

Optimizing the flash extraction process for total flavonoids from *Edgeworthia gardneri* (Wall) Meissn and antioxidant activity of the extracts

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Abstract

In order to efficiently extract natural medicines, response surface methodology (RSM) were used for the modeling and optimization of flash extraction of total flavonoids to determine the antioxidant activity of the extracts obtained from *Edgeworthia gardneri* (Wall). For the optimal combination of the comprehensive yield of total flavonoids (Y), the Box-Behnken design (BBD) was used to improve extract voltage (X_1), extraction time (X_2), ethanol concentration (X_3), and liquid-solid ratio (X_4). Based on the results of single factor test, the amount of total flavonoids extracted from *Edgeworthia gardneri* (Wall) Meissn was used as the evaluation index. The Box-Behnken experimental design was used to optimize the flash extraction process and to investigate the antioxidant activity in vitro. The results showed that the optimal extraction conditions were as follows: extraction voltage 150 V, flash extraction time 100 s, ethanol volume fraction 70%, solid-liquid ratio 1:30 mL/g, and total flavonoids extraction amount of 18.91 (mg/g). The total flavonoids of *Edgeworthia gardneri* (Wall) Meissn had significant scavenging ability against 2,2-diphenyl-1-picrylhydrazyl (DPPH), $O_2^{\cdot-}$ and OH free radicals, and had good antioxidant activity. Furthermore, the HPLC separation conditions of total flavonoids of *Edgeworthia gardneri* (Wall) were explored. Under the set HPLC conditions (Hanbon benatach C₁₈ 250 mm × 4.6 mm, 5 μm, A: acetonitrile-B: 0.2% formic acid aqueous solution, 0→10→50→65→85 min, 5%→10%→30%→45%→90% A gradient elution, 1 mL/min, 314 nm, Column temperature: 25 °C), the main components of total flavonoids of *Edgeworthia gardneri* (Wall) could achieve a good separation, which provide the separation conditions and basis for the identification of total flavonoids from *Edgeworthia gardneri* (Wall) Meissn.

1 Introduction

Edgeworthia gardneri (Wall) Meissn belongs to the genus of *Thymelaeaceae* Juss. is mainly distributed in eastern Tibet and northwestern to western Yunnan. Their flower buds are loose and spherical, covered with light green hairs, with strong fragrant smell and mild medicinal properties^[1]. According to the "Tibetan Medicine Health Picture" records, *Edgeworthia gardneri* (Wall) Meissn has a good prevention and therapeutic effects for diabetes^[2], coronary heart disease, hypertension, high blood fat and vascular inflammation^[3]. Modern pharmacological studies have shown that many compounds, such as flavonoids, coumarins, total polyphenols, polysaccharides, volatile oil and trace elements^[4-7] have hypoglycemic^[8, 9], lipid-lowering^[10], immunity-enhancing, anti-oxidation and anti-tumor effects^[11] and other physiological activities^[12].

Flash extraction is a new technique for crushing the soft and hard tissue of plants^[13-15]. At room temperature, plant roots, stems, leaves, fruits and other materials can be broken into fine particles within a few seconds with a flash extraction device, which will not be damaged by heat and can retain the effective ingredients of the plant to the maximum extent^[16]. It also has the advantages of tool wear resistance, low solvent consumption, short extraction time, high efficiency and low energy consumption.

The Response surface method (RSM) is an optimization method proposed by Box and Wilson in 1951 that integrates experimental design and mathematical modeling. It is commonly used to understand the performance of complex systems and to optimize any type of complex extraction process. RSM has the advantages of less test times, high accuracy, good prediction performance and optimal response. Using this method, a response surface can be obtained by visual inspection of the three-dimensional response surface and thus the interaction of various factors on the target response can be understood, thereby optimizing chemical and physical processes. It is widely used for the extraction of plants, especially herbaceous plants^[17–21].

At present, there are few reports on the study of the chemical constituents of flowers, and are mainly limited to supercritical CO₂ extraction, ethanol extraction, steam distillation, and other traditional methods. There are no reports on the antioxidant properties of flavonoids obtained by flash extraction. Therefore, based on the results of controlling single-factor variable experiment, the RSM was used to optimize the flash extraction process and study the antioxidant activity of the extract, to obtain the best extraction process and a reference for antioxidant activity.

2 Materials And Methods

2.1 Materials

Edgeworthia gardneri (Wall) Meissn was obtained from Qamdo (Xizang, China), and identified by the Jilin Academy of Traditional Chinese Medicine. DPPH and H₂O₂ were obtained from Sigma-Aldrich (St. Louis, MO, USA), HPLC-grade acetonitrile was purchased from Thermo Fisher Scientific (Martha's Vineyard, USA), Unless stated otherwise, all reagents and chemicals were of analytic grade.

2.2 Preparation of Samples

Edgeworthia gardneri (Wall) Meissn was milled using a high-speed multifunctional pulverizer (KC-500, Beijing, China) before being sieved using a 20-mesh screen. The resulting powder was stored at room temperature. The 70% ethanol was measured and 10g of pretreated powder was accurately weighed. The powder was mixed evenly and placed in the shear head of the flash extractor until it was completely immersed. The liquid was centrifuged at 4000 r/min for 30 min to obtain the sample for use. RSM was used to optimize the extraction conditions.

2.3 Test solution

Ethanol (70%) was measured, and 10 g of the pretreated powder was accurately weighed. The powder was evenly mixed and placed in the shear head of the flash extractor until it was completely immersed. The liquid was centrifuged at 4000 r/min for 30 min to obtain the sample for use. RSM was used to optimize the extraction conditions.

2.4 Standard solution

The rutin standard (25 mg) was accurately weighed in a 50 mL volumetric flask, dissolved in anhydrous ethanol at a constant volume, and shaken well to obtain the reference solution.

2.5 Standard curve

Different volumes of reference solutions (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL) were accurately absorbed and placed in a 10 mL volumetric bottle. A total of 5 mL of distilled water was added to each bottle, and 0.6 mL of 5% NaNO₂ was added. After shaking well, the solution was allowed to stand for 6 min, and 0.6 mL of 10% AgNO₃ was added. The bottle was shaken well and allowed to stand for 6 min, 3 mL of 5% NaOH was added, distilled water was added to scale, and the bottle was shaken well and allowed to stand for 20 min. The standard curve for rutin was obtained by measuring the absorbance A at 510 nm, with the absorbance as the abscissa and the rutin concentration (mg/mL) as the ordinate.

2.6 Total flavonoids Extracted from *Edgeworthia gardneri* (Wall) Meissn

The test solution (0.2 mL) was accurately measured and placed in a 10 mL volumetric flask, and the absorbance was determined according to the method described in section 3.4. The total flavonoid concentration was calculated according to the standard curve, and the amount of extracted flavonoids was calculated according to the following formula:

$$W = \frac{\rho \times V}{m}$$

2.7 Single-Factor Experiment

Based on a large number of literature reviews and summaries, the extraction voltage (60, 90, 120, 150, and 180 V), extraction time (60, 80, 100, 120, and 140 s), ethanol concentrations (50, 60, 70, 80, and 90%), and liquid–solid ratio (1:10, 1:20, 1:30, 1:40, and 1:50 mL/g) were selected for single-factor experiments.

