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Research Article

Simultaneous Determination of Content and Antioxidant Activity of Five Components in *Hibiscus mutabilis L* by HPLC-QAMS

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Based on high performance liquid chromatography (HPLC), a method for simultaneous determination of five active components in *Hibiscus mutabilis L* was established by quantitative analysis of multicomponents by single marker (QAMS). This method was used to evaluate the quality of *Hibiscus mutabilis L*. In the study, quercetin was used as the internal standard, and the relative correction factors (RCF) of rutin, protocatechuic acid, catechin, and luteolin were calculated, and the contents of five components were determined simultaneously by quercetin. Compared with the traditional external standard method (ESM), this method had less error and higher feasibility, and the methodological experiments showed that the five components had a great linear relationship within their respective concentration ranges ($r \geq 0.9995$). The average recovery was 96.97%-98.85% (RSD was 0.88%-1.81%), precision (RSD ≦ 1.83%), repeatability (RSD ≦ 1.87%), and stability (RSD ≦ 1.51%) were great. In this experiment, the contents of these five active components in *Hibiscus mutabilis L* from five producing areas were determined. Finally, the antioxidant capacity of *Hibiscus mutabilis L* was determined to determine its antioxidant activity.

1. Introduction

Hibiscus mutabilis L is a Malvaceae plant, mainly produced in East China, South Central, Southwest China, Liaoning, Hebei, Shaanxi, and other places. The roots, leaves, and flowers of *Hibiscus mutabilis L* can be used as medicine. It is also included in the 2020 edition of Chinese Pharmacopoeia [1], which has the effects of cooling blood, detoxification, detumescence, and relieving pain. It mainly treats carbuncle and swelling, scald, eye red swelling and pain, and falling injury [2–4]. *Hibiscus mutabilis L* contains a variety of active ingredients, including flavonoids, organic acids, volatile components, and other components such as stigma, anthraquinone, coumarin, triterpenes, lignans, and inorganic elements [5]. Flavonoids are the most important active components[6].

The quality evaluation index of *Hibiscus mutabilis L* is limited to the content of rutin in the 2020 edition of Chinese Pharmacopoeia and current literature reports [7–9], ignoring the special points of multicomponents and multitargets of traditional Chinese medicine. It contains a variety of components related to the efficacy of *Hibiscus mutabilis L*, the main components are rutin, protocatechuic acid, catechin, quercetin, luteolin, and other components [10–13]. The five components have significant anti-inflammation and analgesia effects [14–16], which directly affect the quality of medicinal materials. The structure of each component is shown in Figure 1.

The components of traditional Chinese medicine are complex and diverse. In addition, it is impossible to achieve satisfactory results only by relying on the quantification of single components. As a consequence, the construction of a multi-

