ORIGINAL ARTICLE

A C G publications

J. Chem. Metrol. 18:2 (2024) 134-145

journal of chemical metrology

Optimization of ultrasound-assisted extraction and quantification of rutin and quercetin in *Flos Styphnolobii japonici imaturi* by validated UHPLC-UV method

Tuyet-Ngan Duong [1], Ngoc-Van Thi Nguyen [2*], Phi-Long Ha [1] and Trung-Kien Nguyen [3]

[1] *College of Traditional Medicine, Can Tho University of Medicine and Pharmacy, Ninh Kieu District, Can Tho City 94000, Vietnam*
[2] *College of Pharmacy, Can Tho University of Medicine and Pharmacy, Ninh Kieu District, Can Tho City 94000, Vietnam*
[3] *College of Medicine, Can Tho University of Medicine and Pharmacy, Ninh Kieu District, Can Tho City 94000, Vietnam*

(Received October 19, 2024; Revised December 05, 2024; Accepted December 09, 2024)

Abstract: This study focuses on enhancing the extraction process of rutin and quercetin from *Flos Styphnolobii japonici imaturi* using ultrasound-assisted extraction (UAE) combined with response surface methodology (RSM). By analyzing 3D surface plots and performing regression analysis on the independent variables, the ideal extraction conditions were determined: 96% methanol concentration, a liquid-to-solid ratio of 19:1 (mL/g, v/w), and an ultrasound duration of 19 minutes. These optimized parameters were then applied to validate the quantification process. The bioactive compounds in the *Flos Styphnolobii japonici imaturi* extract were identified using ultra-high-performance liquid chromatography coupled with an ultraviolet absorption detector. This methodology was employed to quantify the rutin and quercetin content from samples collected across seven different locations in Vietnam. The results revealed that the concentrations of these compounds in the methanol extracts ranged from 0.325% to 35.73%. This study provides a scientific basis for optimizing the extraction process and contributes to the application of *F. Styphnolobii imaturi* in medicinal use.

Keywords: *Flos Styphnolobii imaturi*; rutin; quercetin; response surface methodology.

1. Introduction

Flos Styphnolobii japonici imaturi (Flos S. japonici imaturi) is a flowering plant from the *Fabaceae* family. It is a small tree, growing up to 7 meters tall, with smooth, cylindrical branches. Its flower clusters are white or pale yellow and located at the branch ends. The fruit is bean-shaped, resembling a rosary with knots between the seeds. Native to East Asia, *F. Styphnolobii imaturi* thrives in tropical and subtropical climates, including Vietnam. In traditional medicine, the *Flos S. japonici imaturi* has long been utilized in both food production and the treatment of various conditions, including hematochezia, hemorrhoids, uterine bleeding, hematemesis, epistaxis, liver heat, dizziness, and vertigo [1]. Additionally, *Flos S. japonici*

* Corresponding author E-Mail: ntnvan@ctump.edu.vn

The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology July-December 2024 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.118.2410-3341
Available online: December 21, 2024

imaturi has been shown to protect against the harmful effects of ultraviolet radiation [2]. This protective action is attributed to the flavonoid compounds such as rutin and quercetin present in the plant, which have demonstrated the ability to alleviate oxidative stress [3]. Extensive research has focused on the pharmacological properties of rutin and quercetin derived from *Flos S. japonici imaturi*, with numerous studies published (Liu et al. 2016; Li, Jun, et al. 2022; Li, Jun, et al. 2023; Zhang, Hua, et al. 2023) [4-7]. Notably, *Flos S. japonici imaturi* contains a significant concentration of rutin, ranging from 10% to 28% [8].

Currently, there have been several studies published on the separation and quantification of rutin and quercetin in *S. japonici Flos imaturi* by different methods such as capillary electrophoresis (CE) with an electrochemical detector [9], UV spectroscopy [8], ultra high-performance liquid chromatography (UHPLC) and LC-ESI-Q-TOF MS/MS [4]. In Vietnam, Nguyen Thanh Dat *et al.* conducted a study to determine the rutin content in some medicinal herbs as ingredients in health food preparations by HPLC-UV method [10]; Nguyen Ngoc Tran *et al.* also studied the simultaneous quantification of rutin and luteolin in instant tea functional foods by HPLC-PDA [11]. Among them, high-performance liquid chromatography (HPLC) is a widely adopted analytical technique, recognized for its high accuracy, sensitivity, and reliability, making it well-suited for various laboratory applications.

Extraction yield is significantly affected by several variables, including solvent composition, solvent-to-solid ratio, extraction temperature, and extraction time [12-15]. The studies used a one-element-at-a-time approach, a traditional experiment to investigate condition optimization, in which only one element is varied at a time while the other components are fixed in value. However, this method takes a lot of time and cannot evaluate the interaction of different factors. Therefore, a useful method to solve this problem is the response surface method (RSM). RSM can investigate the association between response values and independent variables and improve procedures or goods when different factors may have an impact on the results [16].