2.8 BBD for Extraction Optimization

The Design-Expert software (Minneapolis, MN, USA) was used for experimental design, the RSM model regression analysis, and RSM model optimization. Based on the single-factor experiment results, the BBD was developed with four variables and three levels. The four independent variables were set at three levels (-1, 0, 1): X_1 (120, 150, and 180 V), X_2 (80, 100, and 120 s), X_3 (60, 70, and 80%), X_4 (1:20, 1:30, and 1:40 mL/g). The details are presented in Table 1. The evaluation value of the total flavonoid extraction yield was chosen as the dependent variable. In the extraction optimization experiments, 29 groups (10 g for *Edgeworthia gardneri* (Wall) Meissn, n = 3) were selected, and the BBD matrix of the 29 experimental standard runs was designed using the software.

Table 1
Factors and levels in Box–Behnken design.

Factors				
levels	X_1	X_2	X_3	X_4
	extract voltage(V)	extraction time(S)	ethanol concentration(%)	liquid–solid ratio(mL/g)
-1	120	80	60	1: 20
0	150	100	70	1: 30
1	180	120	80	1: 40

2.9 Determination of Antioxidant Activity

According to the response surface analysis, optimal process parameters were obtained for flash extraction. The extract solution of total flavonoids obtained was filtered and the volume was reduced to 10 mL after decompression, and 1 mL of distilled water was added to a constant volume of a 100 mL volumetric flask. The mass concentration of the total flavonoids in the sample solution was determined according to the method described in section 3.4. A series of solutions with different concentrations were prepared to analyze the scavenging ability of DPPH, $O_2^{\cdot-}$, and OH radicals.

2.9.1 DPPH·Free Radical Scavenging Ability

The sample and Vc were prepared in solutions of different concentrations. A total of 2 mL of the sample solution was added to 2 mL of 0.2 mg/mL DPPH·, shaken well, and left to stand for 30 min. Anhydrous ethanol was used as the reference solution, and the absorbance A_1 was measured at 517 nm. A total of 2 mL of anhydrous ethanol was added to 2 mL of 0.2 mg/mL DPPH· solution, mixed well, and the absorbance value A_0 was determined at 517 nm. The absorbance value of 2 mL sample solution and 2 mL anhydrous ethanol mixture solution was determined as A_2 . Taking vitamin C as the control, the clearance rate of DPPH· was calculated, and the average value was calculated three times. The calculation formula is as follows:

$$S = \left[1 - \frac{(A_1 - A_2)}{A_0} \right] \times 100\%$$

2.9.2 $O_2^{\cdot-}$ Free Radical Scavenging Ability

The pyrogallol autoxidation method was used. Five 0.5 mL Tris-HCl buffer solutions with pH 8 were prepared, and 1 mL sample solution of different concentrations were added, shaken, and allowed to stand for 20 min. Then, 0.5 mL of 25 mmol·L⁻¹ pyrogallol was added, mixed, and placed in a water bath at 25°C for 15 min. One drop of concentrated hydrochloric acid was added to stop the reaction. The

absorbance was measured at 320 nm and the mean absorbance value A_3 was calculated using three parallel measurements. A volume of 0.5 mL ultrapure water was used to replace the pyrogallol solution, and the absorbance was determined as A_4 . Ultrapure water was used to replace the sample solution, and the above process was repeated to measure the absorbance as A_5 . Vitamin C was used as the control to calculate the scavenging rate of O^{2-} free radicals, which was replicated three times and averaged using the following formula:

$$S = \left[\frac{1 - (A_3 - A_4)}{A_5} \right] \times 100\%$$

2.9.3 OH Free Radical Scavenging Ability

Using a fixed reaction time method, 0.5 mL of 0.1% H_2O_2 , 0.5 mL of 7.5 mmol·L⁻¹ $FeSO_4$, 0.5 mL of 7.5 mmol·L *o*-Phenanthroline, 0.5 mL of phosphate buffer (pH 7.4), and sample solutions of different mass concentrations were added, and absorbance value A_6 was determined at 509 nm. The absorbance value A_7 was determined using 95% ethanol solution instead of phosphate buffer as the reference solution, and the absorbance value A_8 was determined using 95% ethanol solution instead of the sample solution. Taking vitamin C as the control, the clearance rate of hydroxyl radicals was calculated from three replicates and averaged, and the calculation formula is as follows:

$$S = \left[\frac{1 - (A_6 - A_7)}{A_8} \right] \times 100\%$$

2.10 HPLC conditions

HPLC analysis was carried out using a Shimadzu 20 A HPLC with UV detector, equipped with Hanbon benatach C₁₈ (250 mm × 4.6 mm, 5 μm), mobile phase was acetonitrile (A): acetonitrile-0.2% formic acid aqueous solution (B) (0→10→50→65→85 min, 5%→10%→30%→45%→90% A) gradient elution. The flow rate was 1 mL/min. Detection wavelength: 314nm.

3. Results And Discussion

3.1 Drawing of standard curves

Standard curves were drawn with rutin mass concentration as the transverse (X) and absorbance (Y) as the ordinate, and the linear regression equation was

$$Y = 11.99X + 0.041, R^2 = 99.33\%$$

The results showed a good linear relationship between the absorbance and rutin reference solution in the range 0.01–0.08 g/L.

3.2 Single-Factor Experiments

3.2.1 Effect of Extract Voltage

The extraction voltages were set at 60, 90, 120, 150, and 180 V. Other experimental conditions were as follows: extraction time 120 s, liquid–solid ratio 1:30 mL/g, and 70% ethanol was used as the solvent. The effect of extraction voltage on the response value is shown in Fig. 1a. With an increase in the extraction voltage, the response value increased. After the extraction voltage was maintained at 150 V, the response value reached its maximum, followed by a declining trend. Thus, the extraction voltage was set to 120, 150, and 180 V.

3.2.2 Effect of Extraction Time

The experiments in this study were conducted at 40, 60, 80, 100, 120, and 140 s to investigate the influence of extraction time. The extract voltage was set to 150V, the liquid-solid ratio was 30 mL/g, and the ethanol concentration was 70%. All other conditions were equal. The effect of extraction time on the response value is shown in Fig. 1b. When the flash extraction time reached 100 s, the total flavonoids extraction reached the maximum value. When extracting time arrived after 100 s, along with the increasing, the amount of extraction of total flavonoids began to decrease. Possible reason is that the cell membrane of *Edgeworthia gardneri* (Wall) Meissn has not destroyed completely in a certain range of extracting time. With the increase of extraction time, the total flavonoids in *Edgeworthia gardneri* (Wall) Meissn may be destroyed, and other components in cells will flow out, which will increase the impurities in the solution and have a certain influence on the extraction of total flavonoids in *Edgeworthia gardneri* (Wall) Meissn. Therefore, the extraction time was set to 80, 100, and 120 s.

