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Optimization of Phytochemicals Extraction from Fennel (*Foeniculum Vulgare*) Seeds using Response Surface Methodology

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Abstract

Phytochemicals are bioactive chemical compounds found in plants considered to be beneficial to human health. The study aimed to optimize the extraction parameters for phytochemical compounds from fennel seeds using response surface methodology (RSM) based on Box-Behnken Design (BBD). Ethanol concentrations (40%, 60%, and 80%), extraction times (90, 120 and 150 min), and temperatures (30, 50, and 70°C) were selected and optimized to get higher extraction efficiency. The effects of major extraction variables on the levels of phenolic compounds, flavonoids, and antioxidant activity in the extracts were evaluated. Based on these findings, optimal operating conditions were identified to maximize the recovery of these bioactive compounds. Analysis of variance (ANOVA) was applied, and the significant effects of the factors and their interactions were tested at a 95% confidence interval. The optimum conditions obtained using RSM were ethanol concentration of 78%, a temperature of 59.8°C, and a time of 90 min. The phytochemicals were recovered using an optimized system to 48.117 mg GAE/g of total phenolic content (TPC), 17.732 mg QE/g of total flavonoid content (TFC) and 74.14% of antioxidant activity (AA). Hence, solvent extraction is an effective and economically viable method for recovering phytochemicals from fennel seeds.

Keywords:

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Introduction

Medicinal plants have been vital in traditional medicine for centuries, offering bioactive compounds with therapeutic potential (Petrovska, 2012). Culinary spices enhance food flavor and color and are increasingly being used for food preservation and health benefits (Anwar et al., 2009). They contain several valuable phytochemical components with bioactive effects and medicinal and functional properties such as antimicrobial, anti-inflammatory, antioxidant, anti-allergic, and immunomodulatory properties (Kaur et al., 2025). Non-volatile secondary metabolites such as amino acids, lectins, glycoproteins, flavonoids, tannins, quinones, coumarins,

terpenoids, steroids and alkaloids as some of the most important bioactive compounds. These compounds are usually extracted from the plant by solvent extraction, depending on the nature of the targeted compound (Regnier et al., 2012).

Fennel (*Foeniculum vulgare*) is among the oldest cultivated spices, thriving in arid and semi-arid regions. Its wide adaptability, combined with its considerable economic value, has made it an important crop in both traditional and modern agricultural systems (Kooti et al., 2015). Fennel seeds are the dried, aromatic fruits of the fennel plant, widely used as a spice in culinary and traditional medicinal practices. Fennel seeds hold a prominent position in the pharmaceutical and food industries due to their diverse medicinal properties, including digestive, antimicrobial, and anti-inflammatory effects (Zafar et al., 2023). Some of the main phenolic compounds identified in fennel seeds include estragole, anethole, fenchone, rutin, chlorogenic acid, eriocitrin, miquelianin, 1,3-O-dicaffeoylquinic acid, 1,5-O-dicaffeoylquinic acid, 4-O-caffeoylquinic acid, 1,4-O-dicaffeoylquinic acid, 3-O-caffeoylquinic acid, and rosmarinic acid (Noreen et al., 2023). As a result, it is regarded as one of the most versatile and significant medicinal herbs used around the world (Rodríguez-Solana et al., 2014).

Unlocking the therapeutic potential of fennel seeds largely depends on the effective extraction of their phytoconstituents (Kooti et al., 2015). The recovery of these bioactive compounds largely depends on the efficiency of the extraction process. Plant matrices often contain tightly bound phytochemicals, making extraction a critical step for maximizing the yield of functional compounds. Factors such as solvent type, solvent polarity, extraction temperature, and extraction time significantly influence the extraction efficiency of phenolic compounds

(Angelov & Boyadzhieva, 2016). The global fennel extract market was valued at approximately USD 32.2 million in 2024 and is projected to expand at a CAGR of 3.8%, reaching USD 41.5 million by 2032, reflecting sustained demand growth. The commercial relevance of fennel phytochemicals is particularly strong in the food and beverage, nutraceutical and traditional medicine, pharmaceutical and cosmeceutical industries (Rather et al., 2016). The lack of standardized methods for fennel extract production creates quality inconsistencies that challenge market development. Variations in growing conditions, extraction techniques, and post-harvest processing can significantly alter the bioactive compound profile of the final product (Noreen et al., 2023). Previous research on fennel seeds has primarily focused on extracting the essential oil or fennel seed extract using advanced extraction techniques (Malin et al., 2022). These approaches are highly effective for the extraction of lipid-rich oil. Still, they are energy-intensive and inherently poor at extracting the desirable polar compounds, such as phenolics and flavonoids. While high-tech options like modern methods can yield high-quality oil, they are costly (Sinha et al., 2022). The selection of a solvent extraction technique is particularly relevant due to its low cost, operational simplicity, and easy scalability (Azmir et al., 2013). Therefore, there remains a clear research gap in developing cost-effective, food-grade, and statistically optimized extraction strategies for maximizing phenolic content, flavonoid yield, and antioxidant activity from fennel seeds. As a result, the recovery of antioxidant-rich phytochemicals with potential food and nutraceutical value remains underexplored.