The objective of the research is to optimize extraction parameters using RSM with the *Flos S. japonici imaturi* extract having the highest rutin content (RC) and quercetin content (QC). Using ultra-high-performance liquid chromatography coupled with an ultraviolet detector (UHPLC/UV), we developed and validated a method for the quantification of rutin and quercetin of *S. japonici Flos imaturi* extract from Vietnam using optimal extraction conditions.

2. Experimental

2.1. Chemicals and Instruments

Methanol, acetonitrile, and formic acid (Sigma-Aldich, Germany) were HPLC grade and water was purified by a MilliQ system (Millipore Corporation, Bedford, MA, USA). The standards of rutin (98%) and quercetin (98%) used for HPLC analysis were purchased from the Institute of Drug Quality Control - Ho Chi Minh City, Viet Nam.

UHPLC-UV was performed on A Thermo Dionex Ultimate 3000 system, detector UV, all piloted by Chromeleon 7 software.

2.2. Plant Material

S. japonici Flos imaturi was purchased in Can Tho market with a weight of 200 g and a moisture content of less than 13%. Purchased dried flowers are crushed and sieved through 0.9 mm.

2.3. Optimization of the Extraction Procedure

RSM was used to optimize the extraction parameters of rutin and quercetin in *S. japonici Flos imaturi*. The methanol concentration (A), solid-to-liquid ratio (B) and ultrasonic time (C) are the independent variables. For 14 runs of the three stages of the variable optimization process, a rotatable Box-Behnken design was used. Table 1 shows the ranges and numbers of the independent variables in their coded forms. To represent the rutin content (Y1) and quercetin content (Y2) yields as a function of the independent factors, linear models were fitted to the experimental data as follows:

$$Y_n=a_0+a_1A + a_2B + a_3C + a_{11}A^2 + a_{22}B^2 + a_{33}C^2 + a_{12}AB + a_{13}AC + a_{23}BC$$

Where Yn represents the response variables, a_0 is a fixed amount, and $a_1A + a_2B + a_3C$, $a_{11}A^2 + a_{22}B^2 + a_{33}C^2$, and $a_{12}AB + a_{13}AC + a_{23}BC$ represent the linear, quadratic, and interaction coefficients, respectively. The independent variables are A, B and C. Three-dimensional surface response plots were produced by altering the two variables within the experimental range and maintaining the third constant at the center point. The coefficients of the response surface equation were calculated using Design Expert 11. A 95% confidence level ($p < 0.05$) based on the total error criterion was used to test for statistical significance.

Table 1. Experimental design factors and levels of design of experiments.

Independent variable	Units	Experimental value	
		Low(-1)	High(+1)
A: Methanol concentration	v/v,%	60	100
B: Liquid: Solid (L/S)	mL/g	10	30
C: Ultrasonic time	Min	10	20

2.4. Analysis of Samples Through UHPLC-UV

A Thermo Dionex Ultimate 3000 system was used to identify and quantify rutin and quercetin in the crude methanol extracts. The system was controlled using LabSolution software. A 4.6 × 250 mm Agilent C18 column with 5 µm particle size was used to measure phenolic acid and flavonoid components. The complete loop injection option was used to inject 20-µL samples and the sample injection temperature is 30ºC. The mobile phase was used to elute the column at a constant flow rate of 1 mL/min and consisted of HPLC grade acetonitrile (solvent A), HPLC-grade methanol (solvent B), and water/formic acid pH 2.1 (solvent C). The gradient elution program included: 0-5 min, 50% MeOH B and 50% Water/formic acid 0.1% pH 2.1 C; 5-15 minutes, 10% ACN A and 45% MeOH B and 45% Water/formic acid 0.1% (pH 2.1) C. The UV detector was set at 256 nm.

2.5. Standard Solutions

Stock solutions of rutin and quercetin were prepared in methanol at 2000 µg/mL for rutin and at 1000 µg/mL for quercetin. From the stock standard solution, continue to prepare mixed standard solutions of rutin 50 ppm and quercetin 50 ppm. The peak areas and concentrations of each standard were fitted to linear regression and linear regression after square root transformation to select the most suitable regression model.

2.6. Validation of UPLC -UV Method

The quantitative process was validated with expected criteria including system suitability testing, specificity, linearity and linear range, precision, and accuracy according to the Association of Official Analytical Chemists (AOAC) guidelines [17].

2.6.1. Linearity

Linearity was determined for the calibration curves obtained by HPLC analysis on seven concentrations (10–400 µg/mL for rutin and 0.5-20 µg/mL for quercetin). The correlation coefficients were calculated by the linear regression method.

