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Extraction of seven major compounds from *Agastache rugosa* (Fisch. & C.A.Mey.) Kuntze: optimization study using response surface methodology

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Abstract The purpose of this study is to demonstrate the potential enhancement of the flavonoid contents from *Agastache rugosa*, which can be obtained as raw materials for functional products in the food medicine industry by identifying important factors for efficient preparation to save costs and time in terms of economic factors. For this reason, response surface methodology using Box-Behnken design was used to optimize the extraction conditions for the maximum yield of seven major compounds from *A. rugosa*. The optimum conditions were obtained with an ethanol concentration of 60.0%, a temperature of 50 ℃, and an extraction time of 33.6 min, meaning that the regression analysis fits the experimental data well. Under these conditions, the seven major compounds **1**-**7** had observed values of 2.169, 2.135, 0.697, 2.485, 0.105, 1.247, and 0.551%, respectively. These results show that the observed values are in good agreement with the predicted values in the regression model. This process for optimization study exhibited a basic protocol for obtaining stable ingredients from *A. rugosa* that are appropriate for the development of effective functional products.

Keywords *Agastache rugose* · Optimum conditions · Response surface methodology

Introduction

The consumption of special seasonal vegetables, dietary supplements, shampoos, and cosmetics as functional materials in Korea has increased because of their potent biological properties and phytochemicals [1-3]. Additionally, these are food medicines that have been described in ancient documents as medicinal herbal remedies and as components of complex Korean medicinal preparations [http://www.koreantk.com/ktkp2014/prescription/list-by-index.page].

Agastache rugosa, commonly known as Korean mint, is mainly found in East Asia, including Korea. This plant has been widely used as a traditional medicine as well as food [4]. The leaves, stems, and flowers have been used as food ingredients, especially as a herb for fish-based stews in Korea [5]. Recent studies have shown the anti-inflammatory, anticancer, antiviral, antifungal, antioxidant and antimicrobial activities of this plant [6-8]. Previous phytochemical investigations on this plant have resulted in the isolation of several essential oils, flavonoids, diterpenoids, and lignins [9-12]. The main compounds of *A. rugosa* such as rosmarinic acid, tilianin, and acacetin are known to show effective antidiabetic, antioxidant, antiatherogenic, monoamine oxidase, anti-inflammatory and anticancer activities [13-18]. Because *A.*

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rugosa is commonly used for a food medicine as a powder, a pill, and/or alcohol extract, it requires particular attention to maintain the content and efficacy of the traditional substances equivalent [19]. However, the Korea Food and Drug Administration (KFDA) has not yet fully characterized the components of Korean *A. rugosa* as a basis to control the functional labeling system, including extracts and active substances standardized in general foods [20]. Therefore, it is important to correlate the contents of constituents with activities of *A. rugosa*, and it is meaningful to determine changes in the content of the active substances according to the various factors affected by the optimal extraction conditions.

Extraction conditions are an important consideration in recovering compounds from plants for natural product research [21]. Many factors, such as the extraction temperature, solvent, time, and pressure, solid-solvent ratios, and solvent composition, can significantly affect the extraction efficiency. Thus, optimization of extraction conditions is required for maximum efficiency [22-25]. Response surface methodology (RSM) has been widely used for optimizing extraction conditions to get more efficient and accurate approaches of the experimental process in agriculture, biology, food and chemistry [26].

This study was conducted to optimize the extraction conditions for the maximum yield of seven major compounds from *A. rugosa* using a RSM (Box-Behnken design). Therefore, this optimized extraction method can be applied to standardize the labeling system of *A. rugosa* extract as a functional food.

Material and Methods

Plant material

Aerial parts of *Agastache rugosa* (Lot No. SQ-18011-2) were collected at the Yeongcheon farm in Gyeongsang-do, South Korea, in July 2019. The plant was identified (Dr. Hocheol Kim), and the voucher specimen (D190724001) was deposited in the Herb Resource Bank of Traditional Korean Medicine (http://herb-bank.com), Kyung-Hee University (Seoul, Korea). Detection and quantification of sulfur dioxide, pesticide residues and heavy metals were performed according to KFDA guidelines (Ministry of Food and Drug Safety, Cheongju, Korea). The collected samples were dried immediately after sampling and then ground to a powder and stored at −20 °C until further analysis.

