.00	0.00	0.00
5 (CP)	5:5	12.50	125.00	0.00	0.00	0.00
6	5:5	12.50	250.00	0.00	0.00	1.68
7 (CP)	5:5	12.50	125.00	0.00	0.00	0.00
8 (CP)	5:5	12.50	125.00	0.00	0.00	0.00
9	1:0	12.50	125.00	1.68	0.00	0.00
10	5:5	24.00	125.00	0.00	1.68	0.00
11	5:5	1.00	125.00	0.00	-1.68	0.00
12	2:8	5.66	199.33	-1.00	-1.00	1.00
13	5:5	12.50	0.00	0.00	0.00	-1.68
14	2:8	19.34	199.33	-1.00	1.00	1.00
15	2:8	5.66	50.67	-1.00	-1.00	-1.00
16	8:2	5.66	199.33	1.00	-1.00	1.00
17	2:8	19.34	50.67	-1.00	1.00	-1.00
18	0:1	12.50	125.00	-1.68	0.00	0.00
19 (CP)	5:5	12.50	125.00	0.00	0.00	0.00
20	8:2	5.66	50.67	1.00	-1.00	-1.00

carried out to optimize response variables (e.g., concentration and yield of rotenone) which are influenced by several independent variables such as solvent ratio, agitation rate, and extraction time. The experiment includes a series of runs, in which changes are made to the input variables in order to identify the best responses of any output changes occurring throughout those experiments. Therefore, the normal soaking extraction (NSE) was carried out at room temperature ($28 \pm 2^\circ\text{C}$) [9, 10] in accordance with the run order design (Table 1) tabulated using Design Expert 6.0. This central composite rotatable design (CCRD) was comprised of three factors (X_1 : solvent ratio, X_2 : extraction time (hours), and X_3 : agitation rate (rpm)) and five levels ($-\alpha$, -1 , 0 , 1 , and $+\alpha$) that contributed to the total of 20 experiments with eight factorial points, six axial points, and six centre points (CP). The extraction process was conducted by soaking 0.50 g of dried roots in 5 ml of respective solvent systems with solvent-to-solid ratio of 10 ml/g at respective extraction time (hours) and agitation rate (rpm). The liquid crude extract was collected at every respective extraction time (hours) prior to the reversed-phase high performance liquid chromatography (RP-HPLC). The rotenone concentration and yield (dependent variables) were calculated based on the external standard method of RP-HPLC.

2.5. Liquid Crude Extract Collection. The liquid crude extracts were collected according to run order (Table 1) and placed in the labeled vials. There was a total of 20 samples.

Next, the collected samples were placed in a freezer (-18°C) to prevent any thermal degradation.

2.6. Preparation of Fine Debris-Free Liquid Crude Extract.

The collected liquid crude extracts were diluted using analytical grade acetonitrile, Sigma Aldrich, 95% (v/v) with a dilution factor (DF) of 20. Then, the extracts were filtered by using polytetrafluoroethylene (PTFE: $0.45\ \mu\text{m}$ pore size) vacuum filtration to remove any fine debris. A 2 ml vial was used to store the extracts prior to the quantitative analysis [11].

2.7. Quantitative Analysis Using Reversed-Phase High Performance Liquid Chromatography.

Approximately, 20 mg of rotenone standard of Sigma Aldrich, 95% (w/w), was diluted with 50 ml of acetonitrile in a volumetric flask. The stock solution was filtered using Whatman filter paper number 2 with $8\ \mu\text{m}$ pore size. The quantitative analysis was performed by using symmetry C18 $5\ \mu\text{l}$ column and waters with the internal diameter of 4.6 mm and 150 mm in length. The physical parameters involved in the RP-HPLC were as follows: (1) flow rate of 0.7 ml/min; (2) injection volume of $20\ \mu\text{l}$; (3) mobile phase of acetonitrile and deionized water with the ratio of 60 : 40; and (4) photodiode array detector (PDA) wavelength at 294 nm [11, 12].

