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Research Article

Ultrasound-Assisted Extraction of Phenolic Compounds from Moroccan *Lavandula stoechas* L.: Optimization Using Response Surface Methodology

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Secondary plant metabolites, in particular phenolic compounds, are characterized by great diversity in the plant kingdom and are widely used in the medical and industrial fields. The extraction of these compounds represents a critical step, and the differences in extraction conditions strongly affect the yields and the total concentrations of polyphenols in the extracts. In this way, the objective of the present study was to optimize the extraction parameters of the polyphenols from *Lavandula stoechas* using the extraction technique assisted by ultrasound. Yield and the total concentration of polyphenols have been optimized, taking into account three variables, the extraction time (min), the ethanol concentration (%), and the solvent/extract ratio (ml/g). The optimum extraction yield (31.88%) was obtained by ensuring the following parameters: an ethanol concentration of 40%, a liquid/solid ratio of 30 ml/g, and a time processing of 32.62 min. The maximum concentration of total polyphenols (190.14 mg gallic acid equivalents (GAE)/g) was obtained after 21.5 min of extraction, with a liquid/solid ratio of 30 ml/g and a concentration of ethanol at 40%. In order to obtain the maximum yield (24.9%) and the total concentration of polyphenols (190.14 mg GAE/g) simultaneously, the following parameters must be adjusted: an extraction time of 21.5 min, a liquid/solid ratio of 30 ml/g, and a concentration of ethanol at 40%. The experimental values of the yield and the total concentration of the polyphenols were in good agreement with the predicted values, which suggests that the ultrasonic extraction model adopted in this study is validated.

1. Introduction

Therapeutic activities of plants have long been associated with the production of secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, coumarins, and mucilages. Polyphenols are abundant phytochemicals and constitute a large class of compounds which have attracted research attention due to their potential human health benefits [1]. They have attracted the attention of scientists for a long time, and several recent studies have proven the

antioxidant activity [2], anti-inflammatory activity [3], prevention against cardiovascular disease [4], antibacterial [5], antifungal [6], hypoglycemic [7], antiviral [8], anticancer [9] activities, and many other diseases [4].

Lavandula stoechas is one of most plants used traditionally in the Mediterranean countries, including Morocco, for the treatment of painful illnesses such as inflammatory diseases, cystitis, nephritis, and rheumatic arthritis [10, 11]. Several studies have reported that the aqueous and alcoholic extracts of *L. stoechas* are characterized by various biological effects. Indeed, [12, 13], antifungal [14], antioxidant [14, 15], antihyperglycemic activities [16], anticonvulsant, antispasmodic, and sedative effects [17], and anti-inflammatory activity [17–19] have been reported. These activities are related to the phenolic compounds identified in the extracts of *L. stoechas*. Phenolic acids and flavone glycosides are the most reported phenolic compounds in *L. stoechas* extracts [14, 20, 21].

Ultrasound-assisted extraction (UAE) is an innovative and promising technique used in several fields such as cosmetics, pharmaceutical, chemical, and food industries since the second half of the 20th century [22]. Recently, several studies indicate that ultrasound produces more abundant extracts in biomolecules and in a shorter extraction time compared to conventional extraction techniques [22, 23]. A study realized by Chemat et al. [24] showed that the yield of carvone extracted from caraway seeds (*Carum carvi* L.) is best in ultrasound-assisted extraction as those given by the Soxhlet extract. A comparative study on the extraction of phenolic compounds from the flowers and buds of *Acacia confusa* was carried out by ultrasound, maceration, and extraction assisted by heat. This study confirmed that ultrasonic extraction is the most rapid and efficient technique, which allows to significantly increase the rate of phenolic content compared to other methods studied [25]. According to several studies, the quality of the phenolic compounds and the total concentration of polyphenols in several matrices extracted by ultrasound depends on the quantity and the polarity of the solvent used, the ultrasonic intensity [26], the extraction temperature [27], and the extraction time [28].

The aim of this work was to maximize the obtained yield and total phenolic contents from *L. stoechas* extracted by ultrasound method. In order to achieve this objective, we used the Box–Behnken design in conjunction with a response surface methodology (RSM) to optimizing three parameters: the extraction time (min), liquid/solid ratio (ml/g), and ethanol concentration (%).

2. Material and Methods

2.1. Plant Material. The aerial parts (leaves, stems, and flowers) of *L. stoechas* were harvested during the period of April to May 2016 in the commune of Timezgana (34°33′ 02.7″ N 4°40′ 49.3″ W) in Taounate Province (north central region of Morocco), at an altitude of approximately 800 m. The botanical identification was realized by Professor Abdeslam Ennabili, botanist and Head of the Phytology Department in the National Institute of Medicinal and Aromatic Plants, Taounate, Morocco. Authenticated voucher specimens have been deposited in the Herbarium of the National Institute of Medicinal and Aromatic Plants, Morocco.

2.2. The Ultrasound-Assisted Extraction. The extraction method was performed with ultrasound cleaning bath Elma Transsonic TI-H-15 (a frequency of 35 kHz and a nominal power of 100 W). The powder of the dried plants (20 g) was placed in a capped tube (100 ml) and mixed with an appropriate amount of the extraction solution (according to experimental design). The tube with the suspension was immersed in water in the ultrasonic device and irradiated for the preset extraction time. After ultrasonic extraction, the sample was filtered by Whatman paper and evaporated under vacuum at 40°C on a rotary evaporator.