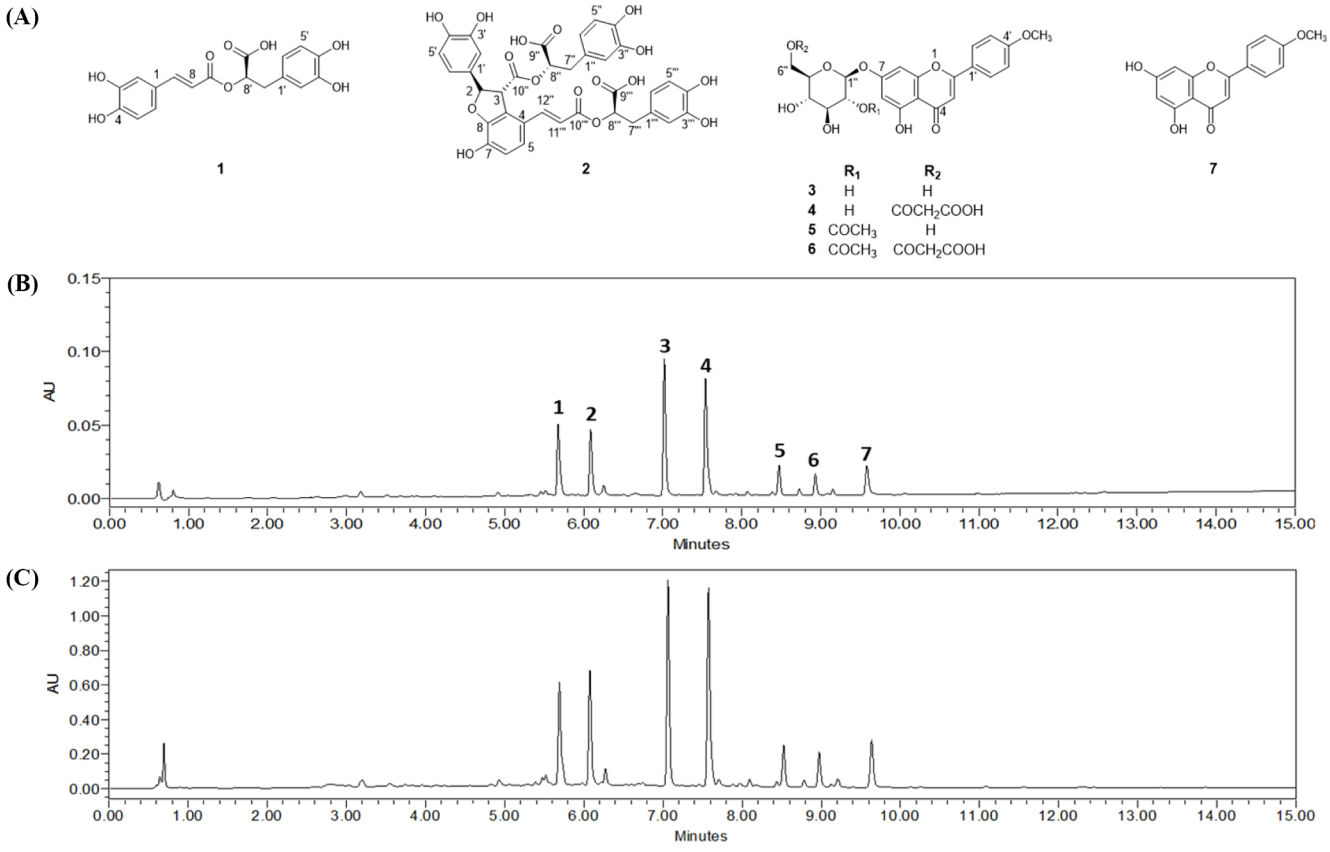

Fig. 1 The chemical structure and UPLC chromatogram of seven major compounds from *A. rugosa*. (A) Chemical structure of compounds **1-7**, (B) UPLC chromatogram of *A. rugosa* extract, (C) UPLC chromatogram of the extract prepared with the optimal extraction yield conditions

UPLC conditions for quantitation of compounds 1-7

Analysis was performed using an ACQUITY UPLC system equipped with a photodiode array. Chromatographic separations were performed on an ACQUITY BEH C_{18} column. Mobile phase A was water with 0.1% formic acid, and B was acetonitrile with 0.1% formic acid. This mobile phase system was run in a gradient elution as follows: 0-1.0 min at 5% B; 1.0-20.0 min from 5 to 100% B; 20.0-21.3 min at 100% B; 21.3-21.4 min from 100 to 5% B; and 21.4-25.0 min at 5% B. The injection volume was 2 µL, and the flow rate was 0.4 mL/min. The wavelength for detection was set at 254 nm (Figs. 1B, 1C).