2.8. Statistical Analysis. The experimental data fit the following second-order polynomial model and the regression coefficients were obtained. The generalized second-order

FIGURE 3: FTIR spectrum of alcohol-based DES.

polynomial model proposed for response surface analysis was given as in (1), where β_0 , β_i , β_{ii} , and β_{ij} are regression coefficients for intercept, linear, quadratic, and interaction term, respectively. X_i and X_j are coded values of independent variables while k equals the number of tested factors ($k = 3$). The analysis of variance (ANOVA) was performed to determine the significant differences between the independent variables. The effect and regression coefficients of individual linear, quadratic, and interaction term were determined. A reduced model involving statistically significant independent variables ($P < 0.05$) was taken into account. The three-dimensional (3D) surface response and contour plots were constructed to represent the interaction between independent variables and responses, while multiple regressions were applied in analysing experimental data to predict the coefficients of the fitted second-order polynomial model.

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

2.9. Verification Model of Optimized Parameters. The verification phase was then carried out based on the results obtained from the optimization phase. The optimal conditions were obtained using second-order polynomial model of RSM (response surface methodology). The suitability of the model equation for predicting the response values (concentration, mg/ml and yield (% w/w)) was verified by conducting the extraction process under the recommended optimal conditions. A numerical optimization method was adopted in identifying the maximized response point and a series of solutions was generated where the solutions to be employed for verification would be selected based on desirability and

suitability. The experimental and predicted values were compared in order to determine the model validity. To confirm the results, the experiments were conducted in triplicate ($n = 3$) under the selected optimized parameters.

3. Results and Discussion

3.1. Characterization of Alcohol-Based Deep Eutectic Solvent.

The prepared DESs with a density and viscosity of 1.38 ± 0.0034 g/ml and 64.15 ± 1.35 cP, respectively, were elucidated using FTIR, ^1H NMR, and ^{13}C NMR. Based on the FTIR spectrum (Figure 3), it could be observed that the broad OH bond stretched at 3294.25 cm^{-1} of choline chloride, ChCl, and 1,4-butanediol along with Sp^3 C-H that reached its stretching peak at 2936.52 cm^{-1} and 2867.96 cm^{-1} . CH_2 and CH_3 bendings were also observed at 1477.51 cm^{-1} and 1416.69 cm^{-1} , respectively, while C-N $^+$ symmetric stretching of choline chloride, ChCl, was observed at 748.24 cm^{-1} . There were several peaks that were represented by 1,4-butanediol. The peaks were C-O-H bond bending (multi-peaks, broad and weak) at a range of $1,300\text{--}1,200\text{ cm}^{-1}$ and C-O bond stretching peak in 1° alcohol at 1048.93 cm^{-1} . Choline chloride, ChCl, was used as hydrogen bond acceptor (HBA), while 1,4-butanediol was used as hydrogen bond donor (HBD). The stretching vibrations of alcohol in both 1,4-butanediol and choline chloride, ChCl, shifted to a lower frequency from $3,340\text{ cm}^{-1}$ in ChCl [13] to 3294.25 cm^{-1} in 1,4-butanediol DES, which was prepared by mixing two components. It was expected that the existence of the main hydrogen bonding in DES was due to the bonding between Cl^- ion in ChCl and hydrogen donor molecule such as urea or ethylene glycol (EG) [14]. Nevertheless, the shift to a lower frequency of

FIGURE 4: ^1H NMR spectrum of alcohol-based DES.

hydroxyl stretching vibration could be attributed to the potential hydrogen bond formation through hydroxyl group in ChCl.

The proton nuclear magnetic resonance, ^1H -NMR (600 MHz) spectrum of DES (Figure 4), was obtained from deuterated methanol (CD_3OD). The ^1H -NMR spectrum showed the mixture of choline chloride, ChCl, and 1,4-butanediol, where the chemical shift for choline chloride, ChCl, appeared at δ (ppm) 3.255 (s, 9H), 3.530–3.592 (m, 2H), and 4.016–4.041 (m, 2H). On the other hand, for 1,4-butanediol, the chemical shift was observed at δ (ppm) 1.591–1.635 (m, 2H) and 3.583–3.592 (m, 2H).

The carbon-13 nuclear magnetic resonance, ^{13}C -NMR (600 MHz) spectrum of DES (Figure 5), was also obtained from deuterated methanol (CD_3OD). The chemical shift for choline chloride, ChCl, appeared at δ (ppm) 53.35 (CH_3), 55.71 (CH_2), and 67.62 (CH_2). As for 1,4-butanediol, the chemical shift was observed at δ (ppm) 28.79 (CH_2) and 61.49 (CH_2). Both ^1H -NMR and ^{13}C -NMR spectrum chemical shifts were comparable to the previous elucidation on DES as according to [15].

