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# Optimization of polyphenol and flavonoid extraction from *Lavandula multifida* using a D-optimal experimental design: Phytochemical screening and yield evaluation

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## ABSTRACT

The research focuses on the effect of solvent polarity on the quantity extracted, chemical composition, and antioxidant capacity of *Lavandula multifida* extracts. The extraction yields were 10.63 for hydroethanolic, 9.86 for aqueous, 7.90 for hydromethanolic and 7.00 for hydroacetonic, confirming the determining effect of solvent polarity. The levels of total polyphenols ranged from 26.3 mg gallic acid equivalent per gram of dry to 12.08 mg EAG/g MS, while total flavonoids were the highest in the aqueous extract (25.35 mg EQ/g MS). Phytochemical screening revealed the presence of quinones, coumarins, flavonoids, alkaloids and tannins, with saponins detected only in the aqueous extract.2,2-diphenyl-1-picrylhydrazyl antioxidant activity showed half maximal inhibitory concentration ranging from 145.44 µg/mL (aqueous) to 351.30 µg/mL (hydromethanolic), and the ferric reducing antioxidant powerassay revealed epicatchin equivalents of 682.62 µg/mL (hydroethanolic) to 950.56 µg/mL (hydroacetonic). Optimization by D-optimal and response surface methodology led to the definition of ideal conditions for increasing the concentration of polyphenols and flavonoids during extraction. These data highlight the pharmacological and antioxidant properties of *L. multifida* and provide a reliable methodology for the targeted extraction of bioactive compounds.

**Keywords:** *Lavandula multifida,* secondary metabolites, polyphenols, flavonoids, antioxidant activity, D-optimal, response surface methodology.

## INTRODUCTION

Medicinal plants have long been an essential source of bioactive molecules used in the pharmaceutical, food and cosmetic sectors (Tlemcani et al., 2025; Chriqui et al., 2024). These natural substances, particularly secondary metabolites such as polyphenols, flavonoids, tannins, and alkaloids, play a key role in defending against diseases associated with oxidative stress and inflammation (Rudrapal et al., 2022; El-Assri et al., 2023). Among these substances, polyphenols and flavonoids stand out for their potential to neutralize free radicals, regulate cell signaling pathways, and stimulate internal antioxidant defenses, thereby contributing to protection against various degenerative diseases (Idrissi et al., 2025; Hmamou et al., 2022).

The variation in the chemical composition and levels of secondary compounds in plants depends on several parameters, such as the part of the plant used, environmental conditions, the type of plant species involved, and, in particular, the extraction technique used (Chamali et al., 2023; Majda et al., 2020). The extraction yield is mainly related to the type of solvent, its polarity and operational parameters such as the solvent/powder ratio as well as the contact time (Zirari et al., 2024). The selection of the solvent therefore represents an important phase, which has a direct effect on the extraction of phenolic compounds and their biological characteristics (Lim et al., 2024). Hydroalcoholic solvents, particularly water-ethanol mixtures, are generally preferred for their ability to efficiently extract polar and semi-polar compounds, while respecting the principles of ecological and sustainable extraction (Naoum et al., 2024).

In this context, optimization strategies such as the response surface methodology (RSM) have proven to be effective tools for improving extraction yield and bioactive compounds content while optimizing the number of experiments needed (Hmamou et al., 2025). Indeed, the experimental design D-Optimal is a relevant strategy to identify the most efficient experimental parameters, allowing reliable modeling while minimizing the number of expiremental tests (Nouioura et al., 2023).

*Lavandula multifida* is a Lamiaceae, belongs to medicinal plants that have been widely studied for its various pharmacological properties, mainly antioxidant, anti-inflammatory and analgesic effects (Allouani et al., 2025; Zirari et al., 2025). This plant is rich in secondary metabolites, which gives it a special therapeutic value in protecting against oxidative stress and helps in the regulation of the respiratory system (Al-Mijalli et al., 2022). On the other hand, the chemical diversity and antioxidant properties of *L. multifida* extracts differ considerably depending on the type of solvent and extraction conditions used, hence the interest in discovering optimized methods to increase the extraction of these metabolites of interest (Lahkimi et al., 2020).

It should be noted that, although initial phytochemical studies have been conducted on *L. multifida*, no research to date has used an experimental D-optimal method to simultaneously refine the extraction parameters for phenolic compounds and predictively model their interactions. This lack of a systematic integrated approach limits the ability to replicate results and hinders the establishment of optimally scientifically validated conditions. There therefore remains a deficit in the use of sophisticated statistical tools to ensure rational optimization and rigorous numerical analysis of the consequences of experimental variables.

For the first time, this study proposes a systematic optimization of the conditions for extracting polyphenols and flavonoids from *L. multifida*, using a D-optimal experimental design. This approach not only offers the possibility of reducing the number of trials required, but also of creating predictive mathematical models that illustrate the individual and interactive impact of the parameters studied. This project is characterized by the implementation of a robust statistical approach, designed to maximize extraction efficiency while ensuring the credibility and repeatability of the results.

This study therefore contributes to advancing knowledge by proposing a scientifically validated and improved method for extracting phenolic compounds, paving the way for more efficient and sustainable use of local plant resources.

## MATERIAL AND METHODES

### Plant material

The aerial part was selected as plant material, harvested in March 2025 in the Fez-Meknes region, located at geographical coordinates 34.074640, -4.989450. The plant has been identified by botanist Eloutassi Nouredinne, a professor at the Regional Center for Education and Training in Fez. A control specimen was at the faculty herbarium under reference number FMF/0124/01/Lm. The collected plant has been carefully cleaned by water in order to ensure purity, the plant material was dried in the shade at ambient temperature, in a well-ventilated room, until obtaining a constant weight. In order to preserve the integrity of heat sensitive secondary metabolites, no high temperature drying was applied. Once dry, the samples were pulverized using an electric grinder until a homogeneous powder was obtained, sieved to a small particle size. The powder obtained was stored in amber glass bottles, hermetically sealed, until its use for various experimental analyses.

### Extraction by maceration

The phenolic compounds and flavonoid were obtained by independent maceration of 30 g of

dried vegetable powder in each of the following four solvents: hydroethanolic, hydromethanolic, aqueous, hydroacetonic (70%; 30%) each maceration was conducted for 48 hours at room temperature, under agitation to promote the diffusion of metabolites in the solvent. The solutions obtained were filtered and the concentrated extracts were kept at a temperature of 4 °C. The yield was determined after extraction using the formula proposed by (Falleh et al., 2008).

