Response Surface Optimized Ultrasonic Assisted Extraction of Total Flavonoids from QingLi Cao and *In Vitro* Antioxidant Activities

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Abstract: An ultrasound-assisted extraction technique was used to extract the total flavonoids from QingLi Cao. The optimal conditions were ethanol concentration 59.20%, liquid-to-solid ratio 31.15 mL/g, extraction time 57.42 min and extraction temperature 58.57°C, which were determined using response surface methodology. The antioxidant activities including reducing power, ABTS+, DPPH, superoxide anion and hydroxyl radical were evaluated, which suggested significant antioxidant activities.

Keywords: Antioxidant, flavonoids, Qing Li Cao, response surface methodology, Ultrasound-assisted extraction.

1. INTRODUCTION

QingLi Cao, a traditional herb has been used for eczema and itching in the minority of Guangxi province, China. It is distributed in Guangxi province and Vietnam. The previous studies show that the extracts are rich in flavonoids, which have been associated with their antioxidant activities. However, insufficient studies have been conducted on flavonoids from QingLi Cao and its antioxidant activity [1].

Ultrasound-assisted extraction (UAE) is an efficient and simple extraction technique. Response surface methodology (RSM) is an effective statistical method for optimizing experimental conditions and investigation of critical processes [2].

The objective of this study was to use RSM to optimize the UAE of total flavonoids from QingLi Cao and evaluate its antioxidant activities. The information obtained will be helpful to further utilization of QingLi Cao.

2. EXPERIMENTAL

2.1. Chemicals and Reagents

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Ruibio, Germany), 1,1,1-Tris (hydroxymethyl) ethane (Tris) (Amresco, USA), 1,1-Diphenyl-1 -picrylhydrazyl (DPPH) (TCI, Japan). The others used purchased from Sinopharm Chemical Reagent Co., Ltd (SCRC, China) and Xiya Reagent Co., Ltd (Xiya, China) used without further purification.

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2.2. Sample Preparation

The QingLi Cao was collected from the Qinzhou City of Guangxi Province in November 2013 and authenticated by Prof. Guangwei Huang. It was dried under shade and ground to powder (40 meshes) in a grinding mill. The powder was kept in refrigerator at 0~5°C until use.

2.3. Ultrasound-Assisted Extraction of Flavonoids

The dried powder of QingLi Cao was mixed with ethanol, and the extraction process used an ultrasonic device according to the method described in references [3]. The sample was centrifuged at 3500 rpm for 10 min to collect the supernatant, UV-Vis analyzed the diluted solution. The UAE device was an ultrasonic device (B2200S, Branson Ultrasonics (Shanghai) Company) with 40 kHz and 120 W.

2.4. Experimental Design

RSM was employed to establish the optimum conditions for extraction parameters. A Box-Behnken experiment was employed and a four independent variable at three levels was used, including ethanol concentration (50-70%), liquid-solid ratio (25:1-35:1), extraction time (40-80min) and extraction temperature (50-70°C) Table **1**.

2.5. Determination of Total Flavonoids

The amount of total flavonoids was measured following a previously reported method [4].

2.6. Evaluation of Antioxidant Activity

2.6.1. Reducing Power

The ability of sample to reduce ferric was determined by the method as is described [5].

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Table 1. Factors and levels in response surface design.

Levels	Independent Variables			
	A Liquid-Solid Ratio / mL/g	B Concentration / %	C Temperature / °C	D Time / min
1	25	50	50	40
0	30	60	60	60
-1	35	70	70	80

2.6.2. ABTS Radical Scavenging Activity

Determination of the scavenging activity of ABTS radical was based on the procedure described in the study [6].

2.6.3. DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity was determined according to the method [7].

2.6.4. Superoxide Radical Scavenging Activity

The scavenging ability of superoxide radical was measured by the previously described [8].

2.6.5. Hydroxyl Radical Scavenging Activity

The hydroxyl radical assay was according to the previously described [9].

2.7. Statistical Analysis

Data for antioxidant activity are expressed as mean ± SD for analysis performed in triplicate. The mean values and standard deviation were calculated with the Excel program from Microsoft Office 2003 package.

3. RESULTS AND DISCUSSION

3.1. Extraction Parameters for Flavonoids

3.1.1. Fitting the RSM

The extraction yield from QingLi Cao was further optimized through the RSM approach. The experimental points were designed as shown in Table **2**. The response value in designed was the average of triplicates.

