Optimizing the Extraction of *Sasa quelpaertensis* Nakai to Develop Natural Cosmetics with Antioxidant and Whitening Activities

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Optimization of the extraction procedure was performed to enhance the antioxidant activity and whitening effect of *Sasa quelpaertensis* Nakai extract using response surface methodology (RSM). The central composite design, a component of RSM, was utilized to optimize and validate the ethanol extract for antioxidant activity and the hot water extract for the whitening effect, respectively. Activities of antioxidant and whitening were determined by DPPH and tyrosinase inhibition assays. The antioxidant activity was notably influenced by ethanol concentration ($p = 0.0344$) more than other factors. The optimal conditions for the antioxidant effect were 54% ethanol concentration, 52 °C, and 3 h extraction time, yielding an antioxidant activity of 83.65±1.56%. On the other hand, the whitening effect was significantly impacted by ultrasonic irradiation time ($p = 0.0175$) compared to other factors. The optimal conditions for whitening were 41 °C, 1:19 of sample-to-solvent ratio, and 8 min of ultrasonic irradiation, achieving a tyrosinase inhibition activity of 51.00±1.80%. High-performance liquid chromatography (HPLC) analysis was conducted to identify compounds such as tricin with antioxidant activity and p-coumaric acid, arbutin with whitening effect under the optimized conditions. The results suggest that the optimized extracts from *S. quelpaertensis* could be utilized as beneficial cosmeceutical materials.

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INTRODUCTION

The demand for natural cosmetic materials, prioritizing safety and functionality, has garnered increasing interest in addressing the side effects of synthetic materials (Hoang *et al.* 2021). Numerous prior studies have explored the use of plant extracts in cosmetic production (Thong-on *et al.* 2021). This study focused on evaluating the antioxidant and whitening properties of *Sasa quelpaertensis* Nakai to establish a database of indigenous natural cosmetic materials in Korea. *S. quelpaertensis* is a member of the *Sasa* genus, found exclusively near Mount Halla on Jeju Island as a local endemic species.

Leaves of *Sasa* species have been used in traditional medicine for their anti-inflammatory, antipyretic, and diuretic properties (Sultana and Lee 2010). Recently,

various biological studies of extracts from *S. quelpaertensis* have been conducted, alongside numerous research efforts to isolate novel bioactive compounds and verify their biological activities (Lee and Lee 2017).

Ethanol and hot water are commonly used solvents for natural product extraction. The constituents and bioactivity of extractives vary depending on the extraction conditions. Phenolic compounds are the most important bioactive compounds in plants (Al-Rajhi *et al.* 2023; Bakri *et al*. 2024). They are known for their active properties, including free radical scavenging, inhibition of oxidative enzymes, and anti-inflammatory effects (Banjarnahor and Artanti 2014).

Also, extracts from *S. quelpaertensis* contain numerous phenolic compounds and flavonoid derivatives with antioxidant and whitening effects (Sultana and Lee 2010; Kang *et al.* 2016; Lee *et al.* 2016). If properly utilized, the results of this study will enhance the industrial use of *S. quelpaertensis*, which grows abundantly in the Jeju region of South Korea, as a cosmetic ingredient. However, in order to improve its industrial use, optimal extraction conditions for process efficiency are required, and this study aimed to explore the optimal extraction conditions.

This study investigated the optimal extraction conditions to enhance the antioxidant and whitening activities of extracts from *S. quelpaertensis* using the central composite design model (CCD) of response surface methodology (RSM), which provides the most information with the least amount of experimentation (Lee *et al.* 2018). The study established the optimal extraction conditions of ethanol extraction for antioxidant properties and hot water extraction for whitening effects and validated the experimental values under the optimized conditions. Furthermore, useful compounds with antioxidant and whitening effects at the optimal conditions were analyzed using high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials

S. quelpaertensis Nakai, grown in June near eastern Jeju-Island, was collected and supplied from Jeju technology application division of the Korea Institute of Industrial Technology, and used as the material for this study. The analytical grade chemical ethanol was purchased from Samchun (Korea). For the antioxidant activity assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma (USA). For the whitening effect evaluation, L-DOPA and the mushroom tyrosinase (1000 U/mg) were purchased from Sigma-Aldrich (USA), and sodium phosphate monobasic anhydrous (NaH2PO4) and sodium phosphate dibasic anhydrous (NaH2PO4) were purchased from Duksan (Korea). For HPLC analysis, phenolic standards such as tricin (95%), p-coumaric acid (98%), and arbutin (98%) were procured from Sigma-Aldrich (USA). To establish the experimental parameters, the central composite design was applied using Design-Expert 12 software.

Extraction

Solvent (ethanol) extraction for antioxidant effect

According to previous researchers, extraction is an important process used for the quantification and isolation of bioactive substances derived from plants. Various factors such as solvent concentration, extraction temperature, and time are generally known to

influence ethanol extraction.

Response surface methodology (RSM) has been utilized for the development and optimization of experiments with high efficiency in biochemical industries (Kalil *et al.* 2000). The process of ethanol extraction from *S. quelpaertensis* was determined based on the central composite design (CCD) for antioxidant activity. CCD is the most commonly used fractional design in the response surface model.

The variables were designed for 17 experimental runs, including 2 at the central point, 8 at the factorial point, and 6 at the axial point, to find the optimum ethanol extraction conditions. The extraction variables included in Table 1 were ethanol concentration (% (v/v)), extraction temperature (°C), and extraction time (h). A 4 g sample powder of *S. quelpaertensis* was used under the conditions of the designed model, with varying ranges of concentration (0 to 88%), temperature (23 to 81°C), and time (1 to 8 h).