## Yield calculation

The yield obtained was determined according to the following equation:

$$Yield(\%) = (\frac{Mex}{Mth}) \times 100 \quad (1)$$

where: Ou $M_{ex}$ corresponds to the amount of dry extract obtained (g) and $M_{th}$ to the initial mass of the dry plant material used.

This function expresses the yield in percent, translate the fraction of extractable material actually recovered compared to the theoretical quantity of the dry plant employed.

## Phytochemical screening

The extracts were examined through initial phytochemical screening, based on several qualitative tests designed to reveal the presence of different secondary metabolites. the screening is established on precipitation and/or staining reactions allowing the identification of the main classes of chemical compounds. Several reagents were used for this purpose, in accordance with the protocols presented in the previous literature (Tlemcani et al., 2025).

## Phytochemical analysis

### *Determination of total polyphenols*

The total polyphenol content of the studied extracts was identified by the Folin ciocalteau method. This reagent, recognized by its yellow color, is formed of a mixed phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolydic acid ($H_3PWo_{12}O_{40}$). In the presence of phenolic compounds, it undergoes a reduction leading to the formation of oxidized complexes of blue color, notably tugstene blue ($W_8O_{23}$) and molybdenum blue ($Mo_8O_3$). The intensity of this staining is directly related to the level of phenolic compounds present in the sample.

Practically speaking, 0.5 ml of extract is reacted with 2.5 ml of Folin-Ciocalteu reagent previously diluted to 1/10 in test tubes. After stirring 4 ml of sodium carbonate ($Na_2CO_3$) at 7.5% to create an alkaline medium favorable for the redox reaction. The mixture is incubated in a 45 °C water bath for a period of 30 minutes. The blue coloration produced formed is quantified by reading the absorbance at 765 nm using a UV-visible spectrophotometer.

### *Determination of total flavonoids*

The quantification of flavonoids was conducted out according to the colorimetric method of Dewanto, using aluminum trichloride ($AlCl_3$) and sodium hydroxide (NaOH) as main reagents. Aluminum trichloride forms a yellow complex with flavonoids, while the addition of sodium hydroxide leads to the formation of a rosé complex, whose absorbance is measured in the visible range at 430 nm. In test tubes, 1ml of each extract with 1 ml of an $AlCl_3$ solution (2% in methanol). Methanol is used instead of the extract to prepare the blanks. The absorbance is read at 430 nm after 10 min of reaction (Lamaison et Carnet 1990).

## Antioxidant activities

### *DPPH free radical scavenging activity*

To study the antioxidant activity of the studied extract, the test 2,2-diphenyl-1-picrylhydrazil (DPPH) was used according to the method established by (Sahin et al., 2004). It involves mixing 2 ml of a methanolic solution containing 0.0023% DPPH (60 µM DPPH in methanol) with 50 µl of each compound at various concentrations. The mixture is shaken well and then allowed to sit in the dark at room temperature for 20 minutes. The absorbance of the mixture is measured at 517 nm. This same method was applied to a control sample, which is DPPH without extracts. Quercetin, at concentrations ranging from 0.38 to 6.09 mg/mL, served as the standard antioxidant. The free radical scavenging capacity of the extracts was calculated as follows:

$$\%I = \frac{A_b - A_s}{A_b} \times 100 \quad (2)$$

where: $\%I$ – percentage of DPPH inhibition (%), $A_b$ – absorbance of the blank, $A_s$ – absorbance of the sample.

*Ferric-reducing antioxidant power test (FRAP)*

The reducing activity of the studied compounds was measured by spectrophotometry according to the Oyaizu (1986) method. Different concentrations of extracts and standard (0.2 ml) were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide ($K_3Fe(CN)_6$). After incubating for 20 minutes at 50 °C, we added 2.5 ml of 10% (w/v) trichloroacetic acid to the mixture. We then took about 2.5 ml of each concentration and added 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride ($FeCl_3$). The intensity of the blue-green color was measured at 700 nm. Catechin (0.65 to 21.39 µg/mL) served as a positive control.

## D-optimal design

In this study, we used a D-optimal experimental design to improve the extraction conditions of phenolic and flavonoid compounds from the plant we examined. This plan, returning to the RSM, to generate 16 experimental trials with the aim of identifying the optimal extraction parameters. The optimization matrix contains two factors (Table 1): (A) maceration time and (B) solvent/powder ratio. This method allowed us to evaluate the interactions between these two factors and their effect on the results obtained. We used the RSM method to find the best combination of these two parameters in order to have the highest levels of total polyphenols and total flavonoids. The second-degree polynomial equations of the D-optimal model helped us to model linear variables, quadratics and interactions between them. This allowed us to determine the optimal conditions. The equations are important to accurately represent the response according to the factors we studied. We identified how the responses varied with the factors using the second-order polynomial model, following this equation:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 \quad (3)$$

where: $Y$ – represents the predicted response (extraction yield, total polyphenols or total flavonoids content), $\beta_0$ is the model constant (ordinate to origin); $\beta_1$ and $\beta_2$ are the coefficients of linear effects of factors $A$ and $B$; $\beta_{12}$ corresponds to the coefficient of the interaction effect between the two factors; while $\beta_{11}$ and $\beta_{22}$ respectively represent the coefficients of the quadratic effects $A$ and $B$.

## Extraction conditions provided by statistical optimization

The extraction conditions were optimized using a D-optimal design of experiment, belonging

**Table 1.** Experimental results and values obtained for total polyphenols and total flavonoids

| Run | Time (h) | Ratio | Dosage of polyphenols (mg GAE/g MS) | Dosage of flavonoids (mg QE/g MS) |
|---|---|---|---|---|
| 1 | 48 | 1.25 | 43.75 | 14.89 |
| 2 | 24 | 1.15 | 43.32 | 15.67 |
| 3 | 5 | 1.1 | 32.16 | 6.51 |
| 4 | 48 | 1.1 | 51.52 | 19.15 |
| 5 | 10 | 1.25 | 28.46 | 6.33 |
| 6 | 5 | 1.15 | 30.48 | 6.44 |
| 7 | 48 | 1.15 | 50.82 | 19.54 |
| 8 | 5 | 1.1 | 32.27 | 6.2 |
| 9 | 24 | 1.25 | 36.94 | 11.17 |
| 10 | 48 | 1.25 | 43.79 | 15.56 |
| 11 | 48 | 1.25 | 42.78 | 14.80 |
| 12 | 48 | 1.1 | 49.53 | 20.22 |
| 13 | 24 | 1.2 | 41.43 | 13.63 |
| 14 | 5 | 1.2 | 26.51 | 4.92 |
| 15 | 10 | 1.25 | 26.97 | 5.08 |
| 16 | 24 | 1.1 | 43.677 | 17.07 |

to the response surface methodology. Two main factors were studied: the maceration time and the solvent/powder ratio. Minimum and maximum values have been defined from the preliminary trials and literature (Mansour et al., 2023).

