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RESEARCH ARTICLE

Optimization of microwave-assisted extraction for picroside I and picroside II from *Picrorrhiza kurroa* using Box-Behnken experimental design

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Abstract The response surface methodology was employed to study the optimization of microwave-assisted extraction of picroside I and picroside II from *Picrorrhiza kurroa* Royle rhizomes. The effects of solid to solvent ratio, and extraction temperature, time and solvent on the yields of picroside I and picroside II have been investigated using Box-Behnken experimental design. The experimental data were fitted to second-order polynomial equations using multiple regression analysis and analyzed using the appropriate statistical method. By solving the regression equation and analyzing 3-D plots, the optimum extraction conditions were found to be: solid to solvent ratio, 10 : 90 (w/v); temperature, 60 °C; and extraction time, 60 s. Under the optimal conditions, the yields of picroside I and picroside II are 41.23 and 6.12 $mg \cdot g^{-1}$ feed respectively, which are in good agreement with the predicted values. The ratio of solid to solvent significantly affects the yields of picroside I and picroside II. Application of microwave-assisted extraction of picroside I and picroside II from *P. kurroa* would dramatically reduce extraction time and solvent consumption.

Keywords microwave-assisted extraction, picroside I, picroside II, *Picrorrhiza kurroa*, Box-Behnken design

1 Introduction

Picrorrhiza kurroa Royle (Scrophulariaceae), commonly known as ‘‘Kutki’’ is a well-known herb in the “ayurvedic medicine”. *P. kurroa* is a low, hairy herb with a perennial woody rhizome. Traditionally, *P. kurroa* has been used to treat disorders of the liver and upper respiratory tract [1]. Its pharmacological studies have revealed hepatoprotective [2], anti-inflammatory [3,4], immune-stimulatory [5,6] and free radical scavenging activity [7]. The hepatoprotective value of the *P. kurroa* has been attributed due to the presence of kutkoside and the iridoid glycosides *viz.* picroside I, picroside II [8,9]. For extraction of picroside I and picroside II from *P. kurroa* rhizomes, numerous methods have been reported: hot percolation [10], cold percolation [11] and ultra-sonication [12,13]. It is well known that conventional solvent extraction methods are tedious and time consuming.

After reviewing the wide scope of picroside I and picroside II in current medical need, we have developed and optimized a microwave-assisted extraction (MAE) process to compare the MAE yield with conventional hot percolation extraction yield. The main advantages of MAE respect to the other techniques are considerable reduction in both extraction time and solvent consumption, if compared to conventional extraction [14]. MAE is a process of using microwave energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent. The ability to rapidly heat the sample solvent mixture is inherent to MAE and the main advantage of this technique [15].

In the present study, MAE method has been optimized by changing the different parameters *viz.* solid to solvent ratio, and extraction temperature, time and solvent. Extraction parameters were optimized through Box-Behnken design. The experimental conditions have been optimized to ensure the maximum yield of picroside I and picroside II. To the best of our knowledge, the use of MAE in picroside I and picroside II from *P. kurroa* rhizomes has not been reported.

The Box-Behnken design is a second-order multivariate technique based on three-level incomplete factorial designs

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that received a wide application for assessment of critical experimental conditions, i.e., maximum or minimum of response function. The number of experiments (N) needed for the development of Box-Behnken matrix is defined as $N = 2k(k - 1) + C_0$, where k is the factor number and C_0 is the replicate number of the central point [16–18].

In the present work, we have investigated the effects of various parameters on the MAE performance using Box-Behnken experimental design method.

2 Material and methods

2.1 Plant material and reagents

The authenticated dried rhizomes of *P. kurroa* were ground to a powder using a pulverizer (K. C. Engineers, Ambala, HR, India). To select uniform particle size, rhizome powder was sifted in a sieve shaker (CIP Machineries, Ahmedabad, GJ, India) with sieves of different sizes (12, 24, 45, 85 and 120 mesh, Swastika electric and scientific works, Ambala, HR, India) for a period of 15 min. The rhizome powder passed through 45 mesh sieve and retained on 85 mesh sieve was collected and used for further extraction experiments. The standard picroside I and picroside II (purity 98% by HPLC) were obtained as gift samples from Natural Product Chemistry Division of Indian Institute of Integrative Medicine (CSIR), Jammu & Kashmir, India. All solvents used for the extraction and the chromatographic purpose were of analytical grade (Finar chemicals ltd., Ahmedabad, GJ, India) and LC-MS grade (Merck, Darmstadt, Germany), respectively.

2.2 LC-MS analysis

The LC-MS analysis of picroside I and picroside II was essentially performed as described earlier [19]. The analysis method was pre-validated before actual analysis.