2.6.2. Precision

A sample was prepared as previously described and analyzed six times on the same day to evaluate intra-day variation and on three consecutive days to assess inter-day variation (nine measurements). The precision was expressed as relative standard deviation (RSD%).

2.6.3. Accuracy

The accuracy was tested by separate spiking with a known amount of the standard rutin (100 µg/mL) and quercetin (5 µg/mL) for 100%. The accuracy was carried out with three level concentration (50%, 100%, 150%) and analyzed in triplicates.

The average recovery was calculated according to the formula:

Recovery (%) = [(net measured amount original amount)/spiked amount] x100.

2.6.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

According to the International Conference on Harmonization Guidelines, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the standard deviation of the response (r) and the slope of the calibration curve (S). LOQ=10 * r/S and LOD = 3.3 * r/S.

2.7. Applications

Seven crude methanol extracts of *S. japonici Flos imaturi* sample collected randomized from different provinces in the Mekong Delta, Vietnam were used. The methanol extracts were analyzed using validated methods for their concentrations of rutin and quercetin to control the quality of herbal products on the market.

2.8. Statistical Analyses

Statistical analyses (recovery percentage, relative standard deviation) were performed using Microsoft Excel. RSM test results of total rutin content and total quercetin content and the correlation of extraction parameters with RC and QC were analyzed for variance through Design Expert 11 software.

3. Results and Discussion

3.1. Optimization of the Extraction Procedure by RSM

The choice of solvent plays a pivotal role in the extraction of rutin and quercetin from *Flos S. japonici imaturi*. The selection primarily depends on the solubility of the target analytes and the interactions between the solvent and sample matrix. Therefore, it is crucial to select a solvent that maximizes the dissolution of the desired compounds while minimizing the co-extraction of impurities. Based on previous studies [4,18,19,20], methanol was selected as the solvent for extracting rutin and quercetin. In this study, three methanol concentrations, 60%, 80% and 100%, were evaluated.

Using an insufficient amount of solvent may result in incomplete extraction of active compounds, while an excessive amount of solvent can lead to increased impurities in the extract. Depending on the characteristics of the raw material, the extraction objective, and the method applied, the ratio of raw material to solvent must be carefully optimized for each extraction protocol. Liu et al. (2016) examined material-to-solvent ratios ranging from 1:10 to 1:400 for flavonoid extraction from *Flos S. japonici imaturi* [4]. They found that a 1:50 ratio achieved the highest extraction efficiency of rutin and quercetin using microwave-assisted extraction with 100% methanol. Similarly, Shan Li et al. (2021) investigated material-to-solvent ratios of 1:20, 1:30, and 1:40 for ultrasound-assisted extraction, with optimal results obtained using 80%

methanol, a 1:30 ratio, and an extraction duration of 40 minutes [21]. In this study, material-to-solvent ratios from 1:10 to 1:30 were evaluated because this is the range of material-to-solvent ratios suitable for using minimize organic solvent.

Extraction time is another critical factor for optimal extraction method. Sanhong Fan et al. (2020) observed that flavonoid content increased during ultrasound-assisted extraction, peaking after 30 minutes, after which it slightly decreased due to the degradation of dissolved flavonoids [20]. Other studies have indicated that optimal extraction times for flavonoids range between 6 and 10 minutes [21]. In this study, ultrasound durations of 10 and 20 minutes were evaluated to determine their effect on flavonoid yields.

3.1.1. Model Fitting

The experimental modeling results showed that RC varied from 7.630 to 30.773 % and QC from 0.325 to 1.085 % (Table 2). The software generated two regression equations that demonstrated the empirical relationship between the response values and extraction parameters of methanol concentration (A), solid-to-liquid ratio (B) and ultrasonic time (C).

Table 2. Rotatable central composite design setting in the original and coded forms of the independent variables (A, B, C) and experimental results of RC (Y1) and QC (Y2)

RUN	Independent variable			Responses	
	A	B	C	RC (%)	QC(%)
1	60	20	10	17.434	0.516
2	80	20	15	30.773	1.085
3	80	10	10	13.637	0.522
4	80	30	20	17.781	0.636
5	60	20	20	11.634	0.564
6	100	30	15	10.920	0.589
7	100	20	20	17.203	0.736
8	100	10	15	13.370	0.581
9	60	10	15	10.515	0.411
10	100	20	10	11.768	0.451
11	80	30	10	7.630	0.325
12	80	10	20	8.205	0.399
13	60	30	15	11.633	0.564
14	60	20	10	17.434	0.516

Table 3. ANOVA for the effect of methanol concentration (X1), solid-to-liquid ratio (X2) and time (X3) of the ultrasound-assisted extraction on RC and QC using a quadratic response surface model.