The optimization software generated different experimental combinations to evaluate the influence and interaction of these parameters on yield as well as on total polyphenol and flavonoid contents. This statistical approach was used to determine the optimal extraction parameters with a minimum number of trials, while ensuring the reliability and consistency of the results in Table 1.

### Statistical analysis

The figures and tables of statistical optimization and response surfaces were created using Design-Expert software version 7.0 (Stat-Ease Inc., Minneapolis, MN, USA). The response results were evaluated using an analysis of variance (ANOVA) to determine the significance of the model's effects. The reliability of the experimental model was confirmed with a confidence level below 5% ($p<0.05$). Finally, the connection between the predicted results and the experimental results by a regression equation was analyzed to prove the validity of the adjustment (Elamraoui, et al., 2025a, 2025b).

## RESULTS

### Extraction yield

The extraction yield is the ratio between the quantity of material extracted by the solvent and the amount of plant material used. This yield changes depending on several factors, including the type of solvent, extraction time, and chemical composition of the sample. The results of the hydroethanolic, aqueous, hydroacetonic and hydromethanolic extracts from the aerial parts of *L. multifida* are presented in Table 2.

The results of the yield of *L. multifida* extracts show that the hydroethanolic extract has the highest yield, at 10.63%. Then the aqueous extract follows with 9.86%, followed by the hydromethanolic extract has a yield of 7.9% and 7% for the hydroacetonic extract. several factors influence the extraction yield, including the duration of the extraction cycle and the solubilization capacity of bioactive compounds.

### Phytochemical screening

the characterization of dominant secondary metabolites in plants allowed us to understand their potential pharmacological activities. we carried out the phytochemical screening on the hydroethanolic, aqueous, hydromethanolic and hydroacetonic extracts of the aerial parts of *L.multifida*. These tests consist in evaluating precipitation and color reactions whose intensity is proportional to the quantity of the studied compound. The results of phytochemical screening are presented in Table 3.

Table 3 displays the phytochemical composition of *L.multifida*, highlighting the diversity and variety of its secondary metabolites. The analyses carried out revealed the permanent presence of quinones, coumarins, terpenes, tannins and free alkaloids in all the analyzed extracts, proving the phytochemical richness of the species.

Indeed, saponins were only determined in the aqueous extract, which could be explained by their strong attachment to aqueous environments. This selective diffusion in terms of solvent polarity highlights the importance of solvent choice in the extraction of secondary metabolites.

The presence of these bioactive substances implies a wide range of potential pharmacological properties, including antioxidant, antimicrobial and anti-inflammatory properties. This diverse phytochemical profile highlights the therapeutic importance of *L.multifida* and proves its ancestral use in traditional medicine.

**Table 2.** Yield of various extracts of *Lavandula multifida*

| Plant | Extract | Mass of the vegetable powder used (g) | Mass of the obtained dry extract (g) | Yield (%) |
|---|---|---|---|---|
| *Lavandula multifida* | Aqueous | 30 | 2.958 | 9.86% |
| | Hydroethanolic | 30 | 3.188 | 10.63% |
| | Hydromethanolic | 30 | 2.371 | 7.9% |
| | Hydroacetonics | 30 | 2.101 | 7% |

**Table 3.** Secondary metabolites present in the aerial parts of *Lavandula multifida* during phytochemical screening studies

| Compounds | Extraction solvent | Secondary metabolites |
|---|---|---|
| Coumarin | Aqueous extract | ++ |
| | Hydroethanolic extract | ++ |
| | Hydromethanolic extract | ++ |
| | Hydroacetonic extract | ++ |
| Free quinones | Aqueous extract | ++ |
| | Hydroethanolic extract | ++ |
| | Hydromethanolic extract | ++ |
| | Hydroacetonic extract | ++ |
| Tannins | Aqueous extract | ++ |
| | Hydroethanolic extract | ++ |
| | Hydromethanolic extract | ++ |
| | Hydroacetonic extract | ++ |
| Terpenes | Aqueous extract | ++ |
| | Hydroethanolic extract | ++ |
| | Hydromethanolic extract | ++ |
| | Hydroacetonic extract | ++ |
| Saponins | Aqueous extract | ++ |
| | Hydroethanolic extract | -- |
| | Hydromethanolic extract | -- |
| | Hydroacetonic extract | -- |
| Alkaloids | Aqueous extract | ++ |
| | Hydroethanolic extract | ++ |
| | Hydromethanolic extract | ++ |
| | Hydroacetonic extract | ++ |

**Note:** (++) detectable; (--) not detectable; (+-) moderately detectable.

## Contents of polyphenols and flavonoids

### *Determination of total polyphenols*

Polyphenols are known for their antioxidant effects and health benefits. This study measures the amount of polyphenols contained in various extracts and their potential as natural sources of antioxidants. To this end, total polyphenols were determined using a calibration curve created with gallic acid as a reference. This was done at different concentrations and tested under the same conditions as the extracts under study. The results are expressed in (mg EAG/g MS).

Among the studied extracts, the highest total polyphenol content, with 26.3 mg EAG/g MS was for the hydroethanolic extract. This shows that it is rich in soluble phenolic substances in ethanol-water solutions. in second place is the aqueous extract with 19.68 mg EAG/g MS, followed by the hydromethanolic extract with 18.3 mg EAG/g MS. the lowest total polyphenol content was for hydroacetone extract with 12.08 mg EAG/g MS. These results indicate to what extent the choice of extraction solvent influences the yield of phenolic compounds.

### *Dosage of flavonoids*

the aluminum colorimetric method was used to determine total flavonoids, using quercetin as a reference standard. A calibration curve was established from different quercetin concentrations, subjected to the similar experimental conditions as the extracts. milligrams of quercetin equivalents per gram of dry matter (mg EQ/g MS) is the frequency with which the results are expressed.

