Optimization of ultrasonic-assisted extraction conditions for polyphenols from Algerian *Phlomis Crinita* (Cav.) by response surface methodology

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Abstract. The aim of this study is to determine the optimal conditions for ultrasonic-assisted extraction (UAE) to maximize the yield, total polyphenols content and antioxidant activity of Algerian *Phlomis crinita* (Cav.) extracts. The effect of extraction parameters is evaluated by response surface methodology (RSM). The antioxidant activity of the extracts is determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay. The maximum yield obtained from ultrasound-assisted extraction is 18.54% (g per 100 g) with a total polyphenols content of 175.75 mg of Gallic acid equivalent (GAE) per gram of extract and an antioxidant activity expressed by the value of the half-maximal inhibitory concentration (IC_{50}) of 7.51 mg L^{-1} of *Phlomis crinita* extracts. The optimum conditions for this result are 60 min for extraction time, ultrasonic power of 40 W, a ratio of 1:20 g mL^{-1} for vegetable matter/solvent and a methanol concentration of 70% (v v^{-1}). The results confirmed the efficiency of ultrasonic extraction and response surface methodology in optimizing the extraction of bioactive compounds from *Phlomis crinita*.

Key words: *Phlomis crinita* (Cav.), antioxidant activity, bioactive compounds, ultrasound, optimal conditions.

Highlights

- *Phlomis crinita* (Cav.), a traditional Algerian medicinal plant, is a promising source of natural antioxidants.
- Ultrasonic-assisted extraction (UAE) was optimized using response surface methodology (RSM).
- Extraction time, ultrasonic power, solvent ratio, and methanol concentration significantly affected polyphenol recovery.
- Optimal UAE conditions yielded 18.54% extract, 175.75 mg GAE g^{-1} polyphenols, and strong antioxidant activity (IC_{50} = 7.51 mg L^{-1}).
- *Phlomis crinita* extracts show potential applications in food, pharmaceutical, and cosmetic industries as natural antioxidants.

INTRODUCTION

In Algeria, the genus *Phlomis* is represented by four species, three of which are endemic (Quezel & Santa, 1963). Among these species, *Phlomis crinita* (Cav.) is widely used as a traditional remedy by local communities (Merouane et al., 2019). *Phlomis crinita* (Cav.) extracts have been the subject of some studies that reported their high antioxidant potential (Amor et al., 2009). A positive correlation between antioxidant activity and total polyphenol content is noticed (Sadeghi et al., 2015). Indeed, many phenolic compounds have antioxidant properties due to their chemical structure that allows them to accept electrons and, thus, keep free radicals at an acceptable concentration and delay the oxidation of a substrate (Wang et al., 2013; Monnier & Schlienger, 2018). Foodstuffs sensory properties are negatively affected by lipid peroxidation resulting from free radical formation which reduces their shelf life as highly reactive and potentially toxic products are generated (Jacobsen et al., 2019; Dangles, 2020). For this reason, the use of antioxidants to neutralize these free radicals is crucial to extend product shelf life. The application of plant extracts as antioxidants in food processing improves their preservation and thus extends their shelf life (Pabón-Baquero et al., 2018). Thus, natural antioxidants, as alternatives to long-controversial synthetic additives for food preservation, are recommended (Santos-Sánchez et al., 2017). The genus *Phlomis* (family Lamiaceae) includes more than 100 species distributed across Africa, Asia and Europe (Zhang & Wang, 2009). These species are often used for the preparation of herbal teas and for culinary purposes (Sarikurkcu & Ćavar, 2020). Their use is mainly due to their antioxidant (López et al., 2010), anti-inflammatory (Shang et al., 2011), antitumour (Alpay et al., 2019) and antimicrobial (El Mokni et al., 2019) effects. Previous studies have shown that plants belonging to the genus *Phlomis* contain different classes of glycosides including iridoids, flavonoids, phenylpropanoids, phenylethanoids and diterpenoids (Dellai et al., 2009). The antioxidant effects of polyphenols in preventing cardiovascular disease and cancer are widely documented in the literature (Brglez Mojzer et al., 2016). Currently, several thousand phenolic compounds have been identified in higher plants, including edible ones (Stagos et al., 2020). Polyphenols can be classified into different groups according to the number of phenol nuclei they contain and on the basis of structural elements that bind these nuclei together (Tazzini, 2014). The main classes include phenolic acids, flavonoids, stilbenes and lignans (Pandey & Rizvi, 2009). The extraction of these bioactive molecules from plants continues to attract increasing interest from researchers (Klavins et al., 2017; Ameer et al., 2017). The extraction method is one of the key parameters determining the extraction performance of polyphenols (Monteiro et al., 2020). Different methods are employed for the extraction of phenolic compounds from plants, such as Soxhlet extraction, maceration, pressurized liquid extraction, supercritical fluid extraction, ultrasonic-assisted extraction and microwave-assisted extraction (Oreopoulou et al., 2019). The traditional method (solvent extraction) intensifies mass transfer by heating, the polyphenols are, thus, released into the solvent water (Tang et al., 2023). Several studies have reported the phenolic content and antioxidant activity of *Phlomis crinita*. Derafa et al. (2022) investigated hydromethanolic and aqueous extracts obtained by

conventional solvent extraction, while Nabti et al. (2023) confirmed bioactive compounds and antioxidant activity in leaf extracts from northern Algeria. Chelgham et al. (2025) further characterized phenolic compounds and antioxidant potential using solvent extraction combined with HPLC analysis. However, these conventional methods often involve long extraction times and large solvent volumes. Consequently, unconventional extraction methods have gained popularity due to their ability to achieve higher yield recovery while minimizing time and solvent usage (Fadil et al., 2024). The application of modern extraction techniques and mathematical models for the optimization of extraction procedures helps to overcome the constraints of conventional methods (Zarins et al., 2018; Savic & Gajic, 2020). In this context, ultrasonic-assisted extraction (UAE) has proven to be a particularly efficient innovative method for the extraction of heat-sensitive bioactive compounds by significantly reducing the temperature, extraction time and amount of solvent (Monnier & Schlienger, 2018). Moreover, UAE exhibits exceptional advantages when extracting bioactive components from plants. Complete extraction with higher purity of the final product can be achieved by ultrasonication in a few minutes with high reproducibility, thus reducing the use of organic solvents (Shen et al., 2023).

The acoustic cavitation produced by ultrasound causes high shear in the matrix and can disrupt the cell walls, allowing the solvent to penetrate the plant material and release the intracellular contents (Goltz et al., 2018). UAE of phenolic compounds from plants depends on many operating parameters that need to be optimized (Jahromi, 2019). Thus, the response surface methodology (RSM) has been applied by several authors to optimize the conditions for UAE of total polyphenols from plants (Benarfa et al., 2020; Khan et al., 2025). RSM was developed by mathematicians to reduce the number of experiments (Bilgin et al., 2018). Moreover, it allows finding all possible combinations of factor levels that produce an optimal response (Majeed et al., 2016). Among RSM designs, the Box-Behnken Design (BBD) efficiently fits quadratic models with fewer experimental runs and avoids extreme experimental conditions, making it particularly suitable for process optimization (Buenaño et al., 2024). Both BBD and central composite design (CCD) have been successfully applied for polyphenol extraction in the food industry (Yolmeh & Jafari, 2017). Compared to CCD, BBD is more economical, requires fewer experiments, and effectively evaluates factor interactions without testing extreme points. The practical applicability of BBD has been further confirmed by Boateng (2023) through several successful polyphenol extraction cases. To the best of our knowledge, no work concerning the optimization of the effect of operational parameters influencing the UAE of phenolic compounds from *Phlomis crinita* (Cav.) by RSM has been reported in the literature. The present study is designed to optimize the effect of extraction time, ultrasonic power, plant material/solvent ratio and methanol concentration on the extraction yield, total polyphenol content and antioxidant activity of extracts from *Phlomis crinita* (Cav.) aerial parts using response surface methodology.