For the analysis of *A. rugosa* extract, 100 mg of powdered *A. rugosa* was extracted with 2 mL of 70% ethanol in DW, and each sample solution was filtered through a 0.23 µm membrane filter before UPLC analysis. Major compounds (**1-7**) of *A. rugosa* were supplied from the Natural Medicine Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB). In the extract, seven compounds were identified by comparing their NMR and MS spectral data (Fig. 1A) and quantified as supplementary data (Supplementary Figs. 1-4, Supplementary Table 1) and a previous report [10]. The yields of compounds **1-7** were expressed as mg of each compound per mg of *A. rugosa* extract.

Extraction

For analysis, 100 mg of powdered *A. rugosa* was extracted into 4 mL of 95% EtOH (v/v, SK Chemicals, Seoul, Korea) with an ultrasonic bath (SDN-900H, SD Ultrasonic Cleaner, Seoul, Korea, 40 kHz, 300 W) at room temperature (23-25 ºC) and centrifuged at 800× g (3000 rpm) for 5 min (Model 5415R, Eppendorf, Hamburg, Germany). This process was repeated three times. Supernatant was filtered through a 0.22 µm polytetrafluoroethylene filter (Thermo Fisher, Waltham, MA, USA), concentrated and measured.

Experimental design

Box-Behnken design (BBD) with three independent variables and three levels was used to optimize the extraction conditions of *A. rugosa*. The design consisted of 15 experimental points with three levels (−1, 0, and 1) for each of the independent variables: ethanol concentration (X_1, %), extraction temperature (X_2, ºC), and extraction time (X_3, min). Based on the preliminary single-variable experiment, the ranges of these independent variables were determined as follows: ethanol concentration, X_1, 60, 80, and 100%; extraction temperature, X_2, 10, 30, and 50 ºC; and extraction time, X_3, 30, 60, and 90 min. The response variables were fit to the following second-order polynomial model equation, which was able to describe the connection between the responses and the independent variables:

$$Y=\beta_0+\sum_{i=1}^{n}\beta_i X_i+\sum_{i=1}^{n}\beta_{ii}X_i^2+\sum_{1\le i\le j}^{n}\beta_{ij}X_iX_j$$

where Y is the dependent variable, X_i and X_j are independent variables, β_0 is a constant coefficient, β_i represents the linear coefficients, β_{ii} represents the quadratic coefficients, β_{ij} represents the interaction coefficients, and n is the number of variables. Analysis of variance for the regression equation and regression coefficients was used to determine the suitability and significance. The fitness of the polynomial equation to the responses was evaluated with the coefficients of determination (R^2), and lack of fit was evaluated using an F-test.

Table 1 Box-Behnken design for independent variables and responses

Run	Coded variables			Actual variables			Observed values						
	X_1	X_2	X_3	EtOH (%)	Temperature (°C)	Time (min)	peak 1 (%) [a]	peak 2 (%) [a]	peak 3 (%) [a]	peak 4 (%) [a]	peak 5 (%) [a]	peak 6 (%) [a]	peak 7 (%) [a]
1	-1	-1	0	60	10	60	1.91	1.77	0.49	2.00	0.07	1.04	0.39
2	1	-1	0	100	10	60	0.21	0.05	0.07	0.12	0.01	0.09	0.17
3	-1	1	0	60	50	60	2.03	2.04	0.67	2.29	0.09	1.10	0.49
4	1	1	0	100	50	60	0.70	0.14	0.24	0.40	0.04	0.31	0.39
5	-1	0	-1	60	30	30	2.05	1.96	0.56	2.20	0.08	1.11	0.41
6	1	0	-1	100	30	30	0.30	0.08	0.10	0.18	0.02	0.13	0.21
7	-1	0	1	60	30	90	1.95	1.89	0.60	2.10	0.09	1.17	0.41
8	1	0	1	100	30	90	0.35	0.08	0.12	0.20	0.02	0.16	0.26
9	0	-1	-1	80	10	30	0.93	0.45	0.19	0.65	0.03	0.35	0.20
10	0	1	-1	80	50	30	1.82	1.14	0.54	1.87	0.08	1.01	0.46
11	0	-1	1	80	10	90	1.36	0.59	0.29	0.95	0.04	0.52	0.31
12	0	1	1	80	50	90	1.99	1.30	0.63	2.00	0.09	1.03	0.49
13	0	0	0	80	30	60	1.42	0.71	0.35	1.11	0.04	0.58	0.34
14	0	0	0	80	30	60	1.66	0.79	0.40	1.34	0.05	0.72	0.39
15	0	0	0	80	30	60	1.72	0.84	0.43	1.45	0.06	0.84	0.43