The flavonoid contents in the aqueous extract showed the highest total polyphenol content, reaching 25.35 mgEQ/g MS, reflecting a high concentration of water-soluble phenolic compounds. The hydroethanolic extract also showed a significant content, of the order of 21.24 mgEQ/g MS, while the hydroacetonic extract at a concentration of 10.28 mgEQ/g MS. The hydromethanolic extract showed the lowest content of 4.15 mgEQ/g MS. Table 4 shows the total polyphenol and flavonoid content of extracts obtained using different solvents from *L. multifida.*

## Antioxidant activities

### *DPPH free radical scavenging activity*

To evaluate the antioxidant activity, we based it on the percentage of DPPH free radical scavenging. In the absence of an absolute measure of antioxidant capacity, results were expressed by reference. The corresponding values are presented in Table 5.

Quercetin, a flavonoid recognized for its antioxidant power, had an $IC_{50}$ value of 5.49 µg/mL, confirming the sensitivity and reliability of the test used. In comparison, extracts of *L.multifida* showed higher $IC_{50}$ values, while retaining significant DPPH radical scavenging activity, attributable to the combined and synergistic action of their phenolic compounds.

For the antioxidant activity in *L.multifida,* the aqueous extract showed the highest activity with an IC50 of 145.44 µg/mL, show significant effectiveness in the fight against free radicals. This power could be attributed to the high content of

**Table 4**. Results of the total polyphenol content of *Lavandula multifida*

| Plant | | Extraction solvent | Total polyphenol content (mg EAG/g MS) |
|---|---|---|---|
| *Lavandula multifida* | Total polyphenol content | Aqueous extract | 19.68$^{b}$± 0.3 |
| | | Hydroethanolic extract | 26.3$^{a}$± 0.17 |
| | | Hydromethanolic extract | 18,3$^{c}$± 0,33 |
| | | Hydroacetonic extract | 12.08$^{d}$± 0.08 |
| | Total flavonoids content | Aqueous extract | 25.35$^{a}$±0.07 |
| | | Hydroethanolic extract | 21.24$^{b}$±0.08 |
| | | Hydromethanolic extract | 4.15$^{d}$±009 |
| | | Hydroacetonic extract | 10.28$^{c}$±0.04 |

water-soluble phenolic substances in the extract, which can act at the same time. The hydroethanolic and hydroacetonic extracts showed average antioxidant activity, with $IC_{50}$ values of 213.82 µg/mL and 276.32 µg/mL, respectively, however the hydromethanolic extract was the least active (351.30 µg/mL). This variation clearly reflects the influence of the solvent on the quantity and nature of the secondary metabolites extracted, especially flavonoids and polyphenols which are responsible for antioxidant activity.

*FRAP test*

The results, (Table 5) used as an antioxidant reference, had an $EC_{50}$ value of 13.90 µg/mL, confirming the effectiveness of the FRAP test. In contrast, *L. multifida* extracts showed higher $EC_{50}$ values while exhibiting significant reducing power, thanks to the variety and interaction of the phenolic compounds they contain.

In comparison, the hydroethanolic and aqueous extracts of *L. multifida* exhibited more moderate antioxidant activities with values successively at 682.62 µg/mL, 712.89 µg/mL. The hydromethanolic extract showed an EC50 of 913 µg/mL, while the hydroacetonic extract showed a value of 950.56 µg/mL, reflecting less moderate antioxidant activity compared to the tested extracts.

## Optimization by D-optimal

### *Modeling and optimization of total polyphenol content*

*Model fitting and analysis of variance (ANOVA).*

Analysis of variance (ANOVA) results for the quadratic model adjusted for polyphenol content are presented in Table 6. The model was highly significant, with F = 279.98 and $p < 0.0001$, indicating that it adequately represents the experimental data (Moussaoui et al., 2024). Factors A (extraction time) and B (solvent/powder ratio), as well as their quadratic terms ($\mathbf{A^2}$ and $\mathbf{B^2}$), exerted significant effects on polyphenol content ($p < 0.01$). In contrast, the AB interaction term was not significant ($p = 0.2467$), reflecting a limited combined influence between time and ratio (El Bourachdi et al., 2025). The lack of a significant adjustment ($p = 0.4715$) shows that the model fits experimental data well.

The statistical parameters related to the model's goodness of fit are summarized in Table 6. The coefficient of determination ($R^2 = 0.9929$) and the adjusted $R^2$ (0.9894) indicate an excellent correlation between the experimental values and those predicted. The predicted $R^2$ (0.9804) is in good agreement with the adjusted $R^2$ (range < 0.2), confirming the predictive reliability of the model (Elamraoui, Asdiou, Boumya, et al., 2025).

**Table 5.** Antioxidant activity of extracts of *Lavandula multifida* using DPPH and FRAP

| Extract and standards | DPPH $IC_{50}$ (µg/ml) | FRAP $EC_{50}$ (µg/ml) |
|---|---|---|
| Aqueous extract | 145.44$^{a}$±2.45 | 712.89$^{b}$±0.66 |
| Hydroethanolic extract | 213.82$^{b}$±4.04 | 682.62$^{a}$±4.40 |
| Hydromethanolic extract | 351.30$^{d}$±4.36 | 913.74$^{c}$±0.49 |
| Hydroacetonic extract | 276.32$^{c}$±2.91 | 950.56$^{d}$±3.48 |
| Quercetin | 5.49 ±0.02 | _ |
| Catechin | _ | 13.90±0.03 |

**Note:** a, b, c and d – values with a significant difference.

**Table 6.** Analysis of variance (ANOVA) for the quadratic model fitted to polyphenol content

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 1086.53 | 5 | 217.31 | 279.98 | < 0.0001 | significant |
| A-Time | 991.57 | 1 | 991.57 | 1277.54 | < 0.0001 | |
| B-Ratio | 165.35 | 1 | 165.35 | 213.03 | < 0.0001 | |
| AB | 1.18 | 1 | 1.18 | 1.51 | 0.2467 | |
| $A^2$ | 49.91 | 1 | 49.91 | 64.30 | < 0.0001 | |
| $B^2$ | 8.30 | 1 | 8.30 | 10.69 | 0.0084 | |
| Residual | 7.76 | 10 | 0.7762 | | | |
| Lack of Fit | 4.01 | 5 | 0.8022 | 1.07 | 0.4715 | not significant |
| Pure Error | 3.75 | 5 | 0.7501 | | | |
| Cor Total | 1094.30 | 15 | | | | |
| $R^2$ | 0.9929 | | | | | |
| Adjusted $R^2$ | 0.9894 | | | | | |
| Predicted $R^2$ | 0.9804 | | | | | |
| Adeq Precision | 43.0155 | | | | | |

In addition, the low coefficient of variation (C.V. = 2.26%) demonstrates high experimental reliability. The adequate precision ratio (Adeq. Precision = 43.02) is much higher than the suggested minimum of 4, indicating a high signal-to-noise ratio. These results prove that the model is statistically sound and suitable for optimizing extraction parameters.

