

OPTIMIZATION OF PHENOLIC COMPOUND RECOVERY AND ANTIOXIDANT ACTIVITY FROM *FUMARIA OFFICINALIS* L. USING RESPONSE SURFACE METHODOLOGY

SABIHA KHAMTACHE-ABDERRAHIM¹, SONIA YAHIAOUI², AMAR OTMANI²,
MOSTAPHA BACHIR-BEY*²

¹Laboratoire de Biotechnologies des plantes et Ethnobotanique, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algérie

²Laboratoire de Biochimie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria

*corresponding author: bachirbeymustapha@gmail.com, mostaphabachirbey@univ-bejaia.dz

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Abstract

The purpose of this study was to optimize the extraction of antioxidant phenolic compounds from *Fumaria officinalis* by two approaches, single-factor experiment and response surface methodology (RSM). After extraction optimization, the content of polyphenols, flavonoids, and tannins was performed. Antioxidant activities of optimal extract were evaluated by DPPH, ABTS, and iron chelating activities. Single-factor experiments showed that all extraction parameters had significant effects ($p < 0.05$) on total phenolic content and free radical scavenging activity. From the optimization process using response surface methodology through Box-Behnken design regarding the three selected significant parameters (solvent concentration, extraction time, and temperature), it was established that the best extraction conditions were 47% methanol, 54.40°C, and 168.02 min, respectively. Under these optimal conditions, the recorded values for TPC and DPPH scavenging activity were successively 1510.10 mg GAE/100g DW and 69.85%. These results were in accordance with model predictions indicating that the model was suitable for the extraction process. Furthermore, higher flavonoids and tannins contents and strong iron-chelating and ABTS scavenging activities were obtained.

Keywords: *Fumaria officinalis*, optimization, single-factor experiments, response surface methodology, phenolic compounds, antioxidant activity

Introduction

There has been growing interest in researching and developing natural of natural antioxidants plant-based extracts to obtain bioactive compounds for pharmaceutical

and food industries (Hrelia and Angeloni, 2020). These compounds have demonstrated antioxidant characteristics that help disease risk reduction and prevent several human pathologies such as cancer, inflammation, and chronic diseases (Teodoro, 2019). In this respect, *Fumaria officinalis* L., which belongs to the family of Fumariaceae and has a wide distribution in Europe and Northern Africa (Suau *et al.*, 2005), is one of the oldest medicinal plants rich in phenolic compounds and alkaloids (Khamtache-Abderrahim *et al.*, 2016). Moreover, many therapeutic activities attributed to *F. officinalis* were validated by intensive recent research and clinical studies, such as antibacterial, analgesic, antioxidant, and beneficial effects in irritable bowel syndrome and biliary disorders (Al-Snafi, 2020), a strong anticancer activity (Adham *et al.*, 2021), aphrodisiac, antidiabetic, and anti-inflammatory effects (Raafat and El-Zahaby, 2020). These activities are due to the diverse bioactive molecules such as phenolic acids, tannins, coumarins, terpenoids, and alkaloids.

Phenolic compounds have gained high interest and attracted more and more researchers and industrials due to their biological activity. The extraction is the first step for the recovery of these bioactive compounds that must be performed adequately. The extraction process can be achieved following the sequential method or with experimental design. The first approach allows studying only one factor at a time and omits the interaction between parameters, however, it permits fixing the lower and upper levels of each factor in order to limit the study range for the experimental design. This last approach has been demonstrated to be a helpful tool that can overcome the difficulties encountered in one-factor experiments, minimizing the number of trials, allowing the relationship between responses and factors to be determined, and also permits the checking of possible interaction between variables (Bezerra *et al.*, 2008; Jorge *et al.*, 2013).

The extraction of phenolic compounds from *F. officinalis* has been performed by different solvents (water alone, methanol, and ethanol or their mixture with water) using different methods such as maceration, sonication, Soxhlet, and water bath during various extraction times (from 15 min to 10 days) with using different temperatures (20-95°C) (Păltinean *et al.*, 2017; Edziri *et al.*, 2020). In order to propose the best procedure for phenolic recovery from fumitory, it is recommended to optimize the extraction process. As far as we know, no study concerned the optimization of extraction of phenolic compounds from *F. officinalis* by response surface methodology (RSM); therefore, the main objective of this study was to optimize phenolic compounds extraction using the sequential procedure followed by RSM. Afterward, the optimal extract was evaluated for its total polyphenols, flavonoid, and tannin contents, and antioxidant activity by DPPH, ABTS, and iron-chelating assays.

Materials and methods

Plant material

The harvest of the aerial part of *Fumaria officinalis* was carried out from the region of Akbou (Bejaia, Algeria) during April 2019. Samples were dried in a free area

and grounded with a crusher (IKA A11, Staufen, Germany). The powder obtained was sieved using an electric sieve and the powder with a diameter from 250 to 45 μm was recovered and stored at 4°C until further analysis.

