EXTRACTION OF 4H-PYRAN-4-ONE, 2,3- DIHYDRO -6-METHYL-, AN ALTERNATIVE ANTIFUNGAL AGENT, FROM *SCHIZOPHYLLUM COMMUNE*: OPTIMIZATION AND KINETIC STUDY

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ABSTRACT. *4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) was believed as a promising alternative in term antifungal activity towards fungal attack in rubberwood. Solid-liquid extraction is performed from basidiomycetes fungus Schizophyllum commune in methanol-water solvent, in order to obtain valuable antifungal agent. Statistical optimization was employed to optimize the extraction condition for maximal total flavonoid content (TFC) and DDMP productivity. The optimum conditions were 70.75% (v/v) methanol, 29 °C, and 145 rpm. The optimization studies were verified and the experimental data fitted well to the selected models with error percentage less than 1%. The extraction kinetics was then investigated using Parabolic diffusion model, Power law model, Peleg's model, and Elovich's model. All empirical models gave a good fit to the experimental data ($R^2 > 0.9$), in which the Power law model having the highest R^2 and lowest RMSD values.*

Keywords. *Schizophyllum commune*; total flavonoid content (TFC); 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP); optimization; extraction kinetics

INTRODUCTION

The interest in the investigation of bioactive compounds, especially total flavonoid content, from natural resources had greatly increased in recent year. According to Das *et al.* (2010), these secondary metabolites were generally synthesized by plants in response to microorganism infection and thus found *in-vitro* to be effective as antimicrobial substance.

Filamentous fungi have a large number of coding for secondary metabolites (Frisvad *et al.*, 2008). At present, the exploitation, conservation, and utilization of fungi belonging to Basidomycetes had gained much attention, which proved beneficial to human and environment. Slana *et al.* (2011) investigated that there might be a variety of flavonoid present in the fungal hosts of saprophytic fungus *Rhizopus nigiricans*, which could exhibit the fungitoxic effect. Moreover, Teoh *et al.* (2012) demonstrated the presence of 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP), a flavonoid fraction compound, in *Schizophyllum commune* during cultured on defined medium. It was important to note that

this flavonoid fraction compound exhibited effective *in-vitro* antifungal activity to the attack of wood-degrading fungi of rubberwood (Teoh & Mashitah, 2013).

Extraction was a very important stage in isolation, identification, and use of total flavonoid content. Several extraction methods had been widely used in pharmaceutical process, such as homogenization in solvent, serial exhaustive extraction, soxhlet extraction, sonication, and also supercritical fluid extraction (Ncube *et al.*, 2008 & Das *et al.*, 2010). Besides, water, methanol, and ethanol were the preliminary used solvent for the investigation of antimicrobial activity of flavonoid. It had been found that flavonoids and most other bioactive compounds were generally soluble in polar solvents such as methanol (Tiwari *et al.*, 2011).

However, literature data about optimization, modeling and simulation of solid-liquid extraction process were scarce. Therefore, there is a need for mathematical modeling, as a useful engineering tool, which considerably facilitates optimization, simulation, design and control of process and contributes to utilization of energy, time and solvent. On the other hand, numerous researches had been conducted to describe the kinetics and mechanism of solid-liquid extraction process for plant tissues (Sturzoiu *et al.*, 2011). However, there is lack of information on the extraction kinetic for filamentous fungus.

In this work, an investigation was carried out to understand the optimum condition for flavonoid and DDMP extraction by *S. commune* using Response Surface Methodology (RSM) coupled with Box-Behnken design (BBD). Then, the applicability of few empirical equations was examined for the extraction process under optimized condition.

MATERIAL AND METHODS

Fungal strain

The wild species fungal strain, *S. commune* was obtained from the Biocomposite and Protection of Timber Forest Products Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Malaysia. The stock culture was grown on malt extract agar (MEA) at 30^{o}C and maintained on agar slant before subsequent studies.

Mycelia suspension preparation

Mycelia suspension was prepared by suspending mycelia discs from 7-day-old culture plates in sampling bottles containing sterilized distiller water, and 0.1%(v/v) Tween 80. The disc of 5 mm diameter was cut on the mycelia mats of the agar plate using a sterilized cork borer. A total of 10 discs for every 100 ml sterilized water were vortexed for 5 min in order to homogenize the mycelia suspensions.

Mycelia extract preparation

In order to produce maximum biomass by *S. commune*, the production medium used containing 18.7 g/l yeast extract, 10.0 g/l malt extract, 38.6 g/l glucose, 1.0 g/l KH_2PO_4, 1.0 g/l K_2HPO_4, 0.59 g/l $MgSO4{\cdot}7H_2O$, and 2.0 g/l $(NH_4)_2SO_4$. Ten milliliters (10%v/v) of the mycelia suspension with 0.5 McFarland standard turbidity was added into 90 ml of medium in 250 ml Erlenmeyer flasks. The medium was sterilized at 121ºC for 15 min before transferring the mycelia suspension into the culture media. The culture was incubated at 30±2ºC, pH 6.5 in an incubator shaker at 200 rpm for 5 d. The culture broth was then harvested and centrifuged at 4,000 *g* for 15 min. The residue was then homogenized for 2 min prior to the extraction process. Meanwhile, the supernatant was evaporated using a rotary evaporator and the residues were maintained in vacuum until the extraction process was carried out.

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Experimental design using design of experiment (DoE)

Response surface methodology (RSM)

The RSM used in this study was three-factor and three-level Box-Behnken design (BBD) experimental plan in order to evaluate the effect of the extraction parameters on the extraction efficiency (Table 1). The results were analyzed using Analysis of Variance (ANOVA) by Design Expert 6.0.6 software. The simultaneous interactions of the three factors can be studied based on the three-dimensional plots. The optimum region was also identified based on the main parameters in the overlay plot. The experiment was then repeated for five times and each result obtained was compared with the predicted values in order to determine the validity of the model.

Table 1: Variables and their levels used for Box-Behnken design for optimization of extraction parameters

Independent Variable	Symbol		Level		
	Actual	Coded	-1	0	+1
Methanol concentration (% v/v)	X1	A	60	70	80
Extraction temperature (°C)	X2	B	25	35	45
Mixing rate (rpm)	X3	C	100	150	200

Verification of the model

The optimum conditions for extraction optimization studies were obtained using RSM coupled with BBD. The experiment was repeated five times and result was compared with the predicted values in order to determine the validity of the model.