[a]peak (%) was expressed as mg peak/mg extract

Results and Discussion

Fitting the model

The effects of the three independent variables, namely, the ethanol concentration (X_1), extraction temperature (X_2), and extraction time (X_3), on the extraction yield of the seven major compounds were investigated using BBD, and the results are shown in Table 1. Based on a preliminary single-factor experiment, the ranges of these variables were determined as follows: ethanol concentration, X_1, 60-100%; extraction temperature, X_2, 10-50 ºC; and extraction time, X_3, 30-90 min. As shown in Table 1, the content of compounds **1-7** changed significantly with the extraction conditions (Supplementary Fig. 5). The content of compounds **1-7** was maximized as 0.21, 0.05, 0.07, 0.12, 0.01, 0.09, and 0.17%, respectively under the extraction conditions of an ethanol concentration of 60%, temperature of 50 ºC, and time of 60 min. On the other hand, it was confirmed that the contents of compounds **1-7** were lowered in other conditions.

The relationship between the content of compounds **1-7** and the extraction variables was expressed in the secondary polynomial regression equation as follows:

$$\text{Compound } \mathbf{1} \text{ yield} = -1.38 + 0.0996X_1 + 0.0036X_2 + 0.0089X_3 - 0.000937X_1^2 - 0.000032X_2^2 - 0.000070X_3^2 + 0.000226X_1X_2 + 0.000062X_1X_3 - 0.000109X_2X_3 \quad (1)$$

Table 2 Regression coefficients and their significance in the second-order polynomial regression equation for compounds **1** and **7** from *A. rugosa*

	Coefficient	Standard error	t	p
[peak 1]				
Intercept	1.600	0.121	13.27	0.000
X_1	-0.7970	0.0739	-10.79	0.000
X_2	0.2654	0.0739	3.59	0.016
X_3	0.0688	0.0739	0.93	0.394
X_1^2	-0.375	0.109	-3.45	0.018
X_2^2	-0.013	0.109	-0.12	0.912
X_3^2	-0.063	0.109	-0.58	0.589
X_1X_2	0.090	0.104	0.86	0.427
X_1X_3	0.037	0.104	0.36	0.735
X_2X_3	-0.065	0.104	-0.62	0.560
[peak 7]				
Intercept	0.3874	0.0226	17.10	0.000
X_1	-0.0820	0.0139	-5.91	0.002
X_2	0.0953	0.0139	6.87	0.001
X_3	0.0242	0.0139	1.74	0.142
X_1^2	-0.0384	0.0204	-1.88	0.119
X_2^2	0.0097	0.0204	0.47	0.656
X_3^2	-0.0282	0.0504	-1.38	0.226
X_1X_2	0.0283	0.0196	1.44	0.208
X_1X_3	0.0134	0.0196	0.69	0.523
X_2X_3	-0.0202	0.0196	-1.03	0.351

$$\text{Compound } \mathbf{2} \text{ yield} = 7.07 - 0.1152X_1 + 0.0131X_2 - 0.0080X_3 + 0.000443X_1^2 + 0.000110X_2^2 + 0.000050X_3^2 - 0.000115X_1X_2 + 0.000034X_1X_3 + 0.000010X_2X_3 \quad (2)$$

$$\text{Compound } \mathbf{3} \text{ yield} = 0.308 + 0.0077X_1 + 0.00338X_2 + 0.00154X_3 - 0.000115X_1^2 + 0.000056X_2^2 + 0.000000X_3^2 - 0.000003X_1X_2 - 0.000006X_1X_3 - 0.000001X_2X_3 \quad (3)$$

$$\text{Compound } \mathbf{4} \text{ yield} = 2.47 + 0.0078X_1 + 0.0143X_2 - 0.0029X_3 - 0.000367X_1^2 + 0.000127X_2^2 + 0.000020X_3^2 - 0.000002X_1X_2 + 0.000050X_1X_3 - 0.000067X_2X_3 \quad (4)$$

$$\text{Compound } \mathbf{5} \text{ yield} = 0.0722 + 0.00024X_1 + 0.00015X_2 + 0.000164X_3 - 0.000010X_1^2 + 0.000013X_2^2 + 0.000003X_3^2 + 0.000004X_1X_2 - 0.000003X_1X_3 - 0.000004X_2X_3 \quad (5)$$