The LC-MS system consisted of a Waters e2695 separation module with auto-sampler, Waters 2489 ultra-violet spectrophotometric detector and the Quattro micro API mass spectrometer (Waters, Milford, MA, USA) equipped with MassLynx data acquisition software, version 4.1. All samples and standards were filtered through 0.45 μm syringe filters (Millipore, Bangalore, India). Separation was achieved on Waters XTerra C-18 column (250 mm × 4.6 mm, 5 μm particle sizes) (Waters, Milford, MA, USA) using a mixture of acetonitrile and water (pH 5.0, 25 : 75 (v/v)) at 0.7 mL·min^{-1} flow rate in isocratic elution. The column temperature was maintained at 30 °C. The UV detection of analytes was carried out at 270 nm. The yield of each compound was expressed as mg·g^{-1} of *P. kurroa* rhizomes on dry weight basis.

A mass spectrometer with ESI interface was used for MS analysis. The analyte infusion experiments were performed using in-built syringe pump of mass spectrometer. ESI parameters were as follows: isonization mode, positive; capillary voltage, 2.1 kV; cone voltage, 23 V; extractor voltage, 1 V; RF lens voltage, 0.1 V; source temperature, 120 °C; dissolvation temperature, 350 °C; dissolvation gas flow rate, 400 L·h^{-1}; and cone gas flow rate, 20 L·h^{-1}. The single ion mode was used to monitor the picroside I at 515 $[M + Na]^+$ and picroside II at 535 $[M + Na]^+$.

2.3 Conventional hot percolation extraction of *P. kurroa*

Conventional hot percolation was used for the maximum recovery of picroside I and picroside II from the *P. kurroa* rhizomes. The powdered material was percolated in deionized water ((98±1) °C for 2 h). After extraction, the extract was filtered and analyzed for picroside I and picroside II content by LC-MS.

2.4 Experimental design and evaluation

A Box-Behnken experimental design with three variables at three levels was used to determine the response pattern and the interaction effect of the independent variables on the response. The three key variables *viz.* solid to solvent ratio (X_1), extraction temperature (X_2) and extraction time (X_3) were selected and their effects on the MAE of picroside I and picroside II were evaluated at the different levels. Variables and levels tested are depicted in Table 1. The experimental design used for the study is shown in Table 2.

Second-order polynomial equation was used to express picroside I and picroside II yield P (mg·g^{-1}), as a function of the independent variables:

$$P = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3. \quad (1)$$

Design-Expert software (version 8.0.6.1, Stat-Ease, Inc., Minneapolis, USA) was used for the analysis of variance

Table 1 Variables and experimental design levels for Box-Behnken experimental design.

Independent variables	Coded symbols	Levels		
		–1	0	1
Solid : solvent ratio /(w·v^{-1})	X_1	1 : 99	5 : 95	10 : 90
Extraction temperature /°C	X_2	50	55	60
Extraction time /min	X_3	1	4	7

Table 2 The Box-Behnken experimental design and the response for the yields of picroside I and picroside II

Run No.	X_1	X_2	X_3	Yield* /(mg·g^{-1})					
				Methanol		Methanol : water/ (50%, v/v)		Water	
				P-I	P-II	P-I	P-II	P-I	P-II
1	1	1	0	21.49	3.82	24.95	3.84	32.28	4.84
2	0	1	1	22.3	3.14	12.91	1.97	15.73	2.61
3	0	–1	–1	15.36	2.21	14.93	2.3	15.23	2.34
4	–1	0	–1	5.79	0.7	2.75	0.52	3.06	0.57
5	0	0	0	18.56	2.81	14.39	2.05	16.61	2.46
6	1	–1	0	32.43	4.7	24.91	3.84	26.82	4.15
7	1	0	1	37.69	5.29	27.48	4.16	29	4.43
8	–1	1	0	6.56	0.71	2.78	0.6	3.15	0.61
9	–1	0	1	3.74	0.82	2.95	0.61	2.91	0.55
10	0	1	–1	16.29	2.4	13.82	2.25	28.47	4.28
11	1	0	–1	15.23	4.25	27.12	4.13	33.15	5.11
12	–1	–1	0	1.98	0.65	3.11	0.62	2.93	0.56
13	0	–1	1	18.27	2.72	13.51	2.12	16.18	2.53
14	0	0	0	18.94	2.79	14.21	2.2	16.51	2.53
15	0	0	0	18.46	2.82	14.43	2.12	16.22	2.48

* Yield is the average of three determinations; P-I, picroside I; P-II, picroside II

of the obtained experimental data. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2 and the values of adjusted-R^2 of models were evaluated to check the model adequacies. The significance of each term in the equation is to estimate the goodness of fit in each case. The analysis of variance tables was generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The p-values of less than 0.05 were considered to be statistically significant. The regression coefficients and regression models were used for statistical calculations and generation of three dimensional plots.