Selected empirical model on the extraction kinetics

In this work, four two-parametric kinetic models were applied for modeling of recovered solutes and were validated based on the following assumptions:

i. The fungal biomass was isotropic and of equal size.
ii. Distribution of antifungal agent within the fungal biomass was uniform and varied only with time.
iii. Net diffusion occurred only towards the external surface of fungal biomass.
iv. Diffusion coefficient of antifungal agent was a constant.

Parabolic diffusion model

Orthogonal polynomial was a useful empirical equation in solid-liquid extraction. The parabolic diffusion equation was often used to indicate the diffusion-controlled process, and had successfully described the pesticide reactions (Spark, 1999). The equation was generally in the form as shown in Eqn. (1).

$$y = \sum_{i=0}^{n} A_i t^{1/2} \quad (1)$$

where A_i is the parameter to be determined, t is the extraction time in minutes, and y represent the antifungal agent extracted.

This empirical equation was corresponded into two-steps extraction mechanisms, which were washing and diffusion steps (Kitanovic *et al.*, 2008). Hence, the above equation could be simplified into Eqn. (2) where A_0 is the washing coefficient, and A_1 is the diffusion rate constant.

$$y = A_0 + A_1 t^{1/2} \quad (2)$$

Power law model

The power law model, which was similar to Freundlich type, was applied widely in the diffusion process of an active agent through non-swelling devices (Kitanovic *et al.*, 2008 & Sturzoiu *et al.*, 2011). It could be applied as in Eqn. (3).

$$y = Bt^n \quad (3)$$

Where y is the antifungal agent extracted, B refers to the constant incorporating the characteristics of the carrier-active agent system, t is the time in minutes, and n is the diffusion exponent, an indicative of transport mechanism. In literature, n was less than 1 ($N < 1$) for extraction from plant or vegetal materials (Sturzoiu *et al.*, 2011). This value was considered for the fungal biomass in this study. The constants for this model were estimated using a regression analysis. In the linearized form, the equation was transformed into Eqn. (4).

$$\ln(y) = n\ln(t) + \ln(B) \quad (4)$$

Peleg's model

In this work, the extraction curve (concentration of total flavonoid content versus time) had similar shape as the sorption curve (moisture content versus time). Hence, it was possible to describe this study using the hyperbolic model proposed by Peleg (Bucic-Kojic *et al.*, 2007 & Sturzoiu *et al.*, 2011). In the case of extraction, the model was adapted and used in the form of Eqn. (5).

$$y = y_o + \frac{t}{K_1 + K_2 t} \quad (5)$$

Where y_0 is the initial yield of product extracted at $t = 0$, t is the extraction time in minutes, K_1 is the Peleg's rate constant, and K_2 is the Peleg's capacity constant. Assuming that the desired product was not found in any extraction process when $t = 0$, the Eqn. (5) was then be simplified and used in the form of Eqn. (6).

$$y = \frac{t}{K_1 + K_2 t} \quad (6)$$

Elovich's model

The Elovich's model has been studied by Paterson *et al.* (1999) in order to fit the leaching curves such as the extraction of polycyclic aromatic hydrocarbons from coal tar-contaminated soil. The relationship assumed that the rate of adsorption and leaching rate decreased exponentially with increasing extraction yield. It was expressed as in Eqn. (7).

$$y = E_0 + E_1 ln(t) \tag{7}$$

Model validation

The profiles from simulation of the experimental data and models were then evaluated using the linear correlation coefficient (R^2) and the root mean square deviation (RMSD) computed as Eqn. (8) (Bucic-Kojic *et al.*, 2007).

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(y_{exp} - y_{calc}\right)^2} \tag{8}$$

Another criterion used to evaluate the best fitting equation was the mean relative percentage deviation (P) value. P value was defined as shown in Eqn. (9).

$$P\ value = \frac{100}{N} x \sum \frac{|Y_{exp} - Y_{calc}|}{Y_{exp}} \tag{9}$$

Where Y and Y_p are experimental and predicted yield of antifungal agent, respectively, and N is the number of experimental data. A model was considered acceptable if the P values are below 10% (Kaymak-Ertekin & Gedik, 2004).

Analytical method

Calculation of extraction yield

The extraction yield was calculated by the percentage ratio between the dry extract residues to the mycelia extract masses, according to Eqn. (10).

$$Extraction\ yield\ (\%) = \frac{g(dried\ biomass\ extract + filter\ paper) - g(filter\ paper)}{g(biomass)} X\ 100\% \tag{10}$$

Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was measured spectrophotometrically by the aluminium chloride colorimetric assay, as presented by Ordonez *et al.* (2006) with slight modification. 100 mg sample (extract biomass) was diluted with 85% ethanol, and 0.5 ml

of the diluted sample was then pipetted into 0.5 ml of 2 %(v/v) $AlCl_3$ ethanol solution (2 g $AlCl_3$ in 100 ml ethanol). Ethanol was used as blank in this study. The absorbance was then measured at 420 nm after 30 min at room temperature. A yellow color indicated the presence of flavonoids. The flavonoid content was expressed as micrograms of quercetin per milligram of sample (µg QE/mg sample). A standard TFC calibration was performed within a range of quercetin concentration from 1 - 20 µg/ml.

Analysis using UV-visible spectrophotometer for determination of DDMP

In this analysis, the DDMP was determined using the method of Cechovska *et al.* (2011). Since the commercially DDMP was not available in the market, the concentration of this compound was determined using a spectrophotometer with a UV-Vis (Model: Evolution 201). Norfuraneol, a pentose-derived analogue of DDMP, which had similar electrochemical properties-half-wave potential at range 0.30 – 0.33 V, was used as a calibration standard for the quantitation of DDMP. A standard DDMP calibration was performed within a range of DDMP concentration from 0 – 10 µg/ml.

RESULTS AND DISCUSSION

Optimization of extraction process using Response Surface Methodology (RSM)

In this work, the box-behnken design (BBD) was used to develop a correlation between the three variables in order to improve the extraction yield and the production of TFC and DDMP from *S. commune* biomass. The complete design matrixes of the variables in coded units together with the values of the corresponding response were obtained from the experimental work (Table 2).

