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RESEARCH ARTICLE

Optimization of extraction process and antioxidant activity of total tanshinones from *Salvia miltiorrhiza* in Guizhou by response surface experiment

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Abstract: Objective: To optimize the extraction process of total tanshinones from Salviae Miltiorrhizae Radix et Rhizoma using Box-Behnken design-response surface methodology (RSM) and evaluate its antioxidant activity. Methods: The content of total tanshinones was determined by ultraviolet spectrophotometry. The antioxidant capacity of S. Miltiorrhizae in vitro was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging assay, hydroxyl radical scavenging capacity, and superoxide anion radical scavenging capacity. Results: The optimal extraction process of total tanshinones from S. Miltiorrhizae was as follows: ethanol concentration of 80%, solid-to-liquid ratio of 1:46 (g: vol), and extraction time of 62 min. Under these conditions, the content of total tanshinones reached 198.41mg/g. The maximum scavenging rates of the four radical systems were 66.20%, 69.49%, 50.30%, and 65.10%, respectively. Conclusion: The extraction process optimized by RSM is stable and feasible with simple operation, which can be used for the extraction of total tanshinones. The total tanshinones extracted from S. Miltiorrhizae exhibit certain antioxidant capacity in vitro and are worthy of further research and development for the prevention and treatment of oxidative stress-related diseases.

Keywords: *Salvia miltiorrhiza*; Total tanshinone; Response surface Methodology; Antioxidant Activity

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1. Introduction

S. Miltiorrhizae contains a variety of chemical components, including but not limited to fat-soluble tanshinones, water-soluble phenolic acids, etc. [1]. Modern pharmacological studies have shown that *S. Miltiorrhizae* has antioxidant [2], anti-atherosclerosis [3], anti-myocardial ischemia, improving cardiac function and other pharmacological effects [4]. It has a good therapeutic effect in cardiovascular and cerebrovascular diseases, cancer, anti-aging and other aspects, and its related preparations have been widely used in clinical [5]. For example, Danshen formula granules can treat cardiovascular and cerebrovascular diseases, anti-tumor [6]; danshen injection has antioxidant effect on patients with stable angina pectoris [7]. In cerebrovascular diseases, the antioxidant, anti-inflammatory and antithrombotic effects of *S. Miltiorrhizae* can effectively reduce brain tissue damage, promote brain tissue recovery, and improve brain blood circulation [8], indicating that *S. Miltiorrhizae* has a wide range of application value in blood circulation-related diseases. At present, many scholars have studied the extraction process of tanshinones in *S. Miltiorrhizae* et al [9] used response surface method and back propagation neural network to study the percolation extraction process of total tanshinones. The optimal extraction process was obtained as follows : the particle size of the medicinal material was the coarsest powder, the ethanol concentration was 95 %, and the solvent dosage was 7 times. The extraction rate of effective components was as high as 85 %. On the basis of single factor experiment, Zhang et al. [10] optimized the supercritical CO_2 extraction process of total tanshinone from *S. Miltiorrhizae* by orthogonal test. The optimum reaction conditions were as follows : pressure 3×10^{-4} kPa, temperature 45℃, time 2.0 h, entrainer flow rate 1.0 mL / min, and the extraction rate was 4.58 %. There are also some reports on the antioxidant activity of tanshinones. For example, Xia et al. [11] found that tanshinones have a good scavenging effect on superoxide anion (·O_2-) and hydroxyl radical (·OH-). The results of Jing et al. [12] showed that the ethyl acetate extract of *S. Miltiorrhizae* had high reducing power and the ability to scavenge DPPH, H_2O_2 and·OH-free radicals. DPPH (a synthetic stable radical), $ABTS^+$ (a cationic

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chromophore), and hydroxyl radicals (·OH, generated via Fenton reactions or mitochondrial oxidative stress) represent distinct oxidative threats. While DPPH and $ABTS^+$ are in vitro proxies for lipid peroxidation and cationic radicals, endogenous ·OH directly damages biomolecules through DNA strand breaks, protein denaturation, and lipid peroxidation. Excessive ·OH and superoxide anions ($\cdot O_2^-$, precursor to ·OH) drive oxidative stress-linked pathologies like neurodegeneration and cardiovascular diseases. Quantifying scavenging efficacy against these radicals validates antioxidant potency, clarifies structure-activity relationships, and informs therapeutic strategies to mitigate ROS-mediated cellular damage, thereby bridging experimental models with physiological relevance. According to the relevant literature [13], tanshinone components are easily decomposed by heat, and the traditional reflux extraction method is used for extraction. The high temperature causes the loss of total tanshinone content, resulting in the waste of *S. Miltiorrhizae* resources. Therefore, the ultrasonic extraction method has obvious advantages in extracting total tanshinone in this experiment, and the active components of total tanshinone extract are better retained. On the basis of single factor experiment, Box-Behnken experimental design-response surface method was used to optimize the extraction conditions with total tanshinone content as the response value. The antioxidant activity of total tanshinone in vitro was evaluated by oxygen free radical scavenging experiment. In order to provide a theoretical reference for the processing of *S. Miltiorrhizae*, the extraction of tanshinones and the evaluation of antioxidation.