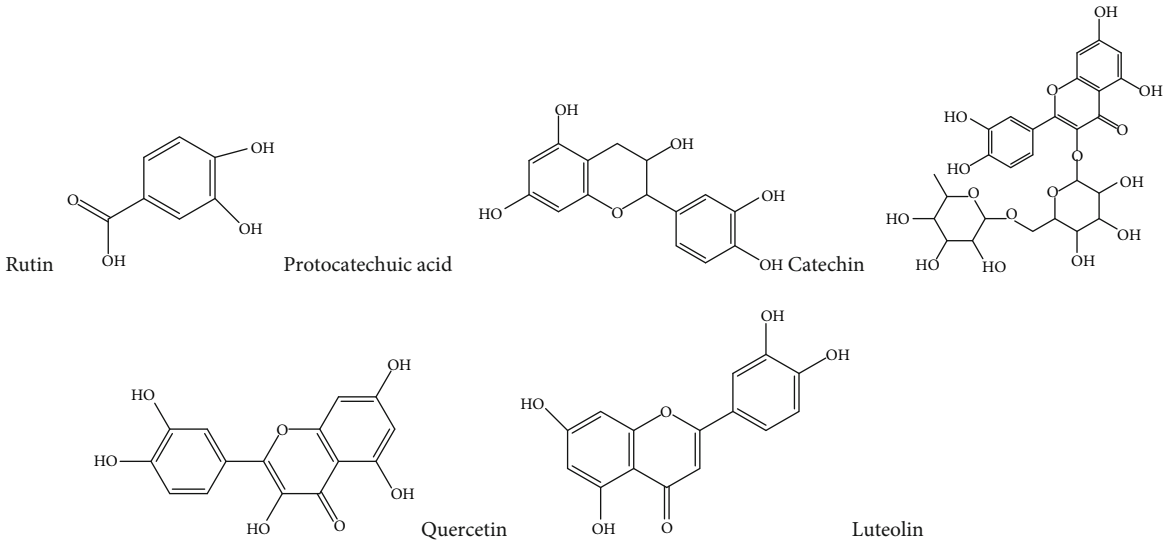

Figure 1: Structure diagram of each component.

index and multicomponent quality control system related to its efficacy has been developed [17–19]. However, traditional Chinese medicine has complex active components, low content, and unstable components, and the high price of traditional Chinese medicine reference substances is due to its own chemical properties and the difficulty of separation. It is always in a state that it cannot be supplied in large quantities, stably and cheaply, so that the analysis of multi-index components is limited to a certain extent, and the quality control evaluation of traditional Chinese medicine is faced with great challenges [20]. In recent years, one test and multiple evaluation (QAMS) is recognized as an effective means to solve this problem. Making use of the inherent functional relationship among the active components contained in traditional Chinese medicine, QAMS [21, 22] realizes the simultaneous determination of multi-index components in traditional Chinese medicine by determining a stable and easily available component in traditional Chinese medicine, which reduces the inspection cost of the traditional multicomponent quality control model. In addition, it effectively avoids the shortcomings of accurate quantitative determination because of the unstable quality of some components.

The contents of rutin, protocatechuic acid, catechin, quercetin, and luteolin in *Hibiscus mutabilis L* were determined simultaneously by HPLC-QAMS method with quercetin as the internal standard, and the feasibility of the method was verified. The results showed that the linear relationship, stability, precision, repeatability, and recovery rate of this method were great, and there was little difference between the determination results and the traditional external standard method. Finally, the antioxidant capacity of *Hibiscus mutabilis L* was determined by DPPH method and $ABTS^{+}$ method, and its antioxidant activity in vitro was determined. This study provides a reference for quality evaluation and clinical application of *Hibiscus mutabilis L*.

2. Materials and Methods

2.1. Instrument. Agilent 1260 high performance liquid chromatograph was purchased from Agilent Technology Co., Ltd.; KQ-250 ultrasonic cleaner was purchased from Kunshan Ultrasonic Instrument Co., Ltd.; FA1004B electronic analytical balance was purchased from Shanghai Youke Instrument Co., Ltd.; AB135-S electronic analytical balance was purchased from Mettler-Toledo International Co., Ltd; and DFY-500 swing multifunction and high-speed traditional Chinese medicine grinder was purchased from Wenzhou Dingli Medical Devices Co., Ltd.

2.2. Test Drugs and Reagents. Hibiscus mutabilis L is derived from *Hibiscus mutabilis L* dried leaves. The specific origin and batch number are shown in Table 1.

Protocatechuic acid (batch number: 110809-201205, content 99.9%), catechin (batch number: 110877-201604, content 99.2%), rutin (batch number: 100080-201811, content 91.7%), quercetin (batch number: 100081-201610, content 99.1%), and luteolin (batch number: 111520-201605, content 99.6%) were purchased from the China Institute for Food and Drug Control.

Methanol was purchased from Saimer Fischer Technology (China) Co., Ltd.; phosphoric acid was purchased from Beijing Chemical Plant; and pure water was purchased from Hangzhou Wahaha Co., Ltd.

2.3. Chromatographic Conditions. The chromatographic column was Phenomenex C_{18} column (4.6 mm × 250 mm, 5 μm), and the mobile phase was methanol (A)-0.1% phosphoric acid (B). Besides, the gradient elution was performed (0 ~ 7 min 5%A 7 ~ 10 min 5% →15%A 10~20 min 15%A 20~25min 15% →31%A 25~32 min 31%A 32~40 min 31% →47%A 40~55 min 47% →56%A 55~55.1 min 56% →100%A 55.1 ~ 60 min 100%A), the flow rate was 1 mL·min-1, the column temperature was 30°C, the injection volume was 10 μL, and the detection wavelength was 265 nm.