2.5 Microwave assisted extraction (MAE) of *P. kurroa* and statistical analysis

A commercial microwave oven with closed-vessel extraction assembly (Model: MicroSYNTH, Max power: 1000 W, M/s Milestone, Shelton, CT, USA) was used for the extraction purposes. The powdered mass of *P. kurroa* rhizomes was suspended in solvent (methanol, methanol : water, and water) and poured into microwave extraction assembly consisting of mono-block rotor and nine cylindrical vessels (height 8.5 cm × internal diameter 3.5 cm). The stirring speed of 400 r·min^{-1} was kept constant throughout the extraction period. The mass was extracted at different extraction conditions of temperature and time. After the pre-defined extraction period, the samples were collected from the extraction assembly, filtered and analyzed for their picroside I and picroside II contents by LC-MS.

Each experiment was performed in triplicates and the mean values were used for drawing the graphs.

3 Results and discussion

3.1 LC-MS analysis and validation

Optimum chromatographic separation of picroside I and picroside II was achieved by acetonitrile : water (pH 5.0, 25 : 75 (v/v)) with a flow rate of 0.7 mL·min^{-1}. The resulting chromatograms showed a retention time of 9.63 min for picroside I and 5.73 min for picroside II (Figs. 1(a–c)). The calibration curves of picroside I and picroside II were linear over the concentration range of 10–200 ng·mL^{-1} (r^2, 0.9842) and 30–600 ng·mL^{-1} (r^2, 0.9901) respectively. Recovery studies were performed using standard addition method. The recoveries of picroside I and picroside II were (94.22±1.35)% and (93.78±2.03)% respectively. The intra-day accuracy in terms of % difference was in the range of –3.47 to + 2.79 for picroside I, and – 3.96 to + 3.17 for picroside II. Inter-day accuracy was in the range of –3.85 to + 3.13 for picroside I, and –4.89 to + 4.07 for picroside II. Intra-day precision (% RSD) was in the range of 2.52 to 4.11 for picroside I, and 2.81 to 4.72 for picroside II. Inter-day precision was in the range of 2.89 to 4.73 for picroside I, and 3.27 to 4.88 for picroside II. Lower limits of quantification for picroside I and picroside II were 10 and 30 ng·mL^{-1}, respectively.

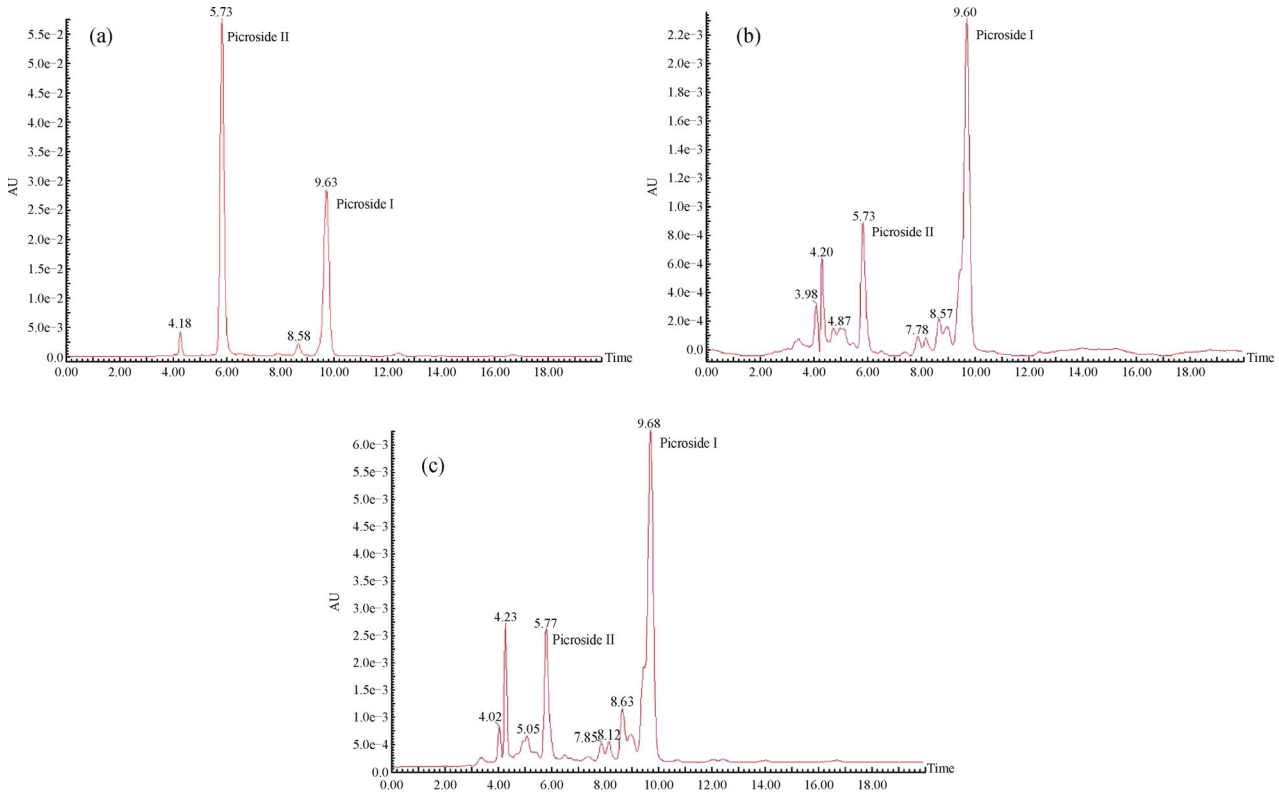

Fig. 1 Chromatographic profiles of (a) standard sample, the extracts obtained by (b) MAE, and (c) by the conventional method

