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Research Article

Optimization of Extraction of Compound Flavonoids from Chinese Herbal Medicines Based on Quantification Theory and Evaluation of Their Antioxidant Activity

Xu Yang,[1] **Dongli Cao**,[1] **Haiyu Ji**,[2] **Yingying Feng**,[2] **Juan Yu**,[2] **and Anjun Liu**[2]

[1]*Tianjin Institute for Food Safety Inspection Technology, Tianjin 300308, China*
[2]*Key Laboratory of Food Nutrition and Safety, Ministry of Education, School of Food Science and Engineering, Tianjin University of Science and Technology, Tianjin 300457, China*

Correspondence should be addressed to Xu Yang; yangxu1009@163.com

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Plant-derived flavonoids have been attracting increasing research interest because of their multiple health promoting effects, where numerous investigations were carried out on the optimization of extraction and bioactivities. This study aims to optimize the extraction process of compound flavonoids (CFs) from Chinese herbal medicines and detect their antioxidant activity in vitro. CFs were extracted from the raw materials named "medicine food homology," composed of hawthorn, lotus leaf, tartary buckwheat, cassia seed, *Lycium barbarum*, and *Poria cocos* in a mass ratio of 4 : 2 : 2 : 1.5 : 1 : 1. L_9 (3^4) orthogonal design, level effect and engineering average estimation, and quantification theory were utilized to improve the extraction method of CFs, and the predictive model for CFs yield was constructed. The 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), hydroxyl radical scavenging rate, and reducing power of CFs were measured. The highest CFs yield was obtained under the following extraction condition: liquid-solid ratio of 35 : 1 mL/g, extraction temperature of 75°C, extraction duration of 75 min, and extraction mode enzyme-assisted extraction. The forecasted yield was 37.62%. The result was accurate and the established prediction equation was reliable ($R = 0.95$). The antioxidant activity of CFs was significantly positively correlated with the concentration from 0.05 to 0.4 mg/mL. The DPPH, ABTS, hydroxyl radical scavenging abilities, and the reducing power of CFs were 81.82 ± 1.75%, 49.35 ± 0.09%, 89.78 ± 0.66%, and 0.232 ± 0.001 at the concentration of 0.4 mg/mL, respectively. CFs could be exploited as natural antioxidants in pharmaceuticals and functional foods.

1. Introduction

Plant flavonoids are an example of the main secondary metabolites with many important biological properties related to human health [1], such as antioxidant [2], antiviral [3], antitumor [4], neuroprotective [5], and immunoregulatory properties [6]. Natural flavonoids usually exist in vegetables, fruits, seeds, and wild plants, and more than 10000 flavonoids have been found so far [7]. Plant flavonoids have a strong antioxidant capacity and are promising antioxidants. More and more attention has been paid to the development of novel plant flavonoids.

Chinese herbal medicines (CHMs) extracted from hawthorn, lotus leaf, tartary buckwheat, cassia seed, *Lycium barbarum*, and *Poria cocos* have high nutritional and medicinal values and are the "medicine food homologous" substances [8]. Numerous research works have been carried out on the extraction optimization and bioactivities of single flavonoids from CHMs [2, 9, 10]. However, the extraction optimization and antioxidant activity of compound flavonoids (CFs) have rarely been explored. CFs are composed of two or more flavonoid ingredients, with many pharmacological activities including anticancer, antivirus, antioxidation, and immune-enhancing functions [11–13]. Furthermore, CFs have stronger radical scavenging capability and immune-modulating effect than single CHM flavonoids [14, 15].

The limiting factors of the yield of flavonoids can be divided into quantitative variables (e.g., liquid-solid ratio, extraction temperature, and extraction duration) and qualitative variables (e.g., extraction mode). Central composite design (CCD), Box-Behnken design (BBD), and response surface method (RSM) are often employed to optimize the extraction process of flavonoids. Under the condition of four factors and three levels, the number of experiments for L_9 (3^4) orthogonal design is only 9, which is remarkably fewer than that of BBD (27) and CCD (30). In addition, RSM cannot be used to assess the effect of qualitative variables [16]. Therefore, in this study, L_9 (3^4) orthogonal design and quantitative theory were adopted to ameliorate the extraction method of CFs and construct the prediction model of CFs yield. We evaluated the antioxidant activities of CFs and single flavonoids in vitro based on DPPH, ABTS, hydroxyl radical scavenging abilities, and reducing power in order to provide a theoretical basis for the application of CFs as a natural antioxidant in such fields as medicine and health food.

2. Materials and Methods

2.1. Materials and Reagents. Hawthorn, lotus leaf, tartary buckwheat, cassia seed, *Lycium barbarum*, and *Poria cocos* were purchased from Bozhou Shenghao Biotechnology Co., Ltd. (Anhui, China). Absolute ethanol was obtained from Gibco (New York, USA). Potassium persulfate was acquired from Fluka Co. (Buchs, Switzerland). Cellulase and Tris-HCl were provided by Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). Rutin, DPPH, ABTS, pyrogallic acid, and potassium ferricyanide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). All other chemical reagents were of analytical grade.

