

Optimization of Polyherbal Extraction Parameters Using Response Surface Methodology: A Study on *Gymnema sylvestre*, *Cuminum cyminum*, and *Allium sativum*

Sridevi J^{1,*}, Manjari V^{1,*}, Janani Syamaroopa Jnanathapaswini², Vishnupriya K³, Kalpana S⁴, Amudha P⁵

¹Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai (Affiliated to TN Dr MGR Medical University, Guindy, Chennai), Tamil Nadu, INDIA.

²Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai (Affiliated to TN Dr MGR Medical University, Guindy, Chennai), Tamil Nadu, INDIA.

³Department of Varma Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai (Affiliated to TN Dr MGR Medical University, Guindy, Chennai), Tamil Nadu, INDIA.

⁴Department of Epidemiology, The Tamil Nadu Dr. MGR Medical University, Chennai, Tamil Nadu, INDIA.

⁵Department of Pharmacology, C.L Baid Metha College of Pharmacy, Thoraipakkam, Chennai, (Affiliated to TN Dr MGR Medical University, Guindy, Chennai), Tamil Nadu, INDIA.

ABSTRACT

Background: The present study aimed to optimize the extraction process of a polyherbal formulation comprising *Gymnema sylvestre*, *Cuminum cyminum*, and *Allium sativum* using the Box-Behnken design (BBD) of Response Surface Methodology (RSM). **Materials and Methods:** Three independent variables-solvent volume (A), solid-to-solvent Ratio (B), and temperature (C)-were evaluated for their influence on Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX). **Results:** The optimized extraction conditions were 51.00 mL solvent, 15.11 g solid-solvent ratio, and 44.99°C temperature, resulting in experimental yields of TFC (30.9±1.09 mg QE/g), TPC (36.9±1.09 mg GAE/g), and AOX (72±0.05%). Statistical analysis revealed all models to be highly significant ($p < 0.0001$), confirming the adequacy of RSM in maximizing phytochemical yield. **Conclusion:** The developed model can serve as a reference for standardizing polyherbal extractions in pharmaceutical and nutraceutical formulations.

Keywords: Polyherbal formulation, *Gymnema sylvestre*, *Cuminum cyminum*, *Allium sativum*, Box-Behnken design, RSM, Antioxidant activity.

Correspondence:

Dr. Sridevi J

PhD Scholar, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47 (Affiliated to TN Dr MGR Medical University, Guindy, Chennai-32), Tamil Nadu, INDIA.
Email: sridevi.jayamani@gmail.com

Dr. Manjari V

Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47 (Affiliated to TN Dr MGR Medical University, Guindy, Chennai-32), Tamil Nadu, INDIA.
Email: drmanjarimd@gmail.com

Received: 21-07-2026;

Revised: 17-08-2026;

Accepted: 08-10-2026.

INTRODUCTION

The use of polyherbal formulations has been a cornerstone of traditional healing systems such as Ayurveda, Siddha, and Unani, where the combination of multiple medicinal plants is believed to yield synergistic therapeutic effects superior to those of individual herbs (Mukherjee *et al.*, 2018). Such formulations are designed to enhance pharmacological efficacy, minimize toxicity, and provide a broader spectrum of bioactivity through

the interaction of diverse phytoconstituents (Raju *et al.*, 2020). In recent decades, the growing global demand for natural and plant-based medicines has renewed scientific interest in the optimization and standardization of polyherbal products to ensure their efficacy, reproducibility, and quality (Rajan *et al.*, 2021). Among the diverse medicinal plants utilized in traditional practice, *Gymnema sylvestre*, *Cuminum cyminum*, and *Allium sativum* occupy an important place due to their well-documented pharmacological profiles.

Gymnema sylvestre (family: Asclepiadaceae) is traditionally referred to as the “sugar destroyer” and is known for its potent antidiabetic, hypolipidemic, and anti-obesity effects. Its gymnemic acids and saponins modulate glucose metabolism and pancreatic function (Singh *et al.*, 2019). *Cuminum cyminum* (family: Apiaceae), commonly known as cumin, exhibits digestive



DOI: 10.5530/ajbls.20260123

Copyright Information :

Copyright Author (s) 2026 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

stimulant, antioxidant, and anti-inflammatory properties owing to its rich composition of terpenoids, flavonoids, and phenolic acids (Basu *et al.*, 2022). *Allium sativum* (family: Amaryllidaceae), or garlic, is widely recognized for its antimicrobial, cardioprotective, antihypertensive, and immunomodulatory activities, primarily attributed to organosulfur compounds such as allicin and S-allyl cysteine. When combined, these plants are expected to deliver complementary pharmacological actions, providing multidimensional therapeutic benefits such as glucose regulation, lipid control, antioxidant protection, and immune modulation (Kumari *et al.*, 2023). However, the bio efficacy of polyherbal formulations is highly dependent on the efficiency of the extraction process, which determines the yield and stability of bioactive compounds such as flavonoids and phenolics (Sharma *et al.*, 2017). Extraction parameters-such as solvent concentration, solid-to-solvent ratio, and temperature-play crucial roles in influencing solute diffusion, solvent penetration, and compound degradation (Dutta *et al.*, 2022). Small variations in these parameters can lead to significant differences in yield and biological activity, thereby necessitating a systematic and quantitative optimization strategy.