2. Instruments and Materials

2.1 Main instruments Ultrasonic cleaning instrument (YQ-1000A, Shanghai Yijing Ultrasonic Instrument Co., Ltd.) ; digital constant temperature water bath (HH-2, Changzhou Huapuda Teaching Instrument Co., Ltd.) ; electric blast drying oven (101-3AB, Tianjin Taisite Instrument Co., Ltd.) ; UV-visible spectrophotometer (TU-1810, Beijing Purkinje General Instrument Co., Ltd.) ; high-speed multifunctional grinder (2000Y purchased from Wuyi Haina Electrical Appliance Co., Ltd.). ***2.2 Reagents*** Tanshinone IIA, batch number : AF21020351, (content ≥ 98.0 %), purchased from Chengdu Efa Biotechnology Co., Ltd. ; ethanol, batch number : 202110228, (purity ≥ 99.0 %), sodium nitrite, batch number : 20210112, (purity ≥ 99.0 %), sodium hydroxide, batch number : 20170302, (purity ≥ 96.0 %), were purchased from Chengdu Jinshan Chemical Reagent Co., Ltd. ; methanol, batch number : 20230629, (purity ≥ 99.0 %), aluminum nitrate, batch number : 20160416, (purity ≥ 99.0 %), purchased from Tianjin Komiou Chemical Reagent Co., Ltd. ; DPPH batch number : S28J12M138964, (purity : 98 %), purchased from Yuanye Biotechnology Co., Ltd. ; ferrous sulfate, batch number : 2021050186, potassium persulfate 2018080166, purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd. ; ABTS, lot number : C14582012 (purity ≥ 98 %), purchased from McLean Biotechnology Co., Ltd. ; H_2O_2, batch number : 20221124, purchased from Jiangxi Ganshan Coral Disinfection Products Co., Ltd. ; 1 × PBS buffer. ***2.3 Materials*** *S. miltiorrhiza* samples were collected from Magu Town, Hezhang County, Guizhou Province, and identified as the roots of *S. miltiorrhiza* by Professor Li Wei, Department of Traditional Chinese Medicine Processing, Guizhou University of Traditional Chinese Medicine. The roots of the collected Radix *S. miltiorrhiza* were placed indoors, dried, allowed to dehydrate naturally, and the attached soil and impurities were removed. They were dried in a blast oven at 60℃ and crushed through a No.4 sieve (65 mesh).

3. Methods and results

3.1 Response surface method to optimize the extraction process of total tanshinone of S.miltiorrhiza

3.1.1 Preparation of test solution : 0.1000 g dried powder sample of *S. miltiorrhiza* was accurately weighed and placed in a 150 mL conical flask. 45 mL of 80 % ethanol solution was added, ultrasounded for 62 min at 53 Hz, and the weight was added. The filtrate was collected as the test solution[14].

3.1.2 Drawing of standard curve : 2.0 mg of tanshinone IIA reference substance was weighed accurately, placed in 10 mL volume, dissolved in 80 % ethanol and diluted to scale to obtain a reference substance with a concentration of 0.2 mol / L for later use. The maximum absorption wavelength is 270 nm at the wavelength of 200-400 nm by UV spectrophotometer [15]. Accurately measure the reference solution 0,0.2,0.4,0.6,0.8,1.0 mL in a 10 mL volumetric flask, add 1 mL 5 % sodium nitrite solution in turn, immediately add 1 mL 10 % aluminum nitrate solution, shake well, and stand for 10 min. 5 mL 1 mol / L NaOH solution was added and diluted to the scale. After standing for 10 min, the absorbance was measured at 270 nm. The standard curve was established with tanshinone IIA concentration as abscissa and absorbance as ordinate. The standard curve equation was $y = 49.486\ x + 0.0416$, r = 0.9993.

3.1.3 Methodological test

Precision test : 3 mL of tanshinone IIA reference solution was taken, and the absorbance was measured at 270 nm after color development according to the conditions under ' 2.1.2 '. The results are shown in Table 1.

Table 1. Precision test results（n=10）

number	absorbance （A）	extraction rate （%）	mean value （%）	*RSD* （%）
1	0.512	17.489		
2	0.507	17.321		
3	0.516	17.623	17.515	1.008
4	0.509	17.388		
5	0.520	17.757		

The RSD of the extraction rate was calculated to be 1.008 % < 3 %, indicating that the instrument had good precision.