Chemicals

Folin-Ciocalteu reagent was obtained from Biochem, Chemopharma (Montreal, Quebec); sodium carbonate was purchased from Biochem, Chemopharma (Cosne Sur Loire, France), gallic acid, acetone (99.78% purity), methanol (99.7% purity), and ethanol (99.5% purity) were from Prolabo (VWR International S.A.S, Fontenay-sous-Bois, France). All other chemicals were purchased from Sigma Chemical (Sigma-Aldrich, Sternheim, Germany).

Extraction of phenolic compounds

Sample powder was weighed (0.2g) and homogenized in 10 mL of extraction solvent (water, 50% methanol, 50% ethanol, and 50% acetone) the mixture was stirred in a water bath shaker equipped with a stirrer (WB 22, Memmert, Schwabach, Germany). After centrifugation at 5000 rpm for 10 min, the mixture was filtered through Whatman 4 filter paper.

Single-factor experiment

Five extraction parameters were considered to optimize the extraction of phenolic compounds and the free radical scavenging activity of *F. officinalis* by a single factor experiment. The optimized parameters were solvent type (50% acetone, 50% ethanol, 50% methanol, and water), solvent concentration (25 – 00 %, v/v), sample to solvent ratio (0.2/10 - 0.8/10 g/mL), extraction temperature (25 - 70°C), and extraction time (30 - 120 min).

Response surface methodology experiment

The variables studied by response surface methodology were methanol concentration (x_1 , %, v/v), extraction temperature (x_2 , °C), and extraction time (x_3 , min). The three considered levels of the studied variables, the lower (-1), the central (0), and the upper (+1), were set for methanol concentration at 20, 50, and 80%, extraction temperature at 35, 55, and 75°C, and extraction time at 50, 120, and 190min. The Box-Behnken design with three central points was adopted for studying two responses, total phenolic content (TPC) and DPPH free radical scavenging activity (FRSA). The responses were given as a second-degree polynomial model according to equation 1.

$$y = a_0 + \sum_{i=1}^3 a_i x_i + \sum_{i=1}^3 a_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=1}^3 a_{ij} x_i x_j (i \neq j) \quad (1)$$

Where a_0 , a_i , a_{ii} , and a_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively, while x_i and x_j are the independent variables.

Verification of the model

The optimum conditions for phenolic extraction and antioxidant activity of *F. officinalis* were obtained using the predictive equations of RSM. In addition,

experimental and predicted phenolic compounds and free radical scavenging activity (DPPH) were compared to check model validity.

Determination of antioxidant parameters

Total phenolic content

TPC was estimated according to the method of Naithani *et al.* (2006). A volume of 100 µL of the extract was mixed with the same volume of the Folin-Ciocalteu reagent (50%) and 2 mL of sodium carbonate (2%, w/v). After 30 minutes of incubation in darkness, the absorbance was measured at 750 nm. Gallic acid was used as a standard and the results were expressed as milligrams of gallic acid equivalent per 100 grams of dry weight (mg AGE/100g DW).

Total flavonoid content

The total flavonoid content (TFC) was measured according to the protocol described by Quettier-Deleu *et al.* (2000). In brief, a volume of 1.5 mL of extract was mixed with an equal volume of aluminum chloride solution (2%, w/v). After incubating for 15 min, the absorbance was measured at 430 nm, and the results were expressed as mg of quercetin equivalent per 100 grams of dry weight (mg QE/100g DW).

Total tannin content (TTC)

The method for estimating the content of condensed tannins is proposed by Hillis and Swain (1959). The vanillin reagent was prepared by dissolving 1 g of the vanillin in 100 mL of sulfuric acid (70%) then 2 mL of this reagent was mixed with 1 mL of extract. After incubation at 50°C for 20 min, the absorbance was measured at 500 nm. The results were expressed in gram catechin equivalent per 100 grams of dry weight (mg CE/100g DW).

Free radical scavenging activity (FRSA)

DPPH assay was performed according to Brand-Williams *et al.* (1995). A volume of 100µL of the extract was mixed with 1000µL of DPPH solution (60 mM in methanol). After 30 min of incubation, the absorbance was measured at 515 nm. The results were expressed as a percentage according to equation 2.

$$\text{DPPH (\%)} = \frac{A_c - A_e}{A_c} \times 100 \quad (2)$$

where Ac: Absorbance of the control, Ae: Absorbance of the extract.

ABTS radical scavenging assay

The scavenging of the cationic radical ABTS^{•+} was determined according to Re *et al.* (1999). Briefly, 0.1 mL of ethanolic solution of ABTS was mixed with 20 µL of extract and the absorbance was read at 734 nm after seven minutes of incubation. The percentage of radical inhibition was calculated using equation 3.

$$\text{ABTS (\%)} = \frac{(A_c - A_e)}{A_c} \times 100 \quad (3)$$

where Ac: Absorbance of the control, Ae: Absorbance of the extract.