2.3. Experiment Design. Response surface methodology (RSM) was used for investigating the influence of three independent variables on yield and total phenols of *L. stoechas* extracts. The extraction time (min, X1), liquid/solid ratio (ml/g, X2), and ethanol concentration (%, X3) were selected as independent variables that should be optimized for the extraction. The samples were kept at room temperature to avoid the degradation of temperature-sensitive compounds. In the study, the experiments were performed on the Box–Behnken design (BBD). The level values of the experimental factors are shown in Table 1.

2.4. Determination of Total Phenolic Content. The Folin–Ciocalteu method was used to determine the total phenolic content (TPC) of the extract with slight modifications [29]. TPC was estimated in milligrams of gallic acid equivalents per gram of dry plant material (mg of GAE/g). 100 μl of the sample (1 mg/ml) was transferred to a 10 ml volumetric flask, to which 500 μl undiluted Folin–Ciocalteu reagent was added subsequently. After 1 min, 1.5 ml of 20% (w/v) Na_2CO_3 was added and the volume was made up to 10 ml with H_2O. After 2 hours of incubation at 25°C, the absorbance was measured at 765 nm and compared to the gallic acid calibration curve.

2.5. Statistical Analysis. The experimental results of the response surface design were analyzed using JMP software (version 10) [30]. The main effect of regression was considered to be statistically significant for p values less than 0.05. All experiments were conducted in triplicate unless otherwise noted in the text.

The complete design was carried out in random order and consisted of 15 combinations, including three replicates in the central point. The data from the Box–Behnken design (BBD) were analyzed by multiple regressions to fit the following quadratic polynomial model:

Table 1: The selected factors and their levels in the Box–Behnken design.

Factors	Unity	Symbol	Factor level		
			−1	0	1
Extraction time	Min	X1	20	30	40
Solvent-to-material ratio	ml/g	X2	20	25	30
Ethanol	%	X3	40	60	80

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + \varepsilon, \quad (1)$$

where Y is the studied response; b_0 represents the average value of responses; b_1, b_2, and b_3 are coefficients of the main terms; b_{11}, b_{22}, and b_{33} are the coefficients of quadratic terms; b_{12}, b_{13}, and b_{23} are the coefficients of the interaction terms; and ε is an error term.

An analysis of variance with F test at a 95% significance level was conducted. The mean squares (MS) were obtained as follows: MS = (SS/DF), where SS is the sum of squares of each variation source and DF is the respective degree of freedom.

The ratio between the mean square regression (MS_R) and the mean square residual (MS_r), $F_{\text{ratio}(R/r)}$, was used in order to establish whether the model was statistically significant [31]. The greater F-value from unity adequately explains the variation of the data around its mean. Besides, the estimated factor effects are real [32].

The quality of fitting the first-order polynomial was also expressed with the coefficient of determination R^2. This coefficient measures the proportion of total variation in the mean response explained by the regression. In fact, it represents the correlation between the observed and predicted responses, and it is often expressed as a percentage [33].

The model coefficients were considered significant for p values <0.05. The statistical significance of the model coefficients was determined by using the t-test (only significant coefficients with p value <0.05 are included).

2.6. Optimization of Operating Conditions. The isoresponse curves were used to find the optimal agreement between operating conditions. These are surface curves that reflect the changes in the response. Moreover, the "desirability" function was used to find the exact optimal combination with a percentage of compromise.

3. Results

3.1. Experimental Design. Table 2 shows the yield and TPC of *L. stoechas* extracts obtained from all experiments.

3.2. Statistical Validation of the Postulated Model. According to the analysis of variance table (Table 3), we can conclude that the main effect of regression is significant for both the studied responses since the significance of the risk (p value) is lower than 0.05. Obviously, for the two studied responses, the calculation of $F_{\text{Ratio}(R/r)}$ (13.93 for the response yield and 6.59 for the response TPC) has shown that it is higher than the theoretical value of $F_{(0.05;9,5)}$ at 95% confidence level which is equal to 4.77.

The linear regression of the model permitted us to evaluate the quality of the established model graphically. It is represented by the linearity of the points measured by the real model according to the points considered by the quadratic model. The goodness of the model is judged if the coefficient of determination is equal to or higher than 80%. The coefficients of determination R^2 are equal to 96% and 92% for the response yield and TPC, respectively. These values give a good agreement between the experimental and predicted values of the adapted model. These results are confirmed by those obtained in the graph (Figure 1), showing a linear curve for the observed values in terms of the predicted ones.

3.3. Study of the Factor's Effects and the Fitted Model for the Response Yield. The effects of all factors studied and the statistical values of Student's t-test and the observed probability (p value) are summarized in Table 4. Student's t-test values are used to determine the significance of the coefficients of each parameter, while p values are defined as the smallest level of importance, leading to rejection of H0 ($b_i = 0$; $\alpha = 0.05$). Thus, Table 4 shows that all factors can be considered as significant since their p values are lower than 0.05 except factor b_1 and factor b_{13}, which can be considered as negligible (p value >0.05).

The equation of the quadratic model used for the modeling extraction conditions of yield, determined according to the results of the JMP 10 software, was represented as follows:

$$Y1 = 21.62 + 4.42X_2 - 2.13X_3 - 3.7X_1^2 - 3.98X_2^2 + 3.44X_3^2 + 2.75X_1X_2 - 3.28X_2X_3 + \varepsilon. \quad (2)$$

3.4. Study of the Factor's Effects and Fitted Model for the Response TPC. The effects of all factors studied and the statistical values of Student's t-test and the observed probability (p value) are summarized in Table 5. These results show that the factors $b_0', b_1', b_3', b_{11}', b_{22}'$ can be considered as statistically significant since their p values are lower than 0.05.

