

## Optimizing the extraction of berberine from the stem of *Tinospora sinensis* (Lour.) Merr by response surface methodology

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### Abstract

*Tinospora sinensis* (*T. sinensis*), whose Tibetan name is “Lezhe”, as a traditional medicine, is widely distributed in Vietnamese. It is used to treat rheumatic arthralgia, sciatica, lumbar muscle strain and diabetes. Several studies have identified berberine as a major alkaloid present in *T. sinensis*, with extensive clinical and experimental evidence highlighting its diverse pharmacological properties, including immunomodulatory, antioxidative, cardioprotective, hepatoprotective, and hypoglycemic activities. The results showed that the optimal conditions for the optimum extraction conditions were the solid/solvent ratio of 1:20 (g/mL) ethanol concentration of 75.21%, extraction time of 23.91 hour and, percolation, the extraction efficiency of berberine was  $28.17 \pm 0.64$  mg/g. These results confirmed the presence of berberine in *T. sinensis* and established an optimized method for its extraction from the stem.

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Antioxidant activity, berberine, extraction optimization, pharmacological properties, *Tinospora sinensis*, traditional medicine.

## 1. Introduction

In recent years, herbal medicines have gained increasing recognition in the pharmaceutical industry due to their effectiveness in treating a wide range of diseases with relatively minimal side effects [1, 2]. Many medicinal plants, including *Tinospora sinensis*, serve dual purposes as both food and medicine, owing to their rich content of bioactive compounds that contribute not only to plant defense but also offer therapeutic benefits to humans [3, 4]. These bioactive substances, often classified as secondary metabolites, are synthesized by plants in response to environmental stress and pathogen attacks, and are

known to exert significant pharmacological effects when incorporated into traditional herbal formulations [5].

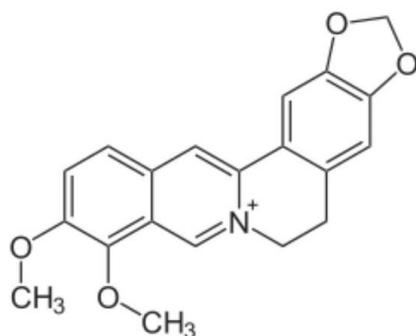
Phytochemistry, the scientific discipline dedicated to studying plant-derived compounds, has progressed rapidly in recent years, shedding light on the structures, biosynthetic pathways, and biological activities of various phytochemicals. This has contributed to the growing validation of herbal medicine as a credible and evidence-based component of healthcare systems [6].

In particular, *Tinospora sinensis*, has long been utilized



in traditional medicine systems for its therapeutic efficacy in the treatment of fever, jaundice, dyspepsia, and diabetes [7, 8]. Among its various bioactive constituents, berberine—a naturally occurring isoquinoline alkaloid—has garnered substantial interest due to its wide spectrum of pharmacological properties, including glucose-lowering activity, enhancement of insulin sensitivity, as well as anti-inflammatory, antioxidant, and antimicrobial effects [9, 10]. It has also been investigated as a promising adjunct therapy in the management of type 2 diabetes, hyperlipidemia, and hypertension [11].

Chemically, berberine is a quaternary isoquinoline alkaloid, possesses a planar and rigid structure with a positively charged nitrogen atom within its isoquinolinium ring system. Its molecular formula is  $C_{20}H_{18}NO_4^+$ , and it is typically found in the form of berberine chloride or sulphate salts. The chemical structure of berberine is shown in Fig. 1.



**Figure 1.** Chemical structure of berberine ( $C_{20}H_{18}NO_4^+$ ), a quaternary isoquinoline alkaloid responsible for diverse biological activities.

This cationic and aromatic nature contributes to its poor water solubility but high affinity for organic solvents such as ethanol, which significantly influences its extraction behavior. The presence of multiple methoxy groups also enhances its lipophilicity, further supporting the use of moderately polar solvents in extraction optimization. To overcome these challenges, recent studies have focused on nanoparticle-based delivery systems to enhance its pharmacokinetic profile and therapeutic efficacy [12].

## 2. Materials and methods

### 2.1. Plant material and preparation of extracts

The stems of *Tinospora sinensis* were collected in Dak

Nong Province, Vietnam, in January 2023. It was air-dried, sliced, and ground into a fine powder (0.2–0.5 mm) before being stored in sealed polyethylene bags at 4°C. For extraction, 10 g of the powder was treated with 150 mL of 96% ethanol for 24 h [13].