Response Surface Methodology (RSM) is a powerful suite of mathematical and statistical tools designed to enhance and fine-tune analytical methods (Khuri & Mukhopadhyay, 2010). This study aims to address the solvent extraction technique applied for the extraction of essential constituents from fennel seeds, and the three-level BBD method was used to optimize the number of test parameters evaluating the effects of ethanol concentration, extraction temperature, and extraction time on total phenolic content, total flavonoid content, and antioxidant activity using RSM.

Materials and Methods

Plant material

Fennel seeds were collected from Dharan, Nepal. The seeds were dried in the cabinet drier at 45°C for about 3-4 h (Lal et al., 2024). Moisture content of the seeds was determined by drying at 100 ± 2°C to a constant mass and was 4%. The dried seeds were milled using a grinder (Baltra Zen-3, New Delhi, India) and sieved to get a particle size of 0.5 mm. This increases the surface area, which improves solvent penetration and extraction efficiency.

Chemicals and instruments

Folin–Ciocalteu reagent (sigma), DPPH (sigma), gallic acid (HiMedia), anhydrous Na₂CO₃ (HiMedia), Quercetin (HiMedia), AlCl₃ (HiMedia), and NaOH (HiMedia) were purchased by Himshikhar Scientific Trading Concern, Itahari, Nepal. All

chemicals used were of analytical grade, and all solutions were prepared using distilled water. The determination of polyphenol content and antioxidant activity was carried out using UV-Vis spectrophotometer (Agilent Cary 60 UV-Vis, model number G6860A, Agilent technologies). Fennel extracts were extracted using a water bath shaker (Water bath incubator shaker, Optics Technology, Delhi, India).

Methods

Preparation of the extract

Extraction of fennel seeds was carried out according to Angelov & Boyadzhieva (2016) with slight modifications. 10 g of the ground fennel seeds was mixed with ethanol-water at concentrations of 40%, 60%, and 80%, maintaining a solid and solvent ratio of 1:10. The resulting mixture was incubated in a water bath shaker at temperatures ranging from 30 to 70°C for a duration of 90 to 150 min at a frequency of 200 rpm. Following this, it was filtered through Whatman filter paper No. 42 (125 mm), collected in a conical flask and stored at 4°C.

Optimization of the parameters of fennel extract

The RSM method was used to optimize the parameters for extracting fennel extract. The BBD using Design Expert (Version 13.0, Stat-Ease Inc., USA) was employed with three coded levels: -1, 0, and +1, as shown in Table 1. To determine the maximum response variables (total phenolic content, total flavonoid, and antioxidant activity), ethanol concentrations (40%, 60%, and 80%), extraction time (90, 120, and 150 min), and temperature (30, 50, and 70°C) were selected based on previous research conducted by (Angelov & Boyadzhieva, 2016; Ashkezari et al., 2021). These parameters were selected because solvent concentration influences solvent polarity and the solubility of phenolic and flavonoid compounds, while temperature enhances mass transfer and facilitates solvent penetration into the plant matrix. Additionally, extraction time determines the duration of contact between the solvent and the plant material, which affects the diffusion and release of bioactive compounds into the solvent (Sayem et al., 2024). These experimental conditions were selected considering that solubility and diffusion of phytochemicals increase with the increase in ethanol concentration, temperature and time, but overheating could be responsible for their decomposition.

Table 1
Response surface factor and level

<i>Factors</i>	<i>-1</i>	<i>0</i>	<i>+1</i>
<i>Time (min)</i>	90	120	150
<i>Temperature (°C)</i>	30	50	70
<i>Ethanol concentration (%)</i>	40	60	80

The response variables obtained from the experimental runs were modelled as functions of the coded variables (X_i, i = 1, 2,

and 3) using the second-degree polynomial expressed in the following equation.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon$$

The polynomial coefficient was depicted by β_0 (constant), β_1 , β_2 and β_3 (linear effects); β_{12} , β_{13} and β_{23} (interaction effects); β_{11} , β_{22} and β_{33} (quadratic effects); and ε (random error). Multiple regression analysis was used to model the data, and ANOVA was used to determine each term's statistical significance (Maitusong et al., 2021).

Total phenolic content

The total phenolic content (TPC) in fennel powder was assessed using the Folin-Ciocalteu reagent method with slight modifications according to the specified protocol given by Kupina et al. (2019). A calibration curve was created utilizing gallic acid solutions in the range of 40–400 mg/ml. An aliquot of 1 ml of the fennel extract was combined with 1 ml of Folin-Ciocalteu reagent and incubated at room temperature for 15 min. After that, 3 ml of saturated Na_2CO_3 was added, and the mixture was incubated for an additional two hours at room temperature. The absorbance was then recorded at a wavelength of 765 nm. The total phenol content was determined using the equation from the standard curve and expressed as mg of gallic acid equivalent (mg GAE / g).

Total flavonoid content

The total flavonoid content (TFC) was determined according to Khan et al. (2018) with slight modifications. A standard curve was established using quercetin solutions with concentrations ranging from 0.25 to 2 $\mu\text{g}/\text{ml}$ in ethanol. An aliquot of 1 ml of the extract was transferred into a test tube containing 5 ml of distilled water, mixed with 0.3 ml of 5% sodium nitrate (NaNO_3), and allowed to sit for 5 min. Then, 0.3 ml of 10% aluminum chloride (AlCl_3) was added to the mixture, stirred, and left to stand for an additional 5 min. This was followed by the addition of 2 ml of 1M sodium hydroxide (NaOH) to the test tube, and the total volume was adjusted to 10 ml. After 5 mins, the absorbance was measured at 510 nm using a reagent blank as a reference. The total flavonoid content was determined using the equation from the standard curve and reported as mg of quercetin equivalents (mg QE / g).