3.2.3 Effect of Ethanol Concentration

Figure 1c shows that when the volume fraction of ethanol is lower than 70%, the extraction amount of total flavonoids increases gradually with the increase of the volume fraction of ethanol. When the volume fraction of ethanol reaches 70%, the extraction amount of total flavonoids reaches the maximum. When the volume fraction of ethanol was increased, the extraction amount of total flavonoids decreased. According to the similarity compatibility principle, when the polarity of extraction agent decreases and the polarity of flavonoids is larger, the solubility of flavonoids also decreases correspondingly, thus reducing the total extraction amount of flavonoids. Therefore, the volume fraction of ethanol selected as the investigation factor in this experiment was 60%, 70%, and 80%.

3.2.4 Effect of Liquid-Solid Ratio

As shown in Fig. 1d, when the liquid-solid ratio was 1:30, the amount of total flavonoids extracted was the maximum. We believe that the extraction of total flavonoids from *Edgeworthia gardneri* (Wall) was not complete at the initial stage of the experiments, and the increase in the liquid-solid ratio might be conducive to improving the molecular diffusion rate; therefore, the amount of total flavonoids extracted

would continue to increase further. When the liquid-solid ratio reached and exceeded 1:30, the difference in solid to liquid concentration was not obvious, and thus the amount of total flavonoids extracted decreased. Therefore, the optimal liquid-solid ratio was 1:20, 1:30, and 1:40.

Finally, four factors and three levels were determined as follows (Table 1): the extract voltage was 120, 150, and 180 V, extraction time was 80, 100, and 120 s ; liquid-solid ratio was 1:20, 1:30, and 1:40 mL/g; the ethanol concentration were 60, 70, 80%, as shown in Table 1.

3.3 BBD Method Optimization of Extraction Conditions

The ranges for the four independent extraction variables, namely, X_1 -extract voltage, X_2 -extraction time, X_3 -ethanol concentration, X_4 -liquid–solid ratio were at three levels (+ 1, 0, -1) for extraction parameter optimization by the BBD method. In the extraction optimization experiment, 29 groups (8 g for *Edgeworthia gardneri* (Wall) Meissn, n = 3) were selected, and the BBD matrix of 29 experimental standard runs was designed by the software, as shown in Table 2.

Table 2
Box-Behnken design (BBD) and results

No.	X_1	X_2	X_3	X_4	Y
1	-1	-1	0	0	12.52
2	1	-1	0	0	15.71
3	-1	1	0	0	6.01
4	1	1	0	0	13.25
5	0	0	-1	-1	9.57
6	0	0	1	-1	9.48
7	0	0	-1	1	13.07
8	0	0	1	1	5.23
9	-1	0	0	-1	9.55
10	1	0	0	-1	12.21
11	-1	0	0	1	5.25
12	1	0	0	1	11.75
13	0	-1	-1	0	14.89
14	0	1	-1	0	11.92
15	0	-1	1	0	11.75
16	0	1	1	0	10.46
17	-1	0	-1	0	8.27
18	1	0	-1	0	14.54
19	-1	0	1	0	5.02
20	1	0	1	0	11.38
21	0	-1	0	-1	15.11
22	0	1	0	-1	12.21
23	0	-1	0	1	12.22
24	0	1	0	1	9.84
25	0	0	0	0	17.63
26	0	0	0	0	17.78

No.	X_1	X_2	X_3	X_4	Y
27	0	0	0	0	18.91
28	0	0	0	0	17.61
29	0	0	0	0	17.88

The data of the value of total flavonoids extraction yield obtained from the 29 experimental runs are shown in Table, analyzed by ANOVA and R^2 interpretation at 95% confidence level ($p < 0.05$) (Table 2) and fitted to a second-order polynomial model ($Y(\text{mg/g})$ -the value of total flavonoids extraction yield, X_1 -extract voltage, X_2 -extraction time, X_3 -ethanol concentration, X_4 -liquid–solid ratio) with insignificant items being removed:

3.4 Model Fitting

3.4.1 RSM Modeling

The experimental data were fitted using a second-order polynomial model^[22]. The generalized second-order polynomial model used in the response surface analysis was as follows:

$$Y = \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1, j \geq 1}^4 \beta_{ij} X_i X_j + \beta_0$$

where Y represents the value of total flavonoids extraction yield, X_i or X_j ($i, j = 1, 2, 3, 4$) are the independent variables: X_1 -Extract voltage, X_2 -extraction time, X_3 -Ethanol concentration, X_4 -Liquid-solid ratio. β_0 is the constant coefficient. β_i , β_{ii} , β_{ij} are constant regression coefficients of the model.

Based on the BBD, 29 experiments were carried out to study the effect of these four factors on the total flavonoid yield. The results of the four independent variables and their levels, as well as the experimental and predictive dependent variables based on the selected BBD, are shown in Table 2. Based on the results, the comprehensive evaluation value of the total flavonoid yield from *Edgeworthia gardneri* (Wall) Meissn extracts ranged from 5.02 to 18.91. The extraction yield of the comprehensive evaluation value can be expressed using the following equation:

$$Y = 17.96 + 2.69X_1 - 1.54X_2 - 1.58X_3 - 0.90X_4 + 1.01X_1X_2 + 0.023X_1X_3 + 0.96X_1X_4 + 0.42X_2X_3 + 0.13X_2X_4 - 1.94X_3X_4 - 4.18X_1^2 - 1.63X_2^2 - 4.17X_3^2 - 4.18X_4^2$$

Analysis of variance (ANOVA) is an effective and important method for evaluating the quality and significance of mathematical models; the results are shown in Table 3. P -values were used to determine the significance of each coefficient. When the " p -value $> f$ " was less than 0.05, 0.01, and 0.001, it

indicated that the model item was significant, extremely significant and highly significant, respectively. When the $p < 0.05$ ($\alpha = 0.05$), the fitted regression equation was considered statistically significant.

Table 3
Results of analysis of variance (ANOVA) of the Box–Behnken design (BBD).