### 2.2. Experimental design

A single-factor experiment was conducted to optimize the recovery of compounds from *Tinospora sinensis* stems by varying with the three factors: ethanol concentration (0%, 45%, 70%, 99.5%), extraction time (12, 24, 36, 48 h), and solid/solvent ratio (1:5, 1:10, 1:15, 1:20 g/mL). The percolation method was used for extraction, and one variable was altered at a time while the others remained constant. The level for each factor was selected based on the results of three response variables.

### 2.3. Box-Behnken experimental design

To evaluate the extraction parameters and optimize the conditions for berberine extraction, a Box and Behnken (1960) [14] design was employed. The experimental design consisted of 15 experimental runs with 3 replicates at the central point. The effects of the independent variables—liquid-to-material ratio (X1), solvent concentration (X2), and extraction time (X3)—were assessed at three different levels during the extraction process (Table 1).

The experiments were randomized to minimize the influence of any unpredictable factors on the responses. The experimental data were analyzed using Design-Expert 13.0.0 software, and the results were fitted to polynomial equations of the form:

$$Y = a_0 + \sum_{i=1}^3 a_i x_i^* + \sum_{i=1}^2 \sum_{j=2}^3 a_{ij} x_i^* x_j^* + \sum_{i=1}^3 a_{ii} x_i^{*2}$$

where Y is the dependent variable or response,  $a_0$  is a scaling constant,  $a_i$  represents the linear coefficients,  $a_{ij}$  is the interaction coefficient,  $a_{ii}$  is the quadratic coefficients, and  $x_i^*$  the independent variables were coded at three levels: -1 (lower limit), 0 (central point), and +1 (upper limit) (Table 1) [15]. An analysis of variance (ANOVA) was conducted to assess the quadratic model's validity and the significance of regression coefficients at a 95% confidence level. The predicted values were calculated and compared with experimental data to validate the model, and the

**Table 1.** Box-Benkhen experimental design with the experimental and predicted values of the responses.

Independent variable	Units	Symbol	Coded values		
			-1	0	1
Solid/Solvent ratio	X1	g/mL	1:10	1:15	1:20
Ethanol concentration	X2	%	45	70	99,5
Time extraction	X3	hour	12	24	48

results were represented as response surface plots.

#### 2.4. Analyses by liquid chromatography-tandem mass spectrometer (LC-MS/MS)

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was employed to quantify berberine in *Tinospora sinensis* stems using an ACQUITY UPLC I-Class / Xevo TQ-XS MS/MS system. Chromatographic separation was carried out on an Acquity Premier BEH C18 column (1.7  $\mu$ m, 2.1  $\times$  100 mm) at 40°C. The mobile phase consisted of 0.1% formic acid in water (phase A) and acetonitrile (phase B), with a flow rate of 0.4 ml/min following a gradient program. Mass spectrometric analysis was performed in positive ion mode using Electrospray Ionization (ESI) and Multiple Reaction Monitoring (MRM) for berberine quantification, with the following parameters: ion source temperature at 150°C, desolvation temperature at 450°C, gas flow rate at 10 L/h, desolvation gas flow rate at 900 L/h, and capillary voltage at 3.2 kV.

#### 2.5. Statistical analysis

All experimental data were analyzed using Excel 2021 and Design-Expert version 13.0.0. The results are presented as mean  $\pm$  standard deviation (SD) of replicate solvent extractions and triplicate assays. One-way analysis of variance (ANOVA) followed by Tukey's test was applied to identify significant differences ( $p < 0.05$ ) between the means. Additionally, Pearson correlation coefficients were calculated to assess the relationships between the antioxidant compound assays and antioxidant capacity assays.