After fitting to the experimental findings, the response extraction yield of total flavonoids and test variables are related by the following second-order polynomial equation:

$$Y=+1.38+0.068A+0.015B-0.015C-0.05D-0.05AB+0.04AC+0.05AD+0.16BC-0.03BD+0.07CD-0.13A^2-0.076B^2-0.11C^2-0.16D^2.$$

Table **3** indicates that the coefficient of determination R-Squared is variability in the data explained. The R-Squared was 0.8658, suggesting that a high correlation was achieved [10]. The F-value of 6.45 and Values of "Prob>F" less than 0.05 indicated the model were significant.

The effects of ethanol concentration, liquid-to-solid ratio, extraction time and extraction temperature on total flavonoids extraction yield of QingLi Cao, as well as their interactions, are shown in Fig. (**1**).

The plots showed interaction effects of two factors on the response while other factors were kept at constant level. When the contour plots are oval, it means the interaction of two independent variables is significant [11]. According to Table **3** and Fig. (**1**), the interaction between extraction time and extraction temperature was significant.

3.1.2. Verification of Predictive Model

The optimal extraction conditions as follows: ethanol concentration 59.20%, liquid-to-solid ratio 31.15 mL/g, extraction time 57.42 min and extraction temperature 58.57°C. The maximum predicted yield of total flavonoids was 13.90 mg/g. The mean value (13.80±0.04 mg/g) obtained from experiment which was close to the predicted result.

3.2. Evaluation of Antioxidant Activity

3.2.1. Reducing Power

The reducing power was evaluated based on the reduction of ferric to divalent iron in which the yellow color of the test solution changes to green or blue, depending on the different reducing power of each sample. Rising absorbance at 700nm indicate an increase in reducing power [12].

As shown in Fig. (**2**), the absorbance of the concentration of 1μg/mL sample and ascorbic acid were 0.106 and 0.397 but sharply increased to 0.425, 0.801 at the concentration of 10 μg/mL, respectively. On reducing power, the extraction had significant effects with increasing concentration in the range of 1-10μg /mL, but compared with the contrast, the effect of sample was slight.

3.2.2. ABTS Radical Scavenging Activity

The abilities of extracts assayed to be scavenging the ABTS radical in comparison with ascorbic acid [13], are

Table 2. Box-Behnken design matrix and the experimental observed responses.

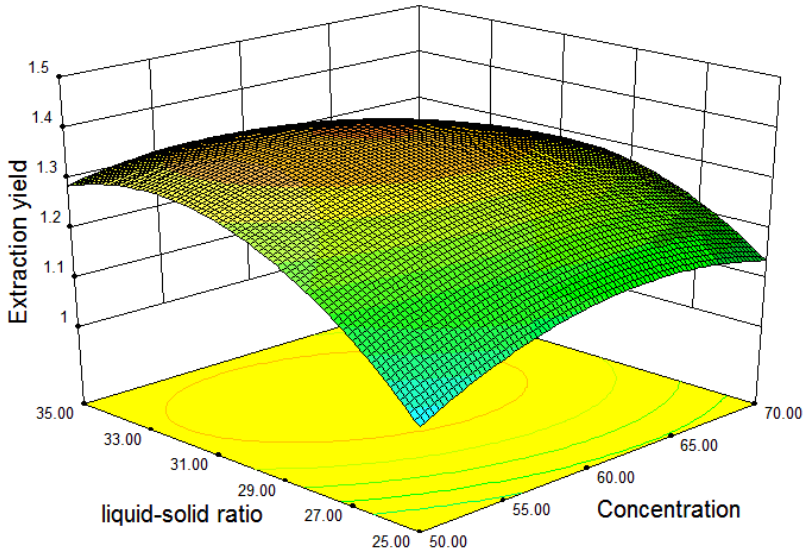
Run	X_1/Liquid-Solid Ratio (mL/g)	X_2/Concentration (%, v/v)	X_3/ Extraction Time (min)	X_4/Temperature (°C)	Total Flavonoids Yield (mg/g)
1	1	0	0	-1	1.19
2	0	1	1	0	1.35
3	0	0	0	0	1.46
4	0	1	0	-1	1.15
5	0	0	1	-1	1.11
6	-1	0	0	1	0.91
7	0	0	0	0	1.21
8	0	0	0	0	1.43
9	-1	1	0	0	1.19
10	0	0	-1	-1	1.22
11	1	1	0	0	1.22
12	0	-1	0	1	1.15
13	-1	0	1	0	0.94
14	0	-1	0	-1	1.09
15	0	0	0	0	1.39
16	0	-1	-1	0	1.37
17	0	1	-1	0	1.09
18	0	-1	1	0	1.01
19	0	1	0	1	1.09
20	-1	0	0	-1	1.21
21	0	0	1	1	1.15
22	1	0	-1	0	1.21
23	1	0	0	1	1.09
24	0	0	0	0	1.39
25	-1	-1	0	0	1.03
26	1	0	1	0	1.22
27	0	0	-1	1	0.97
28	1	-1	0	0	1.24
29	-1	0	-1	0	1.09

Table 3. Variance for response surface quadratic model.