### *Quadratic model regression equation*

The regression equation in terms of coded variables, describing the polyphenol content, is given as follows:

$$TPT\ (mg\ GAE/g\ MS) = 43.61 + 9.82\ A - 3.89\ B + 0.39\ AB - 4.47\ A^2 - 1.81\ B^2 \quad (4)$$

where: $A$ – time; $B$ – ratio (solvent/powder); $AB$ – interaction between time and ratio; $A^2$, $B^2$ – curvature (quadratic effects).

The positive coefficient associated with factor A shows that the polyphenol content increases with extraction time up to a certain optimal threshold, while the negative coefficients of quadratic terms ($A^2$ and $B^2$) indicate that an excess of time or solvent can lead to a decrease in yield, probably due to the degradation of compounds or excessive dilution of the extraction medium.

### *Validation of the model using diagnostic graphs*

The graph of predicted values compared to the experimental values in Figure 1 shows that the experimental points are close to the diagonal, which reflects a strong agreement between the observed and calculated values. This diffusion shows the reliability of the model to predict the polyphenol content in the studied parameters. In addition, Figure 2 shows a random distribution around zero, indicating satisfactory normality and homogeneity of errors (Shamraeva et al., 2022).

### *Analysis of the response surface*

The response surface is presented in Figure 3 where we model the polyphenol content as a function of the extraction time (A) and (B) the solvent/powder ratio. The sub-figure (a) illustrates the response surface in the form of a curve (plan view), which makes it possible to determine the optimal extraction area at the center of the experimental plan, where the polyphenol content values are the most important. The sub-figure (b) shows the same surface in three dimensions, highlighting the cumulative effect of extraction time (A) and solvent/powder ratio (B) (El Bourachdi et al., 2026). The increase in the solvent/powder ratio optimizes the extraction efficiency up to an optimal value close to 1:20, beyond which a significant dilution leads to a decrease in yield. In addition, extend the extraction time exerted a gradual increase in polyphenol content to about 40–45 hours, followed by a slight decrease, this could be due to the partial degradation of certain phenolic compounds. The joint analysis of sub-figures (a) and (b) shows that the quadratic model accurately represents the correlation between the two independent variables and the response,

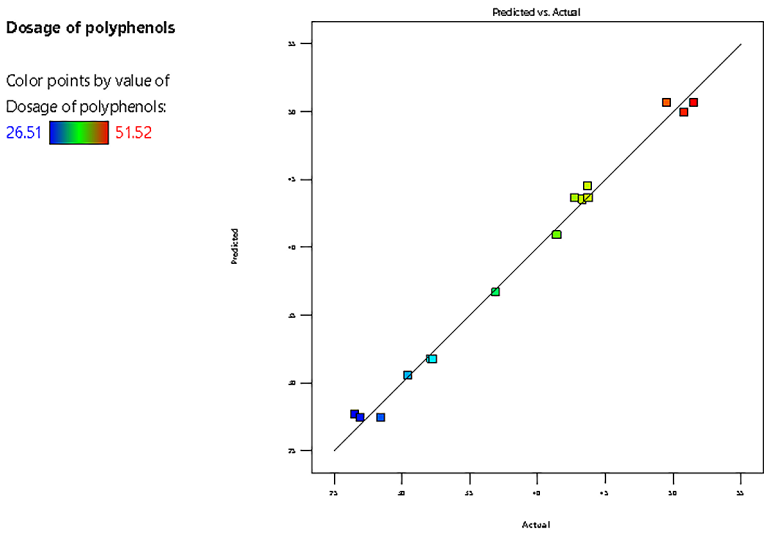



**Figure 1.** Graph of predicted vs.experimental values

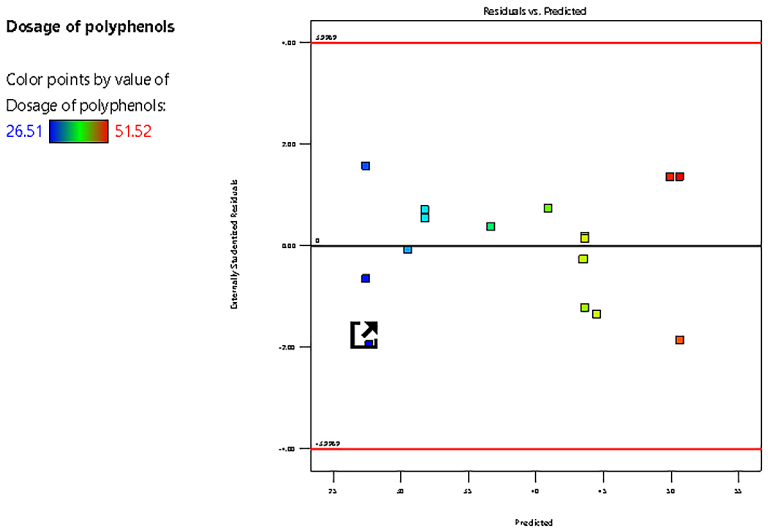



**Figure 2.** Residue graph

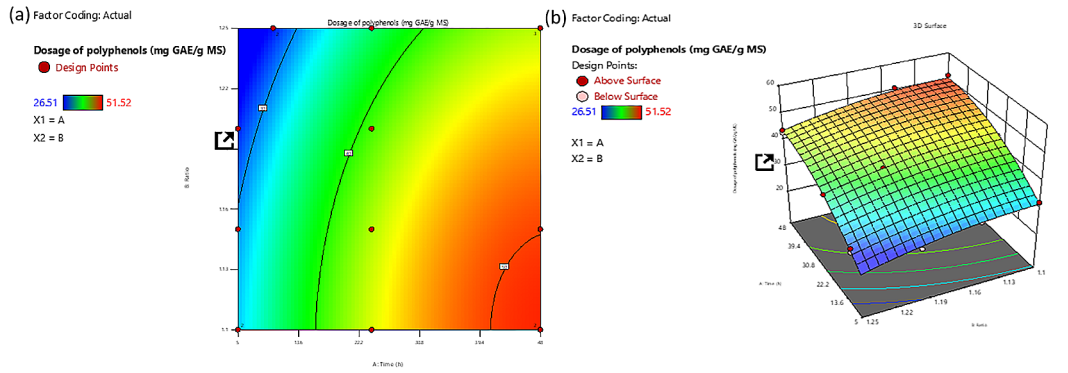
indicating that the optimal extraction conditions are at a solvent/powder ratio of 1:20 and about 40 hours (Benamar et al., 2025).