3.2 Conventional hot percolation extraction of *P. kurroa*

The conventional extraction of *P. kurroa* was carried out to recover the maximum extractable amount of picroside I and picroside II. After the extraction period of 2 h, the picroside I and picroside II yields in resulting extract were 28.94 and 4.64 $mg \cdot g^{-1}$ feed respectively.

3.3 Analysis of experimental design

The experiments were performed employing as extraction solvents methanol, 50% (v/v) aqueous methanol, and water. The best results were obtained by employing water as extraction solvent followed by methanol and 50% (v/v) aqueous methanol. The effects of three process variables *viz.* solid to solvent ratio (X_1), extraction temperature (X_2) and extraction time (X_3) were studied during experimentation. These conditions seemed to be varied depending on the response required. Therefore, an optimum process should be investigated in order to obtain high picroside I and picroside II yields. The results of 15 runs using the Box-Behnken design are presented in Table 2. The Box-Behnken design with three factors and three levels, including three replicates at the center point, was used to fit a second-order response surface to optimize the extraction conditions. The three center point runs were carried out to measure the process stability and inherent variability.

The picroside I and picroside II yields ($mg \cdot g^{-1}$) were selected as the response P-I and P-II respectively. The mathematical model describing the extraction yields of picroside I and picroside II as functions of the coded independent variables (Table 2) in the selected ranges was demonstrated (water as extraction solvent) by the following second order polynomial equations:

$$\begin{aligned}\text{P-I} =& 16.45 + 13.65X_1 + 2.309X_2 - 2.011X_3 \\ & - 1.012X_1^2 + 0.86X_2^2 + 1.595X_3^2 \\ & + 1.31X_1X_2 - 1.0X_1X_3 - 3.423X_2X_3, \end{aligned} \quad (2)$$

$$\begin{aligned}\text{P-II} =& 2.49 + 2.03X_1 + 0.345X_2 - 0.273X_3 \\ & - 0.113X_1^2 + 0.163X_2^2 + 0.288X_3^2 \\ & + 0.16X_1X_2 - 0.165X_1X_3 - 0.465X_2X_3. \end{aligned} \quad (3)$$

The significance of each coefficient was determined using *p*-value, which is used as a tool to check the interaction strength between each independent variable. When a factor and an interaction among variables have a *p*-value less than 0.05, they influence the process in a significant way for a confidence level of 95% [20]. The significance of the *F*- value depends on the number of degrees of freedom (DF) in the model, and is shown in the *p*-value column (95% confidence level). In general, the

effects lower than 0.05 are significant.

The analysis of variance (Table 3) (data for water as extraction solvent) showed that this regression model was highly significant ($p < 0.01$) with F- values of 62.14 and 60.97 for picroside I and picroside II respectively. The F-values of 118.44 and 83.55 for lack of fit implies that they are not significant comparing to the pure error. The fitness of the model was further confirmed by a satisfactory value of determination coefficient, which was calculated to be 0.9911 and 0.991 for two models respectively, indicating that 99.11% and 99.10% of the variability in the response could be predicted by the model. The adjusted R^2 values of two models were determined to be 0.9752 and 0.9747 which showed that only 2.48% and 2.53% of the total variations were not explained by the models. As shown in Table 3, the variable with the largest effect was the X_1 at $p < 0.0001$. It was also observed from Table 3 that the linear coefficient (X_2 and X_3), and the interaction term

Table 3 Analysis of variance for response surface quadratic model of *P. kurroa* extraction determined from Box-Behnken experimental design