The equation of the quadratic model used for the total polyphenols, determined according to the results of the JMP 10 software, was represented as follows:

$$Y2 = 91.86 - 15.45X_1 - 16.22X_3 + 36.63X_1^1 + 27.47X_2^2 + \varepsilon. \quad (3)$$

3.5. Response Surface Optimization. Using the isoresponse curves, we can consider options regarding operating conditions. With manual adjustment of target values of the two

Table 2: Different combinations generated by the Box–Behnken design and the recorded responses for each experiment of *L. stoechas* extracts.

Experiment number	Extraction time (min)	Liquid/solid ratio (ml/g)	Ethanol concentration (%)	Yield (%)	TPC (mg GAE/g)
1	20	20	60	12.33	180.82
2	40	20	60	7.00	144.13
3	20	30	60	15.33	184.36
4	40	30	60	21.03	115.63
5	20	25	40	22.71	149.53
6	40	25	40	26.60	147.48
7	20	25	80	16.60	124.57
8	40	25	80	19.53	108.37
9	30	20	40	14.19	130.53
10	30	30	40	29.93	149.53
11	30	20	80	18.80	118.80
12	30	30	80	21.40	95.52
13	30	25	60	22.33	89.38
14	30	25	60	20.80	95.33
15	30	25	60	21.73	90.87

Table 3: Analysis of variance for the fitted models.

	Yield (%)					TPC				
Model	DF	SS	MS	F	p-value	DF	SS	MS	F	p-value
R	9	434.62	48.29	13.94	0.005	9	12142.34	1349.15	6.60	0.03
R	5	17.32	3.46			5	1022.80	204.56		
Total	14	451.95				14	13165.14			

DF: degrees of freedom; SS: sum of squares; MS: mean square; R: regression; r: residual.

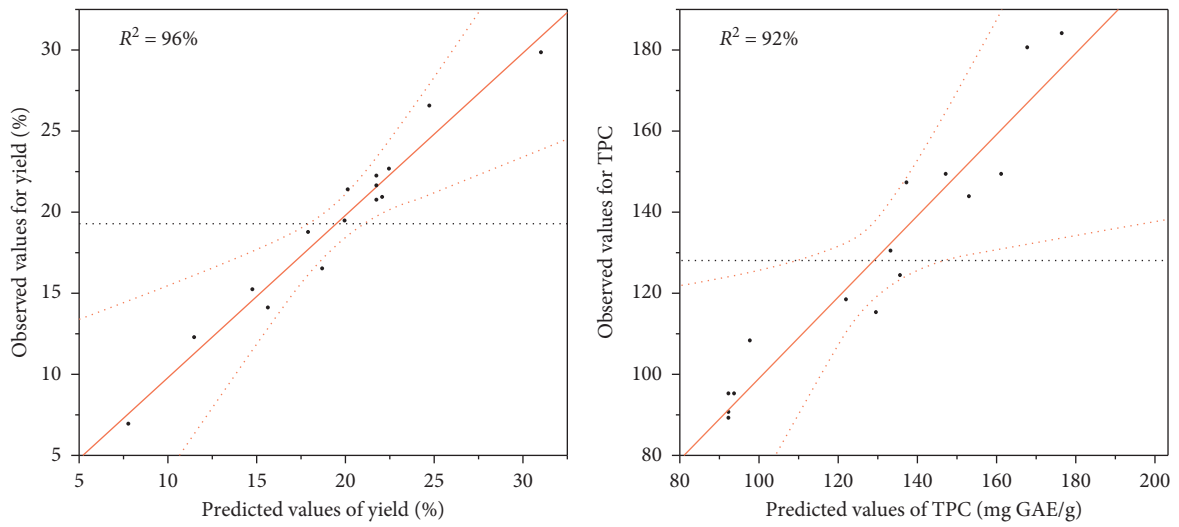


Figure 1: Curves of the observed values in terms of the predicted values for the two studied responses (a) yield and (b) TPC.

studied answers, one is interested in the most advantageous economical experimental conditions. Then, it is advantageous to use the least amount of ethanol in a time interval somewhat weak.

3.5.1. Optimization of the Yield Response. The desirability plot (Figure 2) shows that the maximum yield that we can have is around 31.88%. We can also see that obtaining this value is possible with a desirability of 98.7%. This same plot also shows that the maximal value of yield requires a minimal concentration of ethanol (40%), a maximum solvent-to-material ratio (30 ml/g), and a time processing higher than 30 min. By fixing the concentration of ethanol at 40% in the isoresponse plot, we can see that the required time for obtaining this yield is between 30 and 40 min when the liquid/solid ratio must be between 28 and 30 ml/g (Figure 3(a)). The 3D plot (Figure 3(b)) shows the

Table 4: Estimated regression coefficients for the yield response and their level of significance p value.