Repeatability test : 0.1000 g of *S. miltiorrhiza* sample powder was taken, accurately weighed, and the sample was prepared according to ' 2.1.1 '. After color development according to the conditions under ' 2.1.2 ', the absorbance value was measured at 270 nm. The results are shown in Table 2.

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Table 2. Repeated experimental results（n=10）

number	Sample weight（g）	absorbance（A）	extraction rate（%）	Average content（%）	*RSD*（%）
1	0.1096	0.505	17.254		
2	0.1023	0.511	17.455		
3	0.1021	0.513	17.522		
4	0.1071	0.502	17.153	17.416	1.016
5	0.1039	0.516	17.623		
6	0.1056	0.512	17.489		

The RSD of the extraction rate was calculated to be 1.016 % < 3 %, indicating that the experimental repeatability was good.

Stability test : 0.1000 g of *S. miltiorrhiza* sample powder was taken, accurately weighed, and the solution was prepared according to ' 2.1.1 '. After color development according to ' 2.1.2 ', the absorbance of the sample was measured once according to the time interval in the following table. It was concluded that the sample was stable within 6 h, and the results are shown in Table 3.

Table 3. Experimental results of stability（n=10）

number	Times (min)	absorbance（A）	extraction rate（%）	average content（%）	*RSD*（%）
1	20	0.483	16.516		
2	40	0.486	16.617		
3	60	0.487	16.650		
4	90	0.485	16.583		
5	120	0.484	16.550	16.449	0.907
6	150	0.479	16.382		
7	180	0.478	16.348		
8	240	0.476	16.281		
9	300	0.476	16.281		
10	360	0.476	16.281		

The extraction rate RSD was 0.907 % < 3 %, indicating that the sample had good stability.

Sample recovery test : 0.0500 g of *S. miltiorrhiza* sample powder was accurately weighed, and the test solution was prepared by adding keton IIA at a ratio of 1 : 1. After color development under ' 2.1.2 ', the absorbance value was measured at 270 nm, and the recovery rate was calculated. The results are shown in Table 4. The formula is as follows : [(measured amount-sample amount) / addition amount] × 100 %

Table 4. Experimental results of recovery rate（n=10）

number	Sample weight（g）	content of samples（mg）	Add the amount of reference substance（mg）	Measured content（mg）	recovery rate（%）	average content（%）	RSD（%）
1	0.0503	12.16	11.52	23.52	98.69		
2	0.0503	12.09	11.52	23.52	99.28		
3	0.0506	12.12	11.52	23.49	98.69	97.82	1.25
4	0.0504	12.22	11.52	23.36	96.65		
5	0.0502	12.29	11.52	23.39	96.36		
6	0.0503	12.22	11.52	23.42	97.24		

The calculated RSD was 1.25 % < 3 %, indicating that the sample recovery test error was small, the result was reliable, and the recovery rate of the method was good.

3.2 Determination of total tanshinone content

The 0.5 mL test solution was placed in a 10 mL volumetric flask, and the absorbance was detected at 270 nm to calculate the total tanshinone content. Total tanshinone content (%) = C × dilution multiple × V / m × 100 %, where C (mg / mL) : The total tanshinone concentration was calculated by the regression equation ; v (mL) : extract volume ; m (g) : sample quality.

3.3 Single factor tests

The three factors of extraction solvent concentration, solvent dosage and extraction time were taken as independent variables, and the extraction rate of total tanshinone of *S. miltiorrhiza* was taken as the dependent variable. Two factors were fixed respectively, and a single factor experiment was carried out to investigate the effect of a certain factor on the extraction rate of total tanshinone of *S. miltiorrhiza*.

3.4 Response surface optimization extraction conditions

According to the results of single factor test, the solvent dosage (A), solvent concentration (B) and extraction time (C) were used to establish a three-factor three-level central combination test design to optimize the extraction conditions of total tanshinone from *S. miltiorrhiza*, as shown in Table 5.

Table 2. Repeated experimental results（n=10）

Table 5. Response surface factor level design table

factors	levels		
	-1	0	1
A solvent volume（mL）	35	45	55
B solvent strength（%）	70	80	90
C extraction time（min）	45	60	75

3.5 Antioxidant experiment in vitro

3.5.1 $ABTS^+$ free radical scavenging test : The $ABTS^+$ solution was diluted with phosphate buffer (PBS) until the absorbance at 734 nm reached 0.698-0.702, and the $ABTS^+$ working solution was obtained. 2 mL of test solution with concentrations of 0.0128, 0.0102, 0.0085, 0.0073 and 0.0064 mg / mL was added to 5 test tubes in turn, and then 4 mL of $ABTS^+$working solution was added, fully shaken up, and colored in dark at 25 °C for 6 min. Subsequently, the absorbance was measured at a wavelength of 734 nm, and the $ABTS^+$ scavenging rate of *S. miltiorrhiza* samples was calculated. $ABTS^+$ clearance rate (%) = [1- (A1-A2) / A0] × 100 % (A1 experimental group, A2 control group, A0 blank group).