Source of Variation	Sum of Squares	Variance	Mean Square	<i>F</i>	<i>p</i>	Significance
Model	424.46	14	30.32	37.89	< 0.0001	**
X_1	86.51	1	86.51	108.11	< 0.0001	**
X_2	28.55	1	28.55	35.68	< 0.0001	**
X_3	29.89	1	29.89	37.36	< 0.0001	**
X_4	9.67	1	9.67	12.08	0.0037	**
X_1X_2	4.1	1	4.1	5.12	0.04	*
X_1X_4	3.69	1	3.69	4.61	0.0499	*
X_3X_4	15.02	1	15.02	18.76	0.0007	*
X_1^2	113.46	1	113.46	141.79	< 0.0001	**
X_2^2	17.2	1	17.2	21.5	0.0004	**
X_3^2	112.64	1	112.64	140.77	< 0.0001	**
X_4^2	113.25	1	113.25	141.53	< 0.0001	**
Residual	11.2	14	0.8			
Lack of fit	10.03	10	1	3.42	0.1236	
Cor. Total	435.66	28				

As shown in Table 3, the regression model significance test ($p < 0.0001$) indicated that the model was highly significant. The regression model misfit test $p = 0.1236 > 0.05$, indicated that the model misfit was not significant, and the regression equation fit the test well. The model truly reflected the test data, with coefficient of determination is 97.43% and adjusted R square is 94.86%, indicating that the model had a good fitting degree with the actual test. This model can be used to determine the influence of various factors on the extraction of total flavonoids. The *F* test showed that X_1 -extract voltage, X_2 -extraction time, and X_3 -ethanol concentration had highly significant effects on total extracted flavonoids ($p < 0.001$). X_4 -Liquid–solid ratio was extremely significant for the extraction of total flavonoids ($p < 0.01$). In summary,

within the scope of the experimental design, according to the F value, the order of the degree of influence of each factor on the amount of total flavonoids extracted was as follows: $X_1 > X_3 > X_2 > X_4$.

3.5 RSM of Interaction of Multiple Factors

The Design-Expert 8.0.6 software was used to analyze and obtain the response surface diagram of the total flavonoids extracted from *Edgeworthia gardneri* (Wall) Meissn, and the contour diagram and response surface of the response value of the mutual factors were analyzed.

According to the regression equation, the response surface diagrams were all opened downward, indicating that there was a maximum response value in the investigated range. The change in the response surface and the density of the contour map directly reflected the influence of the interaction among the factors on the extraction of total flavonoids from *Edgeworthia gardneri* (Wall) Meissn. The contour shape reflected the strength of the interaction among the various factors. The steeper the response surface slope, the greater the influence of the factor on the amount of total flavonoids extracted from *Edgeworthia gardneri* (Wall) Meissn, whereas the gentler the response surface slope, the lesser the influence.

According to the response surface diagram in Fig. 2 to Fig. 7, the extraction voltage, extraction time and Ethanol concentration had a greater impact on the extraction amount of Total flavonoids, while the solid-liquid ratio had a relatively small impact on the extraction amount of Total flavonoids. As can be seen from the contour diagram in Fig. 2-Figure 7, the interaction of the four factors is strong, and the contour lines are all elliptic or close to elliptic, indicating that the interaction of the four factors has a significant impact on the extraction amount of total flavonoids. According to the density and shape of contour lines in the contour map, the significance of the influence on the extraction amount of total flavonoids was obtained in descending order: extraction voltage, Ethanol concentration, extraction time and solid-liquid ratio.

According to the response surface diagram in Figs. 2 to Fig. 7, the extraction voltage, extraction time, and ethanol concentration had a greater impact on the amount of total flavonoids extracted than the solid-liquid ratio. According to the contour diagram in Figs. 2 to 7, the interaction of the four factors was strong, and the contour lines were all elliptic or close to elliptic, indicating that the interaction of the four factors had a significant impact on the amount of total flavonoids extracted. According to the density and shape of contour lines in the contour map, the significance of the influence on the amount of total flavonoids extracted was in descending order: extraction voltage, ethanol concentration, extraction time, and solid-liquid ratio.

Based on the BBD and response surface plots, the optimal comprehensive evaluation value was 18.8165 mg/g, and the result from the verification experiment was 18.91 mg/g. The suggested optimal extraction conditions were as follows: extraction voltage 157.98 V, extraction time 91.61 s, ethanol concentration 67.98%, and liquid-solid ratio 1 : 29.63 mL/g. Combined with the results of the single-factor experiments,

the extraction process was determined to have an extraction voltage of 150 V, extraction time of 100 s, ethanol concentration of 70%, and liquid-solid ratio of 1:30 mL/g.

3.6 Determination of Antioxidant Activity

3.6.1 Scavenging ability of DPPH free radical by *Edgeworthia gardneri* (Wall) Meissn's total flavonoids

It can be seen from Fig. 8 that the total flavone and Vitamin C (Vc) at different concentrations showed significant scavenging effects on DPPH free radicals, with a linear increasing trend. With an increase in the concentration of total flavonoids, the scavenging ability of total flavonoids for DPPH radicals increased significantly. When the mass concentration of total flavonoids was 0.42 mg/mL, the scavenging rate was 85.32%, which was 89.71% of the OH radical scavenging rate (95.10%) for the same concentration of Vc. The extracts showed good scavenging ability for DPPH free radical.

3.6.2 Scavenging ability of O^{2-} free radical by *Edgeworthia gardneri* (Wall) Meissn's total flavonoids

It can be seen from Fig. 9 that the total flavonoids and Vc at different concentrations from *L. glossum* showed significant scavenging effects on O^{2-} free radicals, with a linear increasing trend. With an increase in the concentration of total flavonoids, the scavenging ability of total flavonoids for O^{2-} radicals increased significantly. When the mass concentration of total flavonoids was 0.42 mg/mL, the scavenging rate was 59.6%, which was 75.44% of the OH radical scavenging rate (79.0%) for the same concentration of Vc. The extracts showed good scavenging ability for O^{2-} free radicals.

3.6.3 Scavenging ability of OH free radical by *Edgeworthia gardneri* (Wall) Meissn's total flavonoids

As shown in Fig. 10, different concentrations of total flavonoids and Vc showed significant scavenging effects on OH, with a linear increasing trend. With an increase in the concentration of total flavonoids, the scavenging ability of total flavonoids for OH increased significantly. When the mass concentration of total flavonoids was 0.42 mg/mL, the scavenging rate was 68.5%, which was 77.84% of the OH radical scavenging rate (88.00%) for the same concentration of Vc. The extracts showed a good scavenging ability for OH free radicals.

4 Selection And Optimization Of Hplc

In this study, HPLC separation of the main components of *Edgeworthia gardneri* (Wall) Meissn's total flavonoids was performed. The chromatogram of the test solution is shown in Fig. 11. Under these conditions, the chromatographic peaks were well separated, and there was no peak tailing phenomenon.

It can be seen that the chromatographic conditions had a good separation effect on the main chemical components in the extraction of total flavonoids.

5 Conclusions

A single-factor test, four factors, and three levels of the RSM were used to investigate the effect of flash extraction on the yield of total flavonoids. The optimal extraction conditions were as follows: extraction voltage 150 V, extraction time 100 s, ethanol concentration 70%, and liquid-solid ratio 1:30 mL/g, which were close to the results of the prediction model, and had practical application value. The antioxidant activity of the total flavonoids extracted from *Edgeworthia gardneri* (Wall) Meisn was enhanced with an increase in their mass concentration, and the scavenging effect on DPPH, O²⁻ free radicals, and OH free radical free radicals was strong; thus, the flavonoids could be used as potential

Declarations

Authors' contributions: LZ designed the project. MX, ZJ, LJ, ZZ, LL, TJ, CH, and ZZ performed the experiment. MX, ZJ, and LZ analyzed the data and wrote the manuscript draft. ZJ and LZ revised the writing. All the authors have read the final article and approved the submission.