Table 3 Regression coefficients and their significance in the second-order polynomial regression equation for compounds **2-4** from *A. rugosa*

	Coefficient	Standard error	t	p
[peak 2]				
Intercept	0.779	0.103	7.54	0.001
X_1	-0.9135	0.0633	-14.43	0.000
X_2	0.2219	0.0633	3.51	0.017
X_3	0.0289	0.0633	0.46	0.667
X_1^2	0.1774	0.0932	1.9	0.115
X_2^2	0.0442	0.0932	0.47	0.655
X_3^2	0.0447	0.0932	0.48	0.652
X_1X_2	-0.0460	0.0895	-0.51	0.629
X_1X_3	0.0205	0.0895	0.23	0.828
X_2X_3	0.0062	0.0895	0.07	0.948
[peak 3]				
Intercept	0.3911	0.0378	10.35	0.000
X_1	-0.2237	0.0231	-9.67	0.000
X_2	0.1291	0.0231	5.58	0.003
X_3	0.0304	0.0231	1.31	0.246
X_1^2	-0.0460	0.0341	-1.35	0.235
X_2^2	0.0224	0.0341	0.66	0.540
X_3^2	0.0000	0.0341	0.00	1.000
X_1X_2	-0.0012	0.0327	-0.04	0.973
X_1X_3	-0.0038	0.0327	-0.12	0.911
X_2X_3	-0.0004	0.0327	-0.01	0.991
[peak 4]				
Intercept	1.300	0.175	7.45	0.001
X_1	-0.961	0.107	-8.99	0.000
X_2	0.354	0.107	3.31	0.021
X_3	0.044	0.107	0.42	0.695
X_1^2	-0.147	0.157	-0.93	0.393
X_2^2	0.051	0.157	0.32	0.760
X_3^2	0.018	0.157	0.11	0.914
X_1X_2	-0.001	0.151	-0.01	0.996
X_1X_3	0.030	0.151	0.20	0.850
X_2X_3	-0.040	0.151	-0.27	0.800

Compound **6** yield = $1.04 + 0.00694X_1 + 0.0045X_2 + 0.0028X_3 - 0.000203X_1^2 + 0.000004X_2^2 + 0.000011X_3^2 + 0.000104X_1X_2 - 0.000013X_1X_3 - 0.000065X_2X_3$ (6)

Compound **7** yield = $0.036 + 0.00779X_1 - 0.00034X_2 + 0.00378X_3 - 0.000096X_1^2 + 0.000024X_2^2 - 0.000031X_3^2 + 0.000071X_1X_2 + 0.000022X_1X_3 - 0.000034X_2X_3$ (7)

As shown in Table 2, the linear term of ethanol concentration (X_1) and extraction temperature (X_2) and quadratic term of ethanol concentration (X_1^2) exhibited the most significant effects on the yield of compounds **1** and **7**. However, other variables did not have a significant effect. The values of F =15.95 and F =10.48, together with p =0.004 and p =0.009, for compounds **1** and **7**, respectively, supported the suitability of the model (Table 5). The coefficient of determination (R^2) values were 0.9663 and 0.9497 for compounds **1** and **7**, respectively, and the adjusted coefficient of determination (adj. R^2) values were 0.9057 and 0.8590, indicating a high degree of interrelation between the predicted and observed values. Additionally, the p values for lack of fit were 0.338 and 0.783 for compounds **1** and **7**, respectively, which also indicated that the model sufficiently fit the result values. In this study, the statistical analysis matched well between the predicted and experimental values and supported the suitability of this polynomial model for further optimization.