Total antioxidant activity

The antioxidant activity (AA) of the extract was determined using the colorimetric DPPH assay, as described by Abdesslem et al. (2021). To assess the radical scavenging capability of the fennel extract, a 0.1 mM DPPH solution in 80% ethanol was prepared. A volume of 1 ml of the extract was combined with 4 ml of the DPPH solution. The resulting mixture was mixed thoroughly and allowed to sit at room temperature for 30 min. Subsequently, the absorbance was recorded at 517 nm using a spectrophotometer. The antioxidant activity was determined using the following formula:

$$\text{Radical scavenging activity(\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 = the absorbance of the control, A_1 = the absorbance of the extract.

Statistical analysis

All the experiments were performed based on RSM's BBD and repeated three times. Design expert software (version 13, Stat-Ease, Inc., Minneapolis, USA) was used for experimental design, optimization, data analysis and prediction. The statistical analysis was assessed by Analysis of Variance (ANOVA), with p-value less than 0.05. The optimal extraction conditions were analyzed by three-dimensional (3D) response surface plots.

Results and Discussion

RSM analysis

The experimental results presented in Table 2 indicate that the TPC, TFC and AA varied within the ranges of 33.4018 to 53.684 mg GAE/g, 9 to 15.8 mg QE/g, and 62.2% to 83%, respectively. These observed values, obtained through the BBD were analyzed using a second-order polynomial model. The adequacy and statistical significance of the developed response models were evaluated through ANOVA.

$$\text{TPC} = 36.54 + 2.95A + 2.28B - 0.1986C + 1.17AB - 1.24AC + 0.6787BC + 9.68A^2 - 0.4717B^2 + 0.8266C^2$$

$$\text{TFC} = 12.70 + 2.23A + 1.55B - 0.5305C - 0.0096AB + 0.2674AC - 0.9196BC + 0.9029A^2 - 1.11B^2 - 0.6209C^2$$

$$\text{AA} = 81.02 + 5.03A - 0.7775B - 0.5100C - 0.9950AB + 0.9700AC - 1.26BC - 11.73A^2 - 1.34B^2 - 0.2675C^2$$

Where A, B and C are coded values of solvent concentration, time and temperature respectively. A^2 , B^2 , C^2 , AB, AC and BC are model terms.

In the analysis of the regression model (Table 3) for TPC, the concentration of ethanol (A) demonstrated a significant positive effect ($p < 0.05$) on phenolic content, while temperature (B) exhibited a significant positive effect ($p < 0.05$), and time (C) showed a negative effect that was also not significant ($p > 0.05$). The interaction between A and B (AB) had a non-significant positive impact ($p < 0.05$). Likewise, the interaction between A and C (AC) resulted in a negative effect that was not significant ($p > 0.05$), whereas the interaction between B and C (BC) showed a positive effect that was not significant ($p > 0.05$). The quadratic term A^2 had a significant positive impact ($p < 0.05$) on total phenolic content, while the term B^2 resulted in a negative effect that was not significant, and C^2 exhibited a positive effect that was also not significant on total phenolic content.

The regression model was fitted well, as indicated by the value less than 0.0001, and the lack of fit was not significant ($p > 0.05$). With an adjusted R^2 of 0.8569, the predicted R^2 value of 0.9374 was in reasonable agreement. Thus, the TPC was analyzed and predicted using this regression model.

Table 2
Box Behnken design with experimental values

Run	A: Ethanol conc. (%)	B: Temp. (°C)	C: Time (min)	TPC (mg GAE/g)	TFC (mg QE/g)	AA (%)
1	80	70	120	53.6840	15.8233	71.50
2	40	50	90	44.3344	11.6331	65.70
3	60	30	150	33.4018	9.5039	80.24
4	60	70	90	39.0399	14.2662	81.10
5	60	50	120	35.4723	12.5814	83.00
6	60	50	120	33.9540	12.0604	79.00
7	60	30	90	35.7393	8.5087	79.50
8	60	50	120	39.0429	13.2624	80.40
9	80	50	90	49.5797	15.6110	72.60
10	40	30	120	40.1656	9.1361	62.40
11	60	70	150	39.4172	11.5831	76.80
12	80	30	120	46.8558	13.5539	75.68
13	80	50	150	47.2945	14.8676	74.28
14	40	70	120	42.3006	11.4439	62.20
15	40	50	150	46.9908	9.8202	63.50
16	60	50	120	36.8773	12.8676	82.10
17	60	50	120	37.3770	12.7238	80.60

The concentration of ethanol (A) significantly positively ($p < 0.05$) influenced the TFC (Table 4), followed by temperature (B), which also had a positive significant effect ($p < 0.05$), while time (C) demonstrated a significantly negative impact ($p < 0.05$). The interaction between A and B (AB) showed a non-significant negative effect ($p > 0.05$). Likewise, the interaction between A and C (AC) exhibited a positive non-significant impact ($p > 0.05$), whereas the interaction between B and C (BC) had a significantly negative effect ($p < 0.05$). The quadratic term A^2 produced a positive significant effect, B^2 resulted in a negative significant impact, and C^2 had a negative significant effect on the total phenolic content. The regression model demonstrated a good fit, as indicated by a value of less than 0.0001, and the lack of fit was not significant ($p > 0.05$). With an adjusted R^2 of 0.9316, the estimated R^2 value of 0.9701 was in reasonable alignment. Therefore, the total flavonoid content was assessed and forecasted using this regression model.