3.5.2 DPPH free radical scavenging test : 2 mL of test solution with a concentration of 0.0128, 0.0102, 0.0085, 0.0073 and 0.0064 mg / mL was added to 5 test tubes in turn, and then 2 mL of DPPH solution was added. Shake well and color for 30 min in the dark. Another 2 test tubes were replaced with distilled water instead of DPPH solution as a control group, and 2 mL of DPPH solution was taken, and the same volume of 80 % ethanol was added to it as a blank control. Shake well, avoid light for 30 min, calculate the clearance rate. DPPH clearance rate (%) =

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[1- (A1-A2) / A0] × 100 % (A1 experimental group, A2 control group, A0 blank group).

3.5.3 ·OH-free radical scavenging test : 2 mL of test solution with concentrations of 0.0128, 0.0102, 0.0085, 0.0073 and 0.0064 mg / mL was added to 5 test tubes in turn, and 2 mL of 9 mg / mL $FeSO_4$ solution and 2 mL of H_2O_2 solution were added to each test tube in turn for 10 min. Then 2 mL of 10 mg / mL salicylic acid solution was added to each test tube and shaken well. Another 2 test tubes were used to replace salicylic acid solution with distilled water as blank control, and distilled water was used to replace the sample solution to be tested as blank solution. All test tubes were placed in a constant temperature environment of 37 °C for 20 min to complete the color development process. The absorbance was measured at the wavelength of 510 nm and the clearance rate was calculated. ·OH-radical scavenging rate (%) = [1- (A1-A2) / A0] × 100 % (A1 experimental group, A2 control group, A0 blank group).

3.5.4 $\cdot O_2$-scavenging test : 1 mL of 0.0128, 0.0102, 0.0085, 0.0073, 0.0064 mg / mL test solution was added to 5 test tubes in turn, and pH8.2 Tris-HCI solution 4.5 mL was added. After 20 min of water bath balance at 25 °C, 0.4 mL of 7 mg / mL pyrogallol solution was added to accurately react for 4 min (2 drops of hydrochloric acid were used to terminate the reaction), and 2 test tubes were replaced with distilled water instead of pyrogallol solution solution as blank control. Distilled water was used instead of the test solution as the blank solution. After the above operation, the absorbance was measured at 325 nm wavelength and the clearance rate was calculated. $\cdot O_2$-clearance rate (%) = [1- (A1-A2) / A0] × 100 % (A1 experimental group, A2 control group, A0 blank group).

3.6 Single factor test results

3.6.1 Extraction solvent concentration selection

The extraction rate of total tanshinone in *S. miltiorrhiza* increased with the increase of extraction solvent concentration. When the solvent concentration reached 80 %, the extraction rate of total tanshinone reached a peak of 20.026 %, and then decreased. The reason may be that with the increase of ethanol concentration, the total tanshinone compounds reach saturation. At this time, the dissolution of impurities and other components may increase, forming a competitive relationship with the total tanshinone compounds, resulting in a decrease in the extraction rate of total tanshinone. The results are shown in Fig.1. Therefore, 70 ~ 90 % ethanol solution concentration was selected for Box-Behnken test design-response surface method to optimize the extraction process of total tanshinone from *S. miltiorrhiza.*

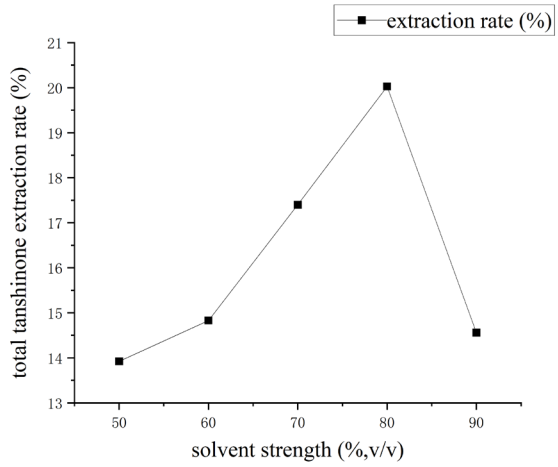


Fig. 1 Effect of extraction solvent concentration on the extraction rate of total tanshinone

3.6.2 Selection of liquid-solid ratio

The extraction rate of total tanshinone reached a maximum of 18.361 % when the liquid-solid ratio was 1 : 450, and then gradually decreased with the increase of solvent volume. The results are shown in Fig. 2. The reason may be that with the increase of the amount of solvent, the dissolution of total tanshinone has reached saturation, and the increase of the amount of solvent will increase the dissolution rate of some impurities, forming a competitive relationship with the total tanshinone compounds, resulting in a decrease in the extraction rate of total tanshinone. Therefore, the liquid-to-material ratios of 1 : 350, 1 : 450 and 1 : 550 were selected for Box-Behnken experimental design.