The effect on the extraction yield of compounds **2**-**6** was investigated as described above. The linear terms of ethanol concentration (X_1) and extraction temperature (X_2) exhibited the

Table 4 Regression coefficients and their significance in the second-order polynomial regression equation for compounds **5** and **6** from *A. rugosa*

	Coefficient	Standard error	t	p
[peak 5]				
Intercept	0.05087	0.00555	9.16	0.000
X_1	-0.02887	0.00340	-8.49	0.000
X_2	0.02058	0.00340	6.05	0.002
X_3	0.00401	0.00340	1.18	0.291
X_1^2	-0.00406	0.00500	-0.81	0.454
X_2^2	0.00533	0.00500	1.07	0.336
X_3^2	0.00233	0.00500	0.47	0.661
X_1X_2	0.00147	0.00481	0.31	0.772
X_1X_3	-0.00175	0.00481	-0.36	0.731
X_2X_3	-0.00216	0.00481	-0.45	0.672
[peak 6]				
Intercept	0.7135	0.0976	7.31	0.001
X_1	-0.4637	0.0598	-7.76	0.001
X_2	0.1817	0.0598	3.04	0.029
X_3	0.0341	0.0598	0.57	0.592
X_1^2	-0.0811	0.0880	-0.92	0.399
X_2^2	0.0016	0.0880	0.02	0.986
X_3^2	0.0101	0.0880	0.11	0.913
X_1X_2	0.0414	0.0845	0.49	0.645
X_1X_3	-0.0075	0.0845	-0.09	0.933
X_2X_3	-0.0391	0.0845	-0.46	0.663

Table 5 Analysis of variance (ANOVA) of the response surface regression equation for compounds **1** and **7**

	Degree of freedom	Sum of square	Mean square	F	P
[peak 1]					
Regression	9	6.26259	0.69584	15.95	0.004
Linear	3	5.68292	1.89431	43.41	0.001
Square	3	0.52447	0.17482	4.01	0.085
Interaction	3	0.05520	0.01840	0.42	0.746
Residual error	5	0.21819	0.04364	-	-
Lack-of-fit	3	0.16573	0.05524	2.11	0.338
Pure error	2	0.05247	0.02623	-	-
Total	14	6.48078	-	-	-
R^2=0.9663, adjusted R^2=0.9057					
[peak 7]					
Regression	9	0.145143	0.016127	10.48	0.009
Linear	3	0.130996	0.043665	28.37	0.001
Square	3	0.008586	0.002862	1.86	0.254
Interaction	3	0.005562	0.001854	1.20	0.398
Residual error	5	0.007695	0.001539	-	-
Lack-of-fit	3	0.002776	0.000925	0.38	0.783
Pure error	2	0.004919	0.002459	-	-
Total	14	0.152838	-	-	-
R^2=0.9497, adjusted R^2=0.8590					

Table 6 Analysis of variance (ANOVA) of the response surface regression equation for compounds **2-4**

	Degree of freedom	Sum of square	Mean square	F	P
[peak 2]					
Regression	9	7.21021	0.80113	24.99	0.001
Linear	3	7.07715	2.35905	73.60	0.000
Square	3	0.12276	0.04092	1.28	0.377
Interaction	3	0.01030	0.00343	0.11	0.952
Residual error	5	0.16027	0.03205	-	-
Lack-of-fit	3	0.15163	0.05054	11.71	0.080
Pure error	2	0.00864	0.00432	-	-
Total	14	7.37047	-	-	-
R^2=0.9783, adjusted R^2=0.9391					
[peak 3]					
Regression	9	0.551560	0.061284	14.30	0.005
Linear	3	0.541191	0.180397	42.09	0.001
Square	3	0.010304	0.003435	0.80	0.544
Interaction	3	0.000065	0.000022	0.01	0.999
Residual error	5	0.021428	0.004286	-	-
Lack-of-fit	3	0.018138	0.006046	3.67	0.221
Pure error	2	0.003291	0.001645	-	-
Total	14	0.572989	-	-	-
R^2=0.9626, adjusted R^2=0.8953					
[peak 4]					
Regression	9	8.51150	0.94572	10.35	0.010
Linear	3	8.40500	2.80167	30.66	0.001
Square	3	0.09636	0.03212	0.35	0.791
Interaction	3	0.01014	0.00338	0.04	0.989
Residual error	5	0.45689	0.09138	-	-
Lack-of-fit	3	0.39685	0.13228	4.41	0.190
Pure error	2	0.06004	0.03002	-	-
Total	14	8.96839	-	-	-
R^2=0.9491, adjusted R^2=0.8574					

most significant effects on the yield of compounds **2-6** (Tables 3 and 4). However, other variables did not show any significant effect. In this study, the R^2 values obtained for compounds **2-6** were 0.9783, 0.9626, 0.9491, 0.9575 and 0.9343, respectively, indicating a good description of the variability of the models. In addition, the lack-of-fit statistics for all parameters that measure the suitability of the model had significant *p*-values (0.08-0.332), and the high *F*-values (2.16-11.71) further confirmed the reliability of the models within the scope of the process conditions evaluated in this study (Tables 6 and 7).

