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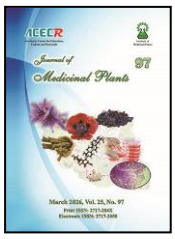

Research Article

Optimization of the extraction of total phenols and flavonoids from *Scutellaria litwinowii*: A green extraction technique based on response surface methodology and macroporous Resins

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ABSTRACT

Background: The flavonoid-rich roots of *Scutellaria litwinowii* have therapeutic potential, but traditional extraction methods are inefficient. This study developed an optimized protocol using modern extraction techniques to maximize extract yield. **Objective:** The roots of *S. litwinowii* contain valuable flavonoid compounds and exhibit biological properties. Response surface methodology and macroporous resins were employed to increase the extraction yield. **Methods:** Extraction of the plant roots was conducted according to a design optimized with Design-Expert® software. A comparative analysis was performed between reflux-assisted extraction (RAE), microwave-assisted extraction (MAE), and traditional maceration. Subsequently, phenolic and flavonoid compounds were enriched using a Diaion HP-20 resin column. **Results:** MAE under optimized parameters yielded a higher total phenolic content compared to conventional 72-hour maceration. Moreover, both MAE and RAE demonstrated significantly higher total flavonoid yields than the maceration method. Furthermore, the Diaion HP-20 resin column increased the amount of active compounds in the *S. litwinowii* extract by approximately 1.75 times. **Conclusion:** This study employed two principal strategies for the enrichment of phenolic and flavonoid compounds. Firstly, RAE and MAE demonstrated significantly higher efficiency compared to traditional maceration. Secondly, a purification step using a Diaion HP-20 macroporous resin column effectively concentrated the target active compounds in the final extract. The application of these novel extraction and purification methods significantly improves the quality of herbal preparations.

Abbreviations: RAE, Reflux-Assisted Extraction; MAE, Microwave-Assisted Extraction; RSM, Response Surface Methodology; CCD, Central Composite Design; HPLC, High Performance Liquid Chromatography

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1. Introduction

The genus *Scutellaria* encompasses over 360 species, containing over 300 bioactive compounds predominantly categorized as flavonoids and phenols with diverse therapeutic properties. Various species within the *Scutellaria* genus were used in traditional Chinese medicine for the treatment of different diseases including fever, cough, inflammation, dysentery, jaundice, and hypertension [1]. The *Scutellaria* genus is characterized by high flavonoid content, specially baicalein, baicalin, wogonin, and oroxylin A, which are well known flavanoids of these genus plant roots. These compounds exhibit a wide range of biological activities, including antioxidant, antimicrobial, anticancer, anti-inflammatory, antidiabetic, and analgesic effects, and regulation of lipid and arachidonate metabolism [2]. A selective extraction method for *Scutellaria* root flavonoids is required to isolate these compounds at high yield without changing in their quality. Therefore, the development of an efficient and high-yield isolation method is critical to maximizing the therapeutic potential of *Scutellaria* flavonoids. This selective method would provide sufficient quantities and high-quality extract for pharmaceutical and nutraceutical applications [3].

Response Surface Methodology (RSM) was utilized, to optimization the extraction methods. This technique involves designing empirical models to understand the relationship between independent variables (e.g., temperature, solvent concentration, and time) and extraction efficiency as the response. Design-Expert® software was used for experimental design, data analysis, and model generation [4-6].

Another way for the selective enrichment and purification of bioactive compounds from natural sources is using macroporous resins. This method is based on the selective adsorption and separation of target molecules within the porous of resin. With this approach, a high pure and concentration extract was obtained. As such, macroporous resin technology represents a powerful tool in natural product chemistry for the isolation of therapeutically relevant compounds [7]. Macroporous resins, such as Diaion HP-20, used in industries to enrich bioactive compounds in plant extracts. Previous studies have applied macroporous resin chromatography to enrich flavonoid compounds from crude plant extracts [8, 9]. This research is designed to establish an optimized protocol for enhancing the flavonoid and phenolic extraction from *S. litwinowii* root. The study first compares the efficacy of two advanced extraction methods (MAE and RAE) against conventional maceration based on efficiency. Additionally, it investigates the performance of Diaion HP-20 macroporous resin for the selective separation of flavonoid compounds from the crude extracts. The findings are expected to contribute to the development of an efficient method for obtaining high-value bioactive constituents from *S. litwinowii* root that used in further pharmacological and industrial applications.

2. Materials and methods

2.1. Raw materials

The roots of *S. litwinowii* were collected from the region between Kang and Zashok (located near Mashhad in Razavi Khorasan province, northeastern Iran) and identified in the herbarium of the Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. All chemicals and reagents used were of analytical grade. Sodium carbonate, aluminium chloride, and ethanol were obtained from Merck (Germany). Diaion® HP-20 macroporous resin was sourced from Mitsubishi Chemical

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Corporation (Japan). Folin–Ciocalteu's phenol reagent, gallic acid, and quercetin were purchased from Sigma-Aldrich.