Term	Df	RC		QC	
Mode		F-ratio	P-value	F-ratio	P-value
Model	9	20.57	0.0151	25.73	0.0109
A	1	0.2288	0.6651	6.21	0.0883
B	1	0.2738	0.6370	2.77	0.1948
C	1	1.04	0.3835	18.39	0.0233
AB	1	1.39	0.3229	2.90	0.1873
AC	1	13.81	0.0339	7.63	0.0700
BC	1	26.55	0.0142	25.64	0.0149
A^2	1	67.78	0.0038	63.65	0.0041
B^2	1	119.44	0.0016	129.22	0.0015
C^2	1	64.47	0.0040	105.74	0.0020
R^2		0.9841		0.9872	
Adj R^2		0.9362		0.9488	

Regression analysis and ANOVA were used to fit the model and examine the statistical significance of the terms. The results of the ANOVA are presented in Table 3. The corresponding coefficients of determination (R^2) for the models were 0.9841 for RC and 0.9872 for QC, indicating excellent model fit and strong explanatory power of the dependent variables. The adjusted coefficient of determination **(**Adjusted R^2) for the models were 0.9362 and 0.9488 for RC and QC, respectively, demonstrating minimal overfitting and model stability. The generated 3D response surface graphs corresponding to all responses showed the interactive effects of the variables (Figure 1).

The study by Sanhong Fan et al. (2020) on *Flos S. japonici imaturi* utilized a Box-Behnken design to optimize the extraction of six flavonoids, including rutin and quercetin. The second-order regression results of the study achieved an R^2 value greater than 0.9798, indicating a high degree of correlation between the experimental data and the predictive model [20].

3.1.2. Effect of the Extraction Variables on RC

The F-ratio for the model regarding RC is 20.57, with a P-value of 0.0151, indicating that the model is statistically significant (Table 3). Significant factors influencing RC include the interaction of AC with a P-value of 0.0339 and BC with a P-value of 0.0142. Notably, the quadratic effects of A^2, B^2, C^2 have very small P-values (< 0.01), suggesting a strong and significant impact on RC. The relationship between RC and variables is described by the following second-order polynomial equation (1):

rutin (Y_1)

$$Y_1 = 30.77 + 0.2557A + 0.2797B + 0.5444C - 0.8925AB + 2.81AC + 3.9BC - 8.23A^2 - 10.93B^2 - 8.03C^2 \quad (1)$$

A 3D response surface (Figure 1A–C) was applied to clarify the interactive effects of the three variables on the RC of *F. Styphnolobii imaturi* extract. Notably, the interaction between B and C (P-value = 0.0142) strongly influences the response surface, creating a high-response region on the graph. The curve demonstrates that as C increases alongside B, RC changes markedly, highlighting the importance of this combination. The interaction between A and C also plays a significant role, with a moderate yet meaningful variation on the response surface (P-value = 0.0339), indicating a combined influence of A and C on RC.

3.1.3. Effect of the Extraction Variables on QC

For QC, the F-ratio of the model is 25.73, with a P-value of 0.0109, also indicating statistical significance. Significant factors influencing QC include C with a P-value of 0.0233, BC with a P-value of 0.0149, and the quadratic effects of A^2, B^2, and C^2, all of which have very small P-values, suggesting a notable impact on QC. The QC model is represented through the following equation (2):

quercetin (Y_2)

$$Y_2 = 1.08 + 0.0378A + 0.0252B + 0.0650C - 0.0365AB + 0.0592AC + 0.1086BC - 0.2263A^2 - 0.3224B^2 - 0.2917C^2 \quad (2)$$

To visualize the effects of the three independent variables on QC from *Flos S. japonici imaturi* extract, 3D response surface plots (Figure 1D–F) were generated according to equation (2). The interaction between B and C (P-value = 0.0149) generates a distinct optimal point on the response surface, indicating that the simultaneous increase of these two variables significantly enhances QC. Figure 1D, E showed A had no significant effect on QC with B and C ($p > 0.05$).