2.4. Preparation of Solution

2.4.1. Mixed Reference Solution. Protocatechuic acid and quercetin were precisely weighed and dissolved with

Table 1: Content of each component in *Hibiscus mutabilis L* of different venues (μg/g, $n = 3$).

(a)

Origin	Batch	Quercetin	Protocatechuic acid			Catechin		
		ESM	ESM	QAMS	RAD/%	ESM	QAMS	RAD/%
S1 (Zhejiang Wenzhou)	20180801	24.090	6.132	6.102	-0.50	63.072	63.843	1.22
S2 (Zhejiang Linan)	20180601	27.263	7.153	7.177	0.34	67.227	67.015	-0.32
S3 (Zhejiang Tonglu)	20180802	25.962	5.872	5.812	-1.02	45.417	46.176	1.67
S4 (Guangxi Lingui)	20180701	26.339	5.991	6.092	1.69	60.931	61.754	1.35
S5 (Yunnan Jinghong)	20180702	19.996	8.127	8.148	0.26	61.569	62.138	0.92

(b)

Origin	Batch	Rutin			Luteolin		
		ESM	QAMS	RAD/%	ESM	QAMS	RAD/%
S1 (Zhejiang Wenzhou)	20180801	782.022	784.269	0.29	26.483	25.959	-1.98
S2 (Zhejiang Linan)	20180601	841.116	849.947	1.05	21.444	21.701	1.20
S3 (Zhejiang Tonglu)	20180802	569.264	577.435	1.44	18.062	18.260	1.10
S4 (Guangxi Lingui)	20180701	971.558	976.806	0.54	24.985	24.714	-1.08
S5 (Yunnan Jinghong)	20180702	700.035	699.334	-0.10	20.671	20.453	1.05

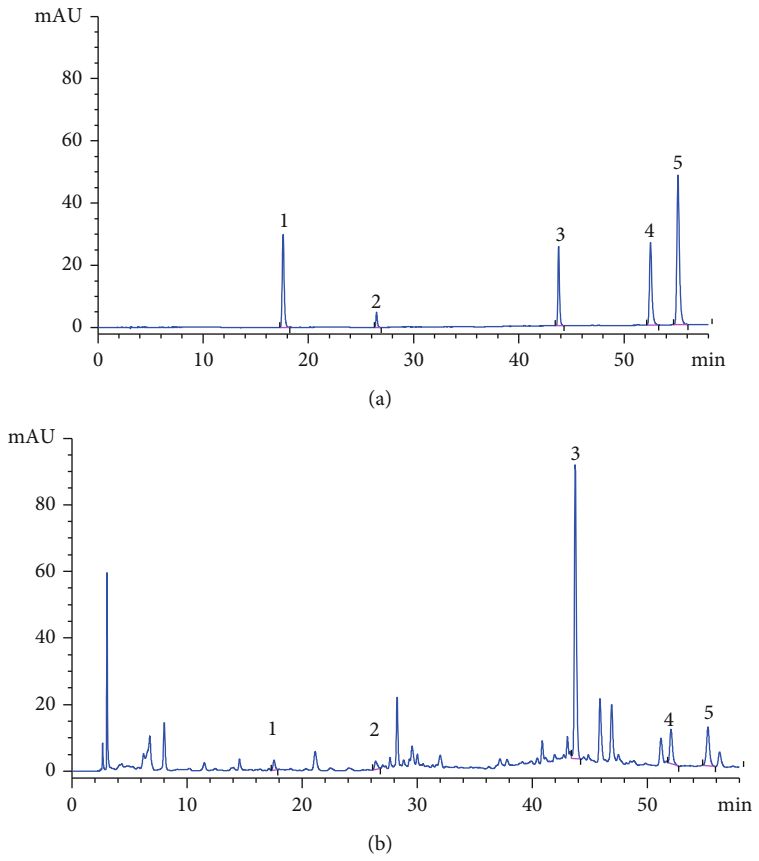

Figure 2: HPLC chromatogram (a) mixed reference substance (b) *Hibiscus mutabilis L*.