Term	Coefficient	Estimation	Standard deviation	p-value
Constant	b_0	21.62	1.075	<0.0001***
Extraction time	b_1	0.899	0.658	0.23
Solvent-to-material ratio	b_2	4.421	0.658	0.001**
Ethanol (%)	b_3	−2.138	0.658	0.023*
Extraction time ∗ solvent-to-material ratio	b_{12}	2.758	0.931	0.031*
Extraction time ∗ ethanol (%)	b_{13}	−0.24	0.931	0.806
Solvent-to-material ratio ∗ ethanol (%)	b_{23}	−3.285	0.931	0.017*
Extraction time ∗ extraction time	b_{11}	−3.709	0.969	0.012*
Solvent-to-material ratio ∗ solvent-to-material ratio	b_{22}	−3.989	0.969	0.009*
Ethanol (%) ∗ ethanol (%)	b_{33}	3.449	0.969	0.016*

*** $p < 0.05$, ** $p < 0.01$, and * $p < 0.001$.

Table 5: Estimated regression coefficients for the response total polyphenol concentration and their level of significance p value.

Term	Coefficient	Estimation	Standard deviation	p-value
Constant	b'_0	91.86	8.258	0.0001***
Extraction time	b'_1	−15.459	5.057	0.028*
Solvent-to-material ratio	b'_2	−3.655	5.057	0.502
Ethanol (%)	b'_3	−16.226	5.057	0.024*
Extraction time ∗ solvent-to-material ratio	b'_{12}	−8.01	7.151	0.314
Extraction time ∗ ethanol (%)	b'_{13}	−3.538	7.151	0.642
Solvent-to-material ratio ∗ ethanol (%)	b'_{23}	−10.57	7.151	0.199
Extraction time ∗ extraction time	b'_{11}	36.634	7.443	0.004**
Solvent-to-material ratio ∗ solvent-to-material ratio	b'_{22}	27.741	7.443	0.014*
Ethanol (%) ∗ ethanol (%)	b'_{33}	3.994	7.443	0.615

*** $p < 0.05$, ** $p < 0.01$, and * $p < 0.001$.

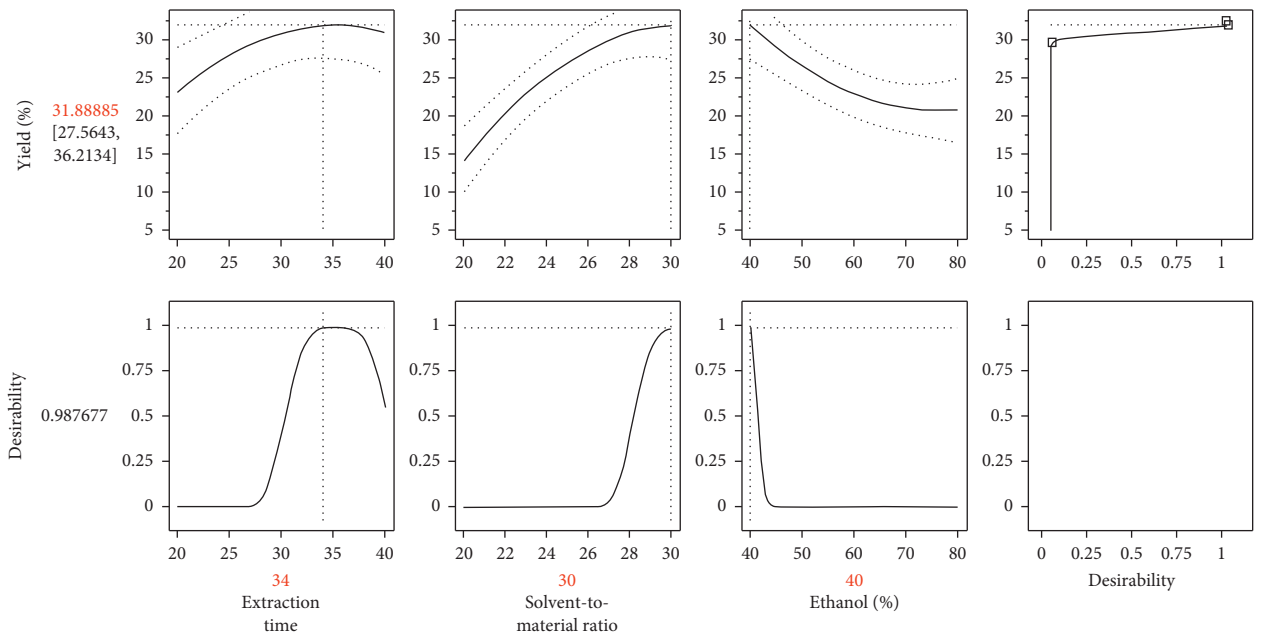
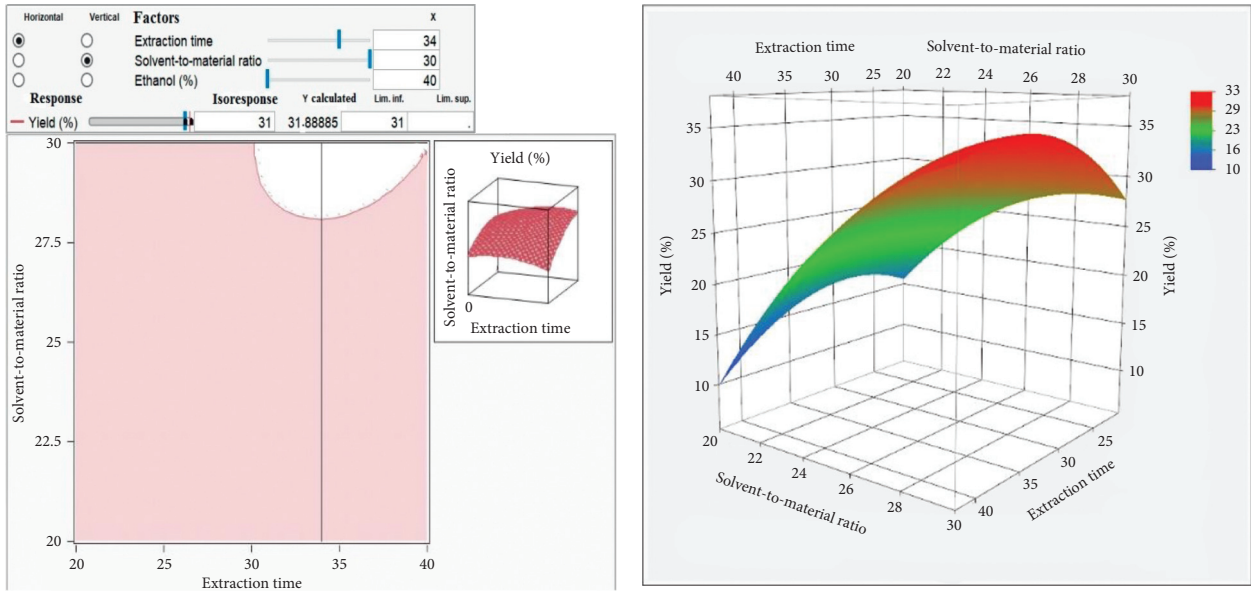