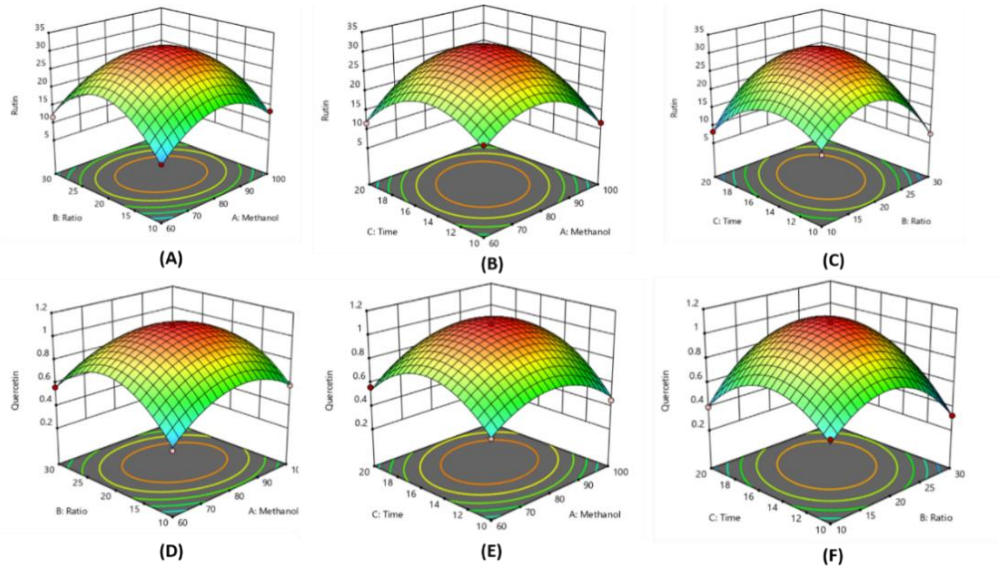

Figure 1. 3D response surface curve showing the influences of independent variables on the RC (A-C) and QC (D-F)

3.2. Optimization of the Extraction Conditions

An experiment was conducted to confirm the reliability of the RSM design under the ideal ultrasound-assisted extraction conditions established through the model. The ideal extraction conditions were determined using the following parameters: 96% methanol concentration, 19:1 (mL/g, v/w) for the liquid-to-solid ratio, and 19 min as the duration of the ultrasound-assisted extraction at optimal conditions. RC and QC were 35.73% and 1.36%, respectively. When compared to Fan Sanhong's study on *Flos S. japonici imaturi*, there is a noticeable variation in both extraction conditions and yield. Sanhong's study, which employed 70% ethanol and a longer extraction time at a higher temperature, resulted in a lower rutin yield (14.61%) but a higher quercetin content (2.80%) [20]. The higher RC suggests that stronger solvents, such as 96% methanol, may be more effective for rutin extraction. This comparison highlights how variations in solvent concentration, extraction time, and liquid-to-solid ratio can significantly affect the yield of bioactive compounds.

3.3. Quantification Method and Validation

3.3.1. System Suitability Testing

System suitability testing was assessed by performing chromatography with a mixed standard solution of rutin and quercetin with 6 successive injections under the appropriate conditions. The % RSD of chromatographic parameters was calculated, and the results were statistically processed. The system was deemed compatible when the % RSD for retention time (Rt) and peak area (S) after 6 injections was less than 2.0%, following AOAC guidelines criteria. The % RSD for both rutin and quercetin were within this limit (≤2%) (Table 4), confirming system compatibility.

Table 4. System suitability testing of the HPLC-UV method

		t_R (min)	**S (mAu*min)**	**As**	**Rs**	**N**
Rutin	Mean	4.53	26.39	1.39	7.61	2507.30
	RSD%	0.59	1.36	1.58	0.52	0.59
Quercetin	Mean	9.70	41.52	1.90	-	2383.20
	RSD%	0.49	0.76	1.82	-	1.35

3.3.2. Selectivity

Selectivity was validated by comparing the retention times and UV spectra of peaks of the crude extract and standards of five analyses at retention times corresponding to the beginning, middle, and end of these peaks. Similar results indicated the selectivity of the method (Figure 2).

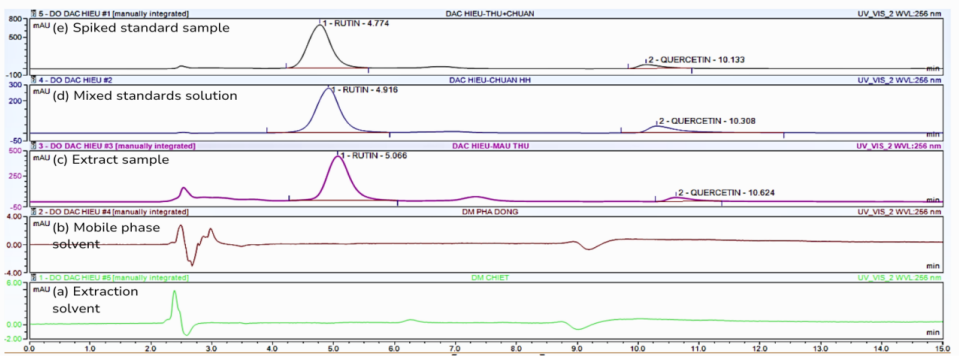

Figure 2. Chromatogram of *F. Styphnolobii imaturi* extract. (a) Extraction solvent; (b) Mobile phase solvent; (c) Extract sample; (d) Mixed standards solution; (e) Spiked standard sample

3.3.3 Linearity and Linear Range

The stock solutions were diluted and mixed into seven concentrations ranging from 10 to 400 µg/mL for rutin and 0.5 to 20 µg/mL for quercetin. To evaluate linearity, each mixed standard sample was injected in triplicate into the HPLC system, and calibration curves were obtained by plotting the average of the peak area responses versus the concentration of each sample. Regression equations rutin and quercetin have square correlation coefficients (R^2) of 0.9996 and 0.9994, respectively (table 5).