Conventional “one-factor-at-a-time” optimization approaches are time-consuming, labor-intensive, and incapable of revealing the interactive effects among variables (Singh *et al.*, 2020). In contrast, Response Surface Methodology (RSM) provides a multivariate statistical technique that efficiently explores the relationships between independent variables and responses, enabling precise optimization with fewer experimental trials (Baskar *et al.*, 2019). Within RSM, the Box-Behnken Design (BBD) is widely employed due to its efficiency, cost-effectiveness, and suitability for fitting second-order quadratic models without requiring extreme factor levels that could lead to degradation of heat-sensitive compounds (Jain *et al.*, 2018). In this context, the present study applies RSM using a Box-Behnken Design to systematically evaluate and optimize the influence of solvent volume, solid-to-solvent ratio, and extraction temperature on the yield of Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX) from a polyherbal formulation composed of *G. sylvestre*, *C. cyminum*, and *A. sativum* (Thakur *et al.*, 2021). By establishing predictive mathematical models and generating three-dimensional response surfaces, this work aims to (i) determine the most significant process parameters, (ii) identify the optimal extraction conditions, and (iii) validate the model through experimental verification (Verma *et al.*, 2019; Kalaiselvi and Venkatachalam, 2023; Mehta *et al.*, 2022).

Ultimately, the findings of this study are expected to contribute to the scientific standardization of traditional polyherbal formulations and provide a reproducible, statistically validated framework for the development of high-quality herbal and nutraceutical products.

MATERIALS AND METHODS

Optimization of Extraction Parameters Using Box-Behnken Design (BBD)

The Box-Behnken Design (BBD) under the Response Surface Methodology (RSM) framework was employed to optimize the extraction conditions for the polyherbal formulation composed of *Gymnema sylvestre*, *Cuminum cyminum*, and *Allium sativum*. This statistical approach was utilized to evaluate the main effects, interaction effects, and quadratic effects of key process variables on the extraction yield and phytochemical responses. Empirical RSM techniques were used to develop multivariate polynomial equations representing the relationship between independent variables and the dependent responses, enabling precise process optimization.

A three-factor, three-level Box-Behnken experimental matrix was designed to investigate the influence of three independent variables: solvent volume (A: 30, 50, and 70 mL), solid-to-solvent ratio (B: 10, 15, and 20 g), and extraction temperature (C: 40, 45, and 50°C). The design comprised seventeen experimental runs, including five replicates at the center point to accurately estimate the pure error and curvature effects of the model. Each experiment was conducted in duplicate to ensure statistical reliability.

The extraction experiments were carried out by systematically varying the levels of the independent variables within the design space. The coded values of the process variables were calculated using the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (1)$$

where x_i represents the coded value of the i -th variable, X_i is the uncoded (actual) value, X_0 denotes the central value, and ΔX indicates the step change between levels of the variable.

The variance, coded levels, and actual values of the independent variables are summarized in Table 1, while the complete experimental design matrix, including the observed and predicted responses for Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX), is provided in Tables 3, 4 and 5.

RESULTS AND DISCUSSION

The extraction of bioactive compounds from the polyherbal formulation containing *Gymnema sylvestre*, *Cuminum cyminum*, and *Allium sativum* was optimized using the Box-Behnken Design (BBD) under Response Surface Methodology (RSM). Three independent variables-solvent volume, solid-to-solvent ratio, and extraction temperature-were evaluated for their influence on the dependent responses, namely Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX) showed in Table 1. The design matrix comprised 17 experimental runs generated according to the BBD, and the resulting data were

analyzed using Analysis of Variance (ANOVA) showed in Table 2. Statistical evaluation confirmed the adequacy and significance of the quadratic model ($p < 0.0001$), with all three responses showing strong correlation coefficients ($R^2 > 0.99$), indicating excellent model predictability. Both linear and interaction terms exhibited significant effects, demonstrating that extraction yield was strongly influenced by the combined action of solvent volume, temperature, and solid-solvent ratio.

Under the optimized conditions-solvent volume 51.00 mL, solid-to-solvent ratio 15.11 g, and temperature 44.99°C-the experimental yields were TFC: 30.9 ± 1.09 mg QE/g, TPC: 36.9 ± 1.09 mg GAE/g, and AOX: $72 \pm 0.05\%$, which closely matched the predicted values. These results confirm the precision and reliability of the RSM approach in optimizing the extraction process. The three-dimensional response surface plots further illustrated the interactive effects between variables, showing dome-shaped surfaces typical of quadratic behavior. The high extraction efficiency observed under optimized conditions demonstrates that moderate temperature and controlled solvent use enhance the diffusion and solubility of phenolic and flavonoid compounds while preventing their thermal degradation.

Similar optimization studies using RSM and BBD for the extraction of polyphenols and flavonoids from medicinal plants have also reported comparable findings, reinforcing the robustness, reproducibility, and scientific reliability of this statistical approach. The established model thus provides a strong framework for standardizing polyherbal extractions and can be effectively applied in the development of phytopharmaceutical and nutraceutical formulations with consistent quality and enhanced bioactivity.