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Figures

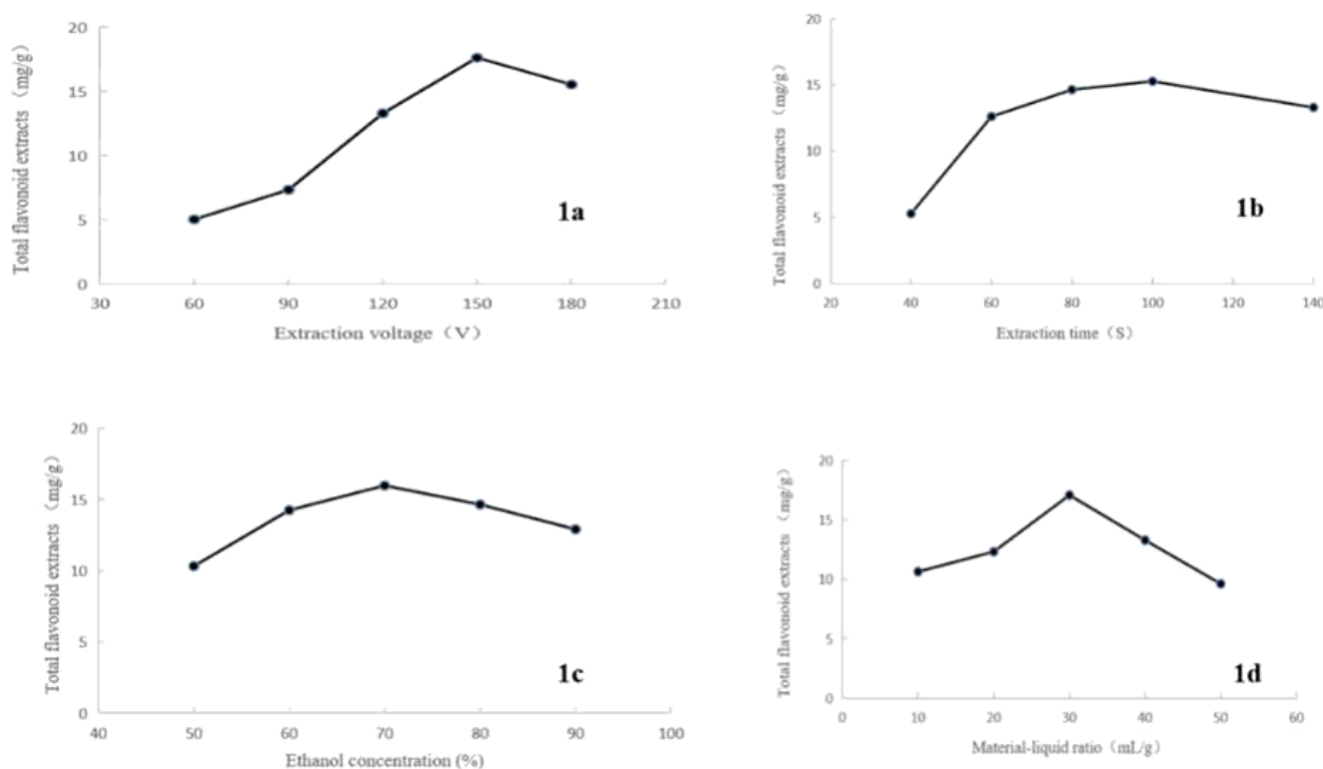


Figure 1

Single-factor experimental results of *Edgeworthia gardneri* (Wall) Meissn extraction process

(1a) Effect of Extract Voltage. (1b) Effect of Extraction Time. (1c) Effect of Ethanol Concentration. (1d) Effect of extraction liquid–solid ratio .

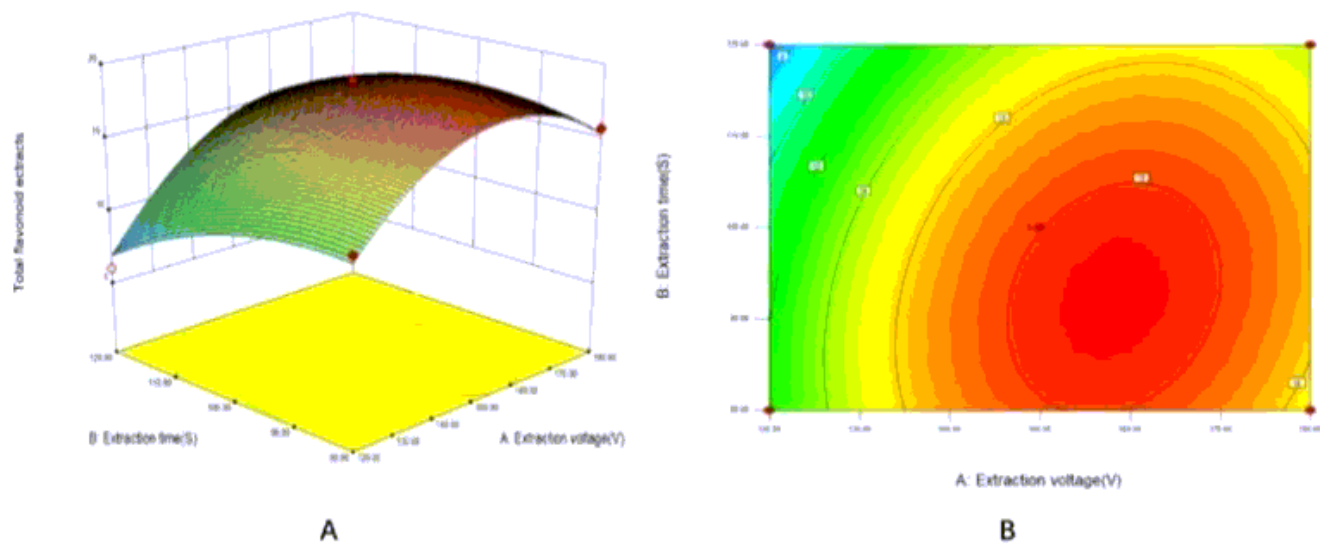


Figure 2

Interaction effect of extraction voltage and extraction time on the *Edgeworthia gardneri*(Wall) Meissn