3.2. Quantitative Analysis. The rotenone concentration and yield (dependent variables) were calculated based on the external standard method of RP-HPLC. Table 2 shows the experiment results of the rotenone concentration and yield based on the run order design. Overall, it can be observed that the rotenone concentration and yield varied depending on the different values of each processing parameter (solvent ratio, extraction time, and agitation rate).

3.3. Response Surface Optimization

3.3.1. Model Fitting: Effect of Processing Parameters on the Concentration and Yield of Rotenone. In this study, central composite rotational design (CCRD) RSM applied had the lower and upper values set at $+\alpha$ ($+\alpha = 1.68$) and $-\alpha$ ($-\alpha = -1.68$) and all the factor levels were selected within the limits that were desirable and practical. The experimental and predicted values of the rotenone concentration and yield obtained from CCRD experimental design are given in Table 2. The results showed that the ranges of the concentration and yield of the rotenone extracted were 0.06 to 0.52 mg/ml and 0.04 to 0.39 (% w/w), respectively. The polynomial equation coefficients for this design were calculated using experimented values and the equation was used in calculating response values (concentration and yield) prediction through analysis of variance (ANOVA). The ANOVA of the resultant linear polynomial model for the rotenone concentration and yield is shown in Table 3. The regression model showed that the model was highly significant due to very low probability value ($P < 0.0001$). In addition, the coefficient of determination (R^2) closer to absolute value for the rotenone concentration (0.9729) and yield (0.9404) including the absence of any lack of fit ($P > 0.05$) had strengthened the model reliability. By applying multiple regression analyses, the relationship between the tested independent variables and those responses (rotenone concentration and yield) was represented by linear polynomial equation suggested by ANOVA in the following:

$$Y = 0.39 - 0.40X_1 + 0.01X_2 - 5.53E - 03X_3, \quad (2)$$

$$Y = 0.31 - 0.33X_1 + 4.20E - 07X_2 + 7.88E - 05X_3. \quad (3)$$

TABLE 2: Three independent factors with two responses and five levels CCRD with the experimented and predicted values under multiple extraction conditions.

Standard order	Independent variables			Dependent variables (responses)			
	X_1^A	X_2^B	X_3^C	$Y_1 = \text{Concentration (mg/ml)}$		$Y_2 = \text{Yield (\%, w/w)}$	
				Experimented	Predicted	Experimented	Predicted
1	8:2	19.34	50.67	0.21	0.22	0.11	0.13
2 (CP)	5:5	12.50	125.00	0.29	0.28	0.19	0.21
3	8:2	19.34	199.33	0.23	0.23	0.13	0.15
4 (CP)	5:5	12.50	125.00	0.26	0.28	0.19	0.21
5 (CP)	5:5	12.50	125.00	0.29	0.28	0.22	0.21
6	5:5	12.50	250.00	0.35	0.35	0.24	0.22
7 (CP)	5:5	12.50	125.00	0.29	0.28	0.21	0.21
8 (CP)	5:5	12.50	125.00	0.28	0.28	0.20	0.21
9	1:0	12.50	125.00	0.06	0.073	0.04	0.04
10	5:5	24.00	125.00	0.39	0.37	0.27	0.26
11	5:5	1.00	125.00	0.15	0.17	0.12	0.16
12	2:8	5.66	199.33	0.37	0.37	0.30	0.29
13	5:5	12.50	0.00	0.32	0.31	0.24	0.20
14	2:8	19.34	199.33	0.49	0.5	0.35	0.34
15	2:8	5.66	50.67	0.35	0.35	0.25	0.27
16	8:2	5.66	199.33	0.13	0.12	0.09	0.09
17	2:8	19.34	50.67	0.45	0.47	0.31	0.33
18	0:1	12.50	125.00	0.52	0.5	0.39	0.38
19 (CP)	5:5	12.50	125.00	0.30	0.28	0.22	0.21
20	8:2	5.66	50.67	0.12	0.11	0.12	0.08

FIGURE 5: ^{13}C NMR spectrum of alcohol-based DES.

TABLE 3: Analysis of variance of the fitted linear equation for rotenone concentration and yield.