2. Materials and Methods

2.1. Experimental design for RSM

The present study evaluate the combined effects of various independent variables on the extraction yields of total phenols and flavonoids using RSM. A Central Composite Design (CCD) with five distinct levels (-α, -1, 0, +1, +α) was used for each factor. The specific coded levels for the independent variables in both the Reflux-Assisted Extraction (RAE) and Microwave-Assisted Extraction (MAE) experiments are detailed in Table 1.

2.2. Reflux-assisted extraction (RAE)

Reflux-assisted extraction (RAE) has been shown to improve extraction yield, reduce time, and lower solvent consumption compared to conventional methods. RAE could be a promising technique for isolating bioactive natural products [10]. According to the designed experiment with Design-Expert® software, twenty distinct experiments were conducted to ensure the consistency of other variables.The RAE procedure was performed using an oil bath for temperature control. For each run, 10 g of powdered *S. litwinowii* root was combined with 100 mL of ethanol at a specified concentration and extracted for a defined duration at a set temperature, as dictated by variations in the three independent variables: temperature, time, and ethanol concentration. The obtained extracts were concentrated using a rotary evaporator and subsequently lyophilized. The designed experiment was described in Table 2.

2.3. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) utilizes microwave energy to induce rapid dipole rotation within polar solvent molecules and generate intense internal heating. This thermal effect promotes the disruption of plant cell walls and enhances the mass transfer of target compounds into the solvent. The extraction efficiency in MAE is primarily controlled by irradiation power and duration. The optimal irradiation time is critical for maximizing yield without thermal degradation of bioactive compounds [11]. In this study, twenty experimental runs (designed by CCD in Design-Expert® software) was performed to evaluate the MAE efficacy. For each run, 10 g of powdered *S. litwinowii* root was extracted with 100 mL of ethanol at a specified concentration (v/v) under defined conditions of microwave power and irradiation time. Following extraction, the solvents were removed using a rotary evaporator, and finally lyophilized. The designed experiment for MAE was described in Table 2.

Table 1. Independent variables used in the RSM design for RAE and MAE

Extraction method	Independent variables	Units	Minimum	Maximum	Coded Low	Coded High
RAE	Time	Min	80.00	120.00	-1 ↔ 90.00	+1 ↔ 110.00
	Temperature	°C	60.00	80.00	-1 ↔ 65.00	+1 ↔ 75.00
	Ethanol	%	70.00	90.00	-1 ↔ 75.00	+1 ↔ 85.00
MAE	Time	Min.	4.00	20.00	-1 ↔ 8.00	+1 ↔ 16.00
	Power	Watt	300.00	700.00	-1 ↔ 400.00	+1 ↔ 600.00
	Ethanol	%	50.00	90.00	-1 ↔ 60.00	+1 ↔ 80.00

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Table 2. Central composite design for independent variables in the reflux and microwave assays

Assay	RAE			MAE		
Run no.	**Time (min)**	**Temperature (°C)**	**Ethanol %**	**Time (min)**	**microwave power (watt)**	**Ethanol %**
Run 1	100	70	80	5	500	90
Run 2	90	75	75	5	500	70
Run 3	110	65	85	5.5	550	60
Run 4	100	70	90	6	500	70
Run 5	100	80	80	5.5	550	80
Run 6	100	70	80	4.5	550	80
Run 7	90	65	85	4	500	70
Run 8	120	70	80	5.5	450	60
Run 9	90	75	85	5	500	70
Run 10	100	70	80	4.5	550	60
Run 11	110	75	75	5	500	70
Run 12	100	70	80	5	500	70
Run 13	100	70	80	5	400	70
Run 14	90	65	75	4.5	450	80
Run 15	100	60	80	5	500	70
Run 16	100	70	80	5	600	70
Run 17	80	70	80	5	500	50
Run 18	100	70	70	4.5	450	60
Run 19	110	75	85	5.5	450	80
Run 20	110	65	75	5	500	70

2.4. Maceration-assisted extraction

For conventional extraction, powdered *S. litwinowii* root (10 g) was subjected to maceration in 70% (v/v) ethanol for 72 hours. The extract was then concentrated using a rotary evaporator and subsequently lyophilized.

2.5. Micro-assay methods for total phenols & flavonoids determination

Total Phenolic Content was quantified using the Folin-Ciocalteu colorimetric assay with gallic acid as the standard [12], adapted for a microplate reader. A gallic acid calibration curve was prepared from serial dilutions (0.025–0.4 mg/ml) in distilled water. For analysis, aliquots of sample or standard were mixed sequentially with diluted Folin-Ciocalteu reagent and sodium carbonate solution. Following incubation, absorbance was measured at 755 nm. Sample extracts were dissolved in ethanol to a working concentration of 1 mg/mL prior to analysis. Phenolic content was expressed as mg of gallic acid equivalents per ml of extract, based on the standard curve.

Total Flavonoid Content was determined by the aluminum chloride colorimetric method, using quercetin as the standard [12]. A quercetin calibration series (0.015–0.5 mg/ml) was prepared in methanol/water (1:4, v/v). In the assay, aliquots were combined with 96 % ethanol, 10 % aluminum chloride, 1 M potassium acetate, and distilled water. The mixture was incubated for 30 minutes at room temperature, and absorbance was read at 415 nm. Sample extracts were prepared at 1 mg/ml in ethanol. Flavonoid content was calculated

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from the quercetin standard curve and expressed as mg of quercetin equivalents per ml of extract.