Table 2: Experimental design for three-level, three variables box-behnken design, and the extraction yield, TFC, and DDMP represented as responses

Run	Independent variables			Responses		
	A, Methanol conc.	B, Temp.	C, Mixing rate	Extraction yield (%)	TFC (µg QE/ mg sample)	DDMP (µg/mg sample)
1	1 (80)	0 (35)	1 (200)	2.9101±0.05	1.076±0.03	0.789±0.03
2	-1 (60)	1 (45)	0 (150)	2.7297±0.05	1.092±0.01	0.828±0.03
3	0 (70)	1 (45)	-1 (100)	2.8992±0.06	1.051±0.03	0.922±0.03
4	0 (70)	0 (35)	0 (150)	3.0742±0.07	1.301±0.01	1.296±0.03
5	-1 (60)	0 (35)	-1 (100)	2.7393±0.03	1.235±0.03	0.792±0.04
6	-1 (60)	0 (35)	1 (200)	2.8506±0.03	1.227±0.03	0.781±0.04
7	-1 (60)	-1 (25)	0 (150)	2.7019±0.03	1.282±0.01	0.882±0.03
8	0 (70)	-1 (25)	1 (200)	2.9193±0.03	1.191±0.01	0.871±0.03
9	1 (80)	1 (45)	0 (150)	2.8426±0.05	1.018±0.05	0.852±0.04
10	0 (70)	0 (35)	0 (150)	3.0835±0.03	1.317±0.03	1.268±0.02

11	0 (70)	0 (35)	0 (150)	3.0729±0.03	1.321±0.03	1.266±0.03
12	1 (80)	0 (35)	-1 (100)	2.9498±0.05	1.238±0.01	0.878±0.04
13	1 (80)	-1 (25)	0 (150)	2.8611±0.05	1.214±0.03	0.801±0.04
14	0 (70)	1 (45)	1 (200)	2.9829±0.03	0.999±0.05	0.818±0.02
15	0 (70)	-1 (25)	-1 (100)	2.8795±0.03	1.301±0.03	0.872±0.02
16	0 (70)	0 (35)	0 (150)	3.0638±0.03	1.321±0.04	1.257±0.03
17	0 (70)	0 (35)	0 (150)	3.0645±0.03	1.315±0.04	1.286±0.03

*Values in parentheses are the actual factors for each independent variable in A: % (v/v), B: ºC, and C: rpm, respectively. The extraction yield, total flavonoid content, and DDMP results are as mean±SD.

By employing the ANOVA with Design Expert 6.0.6 software, the predicted responses Y for extraction yield (Eqn. (11)), TFC (Eqn. (12)), and DDMP (Eqn. (13)) in terms of coded values were obtained. The statistical significance of Eqn. (11), (12), and (13) were confirmed by an F-test and the ANOVA for response surface quadratic model was summarized in Table 3, 4, and 5, respectively.

Table 3: Analysis of variances (ANOVA) for quadratic regression model of extraction yield of *Schizophyllum commune*

Source	Sum of squares	Degree of freedom	Mean square	F value	$(P) > F$
Model	0.25	9	0.027	494.42	<0.0001
A	0.065	1	0.065	1179.18	<0.0001
B	7.633E-05	1	7.633E-05	1.28	0.2781
C	1.026E-03	1	1.026E-03	18.58	0.0035
A^2	0.12	1	0.12	2093.99	<0.0001
B^2	0.061	1	0.061	1106.09	<0.0001
C^2	8.010E-03	1	8.010E-03	145.05	<0.0001
AB	3.822E-04	1	3.822E-04	6.92	0.0339
AC	5.700E-03	1	5.700E-03	103.23	<0.0001
BC	9.303E-06	1	9.303E-06	0.17	0.6938
Residual	3.865E-04	7	5.522E-05		
Lack of fit	1.254E-04	3	4.179E-05	0.64	0.6280
Pure error	2.611E-04	4	6.529E-05		
Cor total	0.25	16			
Std. dev.	0.007			Adj. R^2	0.996
R^2	0.997			Pred. R^2	0.991

$$Y_{\text{extraction yield}} = 2.97 - 0.36A - 0.005B - 0.02C - 0.17A^2 - 0.12B^2 - 0.044C^2 - 0.01AB - 0.038AC - 0.002BC \quad (11)$$

$$Y_{TFC} = 1.23 - 0.14A - 0.11B - 0.08C - 0.052A^2 - 0.11B^2 - 0.068C^2 - 0.002AB - 0.038AC + 0.015BC \quad (12)$$

Table 4: Analysis of variances (ANOVA) for quadratic regression model of TFC of *Schizophyllum commune*

Source	Sum of squares	Degree of freedom	Mean square	*F* value	(*P*) > *F*
Model	0.21	9	0.023	239.73	<0.0001
A	0.019	1	0.019	192.04	<0.0001
B	0.029	1	0.029	304.21	<0.0001
C	0.017	1	0.017	176.60	<0.0001
A^2	0.012	1	0.012	120.08	<0.0001
B^2	0.052	1	0.052	536.80	<0.0001
C^2	0.020	1	0.020	204.43	<0.0001
AB	9.000E-06	1	9.000E-06	0.093	0.7691
AC	5.929E-03	1	5.929E-03	61.35	0.0001
BC	8.410E-04	1	8.410E-04	8.70	0.0214
Residual	6.765E-04	7	9.664E-05		
Lack of fit	4.045E-04	3	1.348E-04	1.98	0.2588
Pure error	2.720E-04	4	6.800E-05		
Cor total	0.21	16			
Std. dev.	0.010			Adj. R^2	0.995
R^2	0.997			Pred. R^2	0.986

Table 5: Analysis of variances (ANOVA) for quadratic regression model of DDMP of *Schizophyllum commune*

Source	Sum of squares	Degree of freedom	Mean square	*F* value	(*P*) > *F*
Model	0.68	9	0.076	137.69	<0.0001
A	0.22	1	0.22	404.11	<0.0001
B	1.734E-03	1	1.734E-03	3.14	0.1198
C	5.430E-03	1	5.430E-03	9.83	0.0165
A^2	0.26	1	0.26	465.96	<0.0001

