Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 883-898.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

OPTIMIZATION OF EXTRACTION PROCESS TO MAXIMIZE PHENOLIC CONTENT, FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITY OF *PROSOPIS FARCTA* USING RESPONSE SURFACE METHODOLOGY

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Prosopis farcta (Banks et Sol.) Eig., is a widely distributed plant, that is rich in health benefits components. This study aims to optimize the ultrasonic-assisted extraction parameters of P. farcta leaves in order to maximize total phenolic content (TPC), total flavonoid content (TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, using response surface methodology (RSM). A Box-Behnken design (BBD) with three levels and three variables was employed, the independent variables were extraction temperature (20, 40 and 60°C), extraction time (20, 40 and 60 minutes) and ethanol concentration (20, 45 and 70%). Results show that all three extraction parameters have great effects on the TPC, TFC, and DPPH scavenging values, the optimal conditions were (extraction temperature: 53.93°C, extraction time: 51.11 minutes and ethanol concentration: 65.45%), under these conditions, the experimental results were (TPC: 109.89 mg GAE/g DW, TFC: 11.287 mg RE/g DW and DPPH: 37.503%), these results are matching well with the theoretical predicted values which proves that RSM models were accurate and reliable. Strong correlations were found between TPC, TFC, and DPPH scavenging activity. This study revealed the importance of P. farcta as a natural source of antioxidants, and highlighted the optimal extraction conditions that can be effectively employed for maximizing production of natural antioxidants from P. farcta leaves.

Keywords: *Prosopis farcta, antioxidant, ultrasonic-assisted extraction, optimization, response surface methodology (RSM)*

INTRODUCTION

Prosopis L., (Family Fabaceae) is a plant genus that has wide distribution across the world in dry and semi-dry regions, it includes about 44 to 50 species. One of these species is *Prosopis farcta* (Banks et Sol.) Eig., which is a short, thorny shrub with a native distribution in the United States, Kuwait, Turkey, Iraq, Iran, Northern Africa, and South Western Asia[1]. *Prosopis farcta* (Syrian mesquite) usually grows up to a height of 0.4-1 m, though, it may grow over 2 m, its various components include leaves, spines, pods, and seeds[2]. Different plant organs of *P. farcta* have been used in traditional medicine for treating some health conditions, which include cold, diarrhea, inflammation, measles, diabetes, skin diseases, prostate disorders, chest pain, interrupt urine, rheumatism, it also has been used as a blood thinner and for treating scorpion stings and open wounds[3-5]. The analysis of the chemical composition of the different plant organs, roots, leaves, pods and seeds, proved that they contain flavonoids, phenols, saponins, alkaloids, tannins, glycosides, and resins[6,7]. The polyphenol content in the leaves were determined using chromatographic methods, where 47 phenolic compounds were identified and characterized, including 13 compounds of phenolic acids, 28 compounds of flavonoids, 4 other polyphenols, a compound of lignans

Received : 4/5/2024 & Accepted : 27/7/2024

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(Schisandrol B), and a compound of stilbenes (3'-Hydroxy-3,4,5,4'-tetramethoxystilbene), chlorogenic acid was the most abundant phenolic acid, while the main flavonoids were catechin and kaempferol[8]. Other investigations identified 39 volatile components in the roots, branches, leaves, flowers and pods[9], proteins and unsaturated fatty acids in the seeds[10]. Several studies have been conducted on the medicinal properties and effects of *P. farcta*, which include antioxidant, antibacterial, anticancer, antidiabetic, antihyperlipidemic, cardioprotective, fertility-enhancing, hepatoprotective, wound healing, and anti-fungal activities[11-20].

Antioxidants are substances that, in low concentration, can prevent the oxidative damage to biomolecules (proteins, nucleic acids, polyunsaturated lipids, and carbohydrates) through free radical mediated reactions[21]. Phenolic compounds play a significant role in promoting human health, particularly through their antioxidant properties[22]. Phenolic compounds are known to have strong chain breaking antioxidant properties, because of their scavenging ability that contributes directly to the antioxidative action[21,23]. Their antioxidant activity is believed to be related to their molecular structure, particularly due to the presence and number of hydroxyl groups, as well as double bond conjugation and resonance effects[21,24]. Phenolic compounds exhibit high DPPH scavenging activity due to their low bond dissociation energies (BDE) of the O–H bond, and their ability to donate hydrogen atom easily[21].

The antioxidant effects of *P. farcta* have already been investigated in many studies, the octanolic extracts from the pods and seeds showed high radical scavenging activity with high TPC values[2], the antioxidant activity of the aqueous fruits extract has been measured and it has been found to be significantly correlated with TPC indicating that phenolic compounds are the significant contributors to the antioxidant activity[25], also, different solvent extracts from the aerial part of *P. farcta* showed promising antioxidant activities[1].

Response Surface Methodology (RSM) is a statistical and mathematical method for designing experiments in order to optimize a desired response that is affected by multiple independent variables. RSM has been used to optimize process parameters and obtain a regression equation that predicts the response based on the submitted parameters[26]. RSM helps to reduce the number of experiments required to identify the optimum conditions[27].

To the best of our knowledge, there are no reports of optimizing the extraction conditions of *P. farcta* leaves to determine the total phenolic and total flavonoid contents, as well as their correlation to the antioxidant activity, in spite of the relatively high phenolic yield reported in studies[28].

The main objective of this study is the optimization of the ultrasonic-assisted extraction parameters in order to maximize total phenolic content (TPC), total flavonoid content (TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of *P. farcta* leaves using response surface methodology, determination the optimal extraction conditions, and determination the Pearson correlation coefficients between TPC, TFC, and the antioxidant potential which represented by the DPPH scavenging activity.

MATERIALS AND METHODS

Chemicals

Ethanol (Sigma-Aldrich), methanol (Sigma-Aldrich), Folin-Ciocalteu reagent (Scharlau S.L.), Na_2CO_3 (Panreac Quimica Sau), gallic acid (Prolabo), $AlCl_3$ (Merck), rutin (Extrasynthese Genay), DPPH reagent (Sigma Aldrich).