Table 1. Independent Variables and their Coded and Actual Values of Ethanol Extraction Condition of *S. quelpaertensis* Nakai

Independent Variable	Units	Symbol	Coded Levels				
			- α	-1	0	1	+α
Concentration	%	x_1	0	18	44	70	88
Temperature	°C	x_2	23	35	52	69	81
Time	H	x_3	1	3	5	7	8

α presented distance circumscribed from axial point to center point (α = 1.682)

Antioxidant Effect of Ethanol Extracts

DPPH (2,2-diphenyl-1-picrylhydrazyl) is commonly used to evaluate the free radical scavenging activity of various compounds. The DPPH radical scavenging activity assay was conducted based on the method developed by Blois with slight modifications (Blois 1958).

A total of 100 μL of sample solutions were diluted to various concentrations ranging from 0.156% to 10%. Subsequently, and the diluted samples were combined with 50 μL of 0.4 mM DPPH solution or 50 μL of ethanol. The mixture was then incubated in a dark room for 10 minutes, and the absorbance was measured at 517 nm. The DPPH radical scavenging activity was determined by calculating the difference in absorbances between the control and experimental groups (1).

$$\text{DPPH radical scavenging (\%)} = \{100-(\text{sample-blank})/(\text{control-blank})\} \times 100 \quad (1)$$

Hot Water Extraction Assisted Ultrasound Pretreatment

The experimental design using RSM was conducted to determine the optimal conditions for both ethanol and hot water extraction. However, ultrasonic pretreatment was only applied to the hot water extraction process. Extraction assisted with ultrasound irradiation induced cavitation by passing ultrasound waves, leading to enhanced extraction compared to conventional methods, such as hot water extraction alone (Macro *et al.* 2020). Extraction processes were applied to RSM analysis using the Central Composite Design (CCD) model to achieve the maximum whitening effect of hydrothermal-assisted ultrasound extraction from *S. quelpaertensis*. A total of 27 experimental runs were

conducted, including 3 at the central point, 16 at factorial points, and 8 at axial points, to determine the optimal extraction conditions. The extraction variables, as shown in Table 2, included extraction time (h), extraction temperature (°C), sample-to-solvent ratio (g/mL), and ultrasonic irradiation time (min).

The 4 g sample powder of *S. quelpaertensis* was subjected to experiments under specific conditions, including varying extraction times (1 to 9 h), temperatures (23 to 95 °C), sample-to-solvent ratios (7 to 23 g/mL), and ultrasonic irradiation times (0 to 32 min).

Table 2. Independent Variables and their Coded and Actual Values of Hot Water Assisted Ultrasound Extraction Condition of *S. quelpaertensis* Nakai

Independent Variable	Units	Symbol	Coded Levels				
			- α	-1	0	1	+α
Time	h	x_1	1	3	5	7	9
Temperature	°C	x_2	23	41	59	77	95
Solvent ratio	g/mL	x_3	7	11	15	19	23
sonic irradiation time	min	x_4	0	8	16	24	32
α presented distance circumscribed from axial point to center point (α = 2)							

Tyrosinase Inhibition Assay of Hot Water Extracts

To determine the inhibitory effect of *S. quelpaertensis* extraction on mushroom tyrosinase activity, an enzyme inhibition experiment was conducted in triplicate, following the method described with slight modifications by Yagi *et al*. (1987).

The sample solution was prepared by dissolving the sample in ethanol and diluting it to an appropriate concentration range (0.156% to 10%) to suppress tyrosinase activity. 40 µL of 10 mM L-DOPA, 80 µL of 67 mM phosphate buffer (pH 6.8), and 40 µL of the same buffer with or without the test sample were added to a 96-well microplate. The mushroom tyrosinase (1000 U/mg) was diluted to 200 U/mL, then, 40 µL of mushroom tyrosinase was mixed in. After incubation, the assay mixture was left at 37 °C for 10 min. The amount of dopachrome produced in the reaction mixture was measured by spectrophotometric analysis of absorbance at 490 nm.

$$\text{Tyrosinase inhibition activity (\%)} = 100 - \frac{(b-b')}{(a-a')} \times 100 \quad (2)$$

where *a* is the absorbance after reaction of the control, *b* is the absorbance after reaction of the sample, and *a'* and *b'* are the corresponding absorbances by substituting buffer in place of enzyme.

Optimal Condition and Model Validation

The optimal methodology has three major steps: firstly, the design of the statistical experiment, called CCD, determines the levels and number of independent variables. Secondly, the regression equation generated by ANOVA analysis is used to model the response surface. Lastly, this model should be verified.

CCD was conducted to determine for the optimal extraction conditions using Design-Expert 12 software (State-Ease, Inc., USA). A CCD design with 3 factors was employed for ethanol extraction, while a CCD design with 4 factors was utilized for hydrothermal-assisted extraction.

The optimized conditions for the extraction were identified to achieve maximum antioxidant activity and whitening effect. The actual values tested were compared with the

predicted values to assess the validity of the model. Additionally, the active compounds were qualitatively analyzed using HPLC under optimal conditions.