2.2. Extraction of CFs. Hawthorn, lotus leaf, tartary buckwheat, cassia seed, *Lycium barbarum*, and *Poria cocos* were dried at 60°C by electrothermal blast drying oven for 1 h to constant weight, smashed in a pulverizer (Model 800C, Yongkang Aizela Electric Appliance Co., Ltd., Zhejiang, China), and sieved (200 meshes), respectively. The fine powders were mixed in a mass ratio of 4 : 2 : 2 : 1.5 : 1 : 1 [17, 18]. The compounds (10 g) were extracted using 60% ethanol solution with a design method under the following condition: liquid-solid ratio of 5 : 1–40 : 1 mL/g, extraction temperature of 40–80°C, extraction duration of 15–120 min, and number of extractions of 1–5. After vacuum filtration, the hydroethanolic extracts were concentrated at 40°C by a rotary evaporator (SY-2000, Yarong Technology and Science Inc., Shanghai, China), then freeze-dried at −20°C using a lyophilizer (FD-1A, Boyikang Experimental Instrument Co., Ltd., Beijing, China), and finally ground into powder (termed CFs). The sodium nitrite-aluminum nitrate method [19] was utilized to determine the content of CFs with a UV-Vis spectrophotometer (UV–2500PC, Shimadzu Co., Kyoto, Japan). The absorbance was measured at 510 nm, and the standard curve with reference substance of rutin was $Y = 7.1105X\text{-}0.004$ ($R^2 = 0.9994$), where Y is the absorbance and X is the concentration of rutin. The CFs yield was calculated as follows:

$$Y_i(\%) = \frac{CNV}{M}, \quad (1)$$

where C (mg/mL) is the solution concentration of CFs, V (mL) is the solution volume of CFs, N is the solution dilution ratio of CFs, and M (mg) is the sample weight.

The ultrasound-assisted extraction (UAE) of CFs was performed by an ultrasonic cleaner (SB25-12DTDN, Xinzhi Biotechnology Co., Ltd., Ningbo, China) with thermostatic control. The ultrasonic power was 400 W and the cellulase amount was 1%. The process of UAE and enzyme-assisted extraction (EAE) was identical to that of ethanol extraction (EE).

2.3. Optimization of Experimental Design. The single-factor experiments were performed to assess the effects of liquid-solid ratio, extraction temperature, extraction duration, and number of extractions on the yield of CFs. The value range of each influence element in the L_9 (3^4) orthogonal design is exhibited in Tables 1 and 2. The range analysis was conducted to determine the optimal combination [20]. The variance analysis was utilized to ascertain the impact of each limiting factor on the yield of CFs [20]. The level effect and engineering average estimation was adopted to estimate the CFs yield corresponding to the optimal combination [21, 22]. According to our previous report [16, 20–22], the quantification theory was applied to establish the prediction model of CFs yield.

2.4. Determination of In Vitro Antioxidant Activity

2.4.1. DPPH Radical Scavenging Capacity. In terms of our past research with some modifications [23], 0.2 mL of DPPH ethanol solution (0.1 mM) was added to 0.2 mL different-concentrations (0.05, 0.1, 0.2, and 0.4 mg/mL) samples prepared with anhydrous ethanol, namely, the CFs, hawthorn flavonoids, lotus leaf flavonoids, *Lycium barbarum* flavonoids, *Poria cocos* flavonoids, cassia seed flavonoids, and tartary buckwheat flavonoids. The mixtures were shaken evenly and kept away from light for 30 min, and the absorbance was measured at 517 nm by a microplate reader (Model 680, Bio-Rad Co., California, USA). Vitamin C (VC) served as the positive control. The scavenging rate of DPPH was calculated by the following formula:

Scavenging rate (%) = $[1 - (A_1 - A_2)/A_0] \times 100\%$, where A_1 represents the absorbance of the sample, A_2 represents the absorbance of the sample except DPPH, and A_0 represents the absorbance of DPPH without the sample

2.4.2. ABTS Radical Scavenging Capacity. On the basis of our published literature with a minor alteration [23], 50.0 mL ABTS solution (7.0 mM) was blended with 50.0 mL potassium persulfate solution (2.45 mM). The mixtures were allowed to stand in the dark at room temperature for 12–16 h

Table 1: Factors and levels of L_9 (3^4) orthogonal experiment.

Level	(A) liquid-solid ratio (mL/g)	(B) extraction temperature (°C)	(C) extraction duration (min)	(D) extraction mode
1	(A_1) 25 : 1	(B_1) 65	(C_1) 45	(D_1) EE
2	(A_2) 30 : 1	(B_2) 70	(C_2) 60	(D_2) UAE
3	(A_3) 35 : 1	(B_3) 75	(C_3) 75	(D_3) EAE

Table 2: Scheme of L_9 (3^4) orthogonal experiment.

Test number	Liquid-solid ratio (mL/g)	Extraction temperature (°C)	Extraction duration (min)	Extraction mode
1	25 : 1	65	45	EE
2	25 : 1	70	60	UAE
3	25 : 1	75	75	EAE
4	30 : 1	65	60	EAE
5	30 : 1	70	75	EE
6	30 : 1	75	45	UAE
7	35 : 1	65	75	UAE
8	35 : 1	70	45	EAE
9	35 : 1	75	60	EE

to acquire the ABTS reaction solution, which was then stored in a brown bottle away from light. Next, 1.0 mL ABTS reaction solution was added to the absolute ethanol and diluted 40- to 50-fold step by step until the absorbance of the solution was 0.7 ± 0.02. Subsequently, 3.9 mL dilution was mixed with 0.1 mL samples in different concentrations. The mixture reacted for 30 min away from light, and the absorbance was determined at 734 nm. The flavonoid solution was substituted with an equal volume of distilled water in the blank group. VC was treated as the positive control. The ABTS scavenging ability was computed as follows:

Scavenging rate $(\%) = [1 - A_1/A_0] \times 100\%$, where A_1 represents the absorbance of the sample and A_0 represents the absorbance of the blank group

2.4.3. Hydroxyl Radical Scavenging Capacity. According to our reported study with a slight correction [23], each flavonoid solution was mixed with $FeSO_4$ (6.0 mM, 2.0 mL) and H_2O_2 (6.0 mM, 2.0 mL) successively and then was incubated for 10 min. After the salicylic acid (6.0 mM, 2.0 mL) was added, the mixture was placed in a thermostatic water bath at 37°C for 30 min. Finally, the absorbance at 510 nm was detected. VC was regarded as the positive control. The hydroxyl radical scavenging capacity was evaluated using the following equation:

Scavenging rate $(\%) = [1 - (A_1 - A_2)/A_0] \times 100\%$, where A_1 represents the absorbance of the sample, A_2 represents the absorbance of the control (distilled water instead of salicylic acid), and A_0 represents the absorbance of the blank (distilled water instead of sample replacement)

2.4.4. Reducing Power. The reducing power was determined using the described method with minor modifications [24]. Each sample solution was mixed thoroughly with phosphoric acid buffer (0.2 M, 2.5 mL, pH 6.6) and potassium ferricyanide (1%, 2.5 mL) and then reacted for 30 min in a thermostatic water bath at 50°C. The mixture was added to trichloroacetic acid (10%, 2.5 mL) to terminate the reaction and then was centrifuged at 3000 r/min for 20 min. Subsequently, 2.5 mL supernatant was mingled thoroughly with 2.5 mL distilled water and $FeCl_3$ (0.1%, 0.5 mL) and stood for 10 min. In the end, the absorbance at 700 nm was measured. VC served as the positive control.

2.5. Level Effect and Engineering Average Estimation. The deviation between the index average corresponding to a certain level of one factor and the population mean is called the level effect and can be calculated using (2). The engineering average is the sum of the level effect in correspondence to the factor with greater influence and the total average value of the index in a combination. The level effect and engineering average can be utilized to estimate the numerical value of the test index for the combination, as shown in (3) [21].

$$EF_{kj} = \frac{r}{N} \sum_{m=1}^{N/r} y_{kj} - \frac{1}{N} \sum_{i=1}^{N} y_i, \tag{2}$$

$$\mu\left(A_i B_j C_k D_m\right) = \frac{1}{N} \sum_{i=1}^{N} y_i + EF_{A_i} + EF_{B_j} + EF_{C_k} + EF_{D_m}. \tag{3}$$

In the above formulas, EF_{kj} is the kth level effect of the jth factor, $\mu(A_iB_jC_kD_m)$ is the engineering average of the combination $A_iB_jC_kD_m$, EF_{A_i} is the ith level effect of factor A, EF_{B_j} is the jth level effect of factor B, EF_{C_k} is the kth level effect of factor C, and EF_{D_m} is the mth level effect of factor D.

2.6. Quantification Theory. The quantification theory belongs to a branch of multivariate statistical analysis. In the quantification theory, both the quantitative and qualitative variables can be considered, and qualitative variables can be transformed into quantitative variables [20, 21]. Therefore,

the quantification theory was adopted for the prediction of CFs yield.

In the quantification theory, the qualitative variable is defined as an item, and the different value of the item is stated as a category. Suppose that there are M items and the Mth item has r_M categories $(c_{M1}, c_{M2}, \ldots, c_{Mr_M})$; the number of categories is $\sum_{j=1}^{M} r_j = h$. $\delta_i(j, k)$ $(i = 1,2, \cdots, Q, j = 1,2, \cdots, M, k = 1,2, \cdots, r_j)$ is the reflection of the kth category of the jth item in the ith sample. When $j = k$, $\delta_i(j, k) = 1$; otherwise, $\delta_i(j, k) = 0$.

For the case of H quantitative variables and M qualitative variables, the quantitative variables in the ith sample are $x_i(u)$ $(u = 1,2, \cdots, H_i, i = 1,2, \cdots, Q)$ and q samples were observed. The measured data formed the reflection matrix as follows:

$$X = \begin{bmatrix} x_1(1), & \cdots, & x_1(H), & \delta_1(1,1) & \cdots & \delta_1(1,r_1) & \cdots & \delta_1(M,r_M) \\ x_2(1), & \cdots, & x_2(H), & \delta_2(1,1) & \cdots & \delta_2(1,r_1) & \cdots & \delta_2(M,r_M) \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ x_q(1), & \cdots, & x_q(H), & \delta_q(1,1) & \cdots & \delta_q(1,r_1) & \cdots & \delta_q(M,r_M) \end{bmatrix}. \tag{4}$$

In the quantification theory, it was supposed that the correlation between the basic variable and the reflection of each item and category conformed to the following linear model:

$$Y_i = d_0 + \sum_{u=1}^{H} b_u x_i(u) + \sum_{j=1}^{M} \sum_{k=1}^{r_j} \delta_i(j,k) b_{jk} + \varepsilon_i, \tag{5}$$

where d_0 is a constant, Y_i is the observed value of the basic variable in the ith sample, b_u and b_{jk} are the unknown coefficients, and ε_i is a random error. In general, the random error can be set as $\varepsilon_i = 0$ to reduce the dimension of (5). By the least square method, d_0, b_u, and b_{jk} are determined. The prediction accuracy of the linear model, similar to the multiple linear regression model, was evaluated using the multiple correlation coefficient R.

2.7. Statistical Analysis. The software SPSS V22.0 was employed for the one-way analysis of variance (ANOVA), Tukey's, and LSD multiple tests and partial correlation analysis, and $P < 0.05$ represents a significant difference.

3. Results and Discussion

3.1. Single-Factor Experiments. The influencing factors of the CFs yield mainly include liquid-solid ratio, extraction temperature, extraction duration, and number of extractions. The single-factor tests were performed to determine the value range of each influence element in the L_9 (3^4) orthogonal experiments, and the results are shown in Figure 1.