In terms of total AA (Table 5), the concentration of ethanol (A) had a significant positive effect ($p < 0.05$), while temperature (B) exhibited a non-significant negative effect ($p > 0.05$), and time

(C) also showed a non-significant negative impact. The interaction between A and B (AB) resulted in a non-significant negative effect ($p > 0.05$). Likewise, the interaction of A and C (AC) demonstrated a positive but non-significant impact ($p > 0.05$), and the interaction of B and C (BC) had a negative but non-significant effect ($p > 0.05$). The quadratic term for A^2 had a statistically significant negative effect, while both B^2 and C^2 exhibited negative but non-significant impacts ($p > 0.05$) on total antioxidant activity. The regression model fit well, as indicated by a p-value of less than 0.0001, and the lack of fit was not significant ($p > 0.05$). With an adjusted R^2 of 0.9599, the estimated R^2 value of 0.9825 aligned reasonably well. Consequently, the antioxidant content was assessed and predicted using this regression model.

Table 3
ANOVA for response surface quadratic model of TPC

Source	Sum of squares	df	Mean square	F-value	p-value
Model	527.30	9	58.59	11.65	0.0019
A-Solvent concentration	69.75	1	69.75	13.87	0.0074
B-Temperature	41.77	1	41.77	8.30	0.0236
C-Time	0.3156	1	0.3156	0.0627	0.8094
AB	5.51	1	5.51	1.09	0.3302
AC	6.10	1	6.10	1.21	0.3070
BC	1.84	1	1.84	0.3663	0.5641
A^2	394.42	1	394.42	78.41	< 0.0001
B^2	0.9370	1	0.9370	0.1863	0.6790
C^2	2.88	1	2.88	0.5720	0.4742
Residual	35.21	7	5.03		
Lack of fit	20.30	3	6.77	1.82	0.2840
Pure error	14.91	4	3.73		
Cor Total	562.51	16			

Figures 1, 2 and 3 represent 3D response graphs for total phenolic content, total flavonoid content and antioxidant activity. The response surface and main effects plot demonstrated the interaction between TPC, TFC, and the AA of fennel seed extract alongside ethanol concentration, temperature, and extraction time.

The highest value of TPC and TFC 53.684 mg GAE/g and 15.8233 mg QE/g was recorded for the combination of 80% ethanol water concentration at 70°C with an extraction time of 120 min. Similar results were observed by Angelov &

Boyadzhieva (2016) and Baiano et al. (2023), and reported that solvent composition, temperature and contact time increase may favour the diffusion and improve the solubilisation of the phenolic compounds, thus increasing the extraction yield of fennel seeds and fennel byproducts.

Table 4
ANOVA for response surface quadratic model of TFC

Source	Sum of squares	df	Mean square	F-value	p-value
Model	73.38	9	8.15	25.22	0.0002
A-Solvent concentration	38.45	1	38.45	118.92	< 0.0001
B-Temperature	19.26	1	19.26	59.58	0.0001
C-Time	2.56	1	2.56	7.93	0.0259
AB	0.0004	1	0.0004	0.0011	0.9740
AC	0.1541	1	0.1541	0.4765	0.5122
BC	3.38	1	3.38	10.46	0.0144
A ²	3.17	1	3.17	9.80	0.0166
B ²	4.90	1	4.90	15.16	0.0059
C ²	1.81	1	1.81	5.61	0.0497
Residual	2.26	7	0.3233		
Lack of fit	1.49	3	0.4982	2.59	0.1900
Pure error	0.7686	4	0.1922		
Cor Total	75.64	16			

Table 5
ANOVA for response surface quadratic model of AA

Source	Sum of squares	df	Mean square	F-value	p-value
Model	823.59	9	91.51	43.60	< 0.0001
A-Solvent concentration	202.61	1	202.61	96.54	< 0.0001
B-Temperature	4.84	1	4.84	2.30	0.1728
C-Time	2.08	1	2.08	0.9915	0.3525
AB	3.96	1	3.9	1.89	0.2119
AC	3.76	1	3.76	1.79	0.2224
BC	6.35	1	6.35	3.03	0.1255
A ²	579.59	1	579.59	276.16	< 0.0001
B ²	7.59	1	7.59	3.62	0.0990

C ²	0.3013	1	0.3013	0.1436	0.7160
Residual	14.69	7	2.10		
Lack of fit	4.96	3	1.65	0.6802	0.6086
Pure error	9.73	4	2.43		
Cor Total	838.28	16			