2.6. Extract adsorption on the macrospores resin

Flavonoid purification was performed using Diaion HP-20 macroporous resin, known for its high selectivity towards these compounds [8]. A column (30 cm × 1.5 cm) was packed with 100 g of the resin. First, the resin was activated by soaking in methanol for 12 hours and then rinsed with distilled water to remove residual methanol. A sample of the 10 g of dry methanolic extract was suspended in distilled water and applied to the column. To ensure complete adsorption, the column was closed and the sample was allowed to interact with the resin for 5 hours. Subsequent elution began with distilled water to collect the unbound aqueous fraction and followed by methanol elution. Methanol fractions were collected continuously until the eluent showed no detectable absorbance, indicating complete desorption of the adsorbed flavonoids.

2.7. High Performance Liquid Chromatography (HPLC) analysis

The HPLC spectrum of extracts (before and after introducing to macrospores resin) were quantified using an Analytical HPLC KNAUER 1500 HPLC system (KNAUER company, German) equipped with a diode array detector. An Onyx Monolithic C8 (100 mm × 4.6 mm) was used. The mobile phase consisted of 0.1 % formic acid-methanol and 0.1 % formic acid-H_2O at a flow rate of 0.8 ml/min with gradient program: 0−4 min, 10 % methanol, 90 % H_2O; 4−10 min, 40 % methanol, 60 % H_2O; 10−13 min, 60 % methanol, 40 % H_2O; 13-17 min, 100 % methanol, 0 % H_2O; 17-19 min, 10 % methanol, 90 % H_2O; 19-21 min, 10 % methanol, 90 % H_2O. The UV detection wavelength was set to 254 nm; injection volume was set to 10 µl, and column temperature was set to 30 °C. Before HPLC analysis, all extracts were filtrated by a 0.22-µm membrane filter.

2.8. Statistical analysis

The statistical analysis of the results related to the RSM method has been done with design-expert® 11 software. In addition, Graphpad Prism 8 software was used for statistical analysis and One-Way ANOVA test was performed and the results were reported as mean ± standard deviation.

3. Results

3.1. Total phenolic content

The total phenolic content of the extracts was quantified using the Folin-Ciocalteu assay, with gallic acid as a standard. Results were expressed as gallic acid equivalents of extract (mg/ml). The corresponding standard curves for the RAE and MAE analyses are presented in Figure 1. The total phenolic content of extracts obtained through RAE and MAE is presented in Table 3. Fig. 3A was compared the phenol yield obtained from reflux extraction (75 % ethanol, 110 min, 65 °C) with the microwave method (80 % ethanol, 5.5 min, 550 W) and the maceration method (74 hours in 70 % ethanol).

3.2. Total flavonoids content

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric assay, with quercetin as the standard. Results were expressed as quercetin equivalents of extract (mg/ml). The corresponding standard curves for the RAE and MAE analyses are presented in Figure 2. The total flavonoid content of extracts obtained through RAE and MAE is presented in Table 4. Figure 3B, was compared the flavonoid yield

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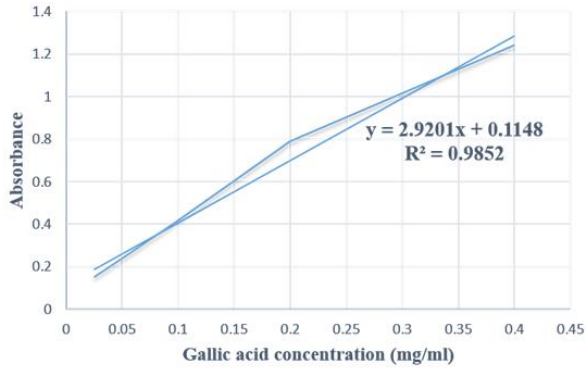
obtained from reflux extraction (80 % ethanol, 100 min, 70 °C) with the microwave method (80 % ethanol, 5.5 min, 550 W) and the maceration method (74 hours in 70 % ethanol).

A

Gallic acid (mg/ml)	Abs.
0.4	1.24
0.2	0.789
0.1	0.418
0.05	0.238
0.025	0.152



B

Gallic acid (mg/ml)	Abs.
0.4	1.37
0.2	0.701
0.1	0.399
0.05	0.234
0.025	0.149

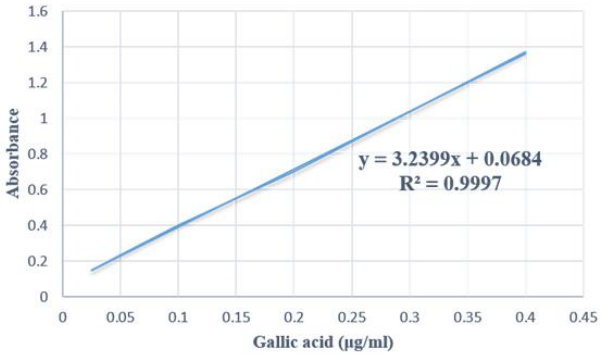


Fig. 1. Gallic acid standard curve for extraction with RAE (A) and MAE (B)