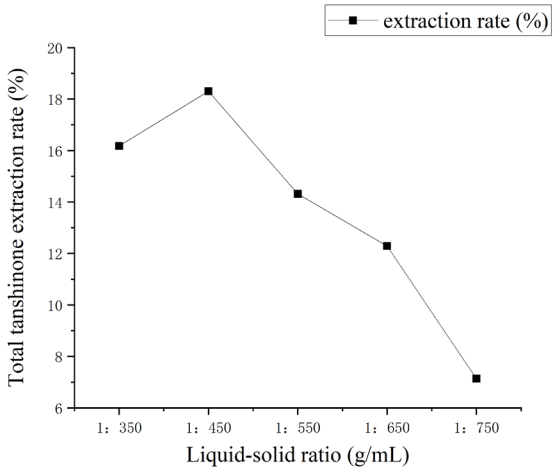


Fig. 2 Effect of liquid-solid ratio on the extraction rate of total tanshinon

3.6.3 Selection of extraction time

As shown in Fig. 3, the extraction rate of total tanshinone in *S. miltiorrhiza* increased first and then decreased with the extraction time, reaching a maximum of 23.033 % at 60 min. The reason may be that during the ultrasonic extraction process, total tanshinone has been competing with impurity components to dissolve until the total

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tanshinone has reached saturation, but some impurity components continue to dissolve with time, resulting in the dissolution of total tanshinone. Therefore, the extraction time of 45 min, 60 min and 75 min were selected for Box-Behnken experimental design.

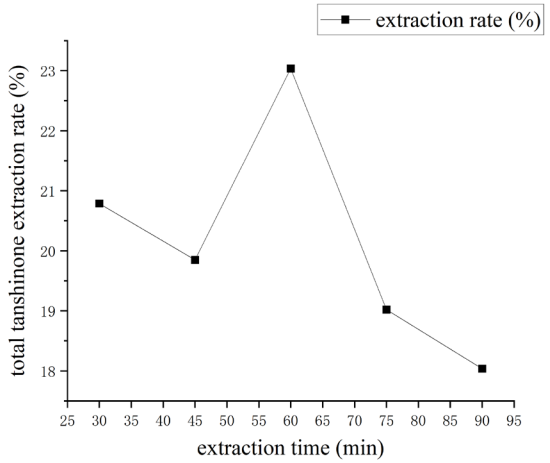


Fig . 3 Effect of extraction time on the extraction rate of total tanshinone

3.7 Optimization of extraction process by response surface methodology

3.7.1 Analysis scheme and test results : Based on the experimental level design table, the software is used to analyze the relevant parameters. Through the in-depth analysis of these data, the feasibility and effectiveness of the scheme are verified, as shown in Table 6.

Table 6. Design Table of Response Surface Factor Levels

Numbers	Factor A : Solvent dosage (mL)	Factor B : Solvent concentration (%)	Factor C : Extraction time (min)	Response value : total tanshinone extraction rate (%)
1	35	70	60	17.981
2	55	70	60	17.936
3	35	90	60	17.324
4	55	90	60	17.923
5	35	80	45	18.147
6	55	80	45	18.236
7	35	80	75	17.923
8	55	80	75	19.031
9	45	70	45	17.925
10	45	90	45	17.571
11	45	70	75	17.912
12	45	90	75	17.934
13	45	80	60	20.026
14	45	80	60	20.304
15	45	80	60	20.004
16	45	80	60	20.206
17	45	80	60	20.113

3.7.2 Fitting regression analysis : Fitting regression analysis was performed on each factor level, and the analysis results are shown in Table 7.