methanol to prepare a solution containing 1.788 mg protocatechuic acid and 2.01 mg quercetin per 1 mL. The amount of catechin, rutin, and luteolin was precisely weighed, put into a 25mL volumetric bottle, and then each 1 mL of the above two solutions was precisely removed and dissolved with methanol to obtain a mixed reference mother liquor containing protocatechuic acid 71.52 μg, catechin 210.0 μg, rutin 977.2 μg, quercetin 80.4 μg, and luteolin 250.4 μg per 1 mL.

Table 2: Linear relationship of components.

Components	Regression equation	Linear range/(μg/mL)	r	LOD (μg/mL)	LOQ (μg/mL)
Protocatechuic acid	$y = 18.537x + 36.306$	1.430~ 71.52	0.9995	0.11	0.36
Catechin	$y = 1.8466x + 9.9157$	4.200~ 210.0	0.9996	0.37	1.22
Rutin	$y = 11.157x - 35.514$	19.544~ 977.2	0.9999	0.21	0.69
Quercetin	$y = 21.435x - 11.379$	1.608~ 80.4	0.9997	0.14	0.46
Luteolin	$y = 28.508x + 152.29$	5.008~250.4	0.9996	0.24	0.79

Table 3: Results of sample recovery of each component.

Components	Original quantity/μg	Adding quantity/μg	Measured quantity/μg	Recovery rate/%	Average recovery rate/%	RSD /%
Protocatechuic acid	15.221	15.09	30.098	98.59	98.85	1.30
	14.954	15.09	29.525	96.56		
	13.113	15.09	27.993	98.61		
	15.228	15.09	30.227	99.40		
	15.876	15.09	30.996	100.20		
	14.953	15.09	30.006	99.75		
Catechin	153.843	154.8	304.799	97.52	97.60	0.88
	155.116	154.8	304.115	96.25		
	149.375	154.8	300.185	97.42		
	150.610	154.8	301.364	97.39		
	148.603	154.8	300.706	98.26		
	148.226	154.8	301.102	98.76		
Rutin	1921.910	1965	3903.125	100.83	98.80	1.81
	1866.231	1965	3810.418	98.94		
	1773.257	1965	3684.005	97.24		
	1833.025	1965	3732.736	96.68		
	2006.813	1965	3990.11	100.93		
	1992.604	1965	3922.047	98.19		
Quercetin	61.705	62.4	122.022	96.66	96.97	1.11
	57.359	62.4	117.072	95.69		
	55.005	62.4	115.081	96.28		
	61.917	62.4	122.972	97.84		
	62.532	62.4	122.899	96.74		
	60.277	62.4	121.816	98.62		
Luteolin	54.998	50.5	105.221	99.45	99.37	1.38
	49.253	50.5	98.403	97.33		
	46.071	50.5	95.733	98.34		
	54.774	50.5	105.915	101.27		
	55.163	50.5	105.534	99.74		
	53.275	50.5	103.806	100.06		

The mixed control solution containing protocatechuic acid 7.152 μg, catechin 21.00 μg, rutin 97.72 μg, quercetin 8.04 μg, and luteolin 25.04 μg per 1 mL was prepared by accurately absorbing the mixed reference mother liquid 1 mL, putting it in a 10 mL measuring bottle and diluting methanol to the scale.

2.4.2. Sample Solution. 5 g of *Hibiscus mutabilis L* was crushed, weighed, and put in a cone bottle with a stopper. Besides, it was added methanol 50 mL, and ultrasonic (250 W☒ 40 kHz) extrassction 30 min with filter, extracting thrice, and combining filtrate; rotating evaporation solvent, transferring to 5 mL measuring bottle, using methanol to

Table 4: Relative correction factors of various constituents.