Tables 3. The total phenolic content of extracts obtained through RAE and MAE

	RAE		MAE	
N.	**Extraction yield (g)**	**Gallic acid (mg/ml)**	**Extraction yield (g)**	**Gallic acid (mg/ml)**
Run1	0.71	0.07541	0.32	0.1613
Run 2	0.83	0.06445	0.54	0.16593
Run 3	0.64	0.0487	0.71	0.14525
Run 4	0.57	0.06582	0.72	0.13939
Run 5	0.79	0.05349	0.59	0.18198
Run 6	0.67	0.07027	0.57	0.17859
Run 7	0.59	0.05657	0.66	0.14556
Run 8	0.76	0.06787	0.77	0.14093
Run 9	0.62	0.09082	0.69	0.1505
Run 10	0.63	0.08089	0.69	0.13074
Run 11	0.61	0.0737	0.64	0.13105
Run 12	0.62	0.07198	0.64	0.16439
Run 13	0.81	0.05897	0.65	0.14618
Run 14	0.78	0.07952	0.56	0.16192
Run 15	0.76	0.06274	0.75	0.16161
Run 16	0.61	0.08911	0.70	0.12056
Run 17	0.74	0.06479	0.77	0.14402
Run 18	0.75	0.07952	0.75	0.12704
Run 19	0.62	0.07507	0.61	0.15513
Run 20	0.76	0.10075	0.70	0.1255

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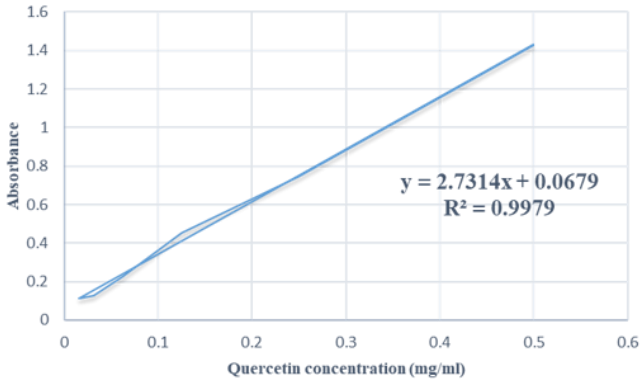
A

Quercetin (mg/ml)	Abs.
0.0156	0.112333
0.03125	0.129333
0.0625	0.227667
0.125	0.453
0.25	0.745667
0.5	1.428



B

Quercetin (mg/ml)	Abs.
0.0156	0.08
0.03125	0.13
0.0625	0.213
0.125	0.367
0.25	0.689
0.5	1.088

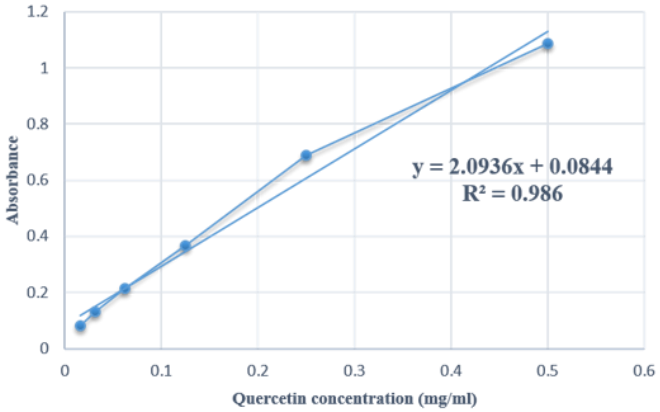


Fig. 2. Quercetin standard curve for extraction with RAE (A) and MAE (B)

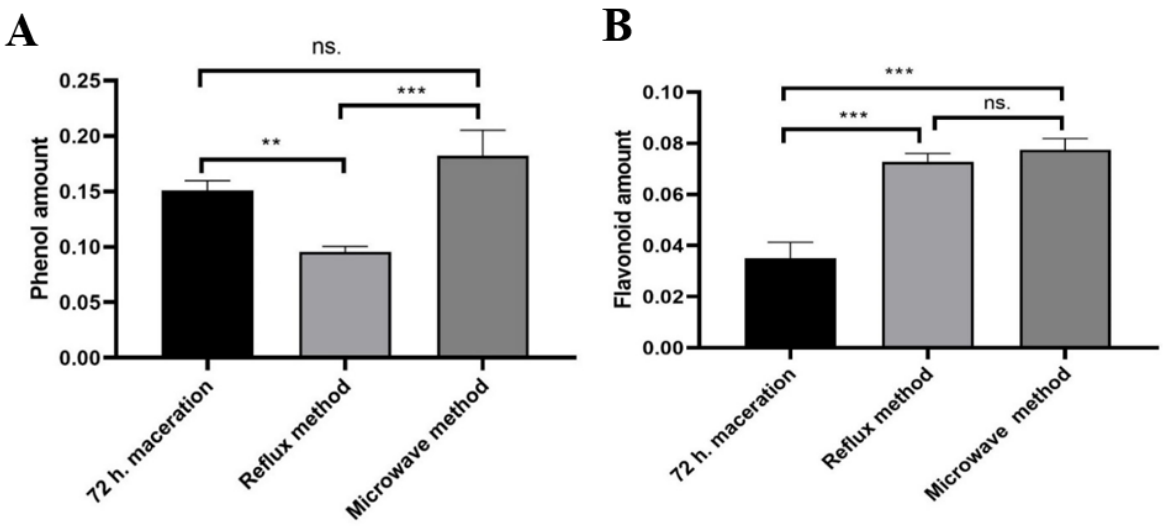


Fig. 3. Statistical comparison of the amount of total phenol (A) and phenolic content (B) in different extracts that obtained by maceration, RAE and MAE ($P < 0.01$**), ($P < 0.001$***)