Apparatus

Ultrasonic cleaner (Model: UC-4120L, frequency: 40 KHz, heating power: 200W, ultrasonic power:120W, voltage: 220 - 240 V, RoHS, China), UV-VIS spectrophotometer (UV-1800 Shimadzu, Japan), water bath (J.P. Selecta, Spain), rotary evaporator (Heidolph Instruments, Germany).

Plant Materials

The leaves of *P. farcta* were gathered in August 2022 from Aleppo university campus in Syria, and identified by professor Ream Nayal (pharmacognosy department); voucher specimens of plant material (PF 35L/22) were deposed in the pharmacognosy department at the faculty of pharmacy, university of Aleppo. Leaves were air-dried in a shelter, ground into a powder, and stored in a cool, dark, and dry place for further procedures.

Extraction Procedures

Powdered leaves (0.5 g) were subjected to ultrasonication for extraction using the solvent with a solid-liquid ratio of 1:50, and the extraction processes were carried out under different conditions, which will be mentioned later. Following the completion of extraction processes, supernatants were collected and made up to the initial volume with the solvent. The resulting supernatants were used for assays, and they were stored at -20°C until analysis.

Determination of Total Phenolic Content (TPC)

The levels of TPC were estimated using spectrophotometric method[29], with some modifications. The reaction mixture was prepared by combining 0.5 ml of extracts solutions, 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in distilled water and 2.5 ml of 7.5% Na_2CO_3. A blank was also prepared simultaneously, consisting of 0.5 ml of distilled water, 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in distilled water and 2.5 ml of 7.5% Na_2CO_3. After mixing and incubating in a water bath at 45°C for 45 minutes, the absorbance of the samples was measured using spectrophotometer at 765 nm. For each analysis, the samples were prepared in triplicate and the mean absorbance value was determined. Gallic acid was used as a standard, five different concentrations of gallic acid solution (0.020, 0.040, 0.060, 0.080, 0.100) mg/ml were used to establish the standard curve shown in **Fig. (1)**, the regression equation was (y = 8.9783x - 0.0665), R^2 value of the regression equation was 0.9975. TPC is expressed as gallic acid equivalents (GAE) per gram of plant dry weight (DW).

Determination of Total Flavonoid Content (TFC)

The levels of TFC were estimated using spectrophotometric method[29]. The reaction mixture was prepared by combining 1 ml of extracts solutions and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. After mixing and incubating at room temperature for 1 hour, the absorbance of the samples was measured using spectrophotometer at 415 nm. For each analysis, the samples were prepared in triplicate and the mean absorbance value was determined. Rutin was used as a standard, five different concentrations of rutin solutions (0.0025, 0.0050, 0.0100, 0.0200, 0.0400) mg/ml were used to establish the standard curve shown in **Fig. (2)**, the regression equation was (y = 16.258x + 0.0073), R^2 value of the regression equation was 0.9983. TFC is expressed as rutin equivalents (RE) per gram of plant dry weight (DW).

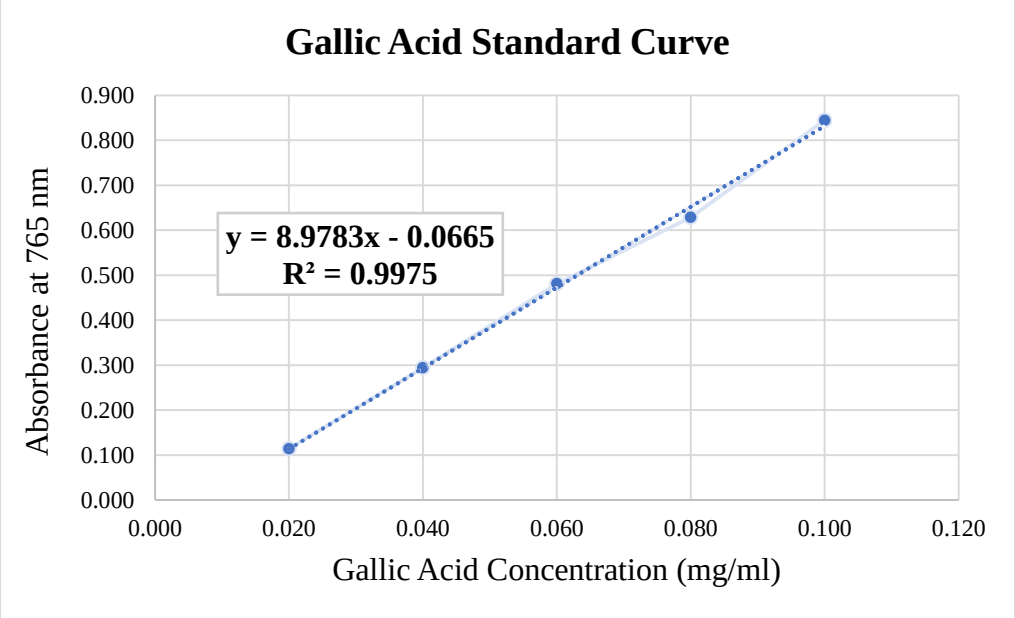

Fig. 1 : Standard Curve of Gallic Acid Used to Determine TPC of *P. farcta* Leaves Extracts.