Table 7. The results of variance analysis of regression equation

Source of variance	mean square sum	degree of freedom	mean square	*F ratio*	*Pratio*	
models	17.960	9	2.000	103.290	<0.0001	significant
A-solvent dosage	0.3830	1	0.383	19.820	0.0030	
B - Solvent concentration	0.1256	1	0.1256	6.5000	0.0381	
C-ultrasonic extraction time	0.1062	1	0.1062	5.5000	0.0515	
AB	0.1035	1	0.1035	5.36	0.0538	
AC	0.2601	1	0.2601	13.46	0.008	
BC	0.0351	1	0.0351	1.82	0.2196	
A_2	3.57	1	3.57	184.62	< 0.0001	
B_2	8.48	1	8.48	438.9	< 0.0001	
C_2	3.23	1	3.23	167.26	< 0.0001	
residual error	0.1352	7	0.0193			
misfit term	0.0722	3	0.0241	1.53	0.3372	non-significant
pure error	0.063	4	0.0158			

According to the statistical analysis results of Table 7, the constructed design model is statistically significant (P <0.05), which is not significant, indicating that the model does not ignore any important variables, and the model as a whole fits well with the actual experiment. Therefore, the model is suitable for predicting the optimal process parameters for the extraction rate of total tanshinone in the extraction process of *S. miltiorrhiza,* and has high prediction accuracy and practicability. There was a certain interaction between the factors, and A, A_2, B_2 and C_2 were extremely significant ($P < 0.01$). B was significant ($P <$ 0.05). Within the scope of the experiment, the influence of three factors on the extraction rate of total tanshinone in *S. miltiorrhiza* was different. The primary and secondary effects on the extraction rate were solvent dosage (A) > solvent concentration (B) > ultrasonic extraction time (C).

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In addition, when considering the interaction between factors, the primary and secondary effects of interaction on the extraction rate were AC > AB > BC. It shows that there is a significant impact on the comprehensive score of the index components. The regression equation is as follows :

$Y = 20.13 + 0.2188A - 0.1253B + 0.11$

3.7.3 Process optimization and verification test

Design-Expert 13 software was used to draw the interaction contour and 3D surface respectively, as shown in Fig.4 and Fig.5.

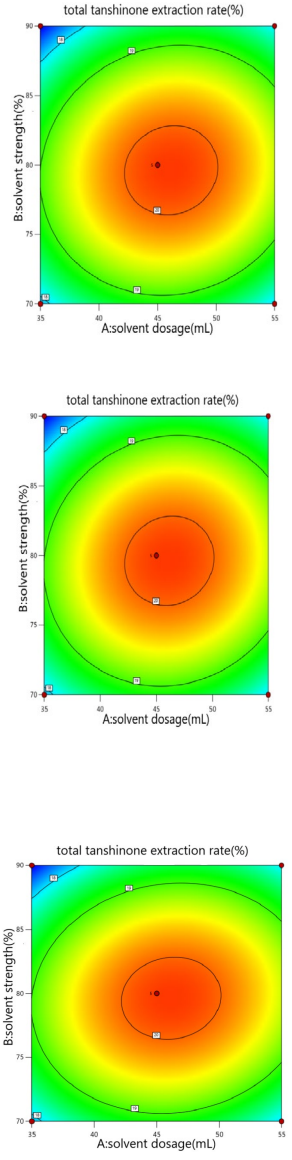


Fig. 4 Interaction contour map

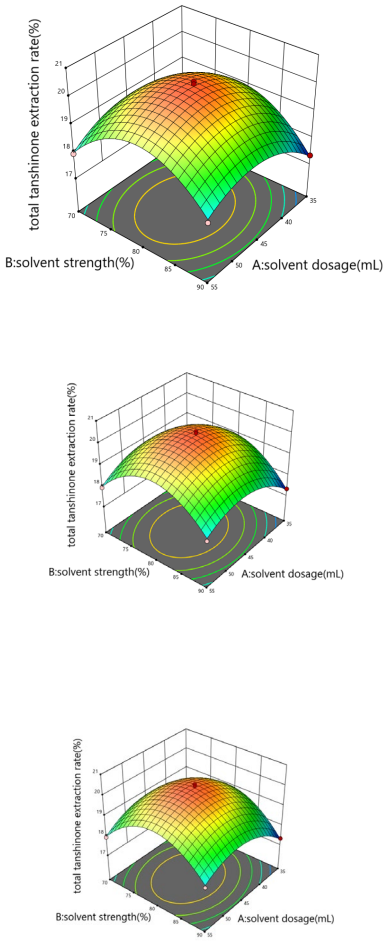


Fig. 5 Interaction 3D surface diagram

From the analysis of Fig.4, it is concluded that the three sets of response surface contour maps drawn by the fitting model are all oval, indicating that the interaction between the respective variables is strong. According to the analysis of fig. 5, the slope of the response surface of the three groups was steep, indicating that the solvent dosage, solvent concentration and extraction time had a significant effect on the extraction rate of total tanshinone. The optimal extraction process parameters of total tanshinones in *S. miltiorrhiza* were analyzed by Design-Expert 13 software. The solvent dosage was 46.27 mL, 79.7 % ethanol solution, and the extraction time was 61.24 min. In order to meet the experimental operation, these parameters were fine-tuned to integers that are easy to operate, that is, 80 % ethanol concentration, 46 mL ethanol dosage, and 62 min extraction time. According to this adjustment scheme, the total tanshinone content was determined, and the total tanshinone content was 198.41 mg / g.