	Sum of squares	DF[a)]	Mean square	F-value	p-value	Significant
For picroside I						
Model	1639.986	9	182.2207	62.1413	0.0001	***
X_1	1490.58	1	1490.58	508.3212	< 0.0001	***
X_2	42.6426	1	42.6426	14.5421	0.0125	*
X_3	32.3610	1	32.3610	11.03581	0.0210	*
$X_1 X_2$	6.8644	1	6.8644	2.3409	0.1866	NS
X_1X_3	4	1	4	1.3641	0.2955	NS
$X_2 X_3$	46.8540	1	46.8540	15.9783	0.0104	*
X_1^2	3.7821	1	3.7821	1.2898	0.3076	NS
X_2^2	2.7335	1	2.7335	0.9322	0.3786	NS
X_3^2	9.3982	1	9.39821	3.2050	0.1334	NS
Residual	14.6618	5	2.9324			
Lack of Fit	14.5798	3	4.8599	118.4381	0.0084	**
Pure Error	0.0821	2	0.0410			
Cor Total	1654.648	14				
R^2	0.9911					
Adj R^2	0.9752					
For picroside II						
Model	36.0462	9	4.0051	60.9701	0.0001	***
X_1	32.9672	1	32.9672	501.8603	< 0.0001	***
X_2	0.9522	1	0.9522	14.4954	0.0125	*
X_3	0.5941	1	0.5941	9.0432	0.0299	*
$X_1 X_2$	0.1024	1	0.1024	1.5588	0.2671	NS
X_1X_3	0.1089	1	0.1089	1.6578	0.2543	NS
$X_2 X_3$	0.8649	1	0.8649	13.1664	0.0151	*
X_1^2	0.0467	1	0.0467	0.7114	0.4375	NS
X_2^2	0.0975	1	0.0975	1.4842	0.2775	NS
X_3^2	0.3052	1	0.3052	4.6459	0.0837	NS
Residual	0.3285	5	0.0657			
Lack of fit	0.3259	3	0.1086	83.5513	0.0119	*
Pure error	0.0026	2	0.0013			
Cor total	36.3746	14				
R^2	0.9910					
Adj R^2	0.9747					

a) Degree of freedom; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$; NS: not significant

coefficient X_2X_3 were significant, with small p-values ($p < 0.05$). The other term coefficients were not significant ($p > 0.05$) on the extraction yield.

3.4 Effect of extraction condition on picroside I and picroside II yields

In the case of extraction from a natural complex matrix, the desired compound or the group of compounds is present in various cells in different parts of the raw material. In the process of leaching these compounds, the solvent has to reach and dissolve them. Solvent usually attacks the cell wall of raw material leads to an enhanced dielectric heating within the cellulosic cell wall. The absorbed solvent gets rapidly heated on exposure to microwave radiation and cellulose itself being ionic conductor, rapidly conducts this heat and consequently undergoes rapid hydrolysis and makes the solvent penetrate it to reach the compounds. The solvent also dissolves various other impurities in the process. Thus for efficient extraction, the solvent penetration through the cell wall should be fast and the selectivity towards desired compound should be high [21,22]. Based on the solubility data of picroside I and picroside II, experiments were conducted with water, methanol and water-methanol mixture as extraction medium.

Equations (2) and (3) allowed the prediction of the effects of the three parameters on picroside I and picroside II yields respectively. The relationship between independent and dependent variables is illustrated in tri-dimensional representation of the response surfaces plots generated by the model for picroside I (Figs. 2(a–c)) and picroside II (Figs. 2(d–e)). Two variables were depicted in one tri-dimensional surface plots while the other variable was kept constant.

3.4.1 Effect of solid to solvent ratio

Generally, a higher ratio of solvent volume to solid matrix may be effective for conventional extraction methods. For MAE, however, a higher ratio may yield lower recoveries, which may be due to inadequate stirring of the solvent by microwaves. An optimum ratio of solvent to solid ensures homogeneous and effective heating. Excessive solvent causes poor microwave heating as the microwave radiation would be absorbed by the solvent and additional power is required. Low ratio of solvent to solid promotes mass transfer barrier as the distribution of active compounds is concentrated in certain regions which limits the movement of the compounds out of cell matrix [23,24].

It was observed that higher yields for picroside I (Figs. 2 (a–b)) and picroside II (Figs. 2(d–e)) were attained by setting solid to solvent ratio higher than 5 : 95 (w/v) with water as an extraction solvent. The effect of increased solid loading on the yield was more noticeable. As shown in Figs. 2(a,d), the maximum yields of picroside I ($41.59\ mg \cdot g^{-1}$) and picroside II ($6.27\ mg \cdot g^{-1}$) were attained at solid to solvent ratio of 10 : 90 (w/v), while keeping temperature and time at optimum conditions of 60 °C and 60 s respectively.

3.4.2 Effect of extraction temperature

Figures 2(a,c) show the effect of extraction temperature on the picroside I yield whereas Figs. 2(d,f) show the effect of extraction temperature on the picroside II yield. The picroside I and picroside II yields increased proportionally with longer microwave irradiation exposure periods. When the temperature was increased from 50 °C to 60 °C at optimum values of solid to solvent ratio, and extraction time (10 : 90 (w/v), 60 s), the yield was improved from 27.51 to $41.59\ mg \cdot g^{-1}$ for picroside I and from 4.33 to $6.27\ mg \cdot g^{-1}$ for picroside II. Inside the experimental domain, the maximum yields of picroside I and picroside II were obtained at an extraction temperature of 60 °C. This is probably due to higher temperature that causes intermolecular interaction within the solvent to increase, giving rise to higher molecular motion and causing higher local temperatures in the solid matrix. The increase in temperature due to microwave may cause opening of cellulose matrix and as a result the availability for extraction increases. Elevated temperature does indeed result in improved extraction efficiencies because desorption of analyte from active sites in the matrix will increase. Additionally solvents have higher capacity to solubilize analytes at higher temperature while surface tension and solvent viscosity decreases with temperature, which will improve sample wetting and matrix penetration respectively [23,24].