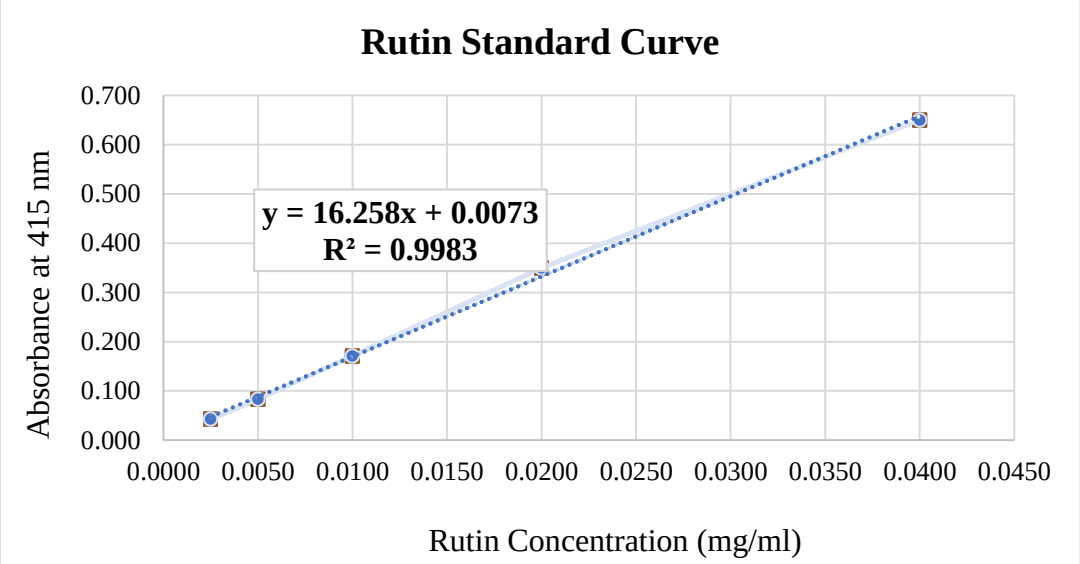

Fig. 2: Standard Curve of Rutin Used to Determine TFC of *P. farcta* Leaves Extracts.

Determination of DPPH Radical Scavenging Activity

Antioxidant activities of the plant extracts were estimated by determining their abilities to scavenge DPPH radical using spectrophotometric method[30]. In brief, 2 ml of extract solution was mixed with 2 ml of 0.16 mM DPPH (2,2-Diphenyl-1-Picrylhydrazyl) solution (dissolved in methanol). The samples were vortexed for 1 minute and then kept at room temperature for 30 min in the dark. The absorbance of the samples was measured using spectrophotometer at 517 nm. For each analysis, the samples were prepared in triplicate and the mean absorbance value was determined. Blank samples (solvent) and control samples (solvent with DPPH) were performed using the same method. All extract solutions have been prepared with equal concentrations before the assay and the results were expressed as percentage of inhibition (scavenging effect) using the following formula:

$$\text{DPPH Inhibition \%} = [(A_{Control} - A_{Sample})/A_{Control}] \times 100$$

Experimental Design

A Box-Behnken design (BBD) with three levels and three variables was used in order to maximize TPC, TFC, and DPPH scavenging activities. The independent variables selected for the study were the extraction temperature (*X1*), the extraction time (*X2*), and ethanol concentration (*X3*), the ranges of these variables were determined based on preliminary experiments examining each single factor, the variables were coded at three levels (-1, 0, and 1) as shown in **Table (1)**.

The complete design included 15 experimental points, with three replications of the central points where all variables were coded as zero, as illustrated in **Table (2)**. The TPC, TFC, and DPPH scavenging activity were chosen as the dependent variables (responses). A regression analysis was subsequently performed to establish a second-order polynomial equation which used to fit the experimental data and to calculate the predicted responses.

The general form of mathematical quadratic response equation was given as:

$$Y = \beta 0 + \sum_{i=1}^{k} \beta iXi + \sum_{i=1}^{k} \beta\, iiXii + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta ijXij$$

where, *Y* represents the response, *β0* is the constant, *βi*, *βii*, and *βij* represent the coefficients of the linear, quadratic, and interactive effects, respectively; *Xi*, and *Xj* are the coded independent variables; and k is equal to the number of the tested factors[31], (k = 3 in this study).

Table 1 : Range of coded and actual values for Box-Behnken design.

	Level		
Factor / Independent Variable	**-1**	**0**	**1**
X1 Extraction temperature (°C)	20	40	60
X2 Extraction time (min)	20	40	60
X3 Ethanol concentration (%, *v/v*)	20	45	70

Table 2 : Box-Behnken design for the independent variables and the observed experimental and predicted responses.

Run	Factors			Responses					
	X1	*X2*	*X3*	TPC (mg GAE/g DW)		TFC (mg RE/g DW)		DPPH scavenging (%)	
	Extraction temperature (°C)	Extraction time (min)	Ethanol concentration (%, *v/v*)	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	40	40	45	96.251 ± 0.009	96.003	9.161 ± 0.025	9.479	32.292 ± 0.012	32.075
2	60	20	45	92.353 ± 0.005	91.866	8.785 ± 0.021	8.731	26.302 ± 0.031	26.270
3	60	40	70	108.689 ± 0.018	109.292	11.285 ± 0.019	11.418	36.198 ± 0.018	36.231
4	20	40	20	48.543 ± 0.023	47.940	2.464 ± 0.028	2.331	9.375 ± 0.028	9.343
5	40	60	20	72.304 ± 0.038	72.420	5.949 ± 0.024	6.028	20.573 ± 0.027	20.573
6	20	60	45	70.448 ± 0.021	70.935	5.584 ± 0.019	5.638	20.182 ± 0.028	20.215
7	40	40	45	96.065 ± 0.019	96.003	9.434 ± 0.033	9.479	32.161 ± 0.023	32.075
8	60	60	45	102.191 ± 0.017	101.611	10.332 ± 0.035	10.233	34.375 ± 0.034	34.147
9	40	20	20	60.424 ± 0.013	60.447	4.081 ± 0.021	4.115	14.844 ± 0.015	14.649
10	40	40	45	95.694 ± 0.008	96.003	9.841 ± 0.032	9.479	31.771 ± 0.021	32.075
11	40	60	70	100.892 ± 0.027	100.869	10.142 ± 0.018	10.108	35.286 ± 0.007	35.482
12	20	20	45	55.412 ± 0.012	55.992	3.391 ± 0.027	3.490	14.453 ± 0.022	14.681
13	60	40	20	76.388 ± 0.025	76.852	6.628 ± 0.017	6.648	19.531 ± 0.036	19.759
14	20	40	70	72.119 ± 0.031	71.655	5.919 ± 0.023	5.899	21.354 ± 0.021	21.126
15	40	20	70	88.269 ± 0.01	88.153	8.450 ± 0.027	8.371	27.995 ± 0.016	27.995

Statistical Methods

Box–Behnken experimental design and data analyzing using response surface methodology were performed using the MINITAB software (Minitab 20). The resulted values were expressed as a mean value of three determinations ± Standard deviation (SD). The analysis of variance (ANOVA) for the regression equations was used to determine significance and suitability. Statistical significance was defined as a *p*-value less than 0.05 (*p*-value ≤ 0.05). The optimal extraction conditions were estimated through the response optimizer function. The correlations between TPC, TFC, and DPPH scavenging were determined using Pearson correlation coefficient.