Table 5. Evaluation results of the linearity and the linear range of rutin and quercetin

Substance	**Rutin**		**Quercetin**	
	Concentrations (ppm)	**S (mau*min)**	**Concentrations (ppm)**	**S (mau*min))**
	10	3,0175	0,5	0,283
	20	6,3211	1	0,6592
	50	16,7109	2.5	1,7186
	100	35,4726	5	4,2828
	150	51,8999	7.5	6,3101
	200	70,717	10	8,728
	400	137,4867	20	18,0617
Regression equation	Y = 0.346x - 0.022		Y = 0.916x - 0.3644	
R^2	0.9996		0.9994	

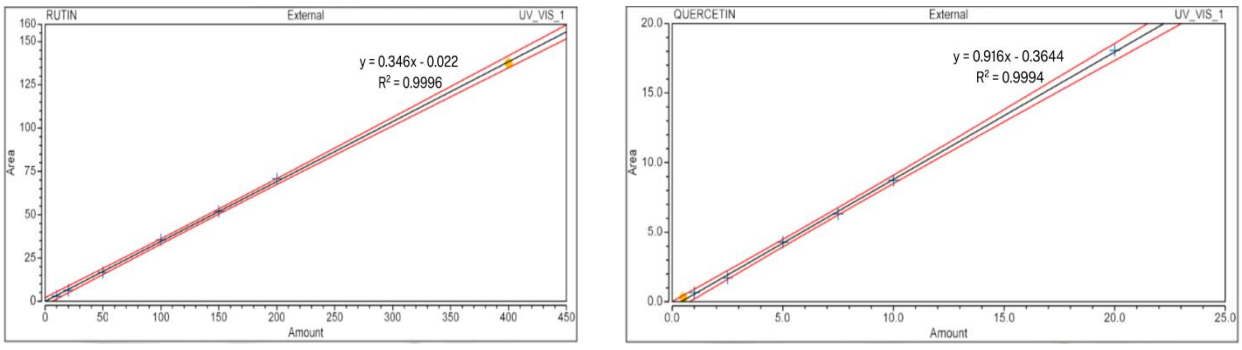

Figure 3. Correlation graph between concentration and peak area of rutin and quercetin

3.3.4. Precisions

Precision was expressed as relative standard deviation (RSD %) values to evaluate repeatability (intraday) and intermediate precision (interday). The results showed that the repeatability and intermediate precision were less than 2% for the six analysts (Table 6). The precision values (≤ 5%) agreed with the AOAC guidelines criteria (Association of Analytical Communities International 2013).

Table 6. Evaluation results of the precisions and the recovery of rutin and quercetin

Substance	**Precision (n=6)**		**Recovery (%)**		
	Intra-day RSD(%)	**Inter-day RSD(%)**	**Low-level**	**Mid-level**	**High-level**
Rutin	0.61	0.85	106.10	105.60	103.86
Quercetin	0.85	1.22	96.58	101.23	102.78

3.3.5. Accuracy

Determine the active ingredient content of the test sample using the validated method. Prepare spiked samples by adding standard solutions at 80%, 100%, and 120% of the analyte concentration in the test sample. Perform chromatography under established conditions to calculate the recovery rate. The method is considered accurate if the recovery of rutin and quercetin falls within the acceptable range of 80 to 110 % as per AOAC guidelines criteria. Based on the results (Table 6) of the recovery test, this method was deemed accurate.

3.3.6. Application

Seven crude methanol extracts of *Flos S. japonici imaturi* samples were collected randomly from various provinces in the Mekong Delta, Vietnam. These samples do not fully represent the species across the country. Using the developed analytical method, rutin content (RC) and quercetin content (QC) were evaluated in samples collected from seven provinces, including Can Tho, Bac Lieu, Ben Tre, Hau Giang, Soc Trang, Vinh Long, and Dong Thap. The results revealed significant variations in rutin and quercetin levels among samples from six provinces: Can Tho, Bac Lieu, Ben Tre, Soc Trang, Vinh Long, and Dong Thap.

Notably, the RC in samples from these provinces exceeded 20%, meeting the standards set by the Vietnamese Pharmacopoeia V, which specifies a minimum RC of 20% for *Flos S. japonici imaturi* [23]. The sample from Can Tho exhibited the highest RC, reaching 36.085%.