HPLC Analysis on Optimal Condition

Phenolic compounds from extracts were analyzed using HPLC (Dionex Ultimate 3000, USA) equipped with binary pumps, an autosampler, a column oven, and a 2998 photodiode array detector (Waters, USA). The compounds tricin and p-coumaric acid were monitored at 280 nm, and arbutin was monitored at 370 nm with a Sunfire TM C18 column (4.6×250mm ID, 5μm particle size; Waters, Milford, MA, USA). The mobile phase consisted of acetonitrile (A) and a 1% acetic acid aqueous solution (B) as follows: 0.0 min, 15% A; 0.0 to 40.0 min, 42.5% A; 40.0 to 40.1 min, 100.0% A; 40.1 to 45.0 min, 100.0% A; 45.0 to 45.10 min, 15.0% A; and 45.10 to 55.0 min, 15% A. Samples were dissolved in methanol/DMSO (1:1, v/v) at a concentration of 1.0 mg/mL, filtered using a 0.45 μm syringe filter, and injected in 10 μL increments *via* an auto-ampler. Standard solutions (1.25, 2.5, 5, 10 mg/ml) of tricin, p-coumaric acid, and arbutin were prepared by diluting them in methanol/DMSO (1:1, v/v). The quantity of each component in the samples was determined by comparing the retention time and absorbance of the three standards.

RESULTS AND DISCUSSION

Establishment of Independent Variables for RSM

This study aimed to determine the optimal extraction conditions for maximizing the efficiency of antioxidant and whitening active compounds from *S. quelpaertensis.* RSM is recognized as an optimization tool that can identify the interrelationship between variables, as commonly utilized in experimental studies on food and plant extractions (Said *et al.* 2015).

The extraction process was carried out using ethanol extraction and hot-water extraction assisted by ultrasonic pretreatment. The independent variables were set differently according to the extraction methods. For ethanol extraction, variables such as ethanol concentration, extraction temperature, and extraction time were selected. In the case of hot water extraction, extraction time, extraction temperature, sample to solvent ratio, and irradiation time of ultrasound pretreatment were set as independent variables.

Experiments were initially conducted one factor at a time to establish the appropriate range of independent variables for the extraction process from *S. quelpaertensis*. Previous research commonly suggested extraction conditions of less than 8 h and temperatures below 95 °C to prevent the decrease of polyphenol compounds derived from natural products due to pyrolysis and polymerization (Vergara-Salinas *et al.* 2012).

The extraction process from *S. quelpaertensis* was carried out under conditions similar to those of hydrothermal extraction, with an extraction temperature ranging from 80 to 95 ℃ and an extraction time of 3 to 5 h. Additionally, ultrasound treatment was employed in bio-separation to overcome the limitations of hydrothermal extraction and enhance the extraction of valuable substances. The bubble-cavitation produced by ultrasound helps to break down the plant cell wall, facilitating the penetration of the solvent into the tissue and the diffusion of physiologically active substances within the cell in a short period of time (Chemat *et al.* 2017).

However, the range of ultrasound irradiation time was set within 30 min due to degradation and hydrolysis over a certain range. Three variables were considered for the ethanol extraction process. The levels of each factor were set as follows: ethanol concentration (20%, 50%, 80%), extraction temperature (25 °C, 50 °C, 70 °C), and extraction time (40 min, 2 h, 4 h, 8 h). Four extraction variables were applied for hot water extraction assisted ultrasound pretreatment. Extraction temperature, extraction time, sample-to-solvent ratio, and irradiation time were used to validate each independent variable.

The experimental conditions for ethanol extraction and hot water extraction were determined based on the antioxidant and whitening effects, respectively. In the ethanol extraction, conditions such as 20% ethanol concentration, 75 °C extraction temperature, and 2 h of extraction time exhibited the highest radical scavenging activity. The condition that included 75 °C extraction temperature, 2 h of extraction time, and a 1:10 solvent ratio demonstrated the most effective whitening effect.

The central composite design (CCD), which is one of the response surface methodology (RSM) models, was established for optimizing the extraction procedure. CCD was designed with three factors: central point, factorial point, and axial point, each with minimum and maximum ranges of factors.

The ethanol extraction process was done as shown in Table 3, and the design variables were ethanol concentration (0 to 88%), extraction temperature (23 to 81 ℃), and extraction time (2 to 8 h) based on previous studies and preliminary experiments for validity verification. Additionally, the hot water extraction-assisted process is detailed in Table 4, with design variables including extraction time (1 to 9 h), extraction temperature (23 to 95 ℃), sample-to-solvent ratio (7 to 23 g/mL), and ultrasonic irradiation time (0 to 32 min).

Table 3. Experimental Model Using Central Composite Design with Three Independent Variables for Ethanol Extraction of *S. quelpaertensis* Nakai

Run	Independent Variables			Dependent Variables
	x_1 (%)	x_2 (°C)	x_3 (h)	y_1 (%)
1	18	35	3	34.07
2	70	35	3	66.79
3	18	69	3	50.40
4	70	69	3	55.80
5	18	35	7	47.80
6	70	35	7	68.22
7	18	69	7	56.70
8	70	69	7	68.64
9	0	52	5	68.88
10	88	52	5	80.91
11	44	23	5	78.74
12	44	81	5	79.26
13	44	52	2	73.03
14	44	52	8	75.62
15	44	52	5	39.55
16	44	52	5	55.29
17	44	52	5	62.91

Independent variable present x_1=Concentration, x_2=Temperature Time, x_3=Time
Dependent variable showed value of antioxidant activity (y_1)

The CCD for ethanol extraction with three factors consisted of 17 random designs, three center points, and six axial points at a distance of α=1.682 from the design center point. The CCD for hydrothermal-assisted ultrasonic extraction with four factors consisted of 27 experimental designs, four center points, and eight axial points at a distance of α=2 from the design center point.