RESULTS AND DISCUSSION

Results

Optimize the Extraction Conditions for TPC, TFC, and DPPH Scavenging:

The extraction parameters were optimized using BBD combined with response surface methodology. The experimental and predicted values for the TPC, TFC and DPPH scavenging activity are given in **Table (2)**.

The effects of different extraction parameters were investigated on the TPC, TFC and antioxidant potential of *P. farcta* extracts. The obtained experimental data were subjected to regression analysis. The significance of each regression coefficient and the interaction between each independent variable were evaluated using their corresponding *p*-values **[Table (3)]**. The relationships between the tested parameters and the responses were explained by the second-order polynomial regression equations. The statistical significance of the equations was examined by ANOVA (Analysis of Variance) method **[Table (4)]**. The coefficient of determination R^2, the lack of fit along with *p*-value at significance level of 0.05 were used to determine the accuracy and validity of the model **[Table (4)]**.

TPC values ranged from 48.543 to 108.689 mg GAE/g DW, applying response surface methodology, the regression equation for TPC is expressed as follows:

$$TPC = -66.27 + 2.7595\ X1 + 1.6083\ X2 + 1.7544\ X3 - 0.024925\ X1X1 - 0.014831\ X2X2 - 0.015358\ X3X3 - 0.003249\ X1X2 + 0.004363\ X1X3$$

The effects of variables on TPC were analyzed as shown in **Table (3)**. The linear terms of extraction temperature (X1) followed by ethanol concentration (X3) had the most positive effects on TPC and were statistically significant (*p*-value < 0.05), while the linear term of extraction time (X2) had a less positive effect and was also statistically significant (*p*-value < 0.05). The quadratic terms of the extraction parameters; extraction temperature ($X1^2$), extraction time ($X2^2$), and ethanol concentration ($X3^2$) on TPC were significantly negative (*p*-value < 0.05). The interaction of extraction temperature and ethanol concentration (X1X3) was significantly positive (*p*-value < 0.05), in contrast, the interaction of extraction temperature and extraction time (X1X2) on TPC was significantly negative (*p*-value < 0.05), while the interaction of extraction time and ethanol concentration (X2X3) was not significant (*p*-value > 0.05).

TFC values ranged from 2.464 to 11.285 mg RE/g DW, applying response surface methodology, the regression equation for TFC is expressed as follows:

$$TFC = -15.05 + 0.4158\ X1 + 0.2532\ X2 + 0.2624\ X3 - 0.003796\ X1X1 - 0.002343\ X2X2 - 0.002218\ X3X3$$

The effects of variables on TFC were analyzed as shown in **Table (3)**. The linear terms of extraction temperature (X1) followed by ethanol concentration (X3) had the most positive effects on TFC and were statistically significant (*p*-value < 0.05), while the linear term of extraction time (X2) had a less positive effect and was also statistically significant (*p*-value < 0.05). The quadratic terms of the extraction parameters; extraction temperature ($X1^2$), extraction time ($X2^2$), and ethanol concentration ($X3^2$) on TFC were significantly negative (*p*-value < 0.05). The interaction of extraction temperature and ethanol concentration (X1X3), the interaction of extraction temperature and extraction time (X1X2), and the interaction of extraction time and ethanol concentration (X2X3) on TFC were not significant (*p*-value > 0.05).

Table 3: Regression coefficients of regression equations for TPC, TFC, and DPPH scavenging of *P. farcta* leaves extracts.

TPC				
Term	Coefficient	Standard Error	T-value	*p*-value
Constant	96.003	0.408	235.41	0.000
X1	16.637	0.250	66.62	0.000
X2	6.172	0.250	24.71	0.000
X3	14.039	0.250	56.21	0.000
$X1^2$	-9.970	0.368	-27.12	0.000
$X2^2$	-5.932	0.368	-16.14	0.000
$X3^2$	-9.599	0.368	-26.11	0.000
X1X2	-1.299	0.353	-3.68	0.014
X1X3	2.181	0.353	6.18	0.002
X2X3	0.186	0.353	0.53	0.621
TFC				
Term	Coefficient	Standard Error	T-value	*p*-value
Constant	9.479	0.144	65.89	0.000
X1	2.4590	0.0881	27.92	0.000
X2	0.9125	0.0881	10.36	0.000
X3	2.0842	0.0881	23.66	0.000
$X1^2$	-1.519	0.130	-11.71	0.000
$X2^2$	-0.937	0.130	-7.23	0.001
$X3^2$	-1.386	0.130	-10.69	0.000
X1X2	-0.161	0.125	-1.30	0.251
X1X3	0.301	0.125	2.41	0.061
X2X3	-0.044	0.125	-0.35	0.738
DPPH Scavenging				
Term	Coefficient	Standard Error	T-value	*p*-value
Constant	32.075	0.170	188.24	0.000
X1	6.380	0.104	61.15	0.000
X2	3.353	0.104	32.13	0.000
X3	7.064	0.104	67.70	0.000
$X1^2$	-5.653	0.154	-36.81	0.000
$X2^2$	-2.593	0.154	-16.88	0.000
$X3^2$	-4.807	0.154	-31.30	0.000
X1X2	0.586	0.148	3.97	0.011
X1X3	1.172	0.148	7.94	0.001
X2X3	0.390	0.148	2.65	0.046

X1 extraction temperature, *X2* extraction time, *X3* ethanol concentration,
$X1^2$, $X2^2$, $X3^2$ quadratic terms of *X1*, *X2*, *X3*, respectively,
X1X2, *X1X3*, *X2X3* interaction terms of *X1* and *X2*, *X1* and *X3*, and *X2* and *X3*, respectively.