These variations are likely influenced by factors such as storage duration, processing methods, local climate, soil and water composition, as well as cultivation and irrigation practices. Furthermore, the levels of rutin and quercetin are affected by the harvest time. The sample from Hau Giang, characterized by a dark, dull appearance and a high proportion of stems and branches, showed the lowest RC (15.5%) and no detectable QC. This aligns with the fact that stems and branches generally contain lower rutin levels, typically around 0.5%–2%.

In a study by Vetrova on *Sophora japonica buds* using subcritical water extraction [24], the reported yields of rutin and quercetin were 29% and 13.7%, respectively. In comparison, *Flos S. japonici imaturi* samples from most provinces in Vietnam exhibited higher RC but significantly lower QC. Liu et al. (2016) utilized microwave-assisted extraction for *F. Sophorae Immaturus*, achieving impressive rutin yields of up to 28% and quercetin yields of 1.73%[4]. Notably, *Flos S. japonici imaturi* from regions such as Can Tho, Soc Trang, and Vinh Long exhibited significantly higher rutin content. However, the quercetin levels remained consistently lower, regardless of geographic origin. Similarly, Sanhong Fan et al. (2020) applied ultrasound-assisted extraction with 70% ethanol, yielding 14.61% rutin and 2.80% quercetin [20]. Despite employing advanced extraction techniques, *Flos S. japonici imaturi* from most provinces in Vietnam consistently demonstrated a superior rutin content while quercetin yields remained notably low. These findings underscore the influence of regional environmental factors and extraction techniques on the yield

of bioactive compounds in plant materials. Optimizing extraction methods and adapting to local environmental conditions are crucial for improving the recovery of these valuable compounds.

Table 6. Rutin and Quercetin content of *F. Styphnolobii imaturi* sample. collected randomized in the Mekong Delta

No.	Place of collected	RC (%)	QC (%)
1	Can Tho City	36.085	0.887
2	Bac Lieu province	21.726	0.576
3	Ben Tre province	31.735	0.830
4	Hau Giang province	15.510	-
5	Soc Trang province	33.866	0.670
6	Vinh Long province	33.434	0.642
7	Dong Thap procince	31.461	0.392

4. Conclusions

In this study, an optimized ultrasound-assisted extraction method was developed to enhance the yields of rutin (RC) and quercetin (QC) from *Flos S. japonici imaturi*. Through 3D surface plots and regression analysis of independent variables, the ideal extraction conditions were identified as follows: 96% methanol concentration, a liquid-to-solid ratio of 19:1 (mL/g, v/w), and an extraction duration of 19 minutes. These optimized conditions were then applied to validate the quantification process. The validated extraction and quantification methods were used to determine rutin and quercetin levels in extracts from seven Mekong Delta provinces. Notably, the sample from Can Tho exhibited the highest concentrations, with 36.1% rutin and 0.9 % quercetin. These results not only offer an effective extraction technique but also enhance the medicinal value of *Flos S. japonici imaturi* in traditional medicine and functional food products.

Acknowledgements

The authors would like to express their hearty gratitude to Can Tho University of Medicine and Pharmacy. The experiment was conducted in Analytical Chemistry – Drug Quality Control Department, Pharmacy College, Can Tho University of Medicine and Pharmacy. Specially, we sincerely thank student group in Pharmacy College: Gia-Ngan Mai Le, Vi-Trang Lam, Nguyen-Trang Ngoc Nguyen, Ngoc-Linh Thi Nguyen, Mong-Tuyen Thi Tran, Nhu-Huynh Nguyen, Nhu-Y Nguyen for their support in helping us complete the experiment of this research project.

ORCID
Tuyet-Ngan Duong: 0000-0002-7373-2873
Ngoc-Van Thi Nguyen:0000-0002-7397-4071
Phi-Long Ha: 0009-0000-6082-2768
Trung-Kien Nguyen: 0000-0002-9395-4764

References

[1] Chinese Pharmacopoeia Commission (2020). Chinese Pharmacopoeia, China Medical Science Press, p. 370.

[2] L. Li**,** T. Huang**,** C. Lan**,** H. Ding, C. Yan and Y. Dou (2019). Protective effect of polysaccharide from *Sophora japonica L.* flower buds against UVB radiation in a human keratinocyte cell line (HaCaT cells), *J. Photochem. Photobiol. B: Biology* **191**, 135-142.

[3] D. Mihaylova and S. Schalow (2013). Antioxidant and stabilization activity of a quercetin-containing flavonoid extract obtained from Bulgarian *Sophora japonica L*., *Brazilian Arch. Biol. Technol*. **56**, 431-438.

[4] J.L. Liu**,** L.Y. Li and G.H. He (2016). Optimization of microwave-assisted extraction conditions for five major bioactive compounds from *Flos Sophorae immaturus* (cultivars of *Sophora japonica L.*) using response surface methodology, *Molecules* **21**, 296.