The Box-Behnken experimental setup allowed simultaneous assessment of both individual and interactive effects of the process variables, providing a detailed understanding of the extraction dynamics. The statistical Analysis of Variance (ANOVA) confirmed the adequacy and significance of the quadratic model ($p < 0.0001$). Both linear and interaction terms were found to have a pronounced influence on extraction efficiency, while the inclusion of central points validated the model's curvature and reproducibility. The developed model successfully described the system behavior with excellent correlation coefficients ($R^2 > 0.99$), demonstrating its predictive strength.

This optimized design provided a systematic, reproducible framework for maximizing the recovery of bioactive phytoconstituents from the polyherbal matrix. The application of RSM-based BBD thus enabled efficient process optimization with minimal experimental trials while ensuring high precision and model reliability for subsequent scale-up and standardization in herbal product development.

Model Development and Statistical Analysis

The yields of Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX) served as the dependent response variables in the optimization of the polyherbal extraction process. The relationship between the independent variables-solvent volume (A), solid-to-solvent ratio (B), and temperature (C)-and the corresponding responses was modeled using a second-order polynomial regression equation. The general mathematical expression used to predict the response is represented as:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i^2 + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j \quad (2)$$

Where Y denotes the predicted response, β_0 represents the intercept, β_i , β_{ii} , and β_{ij} correspond to the coefficients of the linear, quadratic, and interaction terms, respectively, and X_i and X_j are the coded levels of the independent variables.

Regression analysis was performed using Design-Expert Software (Version 13, Stat-Ease Inc., Minneapolis, USA) to estimate the coefficients and generate the predictive equations for each response variable. The adequacy and reliability of the models were verified through Analysis of Variance (ANOVA), which evaluated the significance of linear, quadratic, and interaction terms, as well as the overall model fit. The high F-values and low p-values ($p < 0.0001$) indicated that the developed quadratic models were statistically significant and suitable for describing the behavior of the extraction system. The lack-of-fit test was found to be insignificant, confirming that the models adequately represented the experimental data.

The coefficients of determination ($R^2 > 0.99$) for all three responses (TFC, TPC, and AOX) demonstrated excellent agreement between predicted and experimental values. Additionally, the high values of Adjusted R^2 and Adequate Precision (> 4) further confirmed the reliability and precision of the models. The statistical parameters suggested that the fitted equations could accurately predict the influence of process variables on extraction efficiency and antioxidant potential.

To better interpret the experimental data and visualize the interaction between variables, three-dimensional response surface plots (Figures 1A-1I) were generated. These graphical representations provided insight into the combined effects of solvent volume, solid-to-solvent ratio, and temperature on the extraction yield of bioactive compounds. The surface plots exhibited a dome-shaped curvature characteristic of quadratic models, indicating the presence of optimal points within the studied range.

The optimization results were subsequently used to determine the most favorable extraction conditions in coded values, which were then converted to actual experimental settings for validation. The optimal parameters were identified as solvent volume 51.00 mL, solid-to-solvent ratio 15.11 g, and temperature 44.99°C, yielding

maximum values of TFC (30.9 ± 1.09 mg QE/g), TPC (36.9 ± 1.09 mg GAE/g), and AOX ($72 \pm 0.05\%$). The close agreement between predicted and observed values confirmed the predictive capability of the quadratic model.

These findings emphasize the effectiveness of the Response Surface Methodology in optimizing polyherbal extraction processes. The model established in this study provides a statistically validated and experimentally reproducible framework for maximizing the yield of bioactive phytoconstituents, ensuring consistent quality in herbal and nutraceutical formulations. Moreover, the integration of RSM in process design supports sustainable and efficient extraction strategies, facilitating the development of standardized herbal products with enhanced therapeutic potential.

The 3-D response surface and corresponding contour plots (Figures 1A-1I) generated through the Box-Behnken design provide a comprehensive visualization of how solvent volume (A), solid-solvent ratio (B), and extraction temperature (C) interactively influence the yield of Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX) in the polyherbal formulation. The plots exhibit dome-shaped

curvatures, confirming the quadratic nature of the developed models and the presence of well-defined optimal regions. The curvature indicates that increasing or decreasing the variable beyond the critical point leads to a decline in the response due to degradation, solvent saturation, or limited solute diffusion.

Effect of Solvent Volume and Solid-Solvent Ratio (A $\times$ B)

Figures 1A, 1D, and 1G illustrate the combined influence of solvent volume and solid-solvent ratio while maintaining temperature constant at the center level (45°C).

TFC (Figure 1A): TFC increased progressively with solvent volume up to around 50 mL and solid-solvent ratio ≈ 15 g. Beyond this point, further increments led to a marginal decrease, suggesting that excessive solvent dilutes solute concentration and lowers mass-transfer efficiency.

TPC (Figure 1D): A similar parabolic pattern was observed for TPC, where moderate solvent levels favored phenolic extraction. At higher solvent volumes (>60 mL), the solvent-solute equilibrium shifted, reducing recovery.

Table 1: Levels of different process variables in coded and uncoded form for the TFC, TPC and AOX.

Variables (Independent Variables)	Range and Levels		
	-1	0	+1
Solvent, A (mL)	30	50	70
Solid-solvent, B (g)	10	15	20
Temperature, C ($^\circ\text{C}$)	40	45	50

Table 2: Response surface, Box-Behnken (uncoded) and results of TFC, TPC and AOX.