Source	Sum of Squares	Df	Mean Square	*F* value	*P* value	Significance
Model	0.50	14	0.036000	6.45	0.0006	significant
A	0.057	1	0.057000	10.33	0.0063	

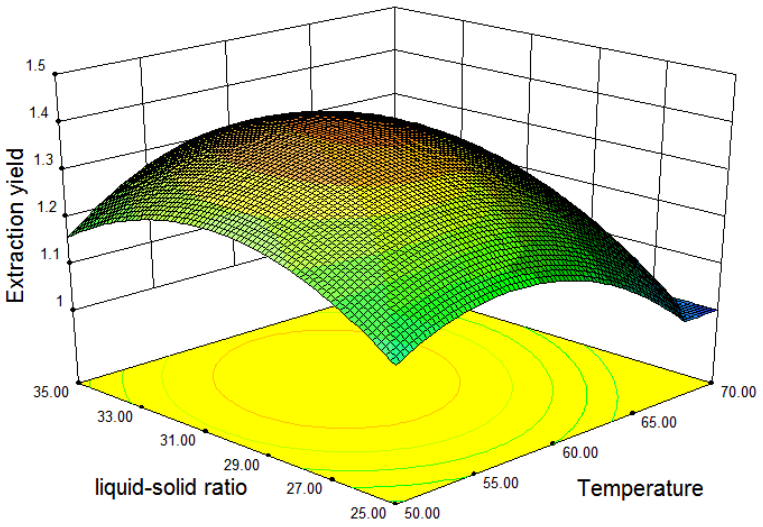
Table 3. contd…

Source	Sum of Squares	Df	Mean Square	*F* value	*P* value	Significance
B	0.002408	1	0.002408	0.43	0.5211	
C	0.0027	1	0.002700	0.49	0.4973	
D	0.030	1	0.030000	5.40	0.0358	
AB	0.011	1	0.011000	1.98	0.1809	
AC	0.0064	1	0.006400	1.15	0.3014	
AD	0.001	1	0.001000	1.80	0.2012	
BC	0.096	1	0.096000	17.29	0.0010	
BD	0.0036	1	0.003600	0.65	0.4344	
CD	0.020	1	0.020000	3.53	0.0814	
A^2	0.11	1	0.110000	19.87	0.0005	
B^2	0.037	1	0.037000	6.65	0.0219	
C^2	0.077	1	0.077000	13.93	0.0022	
D^2	0.16	1	0.160000	29.59	<0.0001	
Residual	0.078	14	0.005559			
Lack of Fit	0.040	10	0.003991	0.42	0.8782	Not significant
Pure Error	0.038	4	0.009480			
Total	0.58	28				

