

Optimizing Polyphenol Extraction from *Lavandula maroccana*: a simplex-Centroid Mixture Design Approach

Oumaima CHATER^{1,*}, Smail AAZZA², Zineb EL JABBOURY¹, Lahsen EL GHADRAOUI¹

¹ Laboratory of Functional Ecology and Environmental Engineering, Faculty of Science and Techniques, University of Sidi Mohamed Ben Abdellah, Fez, Morocco; ² OLMANBGPE, Nador Multidisciplinary Faculty (FPN), Mohammed 1st University, Oujda, Morocco

Received: February 28, 2025; Revised: July 17, 2025; Accepted: July 30, 2025

Abstract

This study aims to enhance the efficiency of extracting polyphenols from *Lavandula maroccana* by employing a simplex-centroid mixture design approach. The efficiency of extracting total phenolic content (TPC), total antioxidant capacity (TAC), and DPPH radical scavenging activity were assessed using various solvent combinations comprising water, methanol, and ethanol. The significance and predictive capability of the models were determined through a detailed analysis using response surface methodology (RSM) and analysis of variance (ANOVA). The findings demonstrated that a well-proportioned combination of water and methanol consistently yielded the maximum extraction efficacy for phenolic compounds and antioxidants in all plant components. The ideal solvent proportions were determined, with water and methanol each accounting for approximately 50% of the combination. The statistical analysis confirmed the reliability and predictive power of the developed models, as reflected by significant F-values along with elevated R², adjusted R², and predicted R² coefficients, all collectively demonstrating a high degree of model fitness and accuracy. These findings provide valuable guidance for optimizing extraction protocols, enhancing both the yield and bioactivity of antioxidant compounds, with promising prospects for application across the nutraceutical, pharmaceutical, and food industries.

Keywords: *Lavandula maroccana*, simplex-centroid mixture design, total phenolic content, antioxidants, response surface methodology, solvent optimization.

1. Introduction

Lavandula maroccana, commonly known as Moroccan lavender, is a native aromatic plant that contains a rich array of phenolic compounds with well-documented antioxidant potential. Consumers and academics are increasingly interested in the health advantages of natural phenolic phytochemicals found in plants, due to their ability to mitigate oxidative stress, which is implicated in the onset of various chronic diseases such as cardiovascular disorders and cancer (Loganayaki et al., 2013). Antioxidants are substances that neutralize free radicals and reactive oxygen species (ROS), thereby preventing lipid peroxidation and cellular damage (Velioglu et al., 1998; Pryor, 1991). In addition to their health benefits, antioxidants are used as food additives to prevent oxidative degradation and have been associated with other biological properties, such as anti-inflammatory, antimicrobial, antiviral, and vasodilatory effects (Shahidi, 2000; Kumar et al., 2010).

Maximizing the efficiency of polyphenol extraction is crucial to increasing both yield and biological activity. Conventional extraction techniques frequently depend on single solvents, which may not effectively extract the full diversity of phenolic compounds. The simplex-centroid mixture design offers a potent alternative, allowing

systematic evaluation of single solvents, and their binary and ternary mixtures and interactions among components. This approach enables a deeper understanding of the individual and synergistic effects of each solvent on extraction performance.

Recent work underscores this potential. Mixture-design or RSM studies have enhanced polyphenol recovery from Moroccan *Cannabis sativa* waste through multivariate optimization (Aazza, 2021) and from *Anacyclus pyrethrum* var. *depressus* roots via simplex-centroid modelling (Chater et al., 2024) as well as from *Ammi visnaga* roots using triangular-surface designs (El Jabboury et al., 2022), *Magnolia × soulangeana* flower buds via Box–Behnken hydro-ethanolic optimization (Zgórka et al., 2023), and broccoli stems with deep-eutectic solvents (Wang et al., 2025). Together, these studies further illustrate the versatility of mixture-design approaches.

Although several authors have optimized polyphenol extraction from *Lavandula* species, to our knowledge no study has yet applied an advanced mixture design to *Lavandula maroccana*. This work is, therefore, the first to employ a simplex-centroid mixture design for that purpose. This study, hence, aims to determine the optimal solvent mixture of water, ethanol, and methanol to maximize total phenolic content (TPC), total antioxidant capacity (TAC), and DPPH radical scavenging activity. By employing response surface methodology (RSM) and

* Corresponding author. e-mail: oumaima.chater@usmba.ac.ma.

analysis of variance (ANOVA), the findings will contribute to the development of efficient, environmentally friendly extraction strategies with potential applications in the nutraceutical, pharmaceutical, and food industries.

2. Materials & Methods

2.1. Plant material

Examples of *L. maroccana* specimens were collected in May 2020 from the Sefrou region located in the Moroccan Middle Atlas. Once gathered, the leaves, flowers, stems, and roots were meticulously divided. Afterward, these plant components were dried in a shaded place to preserve their structure and protect them from damage caused by direct sunshine. Following thorough dehydration, the plant material was finely pulverized, ensuring uniformity and enabling analysis. The powders were stored in opaque pillboxes to protect them from light and other environmental factors, ensuring their purity for future study.

2.2. Solvent Extraction

The samples were prepared in triplicate by combining 1 mL of the solvent (either pure solvents or mixtures) with 50 mg of plant powder. The mixture was then subjected to sonication for 30 minutes in an ultrasonic bath at room temperature. After centrifugation for 10 minutes at 10,000 rpm, the extract was collected and stored in a dark environment at 4 °C. The initial phase of extraction involves a screening process employing solvents of varying polarities, such as water, ethanol, methanol, acetone, ethyl acetate, dichloromethane, chloroform, hexane, di-ethyl ether, and butanol, to determine the most suitable solvent for the subsequent step. The second stage involves extracting the pure specified solvent and their combinations.

2.3. Total phenolic content (TPC)

The spectrophotometric approach was used to quantify the total phenolic content (TPC) by employing the colorimetric technique with the Folin-Ciocalteu reagent (Ma et al., 2010), with some modifications. 50 µL of the sample was combined with 450 µL of a solution of the Folin-Ciocalteu reagent that had been diluted by a factor of 10. Following a 5-minute incubation period at ambient temperature, 450 µL of a solution containing Na₂CO₃ (75 g L⁻¹) was introduced. Subsequently, all samples were placed in a dark environment and incubated for 2 hours at room temperature. The absorbance of the samples was then measured at a wavelength of 760 nm using a spectrophotometer. The calibration curve had a concentration range of 0.062 to 2 mg mL⁻¹ in an ethanolic solution of gallic acid. The equation of the curve was $y = 1.2257x + 0.174$, with a R² value of 0.9988. The experiment was conducted in triplicate, and the results are reported in milligrams of gallic acid equivalent (GAE) per gram of dry plant material.

2.4. Antioxidant activity:

2.4.1. Total Antioxidant Capacity (TAC)

The samples' overall antioxidant activity was evaluated through the creation of the phospho-molybdenum complex (Libbey & Walradt, 1968). 50 µL of the sample solution was mixed with 1 mL of the reagent solution, which

consisted of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixture was subsequently incubated in a water bath at 95 °C for 90 min. The spectrophotometer was used to measure the absorbance of the combination at a wavelength of 695 nm, relative to a blank. The calibration curve was established using an aqueous solution of ascorbic acid. The equation of the curve was $y = 1.4632x + 0.0191$, with a R² value of 0.9997. The concentration of the ascorbic acid in the solution ranged from 1.0 to 0.0625 mg mL⁻¹. The experiment was conducted in triplicate, and the findings represent the average levels of antioxidant activity expressed in grams of ascorbic acid equivalents per gram of dry plant.

2.4.2. Free radical scavenging activity: DPPH

The production of DPPH (2,2-diphenyl-1-picrylhydrazyl) followed the procedure outlined in reference (DiCiulaa et al., 2014). 25 µL of various concentrations of samples or standards were introduced into a 1 milliliter solution of DPPH in ethanol, at a concentration of 60 micromolar. The absorbance readings were taken at a wavelength of 517 nm, following a 60-minute incubation period at room temperature. A negative control was performed by measuring the absorption of a blank sample containing an equal amount of methanol and DPPH solution. The experiment was conducted three times, and the percentage inhibition of the free radical scavenging activity for each extract was determined using the following formula:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100 \quad \text{Eq. 1}$$

2.5. Experimental design and optimization

2.6. Evaluation of solvent effects by simplex axial design

Research was conducted to optimize polyphenol extraction using a mixture approach. Two standard experimental designs are commonly employed for solvent mixture extraction studies: Simplex-Lattice Design and Simplex-Centroid Design. Both methodologies evaluate the triangular response surface at vertex and central locations.