Run	Solvent (Ethanol)	Solid-Solvent	Temperature	TFC	TPC	AOX
1	50	20	50	26.42	32.67	68.08
2	50	10	40	24.34	33.56	68.41
3	30	20	45	26.96	32.05	65.72
4	70	15	40	25.45	33.89	65.43
5	50	15	45	30.93	36.98	72.05
6	50	15	45	30.93	36.98	72.05
7	50	15	45	30.93	36.98	72.05
8	30	15	50	25.75	32.46	64.87
9	70	20	45	26.21	33.25	66.24
10	50	20	40	26.42	33.21	65.87
11	70	10	45	25.56	32.47	68.49
12	50	10	50	27.32	32.34	66.97
13	30	10	45	25.31	32.85	65.76
14	30	15	40	24.59	32.54	66.34
15	70	15	50	26.35	33.21	68.32
16	50	15	45	30.93	36.98	72.05
17	50	15	45	30.93	36.98	72.05

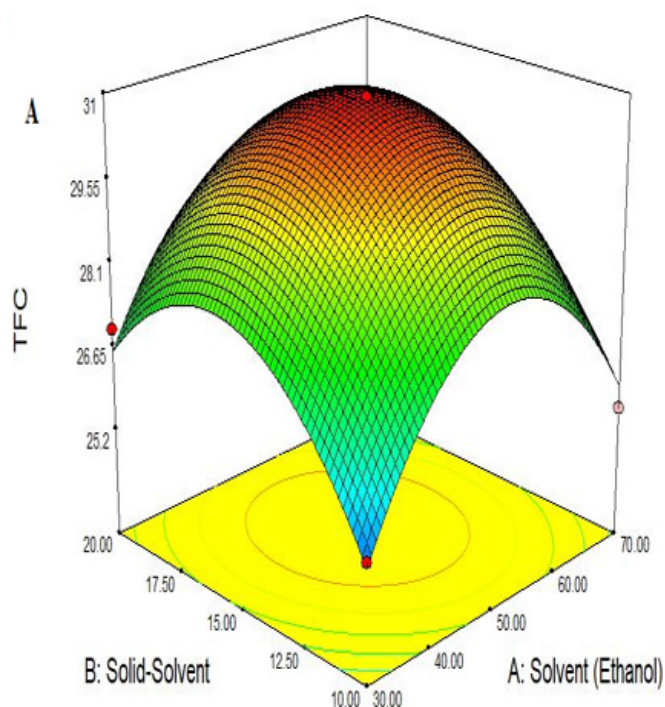
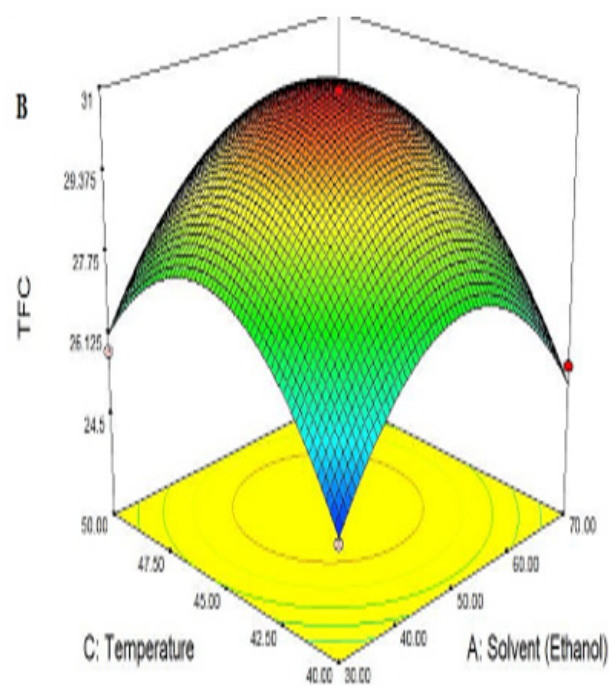
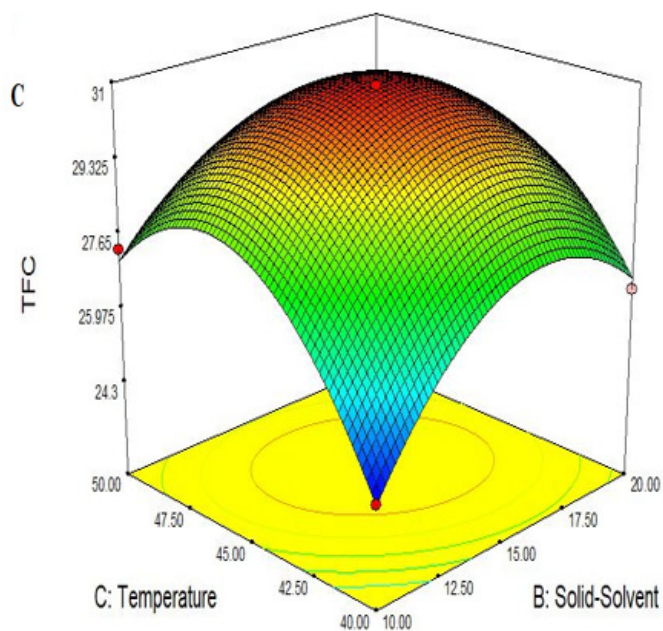
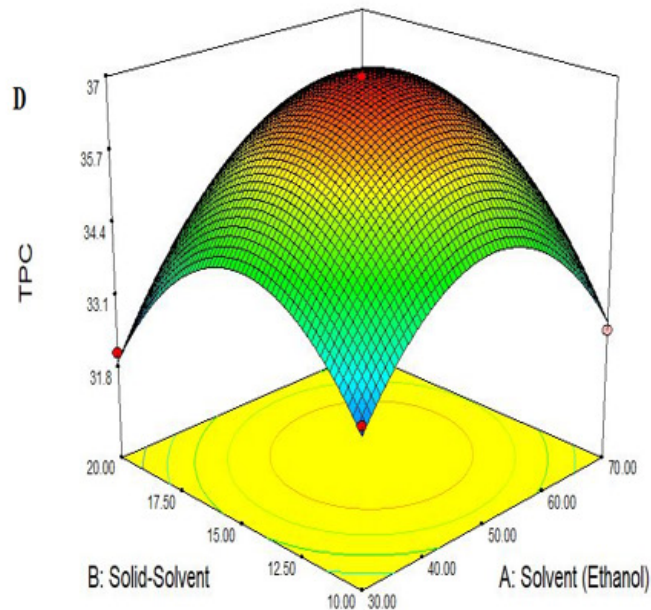
AOX (Figure 1G): The AOX surface displayed a steep rise with increasing solid-solvent ratio and moderate solvent volume, implying that dense extraction media containing sufficient plant mass enhance the availability of antioxidant constituents.