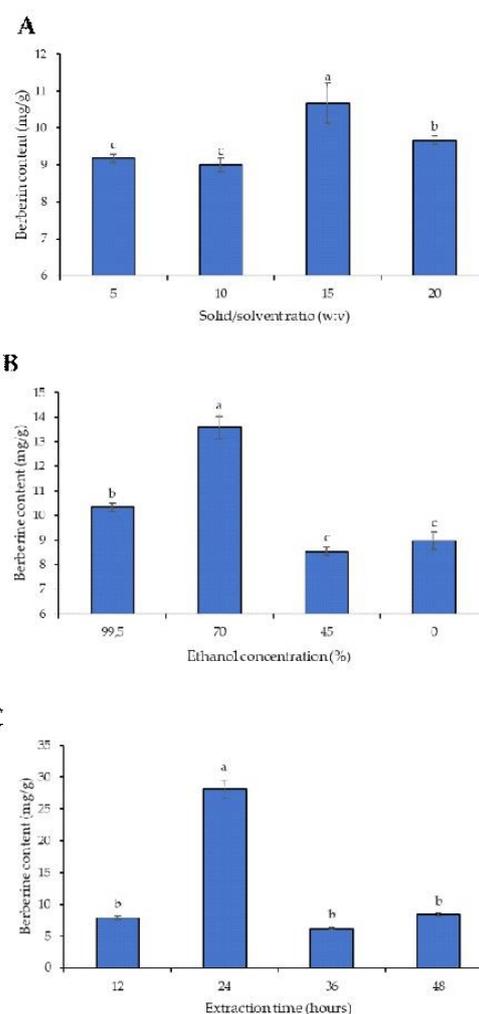
#### 2.6. Verification of model

The experiments were performed under optimal extraction conditions, which were selected based on the highest desirability, to validate the model. The experimental and predicted values were compared to assess the accuracy and validity of the model.

## 3. Results and discussion

### 3.1. Effect of single factor analysis

The influence of the solid-solvent ratio, ethanol concentration, and extraction time on the berberine content extracted from the stem of *T. sinensis* is shown in Fig. 2 A–C.



**Figure 2.** The effect of (A) the solid-solvent ratio, (B) ethanol concentration, and (C) extraction time on the berberine content extracted from the stem of *T. sinensis* ( $n = 3$ ). Values are presented as means  $\pm$  standard deviation of six measurements. Values marked by different lower-case letters (A–C) are significantly different ( $p < 0.05$ ).

#### 3.1.1. Effect of solid-solvent ratio

The proportion of medicinal material to solvent is a crucial factor influencing the efficiency of the

extraction process. During extraction, the immersion of medicinal material in the solvent generates a concentration gradient between the intracellular solutes and the external solvent. This gradient induces a driving force that facilitates the release of bioactive compounds from the cells into the solvent. Numerous studies have demonstrated that modifications to this ratio can substantially affect the yield of extracted compounds. In Fig. 2A, four different solid-solvent ratios (1:5, 1:10, 1:15, and 1:20 g/mL) were examined whilst keeping the other factors constant as follows; 99.5% ethanol concentration, and 24 h extraction time. The results showed that the berberine content extracted was significantly ( $p < 0.05$ ) affected by the solid-solvent ratio. The berberine extract peaked at 1:15 ( $10.67 \pm 0.54$  mg/g,  $p < 0.05$ ). The extracted berberine content exhibited a decreasing trend from 1:5 to 1:10 (g/mL) and from 1:15 to 1:20 (g/mL). Thus, solid-liquid ratios 1:10 to 1:20 (g/mL) were selected for the optimization process. Li *et al.* (2023) highlighted that the ratio of raw material to solvent significantly influences the extraction efficiency of berberine in soil. Their study demonstrated that the berberine content increased progressively as the ratio increased from 1:5 to 1:20 (g/mL) but exhibited a declining trend when further increased to (1:30) g/mL. Finally, 1 : 15, 1 : 20 and 1 : 25 were selected for optimization test [16]. An increased solid-solvent ratio can enhance the concentration gradient, thereby promoting the diffusion of compounds into the solvent and expediting the mass transfer process [17].

### 3.1.2. Effect of the ethanol concentration

Ethanol serves as a key solvent that plays a pivotal role in extraction efficiency and compound selectivity. Its concentration directly affects the solubility of bioactive compounds, where lower ethanol levels are more effective for polar compounds, whereas higher concentrations facilitate the extraction of non-polar substances. Moreover, ethanol enhances mass transfer and diffusion, contributing to improved extraction kinetics. Thus, optimizing ethanol concentration is essential for achieving maximum extraction efficiency. The effect of ethanol concentration, ranging from 0% to 99.5%, on berberine extraction is illustrated in Fig. 2B. The extraction was conducted at a solid-solvent ratio of 1:15 (g/mL), for 24 h. The berberine content