3.8 Evaluation of antioxidant system in vitro

To verify the bioactivity and potential therapeutic relevance of tanshinones, after establishing the optimal extraction parameters, we carried out the evaluation on the antioxidant activity of the obtained tanshinones.

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3.8.1 $ABTS^+$ free radical scavenging test results : As shown in Fig.6, the scavenging efficiency of total tanshinone on $ABTS^+$ free radicals increased with the increase of concentration. The scavenging rate at the maximum concentration was 66.20 %, IC_{50} = 0.0049 mg / mL, and the total tanshinone scavenging rate was lower than that of the positive control group.

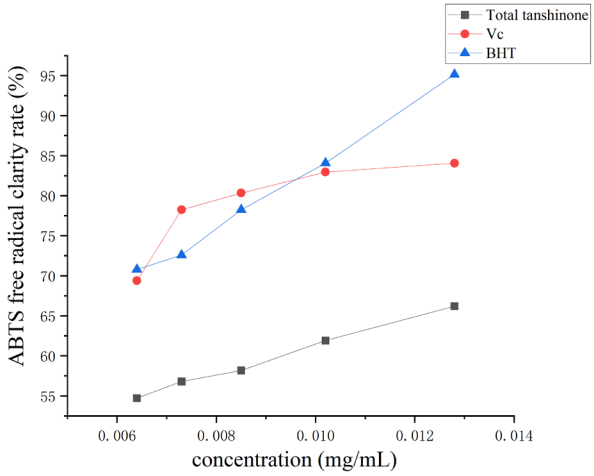


Fig. 6 ABTS free radical scavenging rate experiment

3.8.2 DPPH free radical scavenging test results : As shown in Fig.7, the DPPH free radical scavenging rate of total tanshinone increased with the increase of concentration, and the scavenging rate was 69.49 % at the maximum concentration, IC_{50} = 0.0082 mg / mL. The clearance rate was significantly lower than the positive control group.

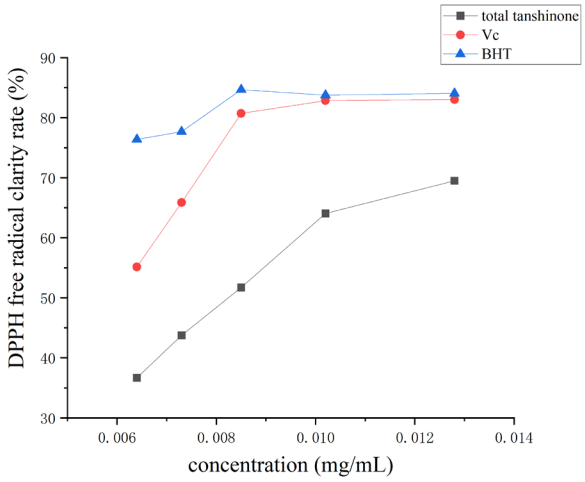


Fig. 7 DPPH free radical scavenging rate experiment

3.8.3 ·OH-free radical scavenging test results : As shown in Fig.8, with the increase of sample concentration, the scavenging rate of total tanshinone on hydroxyl radicals showed a gradual upward trend. At the maximum concentration, the clearance rate was 50.30 %, IC_{50} = 0.0130 mg / mL, which was significantly lower than that of the positive control group.

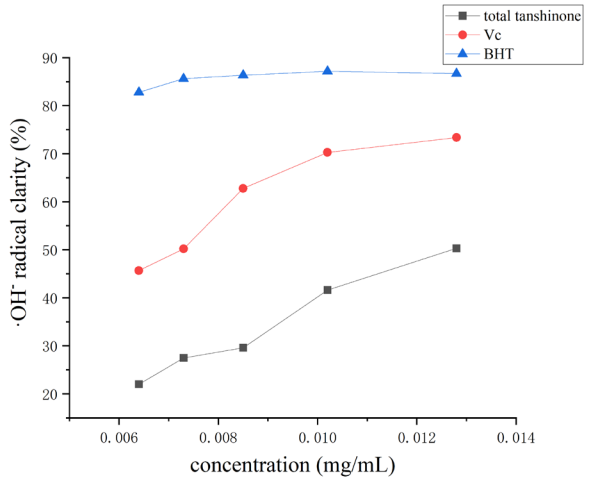


Fig. 8 ·OH free radical scavenging experiment

3.8.4 The results of superoxide anion scavenging test : As shown in fig. 9, the clearance rate of total tanshinone increased with the increase of concentration. When the concentration of total tanshinone reached 0.01 mg / mL, the clearance rate tended to be gentle with the increase of concentration due to the platform effect. The clearance rate at the maximum concentration was 65.10 %, IC_{50} = 0.0019 mg / mL. The clearance rate of total tanshinone group was lower than that of positive control group.