These plots emphasize the importance of maintaining an appropriate solvent-to-solid proportion to achieve maximum extraction yield without excessive dilution or poor solvation.

Effect of Solvent Volume and Temperature ($A \times C$)

Figures 1B, 1E, and 1H: Depict the interaction between solvent volume and extraction temperature.

TFC (Figure 1B): TFC increased with rising temperature up to 45°C owing to improved solute diffusion and solvent penetration. A further rise beyond 50°C reduced TFC, likely due to the thermal degradation of flavonoid structures.



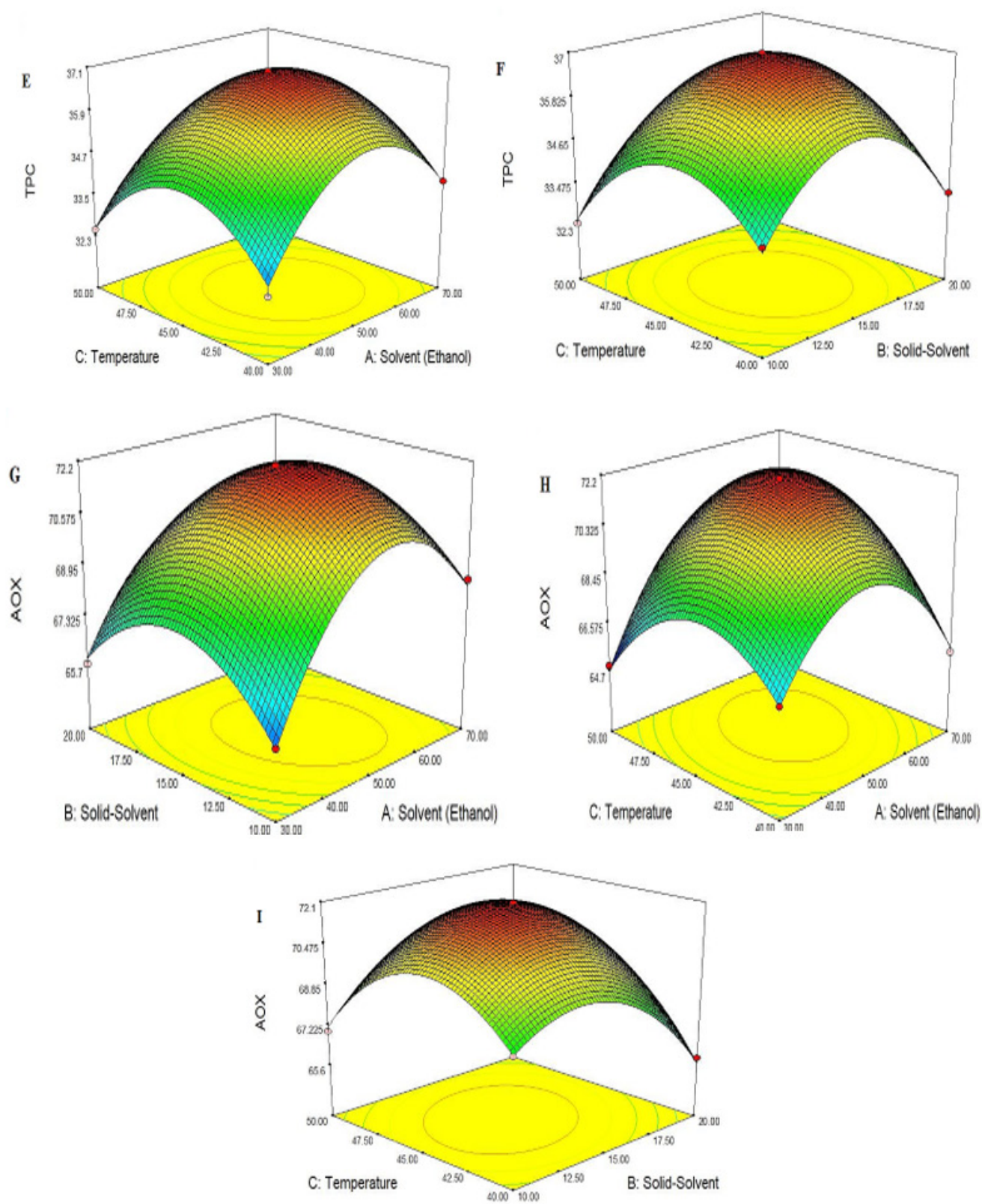


Figure 1: Response surface plots (3-D) (A-I) showing the effect of process variable on the yield of extracted polyherbal formulation (plants are *Gymnema sylvestre*, *Cuminum cimum* and *Allium sativum*).

Table 3: ANOVA for the regression and respective model terms (TFC).

Source	Sum of Squares	d _f	Mean Square	fValue	p-value Prob > f	
Model	62.46691	9	6.940767	147.3177	<0.0001	significant
A-Solvent (Ethanol)	1.0658	1	1.0658	22.62159	0.0021	
B-Solid-Solvent	0.0002	1	0.0002	0.004245	0.9499	
C-Temperature	0.7938	1	0.7938	16.84839	0.0045	
AB	0.6241	1	0.6241	13.24651	0.0083	
AC	0.09	1	0.09	1.910249	0.2094	
BC	0.1156	1	0.1156	2.453608	0.1612	
A ²	18.96845	1	18.96845	402.605	<0.0001	
B ²	20.42529	1	20.42529	433.5265	<0.0001	
C ²	14.13918	1	14.13918	300.104	<0.0001	
Residual	0.3298	7	0.047114			
Lack of Fit	0.3298	3	0.109933			
Pure Error	0	4	0			
Cor Total	62.79671	16				

Table 4: ANOVA for the regression and respective model terms (TPC).

Source	Sum of Squares	d _f	Mean Square	fValue	p-value Prob > f	
Model	119.0628	9	13.2292	444.6257	<0.0001	significant
A-Solvent (Ethanol)	4.190512	1	4.190512	140.8407	<0.0001	
B-Solid-Solvent	1.7298	1	1.7298	58.13756	0.0001	
C-Temperature	0.599512	1	0.599512	20.14926	0.0028	
AB	1.221025	1	1.221025	41.03793	0.0004	
AC	4.7524	1	4.7524	159.7254	<0.0001	
BC	3.330625	1	3.330625	111.9403	<0.0001	
A ²	45.71379	1	45.71379	1536.414	<0.0001	
B ²	20.42529	1	20.42529	686.4819	<0.0001	
C ²	26.63253	1	26.63253	895.1035	<0.0001	
Residual	0.208275	7	0.029754			
Lack of Fit	0.208275	3	0.069425			
Pure Error	0	4	0			
Cor Total	119.2711	16				