### *Optimization and experimental validation*

The numerical optimization approach determined the following optimal extraction parameters (Table 7): a solvent/powder ratio of 1:20 and an extraction time of 40 hours. Under these conditions, the polyphenol concentration predicted by the model was 51.20 0.45 mg GAE/g MS, in contrast, the experimental value obtained was 50.80 mg GAE/g MS, representing a relative deviation of 0.69%. This excellent agreement between the predicted and measured values confirms the reliability and accuracy of the quadratic model for predicting the extraction yield of phenolic compounds (Bayram, 2024).

### *Modeling and optimization of total flavonoid content*

The analysis of variance (Table 8) showed that the proposed quadratic model fits perfectly with experimental data on flavonoid content. The high value of the Fisher ratio ($F = 257.74$; $p < 0.0001$) confirms the overall relevance of the model. The main effects of extraction time (A) and solvent/powder ratio (B) were found to be significant ($p < 0.05$), as were their quadratic effects ($\mathbf{A^2}$ and $\mathbf{B^2}$). On the other hand, their interaction (AB) did not exert a significant influence on the response ($p = 0.3714$). The lack of fit test




(a) : contour representation (flat view) locating the optimal extraction area.

(b): Three-dimensional response surface illustrating the effect of extraction time (A) and solvent/powder ratio (B) on polyphenol content.

**Figure 3.** Response surfaces representing the combined effect of extraction time (A) and solvent/powder ratio (B) on polyphenol content (mg GAE/g MS)

**Table 7.** Comparison between predicted and experimental values at optimal conditions

| Optimization parameters | Conditions/Values |
|---|---|
| Optimal factors | 40 h; 1:20 |
| Value predicted by the model (mg GAE/g MS) | 51.52 |
| Experimental value obtained (mg GAE/g MS) | 50.80 |
| Relative gap (%) | 1.4 |

is not significant (p = 0.4703). This suggests that the model accounts for experimental variability. There is no systematic difference between the observed and predicted values. Statistical indices confirm the robustness of the model. The coefficient of determination **R² (0.9923)** indicates that more than 99% of the observed variability is explained by the model. The adjusted **R² (0.9884)** and the predicted **R² (0.9788)** are close, reflecting excellent consistency between adjustment accuracy and predictive capability. The coefficient of variation (CV = 4.88%) shows a low dispersion of data around the mean, while the value of Adeq Precision (40.91), well above the threshold of 4, shows a satisfactory signal/noise ratio. In general, the results obtained demonstrate that the quadratic model is reliable, robust and well adapted to describe the studied response.

### *Quadratic model regression equation*

The coded variable model equation can be expressed as follows:

**Table 8.** Analysis of variance (ANOVA) of the quadratic model applied to flavonoid content

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 466.49 | 5 | 93.30 | 257.74 | < 0.0001 | significant |
| A-Time | 427.55 | 1 | 427.55 | 1181.13 | < 0.0001 | |
| B-Ratio | 56.16 | 1 | 56.16 | 155.13 | < 0.0001 | |
| AB | 0.3171 | 1 | 0.3171 | 0.8760 | 0.3714 | |
| A² | 34.62 | 1 | 34.62 | 95.65 | < 0.0001 | |
| B² | 3.07 | 1 | 3.07 | 8.47 | 0.0156 | |
| Residual | 3.62 | 10 | 0.3620 | | | |
| Lack of Fit | 1.87 | 5 | 0.3746 | 1.07 | 0.4703 | not significant |
| Pure Error | 1.75 | 5 | 0.3493 | | | |
| Cor Total | 470.11 | 15 | | | | |
| R² | 0.9923 | | | | | |
| Adjusted R² | 0.9884 | | | | | |
| Predicted R² | 0.9788 | | | | | |
| Adeq Precision | 40.9147 | | | | | |

$$TFT\ (mg\ QE/g\ MS) = 15.84 + 6.45\ A - 2.27\ B - 0.20\ AB - 3.72\ A^2 - 1.10\ B^2 \quad (5)$$

where: $Y$ represents the flavonoid content (mg QE/g MS), $A$ is the extraction time (h), and $B$ is the solvent/powder ratio.

The positive sign of the coefficient linked to factor A shows that increasing the extraction time boosts flavonoid enrichment up to an optimal point. On the other hand, the negative coefficients of B, A2, and B2 indicate a limiting effect when these variables go beyond a certain value. This suggests that excessively long extraction times or very high ratios can result in a lower yield. Validation of the model using diagnostic graphs

Diagnostic graphs (Figure 4 and 5) support the statistical validity of the quadratic model. The distribution of residuals follows an almost straight line on the normal probability graph, proving their normality. The scatter plot of "predicted values vs. observed values" confirms an almost perfect correlation, with no apparent bias, while the random distribution of studentized external residuals around zero rules out any effect of heteroscedasticity. These results confirm the reliability of the model fit and the relevance of the regression hypotheses.

### *Validation of the model using diagnostic graphs*

Figure 6 illustrates the three-dimensional response surfaces and contour plots that show how extraction time (A) and solvent/powder ratio (B) affect flavonoid content. The concentration of flavonoids gradually increases over time, reaching a maximum between 13 and 15 hours, before decreasing slightly, probably due to solvent saturation or partial degradation of the substances. In addition, a moderate solvent/powder ratio of 1:15 v/w optimizes extraction, while an excessively high ratio leads to dilution of the solute and reduces flavonoid concentration. These results show a balance between the solvent potential, the stability of the extracted metabolites and the contact time.

### *Optimization and experimental validation*

The modeling highlighted the optimal parameters to have an interesting flavonoid content: a solvent/powder ratio close to 1:15 (v/w) and an extraction time of about 14 hours. A validation experiment carried out under the same conditions gave an experimental content very close to that estimated by the model, thus showing its accuracy and reliability to efficiently guide process optimization. This good affinity between experimental and theoretical values proves that the quadratic model, combined with the D-optimal methodology, is a reliable tool for predicting and optimizing the extraction of secondary metabolites (Table 9).