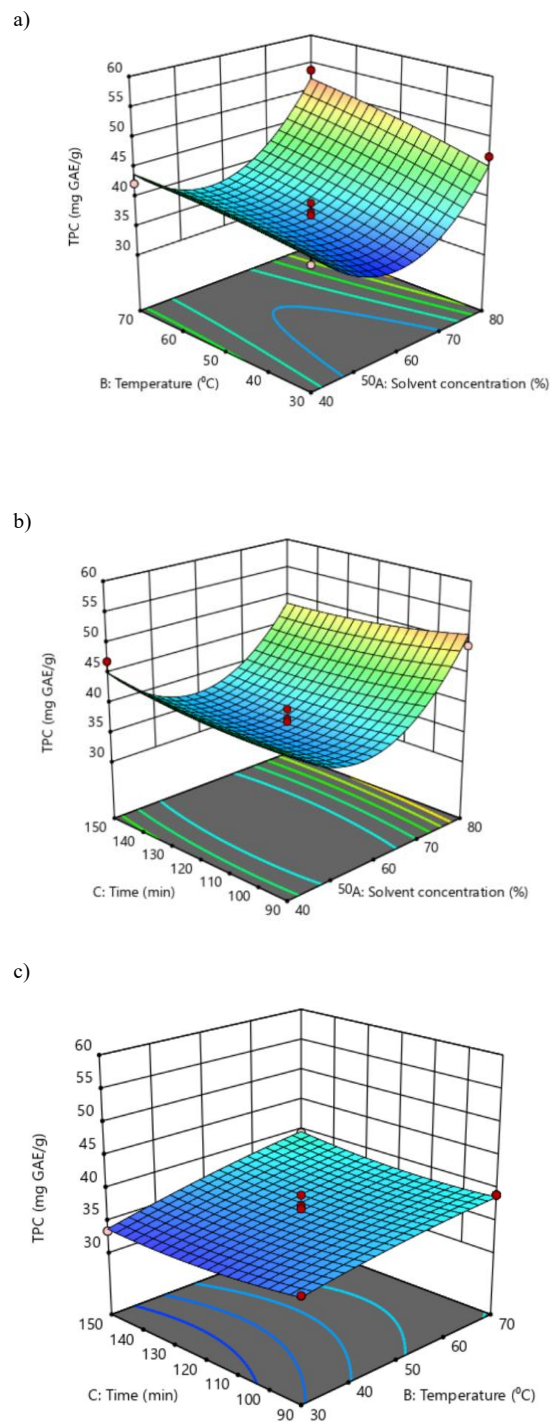


Figure 1
Response surface plot showing the effect of (a) temperature and solvent concentration, (b) time and solvent concentration, and (c) time and temperature on TPC.

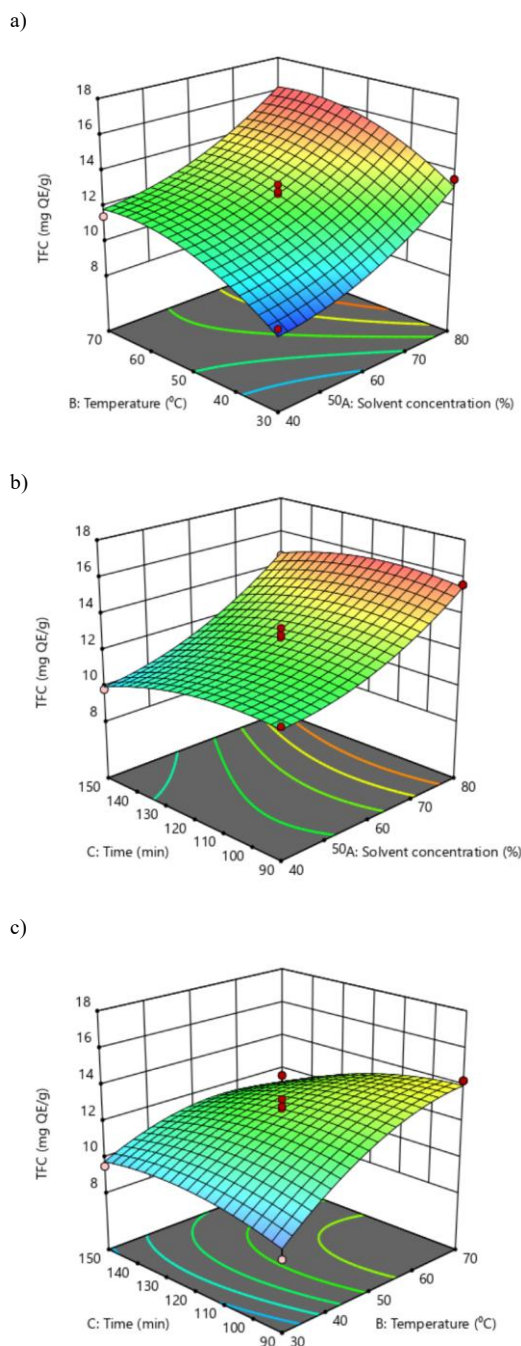


Figure 2
Response surface plot showing the effect of (a) temperature and solvent concentration, (b) time and solvent concentration, and (c) time and temperature on TFC.