B^2	0.15	1	0.15	265.15	<0.0001
C^2	0.20	1	0.20	359.76	<0.0001
AB	2.756E-03	1	2.756E-03	4.99	0.0607
AC	1.521E-03	1	1.521E-03	2.75	0.1411
BC	2.652E-03	1	2.652E-03	4.80	0.0646
Residual	3.868E-03	7	5.526E-04		
Lack of fit	2.853E-03	3	9.511E-04	3.75	0.1172
Pure error	1.015E-03	4	2.538E-04		
Cor total	0.69	16			

Std. dev.	0.024	Adj. R^2	0.988
R^2	0.994	Pred. R^2	0.979

$$Y_{DDMP} = 1.03 – 0.49A + 0.026B - 0.045C – 0.25A^2 – 0.19B^2 – 0.22C^2 + 0.026AB – 0.019AC - 0.026BC \quad (13)$$

The *P* value was used as a tool to check the significance of the model and each coefficient; the smaller the value of *P*, the more significant was the corresponding coefficient. As shown in Table 3 until 5, the ANOVA of the quadratic regression model demonstrated that the model was significant with the *F*-test of a very low probability value ($P > F$) < 0.0001. For example, the model *F*-value of 494.42 for extraction yield implied that the model used was significant. In the study of TFC response, the value of R^2 (0.997) suggested that the sample variation of 99.7% were attributed to the independent variable, and only about 0.3% of the total variation could not be explained by the model. Furthermore, an adequate precision was used to measure the ratio of signal to noise, which is generally desired to be greater than 4. In this study, the value of this ratio for all responses (extraction yield = 64.432, TFC = 41.115, DDMP = 28.016) suggested that the polynomial quadratic model was of an adequate signal, and it could be used to navigate the design space.

Figures 1-3 are three-dimensional surface plots of extraction yield, TFC, DDMP of *S. commune* biomass by extraction based on the effect of methanol-water concentration, temperature and mixing rate, respectively. Figure 1 presents that the maximal extraction yield was attained when methanol concentration was between 70 and 75 %(v/v) and extraction temperature between 28 to 30 ^oC. Uma *et al.* (2010) reported that as the solvent concentration was greater than 60 %(v/v), it gave higher total phenolic yield. According to Guo *et al.* (2013), higher temperature could increase the extraction efficiency which it accelerated the active diffusion. In this present study, the maximal extraction yield was obtained within between 28 to 30 ^oC, in which beyond this level the extraction yield was decreased. This could be due according to Guo *et al.* (2013) the occurrence of oxidation and hydrolysis process at higher temperature, and hence reduced the extraction yield.

Figure 2(a) depicts a higher amount of flavonoid fraction yielded in the region of methanol concentration between 65 and 75% (v/v) and temperature between 25 and 30 ^{o}C. Both methanol concentration and temperature showed significant negative quadratic effects on TFC at $P < 0.0001$ (Table 4). Chan *et al.* (2009) stated that more polar phenolic compounds such as flavonoids fraction could be extracted in higher concentration of methanol and water mixture, following the "like dissolve like" principle. Figure 2(b) denotes the effect of methanol concentration and mixing rate on the TFC produced at fixed temperature of 30 ^{o}C. Methanol concentration demonstrated a pronounced negative influence on the TFC production in a linear and a quadratic manner with $P < 0.0001$ (Table 4). The relationship between the extraction temperatures and mixing rate with TFC production was shown in Figure 2(c). Both factors displayed a significant linear and quadratic effect ($P < 0.0001$) on TFC response (Table 4). Chan *et al.* (2009) reported that moderate heating and mixing rate might weaken the mycelia wall integrity, hydrolyzed the bonds of bound flavonoid compounds, and enhanced the flavonoid fraction solubility. Hence, more flavonoid would be distributed to the solvent.

Figure 3(a) shows that the maximal extraction of *S. commune*'s DDMP occurred within methanol concentration of 65 to 75 % (v/v) and temperature range between 28 to 32 ^{o}C. Result showed that a small increment in temperature caused the DDMP solubility increased, by which according to Guo *et al.* (2013), the solvent viscosity and surface tension decreased as increased the temperature and hence contributed to the sample wetting and matrix penetration. On the other hand, since DDMP had low polarity and was hydrophilic, the mixture of methanol and water had high affinity to extract the compound. Figure 3(b) shows that the optimal mixing rate ranged between 125 to 150 rpm. Beyond to that point, the production of DDMP decreased due to denaturation of internal structure of biomass and resulted in lower product formation. As can be seen in Figure 3(c), once the extraction temperature and mixing rate exceeded 32 ^{o}C and 150 rpm, the extraction of DDMP decreased slightly.

The Design-Expert plot also illustrated the interaction between methanol-water concentrations, extraction temperature and mixing rate corresponding to the extraction yield, TFC and DDMP. The optimum values of the tested variables in uncoded units were 70.75% (v/v) methanol concentration, extraction temperature of 29 ^{o}C, and mixing rate at 145 rpm. In this study, the maximum predicted extraction yield was 3.0741 %, while the maximum predicted production of TF and DDMP was 1.321 µg QE/mg sample, and 1.273 µg/mg sample, respectively. The desirability value for this situation was 0.997, in which supports the application of this model.

Model verification

In order to verify the model adequacy, five sets of experiments were repeated randomly at optimum condition to obtain a maximum extraction yield, and the production of TF and DDMP by *S. commune* experimentally. As shown in Table 6, the percentage error

differences between the experimental and predicted values were in the range of 0.010-0.393 %. Since the differences between actual and predicted responses were always less than 1%, thus providing its validity.