## DISCUSSION

*L.multifida* extracts had the following extraction yields: hydroethanolic 10.63%, aqueous 9.86%, hydromethanolic 7.90%, and hydroacetonics 7.00%. These findings reveal the significance of solvent polarity and bioactive chemical solubility As demonstrated for Ruta montana in Morocco (17.91%), The hydroethanolic extract

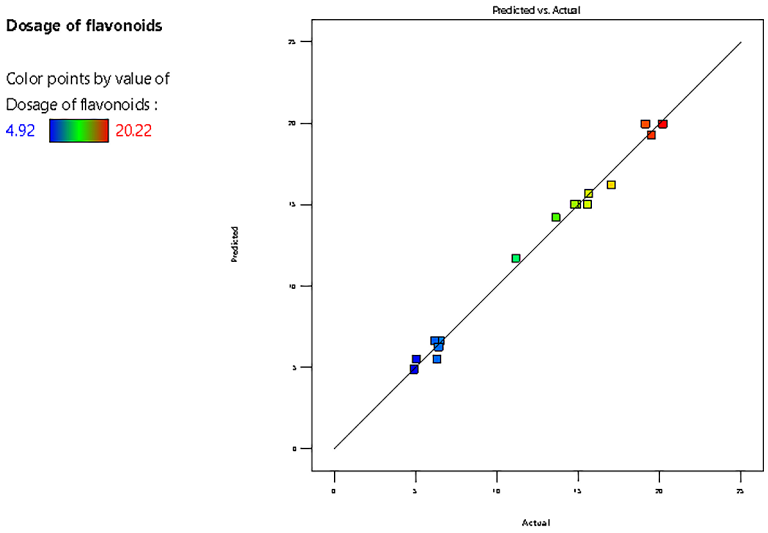



**Figure 4.** Graph of predicted vs. experimental values

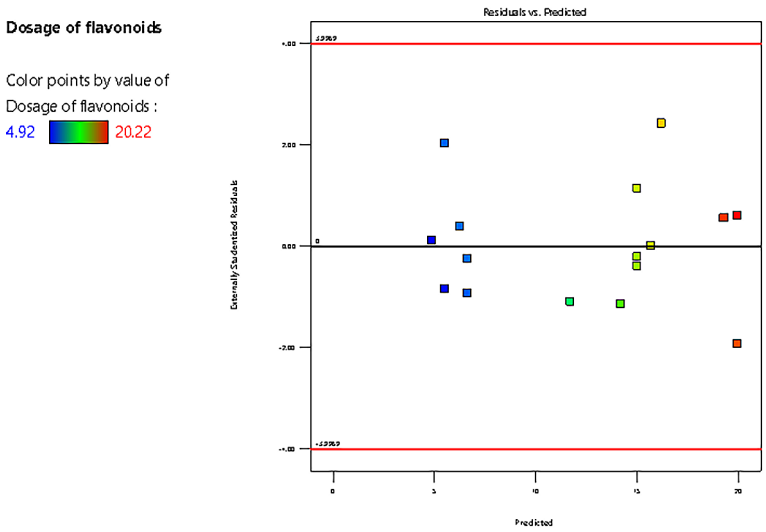
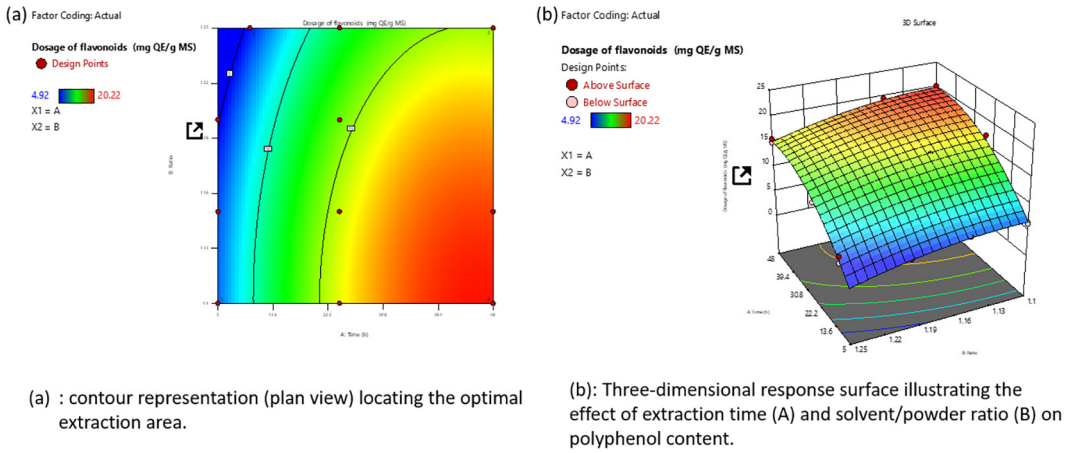



**Figure 5.** Residue graph




(a) : contour representation (plan view) locating the optimal extraction area.

(b): Three-dimensional response surface illustrating the effect of extraction time (A) and solvent/powder ratio (B) on polyphenol content.

**Figure 6.** Response surfaces and contour curves representing the effect of time (A) and solvent/powder ratio (B) on flavonoid content (mg QE/g MS)

**Table 9.** Comparison between experimental and predicted values obtained under optimal extraction conditions

| Optimization parameters | Conditions/ Values |
|---|---|
| Optimal factors | 14 h – 1 :15 |
| Value predicted by the model (mg GAE/g MS) | 15.84 |
| Experimental value obtained (mg GAE/g MS) | 15.67 ± 0.12 |
| Relative gap (%) | 1.07% |

efficiently extract a variety of polyphenols and flavonoids (El Ouardi et al., 2025). While the lower yield of the the hydroaceton extract (7.00%) can be explained by a polarity that is moderately less suited to the solubilization of phenolic compounds. The difference between aqueous and hydromethanol yields (9.86% vs. 7.90%) reflects the combined effect of the solvent and the chemical composition of the plant. Research on Moroccan plants, like *Mentha longifolia,* also demonstrates that the solvent and extraction technique have an impact on yield (Tourabi et al., 2023).