3.1.1. Effect of Liquid-Solid Ratio on the Yield of CFs. Liquid-solid ratio impacts the CFs yield. If the liquid-solid ratio is too small, the extraction of flavonoids is incomplete. In contrast, an excessively large liquid-solid ratio will lead to material waste and high costs. Therefore, an appropriate liquid-solid ratio is significant for the extraction of CFs. The extraction yields of CFs with different liquid-solid ratios (5 : 1, 10 : 1, 20 : 1, 30 : 1, and 40 : 1 mL/g) are displayed in Figure 1(a). The other three extraction parameters, namely, extraction temperature (70°C), extraction duration (60 min), and number of extractions (twice), were fixed. The CFs yield noticeably increased from $21.00 \pm 1.78\%$ to $29.83 \pm 0.62\%$ ($P < 0.05$) with the liquid-solid ratio rising from 5 : 1 to 20 : 1 mL/g. It was mainly because when the solvent dosage was small, the flavonoids in the raw materials were not adequately transferred into the solvent, leading to insufficient extraction and low yield of flavonoids; the increase of solvent dosage could enlarge the concentration gradient between the internal plant cells and the external solvent, contributing to rapid diffusion and dissolution of flavonoids [25]. Nevertheless, the yield of CFs was not enhanced distinctly ($P > 0.05$) with the further increase of the liquid-solid ratio. The reason may be that the contact area of CFs with raw materials and the solubility of CFs peaked [26, 27]. Thus, in this study, 20 : 1 mL/g was selected as the optimal liquid-solid ratio for the extraction of CFs. The results were similar to those in other publications on the extraction optimization of celery leaf flavonoids and *Pinus koraiensis* nuts-coated film flavonoids [28, 29].

3.1.2. Effect of Extraction Temperature on the Yield of CFs. Extraction temperature is an important influence factor of the extraction yield of flavonoids. With the other three extraction parameters, namely, liquid-solid ratio (20 : 1 mL/g), extraction duration (60 min), and number of extractions (twice) fixed, the effect of extraction temperature (40°C, 50°C, 60°C, 70°C, and 80°C) on the CFs yield was explored. As shown in Figure 1(b), the yield of CFs was elevated quickly with the extraction temperature rising from 40°C to 70°C and reached the maximum of $30.82 \pm 0.98\%$ at 70°C. The CFs yield at 70°C was higher than those at 40°C, 50°C, and 60°C ($P < 0.05$). The possible reason is that the higher temperature could decrease the viscosity and surface tension of the solution, accelerating the diffusion of the CFs from the cell wall into the solution and improving their dissolvability [30]. The extraction yield of CFs at 80°C was slightly higher than that at 70°C ($P > 0.05$). In consideration of energy consumption, 70°C was determined as the best extraction temperature. The results are consistent with those in the previous research on the extraction of onion peel flavonoids by Thu et al. [30], where the variation tendency of flavonoids yield with the temperature was identical as well.

3.1.3. Effect of Extraction Duration on the Yield of CFs. Extraction duration is a critical element affecting the extraction efficiency and selectivity of the solvent. Longer durations are better for the extraction of flavonoids. The CFs yields corresponding to extraction durations of 15, 30, 60, 90, and 120 min were exhibited in Figure 1(c), with the liquid-solid ratio, extraction temperature, and number of extractions fixed to be 20 : 1 mL/g, 70°C, and 2, respectively. Figure 1(c) shows that the yield of CFs showed the tendency