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Tables 4. The total flavonoids content of extracts obtained through RAE (A) and MAE (B)

	RAE		MAE	
N.	**Extraction yield (g)**	**Quercetin (mg/ml)**	**Extraction yield (g)**	**Quercetin (mg/ml)**
Run1	0.71	0.05288	0.32	0.06636
Run 2	0.83	0.05947	0.54	0.0445
Run 3	0.64	0.0519	0.71	0.03531
Run 4	0.57	0.05495	0.72	0.0471
Run 5	0.79	0.04714	0.59	0.06429
Run 6	0.67	0.04702	0.57	0.05283
Run 7	0.59	0.0602	0.66	0.05617
Run 8	0.76	0.05617	0.77	0.03022
Run 9	0.62	0.0685	0.69	0.06286
Run 10	0.63	0.04653	0.69	0.03547
Run 11	0.61	0.0458	0.64	0.05267
Run 12	0.62	0.05276	0.64	0.04853
Run 13	0.81	0.05776	0.65	0.0412
Run 14	0.78	0.0519	0.56	0.05617
Run 15	0.76	0.05239	0.75	0.06318
Run 16	0.61	0.07277	0.70	0.05999
Run 17	0.74	0.05361	0.77	0.05251
Run 18	0.75	0.04879	0.75	0.05569
Run 19	0.62	0.05056	0.61	0.06318
Run 20	0.76	0.06228	0.70	0.07735

3.3. Analysis of response surface

The aim of Response Surface Methodology (RSM) is to model and optimize the relationship between multiple independent variables (including temperature, solvent concentration, and extraction time) and the dependent response variables including total phenolic and total flavonoid content of the extracts.

3.3.1. Response surface based on total phenol content

RAE: The optimization of RAE was performed to investigate three variables: extraction time (A), temperature (B), and ethanol concentration (C). The design included a total of 20 runs, including 5 replications of the center points. A reduced two-factor interaction (2FI) model was selected and fitted to the response data. The nonlinear, computer-generated model is given by the following equation:

$$Y\ (\text{phenol amount in RAE}) = 1.93276 + (1.93276A) - (0.041096\ B) - (0.023367\ C) - (0.000135\ AC) + (0.00051BC)$$

The Model F-value of 4.82 indicates that the model is statistically significant. Model terms with p-values less than 0.05 were considered significant. For the reduced 2FI model, the coefficient of determination (R^2) was 0.6326, the adjusted R^2 was 0.5014, and the predicted R^2 was 0.3363. The predicted R^2 of 0.3363 is in reasonable agreement with the adjusted R^2 of 0.5014, as their difference is less than 0.2. Furthermore, an adequate precision value of 9.6246, which measures the signal-to-noise ratio, exceeded the desirable threshold. The lack-of-fit test assesses the variation of the data around the fitted model; a non-significant result ($P > 0.05$) indicates a good model fit. As presented in Table 5, the lack of fit for the reduced 2FI model was not significant ($P = 0.6908$), suggesting that the model adequately describes the experimental data. The effects of the independent parameters (extraction time,

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temperature, and ethanol concentration) on total phenolic content are illustrated in Figure 4. The contour and 3D plots in Figure 4A demonstrate that the total phenolic content in RAE was influenced by ethanol concentration and extraction time.

Table 5. Results of the ANOVA to the response surface reduced 2FI model for RAE and MAE based on total phenol content

Suggested model	Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
RAE							
Reduced two-factor interaction (2FI)	**Model**	0.0020	5	0.0004	4.82	0.0090	significant
	A-time	0.0000	1	0.0000	0.1250	0.7290	
	B-temp	0.0000	1	0.0000	0.0000	1.0000	
	C-ethanol	0.0003	1	0.0003	4.11	0.0621	
	AC	0.0004	1	0.0004	4.31	0.0567	
	BC	0.0013	1	0.0013	15.56	0.0015	
	Lack of Fit	0.0007	9	0.0001	0.7115	0.6908	not significant
MAE							
Reduced two-factor interaction (2FI)	Model	0.0022	3	0.0007	3.29	0.0479	significant
	A-time	0.0013	1	0.0013	5.75	0.0290	
	C-ethanol	3.432E-06	1	3.432E-06	0.0155	0.9024	
	AC	0.0009	1	0.0009	4.10	0.0598	
	Lack of Fit	0.002	11	0.0002	0.5862	0.7862	not significant

