Received: 4 June 2020 | Revised: 4 July 2020 | Accepted: 17 July 2020

DOI: 10.1111/jfpp.14813

ORIGINAL ARTICLE

Journal of Food Processing and Preservation | Institute of Food Science + Technology | ifst | WILEY

Plum (*Prunus domestica* L.) leaves extract as a natural antioxidant: Extraction process optimization and sunflower oil oxidative stability evaluation

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Abstract

The current work aimed to maximize the yield of total phenolic content (TPC) and associated antioxidant activity of plum leaf extract. The feasibility of using the optimal extract as a natural antioxidant for retarding the oxidation process in sunflower oil using rancimat method was also investigated. One-factor-at-a-time (OFAT) method and 3^2 full factorial design were implemented in sequence to optimize the studied factors (extraction time [ET] (10–60 min) and ethanol percentage [EtOH%] (0%–75%)). DPPH scavenging ability of the obtained extracts were positively ($r \geq 0.7493$) and significantly ($p < .0001$) correlated to TPC yield. The optimal conditions for extraction plum leaves were 64.37% EtOH% and 60 min with desirability of 0.856. The optimal plum leaves extract was subjected to HPLC analysis. At the same concentration (200 ppm), the protection factor of BHT and optimal plum leaves extract against sunflower oxidation were insignificantly ($p > .05$) differed.

Practical applications

Plum (*Prunus domestica* L.) leaves are characterized by a high content of phenolic compounds which varied between 66.50 to 143.7 mg GAE/g powder. Plum leaf extracts exhibited high scavenging activity against DPPH radicals. The protection factor of the freeze-dried optimal leaves extract was insignificantly differed than that of BHT at the concentration of 200 ppm. These findings indicate that plum leaves extract could efficiently retard the oxidation process of edible oils; and consequently, improve their quality and extend their shelf life.

1 | INTRODUCTION

Plant leaves are a great source of bioactive materials that are utilized in various food applications due to their functional properties (Bernhoft, 2010). In this context, the antiradical activities of leaf extract of several plants such as olive leaves (Andrikopoulos, Salta, Mylona, Chiou, & Boskou, 2007; Farag, Mahmoud, & Basuny, 2007), murta leaves (Rubilar et al., 2006, 2012), Ginkgo leaves (Kobus et al., 2009), and thyme leaves (Beddows, Jagait, & Kelly, 2000) were evaluated and their abilities to retard edible oils oxidation were determined. The antioxidant activity of plant leaf extracts was attributed to their phenolic compounds content (Moure et al., 2001; Pokorný, 2007).

Phenolic compounds present in all plants and comprise a group of greater than 8,000 identified compounds. These molecules are considered as a secondary metabolite in the plant. They contribute to plant growth, pollination; and protection against ultraviolet radiation, pathogens, and environmental stresses. Polyphenols molecules are characterized by the existence of hydroxyl groups that are attached to a benzene ring. The number of phenolic hydroxyl groups as well as their location determine the antioxidant activity of phenolic compounds (Serra, Almeida, & Dinis, 2018).

Fatty acids composition in addition to the antioxidants that are naturally found in edible oils affect their oxidative stability, which is an important parameter to evaluate the shelf life and quality of oils during processing and storage (Abril et al., 2019). Polyphenols are natural antioxidants that have a valuable role for retarding unsaturated fatty acids oxidation and provide an efficient protection against oxidative stress in the human body (Farhoosh, Khodaparast, & Sharif, 2009).

Plum (*Prunus domestica* L.) is a deciduous tree and belongs to the Rosaceae family. There are more than 2,000 varieties of plum and the major species are found in Europe and Australia (Mocan et al., 2018). Several studies have been conducted to investigate the total phenolic content of plum leaves and its associated antioxidant activity (Gougoulias, 2015; Mocan et al., 2018). However, based on our knowledge, there is no single study has been conducted to optimize the extraction process of plum leaves and evaluate the ability of the obtained extract to extend oxidative stability of edible oils. Therefore, response surface methodology (RSM) using 3^2 full factorial design (FFD) was implemented to maximize the TPC yield of plum leaves extract and its scavenging activity against DPPH radicals. Furthermore, rancimat method was used to investigate the ability of optimal plum leaves extract to extend the oxidative stability of sunflower oil.

2 | MATERIALS AND METHODS

2.1 | Materials

2.1.1 | Plant material and chemicals

Leaves (5 months old) of plum tree (*Prunus domestica* L. SSP Hollywood) were detached from 5 years old trees grafted on "Marianna" plum root stock and grown in sandy soil at the Faculty of Agriculture farm [Latitude 30° 1′ 38.0208″ (N), Longitude 31° 11′ 39.5628″(E)], Cairo University, Egypt in the summer of 2019. Plum trees were received the normal agriculture practices and cultivated under flood irrigation system. Plum leaves were identified by Dr. Ibrahim Hmmam, Pomology Department, Faculty of Agriculture, Cairo University. The leaves were dried at 40°C in a forced air oven (Shel-lab, USA) until constant weight, then they were grinded with analytical mill (Cole-Parmer, USA), sieved up to 50 mesh and stored in a dark place at room temperature till analysis. Free antioxidant sunflower oil was obtained from Cairo Oil and Soap Company (Egypt). Folin–Ciocalteu reagent, Gallic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA).