Ferrous ion chelating activity

The Fe²⁺ chelation of extract was measured according to the procedure described by Wang *et al.* (2008). A volume of extract (500 µL) was mixed with 50 µL of iron (II) chloride (2 mM) and 1.6 mL of distilled water. After 5 min of incubation, 100 µL of ferrozine (5 mM) were mixed and left for an additional 5 min. The absorbance was then measured at 562 nm against a blank. The chelating activity of the sample was calculated by equation 4.

$$\text{Chelating activity (\%)} = \frac{1-(A_1-A_2)}{A_0} \times 100 \quad (4)$$

where A₀: Absorbance of the control (without extract), A₁: Absorbance of the extract, A₂: absorbance without ferrozine.

Statistical analysis

All analyses were carried out in triplicate and the results were expressed as mean ± standard deviation. Statistical analyses were performed using Statistica software (version 5.5.fr, Stat Soft, Inc, Tulsa, USA). One-way analysis of variance (ANOVA) with the LSD (least significant difference) test was used to find out the significant differences ($p < 0.05$) between means. The JMP10 software (statistical analysis system Inc., SAS) was used to analyze the results of the response surface methodology experiment.

Results and discussion

Single-factor experiments

Effect of solvent type on total phenolic content and free radical scavenging

The effect of solvent type on TPC and FRSA from *F. officinalis* extract was represented in Figures 1a and 2a, respectively. It can be seen that solvent type significantly affected polyphenols recovery and DPPH scavenging activity. The four solvents can be ranked according to their extraction efficiency in the following order: 50:50 methanol-water mixture > 50:50 ethanol-water mixture > 50:50 acetone-water mixture > water. The highest TPC and a strong DPPH scavenging activity were obtained using methanol with 1324.92 mg GAE/100g DW and 68.07%, respectively. Our results were in agreement with those obtained by Do *et al.* (2020). Differences in efficiency can be explained by the higher affinity of phenolic compounds for polar solvents. In fact, the solubility of phenolic compounds is influenced by the polarity of the solvent (Naczka and Shahidi, 2006). However, other factors such as swelling of the plant material and weakening of the solute–matrix interactions could also be involved (Zuorro *et al.*, 2014). Therefore, methanol was selected for further experiments.

Effect of methanol concentration on total phenolics content and free radical scavenging

The results of the effect of methanol concentration (25, 50, 75, and 100%, v/v) on TPC and DPPH scavenging activity were presented in Figures 1b and 2b, respectively. The results showed no significant difference between 50 and 75% on FRSA. However, the highest TPC (1345.46 mg GAE/100g DW) of *F. officinalis* was obtained with 50% methanol. On the other hand, the lowest values (908.34 mg

GAE/100g DW for TPC and 47.17 % for FRSA) were obtained using 100% methanol. Our results were in accordance with those obtained by Hashim *et al.* (2016), who found that 50% methanol was the best solvent for phenolic compounds extraction from beetroot peel and pulp. According to Garcia-Salas *et al.* (2010), organic solvent and water mixtures have shown to be more effective in extracting phenolic compounds than mono solvents.

Effect of sample to solvent ratio on total phenolics content and free radical scavenging

Based on previous results concerning the best solvent type and concentration which was 50% methanol, the effect of sample to solvent ratio on TPC and FRSA was evaluated. The extraction was repeated by varying the sample to the solvent ratio (0.2, 0.4, 0.6, and 0.8 g/10 mL) while fixing the extraction time at 30 minutes. The statistical analysis (one-way ANOVA) showed that the sample to solvent ratio had a significant effect ($p < 0.05$) on TPC and FRSA (Figures 1c and 2c). A high TPC and a strong free radical scavenging were obtained with the ratio of 0.2g/10 mL with values of 1339.63 mg GAE/100g DW and 72.54%. These results agreed with those obtained by Chaalal *et al.* (2012), who found that 0.2g/10 mL was the best ratio for phenolic compounds extraction from prickly pear (*Opuntia ficus indica*) seeds. As shown in Figures 1c and 2c, the increase of sample to solvent ratio induced the decrease of TPC and FRSA. This can be explained by the mass transfer principle according to Fick's law based on the assumption of a linear relation between solute dispersive mass flux and concentration gradient existing between solvent and solid matrix (Meziant *et al.*, 2018).

Effect of temperature on total phenolics content and free radical scavenging

In order to study the effect of heating on TPC and DPPH scavenging activity of *F. officinalis*, the extractions were performed with different temperatures ranging from 25 to 70°C (Figures 1d and 2d). Our results indicated that the best extraction temperature for phenolic compounds was 55°C with 1544.22 mg GAE/100g DW. The DPPH followed the same pattern as TPC. The extract obtained at this temperature (55°C) revealed a strong DPPH scavenging activity with a value of 68.81% and then decreased with using 70°C. Our results are in agreement with those obtained by Pinelo *et al.* (2005) and Zhong *et al.* (2019).

It should be noticed that increasing temperature favors the extraction of phenolic compounds by increasing the solubility of the solute and diffusion coefficient, as suggested by Pinelo *et al.* (2005). However, at a certain temperature level, the phenolic yield decrease due to the presence of heat-sensitive compounds (Djabali *et al.*, 2020). From obtained results, the increase of temperature from 55 to 70°C not affected TPC but reduced significantly antioxidant activity.