3.4.3 Effect of extraction time

Extraction time is another parameter to be taken into account. Generally, by increasing the extraction time, the quantity of analytes extracted is increased, although degradation may occur [23,24]. Response surface plots showing the effect of extraction time on the picroside I and picroside II yields are depicted in Figs. 2(b,c) and Figs. 2(e,f). When the extraction time was increased from 1 min to 7 min at optimum values of solid to solvent ratio and extraction temperature (10 : 90 (w/v), 60 °C), the yield was decreased from 41.59 to $28.73\ mg \cdot g^{-1}$ for picroside I and from 6.27 to $4.46\ mg \cdot g^{-1}$ for picroside II. Picroside I and picroside II yields were found to be maximum at optimum extraction time of 60 s. The interaction between temperature and extraction time was statistically significant for picroside I and picroside II yields ($p < 0.05$).

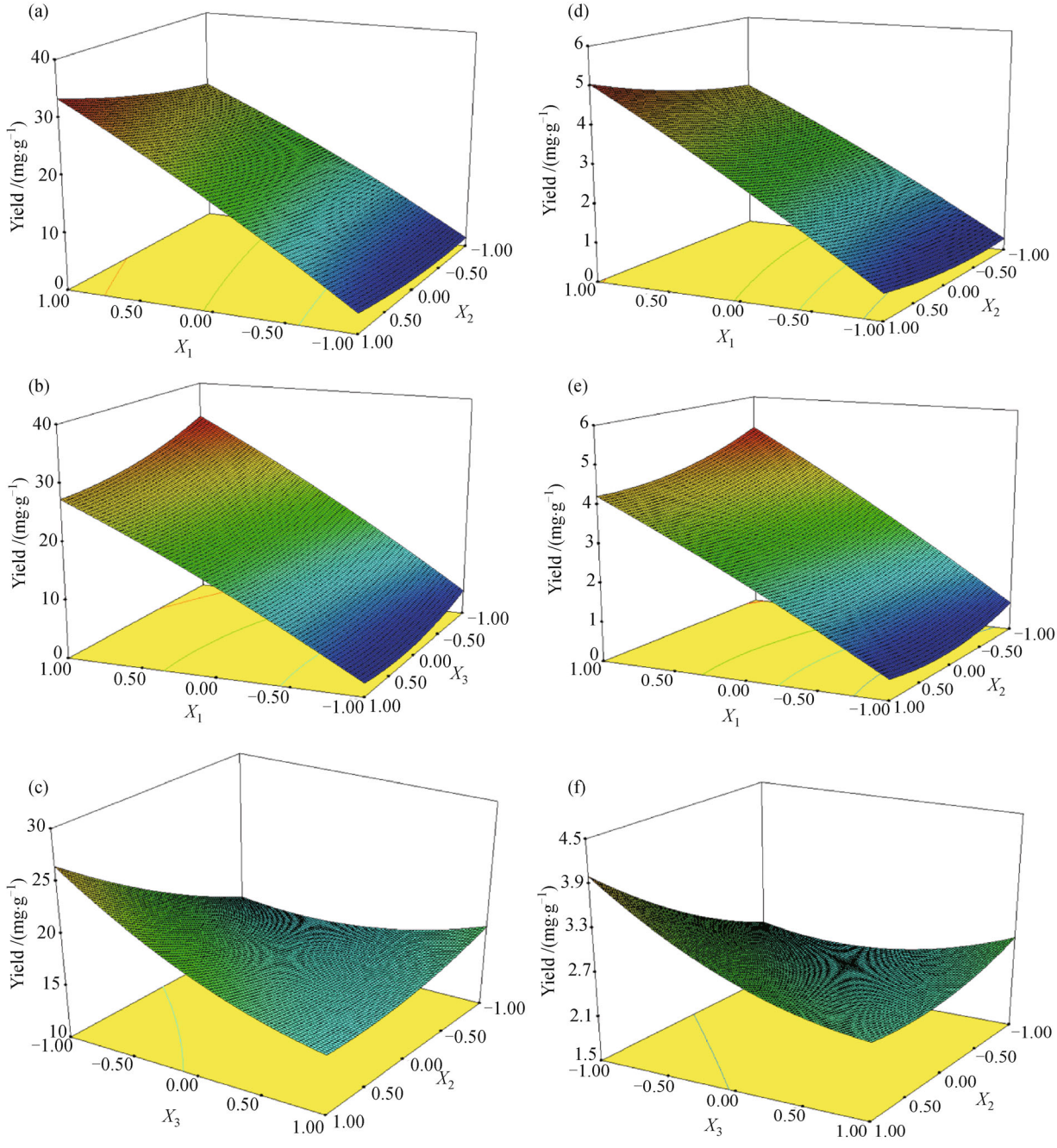

Fig. 2 Response surface plots of picroside I and picroside II showing the effect of (a, d) solid to solvent ratio and extraction temperature at constant extraction time (4 min), (b, e) solid to solvent ratio and extraction time at a constant extraction temperature (55 °C), and (c, f) extraction temperature and extraction time at a constant solid to solvent ratio (5 : 95, w/v).