2.2 | Methods

2.2.1 | Experimental design and statistical analysis

Preliminary extraction experiments

To extract polyphenols, 5 gm *Prunus domestica* dried leaves powder were added to 100 ml aqueous ethanol solution (0%–75%) and stirred for 10–60 min using a benchtop lab stirrer (Heidolph, Germany) at maximum speed. All extraction experiments were conducted at room temperature and repeated three times. The extract was filtered and kept at 8°C for further analysis. The significant effects of studied factors (extraction time [ET] and ethanol percentage [EtOH%]) were estimated using One-way ANOVA followed by Tukey's test at $p < .05$. Degree of association between variables was calculated as Pearson correlation coefficient using XLSTAT 2014.5.03 software (Addinsoft, USA). The three levels of studied factors that significantly resulted in the highest polyphenol yield and radical scavenging activity were estimated and used for further experimental design.

Full factorial design (FFD)

RSM using 3^2FFD was further implemented to maximize the yield of extracted polyphenols and its corresponding antioxidant activity. The chosen three levels of ET (X_1) an EtOH% (X_2) that result in the highest polyphenol yield and radical scavenging activity (Figure 1) were 40, 50, and 60 min and 25%, 50%, and 75%, respectively. Experimental data were subjected to Browne–Forsythe and One-way ANOVA test with post hoc Tukey's test ($p < .05$) to assess responses variance homogeneity and significant effects among various treatments (combinations), respectively. Design-Expert version 11 (Stat-Ease, Inc., USA) was used to implement 3^2 FFD. Reduced cubic model including linear, squared, and interaction terms (Equation 1) was used to fit experimental data.

$$Y = b_0 + \sum_{j=1}^{k} b_j X_j + \sum_{j=1}^{k} b_{jj} X_j^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + \sum_{i<j}^{k} b_{ij} X_i^2 X_j + \sum_{i<j}^{k} b_{ij} X_i X_j^2 + e_i \quad (1)$$

where, Y is the predicted response; b_0 is the intercept; b_j, b_{jj}, and b_{ij} are coefficients of linear, quadratic, and interaction effects of coded independent variables (X_i and X_j), respectively; and e_i is the error. Statistical significance of the model and their various terms were determined using analysis of variance (ANOVA). Lack of fit test in addition to R^2, adjusted R^2 and predicted R^2 values were used to check the adequacy of generated models.

2.2.2 | Total phenolic compounds

Folin–Ciocâlteu assay (Hosseini, Bolourian, Yaghoubi Hamgini, & Ghanuni Mahababadi, 2018) was used to determine Total phenolic compounds (TPC) of dried plum leaves extract and expressed as mg Gallic acid equivalents (GAE)/g powder.

2.2.3 | Determination of antioxidant activity

The antiradical activity of the obtained extracts against DPPH radicals was determined according to the methodology described by Fang et al. (2014). DPPH inhibition percentage (DPPH IN%) was Spectrophotometrically (Unico UV-2000, USA) determined at 517 nm and calculated using Equation (2):