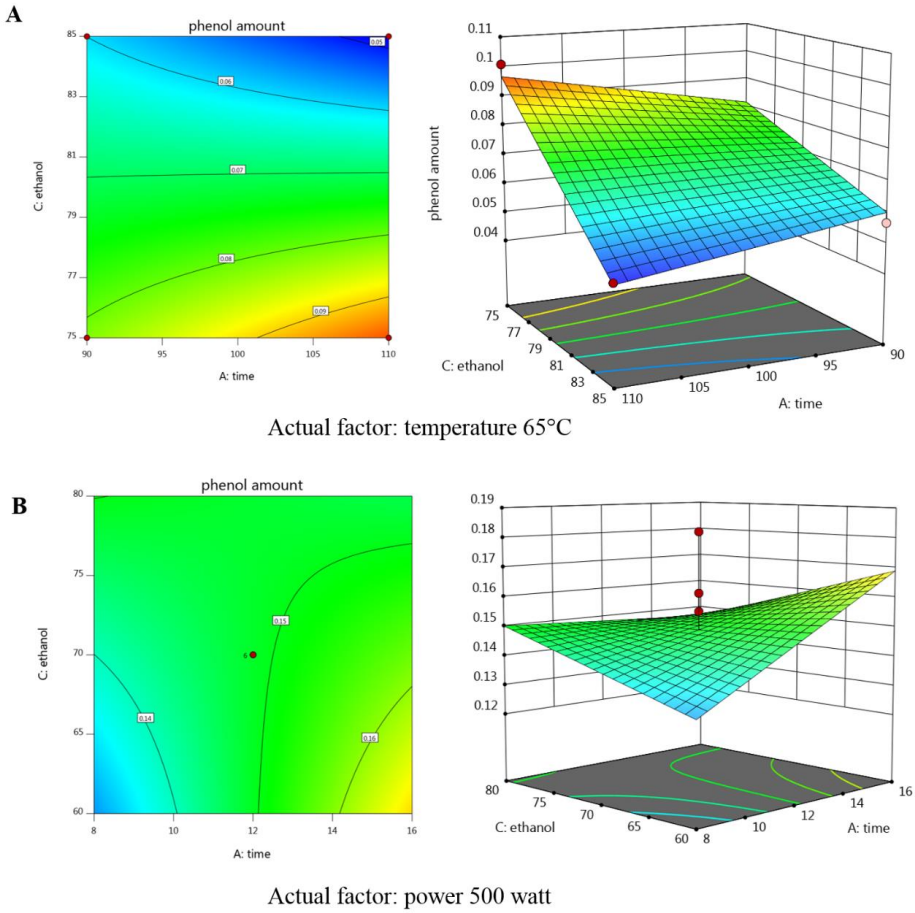

Fig. 4. Contour and 3D response surface plot for RAE (A) and MAE (B) on total phenol amount (mg)

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MAE: The optimization of MAE parameters was performed to evaluate the effects of three variables: extraction time (A), microwave power (B), and ethanol concentration (C). System behavior was modeled using a 2FI approach. The nonlinear, computer-generated model is given by the following equation:

$$Y \text{ (phenol amount in MAE)} = -0.098243 + (0.020864A) + (0.003148\ C) - (0.000266\ AC)$$

A Model F-value of 3.29 indicates that the reduced 2FI model is statistically significant. Model terms with p-values less than 0.05 were considered significant. The R^2, Adjusted R^2, and Predicted R^2 for the reduced 2FI model were 0.3815, 0.2655, and 0.0485, respectively. Moreover, the Adequate Precision value, which measures the signal-to-noise ratio, was 5.884. This value is greater than the desirable threshold of 4. The lack-of-fit test, which evaluates unexplained variation in the data, was not significant (P = 0.7862), as shown in Table 5. This indicates that the model adequately fits the experimental data. The effects of the independent parameters (extraction time, microwave power, and ethanol concentration) on total phenolic content are illustrated in Figure 4. Specifically, the contour and 3D plots in Figure 4B demonstrate that the phenolic content in MAE was dependent on ethanol concentration and extraction time.

3.3.2. Response surface based on total flavonoid content

RAE: The behavior of the system was modeled using a reduced two-factor interaction (2FI) model. Statistical analysis yielded a non-significant model F-value of 1.27 (P = 0.3186), indicating a 31.86 % probability that this result occurred due to noise. Consequently, none of the model terms were statistically significant (P > 0.05).

MAE: For the MAE optimization, three independent variables were investigated, including extraction time (A), microwave power (B), and ethanol concentration (C). A reduced 2FI model was fitted to the experimental data to describe the behavior of the system. The nonlinear, computer-generated model is given by the following equation:

$$Y \text{ (flavonoids amount in MAE)} = 0.0943865 - (0.00058725C)$$

Statistical analysis revealed a significant model with an F-value of 4.66. Model terms with *p*-values less than 0.05 were considered significant. For the reduced 2FI model, the coefficient of determination (R2) was 0.2065, the adjusted R2 was 0.1615, and the predicted R2 was 0.0551. The Adequate Precision value of 6.825 was greater than 4, which is considered desirable. The lack-of-fit test was not significant (P = 0.8382; Table 6), indicating that the model adequately fits the experimental data. The influence of independent parameters (extraction time, microwave power, and ethanol concentration) on total flavonoid content is illustrated in Figure 5. The contour and 3D plots demonstrate that the flavonoid yield in MAE was dependent on ethanol concentration.