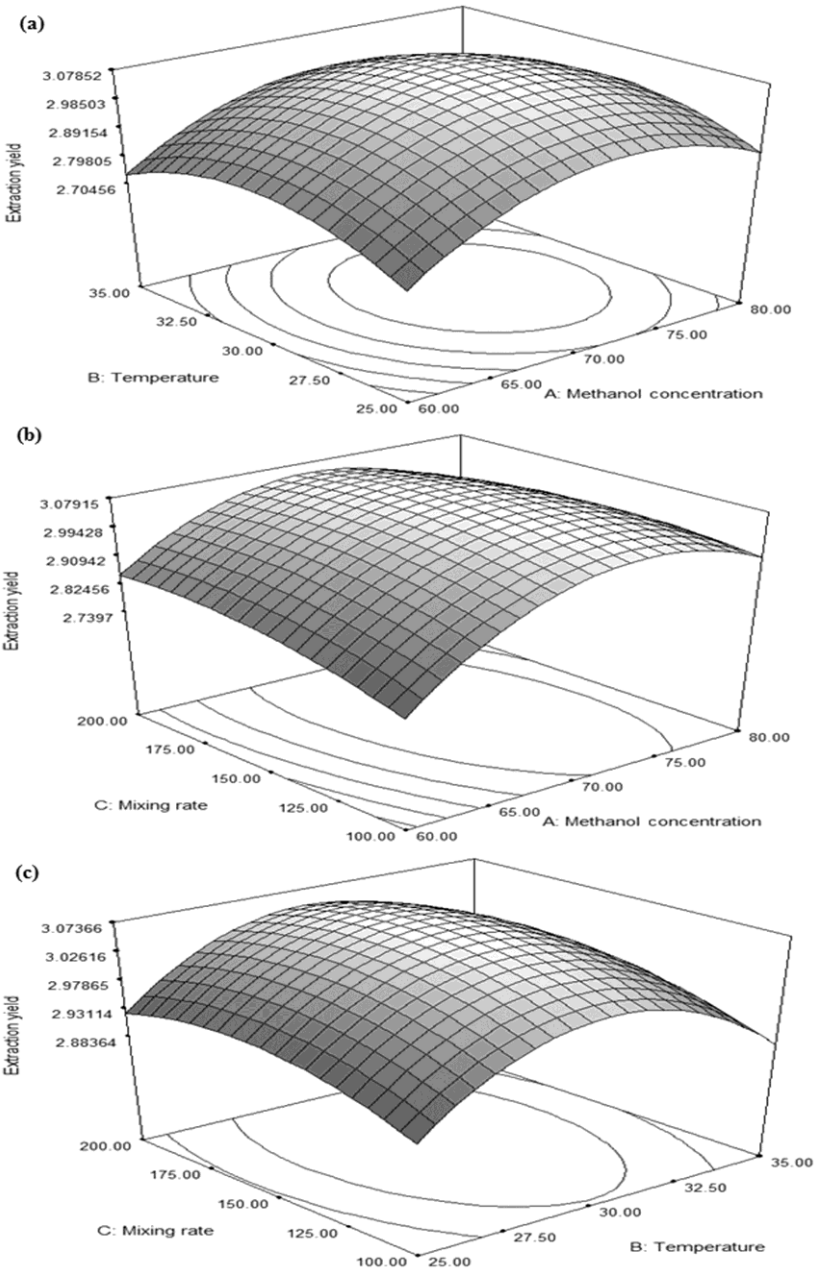

Figure 1: (a) A three-dimensional surface plot of extraction yield as a function of methanol concentration and extraction temperature. (b) A three-dimensional surface plot of extraction yield as a function of methanol concentration and mixing rate. (c) A three-dimensional surface plot of extraction yield as a function of extraction temperature and mixing rate

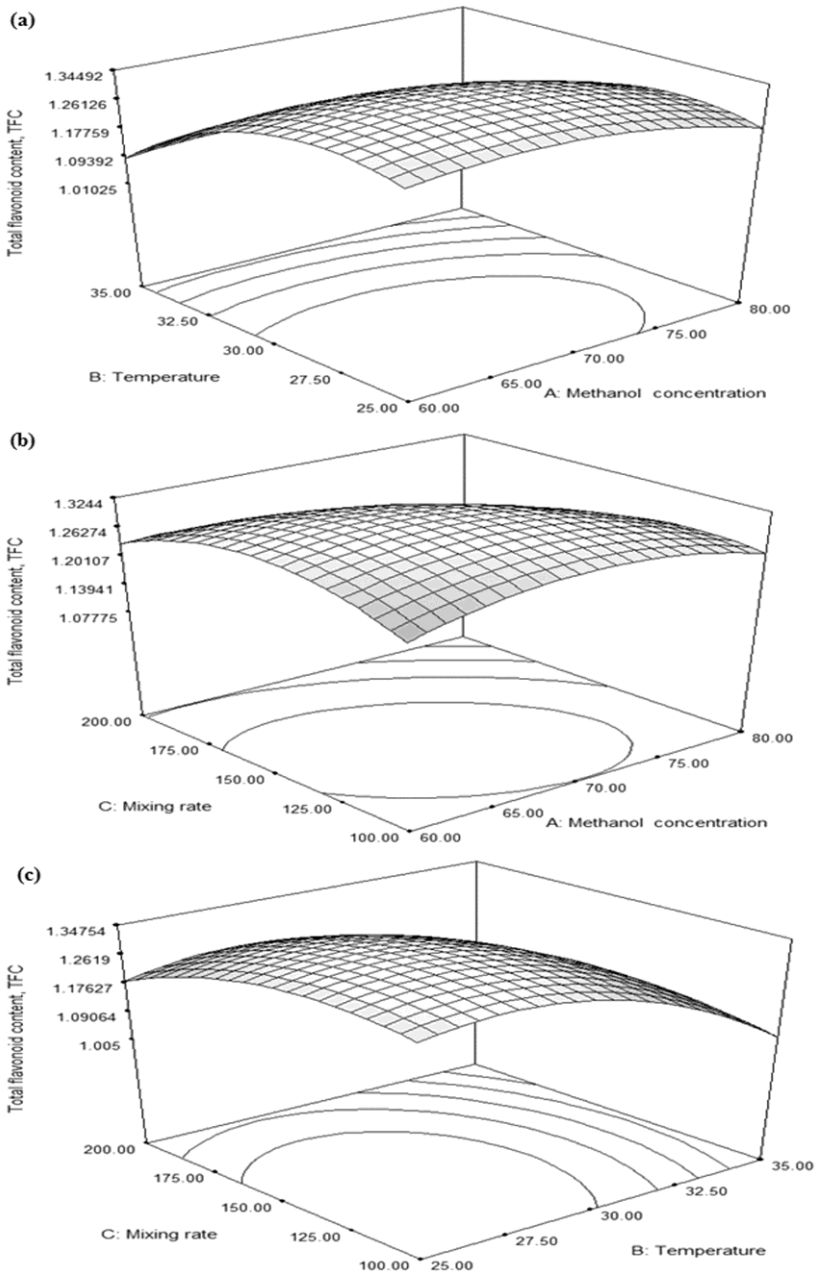

Figure 2: (a) A three-dimensional surface plot of TFC as a function of methanol concentration and extraction temperature. (b) A three-dimensional surface plot of TFC as a function of methanol concentration and mixing rate. (c) A three-dimensional surface plot of TFC as a function of extraction temperature and mixing rate