Table 4. Experimental Model using Central Composite Design with Four Independent Variables for Hot Water Extraction Assisted Ultrasound Extraction of *S. quelpaertensis* Nakai

STD	Independent Variables				Dependent Variables
	x_1 (h)	x_2 (°C)	x_3 (g/mL)	x_4 (min)	y_2 (%)
1	3	41	11	8	34.42
2	7	41	11	8	24.32
3	3	77	11	8	35.82
4	7	77	11	8	42.15
5	3	41	19	8	50.37
6	7	41	19	8	30.80
7	3	77	19	8	36.54
8	7	77	19	8	36.07
9	3	41	11	24	32.50
10	7	41	11	24	27.37
11	3	77	11	24	35.48
12	7	77	11	24	20.26
13	3	41	19	24	35.40
14	7	41	19	24	18.25
15	3	77	19	24	28.95
16	7	77	19	24	33.67
17	1	59	15	16	39.85
18	9	59	15	16	31.17
19	5	23	15	16	22.10
20	5	95	15	16	36.76
21	5	59	7	16	36.12
22	5	59	23	16	23.59
23	5	59	15	0	25.30
24	5	59	15	32	17.44
25	5	59	15	16	27.31
26	5	59	15	16	26.11
27	5	59	15	16	28.88

Independent variables x_1 Time, x_2 Temperature, x_3 Solvent to sample ratio, x_4 Ultrasonic irradiation time; Dependent variable tyrosinase inhibition activity y_2

Optimization of Ethanol Extraction for Antioxidant Activity

The CCD method was employed for reaction surface analysis using Design-Expert software to optimize the ethanol extraction conditions of *S. quelpaertensis*. The adequacy and significance of the design model were determined through analysis of variance (ANOVA), as detailed in Table 5. The antioxidant activity was assessed using the DPPH method.

The model's presented antioxidant activity value was significantly ($p < 0.05$) dependent on ethanol concentration, extraction temperature, and extraction time. The independent variables, x_1 (concentration, %) and x_3 (time, h), had a significant effect on the response value ($p < 0.05$). On the other hand, the independent variable x_2 (temperature, ℃)

did not have a significant effect on the response.

The antioxidant ability was significantly affected by ethanol concentration but not by temperature. This aligns with previous research on ethanol extraction of *Ilex paraguariensis* (Bassani *et al.* 2014), *Fagopyrum esculentum* (Hinneburg and Neubert 2005), and *Rubus coreanus* (Kang and Lee 2021).

The regression equation's R^2 value was used to check the model fit. A model is considered accurate when R^2 is relatively close to 1. In this study, the R^2 value obtained was 0.8788, which can be regarded as suitable. These results generally determine the adequacy of the model and identify optimal conditions.

The regression equation enables the prediction of the factor's effect to assess the main effect, interaction effect, and total satisfaction. The predicted response S for the antioxidant activity of *S. quelpaertensis* Nakai can be expressed by the following polynomial equation in terms of coded values:

$$S = 79.68 + 5.21x_1 - 1.86x_2 - 4.91x_3 + 5.34x_1x_2 \\ -5.02\chi_1x_3 + 2.69x_2x_3 - 11.16{x_1}^2 - 6.22{x_2}^2 - 3.99{x_3}^2 \quad (3)$$

It is necessary to analyze the interaction effect when having two or more independent variables to check the mutual influence between them. The effect of the combination of two factors is shown in Table 5, with the interaction, including ethanol concentration, which is the most influential factor, revealing a relatively large effect. 3D response surface graphs were produced to visually represent the relationship between independent variables and dependent variables of regression using RSM.

Table 5. Analysis of Variance for Ethanol Extraction of *S. quelpaertensis* Nakai as Linear, Quadratic, and Interactions Terms on Response Variables

Source	Sum of squares	Degree of freedom	Mean Square	F-value	*p*-value
Model	2745.40	9	305.04	5.64	0.0164
x_1	371.37	1	371.37	6.86	0.0344
x_2	47.41	1	47.41	0.8764	0.3804
x_3	328.72	1	328.72	6.08	0.0431
x_1x_2	228.25	1	228.25	4.22	0.0791
x_1x_3	202.00	1	202.00	3.73	0.0946
x_2x_3	57.95	1	57.95	1.07	0.3351
x_1^2	1404.04	1	1404.04	25.95	0.0014
x_2^2	436.34	1	436.34	8.07	0.0250
x_3^2	179.79	1	179.79	3.32	0.1111
Residual	378.70	7	54.10		
Lack of fit	376.14	5	75.23	58.67	0.0168
Pure error	2.56	2	1.28		
Total	3124.10	16			
R^2	0.8788				

x_1 Concentration (%), **x_2** Temperature (°C), **x_3** Time (h); Level of significance * $p < 0.05$

Figure 1 shows the response surface plots generated for a concentration range of 0% to 88% and a time range of 1 to 8 hours, with the temperature held constant at 52 °C. The model equation's suitability was assessed using the selected optimal conditions: ethanol concentration of 54%, extraction temperature of 52 °C, and extraction time of 3 h, predicting a maximum response of 82.96%. Additional experiments under these optimal conditions were conducted to test reproducibility. The actual value of 83.65% aligned closely with the predicted value.