3.3.3. Optimization of RAE and MAE parameters

The identification of optimal conditions for RAE and MAE required a systematic multi-response optimization analysis. Design-Expert® software determined the variable settings that maximized the yields of total phenols and flavonoids. The quality of these solutions was analyzed via a desirability function, where an

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index of 1.0 represents a fully ideal outcome. The optimal conditions identified by the software are reported in Table 7.

Table 6. Results of the ANOVA to the response surface reduced 2FI model for RAE and MAE based on total flavonoids content

Suggested model	Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
			RAE				
Reduced two-factor interaction (2FI)	**Model**	0.0002	3	0.0001	1.27	0.3186	not significant
	A-time	0.0000	1	0.0000	0.7508	0.3990	
	B-temp	9.688E-06	1	9.688E-06	0.1953	0.6644	
	AB	0.0001	1	0.0001	2.86	0.1102	
	Lack of Fit	0.0003	11	0.0000	0.3158	0.9484	not significant
			MAE				
Reduced two-factor interaction (2FI)	**Model**	0.0006	1	0.0006	4.66	0.0447	significant
	C-ethanol	0.0006	1	0.0006	4.66	0.0447	
	Lack of Fit	0.0012	13	0.0001	0.5248	0.8382	not significant

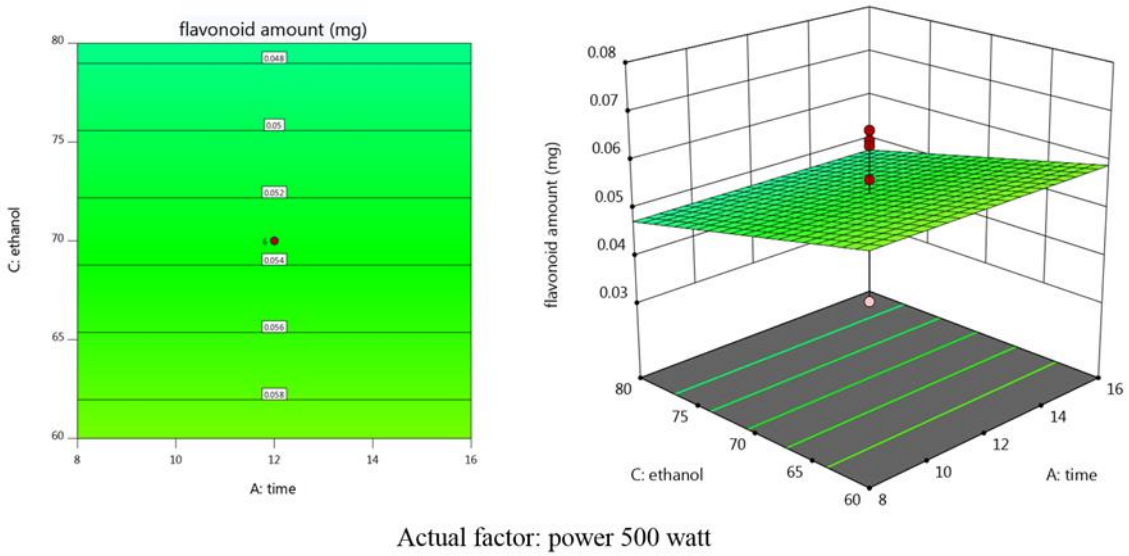

Fig. 5. contour and 3D response surface plot for MAE on total flavonoids amount (mg)

Table 7. The optimum conditions designed by Design-Expert

Independent variables	Time (min)	Temp. (°C)	Ethanol %	Extraction yield (g)	Total Phenol amount (mg)	Total Flavonoid amount (mg)	Desirability
RAE	110.000	65.000	75.000	0.748	0.097	0.058	0.649
Independent variables	**Time (min)**	**Power (watt)**	**Ethanol %**	**Extraction yield (g)**	**Total Phenol amount (mg)**	**Total Flavonoid amount (mg)**	**Desirability**
MAE	16.000	500.000	60.000	0.641	0.169	0.066	0.768

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3.4. Application of extract on macrospores resin

A 10-g of crude methanolic extract was suspended in water and applied to a Diaion HP-20 resin column. After sequential washing, target compounds were eluted with methanol. The eluates were concentrated and dried to yield 5.25 g of purified extract. Chromatographic analysis showed a 1.75-fold enrichment of phenolic compounds relative to the initial maceration extract, confirming the effective concentration of bioactive ingredients (Fig. 6).