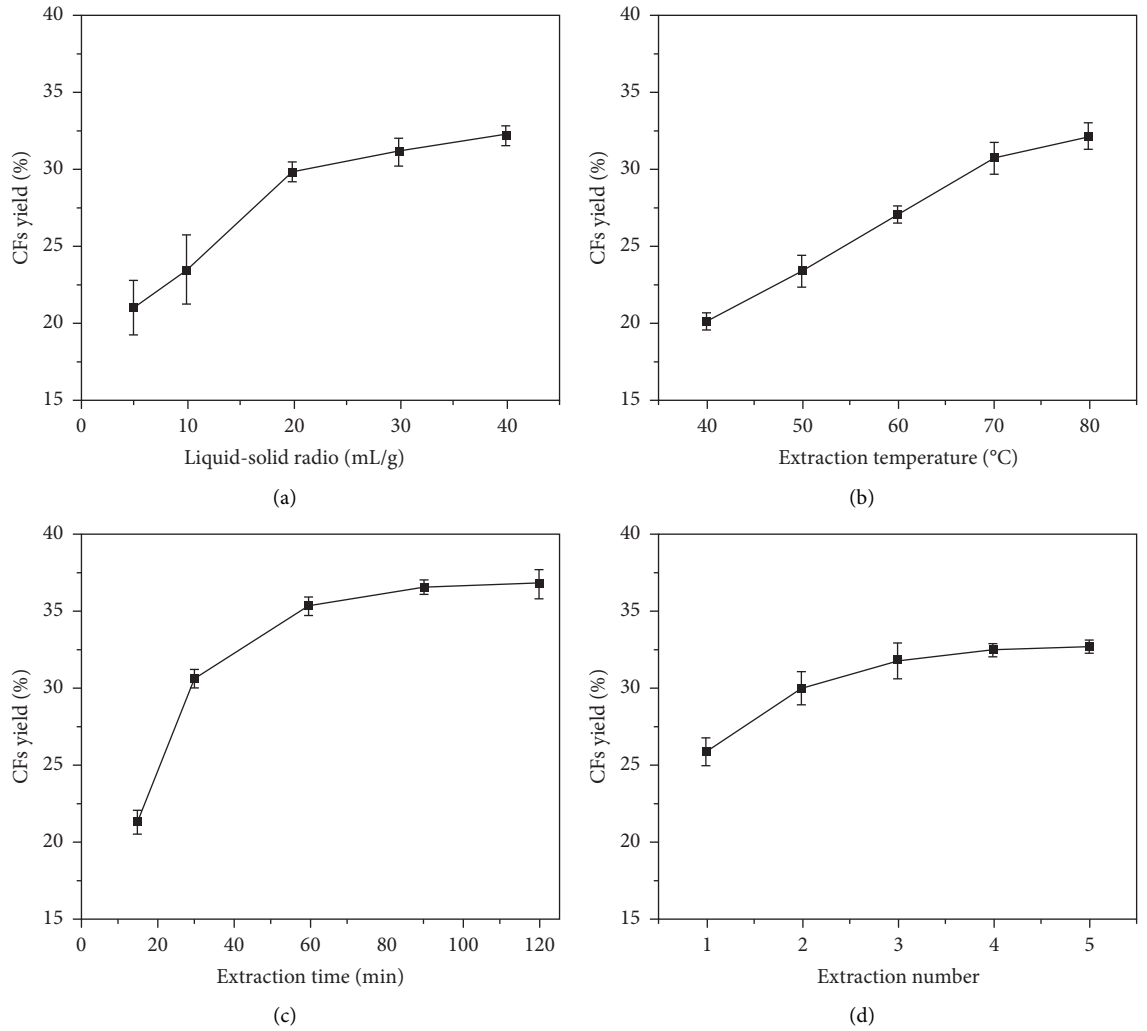

Figure 1: Effects of different extraction parameters on the yield of CFs: liquid-solid ratio (a), extraction temperature (b), extraction duration (c), and number of extractions (d).

of first increasing and then flattening with the extraction duration increasing from 15 to 60 min. The CFs yield reached the peak of $35.27 \pm 0.63\%$ when the extraction duration was 60 min. After 60 min, as time went on, the yield of CFs maintained stability ($P > 0.05$). The variation was identical to that in the report of Shi et al. [26] on total flavonoids extraction from seed skin of *Paeonia lactiflora*. Hence, 60 min was determined as the optimal extraction duration in the current investigation.

3.1.4. Effect of Number of Extractions on the Yield of CFs. The number of extractions is a significant variable influencing the yield of flavonoids. This study characterized the correlation between the number of extractions and the CFs yield. Five numbers of extractions, namely, 1, 2, 3, 4, and 5, were selected. The liquid-solid ratio was fixed to be 20 : 1 mL/g, the extraction temperature was 70°C, and the extraction duration was 60 min. Figure 1(d) shows that there were significant changes in the yield of CFs as the number of extractions increased from 1 to 2. In addition, the CFs yield was $30.00 \pm 1.07\%$ when the number of extractions was 2. There was little augment ($P > 0.05$) with the further increase of the number of extractions. For energy conservation and cost reduction, the optimum number of extractions was determined as 2 in this research, which is consistent with the result in the article about flavonoids extraction [30].

3.2. Extraction Optimization of CFs by Quantification Theory

3.2.1. Range Analysis. Table 3 reveals the range analysis of the orthogonal tests. It was indicated that the ranges R_j of liquid-solid ratio (A), extraction temperature (B), extraction duration (C), and extraction mode (D) were 5.17, 3.96, 3.47, and 2.55, respectively. According to the R_j and k values, which indicate the influence on the yield of CFs, the four factors were in the sequence of $A > B > C > D$. The combination contributing to the highest CFs yield was $A_3B_3C_3D_3$, that is, the liquid-solid ratio of 35 : 1 mL/g, extraction temperature of 75°C, extraction duration of 75 min, and extraction mode of EAE.

Table 3: Range analysis of CFs yield.

Test number	A	*B*	C	*D*	Yield_1 (%)	Yield_2 (%)	Yield_3 (%)	Yield sum (%)
1	1	1	1	1	21.75	22.20	23.45	67.40
2	1	2	2	2	26.86	26.07	25.67	78.60
3	1	3	3	3	33.34	31.38	32.64	97.36
4	2	1	2	3	27.59	26.38	29.76	83.73
5	2	2	3	1	27.38	28.87	29.10	85.35
6	2	3	1	2	27.57	28.45	30.65	86.67
7	3	1	3	2	30.29	33.58	32.70	96.57
8	3	2	1	3	31.41	32.64	29.91	93.96
9	3	3	2	1	31.32	34.40	33.62	99.34
K_1	243.36	247.70	248.03	252.09				
K_2	255.75	257.91	261.67	261.84				
K_3	289.87	283.37	279.28	275.05				
k_1	27.04	27.52	27.56	28.01				
k_2	28.42	28.66	29.07	29.09				
k_3	32.21	31.49	31.03	30.56				
R_j	5.17	3.96	3.47	2.55				
SS_j	128.92	74.99	54.55	29.51				

3.2.2. Variance Analysis. As shown in Table 4, the effects of liquid-solid ratio (*A*), extraction temperature (*B*), extraction duration (*C*), and extraction mode (*D*) on the CFs yield were extremely significant. The variances *F* of the four factors were 36.75, 21.38, 15.55, and 8.41, respectively, indicating that, in terms of the effect on CFs yield, $A > B > C > D$. This result was consistent with that of the range analysis.

3.2.3. Level Effect and Engineering Average Estimation. The optimal combination corresponding to the highest CFs yield was determined through the range and variance analysis and is listed in Table 2. The level effect and engineering average estimation was applied to evaluate the CFs yield of the optimal combination. The level effect of each factor is demonstrated in Table 5.

The CFs yield was $\mu(A_3B_3C_3D_3) = 29.22 + 2.99 + 2.26 + 1.81 + 1.34 = 37.62\%$. Table 4 shows that $SS_e = 31.57$, $df_e = 18$, $F_{0.05}(1, 18) = 4.41$, and n_e (effective number of replicates) = 3.

$\delta_{0.05}(\text{variation range}) = \sqrt{F_{0.05}(1, 18) \times SS_e/df_e \times n_e} = 1.61$. The CFs yield of the combination $A_3B_3C_3D_3$ changed within the ranges of 37.62–1.61 and 37.62 + 1.61, that is, 36.01, 39.32, and the reliability was 95%.