The Simplex-Centroid Design creates a triangular experimental space encompassing various tested conditions. The triangle vertices correspond to individual pure solvents, each constituting 100% of a particular solvent. The edge midpoints represent binary solvent combinations in equal ratios, specifically (1/2:1/2:0; 1/2:0:1/2; 0:1/2:1/2). The central point corresponds to a three-component mixture with uniform proportions of each element (1:1:1).

A mixture model was developed to improve extraction efficiency. The Simplex-Centroid Design incorporating axial points with triplicate runs was selected to determine the optimal solvent blend of water (W), ethanol (E), and methanol (M). This approach allows for evaluation of individual solvent effects (W, E, M), binary interaction effects (WE, WM, EM), and ternary interaction effects (WEM) through linear, quadratic, and cubic models respectively.

The research employed the enhanced simplex centroid design to investigate how various extraction solvents affect

Total Phenolic Content (TPC) and antioxidant properties. The goal was to optimize extraction parameters by examining solvent interactions. Mixture design experiments were conducted and evaluated using STATISTICA software (version 10, StatSoft, Inc., 2013). Ten experimental combinations were tested in total, with all experiments performed in technical triplicates under identical conditions on the same day to ensure repeatability and reduce experimental variation. Data are reported as mean \pm standard deviation and analyzed through analysis of variance (ANOVA). Tukey's post-hoc test determined significant differences at $p < 0.05$. A polynomial function was fitted to each component at every experimental condition. Y represents the predicted response, while β_1 , β_2 , β_3 , β_{12} , β_{13} , and β_{23} are coefficients for linear and interaction terms.

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3$$

Eq.2

3. Results and Discussion

3.1. Extraction solvents screening

Several factors are known to significantly affect extraction yield and efficiency. The solvent is particularly important among these factors and is recognized as having a substantial influence on both the amount extracted and the overall effectiveness of the extraction process. In the first phase of this study, we assessed the impact of solvent polarity on the total phenolic content (TPC) of *L. maroccana*, using ten pure solvents to extract polyphenols from four different plant parts (leaves, flowers, stems, and roots).

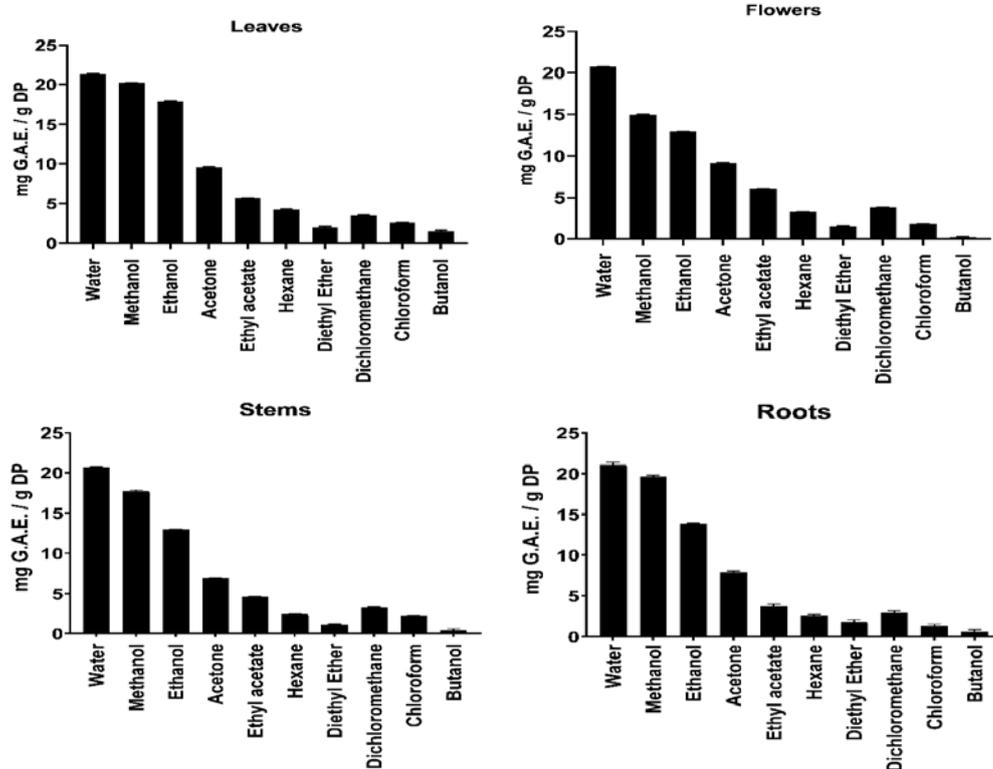


Figure 1: Total phenolic content (TPC) expressed in mg gallic acid equivalent per gram of dry plant material (mg GAE/g d.w.) in leaves, flowers, stems, and roots of *L. maroccana* extracted with different solvents.

Figure 1 illustrates the TPC results expressed in mg gallic acid equivalents (GAE) per gram of dry plant material. This extraction data demonstrates that solvent polarity significantly influences yield across different plant parts, with water achieving the highest extraction yields (20.70-21.37%) and excellent consistency across leaves, flowers, stems, and roots, followed by methanol (14.95-20.22%) and ethanol (12.89-17.86%), both showing particularly strong performance with leaves. Moderately polar solvents like acetone (6.87-9.53%) and dichloromethane (3.04-3.82%) produced intermediate results, while non-polar and less polar solvents including ethyl acetate (3.74-6.02%), hexane (2.43-4.24%), chloroform (1.35-2.54%), and diethyl ether (1.10-1.89%) showed considerably lower extraction efficiency, with butanol performing poorest overall (0.19-1.41%). These

data emphasize the significant impact of solvent extraction effectiveness on the overall yield of phenolic compounds, as the recovery of phenolic compounds is contingent upon the choice of solvent for extraction and its polarity (Boeing et al., 2014). The results highlight the importance of the solvent in determining the amounts of phenolic compounds, with leaves generally providing the highest yields across most solvents, while the clear superiority of polar solvents specifically water, methanol, and ethanol as the most effective solvents for boosting polyphenol productions suggests that the target compounds in these plant materials have predominantly polar characteristics. Based on this preliminary screening, water, methanol, and ethanol were selected for further optimization using a simplex-centroid mixture design, as they demonstrated the highest potential for maximizing polyphenol recovery.

3.2. Extraction using solvent mixtures

The three best solvents were chosen based on the total phenolic compounds (TPC) value. Out of the 10 solvents employed, water, methanol, and ethanol yielded the highest amount of phenolic compounds in comparison to the other solvents.

After evaluating the outcomes of extracting solvents individually, we decided to use a mixture of three solvents

(water, methanol, and ethanol) that showed the highest yields of polyphenols. We conducted experiments using the simplex-centroid mixture design approach to modify the quantities of solvents inside the mixture. The amounts of total phenolic compounds and antioxidant activities of the extracts obtained using the three selected pure solvents (water, ethanol, and methanol) and their mixtures are illustrated in Tables 1, 2, and 3.

Table 1: Simplex axial design and results for mixture tested of TPC

Crude extract	Extract (solvent proportions)	mg Gallic acid / g dry plant			
		Leaves	Flowers	Stems	Roots
1	Water (W) (1)	21.68 ± 0.08	20.71 ± 0.02	20.83 ± 0.12	21.05 ± 0.04
2	Methanol (M) (1)	20.42 ± 0.02	14.84 ± 0.05	17.83 ± 0.02	19.61 ± 0.04
3	Ethanol (E) (1)	17.95 ± 0.04	13.18 ± 0.02	13.05 ± 0.03	13.85 ± 0.05
4	E: M (1/2:1/2)	22.32 ± 0.05	15.65 ± 0.06	19.04 ± 0.06	20.26 ± 0.05
5	W: M (1/2:1/2)	25.24 ± 0.05	24.48 ± 0.05	23.37 ± 0.06	23.65 ± 0.05
6	W: E (1/2:1/2)	22.96 ± 0.03	21.78 ± 0.03	21.06 ± 0.04	21.57 ± 0.04
7	W: E: M (1/3:1/3:1/3)	23.52 ± 0.05	23.14 ± 0.06	21.73 ± 0.07	22.09 ± 0.07
8	W: E: M (1/6:4/6:1/6)	19.10 ± 0.04	16.88 ± 0.03	16.05 ± 0.07	17.82 ± 0.03
9	W: E: M (1/6:1/6:4/6)	24.81 ± 0.03	23.68 ± 0.03	23.01 ± 0.05	24.00 ± 0.04
10	W: E: M (4/6:1/6:1/6)	28.59 ± 0.04	25.43 ± 0.03	24.00 ± 0.04	24.99 ± 0.05

The findings shown in Table 1 indicate that combinations of the "water-ethanol-methanol" ternary mixture (4/6: 1/6: 1/6), predominantly composed of water, produced the highest TPC yields. Positive TPC yields were also observed for other ternary and binary mixtures containing methanol or water. Extracts of pure ethanol showed a low TPC content. However, ethanol showed a better capacity to extract more phenolic chemicals when mixed with water or methanol. In actuality, due to their varying polarity and solubility, no solvent is able to extract all types of bioactive chemicals.