Table 4 : Analysis of variance (ANOVA) of regression equations for TPC, TFC, and DPPH scavenging of *P. farcta* leaves extracts.

TPC					
Source	Degree of freedom	Adjusted Sum of Square	Adjusted Mean Square	F-value	*p*-value
Model	9	4855.29	539.48	1081.24	0.000
Error	5	2.49	0.50		
Lack-of-Fit	3	2.33	0.78	9.67	0.095
Pure Error	2	0.16	0.08		
Total	14	4857.79			
R^2= 0.9995 Adjusted R^2= 0.9986					
TFC					
Source	Degree of freedom	Adjusted Sum of Square	Adjusted Mean Square	F-value	*p*-value
Model	9	106.747	11.8608	191.07	0.000
Error	5	0.310	0.0621		
Lack-of-Fit	3	0.076	0.0254	0.22	0.878
Pure Error	2	0.234	0.1171		
Total	14	107.057			
R^2= 0.9971 Adjusted R^2= 0.9919					
DPPH Scavenging					
Source	Degree of freedom	Adjusted Sum of Square	Adjusted Mean Square	F-value	*p*-value
Model	9	1023.97	113.774	1306.24	0.000
Error	5	0.44	0.087		
Lack-of-Fit	3	0.29	0.096	1.31	0.461
Pure Error	2	0.15	0.073		
Total	14	1024.40			
R^2= 0.9996 Adjusted R^2= 0.9988					

R^2 Coefficient of determination.

The effects of variables on DPPH scavenging were analyzed as shown in **Table (3)**. All the linear, quadratic and interaction terms of DPPH scavenging were statistically significant (*p*-value < 0.05). The linear terms of ethanol concentration (X3) followed by extraction temperature (X1) had the most positive effects on DPPH scavenging, while the linear term of extraction time (X2) had a less positive effect. The quadratic terms of the extraction parameters; extraction temperature ($X1^2$), extraction time ($X2^2$), and ethanol concentration ($X3^2$) had negative effects on DPPH scavenging. The interaction of extraction temperature and ethanol concentration (X1X3), followed by the interaction of extraction temperature and extraction time (X1X2), and the interaction of extraction time and ethanol concentration (X2X3) had the least positive effects on DPPH scavenging.

The coefficients of determination and adjusted coefficients of determination were (R^2=0.9995, adjusted R^2=0.9986) for TPC, (R^2= 0.9971, adjusted R^2= 0.9919) for TFC, and (R^2=0.9996, adjusted R^2=0.9988) for DPPH scavenging, which suggest a good fit. The findings in **Table (4)** demonstrate that the models for TPC, TFC, and DPPH scavenging were established with statistical significance due to the extremely low *p*-values (*p*-value < 0.05). The lack-of-fit test was not statistically significant so it shows that the models for TPC, TFC and DPPH scavenging fit the data well as the *p*-values for lack-of-fit is greater than 0.05 (*p*-value > 0.05).

The contour plots for TPC, TFC, and DPPH scavenging are shown in **Fig. (3), Fig. (4)**, and **Fig. (5)**, respectively.