Phenolics and flavonoids exhibit a higher solubility in solvents with lower polarity, improving the solubility of phenolics, thereby enhancing the extraction efficiency (Lohvina et al., 2022). Similarly, as the temperature for extraction increases up to 70°C, the efficiency of bioactive compounds extraction increases. The diffusivity TPC and TFC increases, allowing the molecules to move more freely and rapidly from the plant matrix into the solvent. Additionally, elevated temperatures promote better penetration and wetting of the plant material by the

solvent, improving mass transfer (Moradi et al., 2020). Extraction time also affected extraction efficiency. Increasing the extraction time generally leads to an increase in TPC up to a time of 120 min. Beyond that point, the phenolics inside and outside the fennel seeds had reached equilibrium and further increases in time i.e., 150 min, did not result in significant changes due to degradation of phenolic compounds (Akhtar et al., 2020).

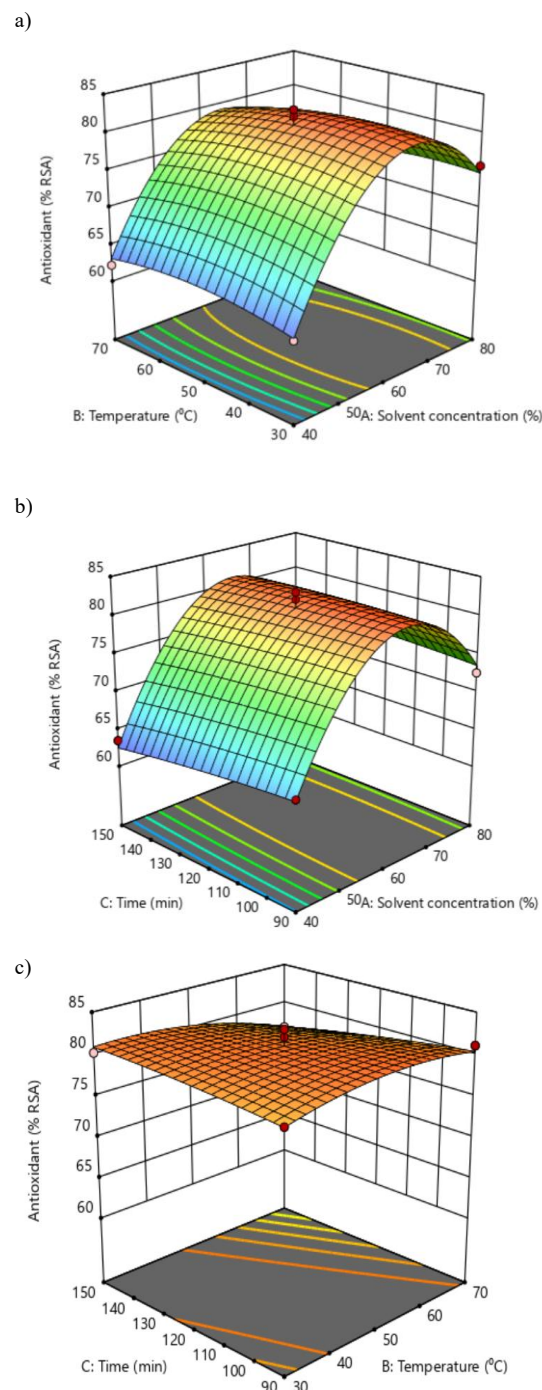


Figure 3
Response surface plot showing the effect of (a) temperature and solvent concentration, (b) time and solvent concentration, and (c) time and temperature on AA.

In contrast, the highest value of AA 83% was recorded for the combination of 60% ethanol water concentration at 50°C with an extraction time of 120 min. Similar results were also reported by Baiano et al. (2023) that 70% ethanol water concentration, 60°C and 90 min for fennel byproducts extraction. The antioxidant activity increased with ethanol concentration up to 60%. However, further increasing the ethanol concentration to 80% caused a significant decrease in antioxidant activity. Hence, 80% ethanol was less efficient in extracting low molecular weight phenolic compounds with high antioxidant capacity from fennel as compared to 60% ethanol. While the yield of phenolic compounds increased as the extraction temperature increased, the antioxidant activity of fennel extract decreased with higher extraction temperatures and time, specifically 70°C and 150 min. This is because of the selective extraction of highly active radical-scavenging compounds at intermediate solvent polarity and the preservation of thermolabile antioxidant constituents at moderate temperature and time (Vern et al., 2025).

Numerical optimization

A numerical optimization method was utilized to determine the best combinations of ethanol concentration, temperature, and time for achieving the highest levels of total phenolics, total flavonoid content, and antioxidant activity as detailed in Table 6.

Table 6

Parameters for extraction optimization

Parameter	Goal	Lower limit	Upper limit
Solvent concentration	In range	40	80
Temperature	In range	30	70
Time	In range	90	150
TPC	Maximize	33.4018	53.684
TFC	Maximize	8.5087	15.8233
AA	Maximize	62.2	83

The independent variables ethanol concentration (40–80%), temperature (30–70°C), and extraction time (90–150 min) were kept within the experimental range, while all response variables were set to maximize. The optimization results predicted that an ethanol concentration of 78%, an extraction temperature of 59.8°C, and extraction time of 90 min would provide the most desirable extraction condition, with a desirability value of 0.91. Under these optimized conditions, the predicted responses were 49.973 mg GAE/g for total phenolic content, 15.992 mg QE/g for total flavonoid content, and 75.203% antioxidant activity. The optimization goals were selected based on the desirability methodology, which assigns a "value" to a set of responses and chooses factor settings that maximize that value (Hoffola et al., 2026). The desirability function was utilized in the process of simultaneous optimization with a focus on three key objectives:

maximizing phenolic compounds, enhancing flavonoid content, and increasing antioxidant activity (Rittisak et al., 2022).