exhibited a rising trend with increasing ethanol concentration, reaching its maximum at 70% ( $13.57 \pm 0.45$  mg/g,  $p < 0.05$ ), followed by a decline from 70% to 90%. Based on these findings, an ethanol concentration ranges of 45% to 99.5% was selected for further optimization. Rojsanga *et al.* (2006) investigated ethanol concentrations ranging from 50% to 80% and found that this range more effectively extract the berberine from the stems of *B. integerrima* and *B. elasticbergii* [18]. A study by Li *et al.* (2023) showed that the highest yield of berberine was obtained at 80% ethanol concentration, the investigated range for the optimal process was 60 to 80% for berberine extraction from soil, which is consistent with our results [16].

### 3.1.3. Effect of extraction time

Extraction time is a critical factor influencing the efficiency of natural compound recovery. Studies have shown that both excessively short and prolonged extraction durations may lead to suboptimal yields. The other independent variables that were kept constant were the maximum 70% ethanol concentration from the second analysis, and a solid-solvent ratio of 1:15 g/mL. The impact of different extraction times on berberine extraction is shown in Fig. 2C, the berberine content increased from 12 to 24 h, reaching its peak at 24 h ( $28.09 \pm 1.42$  mg/g,  $p < 0.05$ ). However, a decline was observed from 24 to 36 h. Consequently, a time range of 12 to 36 h was selected for optimization testing. Sarraf *et al.* (2020) studied the best extraction yield at 24 h berberine extraction time and the range from 12 to 48 h to perform optimization experiment. Extraction time is a key parameter in solid-solvent extraction, influencing analyte solubility and mass transfer, both of which are governed by the molecular structure and weight of the compounds [19]. However, prolonged extraction durations may induce oxidative reactions, epimerization, and degradation of target metabolites [20]. This phenomenon was observed in the present study, as illustrated in Fig. 2C.

### 3.2. Modeling of the total berberine recovery using response surface methodology

Response surface methodology with three independent variables at three levels was applied to evaluate the impact of key extraction parameters,

**Table 2.** The experimental design, actual value, and predicted value of response surface method

Experiment	Solid/Solvent Ratio (g/mL)	EtOH Conc. (%)	Extraction Time (h)	Berberine Content (mg/g)	Berberine content (predict)
	X1			X3	Y
1	10	45	24	20.12	20.95
2	15	45	12	5.93	5.96
3	20	70	12	7.21	6.50
4	15	99.5	12	6.25	6.29
5	15	70	24	28.21	29.13
6	10	99.5	24	25.36	24.26
7	20	70	36	6.22	6.26
8	15	70	24	29.16	29.13
9	15	70	24	30.01	29.13
10	20	99.5	24	24.67	23.84
11	20	45	24	21.18	22.28
12	15	45	36	4.54	4.49
13	10	70	36	4.45	5.15
14	15	99.5	36	5.23	5.19
15	10	70	12	7.52	7.47

Independent variables	Levels		
	-1	0	+1
X1, Solid/Solvent ratio (g/mL)	1:10	1:15	1:20
X2, Ethanol concentration (%)	45	70	99
X3, Time extraction (h)	12	24	36

**Table 3.** Response variables and their corresponding fitted model equations

Symbol	Response variable	Quadratic equation
Y <sub>Berberine (T. sinensis)</sub>	Berberine Content (mg/g)	Y = -102.88 + 3.329*X1 + 0.7824*X2 + 4.469*X3 - 0.1086*X1 <sup>2</sup> - 0.003819*X2 <sup>2</sup> - 0.13931*X3 <sup>2</sup> - 0.00321*X1*X2 + 0.00867*X1*X3 + 0.000283*X2*X3

including the solid–liquid ratio (X<sub>1</sub>), ethanol concentration (X<sub>2</sub>), and extraction time (X<sub>3</sub>), on berberine recovery from *T. sinensis*. The experimental design, along with the corresponding observed and predicted values for 15 experimental runs, is presented in Table 2. The berberine content in *T. sinensis* ranged from 4.45 to 30.01 mg/g.

**3.2.1. Significance of regression coefficients in model evaluation**

The adequacy and significance of the regression model were assessed through analysis of variance (ANOVA) (Table 3). The model exhibited a high coefficient of determination (R<sup>2</sup>), indicating a good fit between the experimental data and the predicted values. The adjusted R<sup>2</sup> was also in close agreement with the R<sup>2</sup>, suggesting the model’s reliability in explaining the variability in berberine content.