No.	Relative correction factor			
	Protocatechuic acid	Catechin	Rutin	Luteolin
1	0.9191	0.0937	0.2002	1.4395
2	0.8937	0.0914	0.1976	1.4029
3	0.9025	0.0942	0.1937	1.4046
4	0.9138	0.0937	0.2015	1.4385
5	0.9044	0.0908	0.1969	1.4232
6	0.8896	0.0914	0.1924	1.4288
Average	0.9039	0.0925	0.1971	1.4229
RSD/%	1.25	1.61	1.80	1.13

fix volume to 5 mL, and getting *Hibiscus mutabilis L* sample solution.

2.5. Study on Antioxidant Activity. 0.5 g *Hibiscus mutabilis L* coarse powder was taken, adding methanol 20 mL with ultrasonic treatment 20 min, filter residue plus 20 mL methanol ultrasonic 20 min, filtering, merging filtrate twice, steaming dry in water bath pot, dissolving residue with appropriate amount of methanol, transferring to a 5mL volumetric flask, adding methanol to calibration, shaking well, and mixing concentration 100 mg/mL solution. Then, it was diluted with methanol into 10 solutions of different concentrations.

2.5.1. Antioxidation Experiment of DPPH Radical. DPPH solution was prepared and weighed 5 mg precisely, dissolving it with methanol, transferring it to a 100 mL volumetric bottle, adding methanol to fix volume, keeping away from light, and preparing when necessary. The absorbance (A) of the reaction solution was determined by adding to 96-well plate and incubating 30 min without light in 517 nm. DPPH clearance rate was calculated.

$$\text{DPPH scavenging rate} = 1 - \frac{(A_1 - A_2)}{A_0 \times 100\%}, \quad (1)$$

where A_0 is equal to 100 μL DPPH + 100 μL methanol, A_1 is equal to 100 μL DPPH + 100 μL sample, and A_2 is equal to 100 μL sample + 100 μL methanol.

2.5.2. Antioxidation Experiment of ABTS Radicasl. 7.5 mg $K_2S_2O_8$ was fixed volume to 10 mL capacity bottle. 38 mg ABTS was fixed volume to 10 mL capacity bottle. The mixed solution of the two was placed in a cool place for 12 hours to 16 hours before use, so that a complete and full reaction occurred between the two. In addition, the original solution was diluted with methanol and detected at the wavelength 734 nm until the final absorbance value was between 0.70 ± 0.2, that is, the preparation of the solution was ompleted.

$$\text{ABTS}^{+}\text{scavenging rate} = 1 - \frac{(A_1 - A_2)}{A_0 \times 100\%}, \quad (2)$$

where A_0 is equal to 150μLABTS + 50 μL methanol, A_1 is equal to 150μLABTS + 50 μL sample, and A_2 is equal to 50 μL sample + 150 μL methanol.

3. Results and Discussion

3.1. System Adaptability. 10 μL of reference solution and 10 μL of test solution were precisely absorbed under "2.4" and analyzed according to the chromatographic conditions of "2.3". As shown in Figure 2, the chromatographic peaks of protocatechuic acid, catechin, rutin, quercetin, and luteolin in mixed control solution and test solution were well separated. In addition, the number of theoretical plates was more than 3000, indicating that this method had great specificity.

1-Protocatechuic acid 2-Catechin 3-Rutin 4-Quercetin5-Luteolin.

3.2. Methodological Experiment

3.2.1. Linear Relation Test. The mother liquor of the mixed reference substance 0.02, 0.04, 0.1, 0.2, 0.4, and 1.0 mL was precisely absorbed and put into the 1 mL measuring bottle, diluted to the scale with methanol, and shaken well. In addition, the mixed reference solution with different mass concentration was prepared. 10 μL of each of the above six solutions were absorbed and injected into the HPLC. With the concentration as the Abscissa (x) and the peak area integral as the ordinate (y), the standard curve was drawn and the regression equation was calculated. The results are shown in Table 2. The five components showed a great linear relationship in the range of their respective mass concentrations.

3.2.2. Precision Test. The 10 μL solution of the same mixed reference substance was precisely absorbed and injected into the HPLC for 6 times, and the chromatographic peak area was recorded. The RSD values of peak areas of protocatechuic acid, catechin, rutin, quercetin, and luteolin were 1.78%, 1.65%, 1.22%, 1.58%, and 1.83%, indicating that the precision of the instrument was great.