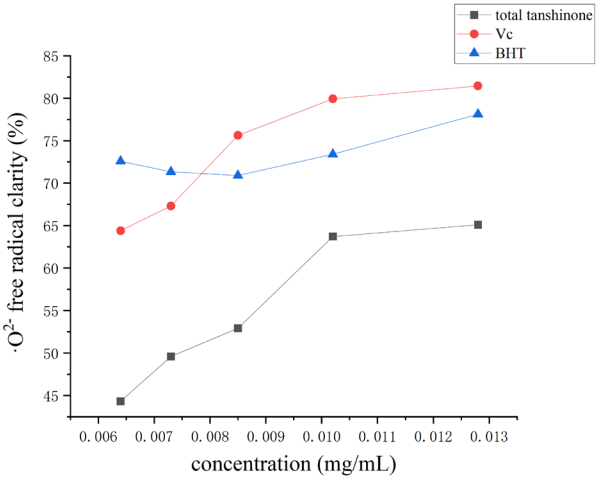


Fig. 9 Superoxide anion free radical scavenging experiment

4. Discussions

Two methods of ultrasonic extraction and reflux extraction were used to investigate the preparation of the test sample. The experimental results showed that the ultrasonic extraction effect was significantly better than the reflux extraction method, which was more efficient and cost-effective. After optimizing the extraction process by response surface method, the extraction rate of total tanshinone was significantly improved, and the total tanshinone content was 19.84 %. The Box-Behnken experimental design-response surface method has high prediction accuracy, simple operation process, low cost, more comprehensive analysis of influencing factors, and avoids excessive waste of medicinal materials resources

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[16]. It can be applied to the extraction process of total tanshinones.

The total tanshinones exhibited variable scavenging capacities across four antioxidant assays, demonstrating moderate efficacy against $ABTS^+$ (IC_{50} = 0.0049 mg/mL), DPPH (IC_{50} = 0.0082 mg/mL), hydroxyl (IC_{50} = 0.013 mg/mL), and superoxide anion (IC_{50} = 0.0019 mg/mL) radicals. While their activity was inferior to synthetic antioxidants (vitamin C and BHT), the results align with prior reports confirming the intrinsic radical-neutralising potential of Salvia miltiorrhiza extracts [11]. Critically, extraction parameters profoundly influenced both tanshinone yield and bioactivity: Ethanol concentration: An 80% ethanol concentration optimally balanced tanshinone solubility (lipophilic nature) and solvent polarity, maximising extraction efficiency (20.026% yield). Higher concentrations (>90%) reduced polarity, favouring co-extraction of non-target impurities that may interfere with antioxidant interactions [17]. Solid-to-liquid ratio: A 1:46 ratio achieved equilibrium between mass transfer efficiency and solvent economy, avoiding dilution effects that reduce radical-scavenging kinetics [18]. Ultrasonic duration: Prolonged extraction (>60 min) induced thermal degradation via cavitation-generated heat, diminishing tanshinone content (23.033% → 17.923%) and destabilising redox-active moieties like conjugated dienes [19]. Low-temperature ultrasonication (53 Hz, 60°C) preserved structural integrity, as evidenced by <3% RSD in stability tests (Table 3). This method minimised thermal decomposition—a known limitation of reflux extraction—while enhancing bioactive retention [18].

These findings underscore that parameter optimisation (80% ethanol, 1:46 ratio, 62 min) is essential to maximise both tanshinone yield (198.41 mg/g) and antioxidant fidelity, providing a robust framework for developing standardised *S. miltiorrhiza*-based nutraceuticals.

In summary, the Box-Behnken experimental design-response surface method is accurate and feasible to optimize the process parameters, which can be used for the extraction of total tanshinone from *S. miltiorrhiza*. *S. miltiorrhiza* total tanshinone has a certain antioxidant capacity in vitro and can be developed and utilized as a natural antioxidant. Therefore, the study on the antioxidant activity of total tanshinone has important potential value for the prevention and management of chronic diseases related to oxidative stress.

5. Conclusion

In conclusion, the Box-Behnken design-RSM effectively optimized the extraction of total tanshinones from Guizhou *S. miltiorrhiza*. Optimal conditions yielded a high content. In vitro antioxidant assays showed certain capacity, despite lower than controls. It's promising for oxidative stress diseases. Future in vivo and clinical studies are needed.

Research Involving Plants

The research did not involve species at risk of extinction.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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