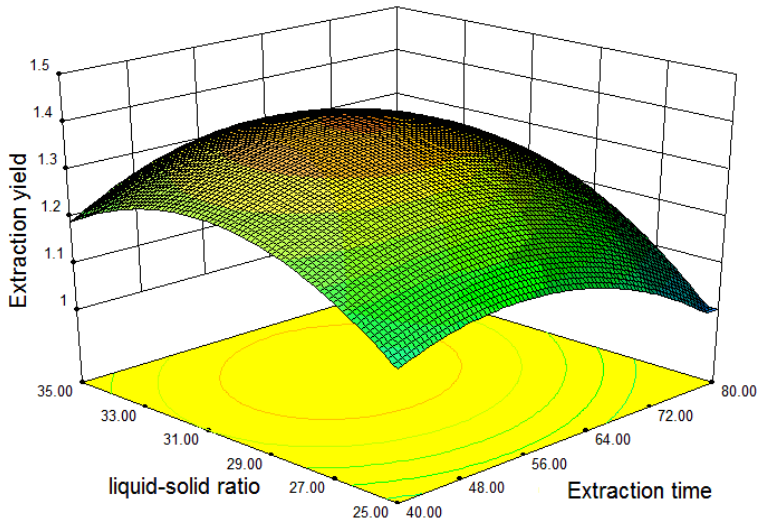


(**A**) Liquid-to-solid ratio and concentration

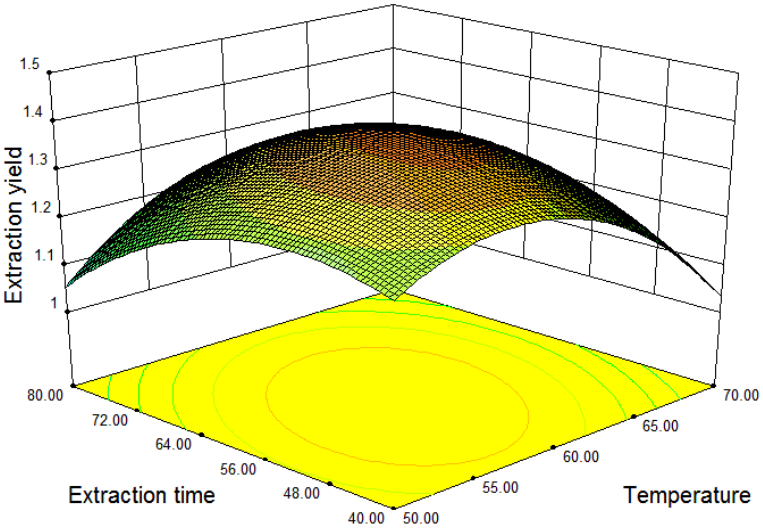
Fig. (**1**). Contd…



(**B**) Liquid-to-solid ratio and temperature

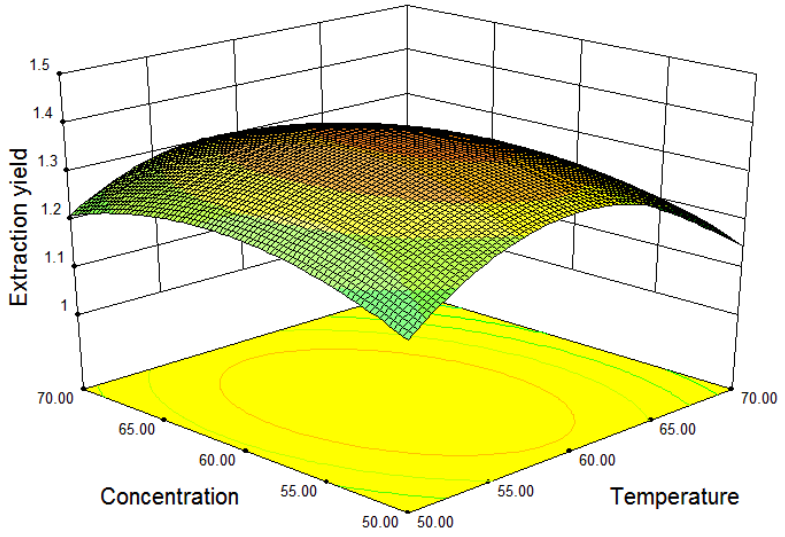


(**C**) Liquid-to-solid ratio and Extraction time

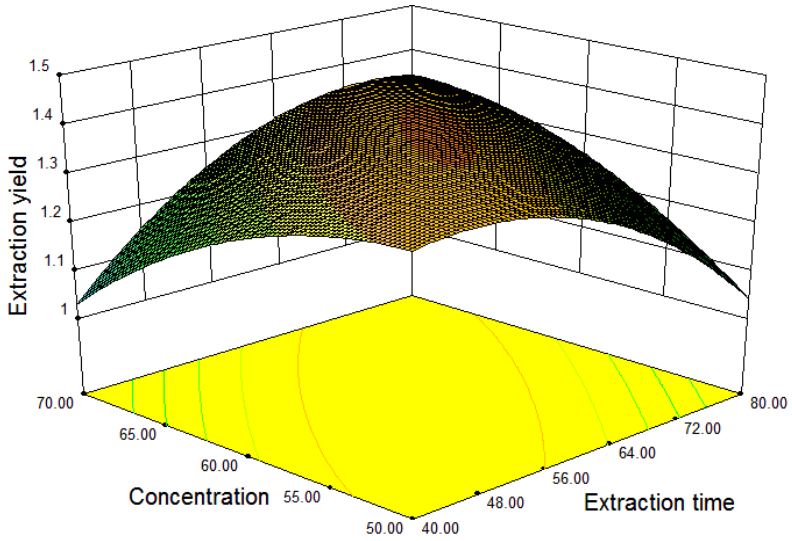


(**D**) Extraction time and temperature

Fig. (**1**). Contd…



(**E**) Concentration and temperature



(**F**) Concentration and Extraction time

Fig. (1). Response surface graphs for the effects of concentration, liquid-to-solid ratio, extraction time and temperature on total flavonoids extraction yield