MATERIALS AND METHODS

Plant material

Phlomis crinita (Cav.) aerial parts (stems, leaves and flowers) were collected during the flowering stage in May 2017 from Mostaganem (35° 56′ 00″ north, 0° 05′ 00″ east), Algeria. The identification of the investigated plant was carried out by botanists of the National High School of Agronomy of Algiers. The samples were dried at room temperature and in the shade for two weeks and then finely ground.

Ultrasonic-assisted extraction

Ultrasonic-assisted extraction (UAE) is performed using an ultrasonic bath (Fisher Bioblock Transonic TI-H, 25-45 kHz) under the following operating conditions: Degas function, frequency of 45 kHz, temperature of 25 °C, extraction times of 30, 45 and 60 min and ultrasonic power of 20, 40 and 60 W. This extraction also depended on the ratio of plant material to solvent (1:5, 1:12.5 and 1:20 (g mL^{-1})) and the concentration of methanol (50, 70 and 90% (v v^{-1})). For each test, 2 g of *Phlomis crinita* (Cav.) powder was placed in a 100 mL beaker and mixed with 10, 25 or 40 mL of methanol at different concentrations. The beaker containing the mixture was then introduced into the ultrasonic bath to undergo sonication. The resulting mixture was filtered through Whatman Grade 1 filter paper and the recovered solvent was placed in a flask and evaporated using a rotary evaporator. The extract obtained was weighed and stored in the dark at 4 °C until further use. The extraction yield (% (g per 100 g)) was calculated using the following equation:

$$Extraction\ yield\ (\%) = \frac{Weight\ of\ extract\ (g)}{Weight\ of\ sample\ (g)} \cdot 100 \qquad (1)$$

Experimental design

The response surface methodology (RSM) was used to optimize the UAE and thus to find the best possible combination of operating parameters to maximize the extraction yield, total polyphenols content and antioxidant activity of *Phlomis crinita* (Cav.) extracts. RSM is a useful tool to study the efficacy of multiple variables on various responses and compute the optimal levels of variables. It results in highly accurate regression equations and shows the interactions between variables in 3D space (Singh & Kumar, 2023).

In this study, the four-factor Box-Behnken design (twenty-seven trials) was chosen to determine the optimal conditions for the extraction process (Box & Behnken, 1960; Ferreira et al., 2007). The independent variables (X_1, extraction time; X_2, ultrasonic power; X_3, plant material/solvent ratio; X_4, methanol concentration) used in the Box-Behnken design and their three levels, (-1, 0, 1), are presented in Table 1.

Table 1. Levels of independent variables used in the Box-Behnken design

Independent variables	Levels		
	-1	0	+ 1
Extraction time (X_1, min)	30	45	60
Ultrasonic power (X_2, W)	20	40	60
Plant material/solvent ratio (X_3, g mL^{-1})	1:5	1:12.5	1:20
Methanol concentration (X_4, % (v v^{-1}))	50	70	90

The second order polynomial equation relating the extraction yield (Y_1), total polyphenols content (Y_2) and antioxidant activity (Y_3) of *Phlomis crinita* (Cav.) extracts to the independent variables of the UAE process is given below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{i=1}^{3} \beta_{ij} X_i X_j \tag{2}$$

where, Y is the dependent variable (response), β_0, β_i, β_{ii} et β_{ij} are the regression coefficients of the intercept, linear, quadratic and interaction terms respectively and X_i et X_j are the coded values of the independent variables.

Determination of total polyphenols content

The quantification of total polyphenols in *Phlomis crinita* (Cav.) extracts was performed by the Folin-Ciocalteu colorimetric method described by Singleton et al. (1999). A volume of 0.25 mL of extract dissolved in methanol was mixed with 1.25 mL of Folin-Ciocalteu reagent. After 3 minutes of reaction time of the mixture, 1 mL of sodium carbonate solution (Na_2CO_3) at a concentration of 75 g L^{-1} was added. After 30 minutes of incubation in the dark and at room temperature, the absorbance was read at 765 nm. The total polyphenols content of the examined extracts was determined by reference to the Gallic acid calibration curve and the values thus obtained were expressed in milligrams of Gallic acid equivalent per gram of extract (mg GAE g^{-1} extract).

Evaluation of antioxidant activity by the DPPH free radical scavenging test

The antioxidant activity of the different extracts examined was determined by the DPPH (1,1-Diphenyl-2-picryhydrazyl) free radical scavenging test. This test was performed according to the method described by Burits & Bucar (2000), where 25 µl of each of the dilutions of the tested extracts was mixed with 975 µl of the methanolic solution of DPPH (60 µM). After a 30 min incubation period at room temperature, the absorbance was read at 517 nm against a blank. A sample containing the same volumes of DPPH and methanol solution was used as a negative control and BHT as a positive control. The percentage of antioxidant activity was given by the following formula:

$$Antioxidante\ activity\ (\%)$$
$$= \frac{absorption\ of\ the\ blank\ sample - absorption\ of\ the\ tested\ sample}{absorption\ of\ the\ blank\ sample} \cdot 100 \tag{3}$$

The antioxidant activity of the extracts was expressed as IC_{50} (concentration of extract required to reduce 50% of the DPPH radical). A low IC_{50} value indicates a high antioxidant activity of the extract.

Statistical analysis

Statistical analysis of the experimental data was performed using Statistica 10 software (StatSoft Inc., USA). In this study, analysis of variance (ANOVA) was performed to assess the descriptive quality of the predicted quadratic models. The significance of the linear, quadratic and interaction terms of the independent variables of the models was determined by Fisher's *F*-test and the probability value ($p < 0.05$). The data were also subjected to regression analysis using the least squares method to generate

the second order polynomial equation that provides the values of the responses as a function of the independent variables. The coefficient of determination (R^2) was used to define the goodness of fit of the models.

RESULTS AND DISCUSSION

Model fitting

The 27 experiments designed according to the Box-Behnken design and the observed response values (extraction yield, total polyphenols content and antioxidant activity) for *Phlomis crinita* (Cav.) extracts are presented in Table 2.