TPC (Figure 1E): TPC followed a similar dome-shaped trend, showing a sharp increment up to the optimal zone (50 mL, 45°C) and a subsequent decline at higher temperatures. Phenolic compounds are sensitive to heat; hence excessive temperature can promote oxidation or polymerization.

AOX (Figure 1H): The antioxidant activity correlated positively with temperature within the moderate range but declined beyond 46-47°C. The observed trend corroborates that controlled heating enhances extraction kinetics but excessive thermal stress compromises antioxidant potency. These results collectively indicate that solvent and temperature synergistically control solubility and stability of bioactive constituents, and their balance is crucial for optimum recovery.

Effect of Solid-Solvent Ratio and Temperature (B × C)

Figures 1C, 1F, and 1I portray the influence of the solid-solvent ratio and temperature on extraction responses.

TFC (Figure 1C): The surface demonstrates that flavonoid yield increased sharply with temperature from 40°C to 45°C and solid-solvent ratio up to 15 g, after which the yield plateaued. Beyond the optimum, a decline was observed due to reduced solvent availability per gram of plant material and potential compound degradation.

TPC (Figure 1F): A pronounced curvature was visible, indicating strong quadratic interaction. Optimal TPC was obtained at ≈15 g solid-solvent ratio and 45°C. Either excessive solid loading

Table 5: ANOVA for the regression and respective model terms (AOX).

Source	Sum of Squares	d _f	Mean Square	fValue	p-value Prob > f	
Model	97.72651	9	10.8585	102.3284	<0.0001	significant
A-Solvent (Ethanol)	0.1152	1	0.1152	1.085622	0.3321	
B-Solid-Solvent	1.5138	1	1.5138	14.26575	0.0069	
C-Temperature	3.1752	1	3.1752	29.92246	0.0009	
AB	0.25	1	0.25	2.35595	0.1687	
AC	0.0169	1	0.0169	0.159262	0.7017	
BC	2.2201	1	2.2201	20.92178	0.0026	
A ²	31.958	1	31.958	301.1659	<0.0001	
B ²	19.73568	1	19.73568	185.9852	<0.0001	
C ²	29.34568	1	29.34568	276.5479	<0.0001	
Residual	0.7428	7	0.106114			
Lack of Fit	0.7428	3	0.2476			
Pure Error	0	4	0			
Cor Total	98.46931	16				

Table 6: Predicted and experimental values of the responses at optimum conditions.