Figure 2: Desirability plot showing the precise operating conditions leading to the optimal yield.

graphical representation of the response yield in terms of extraction time and liquid/solid ratio and by fixing the parameter ethanol concentration (%) on its low level (40%).

3.5.2. Optimization of the Total Phenolic Content (TPC) Response. The desirability plot of this second response (TPC) (Figure 4) shows that the maximum value that we can obtain is around 190.14 mg GAE/g. Obtaining this response



(a) (b)

Figure 3: (a) Isoresponse plot showing the optimal compromise zone leading to the desired yield (31%) obtained by fixing the third parameter (ethanol (%)) in its low level (40%) and varying the other two parameters. (b) 3D plot of the response yield (%) in terms of extraction time and liquid/solid ratio effects and by fixing the concentration of ethanol at 40%.

is possible with 98% desirability. As in the case of the extraction yield, the maximal TPC value that we can get requires a minimal concentration of ethanol (40%), a minimal time processing, and a maximum liquid/solid ratio.

The isoresponse plot (Figure 5(a)) confirms the results of the desirability plot. Thus, this plot shows that the compromised area (white area) is accessible by ensuring a time between 20 and 21.5 min and a liquid/solid ratio between 28 and 30 ml/g and by fixing the concentration of ethanol at 40%. The 3D plot (Figure 5(b)) shows the graphical representation of the response TPC in terms of extraction time and liquid/solid ratio and by fixing the parameter ethanol concentration (%) on its low level (40%).

3.5.3. Optimization of Two Responses Simultaneously. The obtained results for the two responses separately indicate that the maximization of two responses requires a reduction in the ethanol concentration to 40% and an increase in the liquid/solid ratio (30 ml/g). However, this shows disagreement as regards the time factor. When we have a maximum yield (31%) performance requires a time longer than 30 minutes, the TPC response requires a minimal time (just 20 minutes) to reach its maximum. Since the interest of extraction is on TPC, we will adopt the setting that guarantees the maximization of TPC, as shown in the desirability plot (Figure 6). By adopting the optimum setting of maximizing TPC (time: 21.5 min, liquid/solid ratio: 30 ml/g, and ethanol concentration: 40%), we will have a TPC equal to 190 mg GEA/g with a yield of 24% (loss of 7% compared to the maximum possible yield 31%). These results are obtained with a desirability of 99%. The isoresponse plot for both answers shows a very small area (white area) of compromise between the two studied responses (Figure 7).

3.6. Verification of Predictive Model. To compare the predicted result with the experimental result, a control experiment randomly chosen from the experimental domain was performed. Experimental values of yield (14.85 ± 1.02%) and TPC (130.15 ± 2.35 mg GAE/g) were slightly higher than the predicted ones which were equal to 14.29% and TPC at 129.75 mg GAE/g (Table 6). This result suggests that the optimized model explains appropriately the extraction process of yield and phenolic compounds. Moreover, the experimental validation provides preliminary information that the developed model can be successfully applied for predicting the optimal extraction time, the liquid/solid ratio, and the ethanol concentration.

4. Discussion

The objective of this study was to determine the levels of experimental factors that would allow to obtain maximum yield and a high concentration of polyphenols. In fact, to get the maximum yield and TPC, a compromise was found in choosing the optimal adjustment for the concentration of ethanol (40%) and liquid/solid ratio (30 ml/g). However, a disagreement in extraction time was observed for the two responses. Response surface analysis demonstrates that the relationship between the total phenolic content and extraction parameters is quadratic with a good regression