Table 2. Box-Behnken experimental design and observed response values

Run	Independent variables				Responses		
	X_1	X_2	X_3	X_4	Extraction yield (% (g per 100 g))	Total polyphenols content (mg GAE g^{-1} of extract)	Antioxidant activity (DPPH-IC$_{50}$ (mg L^{-1}))
1	0 (45)	0 (40)	-1 (1:5)	-1 (50)	17.75	171.77	8.62
2	0 (45)	0 (40)	-1 (1:5)	1 (90)	18.05	171.92	8.26
3	0 (45)	0 (40)	1 (1:5)	1 (90)	18.37	173.33	8.12
4	1 (60)	1 (60)	0 (1:12.5)	0 (70)	18.51	174.81	7.58
5	1 (60)	-1 (20)	0 (1:12.5)	0 (70)	18.21	173.41	7.79
6	0 (45)	0 (40)	0 (1:12.5)	0 (70)	18.25	173.30	8.57
7	-1 (30)	0 (40)	1 (1:20)	0 (70)	17.77	171.53	8.49
8	-1 (30)	0 (40)	-1 (1:5)	0 (70)	17.52	169.78	8.69
9	1 (60)	0 (40)	1 (1:20)	0 (70)	18.54	175.75	7.51
10	0 (45)	-1 (20)	-1 (1:5)	0 (70)	17.67	171.01	8.75
11	0 (45)	-1 (20)	1 (1:20)	0 (70)	17.91	171.76	8.62
12	0 (45)	1 (60)	-1 (1:5)	0 (70)	17.85	171.63	8.26
13	0 (45)	0 (40)	0 (1:12.5)	0 (70)	18.27	173.26	8.60
14	-1 (30)	-1 (20)	0 (1:12.5)	0 (70)	17.59	169.17	8.71
15	-1 (30)	0 (40)	0 (1:12.5)	-1 (50)	17.63	169.63	8.75
16	0 (45)	-1 (20)	0 (1:12.5)	-1 (50)	17.79	170.88	8.81
17	0 (45)	1 (60)	0 (1:12.5)	-1 (50)	17.92	171.63	8.13
18	0 (45)	-1 (20)	0 (1:12.5)	1 (90)	18.06	170.93	8.49
19	0 (45)	0 (40)	1 (1:20)	-1 (50)	17.94	172.67	8.45
20	0 (45)	0 (40)	0 (1:12.5)	0 (70)	18.23	173.28	8.54
21	-1 (30)	1 (60)	0 (1:12.5)	0 (70)	17.81	169.86	8.23
22	0 (45)	1 (60)	0 (1:12.5)	1 (90)	18.29	172.12	7.97
23	1 (60)	0 (40)	0 (1:12.5)	1 (90)	18.48	174.57	7.58
24	-1 (30)	0 (40)	0 (1:12.5)	1 (90)	17.95	170.26	8.53
25	1 (60)	0 (40)	0 (1:12.5)	-1 (50)	18.21	174.35	7.79
26	0 (45)	1 (60)	1 (1:20)	0 (70)	18.24	172.19	8.07
27	1 (60)	0 (40)	-1 (1:5)	0 (70)	18.04	174.83	7.82

X_1: Extraction time (min); X_2: Ultrasonic power (W); X_3: Plant material/solvent ratio (g mL^{-1}); X_4: Methanol concentration (% (v v^{-1})).

The fit of the models to the observed experimental data is assessed by analysis of variance (ANOVA) and multiple linear regression analysis. Thus, the analysis of variance (ANOVA) is performed to assess the descriptive quality of the quadratic response surface models obtained from the Box-Behnken experimental design. The ANOVA for the quadratic response surface models of extraction yield, total phenol content and antioxidant activity of *Phlomis crinita* (Cav.) extracts is presented in Table 3. Fisher's high *F-test* reveals that the predicted quadratic models are highly significant ($p < 0.001$) for all observed responses. These results confirm that the fitted models adequately explain the relationship between the independent variables and the observed responses. Furthermore, the multiple linear regression models are applied to analyze the data and determine the regression coefficients of the intercept term and the linear, quadratic and interaction terms of the independent variables in order to determine the different second-order polynomial equations.

Table 3. Analysis of variance (ANOVA) of predicted quadratic models

Sources	Sum of squares	Degree of freedom	Mean of squares	F	p
Extraction yield (% (g per100 g))					
$X_1 + X_1^2$	1.203	2	0.601	255.587	<0.0001***
$X_2 + X_2^2$	0.277	2	0.138	58.997	<0.0001***
$X_3 + X_3^2$	0.457	2	0.228	97.120	<0.0001***
$X_4 + X_4^2$	0.349	2	0.174	74.245	<0.0001***
$X_1 X_2$	0.001	1	0.001	0.679	0.425
$X_1 X_3$	0.015	1	0.015	6.639	0.024*
$X_1 X_4$	0.0006	1	0.0006	0.265	0.615
$X_2 X_3$	0.005	1	0.005	2.390	0.148
$X_2 X_4$	0.002	1	0.002	1.062	0.323
$X_3 X_4$	0.004	1	0.004	1.795	0.205
Residual	0.027	12	0.002		
Lack of fit	0.027	10	0.002		
Pure error	0.000	2	0.000		
Total	2.200	26			
Total polyphenols content (mg GAE g^{-1} of extract)					
$X_1 + X_1^2$	63.235	2	31.617	531.741	<0.0001***
$X_2 + X_2^2$	11.020	2	5.510	92.666	<0.0001***
$X_3 + X_3^2$	3.496	2	1.748	29.400	<0.0001***
$X_4 + X_4^2$	3.069	2	1.534	25.814	<0.0001***
$X_1 X_2$	0.126	1	0.126	2.119	0.171
$X_1 X_3$	0.172	1	0.172	2.896	0.114
$X_1 X_4$	0.042	1	0.042	0.706	0.416
$X_2 X_3$	0.009	1	0.009	0.151	0.703
$X_2 X_4$	0.048	1	0.048	0.814	0.384
$X_3 X_4$	0.065	1	0.065	1.093	0.316
Residual	0.712	12	0.059		
Lack of fit	0.712	10	0.071		
Pure error	0.000	2	0.000		
Total	80.244	26			

Table 3 (continued)

Antioxidant activity (DPPH-IC_{50} (mg L^{-1}))

$X_1 + X_1^2$	3.028	2	1.514	198.249	<0.0001***
$X_2 + X_2^2$	0.779	2	0.389	51.023	<0.0001***
$X_3 + X_3^2$	0.141	2	0.070	9.231	0.003**
$X_4 + X_4^2$	0.264	2	0.132	17.340	<0.0001***
$X_1 X_2$	0.018	1	0.018	2.386	0.148
$X_1 X_3$	0.003	1	0.003	0.396	0.540
$X_1 X_4$	0.00002	1	0.00002	0.003	0.955
$X_2 X_3$	0.0009	1	0.0009	0.117	0.737
$X_2 X_4$	0.006	1	0.006	0.837	0.378
$X_3 X_4$	0.0002	1	0.0002	0.029	0.866
Residual	0.089	12	0.007		
Lack of fit	0.089	10	0.008		
Pure error	0.000	2	0.000		
Total	4.194	26			

X_1: Extraction time (min); X_2: Ultrasonic power (W); X_3: Plant material/solvent ratio (g mL^{-1}); X_4: Methanol concentration (% (v v^{-1})); R^2: Coefficient of determination; ***$p < 0.001$ very highly significant; **$p < 0.01$ highly significant; *$p < 0.05$ significant; $p \geq 0.05$ not significant.