Optimum Condition			TFC		TPC		AOX	
Experimental*			Experimental*	Predicted	Experimental*	Predicted		
Predicted								
Sol-vent, (A)	Solid-Sol-vent (B)	Temperature (D) (°C)	30.9±1.09	31.9±2.05	36.9 ±1.09	35±0.05	72±0.05	71±0.05
51.00	15.11	44.99						

*Mean±standard deviation (n=3).

or higher temperatures decreased phenolic recovery, possibly because of inefficient solvent penetration or denaturation of phenolics.

AOX (Figure 11): AOX mirrored the behavior of TPC, reinforcing the dependence of antioxidant potential on phenolic concentration. The highest AOX values were recorded at intermediate ratios and moderate temperatures, reflecting the synergistic effect of efficient diffusion and compound stability.

The convex surfaces in these plots confirm that extraction performance is sensitive to both solid concentration and temperature, with significant interaction ($p < 0.01$) evident in the ANOVA results.

Model Validation and Experimental Verification

To validate the predictive accuracy of the developed quadratic model, experimental extraction was performed under the optimized conditions determined by the Box-Behnken design. The extraction parameters were set at a solvent volume of

51.00 mL, a solid-to-solvent ratio of 15.11 g, and an extraction temperature of 44.99°C. The resulting extracts were analyzed for Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX) to compare the experimental data with the model's predictions.

The experimental results demonstrated a TFC of 30.9±1.09 mg QE/g, a TPC of 36.9±1.09 mg GAE/g, and an AOX of 72±0.05%. These values showed close agreement with the model-predicted responses of 31.9±2.05 mg QE/g (TFC), 35.0±0.05 mg GAE/g (TPC), and 71±0.05% (AOX), respectively (Table 6). The minimal deviation between predicted and observed responses confirmed the reliability and validity of the regression model in accurately describing the influence of extraction parameters on the yield of bioactive compounds.

This strong correlation between predicted and experimental results demonstrates the robustness of the Response Surface Methodology in process optimization. The validated model thus provides a dependable framework for reproducing the optimized

conditions in subsequent experimental or scale-up studies. Furthermore, the close alignment of experimental and theoretical results highlights the suitability of the optimized parameters for achieving maximum extraction efficiency, ensuring batch-to-batch consistency in polyherbal extract preparation for potential pharmaceutical and nutraceutical applications.

Overall Interpretation

Across all plots, the central region—approximately 50 mL solvent, 15 g solid-solvent ratio, and 45°C temperature—yielded maximal responses. The decline in responses beyond these conditions validates the statistical models and supports the experimental optimization results. Excessive solvent volumes dilute extract concentration, while overly high temperatures cause thermal decomposition of thermolabile phytoconstituents such as flavonoids and phenolics. Conversely, insufficient solvent or low temperature restricts diffusion, resulting in incomplete extraction.

Therefore, the response surface analysis conclusively demonstrates that the Box-Behnken-optimized parameters establish a critical balance between solubility, diffusivity, and compound stability, ensuring efficient extraction of the major bioactive components contributing to the polyherbal formulation's antioxidant potential.

CONCLUSION

The present investigation clearly demonstrates that Response Surface Methodology (RSM) employing the Box-Behnken Design (BBD) is an efficient and reliable statistical tool for optimizing extraction parameters in complex polyherbal systems. The polyherbal formulation consisting of *Gymnema sylvestre*, *Cuminum cyminum*, and *Allium sativum* exhibited significantly improved extraction yields of bioactive constituents under optimized conditions. The quadratic models developed for Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Antioxidant Activity (AOX) were statistically significant ($p < 0.0001$) with excellent correlation coefficients ($R^2 > 0.99$), confirming the robustness and predictive capacity of the models.

Optimization studies revealed that a solvent volume of 51 mL, a solid-solvent ratio of 15.11 g, and an extraction temperature of 44.99°C resulted in maximum recovery of flavonoids (30.9 ± 1.09 mg QE/g), phenolics (36.9 ± 1.09 mg GAE/g), and antioxidant activity ($72 \pm 0.05\%$). The close agreement between predicted and experimental values validates the adequacy of the proposed model for practical applications.

The findings emphasize that extraction temperature and solvent volume are critical determinants of extraction efficiency, influencing solute diffusion, solvent penetration, and compound stability. The strong correlation among TFC, TPC, and AOX

further highlights that phenolic and flavonoid constituents are primarily responsible for the antioxidant potential of this formulation.

Overall, this study provides a scientific basis for process standardization of polyherbal extractions, which has traditionally been empirical. The optimized protocol can serve as a reference model for large-scale extraction and formulation development in the pharmaceutical and nutraceutical industries, ensuring batch-to-batch uniformity and product efficacy. Future work should focus on phytochemical profiling of the optimized extract and biological validation of its pharmacological activities to support its application in evidence-based herbal therapeutics.

ACKNOWLEDGEMENT

The authors acknowledge the Indian System of Medicine Laboratory, Sathyabama Institute of Science and Technology, Chennai, for providing necessary facilities and research support.