Source	Rotenone concentration (mg/ml)			Rotenone yield (% w/w)		
	Mean square	F-value	P value	Mean square	F-value	P value
Model	0.09	191.4	<0.0001	0.048	84.18	<0.0001
X_1	0.22	470.59	<0.0001	0.13	232.1	<0.0001
X_2	0.047	100.67	<0.0001	0.011	19.64	<0.0001
X_3	$1.38E-03$	2.93	0.1057	$4.69E-04$	0.82	0.3796
Residual	$4.70E-04$			$5.74E-04$		
Lack of fit	$5.92E-04$	2.89	0.1258	$7.48E-04$	3.94	0.071
Pure error	$2.05E-04$			$1.90E-04$		

The predicted values were consistent with the experimental value. Linear polynomial equation was selected for both responses as there was no interactive term observed between the independent variables towards response values. This shows that each term was highly significant toward the rotenone concentration and yield. The linear term of solvent ratio stood out from the rest of the terms as it showed the highest *F*-value for both responses, followed by the extraction time variables. This indicates that the solvent ratio had the largest effect on the rotenone concentration and yield. However, insignificant linear term of agitation rate showed that the values of response variables were not much affected by agitation rate.

3.4. Analysis of Response Surface Plot. The experimental data gathered from the responses of process parameter analyzed through ANOVA showed that a linear polynomial equation was the best equation in predicting the rotenone concentration and yield as it was fit for all variables involved and insignificant with respect to interaction terms. The effect of each independent variable toward the rotenone concentration and yield was illustrated using three-dimensional (3D) response surface plot (Figure 6) and perturbation plot (Figure 7). The 3D response surface plot illustrates the response in function of two factors and keeps the other constant at its middle level. The predicted response surface showing the effect of solvent ratio and agitation rate on the rotenone concentration at constant extraction time (12.5 hours) appears as a linear plane shape (Figure 6(a)). Figure 6(a) depicts a higher rotenone extract concentration at the solvent ratio of 2:8 and agitation rate of 199.33 rpm. It can be observed that the rotenone concentration mounted up as the DES amount decreased and agitation rate increased. Figure 6(b) presents the effect of solvent ratio and extraction time on the rotenone yield extracted at the fixed agitation rate of 125 rpm. Both factors displayed a significant linear effect and a higher rotenone yield achieved at the solvent ratio of 2:8 and extraction time of 19.34 hours. The rotenone yield increased as the solvent ratio of DES decreased with the increased of extraction time.

In terms of solvent ratio, the viscosity of the binary solvent system played a crucial role in extracting a high concentration and yield of the rotenone. As shown in Table 4, the higher the solvent ratio of DES-to-acetonitrile, the greater the

TABLE 4: Viscosity of DES binary solvent systems at different ratio of DES: acetonitrile.

DES: acetonitrile ratio	Viscosity (Cp)
2:8	0.091 ± 0.006
5:5	18.70 ± 5.09
8:2	25.4 ± 1.38
1:0	64.15 ± 1.35

solvent viscosity and it would eventually limit the extraction process. For that reason, further study has to be carried out on choosing the best solvent ratio in which viscosity of the cocktail medium could be reduced at an applicable level (containing more acetonitrile) and eventually facilitate the diffusion between solutes-to-solvent and improve the extraction process. Rotenone is a nonpolar compound which required a medium polarity solvent to be extracted from *Derris* roots. The green cocktail used (a solvent mixture of DES and acetonitrile) for extraction process contained moderate polarities of 54.76 and 45.6 Kcalmol⁻¹, respectively, in which both chemicals lie in the range of moderate polarity [16]. The present of hydrogen bond donor (butanediol) and hydrogen bond acceptor (choline chloride) could have attracted those molecules to form hydrogen bonding with rotenone's functional groups and, as a result, good extraction efficacy was attained. Thus, this binary solvent system mixture has produced an amicable cocktail in extracting high yield of rotenone and its concentration. As for the positive effect of extraction time toward the rotenone concentration and yield, there was proportionality between extraction times and the responses. The longer the extraction process, the higher the concentration and yield of the rotenone extract until it achieved equilibrium or saturation point where any further increase of extraction time did not affect the rotenone concentration and yield at all. This result showed a similarity with the study conducted by Zubairi et al. [10]. Generally, solute-to-solvent diffusivity relies on the extraction time as the longer the extraction time, the higher the amount of solute that interacts with solvent, thus increasing the concentration and yield of rotenone extract.