Effect of time on total phenolics content and free radical scavenging

The selection of the best extraction time was the final step of single-factor experiments. The effect of extraction time on the phenolic compounds recovery and DPPH scavenging activity was examined for 30, 60, 90, and 120 minutes and the results were shown in Figures 1e and 2e. The recovery of phenolic compounds

in parallel with antioxidant activity significantly increased with extraction time from 30 to 120 min. This duration allowed extraction of 1886.03 mg GAE/100g DW of TPC and expressing 71.21% of antioxidant activity. Hence, the best extraction time of phenolic compounds from fumitory was 120 min.

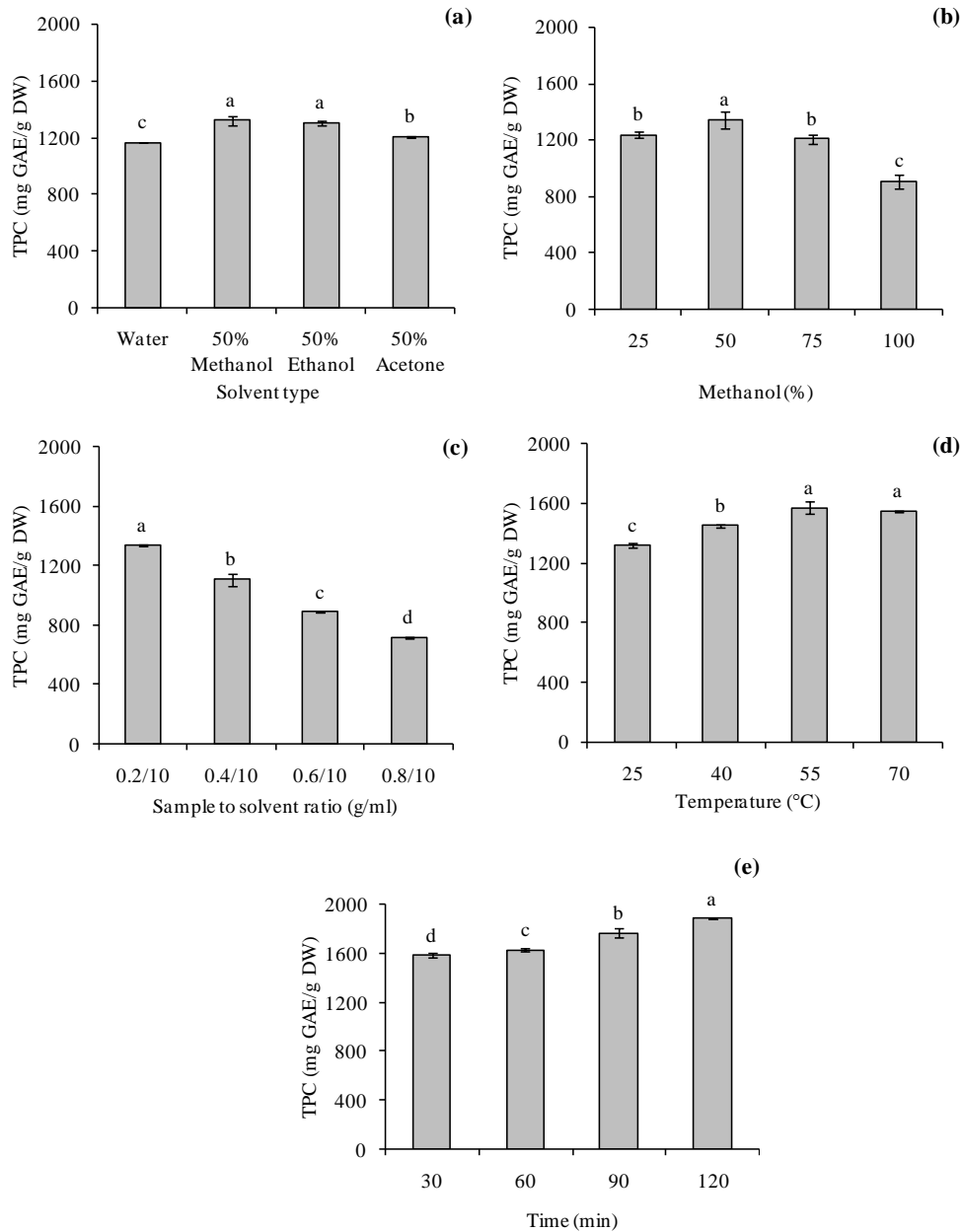


Figure 1. Influences of solvent type (a), solvent concentration (b), sample to solvent ratio (c), temperature (d), and time (e) on total phenolics extraction from *F. officinalis*.