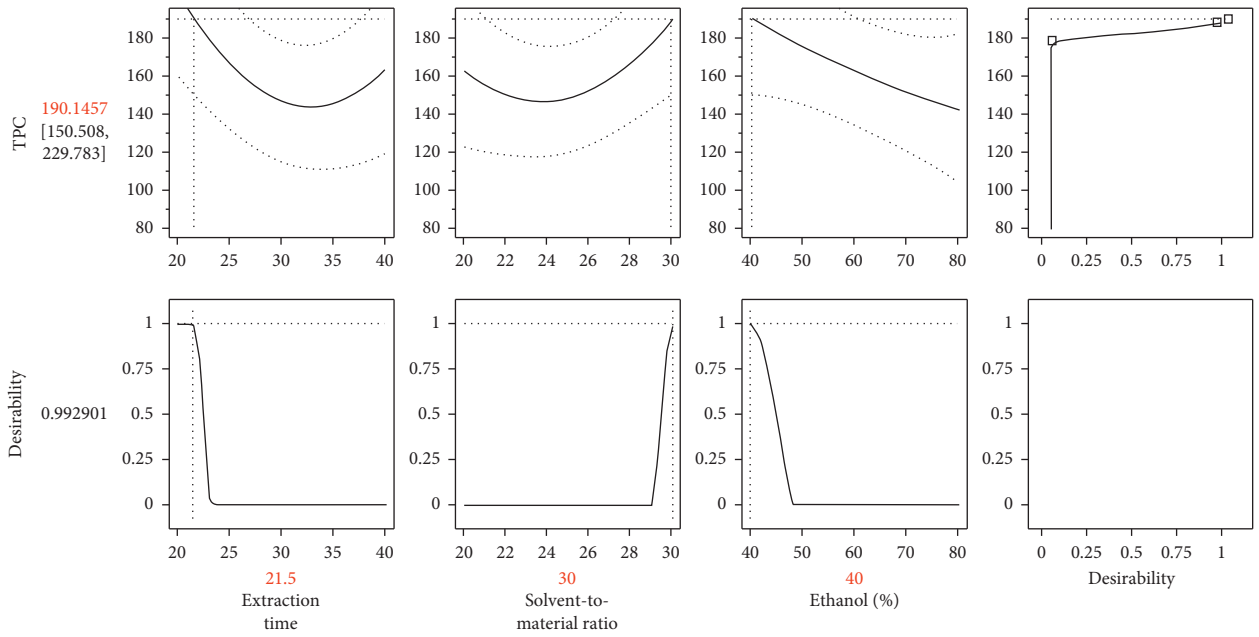
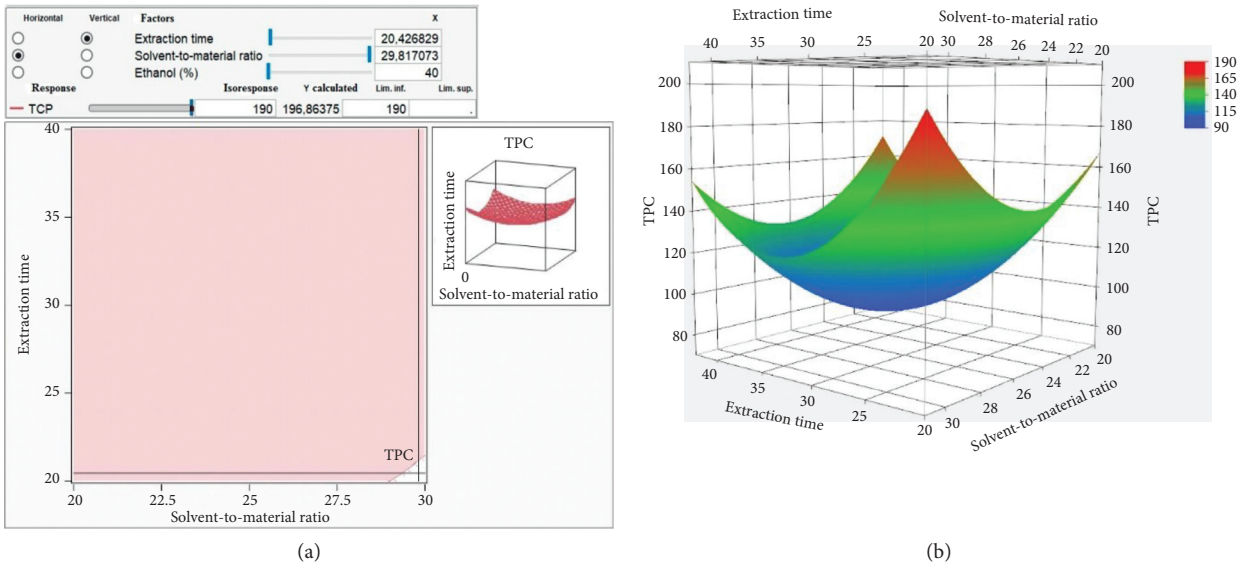


Figure 4: Desirability plot showing the precise operating conditions leading to the optimal TPC.



(a) (b)

Figure 5: (a) Isoresponse plot showing the optimal compromise zone leading to the desired TPC (190 mg EAG/g) obtained by fixing the third parameter (ethanol (%)) in its low level (40%) and varying the other two parameters. (b) 3D plot of the response TPC in terms of extraction time and liquid/solid ratio effects and by fixing the concentration of ethanol at 40%.

coefficient. As the extraction of phenolic compounds depends mainly on the polarity of solvents and compounds, a single solvent might not be effective for the extraction of a bioactive compound. Hence, a combination of alcohol with water is more effective in extracting phenolic compounds than alcohol alone [34]. Ethanol/water was chosen as the unique extraction solvent, instead of others, because of the low price of ethanol, low toxicity, easiness of recycling [35], and good polarity to extract the phenolic compounds. The obtained findings from our study are in agreement with previous studies, which reported that adding water to the alcohol shows a synergistic effect, increasing the extraction performance of phenolic compounds from plant samples [36, 37]. The results of a study carried out by Guerra et al. [38] on the optimization of the parameters influencing the concentration of polyphenols obtained from mango peel by ultrasound-assisted extraction confirmed that the use of 50% ethanol made it possible to obtain optimum phenolic compounds.