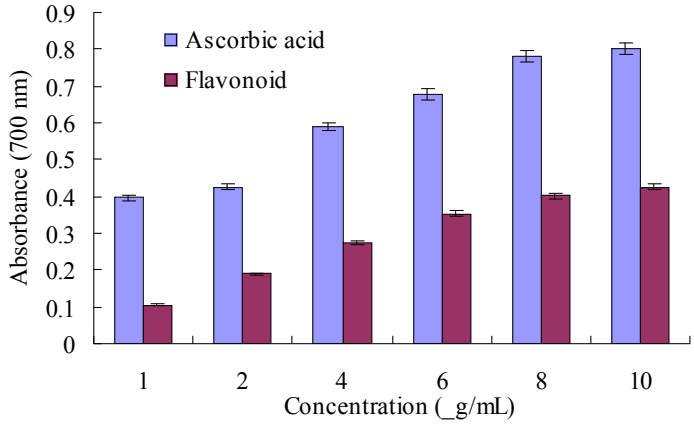


Fig. (2). Reducing power of sample and ascorbic acid.

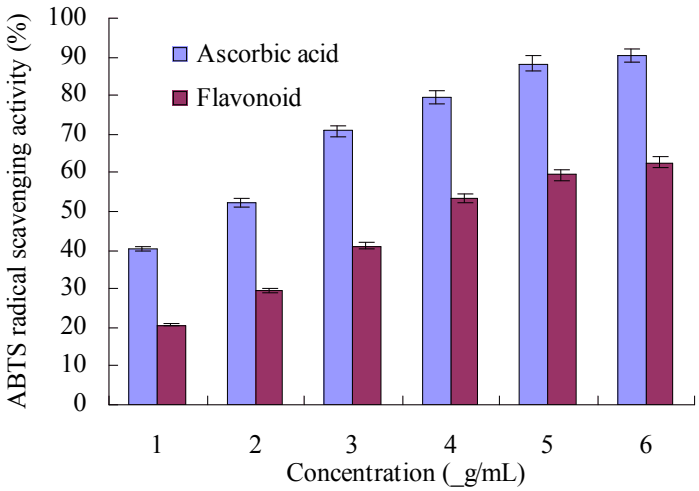


Fig. (3). ABTS radical scavenging activity of sample and ascorbic acid.

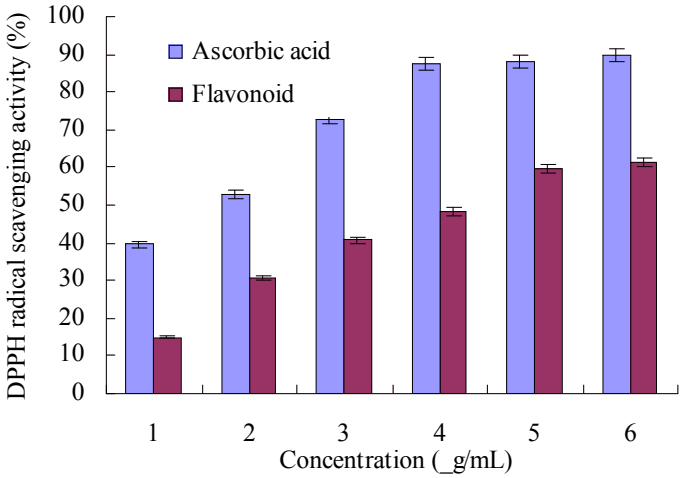


Fig. (4). DPPH radical scavenging activity of sampleand ascorbic acid.

shown in Fig. (**3**). The extracts had effective ABTS in a concentration-dependent manner (1 to10μg/mL), sharply increased from 20.6% to 62.7%. The scavenging effect of the contrast (90.5%) was observed to be higher obviously.

3.2.3. DPPH Radical Scavenging Activity

The absorbance is decreased and the solution changes from purple to light yellow when DPPH concentration is reduced. The mechanism of scavenging DPPH radical is caused by the fact that natural compounds can transfer an electron or a hydrogen atom to DPPH [14].