Model verification

Three additional experimental runs were conducted to assess the adequacy of the regression equations for the model. Table 7 presents a summary of the results obtained from the confirmatory tests.

Table 7

Parameters for extraction optimization

Response	Optimal conditions			Mean value	
	Ethanol conc. (%)	Temp. (°C)	Time (min)	Predicted	Observed
TPC	78	59.8	90	49.985	48.117±0.15
TFC	78	59.8	90	15.995	17.732±0.2
AA	78	59.8	90	75.194	74.14±0.18

This regression model was accurate enough to predict the optimal values since the observed values were very near to the predicted values. So, the optimization results demonstrated that ethanol concentration of 78%, 59.8°C extraction temperature and 90 min extraction time was most suitable for increasing the efficiency of extraction.

Conclusion

The optimization of extraction methods is essential for fully utilizing the therapeutic properties of fennel seeds. The research focuses on identifying the ideal conditions for the batch solvent extraction of fennel seeds to achieve extracts that are rich in antioxidant compounds, polyphenols, and flavonoids. The phytochemicals were obtained using the solvent extraction technique applied with an ideal extraction protocol to 48.117 mg GAE/g, 17.732 mg QE/g, and 74.14% RSA. The best extraction conditions identified using RSM included an ethanol concentration of 78%, a temperature of 59.8°C, and a time of 90 min, facilitating the standardized application of fennel seed extract in various fields, ranging from food to pharmaceutical development.

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Conflict of Interest

The authors declare no conflict of interest.

References

Abdesslem, S. Ben, Boulares, M., Elbaz, M., Ben Moussa, O., St-Gelais, A., Hassouna, M., & Aider, M. (2021). Chemical composition and biological