Three-dimensional response surface plots for each compound yield are shown in Fig. 2. The linear effect of the ethanol concentration was inversely proportional to the extraction yield, consistent with the regression analysis results. As the extraction temperature and time increased, the extraction yield also increased. Generally, the content of extraction yield of *Cnidium monnieri* fruits [22], perilla leaves [23], *Ilex paraguariensis* leaves [26], *Cudrania tricuspidata* fruits [27], *Morus alba* leaves [28], *Cnidium monnieri* fruits [29], and *Eleutherococcus sessiliflorus* (Rupr. & Maxim.) leaves [30] was found to be closely related to temperature and time. Therefore, solvent ratio, temperature, and time show a high correlation with extraction yield, and there are also differences in extraction yield depending on the type of plant compound due to a specific component.

Optimizing and validating the extraction parameters

Verification experiments were performed using the recommended optimal conditions derived from RSM (Table 8). The optimal conditions for the maximum extracted yield of the seven major compounds were determined to be an ethanol concentration of 60.0%, extraction temperature of 50.0 ℃, and extraction time of 33.6 min. The model predicted an extraction of 2.166, 2.204, 0.692, 2.556, 0.099, 1.243, and 0.494% for compounds **1-7**, respectively; under these conditions, the observed values were

Table 7 Analysis of variance (ANOVA) of the response surface regression equation for compounds **5** and **6**

	Degree of freedom	Sum of square	Mean square	F	P
[peak 5]					
Regression	9	0.010421	0.001158	12.53	0.006
Linear	3	0.010183	0.003394	36.72	0.001
Square	3	0.000198	0.000066	0.71	0.585
Interaction	3	0.000040	0.000013	0.14	0.930
Residual error	5	0.000462	0.000092	-	-
Lack-of-fit	3	0.000367	0.000122	2.57	0.292
Pure error	2	0.000095	0.000048	-	-
Total	14	0.010883	-	-	-
R^2=0.9575, adjusted R^2=0.8811					
[peak 6]					
Regression	9	2.03239	0.22582	7.91	0.017
Linear	3	1.99372	0.66457	23.27	0.002
Square	3	0.02545	0.00848	0.30	0.827
Interaction	3	0.01321	0.00440	0.15	0.923
Residual error	5	0.14281	0.02856	-	-
Lack-of-fit	3	0.10908	0.03636	2.16	0.332
Pure error	2	0.03373	0.01686	-	-
Total	14	2.17520	-	-	-
R^2=0.9343, adjusted R^2=0.8162					

2.169, 2.135, 0.697, 2.485, 0.105, 1.247, and 0.551%. These results show that the observed values are in good agreement with the predicted values in the regression model. Therefore, this model can be applied effectively to predict the extraction of seven major compounds from *A. rugosa*. RSM has many advantages compared to the previous simplicity and routine methods for quality control. Fewer experiments are expected to research the all factors effectively, and the optimal combination of all variables can be easily found. It also efficient and takes less time and effort. With all these advantages, it is used in various fields of application including the natural products and food industry.

In the present study, RSM using a BBD method was successfully employed to optimize the extraction of seven major compounds from *A. rugosa*. The quadratic polynomial model provided a satisfactory description of the experimental values and agreement with the predicted values. The results show that the determined optimized conditions simultaneously maximized the content of the seven major compounds, and the ethanol concentration was the most important variable to control the extraction yield from *A. rugosa*. These results will provide useful information for the development of *A. rugosa* in the pharmaceutical and food industries.