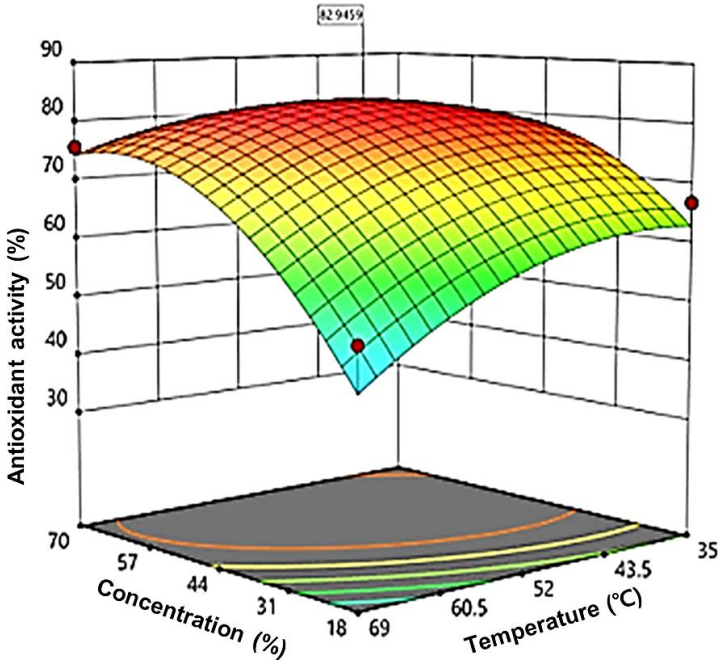

Fig. 1. Response surface for the effect of different extraction parameters (ethanol concentration, temperature) on antioxidant activity

Optimization of Hot Water Extraction for Whitening Effect

The CCD method was adopted to optimize the hot water extraction from *S. quelpaertensis* Nakai for whitening effects. Analysis of variance was applied to determine the adequacy and significance of the model, as detailed in Table 6.

A tyrosinase inhibition assay was performed to evaluate the whitening effect of *S. quelpaertensis* as the dependent variable. The independent variables included x_1 (extraction time), x_2(temperature, ℃), x_3 (sample to solvent ratio, g/mL), x_4 (ultrasonic irradiation time, min). The adequacy and significance of the models were evaluated by analysis of variance (ANOVA) using RSM. The independent variables of ultrasonic irradiation time (x_4) and time (x_1) showed a significant effect ($p < 0.05$) on the tyrosinase inhibition activity response of the linear model.

Whereas the independent variable of sample to solvent ratio (x_3) showed no significant effect on the response, all other independent variables did not show significant terms in the quadratic model, with $p > 0.05$. In Table 7, the adjusted model value showed a significant fit with the quadratic model, with a p-value of 0.0198 ($p < 0.05$) after removing all non-significant independent variables. Regarding the whitening effect on tyrosinase inhibition activity, both ultrasonic irradiation time (x_4) and extraction time (x_1) showed a positive and significant quadratic effect, as shown in Table 7.

These findings imply that tyrosinase inhibition activity depends more on ultrasonic irradiation time and extraction time than other factors. Nonetheless, the results in this study showed a desirability value of 0.6255, which was not as high as predicted. However, similar values were revealed in other reports. For instance, Pinto *et al.* (2021) obtained a value for the extraction of chestnut shells using water and supercritical fluids, respectively. Belwal *et al.* (2016) obtained a value for the extraction of *Berberis asiatica* fruits using methanol. The regression equation for the adjusted quadratic model, after removing the non-significant variable, is as follows.

$$S = 27.43 - 3.08x_1 + 1.87x_2 - 3.10x_4 + 2.96x_1x_2 - 1.03x_3x_4 + 2.62{x_1}^2 + 1.10{x_2}^2 + 1.21{x_3}^2 - 0.9141{x_4}^2 \quad (4)$$

This equation is used to create 3D response surface graphs that visually depict the relationship between independent and dependent variables. Fig. 2 displays the response surface plots generated for ultrasonic irradiation time (ranging from 0 to 32 min) and time (ranging from 1 to 9 h), which are considered to have a positive impact, while maintaining the temperature constant at 1:19.

Table 6. Analysis of Variance for Hydrothermal Assisted Ultrasonic Extraction of *S. quelpaertensis* Nakai as Linear, Quadratic, and Interactions Terms on Response Variables

Source	Sum of squares	Degree of freedom	Mean Square	F-value	*p*-value
Model	971.52	14	69.39	1.55	0.2271
x_1	227.70	1	227.70	5.08	0.0437
x_2	83.78	1	83.78	1.87	0.1967
x_3	2.25	1	2.25	0.0502	0.8265
x_4	230.25	1	230.25	5.13	0.0428
x_1x_2	139.89	1	139.89	3.12	0.1028
x_1x_3	4.34	1	4.34	0.0969	0.7610
x_1x_4	5.02	1	5.02	0.1119	0.7438
x_2x_3	13.46	1	13.46	0.3001	0.5938
x_2x_4	2.12	1	2.12	0.0473	0.8314
x_3x_4	16.82	1	16.82	0.3750	0.5517
x_1^2	146.46	1	146.46	3.27	0.0958
x_2^2	25.85	1	25.85	0.5765	0.4623
x_3^2	31.06	1	31.06	0.6926	0.4215
x_4^2	17.83	1	17.83	0.3975	0.5402
Residual	538.13	12	44.84		
Lack of fit	534.27	10	53.43	27.68	0.0354
Pure error	3.86	2	1.93		
Total	1509.64	26			
R^2	0.6435				

x_1 Time (h), x_2 Temperature (°C), x_3 sample to solvent ratio (g/mL); x_4 Ultrasonic irradiation time (min); Level of significance * $p < 0.05$