- activities of fennel (*Foeniculum vulgare* Mill.) essential oils and ethanolic extracts of conventional and organic seeds. *Journal of Food Processing and Preservation*, 45(1), 1–13. <https://doi.org/10.1111/jfpp.15034>
- Akhtar, I., Javad, S., Ansari, M., Ghaffar, N., & Tariq, A. (2020). Process optimization for microwave assisted extraction of *Foeniculum vulgare* Mill using response surface methodology. *Journal of King Saud University - Science*, 32(2), 1451–1458. <https://doi.org/10.1016/j.jksus.2019.11.041>
- Angelov, G., & Boyadzhieva, S. (2016). Extraction of fennel (*Foeniculum vulgare*) seeds: Process optimization and antioxidant capacity of the extracts. *Chemical and Biochemical Engineering Quarterly*, 30(2), 245–253. <https://doi.org/10.15255/CABEQ.2015.2281>
- Anwar, F., Ali, M., Hussain, A. I., & Shahid, M. (2009). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan. *Flavour and Fragrance Journal*, 24(4), 170–176. <https://doi.org/10.1002/ffj.1929>
- Ashkezari, S. M. M., Bahmanyar, H., Azizpour, H., Najafipour, I., & Mohammadi, M. (2021). Investigation of Operating Parameters on Ultrasound-Assisted Extraction of Anethole in Fennel Essential Oil. *Journal of Chemical and Petroleum Engineering*, 55(2), 339–351. <https://doi.org/10.22059/jchpe.2021.325123.1352>
- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>
- Baiano, A., Romaniello, R., Giametta, F., & Fiore, A. (2023). Optimization of Process Variables for the Sustainable Extraction of Phenolic Compounds from Chicory and Fennel By-Products. *Applied Sciences*, 13(4191), 1–21.
- Hoffola, A. A., Robi, A. G., & Tefera, Z. T. (2026). Optimization of antioxidant extraction from *Citrullus colocynthis* seed using response surface methodology. *Nature*, 16, 1–31.
- Kaur, R., Zhang, S., Berwal, B., Ray, S., Kumar, R., & Varshney, L. R. (2025). From phytochemicals to recipes : health indications and culinary uses of herbs and spices. *Npj Science of Food*, 9(89), 1–12. <https://doi.org/10.1038/s41538-025-00458-z>
- Khan, M. S., Yusufzai, S. K., Rafatullah, M., Sarjadi, M. S., & Razlan, M. (2018). Determination of total phenolic content, total flavonoid content and antioxidant activity of various organic crude extracts of *Licuala spinosa* leaves from Sabah, Malaysia. *ASM Science Journal*, 11(3), 53–58.
- Khuri, A. I., & Mukhopadhyay, S. (2010). Response surface methodology. *Wiley Interdisciplinary Reviews: Computational Statistics*, 2(2), 128–149. <https://doi.org/10.1002/wics.73>
- Kooti, W., Moradi, M., Ali-Akbari, S., Sharafi-Ahvaji, N., Asadi-Samani, M., & Ashtary-Larky, D. (2015). Therapeutic and pharmacological potential of *Foeniculum vulgare* Mill: a review. *Journal of HerbMed Pharmacology*, 4(1), 1–9. <http://www.herbmedpharmacol.com>
- Kupina, S., Fields, C., Roman, M. C., & Brunelle, S. L. (2019). Determination of total phenolic content using the Folin-C assay: Single-laboratory validation, first action 2017.13. *Journal of AOAC International*, 102(1), 320–321. <https://doi.org/10.1093/jaoac/102.1.320>
- Lal, S., Saxena, S. N., Lal, G., & Chudhary, N. (2024). Assessment of different drying methods on sensory , color and quality attributes of fennel (*Foeniculum vulgare* Mill) seeds harvested for chewing purpose. *International journal of seed spice*, 12(2), 16–22.
- Lohvina, H., Sándor, M., & Wink, M. (2022). Effect of ethanol solvents on total phenolic content and antioxidant properties of seed extracts of fenugreek (*Trigonella foenum-graecum* L.) varieties and determination of phenolic composition by HPLC-ESI-MS. *Diversity*, 14(1). <https://doi.org/10.3390/d14010007>
- Maitusong, J., Aimila, A., Zhang, J., Mahinur, B., Maiwulanjiang, M., & Aisa, H. A. (2021). Process optimization for the supercritical carbon dioxide extraction of *Foeniculum vulgare* Mill. seeds aromatic extract with respect to yield and trans-anethole contents using Box-Behnken design. *Flavour and Fragrance Journal*, 36(2), 280–291. <https://doi.org/10.1002/ffj.3643>
- Malin, V., Garofuli', I. E., Repaji', M., Zori'c, Z., Pedisi', S., Sterniša, M., Možina, S. S., & Dragovi'c-Uzelac, V. (2022). Phenolic characterization and bioactivity of fennel seed (*Foeniculum vulgare* Mill.) extracts isolated by microwave-assisted and conventional extraction. *Processes*, 10(510), 1–16.
- Moradi, H., Bahmanyar, H., Azizpour, H., Rezamandi, N., & Ashkezari, S. M. M. (2020). Modeling and optimization of anethole ultrasound-assisted extraction from fennel seeds using artificial neural network. *Journal of Chemical and Petroleum Engineering*, 54(1), 143–153. <https://doi.org/10.22059/jchpe.2020.301561.1312>
- Noreen, S., Tufail, T., Badar, H., Ain, U., & Godswill, C. (2023). Pharmacological , nutraceutical , functional and therapeutic properties of fennel (*Foeniculum vulgare*). *International Journal of Food Properties*, 26(1), 915–927. <https://doi.org/10.1080/10942912.2023.2192436>
- Petrovska, B. B. (2012). Historical review of medicinal plants usage. *Pharmacognosy Reviews*, 6(11), 1–5. <https://doi.org/10.4103/0973-7847.95849>
- Rather, M. A., Dar, B. A., Sofi, S. N., Bhat, B. A., & Qurishi, M. A. (2016). *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. *Arabian Journal of Chemistry*, 9(1), 1574–1583. <https://doi.org/10.1016/j.arabic.2012.04.011>
- Regnier, T., Combrinck, S., & Du Plooy, W. (2012). Essential oils and other plant extracts as food preservatives. In R. Bhat, A.K Alias and G. Paliyath (Eds.), *Progress in Food Preservation* (1st Ed., pp. 539–579). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781119962045.ch26>
- Rittisak, S., Charoen, R., Choosuk, N., & Savedboworn, W. (2022). Response Surface Optimization for Antioxidant Extraction and Attributes Liking from Roasted Rice Germ Flavored Herbal Tea. *Processes*, 10(125), 1–12.
- Rodríguez-Solana, R., Salgado, J. M., Domínguez, J. M., & Cortés-Diéguez, S. (2014). Characterization of fennel extracts and quantification of estragole: Optimization and comparison of accelerated solvent extraction and Soxhlet techniques. *Industrial Crops and Products*, 52(2014), 528–536. <https://doi.org/10.1016/j.indcrop.2013.11.028>
- Sayem, A. S. M., Ahmed, T., Mithun, M. U. K., Rashid, M., & Rana, M. R. (2024). Optimising ultrasound-assisted extraction conditions for maximising phenolic, flavonoid content and antioxidant activity in hog plum peel and seed: A response surface methodology approach. *Journal of Agriculture and Food Research*, 18(1), 1–17. <https://doi.org/10.1016/j.jafr.2024.101312>
- Sinha, D., Mukherjee, S., & Chowdhury, S. (2022). Characterization and therapeutic applications of natural bioactive compounds. In A. Singh (Ed.), *Isolation, Characterization, and Therapeutic Applications of Natural Bioactive Compounds* (Vol. 1, pp. 250–279). IGP Global.
- Vern, N. Y., Khan, M., & Wan Ishak, W. R. (2025). Optimization of aqueous extraction conditions for bioactive compounds and antioxidant properties of overripe banana (*Musa acuminata*) using response surface methodology. *Journal of Agriculture and Food Research*, 20(2024), 1–11. <https://doi.org/10.1016/j.jafr.2025.101775>
- Zafar, S., Khan, M. K., Perveen, S., Iqbal, M., & AL-Huqail, A. A. (2023). Fennel. In M. Zia-Ul-Haq, A. Abdulkreem AL-Huqail, M. Riaz, & U. Farooq Gohar (Eds.), *Essentials of Medicinal and Aromatic Crops* (pp. 483–514). Springer International Publishing. https://doi.org/10.1007/978-3-031-35403-8_19