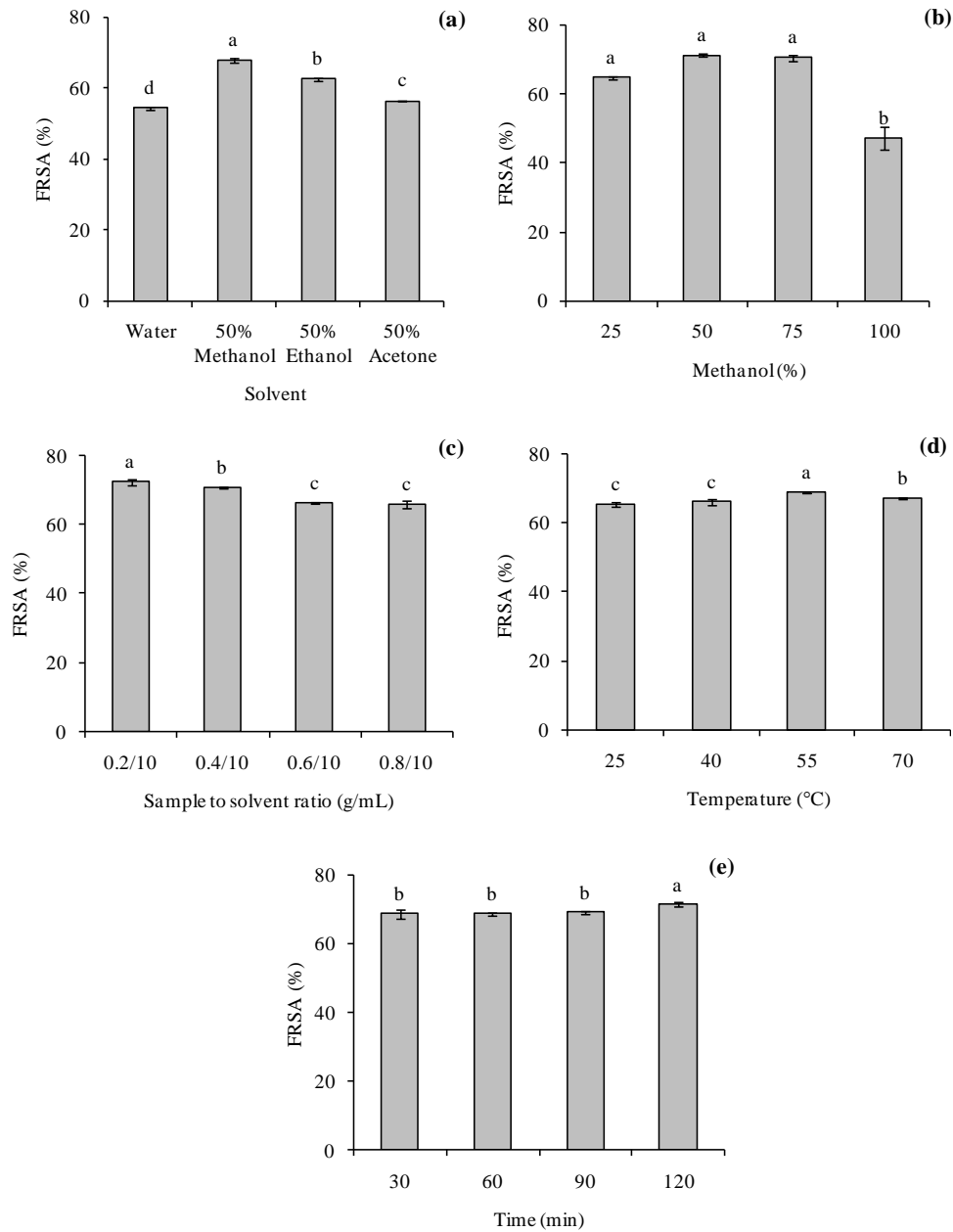


Figure 2. Influences of solvent type (a), solvent concentration (b), sample-to-solvent ratio (c), temperature (d), and time (e) on DPPH free-radical scavenging activity of *F. officinalis*.

The obtained results were in agreement with those reported by Bachir bey *et al.* (2013) and Che Sulaiman *et al.* (2017), who found that 120 min was the optimal extraction time of phenolic compounds from dark dried *Ficus carica* and

Clinacanthus nutans leaves, respectively. In general, prolonged extraction time would lead to more oxygen exposure, representing a potent factor of phenolic compounds oxidation (Naczka and Shahidi, 2006).

Response surface methodology

Model fitting and analysis of variance

Based on the single-factor experimental results, the RSM approach was employed using the Box-Behnken design (BBD) to investigate the effects of solvent concentration, extraction time, and temperature on TPC and FRSA. Fifteen essays, including the three repetitions of central point, in order to check the reproducibility of the extraction process and standard error, were performed. Table 1 presents the design matrices of the actual experiments using BBD and the predicted data for the response variables. Experimental and predicted values for TPC and DPPH scavenging activity were quite similar. The coefficient of determination (R^2) was 0.91 and 0.93 for TPC and FRSA, respectively; in other words, only 9% and 7% respectively of variations are not explained by the model.

Table 1. Experimental design and experimental and predicted values for total phenolic content and free radical scavenging activity of *F. officinalis*.