Tables 2 and 3 show the effects of different solvent combinations on the extracts' overall antioxidant activity

and antiradical activity. The findings highlight how much the type of extraction solvent and its combination affect the antiradical activity of *L. maroccana* extracts. The combinations with the highest total antioxidant capacity (TAC) were the equal ternary combination, the ternary mixture that was primarily made up of water, and the binary mixture that contained extracts of both methanol and water. The extract from the ternary combination that was primarily made up of methanol (1/6 W:1/6 E:4/6 M) showed more DPPH scavenging activity. The existence of polyphenols, which have potent antioxidant properties in plants, may account for this antioxidant activity (Kale et al., 2010).

Table 2: Simplex axial design and results for mixture tested of TAC

Crude extract	Extract (solvent proportions)	mg Ascorbic Acid / g dry plant			
		Leaves	Flowers	Stems	Roots
1	Water (W) (1)	27.22 ± 0.03	16.51 ± 0.02	20.82 ± 0.04	20.55 ± 0.03
2	Methanol (M) (1)	19.44 ± 0.03	12.21 ± 0.02	12.90 ± 0.03	18.36 ± 0.01
3	Ethanol (E) (1)	16.58 ± 0.03	11.45 ± 0.01	11.17 ± 0.03	17.05 ± 0.03
4	E: M (1/2:1/2)	20.65 ± 0.04	15.88 ± 0.04	17.36 ± 0.03	20.22 ± 0.04
5	W: M (1/2:1/2)	33.49 ± 0.03	22.21 ± 0.03	26.54 ± 0.04	25.55 ± 0.06
6	W: E (1/2:1/2)	28.47 ± 0.06	19.39 ± 0.03	23.10 ± 0.04	22.70 ± 0.05
7	W: E: M (1/3:1/3:1/3)	32.33 ± 0.03	22.49 ± 0.04	26.88 ± 0.06	25.94 ± 0.05
8	W: E: M (1/6:4/6:1/6)	18.84 ± 0.06	17.89 ± 0.04	15.33 ± 0.03	19.64 ± 0.03
9	W: E: M (1/6:1/6:4/6)	25.61 ± 0.03	17.36 ± 0.03	25.15 ± 0.06	19.34 ± 0.04
10	W: E: M (4/6:1/6:1/6)	32.83 ± 0.03	21.26 ± 0.02	27.37 ± 0.05	26.86 ± 0.04

Values are expressed as mean ± standard deviation (SD) (n = 3).

Table 3: Simplex axial design and results for mixture tested of DPPH

Crude extract	C	Extract (solvent proportions)	DPPH %			
			Leaves	Flowers	Stems	Roots
1		Water (W) (1)	77.69 ± 0.17	80.67 ± 0.19	70.06 ± 0.11	83.99 ± 0.17
2		Methanol (M) (1)	85.03 ± 0.19	82.83 ± 0.17	76.46 ± 0.17	90.09 ± 0.28
3		Ethanol (E) (1)	78.25 ± 0.23	76.31 ± 0.22	71.58 ± 0.42	88.49 ± 0.22
4		E: M (1/2:1/2)	80.97 ± 0.34	80.26 ± 0.45	73.89 ± 0.34	86.33 ± 0.17
5		W: M (1/2:1/2)	86.00 ± 0.22	84.06 ± 0.17	78.51 ± 0.30	90.99 ± 0.17
6		W: E (1/2:1/2)	86.48 ± 0.34	81.75 ± 0.17	80.67 ± 0.40	89.12 ± 0.17
7		W: E: M (1/3:1/3:1/3)	83.58 ± 0.22	81.34 ± 0.30	75.31 ± 0.22	87.37 ± 0.30
8		W: E: M (1/6:4/6:1/6)	87.34 ± 0.17	78.21 ± 0.30	73.56 ± 0.23	89.35 ± 0.17
9		W: E: M (1/6:1/6:4/6)	89.68 ± 0.36	85.03 ± 0.30	81.45 ± 0.30	91.77 ± 0.23
10		W: E: M (4/6:1/6:1/6)	86.70 ± 0.22	82.27 ± 0.23	77.39 ± 0.26	86.37 ± 0.30

Values are expressed as mean ± standard deviation (SD) (n = 3).

3.3. Validation of experimental models by statistical analysis

The variance analysis (ANOVA) results in Table 4 highlight the high accuracy and significance of the mixing models used to predict Total Phenolic Content (TPC), Total Antioxidant Capacity (TAC), and DPPH radical scavenging activity across leaves, flowers, stems, and roots. The models for TPC and TAC exhibit exceptional predictive power, with F-values ranging from 231.38 to 9553.93 and p-values all at 0.000000, indicating extremely significant models. The R² and adjusted R² values for these models are consistently at or near 1.000, demonstrating their ability to account for nearly all the variability in the data. For DPPH radical scavenging activity, the models also show strong significance, with F-values between 14.30 and 104.74 and p-values below 0.002023. The R² values for DPPH models range from 0.916 to 0.976, and adjusted R² values from 0.881 to 0.965, indicating a slightly less perfect but still very high fit compared to TPC and TAC models. These results confirm the robustness of the mixing models in accurately predicting the antioxidant properties of different plant parts, validating the effectiveness of the solvent mixtures used and providing a solid foundation for optimizing extraction protocols in both research and industrial applications. A significant Lack of Fit indicates that the model is inadequate to

explain the data, and the model should be modified (do Carmo et al., 2018). In this study, however, the Lack of Fit test could not be computed because the degrees of freedom were zero (df = 0), as no solvent composition was repeated independently in the mixture design. Although each experimental condition was tested in triplicate, the design did not include replicated design points, which are required for Lack of Fit analysis. Despite this limitation, the models exhibited very strong statistical validity. The R² and adjusted R² values for TPC and TAC models were extremely high (approaching 1.000), which could raise concerns about potential model overfitting. To address this, we examined the consistency between R² and adjusted R², the very high F-values (Table 5), and the extremely low p-values (p < 0.000001), all of which confirm the robustness of the models. Additionally, the DPPH models, which showed slightly lower R² values (0.916–0.976), reflect inherent variability in experimental responses and help confirm that overfitting is unlikely. Future studies could include external validation or cross-validation approaches to further confirm the generalizability of these models.

Table 4: Variance analysis (ANOVA) results of the fitted mixing models for total phenolic content (TPC), total antioxidant capacity (TAC), and DPPH scavenging activity in different parts of *Lavandula maroccana*.

R^2 and adjusted R^2 values indicate the goodness of fit. High F-values and low p-values confirm the significance of the models.

	F	p	R^2	R^2 Adj
TPC-L	964.26	0.000000	1.000	0.999
TPC-F	977.84	0.000000	1.000	1.000
TPC-S	231.38	0.000000	1.000	1.000
TPC-R	666.09	0.000000	1.000	1.000
TAC-L	9553.93	0.000000	1.000	1.000
TAC-F	3886.58	0.000000	1.000	1.000
TAC-S	5066.89	0.000000	1.000	1.000
TAC-R	3299.48	0.000000	1.000	1.000
DPPH-L	33.27	0.000092	0.916	0.881
DPPH-F	25.39	0.000181	0.933	0.904
DPPH-S	104.74	0.000000	0.976	0.965
DPPH-R	14.30	0.002023	0.948	0.925

Table 5 presents the statistical parameters using response surface methods for models predicting Total Phenolic Content (TPC), Total Antioxidant Capacity (TAC), and DPPH radical scavenging activity across leaves, flowers, stems, and roots. The models exhibit extremely high significance, evidenced by F-values ranging from 25.58 to 96903.08 and p-values consistently at 0.000000, confirming the robustness of these models. Specifically, TPC models for flowers ($F = 29442.33$), TAC models for leaves ($F = 96903.08$), and DPPH models for stems ($F = 94.18$) demonstrate particularly high predictive power. The Sum of Squares (SS) and Mean Squares (MS) values are also substantial, highlighting the models' capacity to explain the variance in the data effectively. Degrees of freedom (df) are consistently at 6, ensuring a reliable distribution of the data points. These statistical parameters validate the efficacy of the response surface methodology in optimizing solvent mixtures for extracting phenolic compounds and antioxidants, providing a solid foundation for efficient extraction protocols in both laboratory and industrial settings. The strong model performance across various plant parts underscores the versatility and reliability of these optimized solvent systems for maximizing extraction yields and antioxidant activity.