3.2.3. Repetitive Test. Six samples of the same batch of *Hibiscus mutabilis L* (serial number: S1) were prepared in parallel according to the method of "2.4.2". 10 μL of each sample was precisely absorbed and injected into the HPLC, and the chromatographic peak area was recorded. The average mass fraction of protocatechuic acid, catechin, rutin, quercetin, and luteolin were 4.088, 42.048, 488.764, 16.060, 14.322 μg/mg, and the RSD was 1.87%, 1.35%, 1.41%, 1.80%, and 1.75%, which indicated that the method had great reproducibility.

3.2.4. Stability Test. 10 μL of each sample solution of the same *Hibiscus mutabilis L* (serial number: S1) was precisely absorbed and injected into the HPLC at 0, 2, 4, 6, 8, 12 h, and 24 h after preparation, and the chromatographic peak area was recorded. The RSD of the average mass fraction of protocatechuic acid, catechin, rutin, quercetin, and luteolin were 1.46%, 1.33%, 1.03%, 1.51%, and 1.11%, indicating that the solution was stable within 24 hours.

3.2.5. Sample Recovery Test. Six known *Hibiscus mutabilis L* sample powders (about 2.5 g each) were precisely weighed and added to 1.006 mg/mL protocatechuic acid 10 μL,

Table 5: Effects of different instruments and columns on relative correction factors.

Instrument	Chromatographic column	Relative correction factor			
		Protocatechuic acid	Catechin	Rutin	Luteolin
Agilent 1260	Agilent ZORBAX SB-C_{18}	0.9045	0.0917	0.1996	1.3902
	Agilent TC-C_{18}	0.9228	0.0931	0.2011	1.4133
	Phenomenex C_{18}	0.9134	0.0938	0.2002	1.4405
Shimadzu LC-2030	Agilent ZORBAX SB-C_{18}	0.8904	0.0892	0.1970	1.4007
	Agilent TC-C_{18}	0.9173	0.0916	0.2003	1.4328
	Phenomenex C_{18}	0.9008	0.0905	0.1975	1.4216
Average		0.9082	0.0917	0.1993	1.4165
RSD/%		1.31	1.83	0.83	1.35

Table 6: Effects of different column temperatures on relative correction factors.

Column temperature	Relative correction factor			
	Protocatechuic acid	Catechin	Rutin	Luteolin
25°C	0.9026	0.0944	0.1999	1.4425
30°C	0.9211	0.0928	0.2023	1.4298
35°C	0.9135	0.0951	0.2021	1.4469
Average	0.9124	0.0941	0.2014	1.4397
RSD/%	1.02	1.25	0.66	0.62

Table 7: Effects of different volume flow rates on relative correction factors.

Flow rate/mL/min	Relative correction factor			
	Protocatechuic acid	Catechin	Rutin	Luteolin
0.9	0.9172	0.0937	0.2016	1.4403
1.0	0.9046	0.0908	0.2004	1.4217
1.1	0.9061	0.0912	0.2015	1.4389
Average	0.9093	0.0919	0.2012	1.4336
RSD/%	0.76	0.76	0.33	0.72

1.032 mg/mL catechin 100 μL, 1.350 mg/mL rutin 1 mL, 1.010 mg/mL quercetin 40 μL, and 1.010 mg/mL luteolin 40 μL in the same cone bottle. It was supplemented with methanol to 20 mL, and then, the sample solution was prepared according to the method of "2.2.2" and injected into the sample for analysis. The results showed that the average recoveries of protocatechuic acid, catechin, rutin, quercetin, and luteolin were 98.85%, 97.60%, 98.80%, 96.97%, 99.37%, and RSD were 1.30%, 0.88%, 1.81%, 1.11%, and 1.38%, respectively, indicating that the method was accurate (Table 3).

3.3. QAMS Method

3.3.1. Calculation of RCF. The mixed reference solution of item "2.4.1" was determined, and the relative correction factor $f_{k/s}$ of the other four components was calculated with quercetin as the internal standard material. The formula was $f_{k/s} = f_k/f_s = (W_k A_s)/(W_s A_k)$, where (W_k) was the mass concentration of internal standard, A_k was the peak area of internal standard, W_s was the mass concentration of other components, and A_s was the peak area of other components. The results were shown in Table 4.