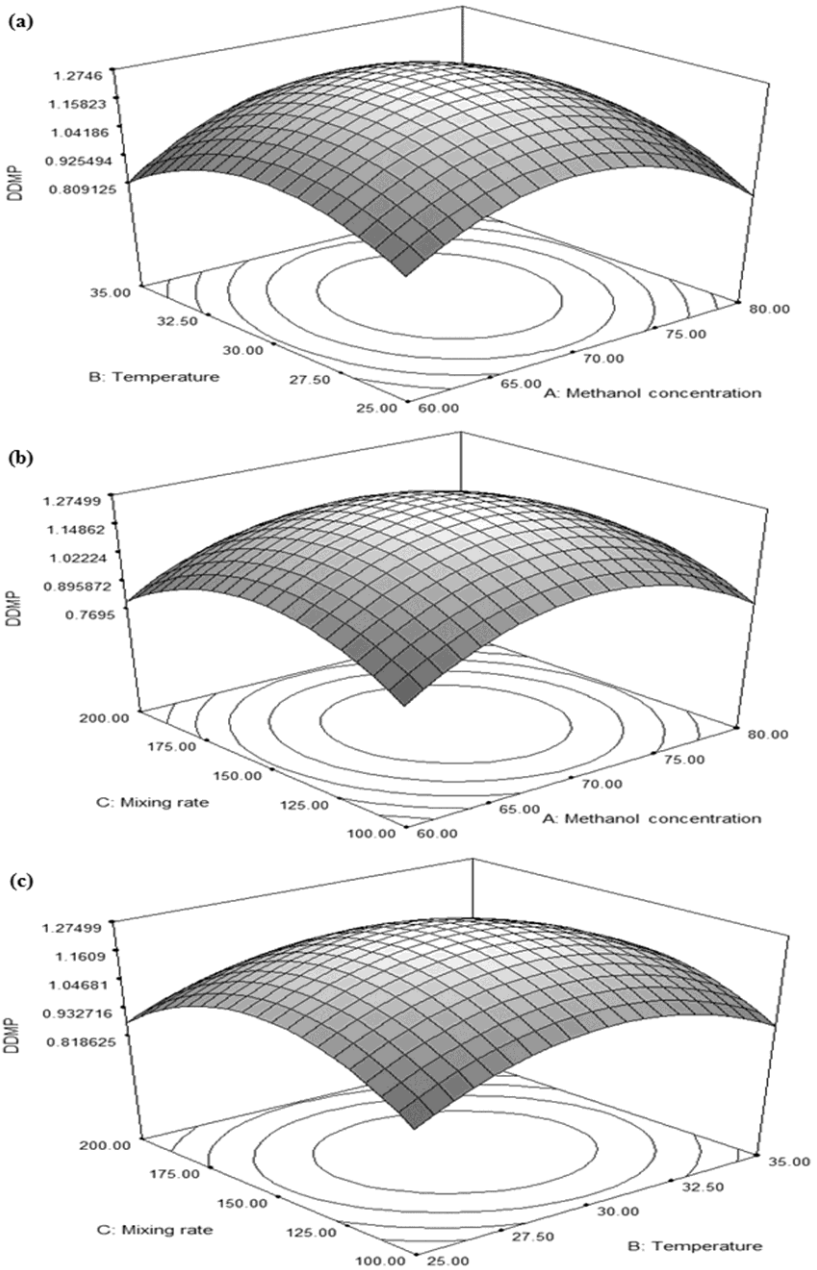

Figure 3: (a) A three-dimensional surface plot of DDMP as a function of methanol concentration and extraction temperature. (b) A three-dimensional surface plot of DDMP as a function of methanol concentration and mixing rate. (c) A three-dimensional surface plot of DDMP as a function of extraction temperature and mixing rate

Table 6: Validation of the data and model constructed

Run	Extraction yield (%)			TFC (μg QE/mg sample)			DDMP (μg/mg sample)		
	Exp.	Pred.	Error (%)	Exp.	Pred.	Error (%)	Exp.	Pred.	Error (%)
1	3.0712	3.0741	0.094	1.318	1.321	0.227	1.273	1.273	0.393
2	3.0745	3.0741	-0.013	1.323	1.321	-0.151	1.269	1.273	0.314
3	3.0738	3.0741	0.010	1.318	1.321	0.227	1.275	1.273	-0.157
4	3.0750	3.0741	-0.029	1.322	1.321	-0.076	1.278	1.273	-0.393
5	3.0734	3.0741	0.017	1.317	1.321	0.303	1.270	1.273	0.236

Selected empirical kinetic models for the extraction of antifungal agent from *Schizophyllum commune* biomass

The common extraction kinetic models consisted of two periods: a fast washing action in the very beginning, and a slow-diffusion-controlled extraction in the last period. Under certain operating conditions, the extraction of easily accessible extractive substance was so fast that it was difficult to observe the first period of washing (Kitanovic *et al.*, 2008). Figure 4(a) represents the profile of extraction yield versus time for the *S. commune* extract obtained under the optimized condition (70.75 %(v/v) methanol, extraction temperature of 29 ^{o}C, and mixing rate at 145 rpm) as described in above section. A typical trend was obtained in this study which was similar as those reported in the literature on the solid-liquid extraction profile. Similar trend was also observed for the TFC and DDMP profile, as shown in Figure 4(b) and 4(c), respectively. Due to that, four empirical kinetic models were selected from the literature, which were usually used to fit the experimental data of extraction curves, such as parabolic diffusion model, power law model, Peleg's model, and Elovich's model.