3.2.4. Predictive Model of CFs Yield. According to Table 3, the liquid-solid ratio (*A*), extraction temperature (*B*), and extraction duration (*C*) are quantitative factors, and the extraction mode (*D*) is a qualitative factor. Category 1 is the liquid-solid ratio of 25 : 1 mL/g ($\delta_i(1,1)$), Category 2 is the liquid-solid ratio of 30 : 1 mL/g ($\delta_i(1,2)$), Category 3 is the liquid-solid ratio of 35 : 1 mL/g ($\delta_i(1,3)$), Category 4 is the extraction temperature of 65°C ($\delta_i(2,1)$), Category 5 is the extraction temperature of 70°C ($\delta_i(2,2)$), Category 6 is the extraction temperature of 75°C ($\delta_i(2,3)$), Category 7 is the extraction duration of 45 min ($\delta_i(3,1)$), Category 8 is the extraction duration of 60 min ($\delta_i(3,2)$), Category 9 is the extraction duration of 75 min ($\delta_i(3,3)$), Category 10 is the extraction mode EE ($\delta_i(4,1)$), Category 11 is the extraction mode UAE ($\delta_i(4,2)$), and Category 12 is the extraction mode EAE ($\delta_i(4,3)$). 27 extraction-optimization experiments were executed to determine the values of the parameters of the predictive model (Equation (5)). The prediction equation of CFs yield was acquired as follows:

$$\begin{aligned} Y_i = {} & 37.60 - 5.17\delta_i(1,1) - 3.79\delta_i(1,2) - 3.96\delta_i(2,1) \\ & - 2.83\delta_i(2,2) - 3.47\delta_i(3,1) - 1.96\delta_i(3,2) \\ & - 2.55\delta_i(4,1) - 1.47\delta_i(4,2). \end{aligned} \quad (6)$$

The multiple correlation coefficient *R* is 0.95, and the partial correlation coefficients of the four factors were indicated as follows: r_1 (liquid-solid ratio) = 0.896, r_2 (extraction temperature) = 0.839, r_3 (extraction duration) = 0.796, and r_4 (extraction mode) = 0.695. The *t*-test of the partial correlation coefficient was adopted to verify the accuracy of the established model.

$t = r_4 \cdot \sqrt{n - m - 1/1 - r_4^2} = 0.695 \quad \sqrt{22/1 - 0.695^2} = 4.534 > t_{0.05} = 2.074$, implying that the influences of the four factors on the yield of CFs were significant, and $A > B > C > D$, which was consistent with the range and variance analysis results. The CFs yield of the best combination $A_3B_3C_3D_3$ was $Y_i = 37.62 \in (36.01, 39.32)$. The prediction model of CFs yield constructed by the quantification theory is reliable and the prediction result is accurate.

3.3. In Vitro Antioxidant Activities of CFs

3.3.1. DPPH Radical Scavenging Ability. DPPH is a very stable nitrogen-centered radical. DPPH radical scavenging can reduce peroxidative radicals and interrupt lipid peroxidation chain reaction through hydrogen supply and is characterized by simplicity, rapidity, and sensitivity. DPPH radical scavenging capacity is widely used to indicate the oxidation resistance of various antioxidants [31]. The comparison of the DPPH radical scavenging ability of CFs and six single-ingredient flavonoids with that of VC is exhibited in Figure 2(a). It was suggested that the DPPH

Table 4: Variance analysis of CFs yield.

Source of variance	Sum of deviation squares	Freedom	Mean square	F value	Critical value	Significance level
A	128.92	2	64.46	36.75	$F_{0.01}(2, 18) = 6.01$	***
B	74.99	2	37.50	21.38		***
C	54.55	2	27.27	15.55	$F_{0.05}(2, 18) = 3.56$	***
D	29.51	2	14.75	8.41		***
e (error)	31.57	18	1.75		$F_{0.1}(2, 18) = 2.62$	
Sum	319.54	26				

***Significant level $P < 0.01$. **Significant level $P < 0.05$.

Table 5: Effect values of factors and levels for CFs yield.

Factor	Level	Effect value
A	A_1 (25 : 1 mL/g)	−2.18
	A_2 (30 : 1 mL/g)	−0.80
	A_3 (35 : 1 mL/g)	2.99
B	B_1 (65°C)	−1.70
	B_2 (70°C)	−0.56
	B_3 (75°C)	2.26
C	C_1 (45 min)	−1.66
	C_2 (60 min)	−0.15
	C_3 (75 min)	1.81
D	D_1 (EE)	−1.21
	D_2 (UAE)	−0.13
	D_3 (EAE)	1.34

radical scavenging capacities of CFs and six single-ingredient flavonoids were enhanced gradually with the concentration of flavonoids increasing from 0.05 to 0.4 mg/mL. All samples significantly influenced DPPH radical scavenging, especially those at high concentrations. When the sample concentration was 0.4 mg/mL, CFs and six single-ingredient flavonoids were ranked in descending order of DPPH radical scavenging activity as CFs > hawthorn flavonoids (HTFs) > tartary buckwheat flavonoids (TBFs) > lotus leaf flavonoids (LLFs) > *Poria cocos* flavonoids (PCFs) > cassia seed flavonoids (CSFs) > *Lycium barbarum* flavonoids (LBFs). It was speculated that there might be a strong synergistic effect among the individual components of CFs. The results agree with those in the previous research on buckwheat hull flavonoids [14]. The DPPH radical scavenging rate of CFs achieved 81.82 ± 1.75%, 12.62% lower than that of VC (94.44 ± 1.43%), and is similar to DPPH radical scavenging capability of Fengdan peony flavonoids [32]. The DPPH radical scavenging activities of HTFs and TBFs did not show significant differences ($P > 0.05$), but they were dramatically different from those of LLFs, PCFs, CSFs, and LBFs ($P < 0.01$). The DPPH radical scavenging capacity of LLFs was observably stronger than those of PCFs, CSFs, and LBFs ($P < 0.05$). The DPPH radical scavenging rate of PCFs showed a prominent difference from that of CSFs ($P < 0.05$), and the latter was remarkably higher than the DPPH radical scavenging rate of LBFs ($P < 0.01$), which was merely 43.43 ± 0.71%. The DPPH radical scavenging rate of LBFs was identical to that in the previous research (40.5–54.9%) [10]. It was implied that CFs had a strong DPPH radical scavenging ability because of easily providing the hydrogen atom or electron [33].