All *L. multifida* extracts are rich in secondary metabolites, according to phytochemical screening. The chemical diversity of this species is demonstrated by the constant presence of coumarins, free quinones, tannins, terpenes, and alkaloids extracts. The selection of saponins only in the aqueous extract shows the significance of solvent polarity in the extraction of certain chemical compounds. These findings are in line with previous research conducted in Moroccan. For instance (Mechqoq et al., 2022) found that extracts of numerous native Moroccan plants consistently included flavonoids,

tannins, coumarins, and saponins. In a similar vein (Bessi et al., 2025) showed the recurrent presence of these metabolites in a variety of solvents tested on Moroccan aromatic medicinal plants. The selective distribution of metabolites according to solvent, in particular the absence of saponins in hydroethanolic, hydromethanolic and hydroacetonic extracts, reflects the partition of plant compounds depending on the polarity of the solvent and their binding to the plant matrix. This confirms that the optimization of targeted extraction requires a solvent choice guided by the chemical nature and localization of compounds in tissues. Thus, the reported phytochemical profile supports the potential therapeutic significance. *L. multifida* and validates its customary application in Moroccan traditional medicine.

The overall polyphenol content has a similar parretn. There is 26.3 mg of EAG/g MS in the hydroethanolic extract, 19.68 mg of EAG/g MS in the aqueous extract, 18.30 mg of EAG/g MS in the hydromethanolic extract, and 12.08 mg of EAG/g MS in the hydroacetonics extract. These results show that the recovery of phenolic compounds is significantly influenced by the solvent selection. The hydroethanolic extract's high yield illustretes ethanol-water combinations dissolve and release these beneficial compounds. These findings align with Moroccan research on Papaver rhoeas (Hmamou et al., 2023) and Cynara humilis (El Khomsi et al., 2022), which indicate significant polyphenol concentrations in hydroalcoholic extracts. according to previous Moroccan investigations, the polarity of the solvent, the position of the chemicals in the tissues, or the plant matrix can all account for the slow decline in the hydroethanolic extract's contents to hydroacetone (Ezaouine et al., 2022).

The total flavonoid assay revealed that the aqueous extract had the highest content (25.35 mg EQ/g MS). followed by the hydroethanolic extract (21.24 mg EQ/g MS), the hydroacetonics extract (10.28 mg EQ/g MS), and the hydromethanolic extract (4.15 mg EQ/g MS). This variation demonstrates how the solvent affects the extraction of flavonoids, which are primarily polar and more soluble in aqueous or hydroalcoholic solvents. These results are support studies on *Juncus acutus* (Hammouti et al., 2023) and *Hammada scoparia* (Nounah et al., 2019), confirming the critical significance of solvent selection in flavonoid extraction. The hydromethanolic extract's low flavonoid concentration shows how intermediate polarity restricts the extraction of highly polar or cell matrix-bound chemicals. Thus, to maximize the content of flavonoids and exploit their antioxidant or therapeutic potential, the aqueous extract, followed by hydroethanolic, appears as the optimal choice.

Antioxidant activity measured by the DPPH test showed IC values of 145.44 µg/mL for the aqueous extract, 213.82 µg/mL for the hydroethanolic extract, 276.32 µg/mL for the hydroacetone extract, and 351.30 µg/mL for the hydromethanol extract. These results show that the aqueous extract, rich in water-soluble compounds, has the best ability to neutralize free radicals. Moroccan studies confirm this trend, for example on *Thymus satureioides* (Labiad et al., 2017) and different species of Thymus (Ouknin et al., 2025), highlighting that highly polar solvents allow the efficient extraction of water-soluble antioxidants. The decrease in antioxidant activity with the increase in CI for less polar extracts suggests that these compounds are less well extracted or present in lower amounts.

The FRAP method provided EC values of 682.62 µg/mL for the hydroethanolic extract, 712.89 µg/mL for the aqueous extract, 913.74 µg/mL for the hydromethanolic and 950.56 µg/mL for the hydroacetone, with the reference catechin having 13.90 µg/mL. These differences show that the polarity of the solvent and the nature of the compounds condition the ability to reduce ferric ions. The most polar extracts (hydroethanolic then aqueous) with the lowest values reflect an efficient extraction of molecules capable of reducing $Fe_3$ /$Fe_2$, corroborated by studies on *Cistus monspeliensis* (Haida et al., 2021). Analysis of variance (ANOVA) showed that the quadratic models adjusted for total polyphenols and flavonoids content are highly significant ($F > 250$; $p < 0.0001$), with significant linear and quadratic effects for "extraction time" and "solvent/powder ratio", while their interactions were not significant. The fit parameters ($R^2 > 0.99$, $R^2$ adjusted 0.99, $R^2$ predicted > 0.97, C.V. < 5%, Adeq. Precision > 40) confirm the robustness and predictive capacity of the models.

Regression equations indicate that increasing the extraction time favors maximum recovery of polyphenols and flavonoids to an optimal level, after which an excess lead to a slight decrease in yield, probably due to excessive degradation or dilution. The best conditions for each metabolite were found by response surfaces analysus:

around 40 hours with a solvent/powder ratio of 1:20 for polyphenols, and 14 hours with a ratio of 1:15 for flavonoids. The model's validity was confirmed by the experimental findings under thes conditions, which were extremely near to the predicted values (relative deviation < 1.5%). These results are consistent with prior research on *cornouaille cherry* (Frumuzachi et al., 2025) extraction optimization utilising the optimum D plan and RSM, and *Coriandrum sativum* (Azahar et al., 2017) demonstrating that the D-optimal strategy combined with RSM is a dependable method for raising *L. multifida* polyphenol and flavonoid yields while lowering the number of tests needed.

## CONCLUSIONS

The study of *L. multifida* extracts showed that the yield, chemical screening and biological activities of the extracts are strongly related to the type of secondary metabolites present in the plant and also to the polarity of the solvent. Hydroethanolic and aqueous extracts have proven to be the most effective for extracting polyphenols and flavonoids due to their solubility in polar or hydroalcoholic solvents. Phytochemical screening includes alkaloids, flavonoids, tannins, coumarins and quinones, shows the chemical richness of this plant and justifies its traditional use in Morocco. The results of DPPH and FRAP antioxidant tests show that extracts rich in water-soluble compounds show higher antioxidant activity. This highlights the importance of selecting the best solvent to increase biological efficiency. Finally, the optimization helped us identify the extraction conditions to maximize the concentration of polyphenols and flavonoids using the D-optimal and RSM methods while minimizing the number of experiments. These results highlight that *L. multifida* is a potential source of natural secondary metabolites. The applied methods can also be used to optimize the extraction conditions of important bioactive compounds in other medicinal plants.

### Acknowledgment

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