Furthermore, the p-value of the model was less than 0.05, confirming its statistical significance at the 95% confidence level.

The lack-of-fit test was found to be non-significant (p > 0.05), implying that the model adequately fit the data and that the variation not explained by the model was within the range of experimental error.

The analysis of the regression coefficients provided insights into the influence of each factor. Larger absolute values of the coefficients indicate stronger effect on the response variable. Positive coefficients suggested a direct relationship with berberine content, whereas negative coefficients indicated an inverse relationship. These findings enable the identification of key factors and interactions that most significantly affect the extraction efficiency.

**Table 4.** Analysis of variance (ANOVA) for the fitted quadratic model of *T. sinensis*

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	1524.64	9	169.40	88.60	< 0.0001	Significant
A-Solid/Solvent ratio	0.4186	1	0.4186	0.2189	0.6595	
B-Ethanol concentration	11.86	1	11.86	6.20	0.0551	
C-Time extraction	5.23	1	5.23	2.74	0.1590	
AB	0.7656	1	0.7656	0.4004	0.5547	
AC	1.08	1	1.08	0.5657	0.4859	
BC	0.0342	1	0.0342	0.0179	0.8988	
A <sup>2</sup>	27.23	1	27.23	14.24	0.0130	
B <sup>2</sup>	47.28	1	47.28	24.73	0.0042	
C <sup>2</sup>	1485.92	1	1485.92	777.13	< 0.0001	
Residual	9.56	5	1.91			
Lack of Fit	7.94	3	2.65	3.26	0.2433	not significant
Pure Error	1.62	2	0.8108			
Cor Total	1534.20	14				

**Table 5.** Fit statistics

Std. Dev.	Mean	C.V. %	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	Adeq Precision
1.38	15.07	9.18	0.9938	0.9826	0.9148	22.8142

### 3.3. ANOVA for the quadratic model

The ANOVA confirmed the suitability and significance of the quadratic regression model, showing a strong correlation between the response and experimental factors. The intercept, linear, interaction, and quadratic terms contributed significantly. Details of the ANOVA are presented in Table 4. Based on these findings, the model was validated for predicting berberine content across 15 experimental runs.

The analysis of variance (ANOVA) results indicated that the quadratic model was highly significant ( $p < 0.0001$ ) (Table 4), with an F-value of 88.60, demonstrating the model's strong predictive power for berberine content. Among the linear terms, ethanol concentration (B) showed a moderate influence ( $p = 0.0551$ ), whereas the solid/solvent ratio (A) and extraction time (C) were not significantly different ( $p > 0.05$ ). The interaction effects between the variables (AB, AC, and BC) were also not statistically significant, suggesting minimal synergistic effects. However, the quadratic terms A<sup>2</sup>, B<sup>2</sup>, and particularly C<sup>2</sup> were all significant, with C<sup>2</sup> contributing most strongly ( $p < 0.0001$ ), indicating that extraction time has a pronounced nonlinear effect on berberine yield.

The lack-of-fit test was not significant ( $p = 0.2433$ ), confirming that the model adequately fit the experimental data without systematic errors.