3.5. Optimization Condition for Rotenone Extraction. The previous study on rotenone extraction emphasized three

FIGURE 6: Response surface plot corresponding to rotenone concentration (a) as a function of solvent ratio and agitation rate and rotenone yield (b) as a function of extraction time and solvent ratio.

FIGURE 7: Perturbation plot of independent variables (A: solvent ratio; B: extraction time; C: agitation rate) and response variables (rotenone yield and concentration).

TABLE 5: Processing parameters used in the rotenone extraction process.

Processing parameters	
Solvent-to-solid ratio (mg/ml)	10 ml/g
Weight of raw materials (g)	0.50 g
Raw material particles size (mm)	0.86 ± 0.20 mm
Temperature ($^{\circ}$ C)	$28 \pm 2^{\circ}$ C

TABLE 6: Processing parameters used in the rotenone extraction process.

Solvent ratio	Extraction time (hours)	Agitation rate (rpm)	Concentration (mg/ml)	Yield (% w/w)	Desirability
2 : 8	19.34	199.32	0.5	0.34	0.91

TABLE 7: Processing parameters used in the rotenone extraction process.

Solvent ratio	Extraction time (hours)	Agitation rate (rpm)	Concentration (mg/ml)				Yield (% w/w)			
			REP 1	REP 2	REP 3	Average	REP 1	REP 2	REP 3	Average
2 : 8	19.34	199.32	0.41	0.51	0.55	0.49 ± 0.07	0.29	0.348	0.41	0.35 ± 0.06

* REP: replication.

optimal conditions for a high rotenone extraction which included solvent-to-solid ratio, particle size, and solvent types (Table 5) [7]. Thus, the optimal conditions were applied in this study with an addition of three different optimal independent variables, solvent ratio, extraction time, and agitation rate. The rotenone extraction process with the desired characteristic of higher concentration and yield can be considered as the optimum extract formulation. Under the optimum conditions suggested by the model (Table 6), the process parameters involving the solvent of the ratio of 2 : 8, extraction time of 19.34 hours, and agitation rate of 199.32 rpm yielded the predicted response values of the rotenone concentration and yield that were estimated to be 0.50 (mg/ml) and 0.34 (% w/w). The extraction process was prepared under the recommended optimum conditions and the resulting responses were compared to the predicted values.

3.6. Verification of Predictive Model. The verification of the predicted model was completed to test the adequacy of the predicted response values in triplicate ($n = 3$). Table 7 shows that there were no significant differences between the actual and predicted values. This implies that there was a high fit degree between the values observed in the experiment and the value predicted from the regression model. Hence, the response surface modeling could be applied effectively to predict the extraction of rotenone from *Derris elliptica* root. The experimental values obtained for the rotenone concentration and yield under the optimal condition were 0.49 ± 0.07 mg/ml and 0.35 ± 0.06 (% w/w), respectively, which has resulted in a 3.5-fold increase as compared to the control extract (acetone only: 0.10 ± 0.03 (% w/w)).

4. Conclusion

The results from RSM showed that the rotenone concentration and yield were mostly affected by solvent ratio,

followed by extraction time and agitation rate. Using the numerical optimization method, the optimum conditions for the maximum rotenone concentration and yield were 2 : 8 (DES : acetonitrile) of solvent ratio, 19.34 hours of extraction time, and 199.32 rpm for the agitation rate. Under those optimized conditions, the rotenone concentration (0.49 ± 0.07 mg/ml) and yield (0.35 ± 0.06 (% w/w)) were observed to be comparable to the predicted values of selected response variables. In fact, it has resulted in a 3.5-fold increase as compared to the control extract (acetonitrile only: 0.10 ± 0.03 (% w/w)). The linear polynomial equations showed insignificant interaction between independent variables used but significantly correlated only to each independent variable against those dependent variables (rotenone concentration and yield). In conclusion, the optimal extraction condition using alcohol-based deep eutectic solvent (DES) as a green additive in the extraction medium cocktail has shown its potential capability to enhance the yield of extracted bioactive constituents besides conserving the environment.

Competing Interests

The authors declare that they have no competing interests.

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