3.3.2. Durability Inspection

(1) Different Instruments and Chromatographic Columns. The mixed reference solution of item "2.4.1" was determined in accordance with the law. Besides, the effects of chromatograph and chromatographic column on the relative correction factor were investigated. The results were shown in Table 5, which showed that there was no significant effect.

(2) Column Temperature. The mixed reference solution of item "2.4.1" was determined in accordance with the law. In addition, the effects of column temperature at 25°C, 30°C, and 35°C on the relative correction factor were investigated, respectively. The results were shown in Table 6, which showed that there was no significant effect.

(3) Volume Flow. The mixed reference solution of item "2.4.1" was determined in accordance with the law. In addition, the effects of 0.9, 1.0, and 1.1 mL/min volume flow rate on the relative correction factor were investigated, respectively. The results were shown in Table 7, which showed that there was no significant effect.

3.3.3. Location of the Chromatographic Peak of Measured Components. The mixed reference solution of item "2.4.1" was determined according to the law. Moreover, the relative retention time ($t_{k/s}$) method was used to locate the chromatographic peaks of the other five components with Quercetin as the internal standard. The results were shown in Table 8, which showed that different liquid chromatographic instruments and chromatographic columns from different manufacturers had no obvious effect on the relative retention time of the components to be tested.

3.4. Comparison of the Results between QAMS and ESM. *Hibiscus mutabilis L* from Zhejiang, Guangxi, and Yunnan provinces in China was selected and the contents of protocatechuic aldehyde, catechin, rutin, quercetin, and luteolin were analyzed. The content and relative error (RAD) are calculated by one test multiple evaluation method (QAMS) and

Table 8: Relative retention values of various constituents.

Instrument	Chromatographic column	Relative retention values			
		Protocatechuic acid	Catechin	Rutin	Luteolin
Agilent 1260	Agilent ZORBAX SB-C_{18}	2.9839	1.9830	1.1995	0.9527
	Agilent TC-C_{18}	2.9846	1.9964	1.2021	0.9608
	Phenomenex C_{18}	2.9735	1.9803	1.1992	0.9588
Shimadzu LC-2010	Agilent ZORBAX SB-C_{18}	2.9817	1.9932	1.2037	0.9521
	Agilent TC-C_{18}	2.9742	1.9837	1.1926	0.9594
	Phenomenex C_{18}	2.9801	1.9929	1.2134	0.9625
Average		2.9797	1.9883	1.2018	0.9577
RSD/%		0.16	0.34	0.57	0.45

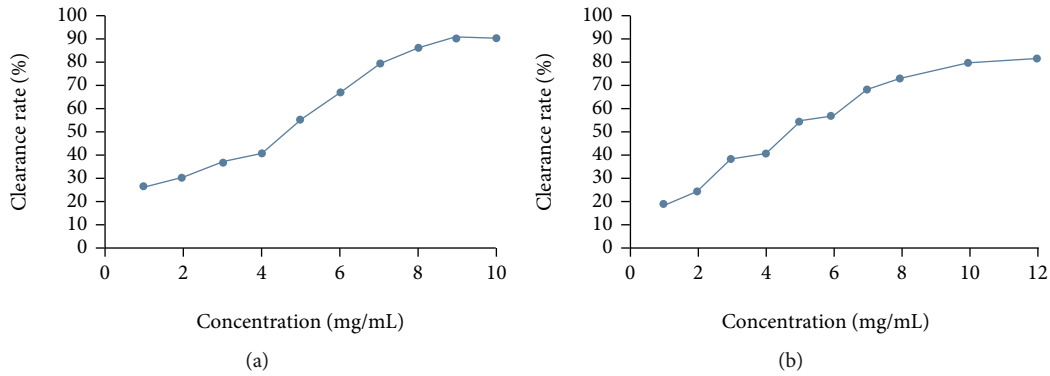

Figure 3: Clearance of DPPH·(a) and $ABTS^{+}$·(b) by *Hibiscus mutabilis L.*

external standard method (ESM), respectively, and the formula is RAD% = [(QAMS calculated value − ESM measured value)/ESM measured value] × 100%. The results were shown in Table 1. It can be known that there was no significant difference between the two methods.