Table 4. Regression coefficients of predicted quadratic models

Sources	Extraction yield (% (g per 100 g))	Total polyphenols content (mg GAE g^{-1} of extract)	Antioxidant activity (DPPH-IC_{50} (mg L^{-1}))
Model	18.250***	173.280***	8.570***
Linear			
X_1	0.310***	2.290***	-0.444***
X_2	0.115***	0.423***	-0.244***
X_3	0.157***	0.524***	-0.095**
X_4	0.163***	0.183*	-0.133***
Quadratic			
X_1^2	-0.096***	-0.220	-0.352***
X_2^2	-0.147***	-1.289***	-0.109*
X_3^2	-0.172***	-0,1933	-0.078
X_4^2	-0.074***	-0.707***	-0.098*
Interaction			
$X_1 X_2$	0.020	0.177	0.067
$X_1 X_3$	0.062*	-0.207	-0.027
$X_1 X_4$	-0.012	-0.102	0.002
$X_2 X_3$	0.037	-0.047	-0.015
$X_2 X_4$	0.025	0.110	0.040
$X_3 X_4$	0.032	0.127	0.007
R^2	0.987	0.985	0.978
R^2 adjusted	0.972	0.978	0.953
R^2 predicted	0.953	0.940	0.921

X_1: Extraction time (min); X_2: Ultrasonic power (W); X_3: Plant material/solvent ratio (g mL^{-1}); X_4: Methanol concentration (% (v v^{-1})); R^2: Coefficient of determination; ***$p < 0.001$ very highly significant; **$p < 0.01$ highly significant; *$p < 0.05$ significant; $p \geq 0.05$ not significant

The results of multiple linear regressions of the predicted quadratic models for extraction yield, total polyphenols content and antioxidant activity of *Phlomis crinita* extracts are given in Table 4. The coefficients of determination (R^2) obtained for

extraction yield, total polyphenols content and antioxidant activity are 0.987, 0.985 and 0.978 respectively. These high values argue for a high quality of the fitted models. The adjusted R^2 values of 0.972, 0.978 and 0.953, found for extraction yield, total polyphenols content and antioxidant activity of the extracts, respectively, indicate that the values predicted by the models are very close to the experimental values.

In the present study, the robustness of the goodness of fit of the quadratic models is justified by the values of the coefficients of determination (R^2) very close to 1 (Azahar et al., 2019; Jagadeesan et al., 2019). The R^2 is defined as the ratio of the sum of squares due to the regression to the total sum of squares and is interpreted as the proportion of the variability in the data explained by the ANOVA (Anuar et al., 2013). It is possible to understand the increasing or decreasing influences of the independent variables on each response by considering the positive and negative signs of each relevant regression coefficient for each factor (Cebi et al., 2019). According to Wani et al. (2017), the best way to visualize the effect of independent variables on responses is to plot the response surfaces of the models. The regression analysis of the results obtained from the implementation of these models generates polynomial equations (using the values of the estimated regression coefficients), from which the optimal conditions of the process are determined (Medina-Torres et al., 2017). The significance of each coefficient was determined by Fisher's *F-test* and *p-value*, and the larger the F-value and the smaller the p-value, the more significant the corresponding coefficient (Anuar et al., 2013).

Effect of ultrasonic-assisted extraction conditions on responses
Effect on extraction yield and total polyphenols content
According to the results reported in Table 2, the extraction yield of *Phlomis crinita* (Cav.) ranged from 17.52 to 18.54% (g per 100 g). The linear, quadratic and interaction effects of the UAE independent variables on the yield of *Phlomis crinita* (Cav.), at a 95% confidence level, are shown in the Pareto chart (Fig. 1). Thus, the extraction time (X_1) is the most influential factor on extract yield, followed by methanol concentration (X_4), plant material/solvent ratio (X_3) and ultrasonic power (X_2).
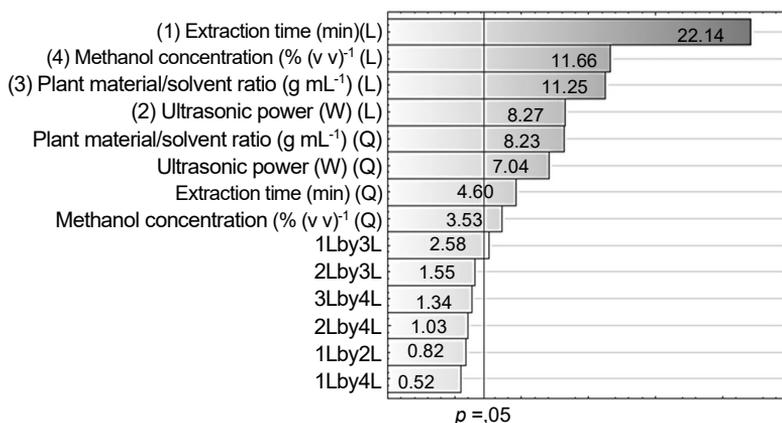
Figure 1. Pareto chart showing effects of ultrasonic-assisted extraction conditions on yield of *Phlomis crinita* (Cav.) extracts.

On the other hand, the regression coefficients given in Table 4 reveal that the extraction yield is affected by all linear and quadratic terms of the models ($p < 0.001$) as well as by the interaction term of extraction time and plant material/solvent ratio (X_1X_3) with low probability ($p < 0.05$). Whereas the rest of the interaction terms (X_1X_4, X_2X_3, X_2X_4 and X_3X_4) had no effect on the extraction yield of *Phlomis crinita* (Cav.) ($p > 0.05$). The mathematical expression of the relationship between the yield of *Phlomis crinita* (Cav.) extracts and the independent variables of the UAE process is presented by the following equation:

$$\begin{aligned} Y_1 = {} & +18.250 + 0.310\,X_1 + 0.115\,X_2 + 0.157\,X_3 + 0.163\,X_4 - 0.09\,X_1^2 \\ & - 0.147\,X_2^2 - 0.172\,X_3^2 - 0.07\,X_4^2 + 0.02\,X_1X_2 + 0.06\,X_1X_3 \quad (4) \\ & - 0.01\,X_1X_4 + 0.03\,X_2X_3 + 0.02\,X_2X_4 + 0.03\,X_3X_4 \end{aligned}$$

where Y_1 is the predicted extraction yield expressed in % (g per 100 g); X_1 is the extraction time (min); X_2 is the ultrasonic power (W); X_3 is the plant material/solvent ratio (g mL^{-1}); X_4 is the methanol concentration (% (v v^{-1})) in coded values.