3.5 Optimized sample preparation conditions and validation of the model

Figure 3(a) gives information about picroside I yield by comparing the experimental data against the model predicted values. Figure 3(b) implies the experimental data versus the predicted ones derived by model for picroside II yield. The Fig. 3 shows that the data obtained from the model are in a very good agreement with the laboratory results. The predictions that match measured values should fall on the diagonal line. Almost all data fall close to this line, which confirms the accuracy of the model.

The optimum values of selected variables were obtained using response surface. In summary, the optimal conditions of MAE process to obtain the highest picroside I and

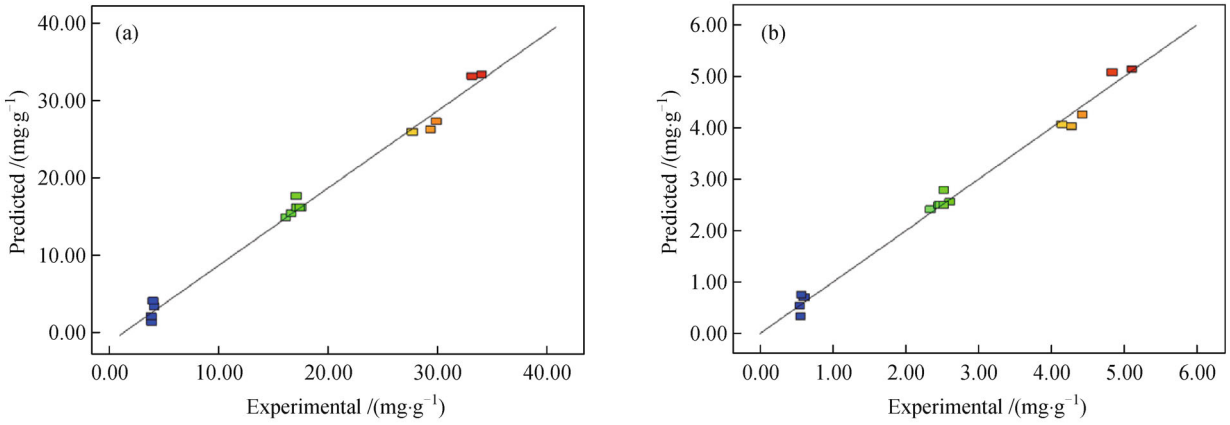

Fig. 3 Scatter plot of experimental values *vs.* predicted values of (a) picroside I and (b) picroside II

picroside II yields were 10 : 90 (w/v) solid to solvent ratio, 60 °C temperature and 60 s extraction time.

The suitability of the model equation for predicting the optimum response values was tested by executing three experiments under the optimal conditions. The experimental yields of picroside I and picroside II in resulting extract were 41.23 and 6.12 mg·g^{-1} feed respectively which were close to the predicted yields (41.59 and 6.27 mg·g^{-1} feed). There was no statistical difference at 5% level of significance between the experimental and predicted values. The results indicated that the experimental values were in good agreement with the predicted values and the regression model was accurate and adequate for the extraction process.

3.6 Comparison of conventional extraction with MAE on the basis of yield and extraction time

The conventional hot percolation assisted water extract of *P. kurroa* powder resulted in 28.94 and 4.64 mg·g^{-1} yields of picroside I and picroside II, respectively after 2 h of extraction. The MAE showed 41.23 and 6.12 mg·g^{-1} recoveries of picroside I and picroside II, respectively after an extraction period of 60 s. The comparison of yield and the time required for the extraction of picroside I and picroside II demonstrated that MAE is more efficient than the conventional method.

4 Conclusions

In this study, the microwave assisted extraction process was developed for the extraction of picroside I and picroside II from *P. kurroa*. The proposed models were able to indicate operational conditions, allowing superior yields. The process optimization results demonstrated that the effects of solid to solvent ratio, extraction temperature, and extraction time on the yields of picroside I and picroside II are significant. MAE with water as solvent can be used for the extraction of picroside I and picroside II with high efficiency and reduced extraction time for quantitative recovery.