[5] J. Li, Y. Gong, J. Li, and L. Fan (2022). In vitro xanthine oxidase inhibitory properties of *Flos Sophorae Immaturus* and potential mechanisms, *Food Biosci*. **47**, 101711.

[6] J. Li, Y. Gong, J. Li, and L. Fan (2023). Improving the xanthine oxidase and adenosine deaminase inhibitory activities of *Flos Sophorae Immaturus* by ultrasound-assisted heating treatments, *Food Biosci*. **51**, 102245.

[7] H. Zhang**,** Q. Zhao**,** J. Qiu**,** Z. Wang and X. Yang (2023). Synthesis of a magnetic micelle molecularly imprinted polymers to selective adsorption of rutin from *Sophora japonica*, *J. Chromatogr. B*. **1214**, 23492.

[8] Y.B. Ji, H. J. Zhu, G.S. Xin and C. Wei (2017). The influence of different processing methods on component content of *Sophora japonica*, *IOP Conf. Ser. Earth Environ. Sci*. 012049.

[9] X. Li, Y. Zhang and Z. Yuan (2002). Separation and determination of rutin and quercetin in the flowers of *Sophora japonica L.* by capillary electrophoresis with electrochemical detection, *Chromatographia* **55**, 243-246.

[10] T.D. Nguyen, T.H.H. Nguyen**,** T.T. Dam**,** T.T. Chu and T.N. Hoang (2019). Determination of rutin content in some *Sophora japonica* flower extracts used as raw materials in health food preparations by HPLC-UV method, *J. Food Testing Safety* **3**, 51-55.

[11] N.N. Tran, N. T. N. Van, D.T. Ngan and D.N. Chau (2021). Simultaneous determination of rutin and luteolin in soluble tea dietary supplements by HPLC-PDA, *Can Tho J. Medicine Pharmacy* **43**, 139-146.

[12] R. Chirinos, H. Rogez, D. Campos, R. Pedreschi and Y. Larondelle (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers, *Separat. Purificat. Technol.* **55**, 217–225.

[13] K. Ghafoor, Y.H. Choi, J.Y. Jeon and I.H. Jo (2009). Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from grape (*Vitis vinifera*) seeds, *J. Agric. Food Chem*. **57**, 4988–4994.

[14] M. Omwamba and Q. Hu (2009). Antioxidant capacity and antioxidative compounds in barley (*Hordeum vulgare* L.) grain optimized using response surface methodology in hot air roasting, *Eur. Food Res. Technol.* **229**, 907–914.

[15] T.T.T. Nguyen, M. Chollet-Krugler**,** F. Lohézic-Le Dévéhat**,** I. Rouaud and J. Boustie (2015). Mycosporine-like compounds in chlorolichens: Isolation from *Dermatocarpon luridum* and *Dermatocarpon miniatum*, and their photoprotective properties, *Planta Med. Lett.* **2**, e1-e5.

[16] D. Bas and I.H. Boyaci (2007). Modeling and optimization I: Usability of response surface methodology, *J. Food Engineering* **78**, 836–845.

[17] Association of Official Analytical Chemists International (2013). Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals, AOAC International, 1–38.

[18] P.G. Dien (2021). Refinement technology process for rutin. Proceedings of the 3rd National Conference on Organic Chemistry and Technology.

[19] N.T.T. Huyen (2010). Investigation of raw materials, enhancement of extraction efficiency, and quality improvement of rutin from *Sophora japonica buds* in Vietnam. Hanoi University of Science and Technology.

[20] S. Fan, G. Yang, J. Zhang, J. Li and B. Bai (2020). Optimization of ultrasound-assisted extraction using response surface methodology for simultaneous quantitation of six flavonoids in *Flos Sophorae Immaturus* and antioxidant activity, *Molecules* **25**, 1767.

[21] Q. Mou, J. He, R. Yin, B. Yang, M. Fu, J. Fang and H. Li (2017). Response surface optimized infrared-assisted extraction and UHPLC determination of flavonoid types from *Flos Sophorae*, *Molecules* **22**, 1000.

[22] S. Li, ,M. Lu , L. Yao, X. Mo, G. Yang and H. Zeng (2021). Optimization of the extraction process of rutin from *Flos Sophorae* by orthogonal experiments, *Agric. Biotechnol.* **10**, 120-126.

[23] Pharmacopoeia Council (2018). Vietnamese Pharmacopoeia V, Medical Publishing House.
[24] E.V. Vetrova**,** E.V. Maksimenko**,** S.N. Borisenko, A. V. Lekar, N. I. Borisenko and V. I. Minkin (2017). Extraction of rutin and quercetin Antioxidants from the Buds of *Sophora Japonica* (*Sophora japonica L.*) by subcritical water, *Russ. J. Physic. Chem. B.* **11**, 1202–1206.