Table 5: Summary of statistical parameters for the fitted response surface models using ANOVA.

SS: sum of squares, df: degrees of freedom, MS: mean square, F: Fisher value, p: significance level. High F-values and extremely low p-values ($p < 0.000001$) indicate the strong statistical significance of the models

Model	SS	df	MS	F	p
TPC-L	98.45	6	16.41	6470.60	0.000000
TPC-F	360.60	6	60.10	29442.33	0.000000
TPC-S	206.37	6	34.40	8098.03	0.000000
TPC-R	175.90	6	29.32	11619.26	0.000000
TAC-L	786.26	6	131.04	96903.08	0.000000
TAC-F	364.15	6	60.69	69609.75	0.000000
TAC-S	706.86	6	117.81	74392.90	0.000000
TAC-R	209.49	6	34.91	20986.23	0.000000
DPPH-L	259.06	6	43.18	25.58	0.000001
DPPH-F	131.42	6	21.90	32.46	0.000000
DPPH-S	295.95	6	49.33	94.18	0.000000
DPPH-R	123.00	6	20.50	42.26	0.000000

3.4. Analysis of mixture optimization by response surface methodology

3.4.1. Total phenolic compound extraction

A potent statistical technique for modeling and optimizing the impact of several variables on a response variable of interest is surface response methodology (SRM). It is frequently employed to maximize the phenolic compound extraction process from natural sources (Weremfo et al., 2023). It is advised to utilize a combination of solvents with varying compositions and polarity for mixture design extraction to maximize the extraction efficiency of phenolic compounds and phytochemicals (Santos Felix et al., 2018). The mixture design diagram for the special cubic model describing the interaction effects and the different proportions of pure solvents and their mixtures compared to TPC values is shown in Fig.2.

Special linear, quadratic, and cubic models were tested. ANOVA assessed the lack of fit for these surfaces. The selected special cubic model is described by the following equations, where x, y, and z represent the proportions of water, methanol, and ethanol, respectively. Subscripts indicate the plant part studied: L (leaves), F (flowers), S (stems), and R (roots).

$$TPC_L = 21.68x + 20.42y + 17.94z + 16.74xy + 12.59xz + 12.54yz - 31.12xyz$$

$$TPC_F = 20.71x + 14.84y + 13.17z + 26.81xy + 19.35xz + 6.54yz - 28.11xyz$$

$$TPC_S = 20.83x + 17.82y + 13.04z + 16.16xy + 16.48xz + 14.41yz - 19.72xyz$$

$$TPC_R = 21.04x + 19.61y + 13.85z + 13.28xy + 16.48xz + 14.09yz - 25.79xyz$$

These equations describe the contribution of each solvent and their interactions to the total phenolic content in different parts of *L. maroccana*.

According to the equations above, TPC extraction was mostly and positively affected by the binary mixture "water-methanol" for flowers. The linear coefficient for water is higher than other pure solvents, indicating a larger positive effect on TPC extraction. Meanwhile, in ternary

interaction “water-methanol-ethanol” mixture had a negative effect. Also, the mixture of the two organic

solvents “ethanol-methanol” resulted in the lowest positive effect.

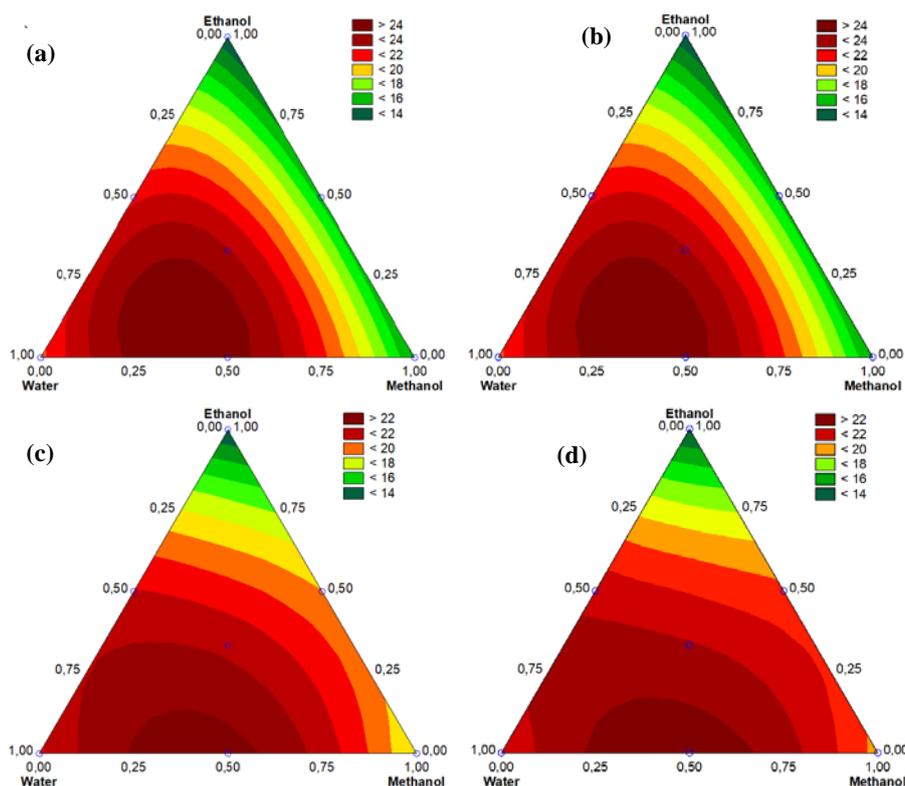


Figure 2: Response surface of the Special Cubic Model predicts TPC based on the proportions of Water, Ethanol, and Methanol of leaves (a), flowers (b), stems (c), and roots (d).

The response surface of the special cubic model (2D) for Total Phenolic Content (TPC) extraction is illustrated in Figure 2. This figure reveals that pure ethanol and methanol are the least effective solvents for extracting TPC from leaves and flowers, while pure ethanol is the least effective for stems and roots. Similar findings have shown that too low or too high ethanol concentration in the extraction solvent is unfavorable for the extraction of total phenolic and (TPC) (Chater, Aazza, Silva, et al., 2024; Liao et al., 2022). In contrast, pure water extracted higher amounts of TPC from all four plant parts. The mixture design shows a dark region corresponding to the highest TPC values, which is located around the area representing equal portions of water and methanol, as well as the region for 75% of water. This indicates that the optimal solvent composition for extracting phenolic compounds involves a mixture of water and methanol in equal proportions or 75% water and 25% methanol, as these combinations achieve the highest extraction efficiency.

3.4.2. Total Antioxidant Capacity (TAC)

The response surface analysis using the Special Cubic Model reveals the optimal solvent proportions for extracting Total Antioxidant Capacity (TAC) from leaves, flowers, stems, and roots. The equations below show that water (x) has the highest individual contribution to TAC across all plant parts, followed by methanol (y) and ethanol (z). However, the significant positive coefficients for the interaction terms, especially the three-way interaction (xyz), indicate that a balanced mixture of these

solvents maximizes TAC extraction. For instance, in leaves, the term 71.52xyz substantially boosts TAC, similar patterns are observed in flowers (36.63xyz), stems (56.36 xyz), and roots (46.62 xyz). This analysis highlights that the synergistic effects of combining water, methanol, and ethanol are crucial for efficient antioxidant extraction, providing valuable guidance for developing optimal extraction protocols in both research and industrial applications.

$$TAC_L = 27.21x + 19.44y + 16.57z + 40.63xy + 26.27xz + 10.55yz + 71.52xyz$$

$$TAC_F = 16.37x + 12.05y + 11.31z + 31.99xy + 22.16xz + 16.79yz + 36.63xyz$$

$$TAC_S = 20.81x + 12.89y + 11.10z + 38.74xy + 28.43xz + 21.29yz + 56.36xyz$$

$$TAC_R = 20.54x + 18.35y + 17.04z + 24.39xy + 15.61xz + 10.06yz + 46.62xyz$$

The response surface analysis of the Special Cubic Model predicting Total Antioxidant Capacity (TAC) based on the proportions of water, ethanol, and methanol for leaves, flowers, stems, and roots (Fig.3) reveals a consistent pattern in solvent efficiency. The highest TAC values are consistently achieved with a balanced mixture of water and methanol, demonstrating the synergistic effect of these two solvents. This combination outperforms the use of pure solvents across all plant parts. Specifically, pure ethanol is the least effective, consistently yielding the

lowest TAC values, likely due to its limited solubility for certain antioxidant compounds. On the other hand, pure water and pure methanol provide intermediate effectiveness but do not match the efficiency of their mixture. The results suggest that an equal mixture of water and methanol significantly enhances the extraction

efficiency of antioxidants, making it the optimal choice for extracting total antioxidant capacity from plant materials. This approach can be particularly beneficial in both laboratory and industrial settings where maximizing antioxidant yield is crucial, providing a clear strategy for solvent selection to achieve superior extraction results.