A: The response surface figure, B: Contour map

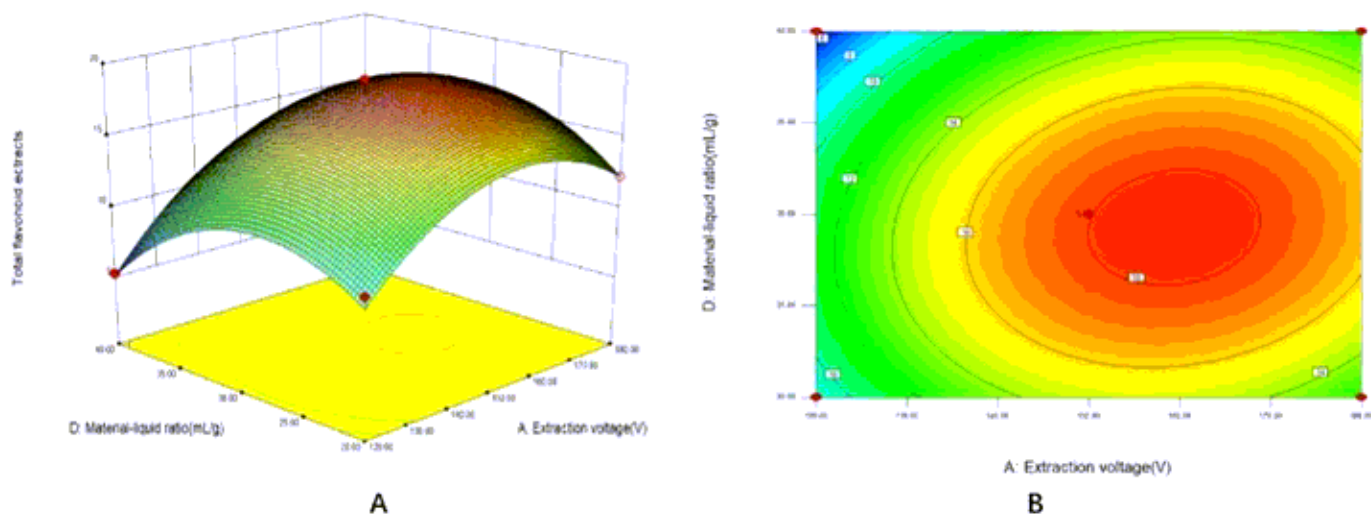


Figure 3

Interaction between extraction voltage and solid-liquid ratio of total flavonoids extracted from the *Edgeworthia gardneri*(Wall) Meissn

A: The response surface figure ,B: Contour map

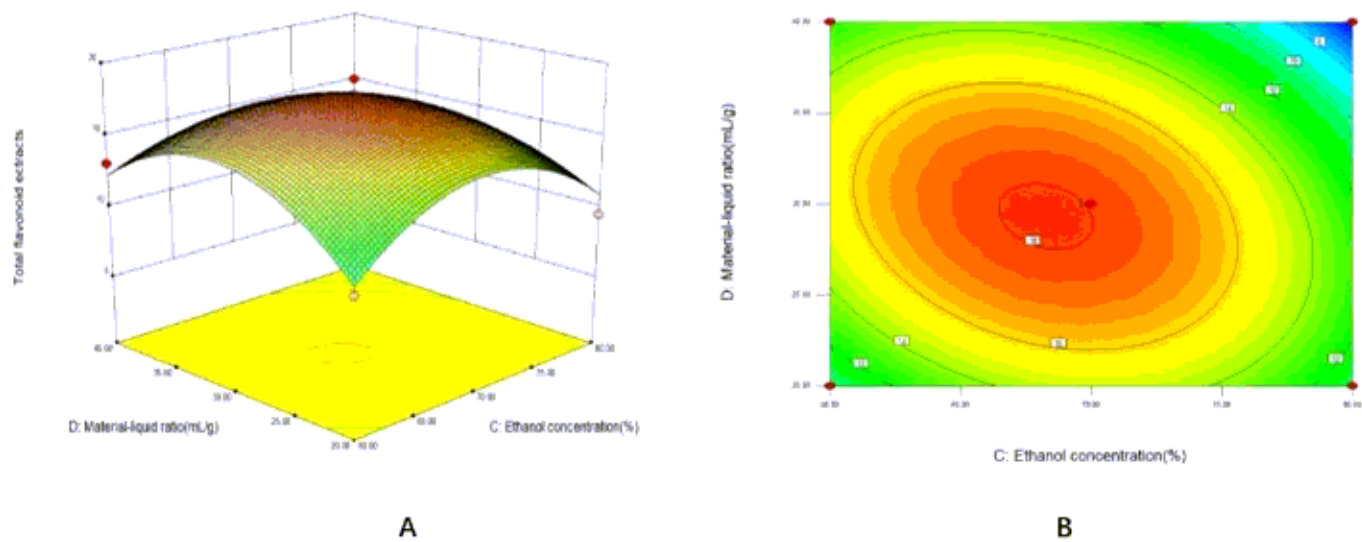
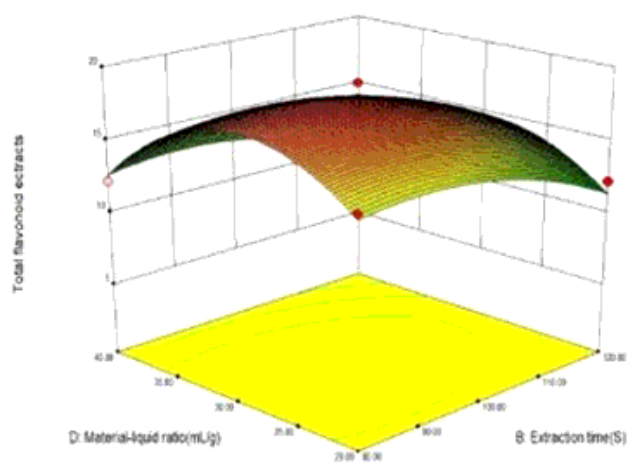


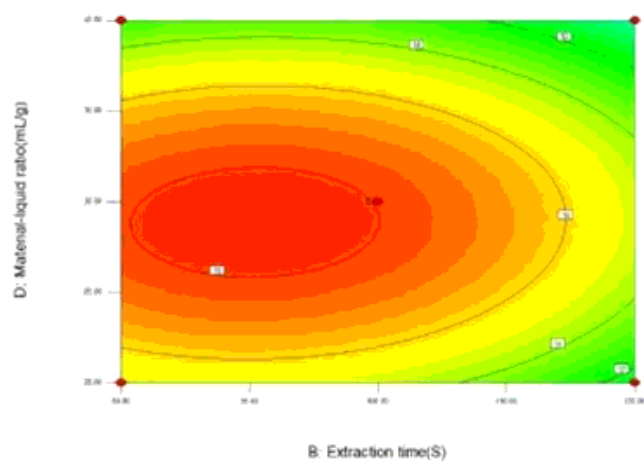
Figure 4

Interaction between ethanol concentration and solid-liquid ratio of total flavonoids extracted from the flower of Rhododendron L e on the *Edgeworthia gardneri*(Wall) Meissn