#### 3.3.1. Fit statistics

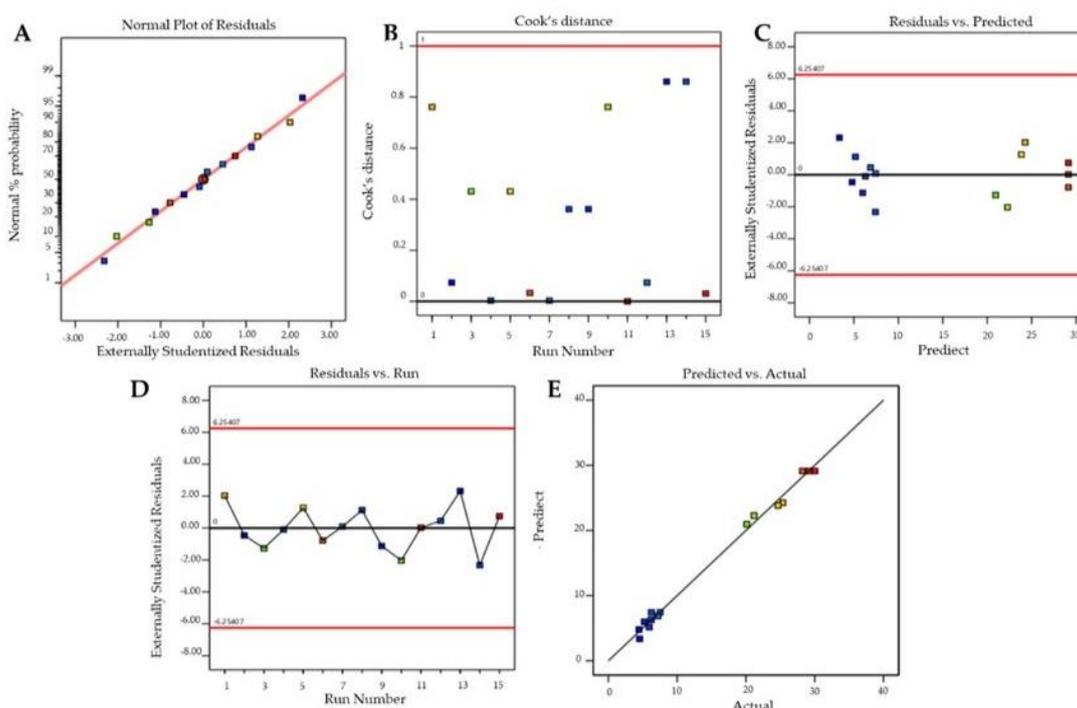
In addition to the ANOVA, model evaluation metrics supported its robustness and predictive power. The R<sup>2</sup> value of 0.9938, along with a high adjusted R<sup>2</sup> (0.9826) and predicted R<sup>2</sup> (0.9148), confirmed excellent model fit and generalization ability. Adequate precision (22.81) indicated a strong signal-to-noise ratio. The low coefficient of variation (9.18%) and standard deviation (1.38) reflected good reproducibility and consistency (Table 5).

The regression coefficients indicated that ethanol concentration had the most positive linear effect on berberine yield, while extraction time had a slight negative influence (Table 6). Interaction effects were minimal, as shown by the low coefficients and high standard errors. Notably, the quadratic terms—especially C<sup>2</sup> (−20.06)—were strongly negative, confirming a significant nonlinear relationship, with extraction time being the most sensitive parameter.

The diagnostic plots collectively validate the robustness and adequacy of the regression model (Fig. 3 A-E).

**Table 6.** Coefficients in terms of coded factors

Factors	Coefficient estimate	df	Standard error
Intercept	29.13	1	0.7983
A-Solid/Solvent ratio	0.2287	1	0.4889
B-Ethanol concentration	1.22	1	0.4889
C-Time extraction	-0.8088	1	0.4889
AB	-0.4375	1	0.6914
AC	0.5200	1	0.6914
BC	0.0925	1	0.6914
A <sup>2</sup>	-2.72	1	0.7196
B <sup>2</sup>	-3.58	1	0.7196
C <sup>2</sup>	-20.06	1	0.7196



**Figure 3.** (A) Normal probability plot of externally studentized residuals for the reduced polynomial model. (B) Cook's distance for the reduced polynomial model. (C) The plot of residuals against predicted values. (D) The plot of residuals against run number. (E) Plot of predicted values against the observed values.

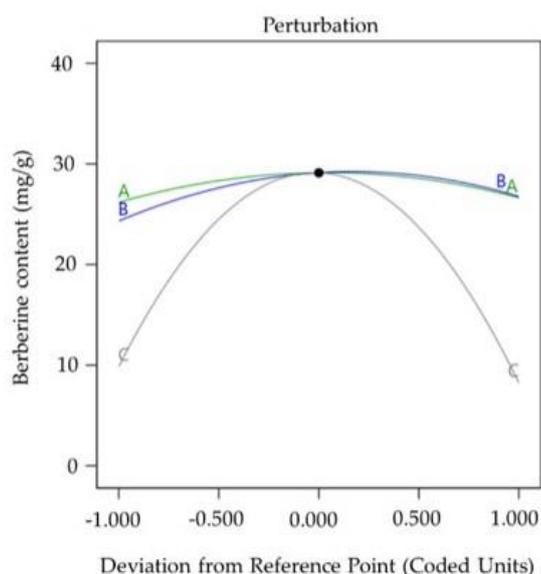
In the normal probability plot of residuals (Fig. 3 A), the data points closely follow a straight line, indicating that the residuals are approximately normally distributed—a key assumption in regression modeling. The Cook's distance plot (Fig. 3 B) shows that all values remain well below the cutoff threshold of 1, suggesting that none of the observations unduly influence the model's parameter estimates. The residuals versus predicted values plot (Fig. 3 C)