Table 7. Adjusted Analysis of Variance for Hot Water Extraction Assisted Ultrasound Extraction of *S. quelpaertensis* Nakai as Linear, Quadratic, and Interaction Terms on Response Variables

Source	Sum of squares	Degree of freedom	Mean Square	F-value	*p*-value
Model	944.32	9	104.92	3.16	0.0198
x_1	227.70	1	227.70	6.85	0.0180
x_2	83.78	1	83.78	2.52	0.1309
x_4	230.25	1	230.25	6.92	0.0175
x_1x_2	139.89	1	139.89	4.21	0.0560
x_3x_4	16.82	1	16.82	0.5058	0.4866
x_1^2	146.46	1	146.46	4.40	0.0511
x_2^2	25.85	1	25.85	0.7775	0.3902
x_3^2	31.06	1	31.06	0.9340	0.3474
x_4^2	17.83	1	17.83	0.5361	0.4740
Residual	565.32	17	33.25		
Lack of fit	561.46	15	37.43	19.39	0.0501
Pure error	3.86	2	1.93		
Total	1509.64	26			
R^2	0.6255				

x_1 Time (h), x_2 Temperature (°C), x_4 Ultrasonic irradiation time (min); Level of significance * $p < 0.05$

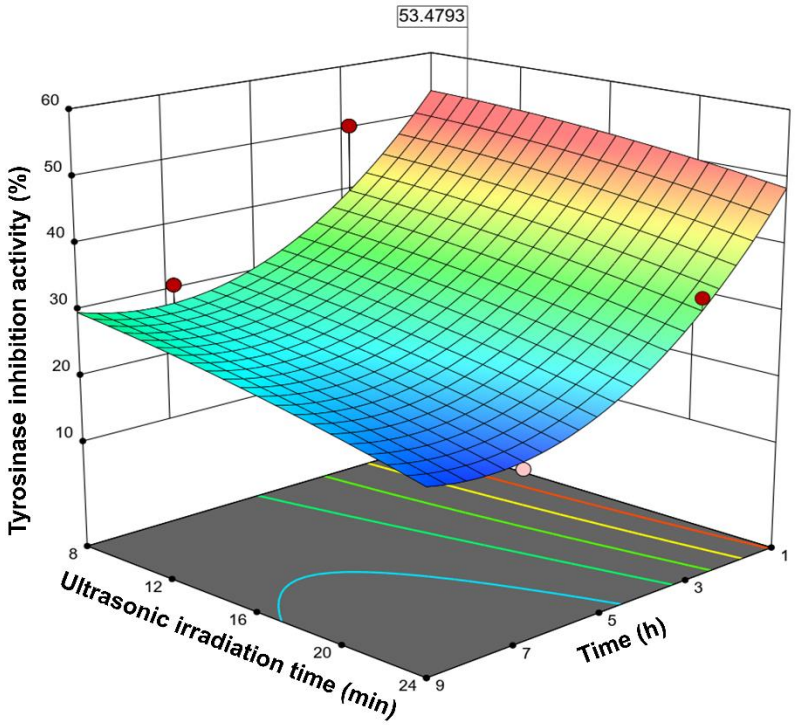

Fig. 2. Response surface for the effect of different extraction parameters (Ultrasonic irradiation time, time) on Tyrosinase inhibition activity

These optimal conditions were determined and validated according to the above process. The optimal conditions were selected as follows: extraction time (1 h), extraction temperature (41 °C), sample to solvent ratio (1:19), and ultrasonic irradiation time (8 min). Under these conditions, tyrosinase activity was expected to be 53.47%. Additional experiments were conducted in triplicate for reproducibility, and the observed values were similar to the predicted values, shown as 51.00±1.80%.

Phenolic Compounds Screening by HPLC analysis

Groups of natural compounds isolated from plants include flavonoids, lignans, terpenes, phenylpropanoids, alkenes, and organic acids. Phenolic compounds are representative antioxidants widely distributed in plant tissues (Ko *et al.* 2020). In a previous study, p-coumaric acid, tricin, and arbutin were selected as indicator components of *S. quelpaertensis*.

Qualitative and quantitative analysis of phenolic compounds from *S. quelpaertensis* extract under optimal conditions was conducted using high-performance liquid chromatography (HPLC). The phenolic compounds, such as p-coumaric acid and tricin, were selected as indicator substances, and arbutin, indicating a whitening effect, was tested and detected at different wavelengths. Among all the compounds detected and presented in Tables 8 and 9, p-coumaric acid and tricin were found in the ethanol extract, while p-coumaric acid, tricin, and arbutin were found in the hot water extraction extracts, respectively.