A: The response surface figure ,B:Contour map



A

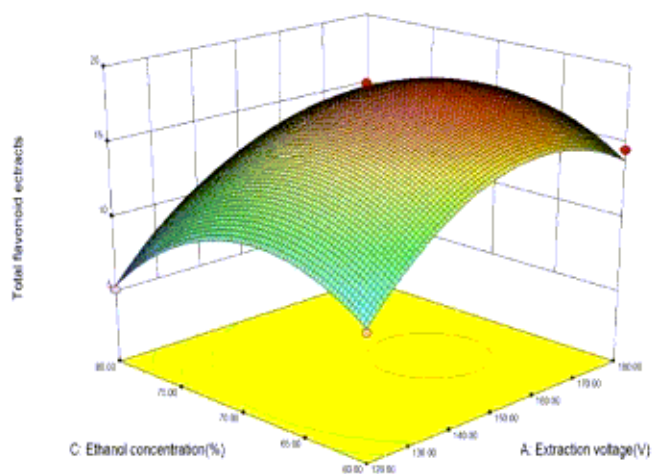


B

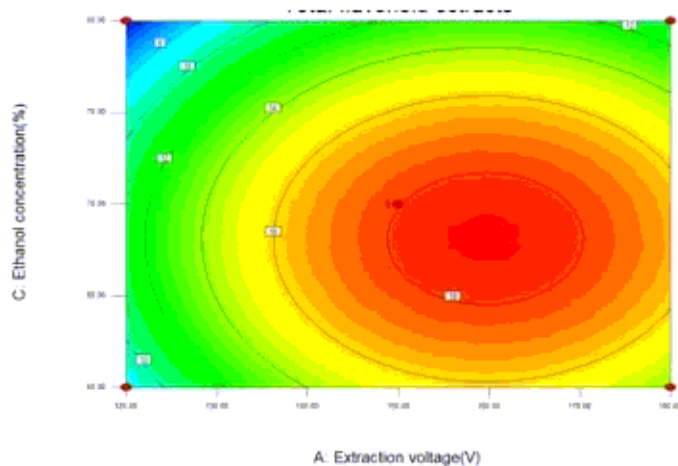
Figure 5

Interaction between extraction time and solid-liquid ratio of total flavonoids extracted from the flower of *Rhododendron L e* on the *Edgeworthia gardneri*(Wall) Meissn

A: The response surface figure, B:Contour map



A



B

Figure 6

Interaction between extraction voltage and ethanol concentration of total flavonoids extracted from the flower of Rhododendron L e on the *Edgeworthia gardneri*(Wall) Meissn

A: The response surface figure, B:Contour map

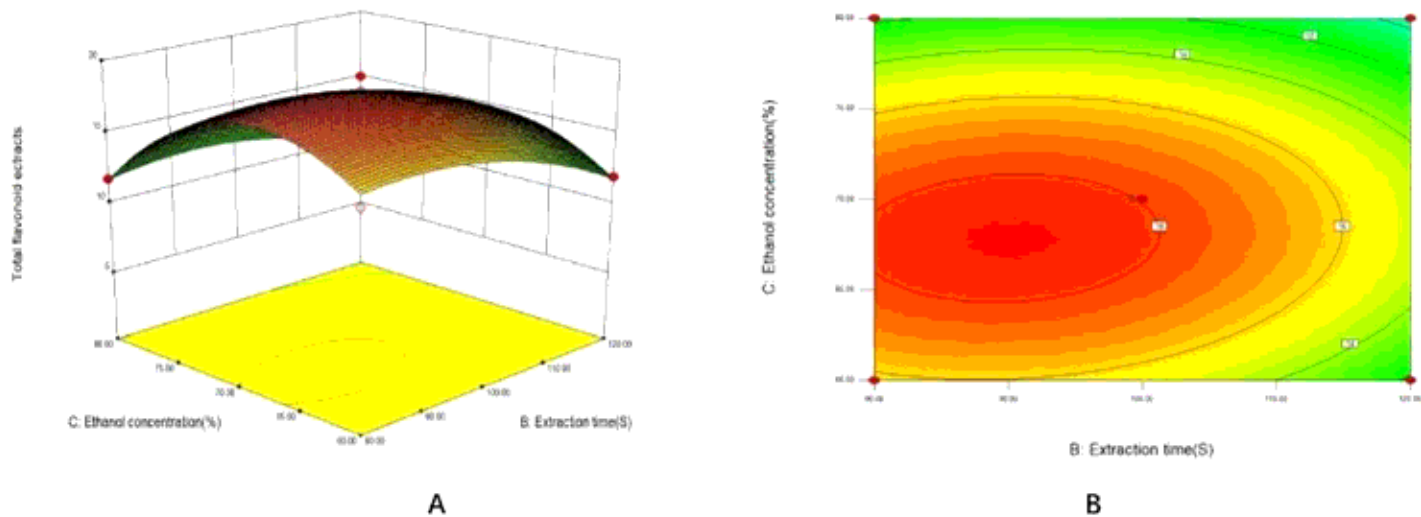


Figure 7

Interaction between extraction time and ethanol concentration of total flavonoids extracted from the flower of Rhododendron L e on the *Edgeworthia gardneri*(Wall) Meissn