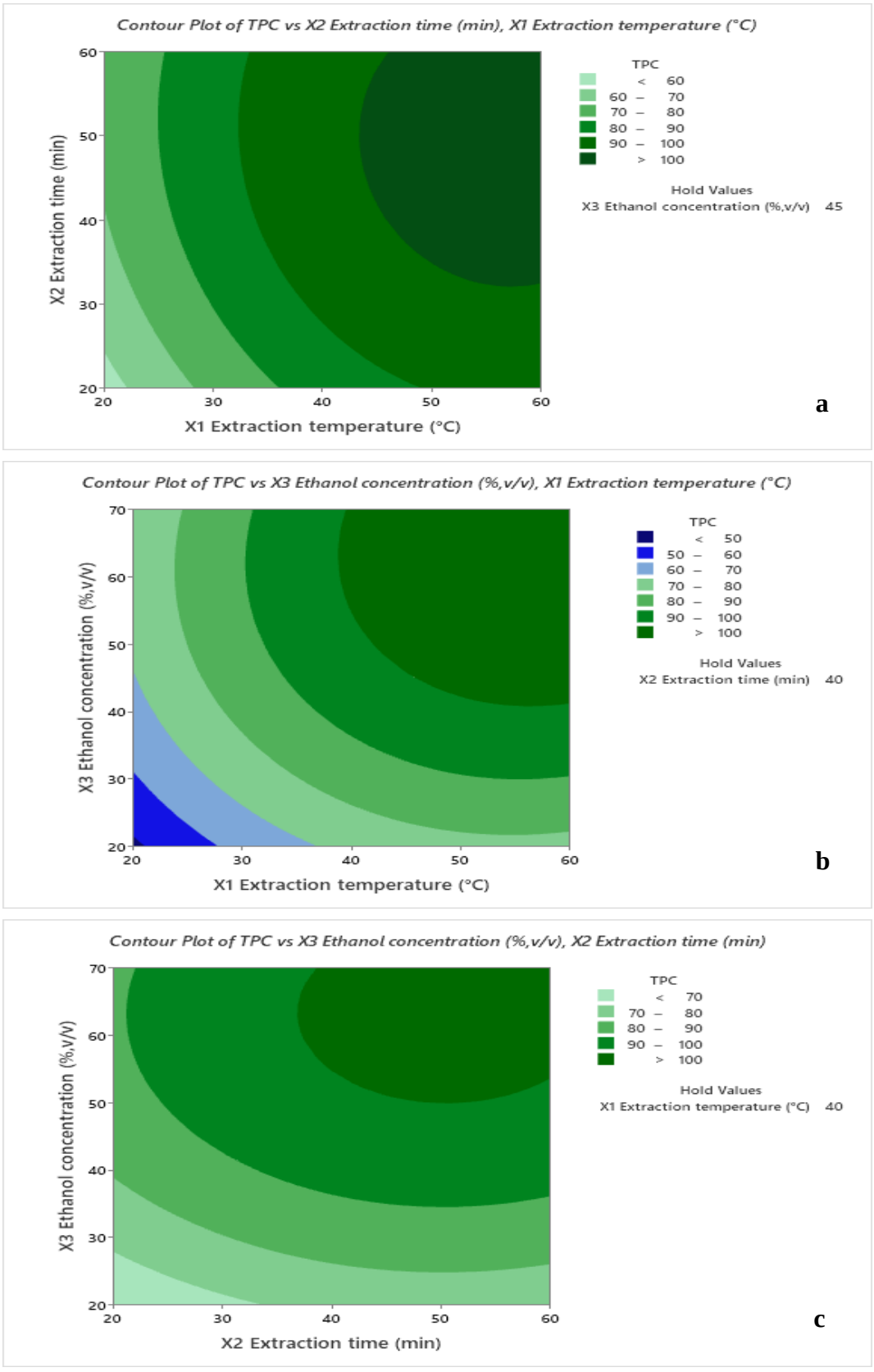

Fig. 3: Contour plots of TPC.

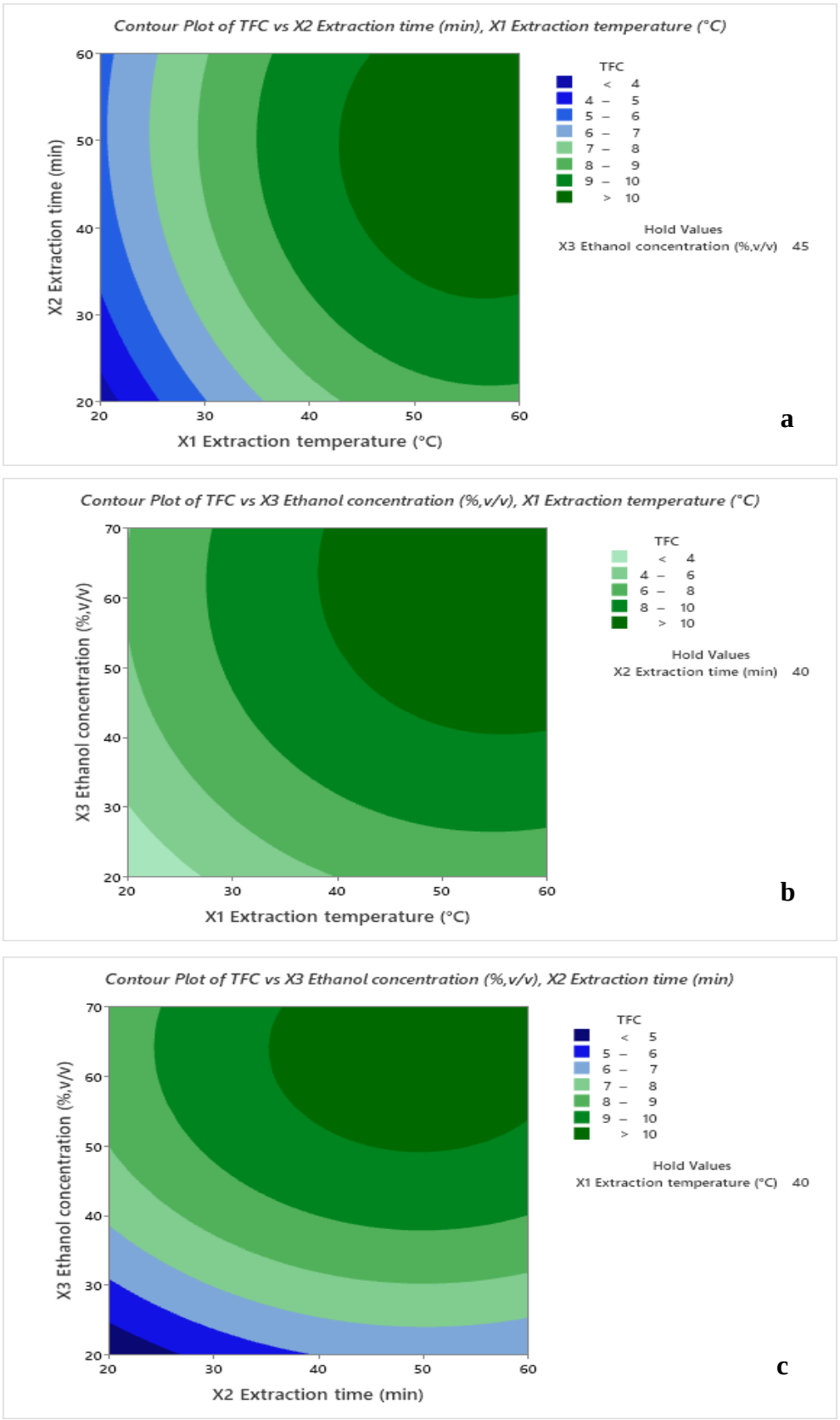

Fig. 4 : Contour plots of TFC.