Table 8. Phenolic Profile of *S. quelpaertensis* Nakai Extract at Optimized Ethanol Extraction Condition

Compound	Retention Time (min)	UV Band (nm)	Concentration (μg/L)
p-coumaric acid	18.90	280	2.41±0.10
Tricin	33.59	370	0.68±0.01

Table 9. Phenolic Profile of *S. quelpaertensis* Nakai Extract at Optimized Hydrothermal Extraction Condition

Compound	Retention Time (min)	UV Band (nm)	Concentration (μg/L)
Arbutin	5.42	280	41.36±1.21
p-coumaric acid	18.93	280	25.34±0.89
Tricin	33.60	370	0.22±0.02

The contents of p-coumaric acid and tricin purified from ethanol extraction were estimated to be 2.408 and 0.683 μg/L, respectively, as shown in Figs. 3 and 4. The compounds tricin, p-coumaric acid, and arbutin purified from hot water extraction were estimated to be 0.22, 25.3, and 41.4 μg/L, as shown in Figs. 5 and 6. Polyphenolic compounds such as tricin and p-coumaric acid have been reported for their biological activities, including antioxidant properties and inhibition of cellular melanogenesis, according to previous research (Boo 2019). Although the concentration of p-coumaric acid was lower than 3 μM that was reported as a 50% inhibition activity concentration (IC50), the concentration could be increased through a concentration process (Boo 2019). Thus, the effect of treatment from *S. quelpaertensis* extract may be due to the combination of these phenolic compounds.

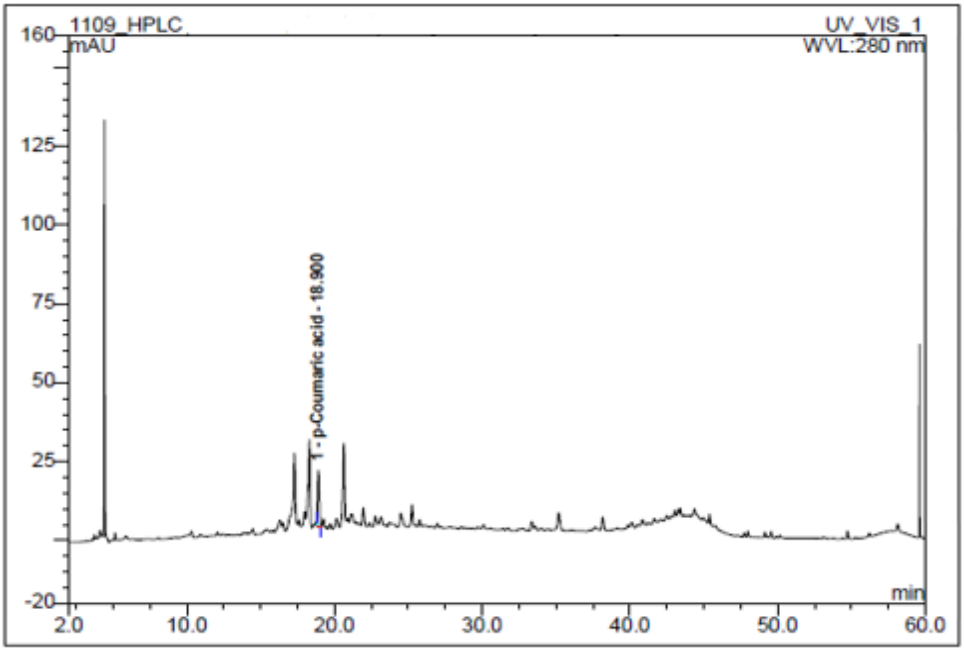

Fig. 3. HPLC chromatogram at 280 nm of *S. quelpaertensis* at optimized ethanol extraction

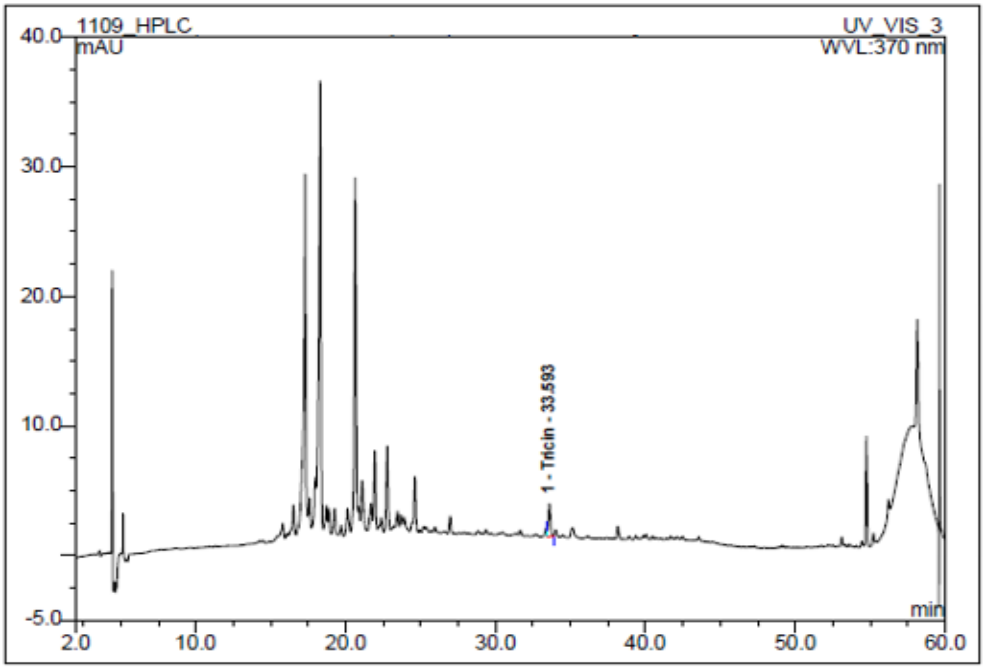

Fig. 4. HPLC chromatogram at 370 nm of *S. quelpaertensis* at optimized ethanol extraction