Also, three-dimensional (3D) response surface curves of the fitted models are constructed by varying two parameters within the limits of the experimental design and keeping the other two constant at their central values in order to highlight the effect of the different independent variables of the UAE on the yield of *Phlomis crinita* (Cav.). Fig. 2 shows the 3D response surfaces of extraction yield as a function of the interaction between two parameters: (a) extraction time and ultrasonic power (X_1X_2), (b) extraction time and plant material/solvent ratio (X_1X_3), (c) extraction time and methanol concentration (X_1X_4), (d) ultrasonic power and plant material/solvent ratio (X_2X_3), (e) ultrasonic power and methanol concentration (X_2X_4) and (f) plant material/solvent ratio and methanol concentration (X_3X_4). According to Fig. 2, it is observed that increasing the values of extraction time from 30 to 60 min, ultrasonic power from 20 to 40 W, plant material/solvent ratio from 1:5 to 1:20 and methanol concentration from 50 to 70% (v v^{-1}) rapidly increases the extraction yield of *Phlomis crinita* (Cav.). However, increasing the ultrasonic power from 40 to 60 W and the methanol concentration from 70 to 90% (v v^{-1}) causes the extraction yield to decrease.
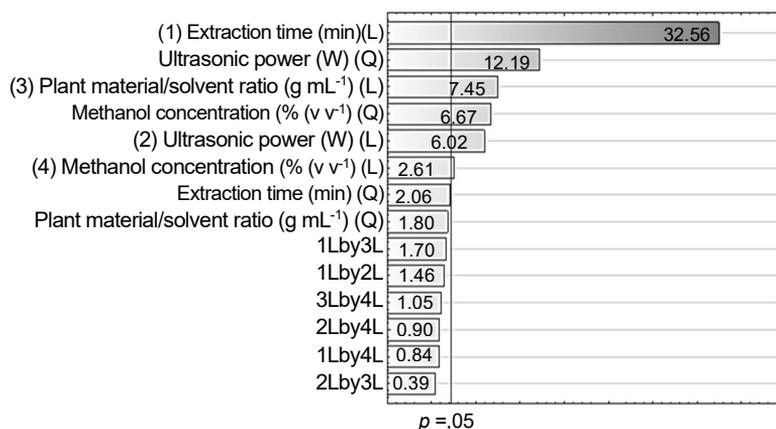
The total polyphenols content of *Phlomis crinita* (Cav.) extracts ranged from 169.17 to 175.75 mg GAE g^{-1} extract (Table 2). The effect of UAE parameters on the total polyphenols content of *Phlomis crinita* (Cav.) extracts is illustrated in Fig. 3, 4. It is found that the extraction time (X_1) is the factor with the strongest influence on the total polyphenols content of the examined extracts, followed by the plant material/solvent ratio (X_3), the ultrasonic power (X_2) and the methanol concentration (X_4). These results are confirmed by the regression coefficients given in Table 4 which indicate the significant contribution of all linear terms (X_1, X_2, X_3 and X_4) and quadratic terms of ultrasonic power (X_2^2) and methanol concentration (X_4^2) on the total polyphenols content of *Phlomis crinita* (Cav.) extracts ($p < 0.05$). However, the quadratic terms of extraction time (X_1^2) and plant material/solvent ratio (X_3^2) as well as all interaction terms (X_1X_3, X_1X_4, X_2X_3 and X_2X_4) showed no effect on the total polyphenols content of *Phlomis crinita* (Cav.) extracts ($p > 0.05$).
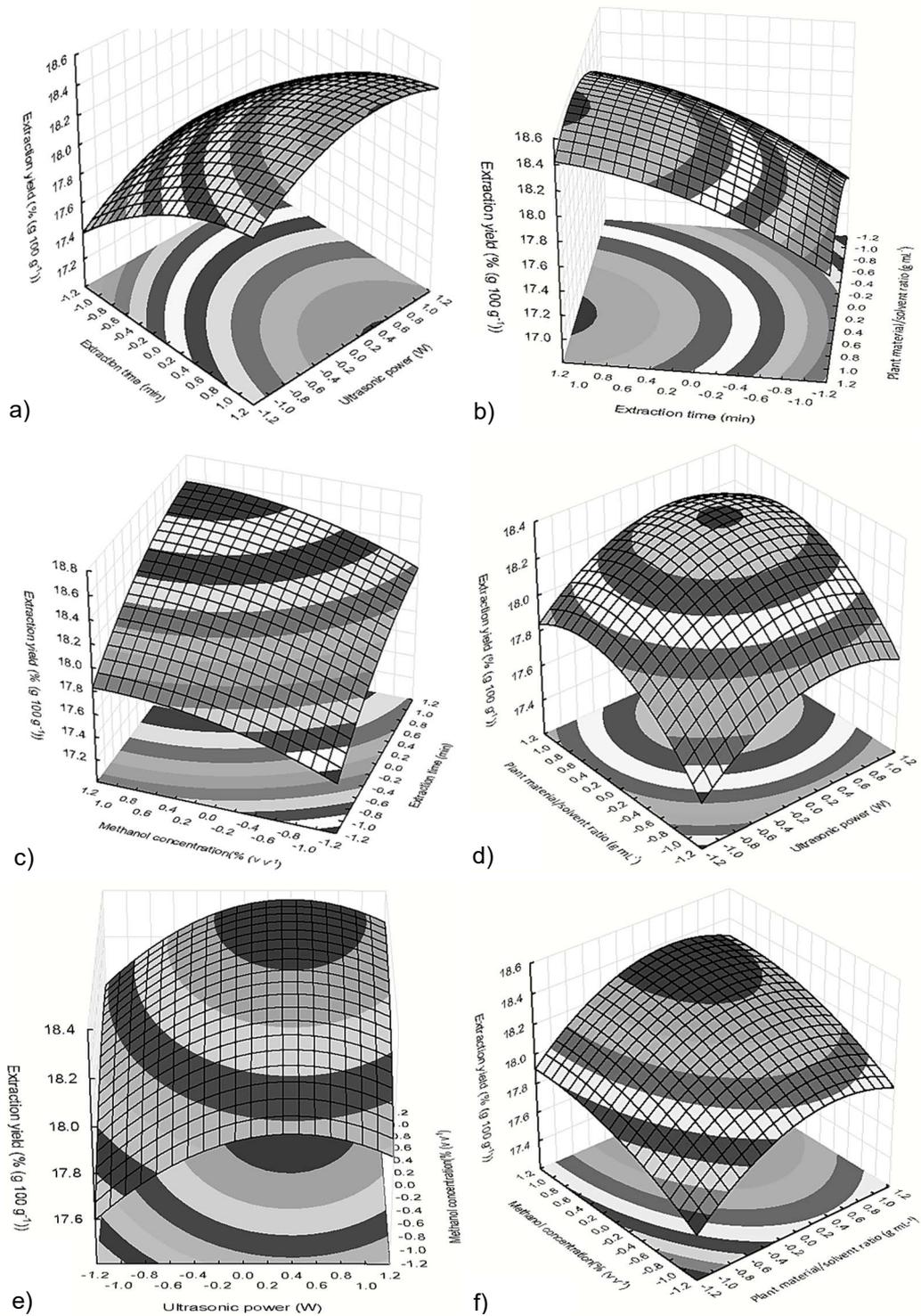
Figure 2. 3D plots of *Phlomis crinita* (Cav.) extraction efficiency as a function of the combined effect of independent variables.