The extraction time is an important parameter to minimize the energy cost of the process [39]. In our study, the total phenolic concentration increased with prolonged

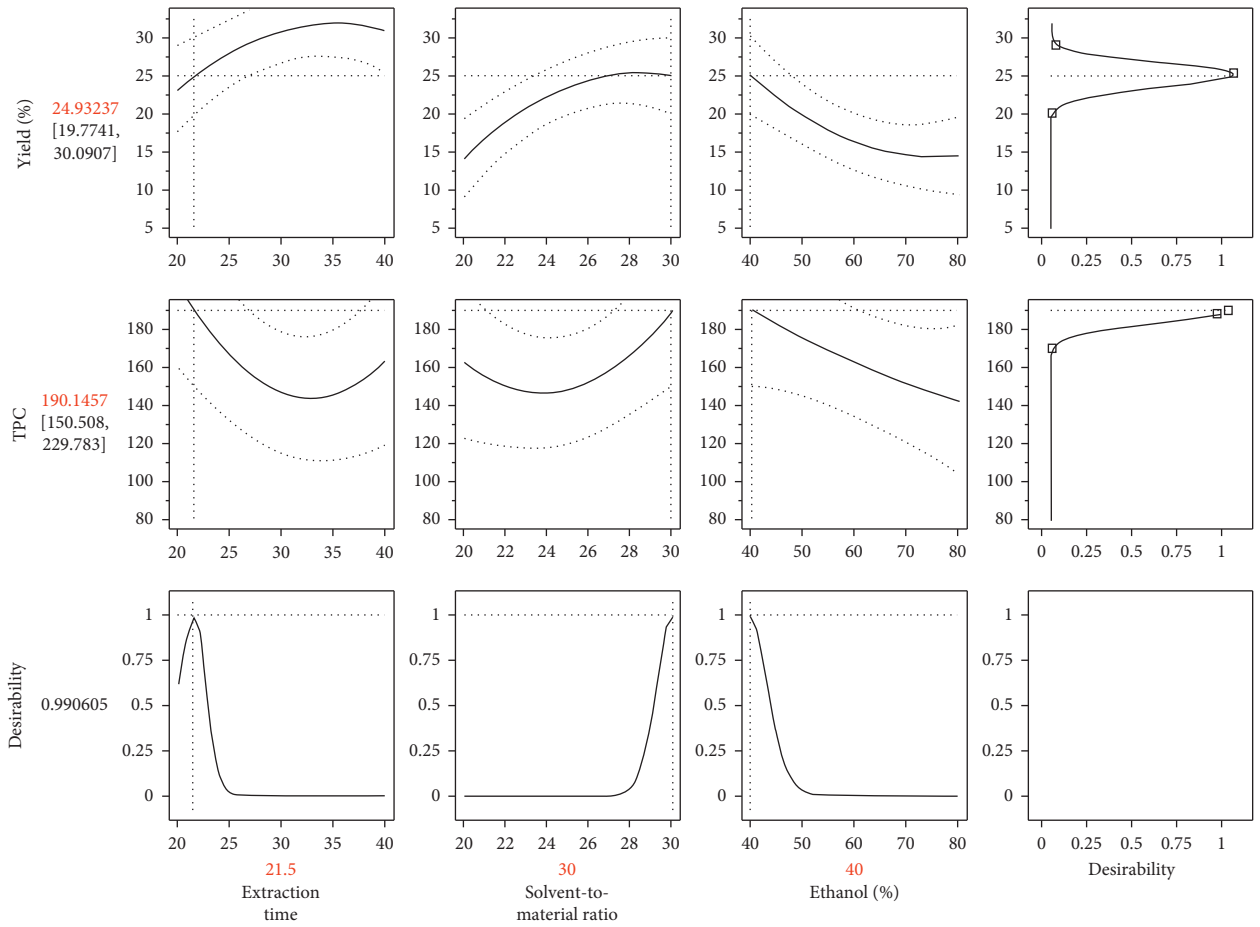


Figure 6: Desirability plot showing the precise operating conditions leading to the optimization of both yield and TPC responses, simultaneously.

extraction time from 20 to 40 min. This observation was understandable because an extended extraction time favors the extraction of phenolic compounds [40]. Also, another study realized by Hammi et al [41] reported similar results while investigating the effect of time on the extraction efficiency of polyphenols from *Zizyphus lotus*, while other studies revealed a shorter extraction time to obtain the optimum phenolic compounds using ultrasound-assisted extraction. The optimal time for the extraction of phenolic compounds (1225.7 mg EAG/100 g of extract) from the peel of *Litchi chinensis* extracted by ultrasound was 30 minutes [42]. The maximum of phenolic compounds (345.81 ± 2.82 mg EAG/100 g of the extract) from the stems of *Bactris gasipaes* (Arecaceae family) was obtained during 30 min of extraction [43]. Recently, Iftikhar et al. [44] evaluated the total concentration of polyphenols in *Secale cereale* ethanolic extract (Poaceae), the extraction time to obtain the optimum TPC (245.74 mg GAE/100 g of extract) was 29 minutes. During these two studies, the extraction temperatures were 60 and 66°C, respectively, which could explain that the researchers obtained the optimum TPC in a reduced time compared to our study (temperature of extraction at 25°C).