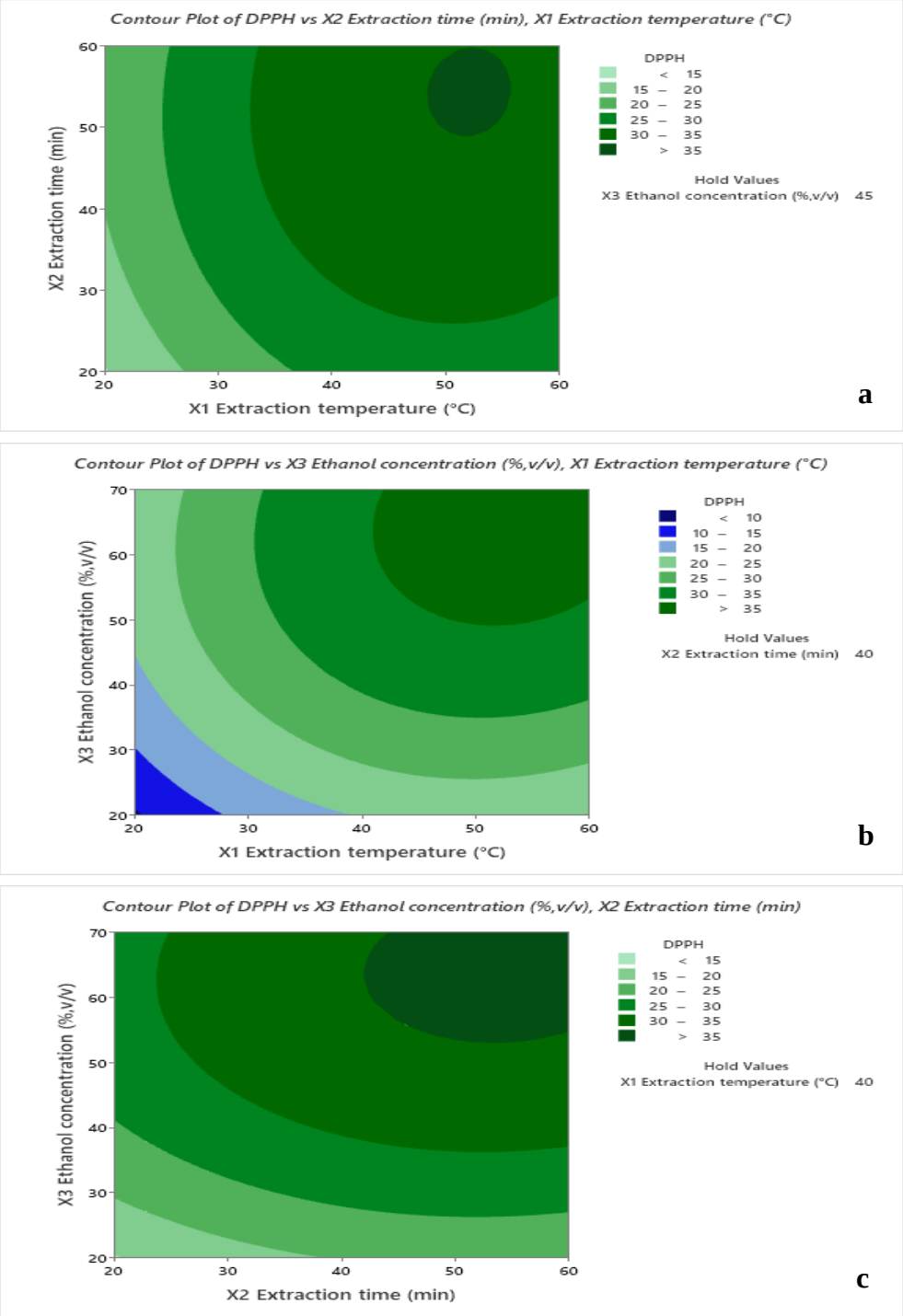

Fig. 5 : Contour plots for DPPH.

Determination of the Optimal Extraction Conditions

The optimal extraction conditions for TPC, TFC, and DPPH scavenging of *P. farcta* leaves extracts were estimated through the response optimizer function in Minitab software. The optimal extraction conditions, the predicted and experimental values under optimized conditions are given in **Table (5)**.

Determination of TPC, TFC, and DPPH Scavenging Correlations

Pearson correlation coefficients between TPC, TFC, and DPPH scavenging were estimated in **Table (6)**. The results show strong correlations among the three responses and these correlations are statistically significant. (p-value < 0.05).

Table 5 : Optimal extraction conditions, predicted and experimental values under optimized conditions for TPC, TFC, and DPPH scavenging of *P. farcta* leaves extracts.

Factors			**Responses**					
X1	***X2***	***X3***	**TPC (mg GAE/g DW)**		**TFC (mg RE/g DW)**		**DPPH scavenging (%)**	
Extraction temperature (°C)	Extraction time (min)	Ethanol concentration (%, *v/v*)	Predicted Value	Experimental Value	Predicted Value	Experimental Value	Predicted Value	Experimental Value
53.93	51.11	65.45	110.240	109.89±0.012	11.538	11.287±0.027	38.471	37.503±0.021

Table 6 : Pearson correlation coefficients between TPC, TFC, and DPPH scavenging of *P. farcta* leaves extracts.

Correlation	**Pearson correlation coefficient (r)**	***p*-value**
TPC, TFC	0.998	0.000
TPC, DPPH	0.986	0.000
TFC, DPPH	0.984	0.000

Discussion

Results show that all three extraction parameters have major effects on TPC, TFC, and DPPH scavenging values, the data analysis indicates that extraction temperature followed by ethanol concentration had the greatest effect on TPC and TFC, while extraction time had a moderate effect. The ethanol concentration followed by extraction temperature had the greatest effect on DPPH scavenging, while extraction time had a moderate effect. The interaction of extraction temperature and ethanol concentration on TPC was significantly positive as shown in **Fig. (3b)**, in contrast, the interaction of extraction temperature and extraction time on TPC was significantly negative indicating that high extraction temperature or longer extraction time are not necessary for TPC recovery. All the interactions between the three extraction parameters had no significant effects on TFC, while they had significant effects on DPPH scavenging. Thus, the TPC, TFC, and DPPH scavenging values increased gradually with increasing extraction parameters within the studied range as shown in the contour plots **[Fig. (3),(4),(5)]**, the highest values were obtained at a point close to the highest extraction parameters values, which were determined to be the optimal values based on the results in **Table (5)**. These findings are consistent with a previous study that has investigated the influence of extraction temperature and time on polyphenolic compounds of garlic, oregano, and parsley, the study results have shown that temperature has a greater effect on the extraction yield of phenolic compounds than that of time, so TPC and TFC values increased with increasing extraction temperature because it softens the plant tissues, reduces solvent viscosity, enhances the efficiency of mass transfer of polyphenolic compounds and breaks down the cellular components of the plant cells[32]. Additionally, TPC and TFC values increased with increased ethanol concentration which may be related to increases the solubility of solutes during ultrasonic assisted extraction[33]. The influence of time could be explained by the fact that long extraction time enhances the extraction of polyphenols, because prolonged exposure of sample permits solvent molecules to penetrate the plant tissues and cells and dissolve further of the phytochemical compounds[34]. The high correlations between TPC, TFC, and DPPH scavenging suggest that DPPH scavenging increases may be explained by the increases of TPC and TFC yields. However, **Fig. (5a)** shows a noticeable decrease in the DPPH scavenging activity at temperatures beyond 55.7 °C, which may be attributed to the degradation of thermally liable phenolic components[32].