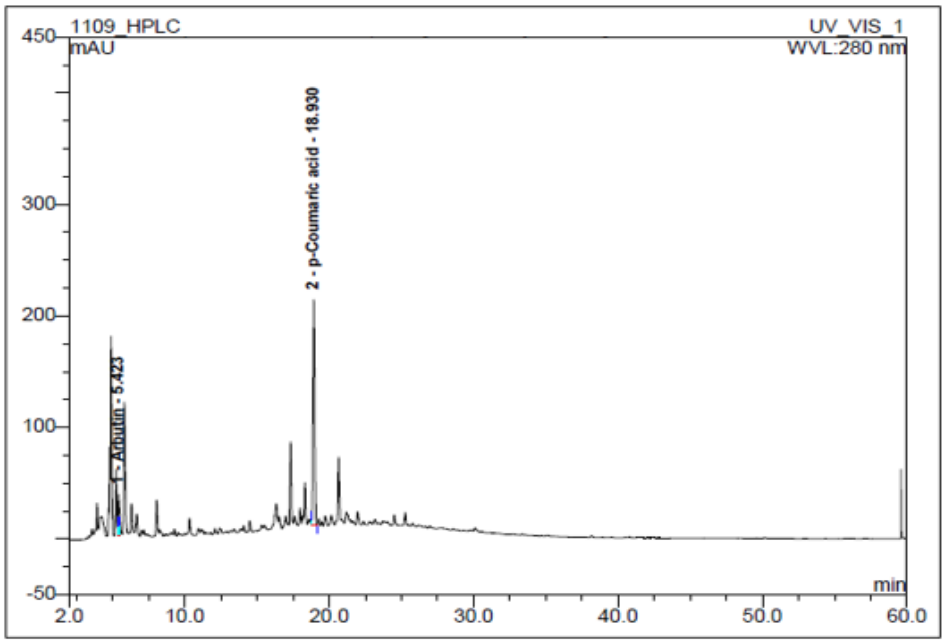

Fig. 5. HPLC chromatogram at 280nm of S. *quelpaertensis* at optimized hydrothermal extraction

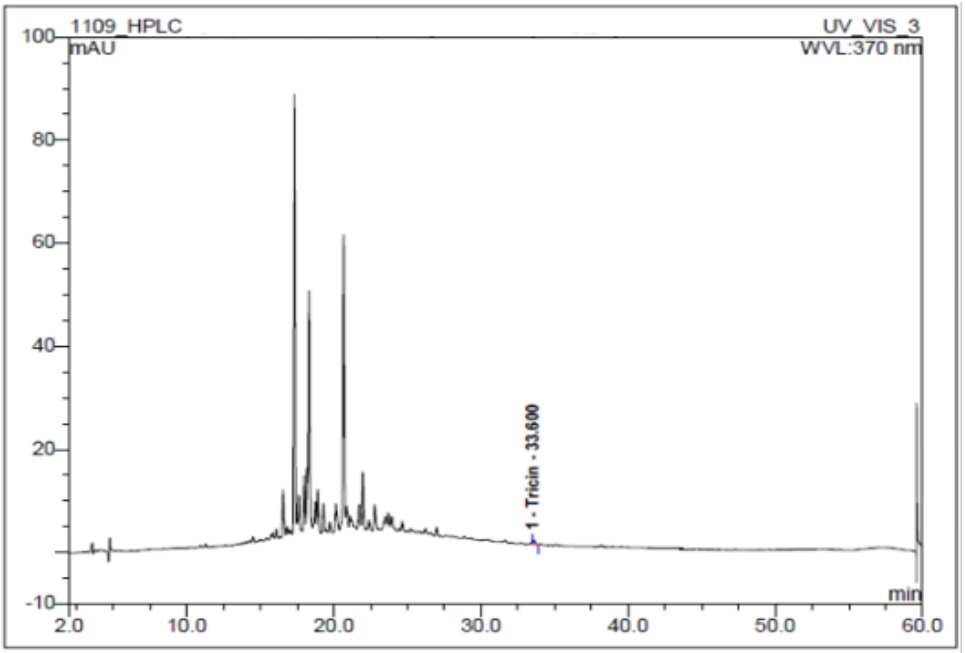

Fig. 6. HPLC chromatogram at 370nm of S. *quelpaertensis* at optimized hydrothermal extraction

CONCLUSIONS

1. The extraction process of *Sasa quelpaertensis* Nakai was optimized using central composite design of response surface methodology (RSM) to obtain optimal extract conditions with strong antioxidant activity using ethanol solvent or whitening effect using hydrothermal solvent. Two quadratic models were designed and validated to optimize the extraction process. The combination of 54% ethanol concentration, 52°C extraction temperature, and 3 h extraction time was established as the optimal conditions to achieve the highest antioxidant activity.
2. The optimal conditions for achieving the highest whitening effect were determined as follows: extraction time of 1 h, extraction temperature of 41 °C, sample to solvent ratio of 1:19 g/mL, and ultrasonic irradiation time of 8 minutes. Under these optimal conditions, the experimental antioxidant activity was measured at 83.65±1.56, and the whitening effect was found to be 51.00±1.80. These experimental values were highly consistent with those predicted by the regression equation and the 3D graph.
3. The phenolic compounds were identified under optimum conditions. Tricin was confirmed in ethanolic extracts and p-coumaric acid and arbutin were determined by high performance liquid chromatography (HPLC) analysis.
4. Optimal conditions for ethanol and hot water extractions were identified by RSM analysis, and it is expected that this can be applied to the industrial utilization of *S. quelpaertensis* extract as cosmetic ingredient.

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