The increased extraction of total phenolic content was observed with an increased solvent-to-material ratio from 20 to 30 ml/g. This is probably due to the fact that more solvent can enter cells while more phenolic compounds can permeate into the solvent under the higher solvent-to-material ratio conditions [45]. A comparison of the literature has shown that extraction assisted by ultrasound contributes to the increase in yields of polyphenols, in addition to the preservation and growth of the biological activities of polyphenol extracts compared to traditional techniques [46]. A recent study conducted by Palsikowski et al. (2020) compared the efficiency of the extraction of leaves of *Bauhinia forficata* (Fabaceae family) by ultrasound and maceration methods using alcoholic solvents of different polarity

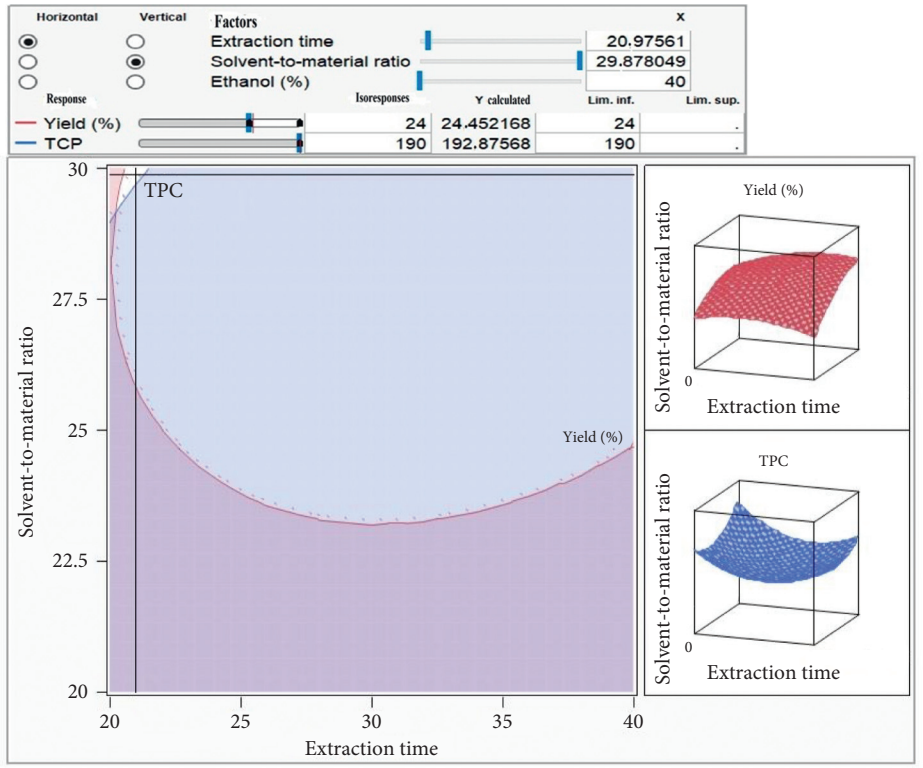


Figure 7: Isoresponse plot showing the precise operating conditions leading to the optimization of both yield and TPC responses, simultaneously.

Table 6: Theoretical and experimental responses recorded for the selected operating conditions.

Extraction conditions		Yield (%)		TPC (mg GAE)/g dry matter)	
		Predicted yield (%)	Experimental yield (%)	Predicted TPC	Experimental TPC
Time	30 min				
Liquid/solid ration	20 ml/g	14.29	14.85 ± 1.02	129.75	130.15 ± 2.35
Concentration of ethanol	45%				

(hexane, ethyl acetate, and ethanol). During this study, the researchers obtained an optimum yield and total concentration of polyphenols of 8.33% and 59.47 mg EAG/g, respectively. These results were obtained according to the following conditions: the temperature at 41°C, the ratio solvent-material at 1/20 (w/v), and a power value of 80%. The scientists proved that the ultrasonic extraction could reduce the extraction time compared to the extraction by maceration while obtaining a better concentration of phenolic compounds. Besides, ethanol was the most suitable solvent for the extraction of phenols [47].

5. Conclusion

The response surface methodology was successfully employed to optimize the yield and the phenolic compound extraction from Moroccan *L. stoechas*. The BBD proved to be a powerful tool for the optimization of ultrasonic-assisted extraction parameters. This study has permitted to define the optimal extraction process for yield response as follows: an extraction time of 34 min, a liquid/solid ratio of 30 ml/g, and an ethanol concentration of 40%. The optimal conditions to obtain the highest polyphenols content, 190.14 mg GAE/g, are as follows: extraction time of 21.5 min, liquid/solid ratio of 30 ml/g, and ethanol concentration of 40.5% (v/v). However, for the optimization of two responses simultaneously to get a yield of 24.2% and a total concentration polyphenols of 190.24 mg GAE/g, the following parameters must be respected: extraction time of about 20.73 min, liquid/solid ratio of 29.72 ml/g, and ethanol concentration of 40.5% (v/v).

The results indicated that the UAE-RSM approach was effective for maximizing the yield extraction and TPC, and the knowledge gained from this study should be useful for further exploitation and application of the phenolic compounds.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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