displays a random scatter around zero, confirming the homoscedasticity (constant variance) of residuals and the lack of systematic error across the prediction range. The residuals versus run number plot (Fig. 3 D) further supports the independence of residuals, as no obvious trend or cyclic pattern was observed throughout the experimental runs. This implies that the experimental order does not introduce any time-dependent bias or autocorrelation. Finally, the

predicted versus actual values plot (Fig. 3 E) demonstrated a strong correlation between the observed and predicted values, with data points clustering closely along the diagonal line. This confirms the model's high predictive accuracy and goodness of fit.

### 3.4. Optimization of the extraction conditions

The perturbation plot (Fig. 4) provides a comparative visualization of the influence of three independent variables—solid/solvent ratio (A), ethanol concentration (B), and extraction time (C)—on the berberine content. Among these, extraction time (C) exhibited the most significant impact, as indicated by its steep curvature. The berberine content increased with time initially, reaching a maximum around the center point, but decreased beyond this optimum, possibly due to degradation of the compound or saturation of the extraction medium. In contrast, the effects of ethanol concentration and solid/solvent ratio were comparatively moderate, with relatively flat curves near the center point, suggesting a more stable response to changes in these parameters. These findings underscore the critical role of extraction time in maximizing berberine recovery, while also highlighting the necessity of optimizing all parameters to achieve optimal content.

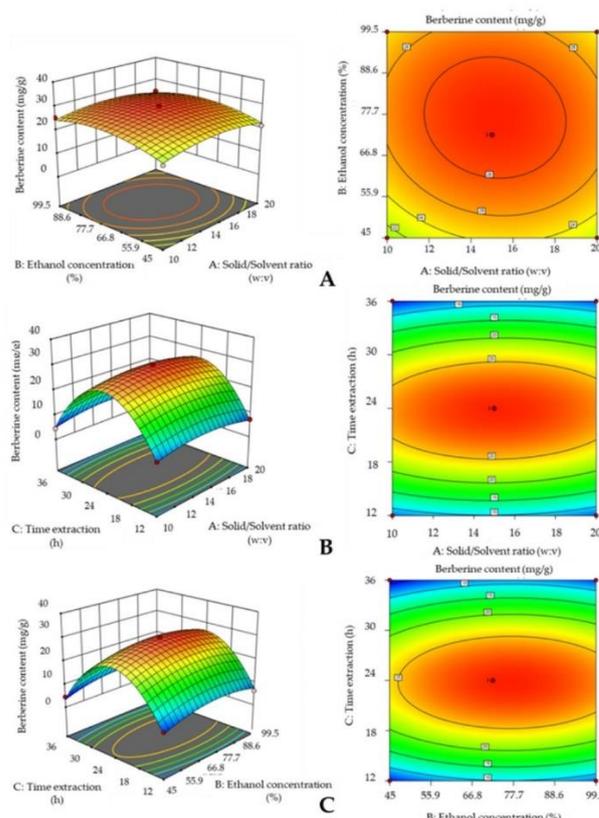


**Figure 4.** The perturbation plot displaying the impact of all the independent factors on the berberine content at the center. (A) solid-liquid ratio; (B) ethanol concentration; (C) extraction time.

### 3.5. Analysis of the surface plots

The interaction of two of the three variables and their

effect on berberine content while the rest of the three variables were kept constant is illustrated in the three-dimensional RSM plot (Fig. 5 A–B).



**Figure 5.** The interactive effect of (A) of solid-liquid ratio and ethanol concentration; (B) extraction time and solid-liquid ratio; (C) extraction time and ethanol concentration.