Run	Independent variable			TPC (mg GAE/100g DW)		FRSA (%)	
	Solvent Concentration (%)	Temp. (C°)	Time (min)	Experimental	Predicted	Experimental	Predicted
1	50	75	50	1231.80	1237.81	70.70	74.34
2	50	35	190	1381.88	1375.87	62.98	59.34
3	50	55	120	1511.56	1510.59	71.96	71.93
4	50	55	120	1511.56	1510.59	71.92	71.93
5	20	55	190	1524.67	1477.86	64.02	67.43
6	50	75	190	1045.3	1124.89	71.18	72.63
7	80	35	120	1220.15	1252.94	38.54	43.39
8	80	75	120	1297.37	1244.55	62.46	62.23
9	50	35	50	1287.17	1207.58	53.82	52.38
10	80	55	190	1501.36	1474.59	68.75	67.54
11	50	55	120	1508.65	1510.59	71.92	71.93
12	20	35	120	1402.28	1455.10	45.70	45.93
13	20	75	120	1275.52	1242.74	67.19	62.34
14	20	55	50	1520.30	1547.07	65.02	66.23
15	80	55	50	1303.20	1350.01	66.88	63.47

TPC, total phenolic content; FRSA, free radical scavenging activity.

According to Jumbri *et al.* (2015), the more the R^2 value is close to 1, the more empirical model has a strong explanation. Indeed, the adjusted coefficients of

determination (Adj R^2) values were 0.75 for TPC and 0.79 for FRSA. The adjusted R-square (Adj. R^2) showed the descriptive power of the regression models while including the significant terms (Che Sulaiman *et al.*, 2017). ANOVA analysis showed that the p -values of the two models were less than 0.05 (Table 2). p -values were used as a measure to check the significance of each coefficient. When the p -value is small, the corresponding coefficient is more significant. Another way to verify and confirm the validity of the model is F values. A large F value is indicative that the independent variables have a significant impact on the respective response variables. Our results showed that F values of 5.71 and 7.00 for TPC and DPPH scavenging activity, respectively, indicated that the models were significant (Tan *et al.*, 2016).

Table 2. Estimation of regression coefficients, Standard error, and Student's t-test results of response surface for total phenolic content and free radical scavenging activity.

Parameter	Estimate	Std. error	t Ratio	Prob. > t
Total phenolic contents				
Model				0.0347*
$R^2= 0.91$	Adj. $R^2= 0.75$			
Intercept	1510.591	41.866	36.08	<0.0001*
x_1	-50.086	25.638	-1.95	0.1082
x_2	-55.186	25.638	-2.15	0.0840
x_3	13.842	25.638	0.54	0.6124
$x_1 \times x_2$	50.997	36.257	1.41	0.2186
$x_1 \times x_3$	48.447	36.257	1.34	0.2391
$x_2 \times x_3$	-70.303	36.257	-1.94	0.1102
$x_1 \times x_1$	7.041	37.738	0.19	0.8593
$x_2 \times x_2$	-218.803	37.738	-5.80	0.0022*
$x_3 \times x_3$	-55.247	37.738	-1.46	0.2031
Free radical scavenging activity				
Model				0.0226*
$R^2= 0.93$	Adj. $R^2= 0.79$			
Intercept	71.932	2.632	27.33	<0.0001*
x_1	-0.661	1.611	-0.41	0.6984
x_2	8.811	1.611	5.47	0.0028*
x_3	1.313	1.611	0.81	0.4523
$x_1 \times x_2$	0.607	2.279	0.27	0.8005
$x_1 \times x_3$	0.716	2.279	0.31	0.7661
$x_2 \times x_3$	-2.17	2.279	-0.95	0.3848
$x_1 \times x_1$	-8.481	2.372	-3.57	0.0160*
$x_2 \times x_2$	-9.978	2.372	-4.21	0.0084*
$x_3 \times x_3$	2.716	2.372	1.14	0.3041

x_1 , solvent concentration (%); x_2 , temperature ($^{\circ}\text{C}$); x_3 , time (min).* Significant at $p < 0.05$; R^2 , coefficient of determination; Adj. R^2 , adjusted coefficient of determination.

Effect of regression parameters

Table 2 presents the effects of independent variables on TPC and DPPH scavenging activity. The statistical analysis showed that TPC was significantly

affected only by the quadratic term of temperature (x_2^2 , $p < 0.05$), while the DPPH scavenging activity was significantly influenced by the linear term of temperature (x_2 ; $p < 0.05$) and the quadratic terms of both methanol concentration and temperature (x_1^2 ; x_2^2 , $p < 0.05$). Applying multiple regression analysis on experimental data and considering the significant terms, the fitted models can be presented as quadratic polynomial equations (Equation 5 and 6).

$$\text{TPC} = 1510.591 - 218.803x_2^2 \quad (5)$$

$$\text{FRSA} = 71.932 + 8.811x_2 - 8.481x_1^2 - 9.978x_2^2 \quad (6)$$

where x_1 : methanol concentration, x_2 : extraction temperature.