A: The response surface figure, B:Contour map

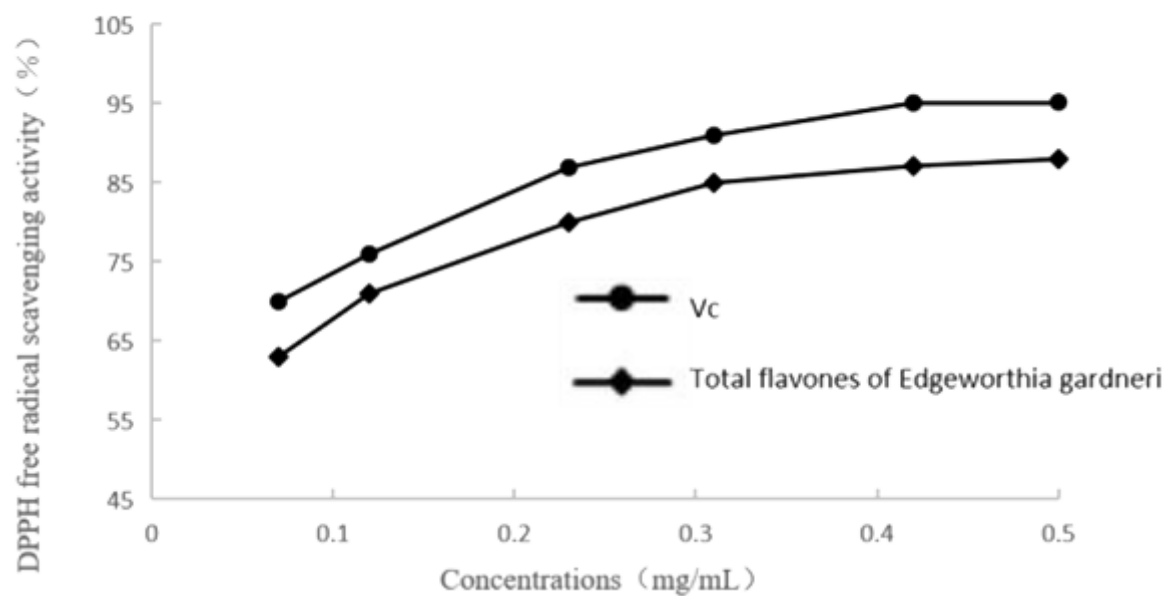


Figure 8

DPPH radical scavenging activity curve

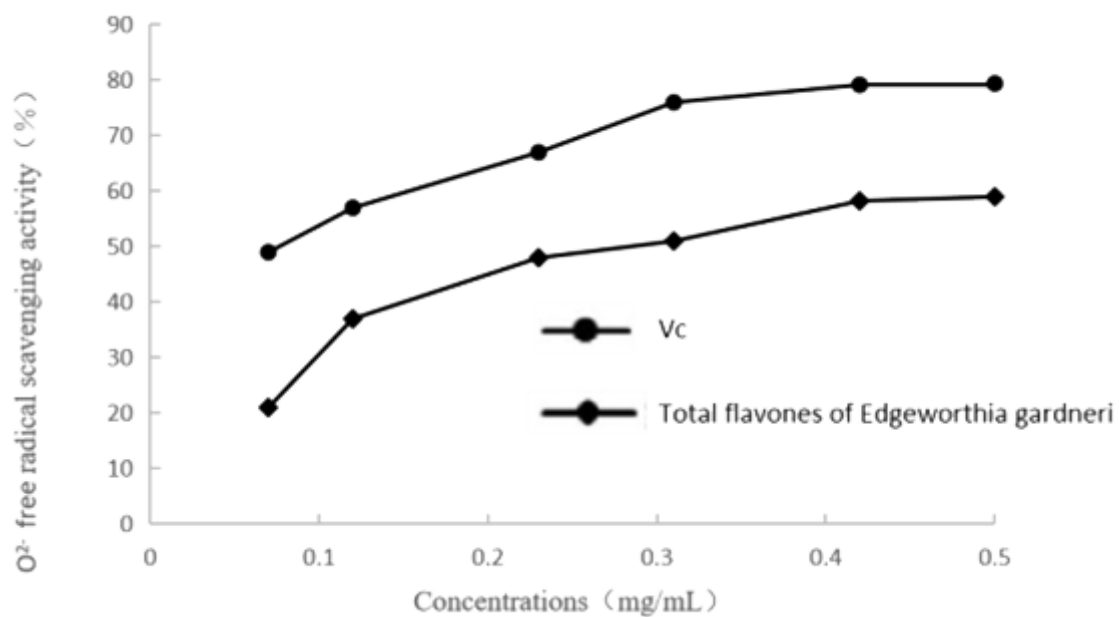


Figure 9

O_2^- radical scavenging activity curve

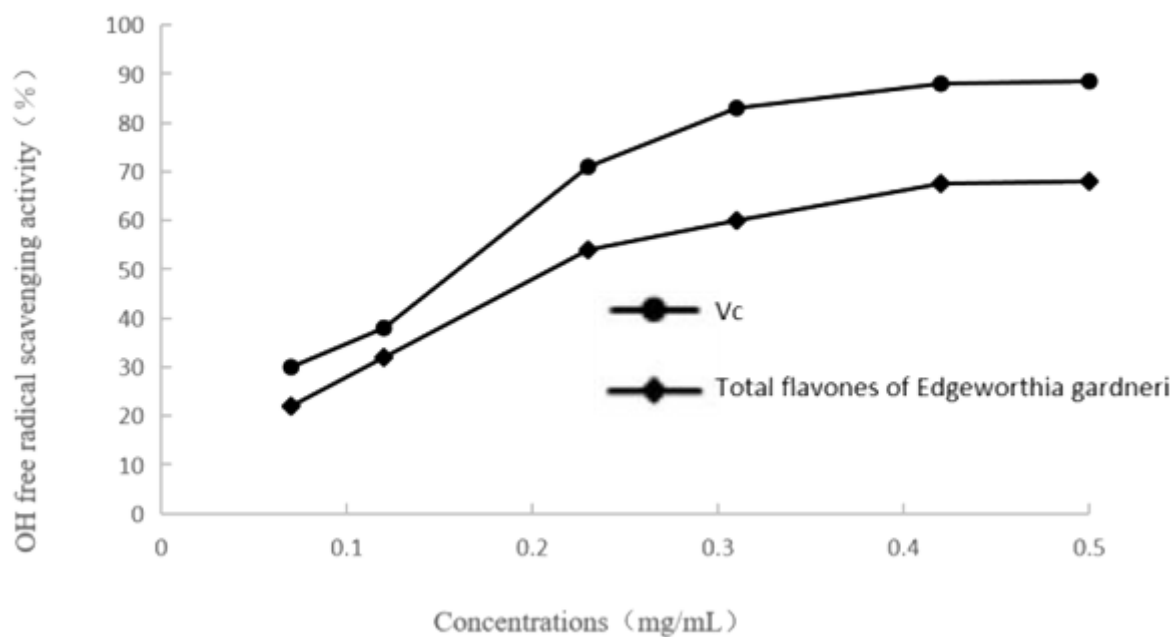


Figure 10

OH radical scavenging activity curve

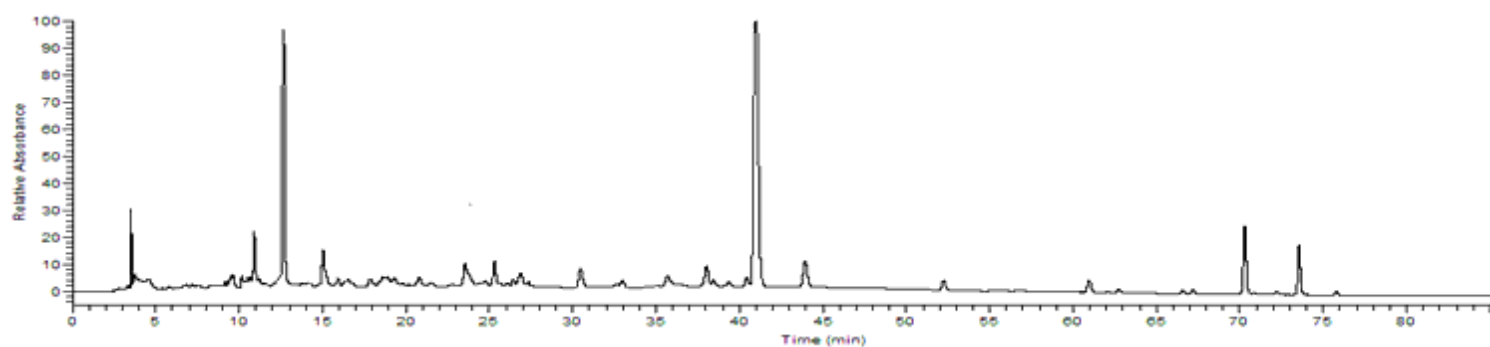


Figure 11

HPLC of total flavone extract from *Edgeworthia gardneri* (Wall) Meissn