3.3.2. ABTS Radical Scavenging Ability. ABTS radical scavenging capacity is broadly applied to indicate the total antioxidant activity of flavonoids [31]. As displayed in Figure 2(b), the ABTS radical scavenging abilities of CFs, six single-ingredient flavonoids, and VC were related to the sample concentration from 0.05 to 0.4 mg/mL. In terms of ABTS radical scavenging activity at 0.4 mg/mL, CFs and six single-ingredient flavonoids were sequenced as LLFs > CFs > TBFs > HTFs > PCFs > CSFs > LBFs. ABTS radical scavenging rate of LLFs reached 69.55 ± 0.52%, and the ABTS radical scavenging rates of all samples were weaker than that of VC (92.89 ± 0.29%). ABTS radical scavenging power of CFs, TBFs, HTFs, PCFs, CSFs, and LBFs showed significant discrepancy ($P < 0.01$). The ABTS radical scavenging rates of CFs and HTFs were 49.35 ± 0.09% and 41.69 ± 0.09% at the concentration of 0.4 mg/mL, respectively, and the former was 1.8 times greater than the latter. This result is in agreement with the ABTS radical scavenging capacity of two *Rubia* fruits flavonoids obtained by Chen et al. [1].

3.3.3. Hydroxyl Radical Scavenging Ability. The hydroxyl radical is the most harmful among all reactive oxygen species. It can destroy macromolecules in the human body, such as carbohydrates, lipids, proteins, amino acids, nucleic acids, and DNA, leading to cell death, tissue disorder, and apoptosis [16]. The hydroxyl radical scavenging helps protect the body from oxidative damage. The comparison of hydroxyl radical scavenging capacities of CFs and six single-ingredient flavonoids with that of VC is manifested in Figure 2(c). In terms of the hydroxyl radical (concentration = 0.4 mg/mL) scavenging activities, the order is CFs > LLFs > CSFs > LBFs > HTFs > TBFs > PCFs. The hydroxyl radical scavenging rate of CFs was 89.78 ± 0.66%, 6.38% higher than that of VC and 1.18 times higher than that of HTFs. There was an obvious difference in the hydroxyl radical scavenging power among LLFs, CSFs, LBFs, and HTFs ($P < 0.01$). The hydroxyl radical scavenging ability of HTFs was distinctly superior to those of TBFs and PCFs ($P < 0.01$). The difference in the hydroxyl radical scavenging rates of TBFs and PCFs was not significant ($P > 0.05$). CFs showed excellent hydroxyl radical scavenging capability at all concentrations in a dose-dependent manner, which surpassed the scavenging capabilities of tremella flavonoids (lower than 30%) [34], peony seed meal flavonoids (70.33%) [35], kale flavonoids (under 60%) [36], and bear bile grass flavonoids (73.79%) [37].