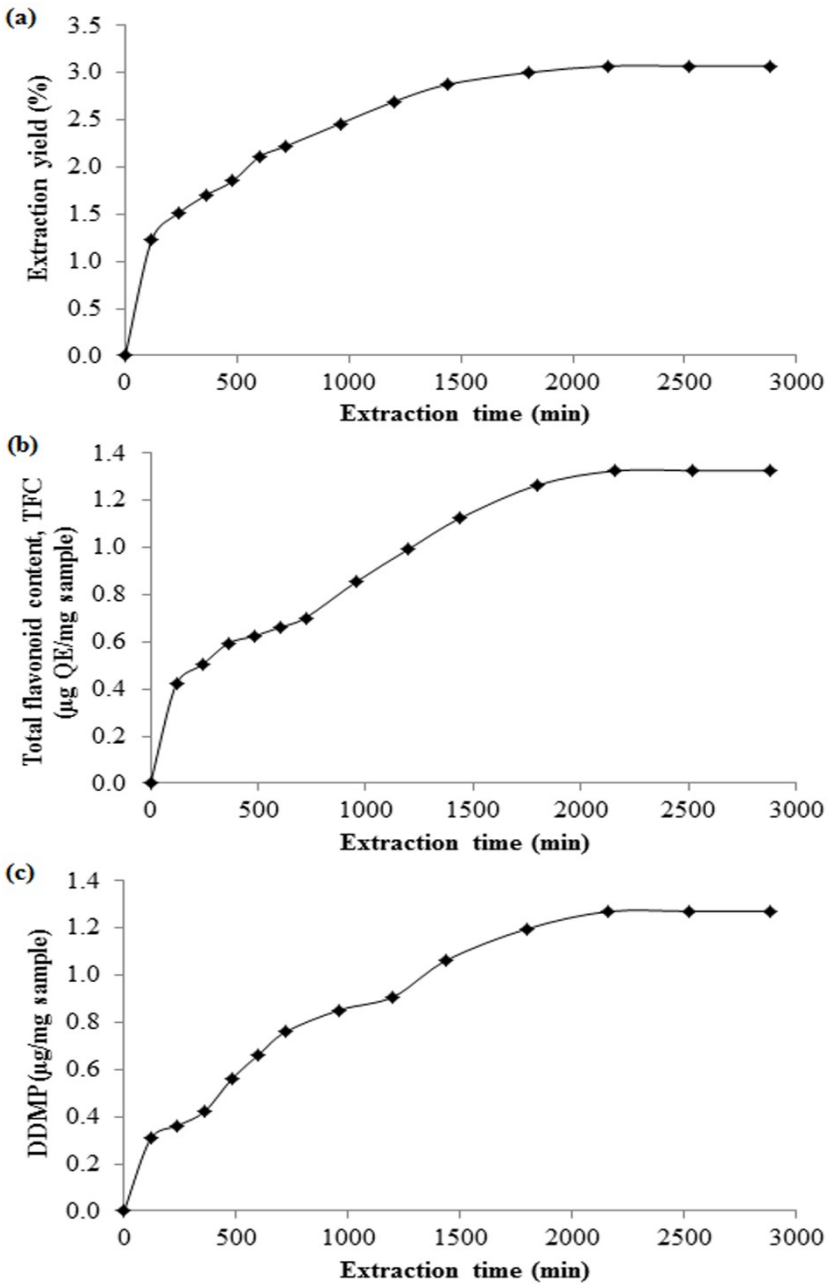

Figure 4: Extraction profile for (a) extraction yield, (b) TFC, and (c) DDMP from *Schizophyllum commune* extract under the optimized condition (70.75 %(v/v) methanol, temperature of 29 ºC, mixing rate 145 rpm)

The experimental results of antifungal agent extraction were then analyzed using the linearized equations of the selected empirical kinetic models as summarized in Table 7. Model parameters were then calculated by linear regression using Microsoft Excel software.

Table 7: Selected empirical extraction kinetic models in linearized forms

Model	Model equation	Linearized form
Parabolic diffusion model	$y = A_o + A_1 t^{1/2}$	-
Power law model	$y = Bt^n$	$ln(y) = nln(t) + ln(B)$
Peleg's model	$y = t/(K_1 + K_2 t)$	$(t/y) = K_1 + K_2 t$
Elovich's model	$y = E_0 + E_1 ln(t)$	-

Table 8 summarizes the calculated parameters for the selected empirical models, and the corresponding statistical correlation values. For parabolic diffusion model, it was found that the washing coefficient (A_0) in term of TFC and DDMP production was lower compared to the extraction yield, in which indicated the lower solubility of the TFC and DDMP (called as extractive substance) in the 70.75 %(v/v) methanol concentration at time t=0. During the extraction process, the diffusion rate (A_1) of extraction yield was 0.0462 $1/min^{0.5}$, while the diffusion rate of TFC (0.0246 $1/min^{0.5}$) was almost a similar rate as in DDMP (0.0259 $1/min^{0.5}$). This indicated that TFC and DDMP had lower diffusion coefficient in the extraction process. Similar trend was also observed for the kinetic parameters obtained using power law and Elovich's model, in which the extraction yield always provide a higher diffusion rate during solid-liquid extraction of *S. commune* biomass compared to other two extractive substances (e.g., TFC and DDMP). On the other hand, the K_1 and K_2 value in Peleg's model was related to the extraction rate and the equilibrium concentration, respectively. In this study, the extraction of DDMP showed the highest K_1 and K_2 values with 524.28 min.g/mg and 0.5851 g/mg, respectively. As mentioned by Karacabey *et al.* (2013), as K_1 and K_2 values increased, the extraction of extractive substances were also accelerated, thus achieving higher equilibrium concentration.