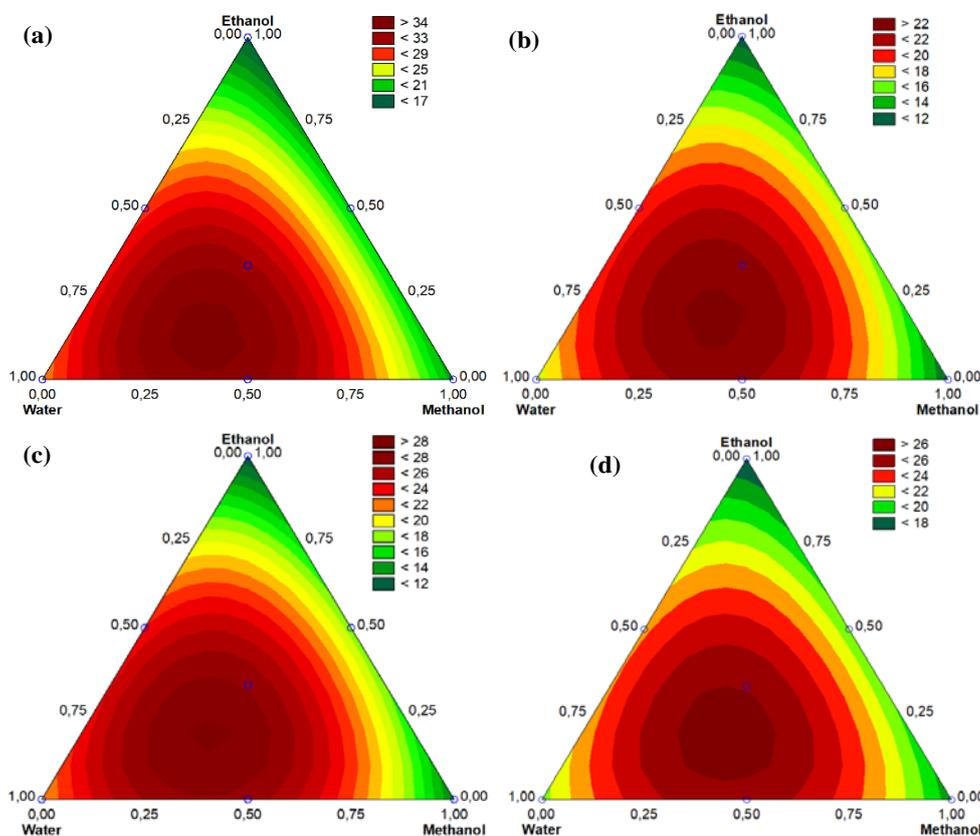


Figure 3: The response surface of the Special Cubic Model predicts TAC based on the proportions of Water, Ethanol, and Methanol of leaves (a), flowers (b), stems (c), and roots (d).

3.4.3. DPPH scavenging activity

One of the two most popular spectrophotometric techniques for determining how well meals, vegetables, and herbs scavenge free radicals is the DPPH test method. Stable radicals are used in this technique, which is highly sensitive and simple to employ. It works especially well for figuring out how antioxidant-active different pure chemicals, fruits, vegetables, and tea extracts (Arnao, 2000).

The response surface analysis using the Special Cubic Model equations below for predicting DPPH radical scavenging activity indicates that water (x), methanol (y), and ethanol (z) each contribute positively to antioxidant activity across leaves, flowers, stems, and roots. However, the highest DPPH activities are generally achieved with water and methanol mixtures, as shown by the equations' coefficients. For example, the highest individual positive effects are seen in roots (DPPH-R) with ethanol contributing significantly, yet the three-way interaction (xyz) negatively impacts the overall DPPH activity, particularly in stems (DPPH-S). The analysis suggests that the combination of water and methanol is optimal for maximizing antioxidant extraction, while ethanol's

inclusion may reduce effectiveness due to competitive or inhibitory interactions. The incorporation of ethanol into the solvent system may compromise extraction efficiency, attributable to its comparatively lower polarity relative to water and methanol. This reduced polarity diminishes the solubility of highly hydrophilic phenolic compounds and may interfere with the hydrogen-bond network responsible for stabilizing polyphenol-solvent interactions, ultimately leading to a less favorable extraction environment for polar bioactive molecules (Lee et al., 2024). Consequently, favoring water and methanol as solvents provides the most efficient extraction strategy for achieving high DPPH radical scavenging activity, offering valuable guidance for both laboratory and industrial applications. Future work should examine bioactivity-guided fractionation, the use of greener solvents (e.g., deep-eutectic systems), and pilot-scale validation to confirm process scalability and sustainability.

$$\begin{aligned} \text{DPPH}_L &= 77.69x + 79.02y + 78.24z + 30.54xy + 34.04xz + 9.31yz - 46.71xyz. \\ \text{DPPH}_F &= 80.67x + 76.83y + 76.31z + 21.23xy + 13.03xz + 14.75yz - 82.35xyz. \\ \text{DPPH}_S &= 70.05x + 70.46y + 71.58z + 33.01xy + 39.4xz + 11.47yz - 147.38xyz. \end{aligned}$$

$$\text{DPPH}_R = 83.98x + 84.00y + 88.49z + 27.79xy + 11.54xz + 0.15yz - 52.41xyz.$$

The response surface analysis of the Special Cubic Model predicting DPPH radical scavenging activity based on the proportions of water, ethanol, and methanol for leaves, flowers, stems, and roots (Fig.4) reveals that the highest antioxidant activities are consistently achieved with a balanced mixture of water and methanol or a higher proportion of methanol. This combination significantly outperforms pure ethanol, which consistently yields the lowest DPPH values. Specifically, leaves and stems show

optimal DPPH activity around a 0.5:0.5 ratio of water to methanol, while flowers and roots achieve higher activity with a methanol-rich mixture. The consistent pattern across all plant parts underscores methanol's crucial role in enhancing antioxidant activity. Therefore, for optimal extraction of antioxidants exhibiting high DPPH radical scavenging activity, a mixture of water and methanol is recommended. This insight is valuable for developing efficient extraction protocols in both laboratory and industrial settings, ensuring maximum yield and efficacy of antioxidants.

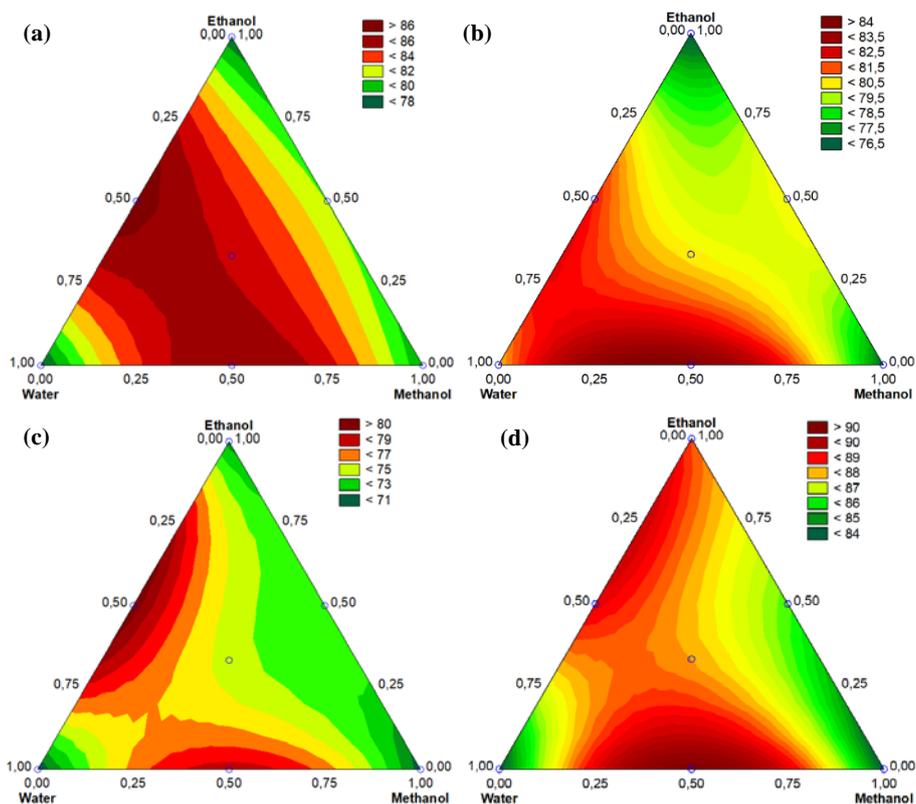


Figure 4: The response surface of the Special Cubic Model predicts DPPH based on the proportions of Water, Ethanol, and Methanol of leaves (a), flowers (b), stems (c), and roots (d).