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The second-order polynomial equation representing the total polyphenols content of *Phlomis crinita* (Cav.) extracts as a function of the independent variables is as follows:

$$Y_2 = +173.280 + 2.290\,X_1 + 0.423\,X_2 + 0.524\,X_3 + 0.183\,X_4 - 0.220\,X_1^2$$
$$- 1.289\,X_2^2 - 0.193\,X_3^2 - 0.707\,X_4^2 + 0.177\,X_1X_2 - 0.207\,X_1X_3 \quad (5)$$
$$- 0.102\,X_1X_4 - 0.04\,X_2X_3 + 0.110\,X_2X_4 + 0.127\,X_3X_4$$

Figure 3. Pareto chart showing effects of ultrasonic-assisted extraction conditions on total polyphenols content of *Phlomis crinita* (Cav.) extracts.
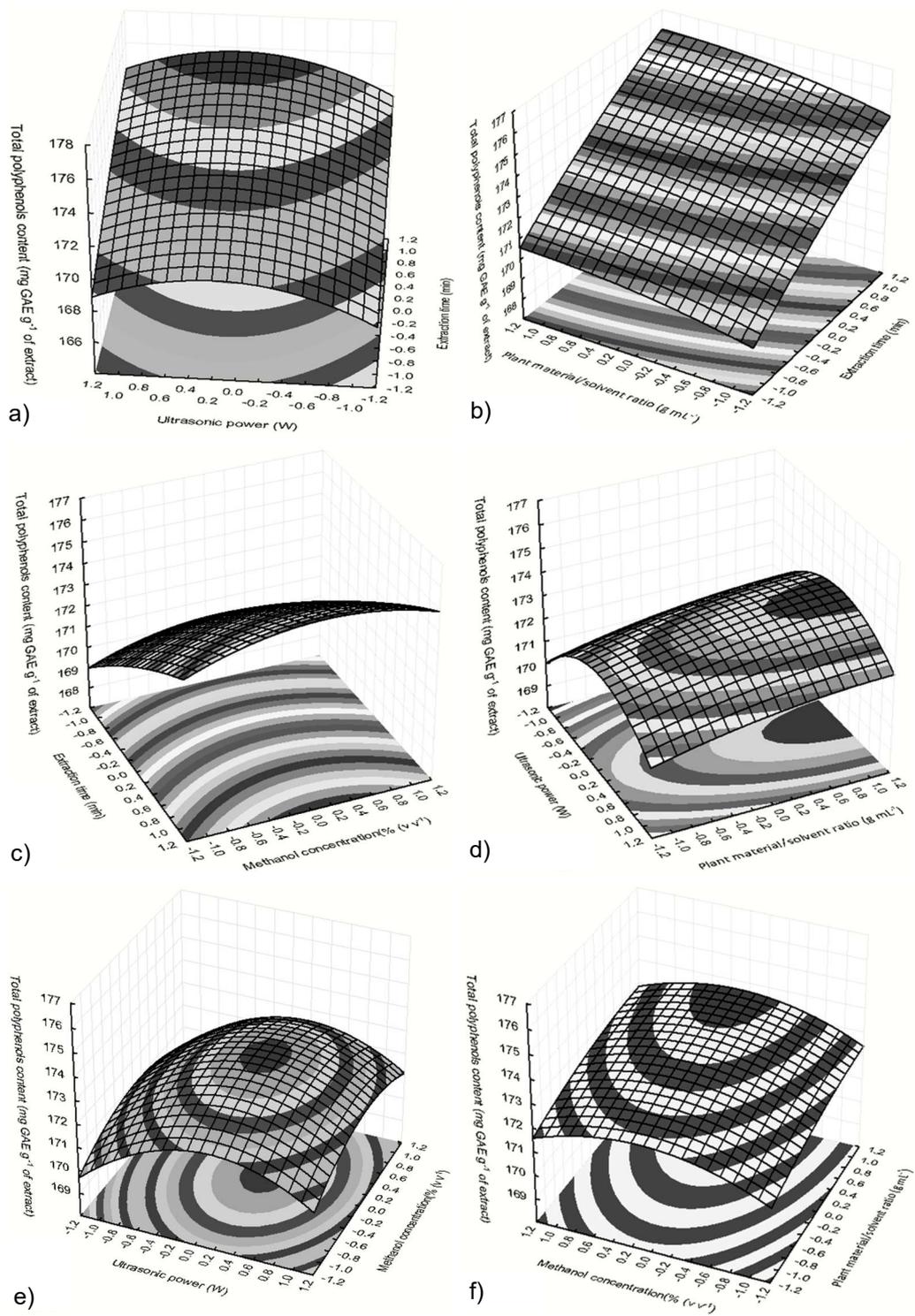
Effect on antioxidant activity

The IC_{50} values of the different *Phlomis crinita* (Cav.) extracts vary between 7.51 and 8.81 mg L^{-1} (Table 2). The positive or negative effect of the UAE independent variables on the antioxidant activity of *Phlomis crinita* (Cav.) extracts is shown in Figs 5, 6. We can notice that the extraction time (X_1) is the most influential on the antioxidant activity of *Phlomis crinita* (Cav.) extracts, followed by the ultrasonic power (X_2), the methanol concentration (X_4) and the plant material/solvent ratio (X_3). Table 4 of the multiple linear regression analysis shows the significant contribution of all linear terms (X_1, X_2, X_3 and X_4) ($p < 0.01$) and quadratic terms of extraction time (X_1^2), ultrasonic power (X_2^2) and methanol concentration (X_4^2) ($p < 0.05$) on the antioxidant activity of the extracts examined. Whereas, the quadratic term of plant material/solvent ratio (X_3^2) as well as all interaction terms (X_1X_3, X_1X_4, X_2X_3 and X_2X_4) revealed no effect on the free radical scavenging activity of *Phlomis crinita* (Cav.) extracts ($p < 0.05$).

The second-order polynomial equation for the antioxidant activity of *Phlomis crinita* (Cav.) extracts as a function of the independent variables is described below:

$$Y_3 = +8.570 - 0.444\,X_1 - 0.244\,X_2 - 0.09\,X_3 - 0.133\,X_4 + 0.352\,X_1^2 - 0.109\,X_2^2$$
$$+ 0.078\,X_3^2 + 0.098\,X_4^2 - 0.09\,X_1X_2 + 0.06\,X_1X_3 - 0.02\,X_1X_4 \quad (6)$$
$$- 0.015\,X_2X_3 + 0.04\,X_2X_4 + 0.007\,X_3X_4$$

The influence of different UAE parameters on the yield, total polyphenols content and antioxidant activity of some plant extracts has been the subject of several studies (Anaya-Esparza et al., 2019; Bouaoudia-Madi et al., 2019). According to Liyana-Pathirana & Shahidi (2005), the extraction time is an important parameter to optimize the recovery of phenolic compounds and the antioxidant capacity of extracts.

Figure 4. 3D plots of polyphenols content of *Phlomis crinita* (Cav.) extracts as a function of the combined effect of independent variables.