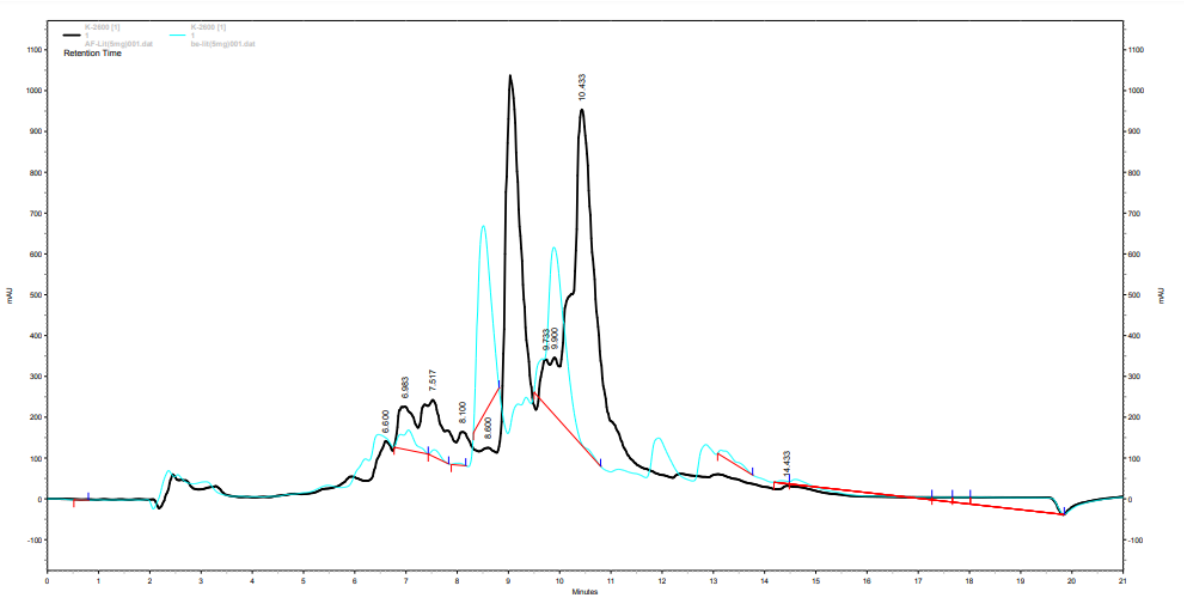

Fig. 6. HPLC chromatogram of the 5 mg plant extract before (blue) and after (black) undergoing column filtration at a wavelength of 254 nm.

4. Discussion

Flavonoids and phenolic compounds are bioactive compounds widely studied for their health benefits, including antioxidant, anti-inflammatory, and anticancer properties. Efficient extraction of these compounds is a critical step for pharmaceutical and nutraceutical applications. MAE is one of the most effective methods for flavonoid extraction, which use microwave radiation (300 MHz to 3000 GHz) to rapidly heat the sample. This technique achieves high extraction yields in a short time (15 – 30 minutes) while significantly reducing solvent consumption [13, 14]. Furthermore, based on previous studies, MAE produces greater flavonoid yields comparable to ultrasound-assisted extraction [15].

In this study, RAE and MAE were compared with conventional 72-hour maceration for extracting phenolic and flavonoid compounds. MAE achieved phenol yields comparable to maceration but in significantly less time (5.5 minutes vs. 72 hours). Conversely, for flavonoids, both MAE and RAE produced significantly higher yields than maceration ($P < 0.001$). Rapid micro-assay techniques were also validated for quantification. Overall, MAE showed to be a superior alternative to maceration, offering high yields with minimal time and solvent consumption, particularly for flavonoid recovery.

This study effectively utilized RSM-CCD to optimize the extraction parameters for RAE and MAE. The influence of independent variables

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on flavonoid extraction has been investigated in previous studies [16, 17]. Furthermore, Gan et al. optimized the extraction of total flavonoids from *Scutellaria baicalensis* to enhance yield, composition, and antioxidant activity. They used RSM to increase flavonoid extraction for the industrial production and bioactive utilization of *S. baicalensis* flavonoids. The optimal conditions were 56 % ethanol concentration, a 40:1 liquid-to-solid ratio, 50 °C temperature, and 1 hour of extraction time [18]. In the RAE model, extraction time was identified as the most influential parameter for total phenol extraction (+0.010901), whereas the model for total flavonoids was statistically insignificant. In contrast, both MAE models were statistically significant. Extraction time remained the most substantial factor, with positive coefficients for total phenols and total flavonoids of +0.020864 and +0.010726, respectively. These results highlight the critical role of extraction time in optimizing these advanced extraction techniques. Furthermore, the use of Diaion HP-20 resin for *S. litwinowii* extracts has proven to be a beneficial strategy for enhancing the concentration of bioactive compounds, particularly phenolic compounds and flavonoids. HPLC analysis demonstrates that incorporating resin particles can elevate the levels of bioactive compounds in plant extracts by approximately 1.75-fold.

5. Conclusion

This study focused on the optimization of phenol and flavonoid extraction from *S. litwinowii* root. The findings clearly establish that modern techniques, particularly MAE, offer substantial advantages over traditional maceration by significantly reducing extraction time and enhancing efficiency. RSM analysis demonstrated that extraction duration was the important factor for maximizing yield, whereas temperature, power, and ethanol concentration were less critical. Furthermore, purification with Diaion HP-20 resin enhanced the concentration of active compounds in the final extract by 1.75-fold. These optimized methods enhance the therapeutic quality of the herbal extract while advancing green chemistry through reduced solvent consumption. This work presents a scalable model for sustainable phytopharmaceutical development.

Authors' contributions

MTB contributed to data collection and initial draft; JA and SNE contributed to conceptualization and methodology; MB was involved in initial draft, data analysis, supervision and revision. All authors read and approved the manuscript.

Conflicts of interest

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

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