Table 8: Summary of model parameters and statistical correlation values for each empirical extraction kinetic model

Model parameters	Statistical correlation values	Extraction data		
		Extraction yield	TFC	DDMP
Parabolic diffusion model				
A_0 (1/min$^{0.5}$)		0.9002	0.1176	0.0132
A_1 (1/min$^{0.5}$)		0.0462	0.0246	0.0259
	R^2	0.941	0.967	0.969
	RMSD	0.146	0.057	0.059
	P value (%)	5.839	5.404	6.619
Power law model				
n		0.312	0.411	0.510
B (1/min^{n})		0.2802	0.0528	0.0244
	R^2	0.980	0.968	0.975
	RMSD	0.080	0.058	0.046
	P value (%)	2.650	5.362	5.778
Peleg's model				
K_1 (g.min/mg)		99.98	428.55	524.28
K_2 (g/mg)		0.2865	0.5735	0.5851
	R^2	0.976	0.963	0.973
	RMSD	0.128	0.086	0.050
	P value (%)	5.048	7.200	6.651
Elovich's model				
E_0		2.1021	1.3775	1.6021
E_1		0.6651	0.3386	0.3627
	R^2	0.975	0.919	0.952
	RMSD	0.099	0.082	0.062
	P value (%)	4.094	7.937	7.349

Figure 5 illustrates the profiles of experimental and simulated data for the extraction of antifungal agent from *S. commune* biomass under the optimized condition of 70.75 %(v/v) methanol concentration, at temperature of 29 ^{o}C, and mixing rate 145 rpm using the parabolic diffusion model, power law model, Peleg's model, and Elovich's model, respectively. The predicted results gave a relatively good agreement to the experimental data, with the linear correlation coefficient (R^2) values above 0.9. In fact, the R^2 values were higher for all the empirical models tested, ranging between $0.919 < R^2 < 0.980$, which showed that the selected empirical models were sufficient to describe both the

fast-washing action and slow-diffusion of the extraction process for the antifungal agent extraction process of this study. Generally, the R^2 value was frequently used to judge whether the model correctly represented the data, implying that if R^2 closer to 1, then the regression model was correct. However, there are many examples exist where the R^2 value is close enough to 1 but the model is still not appropriate. Thus, the root mean square deviation (RMSD) was used with the R^2 value for the comparison of various empirical models. As reported by Kitanovic *et al.* (2008), the higher the value of R^2 and the lower the value of RMSD, the better the goodness of fit. In this study, the RMSD results ranged within 0.046 – 0.146, which implied a good agreement between the experimental and simulated data.

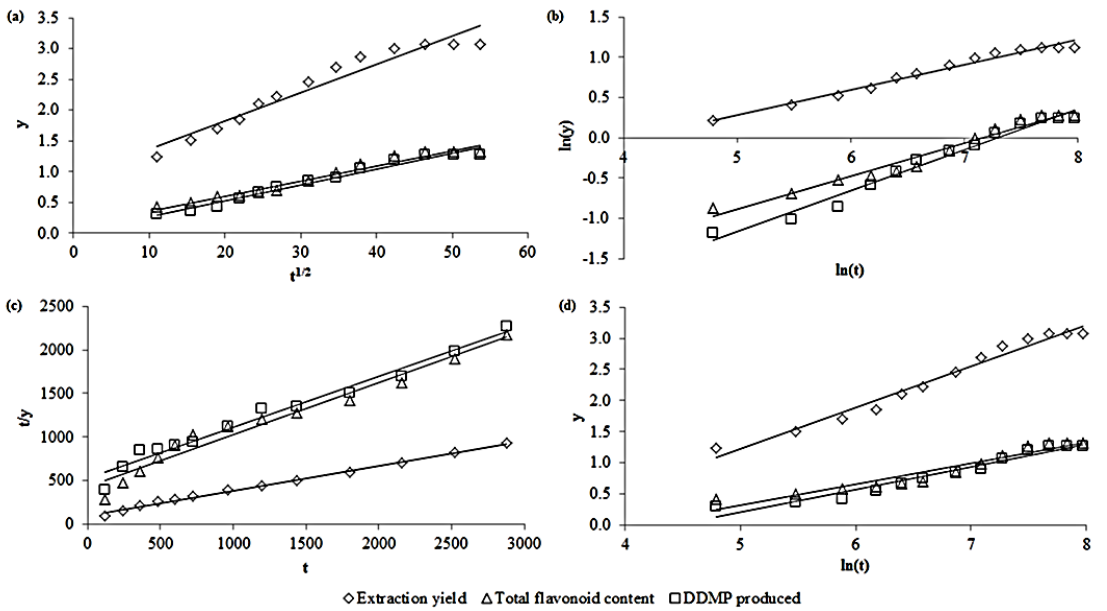

Figure 5: Comparison between the experimental (symbol) and simulated (line) data for the extraction of antifungal agent under optimized condition based on the linearized form of kinetic equations: (a) Parabolic diffusion model, (b) Power law model, (c) Peleg's model, and (d) Elovich's model.

In order to further evaluate the best fitting models for predicting the antifungal agent extraction process, *P* values corresponding to each selected empirical model were calculated. Kaymak-Ertekin & Gedik (2004) suggested that a model was acceptable if the *P* value was less than 10%. Observing the data shown in Table 8 of the present study, it was found that all selected models used in this study were adequate in describing the kinetics or behavior of antifungal agent extraction with the calculated *P* values ranged from 2.650 to 7.937%. In fact, as shown in Table 8, the Power law having the highest value of R^2, the lowest value of RMSD, and the smallest *P* value in all extraction process

either for the extraction yield, TF or DDMP production. Hence, in this study, the Power law was selected as the best empirical model for antifungal agent extraction from *S. commune* biomass.

CONCLUSION

In order to obtain the highest bioactive compounds from *S. commune* biomass, the extraction parameters that affected the extraction process was optimized using RSM coupled with BBD. The optimum values of the tested variables were 70.75% (v/v) methanol concentration, extraction temperature of 29 ºC, and mixing rate at 145 rpm. The results showed that the maximum predicted extraction yield and the production of TFC and DDMP were 3.0741 %, 1.321 µg QE/mg sample, and 1.273 µg/mg sample, respectively. For the extraction kinetics study, Power law described the extraction process well with the highest R^2, the lowest RMSD, and also the smallest *P* values for all tested variables. Hence, the Power law was selected as the best empirical model for antifungal agent extraction from *S. commune* biomass.

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LIST OF SYMBOLS

A_i	Extraction kinetic parameter related to parabolic diffusion model ($1/min^{0.5}$)
A_0	Washing coefficient related to parabolic diffusion model ($1/min^{0.5}$)
A_1	Diffusion rate constant related to parabolic diffusion model ($1/min^{0.5}$)
B	Parameter of the power law model incorporating the characteristics of the extraction system ($1/min^n$)
E_0, E_1	Extraction kinetic parameters of Elovich's model
K_1	Peleg's rate constant (min.g/mg)
K_2	Peleg's capacity constant (g/mg)
n	Diffusion exponent of the power law model
P value	Mean relative percentage deviation (%)
R^2	Linear correlation coefficient
t	Time (min)

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