3.5. Pareto Analysis

Pareto's Graph analysis of standardized effects for Total Phenolic Content (TPC) from leaves, flowers, stems, and roots (Fig.5) demonstrates that water and methanol are the most effective solvents. Water consistently shows the highest standardized effect across all plant parts, with values of 745.7123 for leaves, 794.0229 for flowers, 553.9374 for stems, and 725.824 for roots. Methanol also exhibits substantial positive effects, closely following

water with values of 702.4962 for leaves, 569.0358 for flowers, 473.8442 for stems, and 676.1282 for roots. Ethanol, while contributing positively, has lower standardized effects compared to water and methanol, with effects ranging from 346.7823 to 617.9735 across different plant parts. The interaction effects (AB, AC, BC) are positive but significantly less impactful than the individual solvent effects, and the three-way interaction (ABC) is minimal, with a slight negative effect observed.

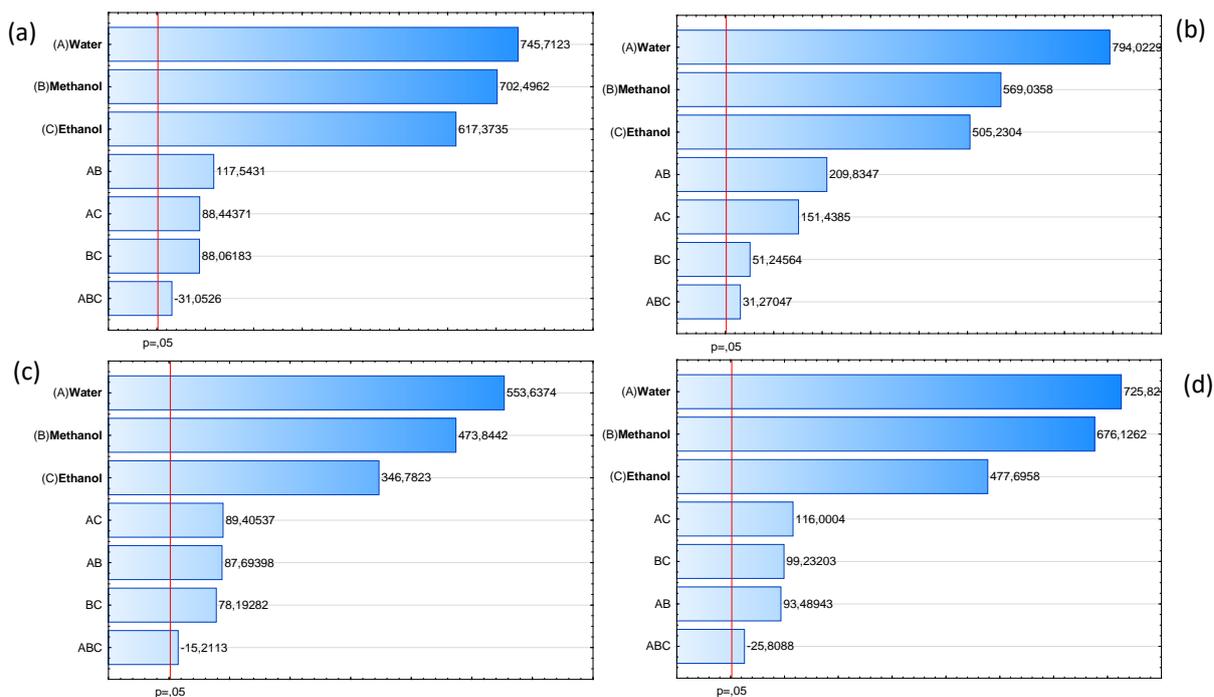


Figure 5: Pareto's Graph analysis of standardized effects for TPC of leaves (a), flowers (b), stems (c), and roots (d).

The Pareto's Graph analysis of standardized effects for Total Antioxidant Capacity (TAC) of leaves, flowers, stems, and roots (Fig.6), based on the solvent proportions of water (A), methanol (B), and ethanol (C), highlights the dominant role of water and methanol in enhancing TAC. For leaves, water has the highest effect (1281.949), followed by methanol (915.6286), and ethanol (780.8605). Interaction effects like AB (390.6511) and AC (252.5784) are notable, but the three-way interaction (ABC) is minimal (97.7442). For flowers, water also shows the highest effect (960.724), with methanol (707.0931) and ethanol (663.5295) contributing significantly. Interaction terms like AB (383.0815) and AC (265.353) are considerable, whereas ABC is lower (62.34244). In stems, water remains the most effective (906.0007), followed by methanol (561.3489) and ethanol (486.1904), with significant interaction terms AB (344.2363) and AC (252.672) but minimal ABC (71.18209). For roots, water (872.521) and methanol (779.4588) lead, followed by ethanol (723.931), with notable interaction effects AB (211.4467) and AC (135.3828), and minimal ABC (57.44113). These findings consistently show that water and methanol are the most effective solvents for TAC extraction across all plant parts, with individual and binary interactions contributing more significantly than three-way interactions, emphasizing the efficacy of using water and methanol mixtures for optimal antioxidant extraction.

water remains the most effective (906.0007), followed by methanol (561.3489) and ethanol (486.1904), with significant interaction terms AB (344.2383) and AC (252.872) but minimal ABC (71.18209). For roots, water (872.521) and methanol (779.4588) lead, followed by ethanol (723.931), with notable interaction effects AB (211.4467) and AC (135.3828), and minimal ABC (57.44113). These findings consistently show that water and methanol are the most effective solvents for TAC extraction across all plant parts, with individual and binary interactions contributing more significantly than three-way interactions, emphasizing the efficacy of using water and methanol mixtures for optimal antioxidant extraction.

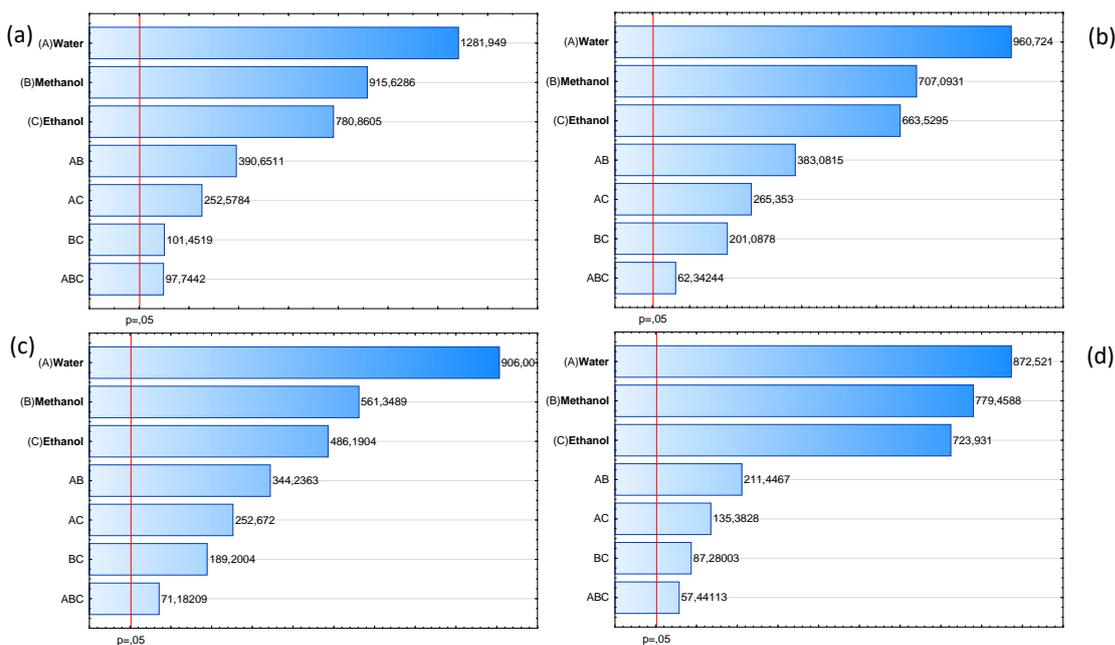


Figure 6: Pareto's Graph analysis of standardized effects for TAC of leaves (a), flowers (b), stems (c), and roots (d).