References

1. Luper S. A review of plants used in the treatment of liver disease: Part 1. Alternative Medicine Review, 1998, 3(6): 410–421
2. Dhawan B N. Picroliv—a new hepatoprotective agent from an Indian medicinal plant, *Picrorhiza kurroa*. Medicinal Chemistry Research, 1995, 5(8): 595–605
3. Singh G B, Bani S, Singh S, Kaul A, Khajuria A, Sharma M L, Gupta B D, Banerjee S K. Anti-inflammatory activity of the iridoids kutkin, picroside-I and kutkoside from *Picrorhiza kurroa*. Phytotherapy Research, 1993, 7(6): 402–407
4. Recio D C, Maria M, Ginger R, Manez S, Rios J L. Structural considerations on the iridoids as anti-inflammatory agents. Planta Medica, 1994, 60(3): 232–234
5. Atal C K, Sharma M L, Kaul A, Khajuria A. Immunomodulating agents of plant origin. I: Preliminary screening. Journal of Ethnopharmacology, 1986, 18(2): 133–141
6. Puri A, Saxena R P, Sumati Guru P Y, Kulshreshtha D K, Saxena K C, Dhawan B N. Immunostimulant activity of picroliv, the iridoid glycoside fraction of *Picrorhiza kurroa*, and its protective action against *Leishmania donovani* infection in Hamsters. Planta Medica, 1992, 58(6): 528–532
7. Chander R, Kapoor N K, Dhawan B N. Picroliv, picroside-I and kutkoside from *Picrorhiza kurrooa* are scavengers of superoxide anions. Biochemical Pharmacology, 1992, 44(1): 180–183
8. Girish C, Koner B C, Jayanthi S, Ramchandrarao K, Rajesh B, Pradhan S C. Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. Fundamental & Clinical Pharmacology, 2009, 23 (6): 735–745
9. Kiso Y, Tohkin M, Hikino H. Assay method for antihepatotoxic activity using galactosamine induced cytotoxicity in primary

cultured hepatocytes. Journal of Natural Products, 1983, 46(6): 841–847

10. Kitagawa I, Hino K, Nishlmura T, Iwata E, Yosioka I. On the constituents of *Picrorhiza kurrooa*. (1). The structure of picroside I, a bitter principle of the subterranean part. Chemical & Pharmaceutical Bulletin, 1971, 19(12): 2534–2544
11. Tiwari S S, Pandey M M, Srivastava S, Rawat A K S. TLC densitometric quantification of picrosides (picroside-I and picroside-II) in Picrorhiza kurroa and its substitute Picrorhiza scrophulariiflora and their antioxidant studies. Biomedical Chromatography, 2012, 26 (1): 61–68
12. Gaikwad P S, Bhope S G, Kuber V V, Patil M J. Validated TLC method for simultaneous quantitation of kutkoside and picroside-I from kutki extract. Phytochemical Analysis, 2011, 22(1): 36–41
13. Bhandari P, Kumar N, Singh B, Kaul V K. Simultaneous determination of sugars and picrosides in *Picrorhiza* species using ultrasonic extraction and high-performance liquid chromatography with evaporative light scattering detection. Journal of Chromatography. A, 2008, 1194(2): 257–261
14. Wakte P S, Sachin B S, Patil A A, Mohato D M, Band T H, Shinde D B. Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from *Curcuma longa*. Separation and Purification Technology, 2011, 79 (1): 50–55
15. Eskilsson C S, Bjorklund E. Analytical-scale microwave assisted extraction. Journal of Chromatography A, 2000, 902(1): 227–250
16. Box G E P, Hunter W G, Hunter J S. Statistics for Experimenters. New York: Wiley, 1997
17. Montgomery D C. Design and Analysis of Experiments. 4th ed. New York: Wiley, 1997
18. Patil A A, Bhusari S S, Wakte P S, Shinde D B. Optimization of sample preparation variables for wedelolactone from *Eclipta alba* using Box-Behnken experimental design followed by HPLC identification. Annales Pharmaceutiques Francaises, 2013, 71(4): 249–259
19. Patil A A, Bhusari S S, Wakte P S, Shinde D B. Study of supercritical fluid extraction and ionic liquids as an additive on picroside I and picroside II recovery from *Picrorhiza scrophulariiflora* rhizomes. Journal of Pharmaceutical Investigation, 2013, 43 (3): 215–228
20. Machmudah S, Kawahito Y, Sasaki M, Goto M. Supercritical CO_2 extraction of rosehip seed oil: Fatty acids composition and process optimization. Journal of Supercritical Fluids, 2007, 41(3): 421–428
21. Murphy E J. Ionic conduction in hydrogen bonded solids. Annals of the New York Academy of Sciences, 1965, 118(19 Ionic Conduc): 728–738
22. Pethig R. Dielectric and Electronic Properties of Biological Materials. Chichester: Wiley, 1979
23. Chan C H, Yusoff R, Ngoh G C, Kung F W L. Microwave-assisted extractions of active ingredients from plants. Journal of Chromatography A, 2011, 1218(37): 6213–6225
24. Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviews, 2007, 1(1): 7–18