From those results, we notice that the extraction yield, total polyphenols content and antioxidant activity of *Phlomis crinita* (Cav.) extracts are significantly influenced by the extraction time. Indeed, an increase in the extraction time towards the upper level of its range (60 min) led to higher responses. Our results are in agreement with those found by Falleh et al. (2012), who found that *Mesembryanthemum edule* (L.) extract obtained by longer sonication time exhibited better total polyphenols content and high antiradical activity. Chooklin et al. (2016) observed a substantial increase in phenolic compound contents and antioxidant activities of native plant extracts with an increase in extraction period from 5 to 60 minutes. Altemimi et al. (2015) reported that the extraction yield, total polyphenols content and antioxidant activity of spinach extracts increased gradually with increasing the exposure time from 5 to 30 min at a temperature of 40 °C and a power setting of 50% for frequencies of 37 and 80 kHz. Liu et al. (2018a) noted that a long extraction time increased the total polyphenols content of *Sorghum* extracts. As the extraction time increases, the probability of phenolic compounds coming into contact with the solvent also increases, leading to higher extraction efficiency (Gam et al., 2020). Also, ultrasound propagating through the solvent produces cavitation bubbles that accelerate solute dissolution and diffusion as well as heat transfer, which improves extraction efficiency (Zhang et al., 2018). Furthermore, Pareto plots (Fig. 1, 3, 5) showed that extraction time was the most influential factor on the extraction yield, total polyphenols content and antioxidant activity of *Phlomis crinita* (Cav.) extracts. These results are in agreement with those obtained by Kaleem et al. (2019) who reported that extraction time had the maximum impact on the extraction of phenolic compounds from *Perlette grape*. Therefore, extraction time is an important variable in the ultrasonic extraction process of phenolic compounds from plant materials (Ilghami et al., 2015; Sepelevs et al., 2020).
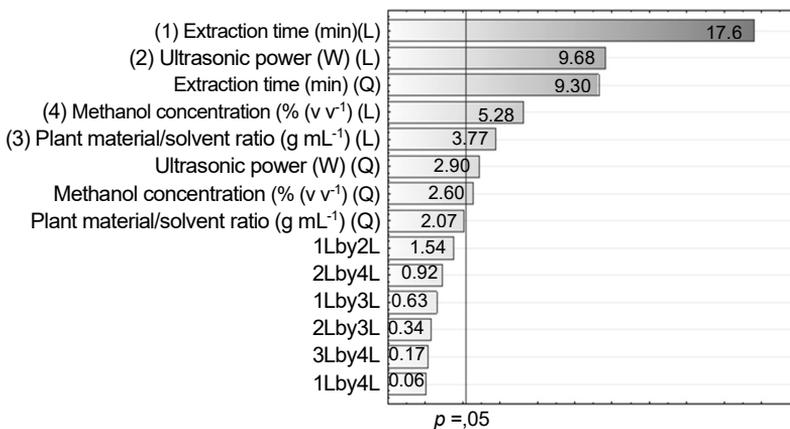
Figure 5. Pareto chart showing effects of of ultrasonic-assisted extraction conditions on antioxidant activity of *Phlomis crinita* (Cav.) extracts.

The effect of ultrasonic power on total polyphenols content and antioxidant activity has been confirmed by several authors (Yang et al., 2017; Pham et al., 2020). According to Mahalleh et al. (2020), the intensification of the polyphenols extraction process could

be explained by the cavitation effects of ultrasound which can enhance the hydration and fragmentation process while increasing the mass transfer of solutes to the extraction solvent. Our results showed that the observed responses improved with increasing ultrasonic power up to its central value. Above 40 W, increasing the ultrasonic power has no effect on the responses. The results obtained are in line with those of Bakht et al. (2019), who reported that 40% ultrasonic power resulted in the highest extraction yield, total polyphenols content and free radical scavenging activity in the tea extracts examined. Similar effects were also observed in some studies regarding the total polyphenols content of *Cimicifug aerhizome* (Liu et al., 2015) and *Citrus hystrix* (Liew et al., 2020) extracts. The decrease in responses recorded in the range of 40–60 W is probably due to the degradation of thermally unstable phenolic compounds caused by the high temperature and pressure produced during the collapse of cavitation bubbles (Ravanfar et al., 2020).

The solid/liquid ratio is an important factor influencing the extraction of the target compound as it relates to the contact area between the raw material and the solvent (Wang et al., 2016). According to Yang et al. (2020), this ratio plays a considerable role in the extraction results. The solid-liquid ratio can significantly increase the extraction of polyphenols but, on the other hand, the amount of solvent is an important factor that needs to be emphasized, especially to reduce process costs and residual materials (Carniel et al., 2018). We found from our results that the extraction yield, total polyphenols content and antioxidant power of *Phlomis crinita* (Cav.) extracts are proportional to the increase of the plant material/solvent ratio from 1:5 to 1:20. These results are in agreement with those of Cui et al. (2017), who reported that increasing the solid/liquid ratio from 1:15 to 1:25 resulted in an improvement in the total polyphenols content of *Perilla* leaf extracts. Mehmood et al. (2018) found that the ratio solute to solvent of 1:30 g mL^{-1} resulted in higher values of yield, total polyphenols content and antioxidant activities of *Sunflower* extracts. According to Zhang et al. (2020), this is due to the fact that higher values of the plant material/solvent ratio result in higher concentration differences, which facilitates the diffusion of the components in the solvent and accelerates mass transfer. In general, the higher the solute-to-solvent ratio, the higher the amounts of phenolic compounds dissolved in the solvent (Chen et al., 2020). In a study about extraction of *Thuya orientalus* leaves, a solvent-to-sample ratio of 35 mL g^{-1} resulted in the highest TFC value, while a ratio of 25 mL g^{-1} resulted in the lowest TFC value (Imtiaz et al., 2023), therefore, the authors concluded that a higher solvent-to-sample ratio can increase the solubility and diffusion of flavonoids from the plant matrix, leading to higher TFC values.

Nayak et al., noted that at a higher solvent-to-sample ratio, more solvent is available for a certain amount of the feed causing greater solubilization of the target phytochemicals such as TPC content (Nayak et al., 2015).