In the present investigation, a comparison of sample and ascorbic acid is shown in Fig. (**4**). DPPH radical scavenging abilities of sample sharply increased from 15.0% to 61.3%, when the concentration was increased from 1 to 10 μg/mL. The results show that sample showed excellent percent inhibition of DPPH activity at the concentration of 10μg/mL but significantly lower than that of the contrast (89.6%).

3.2.4. Hydroxyl Radical Scavenging Activity

Hydroxyl radical exhibits the strongest oxidative activity in terms of its very high redox potential and extremely fast kinetics [15]. Thus, it is an important parameter for evaluating the antioxidant activity of sample extracts.

As shown in Fig. (**5**), hydroxyl radical scavenging effect of sample increased with concentrations. At concentration of 10μg/mL, it was 60.7% and 89.7% respectively for sample and ascorbic acid. Results indicated that sample had strong capability of scavenging hydroxyl radical but significantly lower than contrast.

3.2.5. Superoxide Anion Radical Scavenging Activity

Superoxide anion radical is considered as an initial free radical and formed from mitochondrial electron transport system [16].

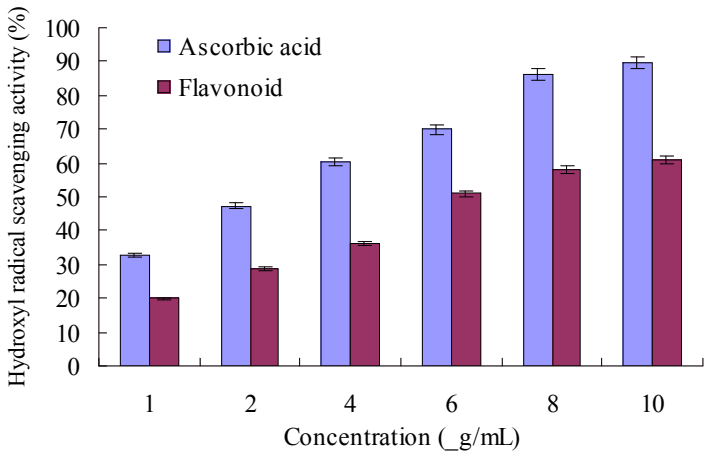


Fig. (5). Hydroxyl radical scavenging activity of sample and ascorbic acid.

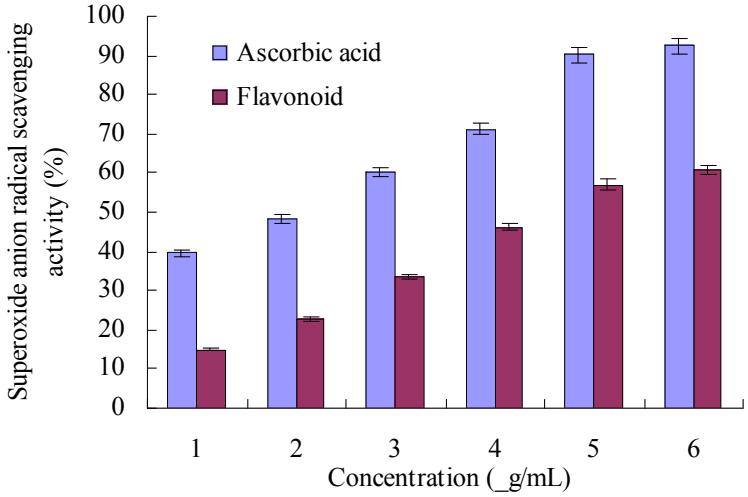


Fig. (6). Superoxide anion radical scavenging activity of sample and ascorbic acid.

Fig. (**6**) shows the percentage inhibition of superoxide radical generation from 1 to 10 μg/mL concentration of sample and ascorbic acid. In this study, superoxide anion radicals were scavenged by sample and ascorbic acid in a concentration dependent manner. The inhibition of superoxide anion radical scavenging at 10μg/mL of sample was 60.7%, but ascorbic acid showed stronger that is 92.4%.

CONCLUSION

In this study, the RSM was successfully employed to optimize the ultrasonic-assistant extraction of total flavonoids from QingLi Cao and evaluated. The results showed that all the factors had significant effects on the extraction rate of total flavonoids. The optimum extraction conditions were obtained at ethanol concentration 59.20%; liquid-to-solid ratio 31.15mL/g; extraction time 57.42 min and extraction temperature 58.57°C. The extraction rate of total flavonoids was in agreement with the predicted ones.

The antioxidant activities including reducing power, ABTS+ radical scavenging activity, DPPH radical scavenging activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity were evaluated, which suggested significant antioxidant activities.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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