The Pareto's Graph analysis of standardized effects for DPPH radical scavenging activity of leaves, flowers, stems, and roots (Fig.7) demonstrates that methanol, ethanol, and water each significantly contribute to antioxidant activity, with slight variations in their effectiveness across different plant parts. For leaves, methanol exhibits the highest effect (105.3936), closely followed by ethanol (104.3261) and water (103.5811). For flowers, water shows the highest effect (170.102), followed by methanol (162.0053) and ethanol (160.9137). Stems display the highest effect from ethanol (171.3225), followed by methanol (168.6395) and water (167.6679). Roots show ethanol as the most effective (220.064), with

methanol (209.1283) and water (208.8577) close behind. Interaction effects (AB, AC, BC) are generally minor compared to the individual solvent effects, and the three-way interaction (ABC) has a negligible or slightly negative impact, indicating minimal competitive interactions when all three solvents are combined. These findings suggest that all three solvents are effective for maximizing DPPH activity, with methanol and ethanol being particularly impactful in different contexts. Therefore, combining these solvents in varying proportions can optimize antioxidant extraction, providing valuable insights for developing efficient extraction protocols.

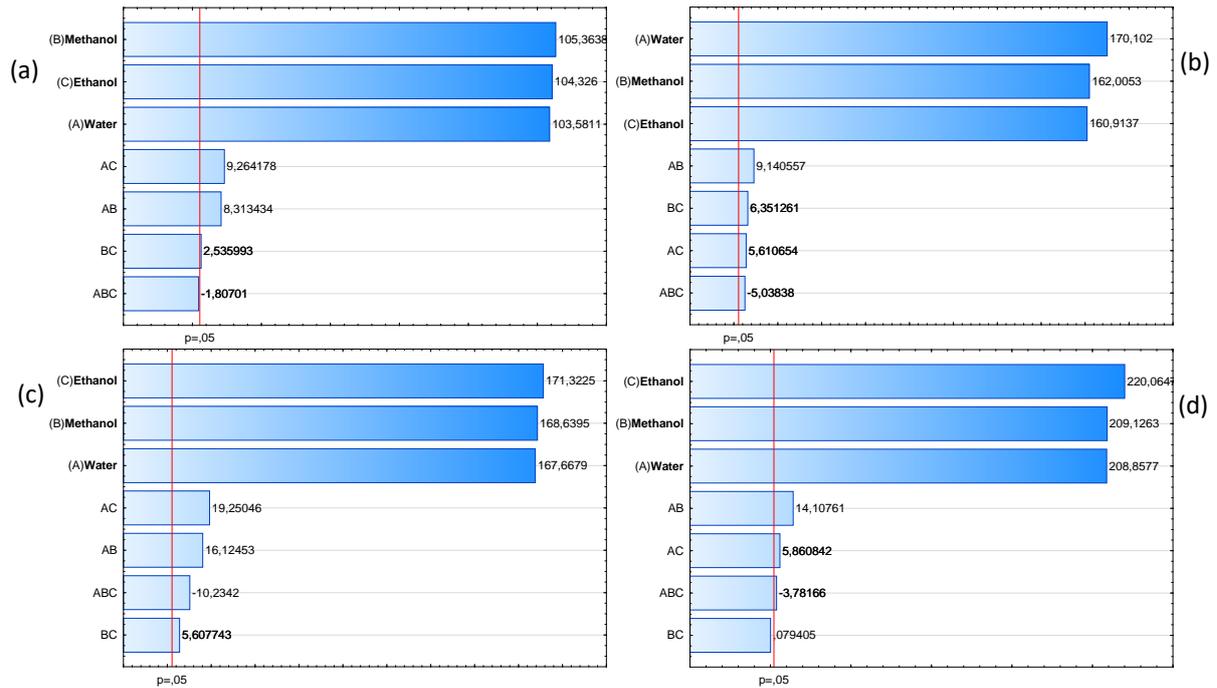


Figure 7: Pareto's Graph analysis of standardized effects for DPPH of leaves (a), flowers (b), stems (c), and roots (d).

3.6. Desirability analysis

The desirability function is a widely employed tool for optimizing a large number of responses based on specific

criteria. The present investigation employed this approach to ascertain optimal conditions utilizing a predetermined desirability value (Vera Candiotti et al., 2014).

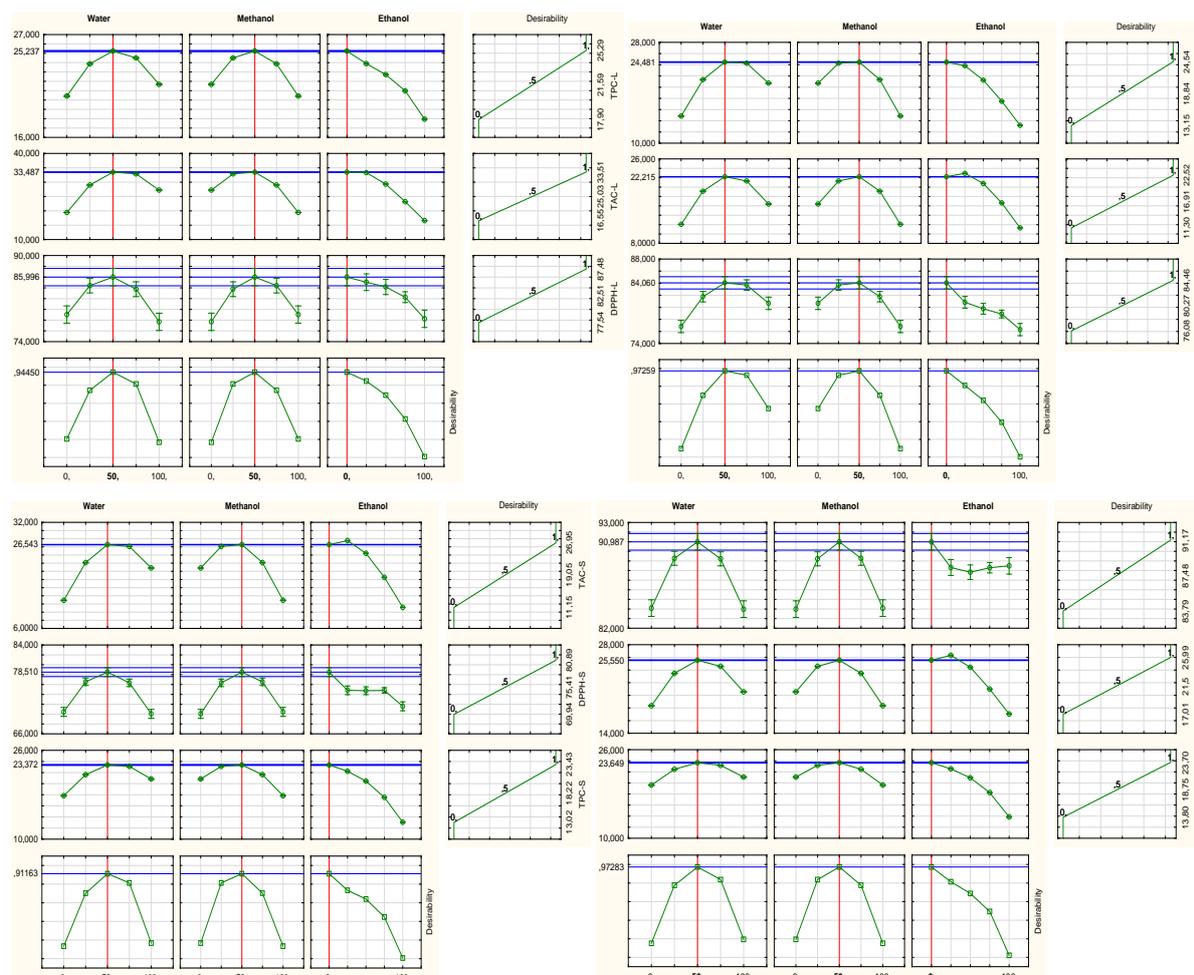


Figure 8: Optimization of Desirability function values of the phenolic compounds and the antioxidant activities obtained as a function solvent mixture.