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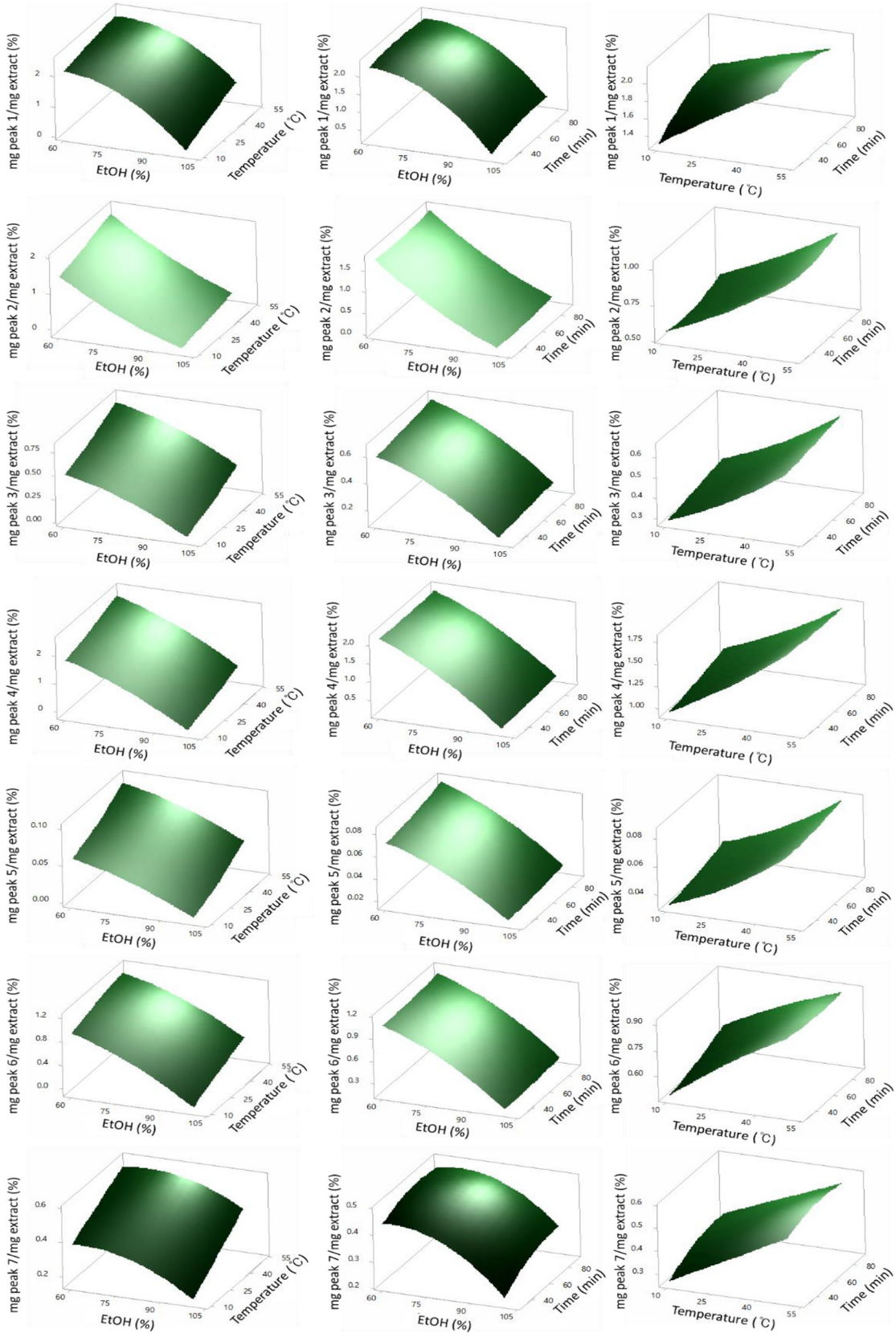

Fig. 2 Response surface plot analysis of the effect of the ethanol concentration, temperature, and extraction time on seven major compounds from *A. rugosa*

Table 8 Predicted and observed values of responses tested at optimal maximum extraction yield conditions

Parameters	Predicted	Observed
EtOH (%)	60.0	60.0
Temperature (°C)	50.0	50.0
Time (min)	33.6	34.0
Peak 1 (%)	2.166	2.169
Peak 2 (%)	2.204	2.135
Peak 3 (%)	0.692	0.697
Peak 4 (%)	2.556	2.485
Peak 5 (%)	0.099	0.105
Peak 6 (%)	1.243	1.247
Peak 7 (%)	0.494	0.551

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