In order to illustrate the effects of the three studied factors (x_1 , x_2 , and x_3) on TPC and DPPH scavenging activity, the three-dimensional (3D) response surface plots were generated with varying two factors in the experimental field and keeping the third at the central level. Figure 3a illustrated the combined effect of solvent concentration (x_1) and temperature (x_2) on the TPC and FRSA. Extraction temperature showed a significant quadratic effect on TPC and both linear and quadratic effects on DPPH scavenging activity. Therefore, the TPC progressively increased with increasing temperature until reaching 54.40°C and then began to decrease. Increasing temperature promotes extraction by improving both solubility of solutes and the diffusion coefficient. However, elevated temperatures can degrade phenolic compounds (Hismath *et al.*, 2011).

Our results showed that the temperature had a significant negative quadratic effect on the extraction of phenolic compounds; this can be explained by the thermo-sensitivity of certain compounds in these extracts. Therefore, high temperatures resulted in the degradation of these compounds. This result was similar to that obtained by Bachir bey *et al.* (2014), who found that extraction temperature exerts positive linear and negative quadratic effects on extracting phenolic compounds from dried fig. This indicates that at relatively low temperatures, the extraction of phenolics was enhanced by heating, but at high temperatures, the degradation of these compounds will occur. Figure 3b depicted the effects of solvent concentration (x_1) versus time extraction (x_3). The TPC and DPPH scavenging activity slightly increased with methanol concentration until reaching 1510.10 mg GAE/g DW and 71.85%, respectively. This observation could be explained by the increase of solvent polarity with the addition of water until obtaining an adequate polarity for phenolic solubilization (Iglesias-Carres *et al.*, 2019). Previous studies have reported that organic solvent /water mixtures favor the extraction of phenolic compounds from plants compared to pure solvents (Benkerrou *et al.*, 2018; Saci *et al.*, 2018; Ezzoubi *et al.*, 2021).

Figure 3c represented the effects of extraction temperature (x_2) and time (x_3) on the two responses at a central level of methanol concentration. The highest phenolic compounds content was obtained at 168.02 min. The extraction time showed no linear or quadratic effect on TPC and DPPH scavenging activity.