Solvent concentration is considered as one of the important parameters in UAE (Zhou et al., 2019). Many reports indicate that aqueous mixtures of organic solvents are the most suitable for the extraction of phenolic compounds from plants (Venkatesan et al., 2019). Our results showed that the extraction yields, total polyphenols content and antioxidant capacity of *Phlomis crinita* (Cav.) extracts increased with increasing methanol concentration to its central level (70% (v v^{-1})).
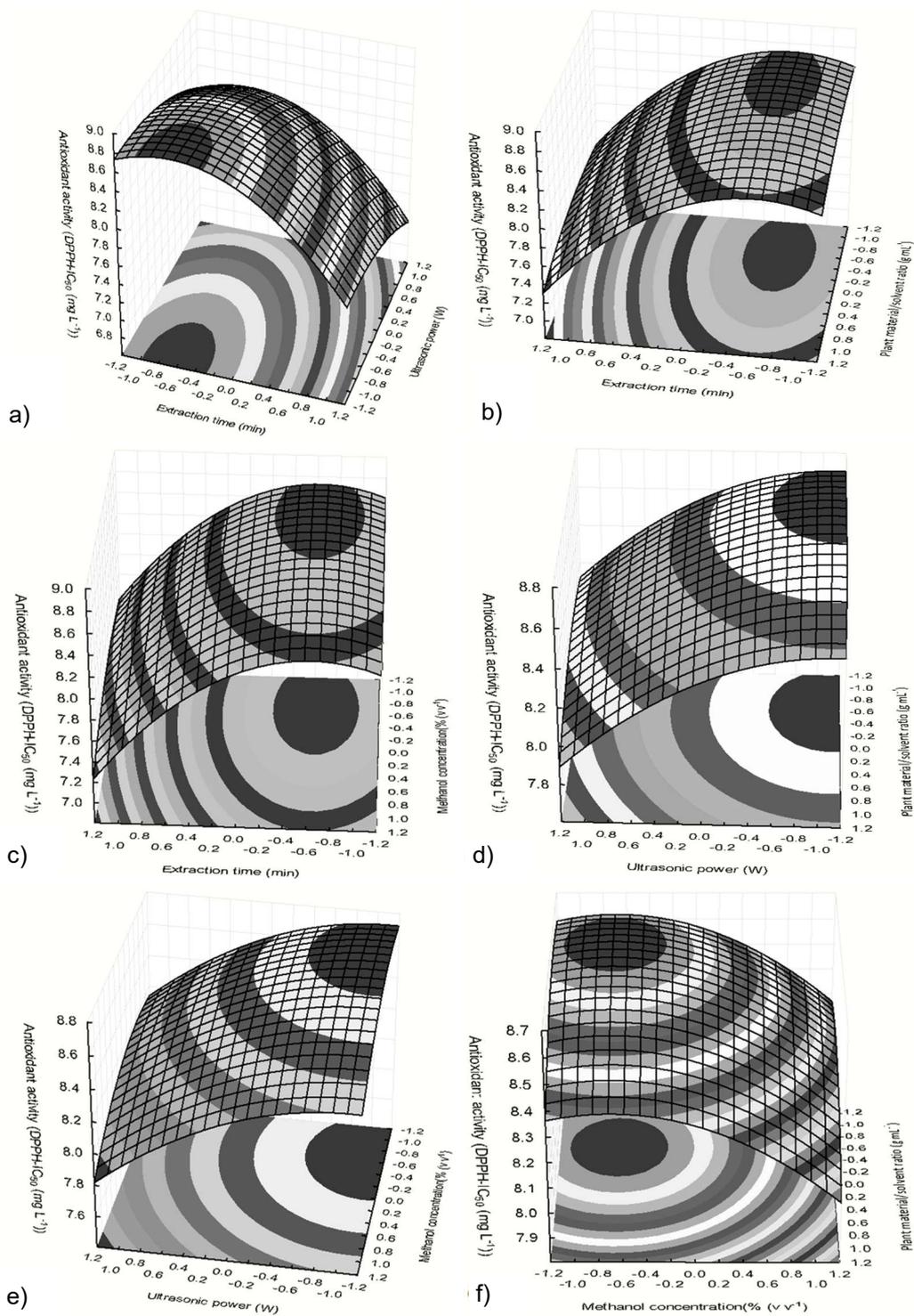
Figure 6. 3D plots of antioxidant activity of *Phlomis crinita* (Cav.) extracts as a function of the combined effect of independent variables.

However, once this level was exceeded, the observed response values decreased. These results are similar to those obtained by Liu et al. (2018b), who reported that the extraction yield increased as the solvent concentration increased but decreased when the concentration reached its central value due to the decrease in the polarity of the extraction solution and the fact that other substances compete for extraction from *Phyllanthus urinaria*. According to Thouri et al. (2017), the change in the polarity of the solvent influences the dissolution of some antioxidant compounds and the estimation of antioxidant activity. According to Palma et al. (2023), several authors have indicated that a mixture of alcohol and water is more effective for phenolic compounds extraction than alcohol alone, this is due to the alcohol dielectric constant of the extraction solvent, allowing the analytes to attach to the solvent molecules, while water creates a more polar medium and the breaking of hydrogen bonds facilitates the extraction of the most and least polar polyphenolic compounds (Palma et al., 2023).

Optimization of UAE parameters by RSM led to an extraction yield of 18.54% (g per 100 g)), a total polyphenols content of 175.75 mg GAE g^{-1} extract and a high antiradical activity with an IC$_{50}$ of 7.51 mg L^{-1} of *Phlomis crinita* (Cav.) extracts. We noticed that the total polyphenols content of our extracts is higher than that reported by Merouane et al. (2018) (81.80 ± 2.25 µg GAE mg^{-1} extract) for the extract obtained by maceration of *Phlomis crinita* (Cav.) leaf powder. We also found that the antioxidant activity of the examined extracts is higher than that of BHT (IC$_{50}$ of 27.99 mg L^{-1}), used as a positive control for this study. Furthermore, the DPPH free radical scavenging activity of our extracts is significantly higher than Tamert & Latrech (2016) results for extracts obtained by maceration (IC$_{50}$ = 1.12 ± 0.001), decoction (IC$_{50}$ = 1.78 ± 0.001) and infusion (IC$_{50}$ > 2) of *Phlomis crinita* (Cav.) powder. The high free radical scavenging capacity of the examined extracts could be attributed to their high content of total polyphenols. According to Johari & Khong (2019), high total polyphenols content provides high antioxidant capacity due to the positive linear correlation between the two parameters. Thus, phenolic compounds are important antioxidants responsible for deactivating free radicals due to their ability to donate hydrogen atoms to free radicals (Aryal et al., 2019).

Evaluation and verification of optimal conditions

According to the RSM results, the optimal UAE conditions leading to the best responses are an extraction time of 60 min, an ultrasonic power of 40 W, a plant material/water ratio of 1:20 g mL^{-1} and a methanol concentration of 70% (v v^{-1}).

Table 5. Predicted and experimental values of responses obtained under optimal extraction conditions

	Extraction yield (% (g per 100 g))	Total polyphenols content (mg GAE g^{-1} of extract)	Antioxidant activity (DPPH-IC$_{50}$ (mg L^{-1}))
Predicted values	18.50	175.46	7.56
Experimental values	18.54	175.75	7.51

In order to confirm the relevance of these results, the values of the experimental responses obtained under the optimal extraction conditions are compared with those predicted by the fitted quadratic models (Table 5). It is found that the difference between the predicted and experimental values is very small, which confirms the

reliability and validity of the quadratic response surface models obtained from the Box-Behnken experimental design in predicting the extraction yield, polyphenols content and antioxidant activity of *Phlomis crinita* (Cav.) extracts.

CONCLUSIONS

The interest of the present work lies in the search for efficient methods to optimize the extraction of natural bioactive compounds, endowed with strong antioxidant power, from Algerian medicinal flora. The optimization of the UAE of *Phlomis crinita* (Cav.) extracts by RSM revealed that the extraction yield, total polyphenols content and antioxidant activity increase rapidly with increasing extraction time from 30 to 60 min, ultrasonic power from 20 to 40 W, plant material/solvent ratio from 1:5 to 1:20 and methanol concentration from 50 to 70% (v v^{-1}). The optimal conditions provided by the Box-Behnken design, giving the best responses, were an extraction time of 60 min, an ultrasonic power of 40 W, a plant material/solvent ratio of 1:20 g mL^{-1} and a methanol concentration of 70% (v v^{-1}). In this study, the RSM was able to effectively predict the optimal responses of the fitted quadratic models. Also, UAE is a suitable technique to maximize the extraction yield (18.54% (g per 100 g)), polyphenols content (175.75 mg GAE g^{-1} extract) and free radical scavenging activity (IC_{50} of 7.51 mg L^{-1}) of the investigated plant extracts, while reducing the extraction time compared to conventional methods. Regarding antioxidant activity, the results showed that *Phlomis crinita* (Cav.) extracts exhibit high DPPH free radical scavenging activity due to their high content of total polyphenols. These extracts could be used as natural antioxidants in the food, pharmaceutical, and cosmetic industries, serving as functional food ingredients, nutraceuticals, or natural preservatives, providing a safe alternative to synthetic additives while promoting health and extending product shelf life.

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