The desirability profile for optimizing solvent reference mixtures, as illustrated in Figure 8, reveals that the optimal solvent proportions for maximizing extraction efficiency and desired responses—such as total phenolic content (TPC), total antioxidant capacity (TAC), and DPPH scavenging activity—in leaves, flowers, stems, and roots of *L. maroccana* generally peak around a balanced mixture of water and methanol. Each solvent's desirability function peaks near 50% across all plant parts, indicating that an equal proportion of these solvents yields the highest overall desirability. This balanced mixture strategy is consistently observed, with pure or minimal amounts of any single solvent resulting in lower desirability scores. The consistent trend across all graphs underscores the importance of using mixed solvent systems rather than relying on a single solvent. This approach enhances the extraction process, ensuring the highest possible yield and activity of extracted compounds. Thus, for optimal extraction of antioxidants and other valuable compounds from plant materials, employing a balanced mixture of water and methanol is recommended. This strategy is crucial for both laboratory and industrial applications, providing a robust framework for developing efficient and effective extraction protocols.

4. Conclusion

The optimization of polyphenol extraction from *Lavandula maroccana* using a simplex-centroid mixture design has shown that a well-balanced combination of water and methanol is the most efficient solvent mixture for maximizing the total phenolic content, total antioxidant capacity, and DPPH radical scavenging activity. The response surface methodology and ANOVA findings have proven the strong significance and predictive capability of the models, hence proving the effectiveness of the improved solvent systems. These findings establish a strong basis for creating effective extraction techniques in both laboratory and industrial environments, guaranteeing optimal production and effectiveness of antioxidants and other important chemicals from plant materials.

References

- Aazza, S. (2021). Application of Multivariate Optimization for Phenolic Compounds and Antioxidants Extraction from Moroccan Cannabis sativa Waste. *J. Chem.*, 2021, 1–11. <https://doi.org/10.1155/2021/9738656>
- Arnao, M. B. (2000). Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends Food Sci. Technol.*, 11(11), 419–421. [https://doi.org/10.1016/S0924-2244\(01\)00027-9](https://doi.org/10.1016/S0924-2244(01)00027-9)

- Boeing, J. S., Barizão, É. O., e Silva, B. C., Montanher, P. F., de Cinque Almeida, V. & Visentainer, J. V. (2014). Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: application of principal component analysis. *Chem. Cent. J.*, 8(1), 48. <https://doi.org/10.1186/s13065-014-0048-1>
- Chater, O., Aazza, S., Bouzaid, H. & El Ghadraoui, L. (2024). Optimizing polyphenol extraction from *Anacyclus pyrethrum* var. depressus (Ball) Maire roots: a simplex-centroid mixture design approach. *J. Med. Plants.*, 23(90), 31–51. <https://doi.org/10.61186/jmp.23.90.31>
- Chater, O., Aazza, S., Silva, H., Harrach, A. & El Ghadraoui, L. (2024). Multivariate Sonication-Based Extraction Optimization to Improve the Antioxidant and Anti-Inflammatory Properties of *Anacyclus pyrethrum* var. *Pyrethrum* Root Extracts. *Chem. Afr.* <https://doi.org/10.1007/s42250-024-00910-9>
- DiCiaulaa, M. C., Lopesa, G. C., Scarminiob, I. S. & Melloa, J. C. P. de. (2014). Optimization of solvent mixtures for extraction from bark of *schinus terebinthifolius* by a statistical mixture-design technique and development of a uv-vis spectrophotometric method for analysis of total polyphenols in the extract. *Quim. Nova*, 37(1), 158–163.
- do Carmo, M. A. V., Pressete, C. G., Marques, M. J., Granato, D. & Azevedo, L. (2018). Polyphenols as potential antiproliferative agents: scientific trends. *Curr. Opin. Food. Sci.*, 24, 26–35. <https://doi.org/10.1016/j.cofs.2018.10.013>
- Kale, A., Gaikwad, S., Mundhe, K., Deshpande, N. & Salvekar, J. (2010). Quantification of phenolics and flavonoids by spectrophotometer from *Juglans regia*. *Int. J. Pharma Bio Sci.*, 1(3).
- Lee, J.-E., Jayakody, J., Kim, J.-I., Jeong, J.-W., Choi, K.-M., Kim, T.-S., Seo, C., Azimi, I., Hyun, J. & Ryu, B. (2024). The Influence of Solvent Choice on the Extraction of Bioactive Compounds from Asteraceae: A Comparative Review. *Foods*, 13(19), 3151. <https://doi.org/10.3390/foods13193151>
- Liao, J., Xue, H. & Li, J. (2022). Extraction of phenolics and anthocyanins from purple eggplant peels by multi-frequency ultrasound: Effects of different extraction factors and optimization using uniform design. *Ultrason. Sonochem.*, 90, 106174. <https://doi.org/10.1016/j.ultsonch.2022.106174>
- Libbey, L. M. & Walradt, J. P. (1968). 3,5-di-Tert-butyl-4-hydroxytoluene (BHT) as an artifact from diethyl ether. *Lipids*, 3(6), 561–561. <https://doi.org/10.1007/BF02530903>
- Loganayaki, N., Siddhuraju, P. & Manian, S. (2013). Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *J. Food Sci. Technol.*, 50(4), 687–695. <https://doi.org/10.1007/s13197-011-0389-x>
- Ma, T., Wang, Q. & Wu, H. (2010). Optimization of extraction conditions for improving solubility of peanut protein concentrates by response surface methodology. *LWT Food Sci. Technol.*, 43(9), 1450–1455. <https://doi.org/10.1016/j.lwt.2010.03.015>
- Pryor, W. A. (1991). The antioxidant nutrients and disease prevention--what do we know and what do we need to find out? *Am. J. Clin. Nutr.*, 53(1), 391S-393S. <https://doi.org/10.1093/ajcn/53.1.391S>
- Santos Felix, A. C., Novaes, C. G., Pires Rocha, M., Barreto, G. E., do Nascimento, B. B. & Giraldez Alvarez, L. D. (2018). Mixture Design and Doehlert Matrix for the Optimization of the Extraction of Phenolic Compounds from *Spondias mombin* L Apple Bagasse Agroindustrial Residues. *Front. Chem.*, 5. <https://doi.org/10.3389/fchem.2017.00116>
- Shahidi, F. (2000). Antioxidants in food and food antioxidants. *Nahrung/Food*, 44(3), 158–163. [https://doi.org/10.1002/1521-3803\(20000501\)44:3<158::AID-FOOD158>3.0.CO;2-L](https://doi.org/10.1002/1521-3803(20000501)44:3<158::AID-FOOD158>3.0.CO;2-L)
- Velioglu, Y. S., Mazza, G., Gao, L. & Oomah, B. D. (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.*, 46(10), 4113–4117. <https://doi.org/10.1021/jf9801973>
- Vera Candiotti, L., De Zan, M. M., Cámara, M. S. & Goicoechea, H. C. (2014). Experimental design and multiple response optimization. Using the desirability function in analytical methods development. *Talanta*, 124, 123–138. <https://doi.org/10.1016/j.talanta.2014.01.034>
- Wang, B., Chen, P., Zhang, H., Chen, Y. & Chen, L. (2025). Optimization of polyphenols extraction by deep eutectic solvent from broccoli stem and characterization of their composition and antioxidative effects. *Sci. Rep.*, 15(1), 16066. <https://doi.org/10.1038/s41598-025-00632-z>
- Weremfo, A., Abassah-Oppong, S., Adulley, F., Dabie, K. & Seidu-Larry, S. (2023). Response surface methodology as a tool to optimize the extraction of bioactive compounds from plant sources. *J. Sci. Food Agric.*, 103(1), 26–36. <https://doi.org/10.1002/jsfa.12121>
- Zgórka, G., Adamska-Szewczyk, A. & Baj, T. (2023). Response Surface Methodology in Optimising the Extraction of Polyphenolic Antioxidants from Flower Buds of *Magnolia × soulangeana* Soul.-Bod. var. 'Lennei' and Their Detailed Qualitative and Quantitative Profiling. *Molecules*, 28(17), 6335. <https://doi.org/10.3390/molecules28176335>
- Zineb El Jabboury, Smail Aazza, Driss Ousaaid, Oumaima Chater, Wafae Squalli, Ouafae El Ghadraoui, Meryem Benjelloun, L. E. G. (2022). Optimisation of Total Phenolic Compound Extraction and Antioxidant Activity from Dried Inflorescence of *Ammi Visnaga* Using Mixture Design and Triangular Surfaces. *Jordan J. Biol. Sci.*, 15(04), 679–687. <https://doi.org/10.54319/jjbs/150417>