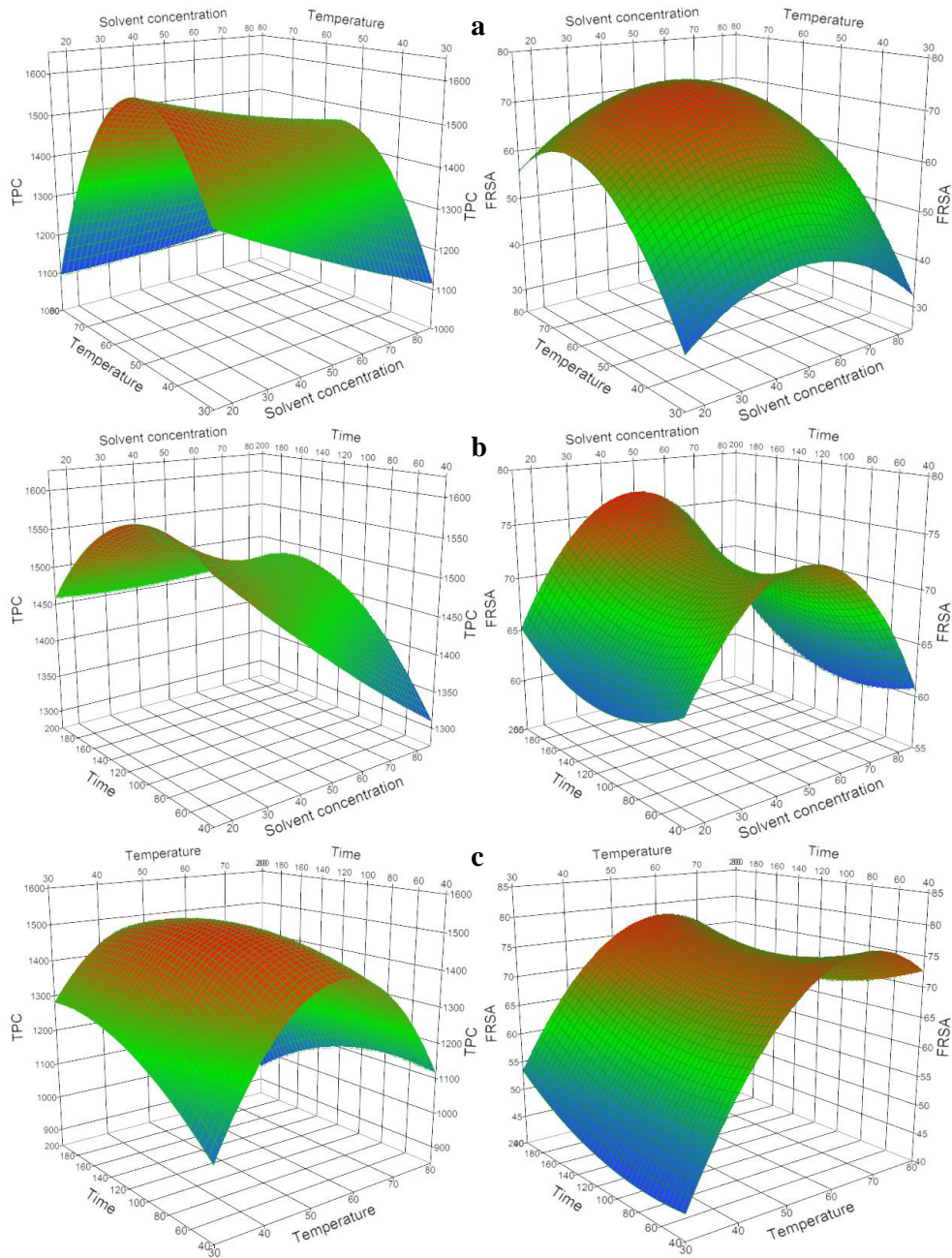


Figure 3. Response surface plots showing the effects of solvent concentration and time (a), solvent concentration and temperature (b), temperature and time (c) on TPC (total phenolic content) and FRSA (free radical scavenging activity) of *F. officinalis*.

Optimal conditions

The optimal conditions proposed by the developed models were 47% methanol, 54.40°C, and 168.02 min with predicted values for the responses of 1498.97 mg GAE/100g DW (TPC) and 73.81% (antioxidant activity). An experimental test was carried out by applying the theoretical optimal conditions of the three optimized parameters in order to validate these required conditions and obtain responses. The practical results obtained under the optimal conditions for the responses were 1510.10 mg GAE/100g (TPC) and 71.85% (FRSA), denoting that these experimental findings were close to the predicted values. Therefore, these results confirmed the validity of the quadratic models and demonstrated that the response surface methodology could be applied effectively to optimize the extraction of phenolic compounds from fumitory (*Fumaria officinalis* L.).

Bioactive contents and antioxidant activities from the optimal extract

After the validation of optimal conditions of antioxidants extraction from *Fumaria officinalis*, the obtained optimal extract was then used to estimate total flavonoid and tannin contents as well as ABTS scavenging and iron-chelating activities (Table 3). TPC for aerial part of *F. officinalis* extract was 1510.10 ± 2.52 mg GAE/100g DW, higher than obtained by Păltinean *et al.* (2017) and Safari *et al.* (2018) and lower than found by Edziri *et al.* (2020). TFC from the optimal extract of *F. officinalis* was 629.30 ± 34.84 mg QE/100g. This result was higher than those of Safari *et al.* (2018) and lower than Edziri *et al.* (2020). However, the total tannin content was estimated to be 706.67 ± 32.15 mg QE/100g.

Furthermore, *F. officinalis* methanol extract has been subjected to the antioxidant activities tested by three different methods, DPPH and ABTS scavenging activities and iron-chelating test. *F. officinalis* extract exhibited a good DPPH radical scavenging (71.85%) and a moderate ABTS scavenging activity (41.95%). Our results were lower than those obtained by Jaberian *et al.* (2013) and Stanojević *et al.* (2018). However, a strong activity was obtained regarding the iron-chelating assay (87.85%), and according to our knowledge, this is the first study that evaluated the iron-chelating activity of *F. officinalis* extract.

Table 3. Bioactive contents and antioxidant activities of optimal extract.

	Bioactive contents		Antioxidant activities
TPC	1510.10 ± 2.52	DPPH ^o scavenging activity (%)	71.85 ± 0.92
TFC	629.30 ± 34.84	ABTS ^{o+} scavenging activity (%)	41.95 ± 0.64
TTC	706.67 ± 32.15	Fe ²⁺ chelating activity (%)	87.85 ± 2.19

TPC, total phenolic content (mg GAE/g 100g DW); TFC, total flavonoid content (mg QE/100g DW); TTC, total tannin content (mg CE/100g DW).

Conclusions

In this study, RSM was developed to maximize the extraction of phenolic antioxidants from *F. officinalis* using the Box-Behnken design. The optimal

conditions for phenolic compounds extraction were 47% methanol, using 54.40°C for 168.02 min. Under these conditions, the experimental values of TPC and DPPH scavenging activity were 1510.10 mg GAE /100g DW and 71.85%, respectively. The values of these responses were in accordance with the predicted values (1498.97 mg GAE /100g DW for TPC and 73.81% for FRSA). These findings confirmed the validity of the developed model for the extraction of phenolic compounds and the free radical scavenging activity in the experimental conditions used. Furthermore, RSM was able to determine the optimal conditions for the extraction of phenolic compounds and antioxidant activity. In addition, strong iron-chelating and interesting ABTS scavenging activities, as well as related bioactive compounds, were obtained from the optimal extract. These extracted compounds were particularly interesting for their role in diverse biological activities. Moreover, identification and characterization methods should be applied to identify the phenolic compounds present in this extract.

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