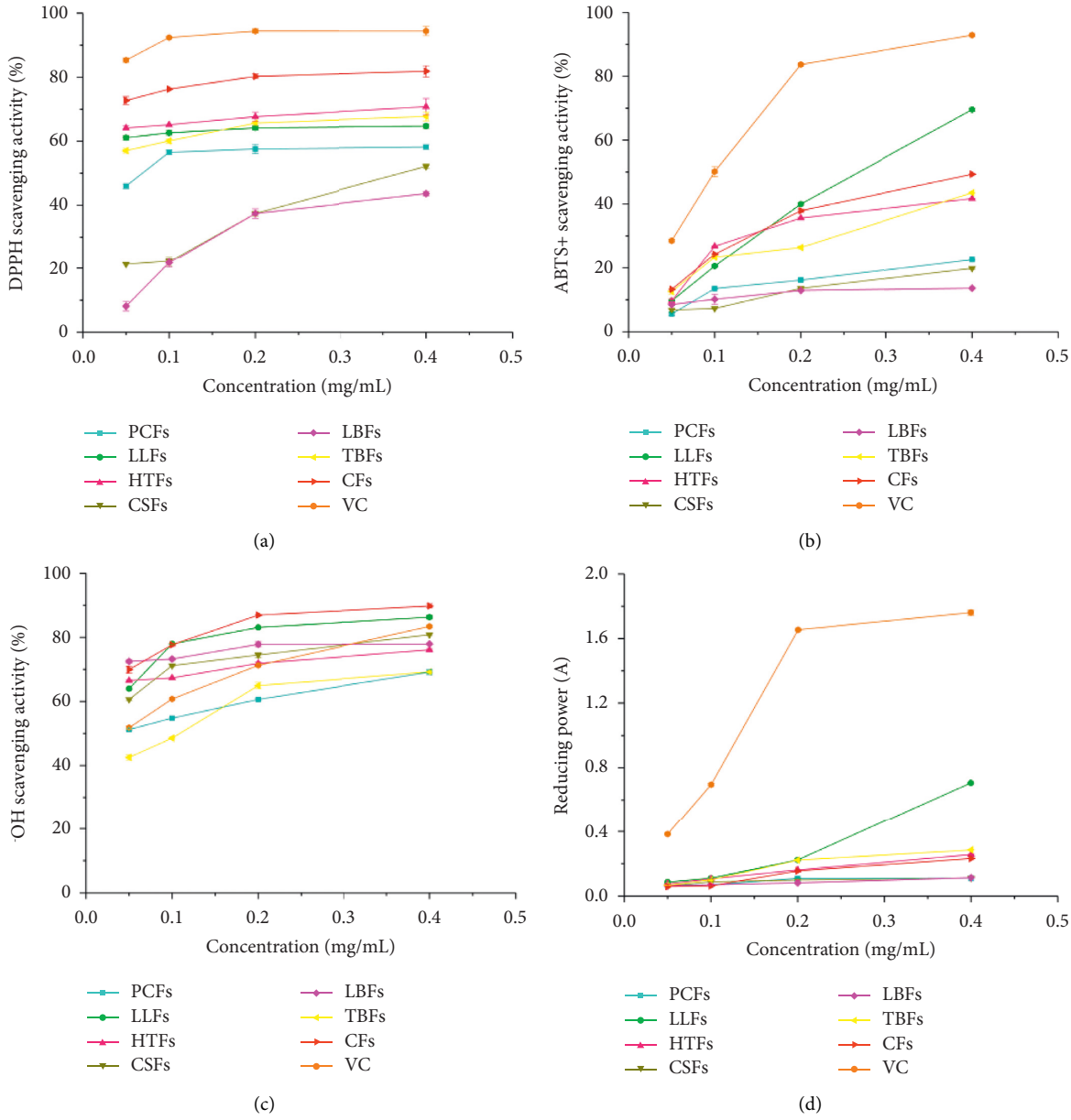

Figure 2: Antioxidant activities of CFs, PCFs, LLFs, HTFs, CSFs, LBFs, TBFs, and VC. DPPH radical scavenging ability (a), ABTS radical scavenging ability (b), hydroxyl radical scavenging ability (c), and reducing power (d).

3.3.4. Reducing Power. The reducing power reflects the potential activity of natural products and phytochemicals. Specifically, a stronger reducing power indicates a higher antioxidant activity. In addition, the absorbance is positively correlated with the reducing power [16]. As shown in Figure 2(d), in terms of the absorbance at the concentration of 0.4 mg/mL, the samples were ranked in descending order as LLFs > TBFs > HTFs > CFs > LBFs > CSFs > PCFs. The corresponding absorbances were 0.706 ± 0.003, 0.285 ± 0.002, 0.259 ± 0.005, 0.232 ± 0.001, 0.112 ± 0.003, 0.111 ± 0.002, and 0.110 ± 0.001. There was a prominent difference in the reducing power among TBFs, HTFs, and CFs ($P < 0.01$). The differences in the reducing power of LBFs, CSFs, and PCFs were not significant ($P > 0.05$). The absorbance of CFs was only 13.17% of that of VC (1.732 ± 0.017), indicating that the reducing power of CFs was relatively low, which is in accordance with the result in the previous report on cherry flavonoids [38]. It was reported that the scavenging free radical activity is not positively correlated with the reducing power capacity because of the influence of impurity and other components, such as sugars and proteins from plant species, which could be involved in the reaction of reducing power assay as well [39, 40].

The changing curve of antioxidant activity and CFs concentration (Figure 2) indicated the correlation between antioxidant capacity and concentration of CFs (Table 6). The consequences hinted the relationship between the

Table 6: The correlation between antioxidant activity and concentration of CFs.

Antioxidant arrays	Optimal fitting functions	Determination coefficients (R^2)
DPPH	$Y_a = 4.5121\text{Ln}(X_c) + 86.613$	0.9707
ABTS	$Y_a = 17.607\text{Ln}(X_c) + 65.62$	0.9983
Hydroxyl	$Y_a = 9.9148\text{Ln}(X_c) + 100.5$	0.9623
Reducing power	$Y_a = 0.5229X_c + 0.0288$	0.9585

X_c is the concentration of CFs and Y_a is the antioxidant activity corresponding to X_c.

scavenging ability for DPPH, ABTS, hydroxyl radical, and the CFs concentration, which kept the logarithmic functions with R^2 of 0.9707, 0.9983, and 0.9623, severally. Furthermore, there was a significant linear relation between the reducing power and the concentration of CFs ($R^2 = 0.9585$).

4. Conclusions

The single-factor experiments, orthogonal tests, and quantification theory were adopted to optimize the extraction procedure of CFs in this study. The influences of limiting factors (liquid-solid ratio, extraction temperature, extraction duration, and extraction mode) on the CFs yield were significant ($P < 0.05$), of which the order was liquid-solid ratio > extraction temperature > extraction duration > extraction mode. The optimal combination of CFs yield was as follows: liquid-solid ratio of 35 : 1 mL/g, extraction temperature of 75°C, extraction duration of 75 min, and extraction mode of enzyme-assisted extraction. The forecasted yield of CFs was 37.62%, which was consistent with the level effect and engineering average estimation. It was indicated that the predictive model was accurate and reliable ($R = 0.95$). In addition, the scavenging capacities of CFs against DPPH, ABTS, and hydroxyl radical were remarkable and CFs had a certain reducing power, which were $81.82 \pm 1.75\%$, $49.35 \pm 0.09\%$, $89.78 \pm 0.66\%$, and 0.232 ± 0.001 at a concentration of 0.4 mg/mL, respectively. The consequences demonstrated that CFs possessed application potential in such fields as functional food and medicine and laid a foundation for further in vivo antioxidant experiments.

Data Availability

The data are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Xu Yang, Haiyu Ji, and Anjun Liu conceived and designed the research. Haiyu Ji, Yingying Feng, and Juan Yu performed the experiments. Xu Yang, Haiyu Ji, and Yingying Feng analyzed the data. Dongli Cao, Haiyu Ji, and Yingying Feng wrote the original draft. Juan Yu and Anjun Liu reviewed and edited the manuscript. Xu Yang, Haiyu Ji, and Yingying Feng contributed equally to this work.

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