The regression models developed for TPC, TFC, and DPPH scavenging exhibit good fit and strong association between the experimental and predicted values, as demonstrated by the high values of R^2 and adjusted R^2 which are very close to 1, therefore these models can represent the actual relationship between the responses and extraction parameters very well. Additionally, the lack of fit values for TPC, TFC, and DPPH scavenging models are not statistically significant, the *p*-values that are greater than 0.05 indicate that the models are able to accurately describe the experimental data.

Under the optimized conditions, the experimental results shown in **Table (5)** were consistent with the predicted values confirming that the TPC, TFC, and DPPH scavenging models were accurate, reliable and successful for determination the optimal extraction conditions.

Antioxidant potential for the leaves extracts which represented by their DPPH scavenging activity exhibited very strong and significant correlations with TPC and TFC, indicating that the polyphenolic compounds are the significant contributors to the antioxidant activity of *P. farcta* leaves extracts.

Conclusion

In the present study, response surface methodology using a BBD method was successfully employed to evaluate the effect of three extraction parameters, namely, temperature, time and ethanol concentration on the extraction yield of TPC, TFC, and the antioxidant potential represented by DPPH scavenging activity of *P. farcta* leaves extracts. It was found that all three extraction parameters have significant effects on TPC, TFC and DPPH scavenging activity. The obtained regression models can represent the actual relationship between the responses and extraction parameters very well. The optimum extraction conditions for TPC, TFC, and DPPH scavenging were extraction temperature 53.93°C, extraction time 51.11 minutes and ethanol concentration 65.45 %. Under these optimum conditions, the obtained experimental results were consistent with the predicted values proving that RSM models were accurate, reliable and successful for the optimization of the extraction conditions, leading to more consistent results and savings in time and resources. Furthermore, highly positive correlations were found between TPC, TFC and DPPH scavenging activity, indicating that the polyphenolic compounds are the significant contributors to the antioxidant activity of *P. farcta* leaves extracts.

This study provides a deeper understanding of the effect of extraction parameters on the extraction of bioactive compounds from *P. farcta* leaves and can serve as a reference for further studies on the extraction and isolation of valuable phytochemicals, moreover it highlights the importance of the potential applications of the optimized extraction conditions for the production of natural antioxidants from *P. farcta* leaves extracts.

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Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 883-898.

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تدقيق عملية الاستخلاص لتحقيق أفضل قيم لمحتوى المركبات الفينولية والفلافونوئيدية والفعالية المضادة للأكسدة لنبات بروسوبيس فاركتا (المسكيت السوري) باستخدام منهجية سطح الاستجابة

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يعتبر نبات بروسوبيس فاركتا (المسكيت السوري) من النباتات واسعة الانتشار ويتميز بغناه بالمكونات ذات الفوائد الصحية. تهدف هذه الدراسة إلى إجراء تحسين لشروط عملية الاستخلاص بالأمواج فوق الصوتية لأوراق نبات بروسوبيس فاركتا وذلك لتحقيق أفضل قيم ممكنة لكل من المحتوى الكلي للفينولات TPC، والمحتوى الكلي للفلافونوئيدات TFC، وتثبيط جذر DPPH باستخدام منهجية سطح الاستجابة .RSMاستُخدِمَ تصميم Box-Behnken (BBD) وتضمن ثلاث متغيرات مستقلة وثلاثة مستويات لكل متغير وهي درجة حرارة الاستخلاص (٢٠ و٤٠ و٦٠ °م)، وزمن الاستخلاص (٢٠ و٤٠ و٦٠ دقيقة) ، وتركيز الإيتانول (٢٠ و٤٠ و٧٥%). أظهرت النتائج أن لجميع متغيرات الاستخلاص الثلاثة تأثيرات كبيرة على قيم عوامل الاستجابة TPC وTFC وتثبيط DPPH، كانت شروط الاستخلاص المثلى هي (درجة حرارة الاستخلاص: ٥٣.٩٣ °م، وزمن الاستخلاص: ٥١.١١ دقيقة، وتركيز الإيتانول ٦٥.٤٥%)، كانت النتائج التجريبية في ظل هذه الشروط هي (TPC: 109.89 ملغ مكافئ من حمض الغاليك/غ وزن جاف، TFC: 11.287 ملغ مكافئ من الروتين/غ وزن جاف، (DPPH: 37.503%، وقد توافقت هذه النتائج بشكل جيد مع القيم النظرية المتوقعة مما يثبت أن نماذج RSM كانت دقيقة وموثوقة. أشارت النتائج إلى وجود ارتباطات قوية ما بين عوامل الاستجابة TPC وTFC وتثبيط .DPPH بينت هذه الدراسة أهمية نبات بروسوبيس فاركتا كمصدر طبيعي للمواد المضادة للأكسدة، بالإضافة إلى أنها سلطت الضوء على شروط الاستخلاص المثلى وإمكانية تطبيقها لتحسين إنتاج مضادات الأكسدة الطبيعية من أوراق بروسوبيس فاركتا.