ABBREVIATIONS

ANOVA: Analysis of Variance; **AOX:** Antioxidant activity; **BBD:** Box-Behnken design; **Cor Total:** Corrected total; **df:** Degrees of freedom; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent; **RSM:** Response Surface Methodology; **TFC:** Total flavonoid content; **TPC:** Total phenolic content.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- Baskar, G., Chandhuru, J., Renuka, S., & Rajeshkumar, R. (2019). Application of RSM in the extraction and quantification of bioactive compounds from medicinal plants. *Industrial Crops and Products*, 138, Article 111454. <https://doi.org/10.1016/j.indcrop.2019.111454>
- Basu, A., Ghosh, S., & Dutta, M. (2022). Response surface optimization and kinetic modeling for enhanced recovery of phenolics and flavonoids from herbal matrices. *Journal of Food Process Engineering*, 45(6), Article e13981. <https://doi.org/10.1111/jfpe.13981>
- Dutta, D., Chatterjee, S., & Saha, S. (2022). Application of Box-Behnken design in optimization of polyherbal formulation for maximum antioxidant activity. *Journal of Ayurveda and Integrative Medicine*, 13(2), Article 100535. <https://doi.org/10.1016/j.jaim.2022.100535>
- Jain, T., Jain, V., Pandey, R., & Vyas, A. (2018). Optimization of extraction conditions for flavonoids from *Gymnema sylvestre* using Box-Behnken design and evaluation of its pharmacological activities. *Pharmacognosy Magazine*, 14(56), 338–345. https://doi.org/10.4103/pm.pm_338_17
- Kalaiselvi, P., & Venkatachalam, R. (2023). Design Expert-based optimization of phytochemical extraction from *Gymnema sylvestre* and evaluation of its antioxidant potential. *Biocatalysis and Agricultural Biotechnology*, 50, Article 102786. <https://doi.org/10.1016/j.bcab.2023.102786>
- Kumari, P., Sharma, A., Rathore, M., & Prasad, N. (2023). Green extraction optimization of phytoconstituents from *Gymnema sylvestre* using RSM and its evaluation for antioxidant and antidiabetic properties. *South African Journal of Botany*, 157, 67–75. <https://doi.org/10.1016/j.sajb.2023.04.015>
- Mehta, S., Chauhan, N., & Kumar, D. (2022). Response surface optimization of herbal extraction and development of standardized polyherbal antioxidant formulation. *Pharmacognosy Journal*, 14(4), 835–842. <https://doi.org/10.5530/pj.2022.14.104>
- Mukherjee, P. K., Bahadur, S., Chaudhary, S. K., Kar, A., Khajuria, R. K., & Saha, B. P. (2018). Application of response surface methodology in herbal drug standardization: An overview. *Phytochemical Analysis*, 29(3), 289–301. <https://doi.org/10.1002/pca.2742>

- Rajan, R., Jayalakshmi, S., & Chandran, S. (2021). Optimization of flavonoid extraction from *Gymnema sylvestre* leaves by Box-Behnken design and assessment of its antioxidant potential. *Journal of Applied Research on Medicinal and Aromatic Plants*, 25, Article 100338. <https://doi.org/10.1016/j.jarmap.2021.100338>
- Raju, L., Shanmugam, G., & Manikandan, R. (2020). Optimization of polyphenol extraction from *Cuminum cyminum* seeds using response surface methodology and evaluation of its antioxidant activity. *Industrial Crops and Products*, 152, Article 112548. <https://doi.org/10.1016/j.indcrop.2020.112548>
- Sharma, S., Rani, S., & Gill, B. S. (2017). Optimization of solvent extraction conditions for total phenolic and flavonoid contents from *Allium sativum* using response surface methodology. *Journal of Food Science and Technology*, 54(8), 2403–2411. <https://doi.org/10.1007/s13197-017-2698-8>
- Singh, A., Kumar, R., & Pandey, A. K. (2019). Phytochemical profiling and antioxidant potential of *Allium sativum* extract optimized by response surface methodology. *Food Chemistry*, 278, 682–691. <https://doi.org/10.1016/j.foodchem.2018.11.045>
- Singh, J., Kaur, M., & Kaur, S. (2020). Statistical optimization of phenolic extraction from *Cuminum cyminum* and evaluation of its antioxidant and antimicrobial properties. *Food Bioscience*, 36, Article 100611. <https://doi.org/10.1016/j.fbio.2020.100611>
- Thakur, M., Singh, D., & Kumar, A. (2021). RSM-based optimization of antioxidant extraction from polyherbal formulations and correlation with in vitro radical scavenging activity. *BMC Complementary Medicine and Therapies*, 21(1), Article 141. <https://doi.org/10.1186/s12906-021-03310-6>
- Verma, R., Sharma, A., & Gupta, P. (2019). Process optimization of polyphenolic extraction from *Allium sativum* and *Cuminum cyminum* using response surface methodology. *Journal of Food Biochemistry*, 43(11), Article e13047. <https://doi.org/10.1111/jfbc.13047>

Cite this article: Jayamani S, Venkatraman M. Optimization of Polyherbal Extraction Parameters Using Response Surface Methodology: A Study on *Gymnema sylvestre*, *Cuminum cyminum*, and *Allium sativum*. *Asian J Biol Life Sci.* 2026;15(3):659-68.