3.5. Study on Antioxidant Activity

3.5.1. Determination of Scavenging Ability of Hibiscus mutabilis L to DPPH Radical. As shown in Figure 3(a), the scavenging rate of DPPH increased with the increase of mass concentration of *Hibiscus mutabilis L*, and the curve tended to smooth when the concentration reaches 10 mg/mL, indicating that in a certain concentration range, there was a dose-effect relationship between the scavenging ability of DPPH and *Hibiscus mutabilis L* concentration. The maximum clearance rate was 91.39%.

3.5.2. Determination of Scavenging Ability of Hibiscus mutabilis L to $ABTS^{+}$ Radical. As shown in Figure 3(b), in the range of experimental concentration, with the increase of *Hibiscus mutabilis L* concentration, the scavenging ability of $ABTS^{+}$ showed a logarithmic trend, the maximum scavenging rate was 86.47% (*Hibiscus mutabilis L* concentration was 12 mg/mL).

3.6. Optimization of Sample Preparation. The extraction method, extraction solvent, extraction solvent volume, and extraction time were investigated by a single-factor method in the aspect of extraction process optimization. In the investigation of extraction methods, the commonly used extraction methods of thermal reflux method and ultrasonic extraction method were compared, and it was found that the extraction effect of the two methods was similar. However, the extraction time of ultrasonic extraction method was shorter and the operation was simpler. In the selection of extraction solvents, the extraction effects of commonly used extraction solvents such as methanol, ethanol, water, 90% ethanol, 90% methanol, and chloroform were investigated. Besides, it was found that the extraction effect of methanol was better. 30 mL, 40 mL, 50 mL, and 60 mL were investigated in the volume of extraction solvent, the extraction times were 2, 3, and 4 times, and the extraction time was 10 min, 20 min, 30 min, and 40 min. The best extraction solvent 50 mL, extraction time 30 min, and extraction times 3 times were determined. This method not only had a great extraction effect but also had high efficiency.

3.7. Optimization of Chromatographic Method. DAD detector [23] was used to investigate the absorption of protocatechuic acid, catechin, rutin, quercetin, and luteolin at the detection wavelength of 225, 256, 265, 324, and 374 nm. The results showed that under the detection wavelength of 265 nm, the five components to be tested all had large absorption. As a consequence, 265 nm was chosen as the detection wavelength in this experiment.

A variety of mobile phase systems such as acetonitrile-water, acetonitrile-0.1% phosphoric acid solution, methanol-water, and methanol-0.1% phosphoric acid solution were investigated[24]. As a result, compared with acetonitrile-water, acetonitrile-0.1% phosphoric acid solution, and methanol water, taking methanol-0.1% phosphoric acid solution as mobile phase, under the condition of "2.3" gradient elution, the baseline was stable, the peak shape of each component to be tested was great, and the degree of separation was high. As a consequence, methanol-0.1% phosphoric acid solution was selected as the *Hibiscus mutabilis L* mobile phase system.

4. Conclusion

Hibiscus mutabilis L is a safe, nontoxic, and commonly used traditional Chinese medicine with good efficacy. For the first time, HPLC-QAMS was used to determine the contents of rutin, protocatechuic acid, catechin, quercetin, and luteolin in *Hibiscus mutabilis L*. In addition, the quality control method of traditional Chinese medicine *Hibiscus mutabilis L* was established. This method is accurate and efficient, and only one standard can be used for the determination of five components, which greatly saves the cost and greatly improves the quality control method of the original 2020 edition of Chinese Pharmacopoeia. Through the experimental results, it is found that the quality and producing area have little influence. As a consequence, the producing area can be taken as the secondary index in the selection. The antioxidant capacity of *Hibiscus mutabilis L* was determined by DPPH and ABTS in vitro, which is of reference significance for the pharmacodynamic study and product application of *Hibiscus mutabilis L*.

Data Availability

The main table and figure data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no competing financial interests.

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