The three-dimensional response surface plots (Fig. 5 A–C) clearly illustrated the interactive effects of ethanol concentration, extraction time, and solid-to-solvent ratio on berberine yield from *T. sinensis*. In Fig. 5 A, the interaction between ethanol concentration and solid-to-solvent ratio revealed a significant quadratic effect, where berberine recovery increased with both parameters up to an optimum, beyond which the yield plateaus. The highest response is observed near 75% ethanol and a 1:15 to 1:20 solid-to-solvent ratio. Fig. 5 B demonstrates a similar trend for the interaction between extraction time and solid-to-solvent ratio, with berberine content rising steadily as both variables increased, reaching a peak at approximately 24 h and a ratio of 1:20. Fig. 5 C highlights the combined influence of ethanol concentration and extraction time, where moderate ethanol levels (~75%) and prolonged extraction (~24 h)

**Table 7.** Predicted vs. experimental optimal values of berberine content

Response	Optimal conditions			Maximum content	
	X1	X2	X3	Predicted	Actual
Berberine	20	75.21	23.91	26.68 mg/g	28.17 ± 0,64 mg/g

X1: Solid-solvent ratio (g/mL); X2: Ethanol concentration %; X3: Time extraction (h). Experimental results were expressed as mean values ± standard deviation (n=6)

favor berberine recovery, while excessive ethanol concentration or time did not further improve extraction efficiency. These trends confirm the strong synergistic interactions among the extraction variables, and the elliptical contour patterns validate the suitability of the quadratic polynomial model. The results of this study, particularly the interactive effects of ethanol concentration, extraction time, and solid-to-solvent ratio on berberine yield, are consistent with the findings previous studies in the field of plant extraction optimization. For instance, Satija *et al.* (2015) utilized a central composite design to optimize the extraction of bioactive compounds from *Tinospora cordifolia* and observed similar trends in berberine recovery, where an optimal ethanol concentration and solid-to-solvent ratio maximized the yield [21]. In our study, the highest berberine yield was achieved near 75% ethanol and a 1:15 to 1:20 solid-to-solvent ratio, which aligns with the optimal conditions reported by Xu *et al.* (2017), who found that moderate ethanol concentrations favored the recovery of berberine from *Coptis chinensis* extracts [22]. These results validate the applicability of response surface methodology (RSM) in optimizing extraction conditions and support the robustness of the quadratic polynomial model for predicting optimal extraction parameters.

### 3.6. Determination of optimal conditions and validation of model

To validate the predictive accuracy of the model, experimental trials were conducted under the identified optimal extraction conditions. The observed values closely matched the predicted results, confirming the reliability of the optimization process. The developed quadratic polynomial regression model yielded optimal conditions with a desirability value of 1.000. In desirability function analysis, values range from 0 to 1, where 0 indicates an unacceptable outcome and 1 represents the most favorable response for the target variable. The optimal conditions for

extracting berberine from *T. sinensis* extracts were as follows: a solid-solvent ratio of 1:20 (g/mL), time extraction of 23.91 h, and ethanol concentration of 75.21%. Under these conditions, the berberine content reached 28.17 ± 0,64 mg/g (Table 7). To validate the predictive accuracy of the model, six replicate verification experiments were conducted. The resulting berberine content was 28.17 ± 0.64 mg/g, which closely matched the predicted value.

## 4. Conclusions

The berberine was successfully extracted from the stem of *T. sinensis* using a maceration method. The effects of ethanol concentration, extraction time, and solid-to-liquid ratio on berberine recovery were found to be statistically significant ( $p < 0.05$ ). Optimization using response surface methodology identified the optimal extraction conditions as a solid-solvent ratio of 1:20 (g/mL), an extraction time of 23.91 h, and an ethanol concentration of 75.21%. Under these conditions, the berberine content reached 28.17 ± 0.64 mg/g, demonstrating excellent agreement with the model's prediction, and confirming the reliability of the optimization approach. The optimized berberine extraction from *T. sinensis* demonstrates scalability for industrial applications, with promising yield and stability, suggesting potential for commercial development.

## Authors' contributions

Designed and supervised the project, N.V.T.T; Performed the experiments, L.L.Q; D.N.H; Analyzed the data, D.D.T.H.; N.T.T.M; Contributed to data interpretation and statistical analysis, K.T.T; T.N.T.T; Wrote the manuscript and critically revised the paper, N.V.T.T.

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## Availability of data and materials

All data will be made available on request according to the journal policy.

## Conflicts of interest

The authors declare that they have no financial and commercial conflicts of interest.

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