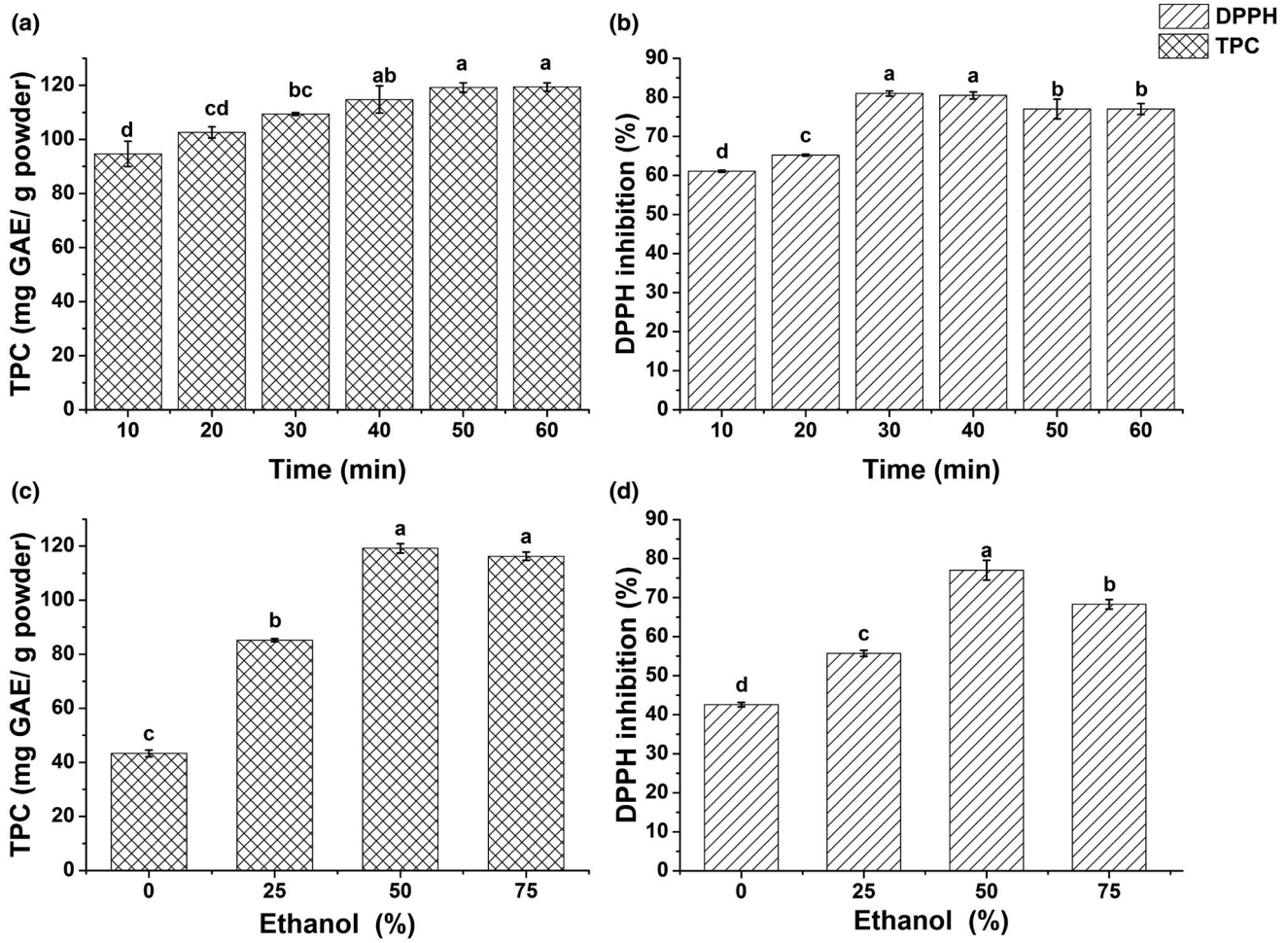

FIGURE 1 Effect of extraction time and ethanol percentage on total polyphenols content [TPC (mg GAE/g dried powder] of plum leaves and its associated DPPH radical scavenging activity [DPPH IN%]

$$\text{DPPH IN\%} = \left(\frac{A_{517\text{nm of DPPH solution}} - A_{517\text{nm of DPPH and extract solution}}}{A_{517\text{nm of DPPH solution}} \right) \times 100 \quad (2)$$

2.2.4 | HPLC analysis

The optimal plum leaves extract was analyzed using an Agilent 1260 series HPLC system (Agilent technologies Inc. CA, USA). The separation was carried out using C18 column (100 mm × 4.6 mm i.d., 5 µm). The mobile phase consisted of (A) water 0.2% H_3PO_4, (B) methanol, and (C) acetonitrile at a flow rate 0.6 ml/min. Gradient elution was according to the following scheme: 0–11 min (96% A, 2% B); 11–13 min (50% A, 25% B); 13–17 min (40% A, 30% B); 17–20.5 min (50% B, 50% C), and 20.5–30 min (96% A, 2% B). Detection wavelength was set at 284 nm. The injection volume was 20 µl and the column temperature was maintained at 30°C. Compounds were identified by comparing their retention time with those from authentic standards. Calibration curves were used to calculate the compound amounts.

2.2.5 | Physical and chemical properties of sunflower oil

Refractive index, acid value, and peroxide value of sunflower oil were determined according to AOCS official methods (AOCS, 2009).

2.2.6 | GC analysis of fatty acids

Fatty acids methyl esters (FAMEs) preparation

The FAMEs were prepared using a cold saponification method according to ISO standard No. 12966-2 (ISO, 2011).

Identification of FAMEs

The FAMEs were analyzed by an Agilent 6,890 series gas chromatography equipped by a flam ionization detector and DB23 (60 m × 0.32 mm × 0.25 µm) capillary column (Agilent technologies Inc. CA, USA). The carrier gas was N_2 at a flow rate of 1.6 ml/min and split ratio of 50:1. The column oven temperature was programed at

initial temperature of 150°C for 1 min; rising at 10°C/min to 170°C and held for 5 min; rising at 5°C/min to 220°C and held for 3 min. The injector and detector temperature were set at 250 and 270°C, respectively. Gases flow rates for the detector were 450, 40, and 25 ml/min for air, H_2, and N_2, respectively. Fatty acid standards were used to identify the peaks.

2.2.7 | Rancimat test

The optimal plum leaves extract was first concentrated using rotary evaporator (EYELA rotary evaporator N-1000, Japan) at 40°C and then, freeze-dried (Edward freeze dryer (3,983), England). The lyophilized extract at concentration of 100, 200, and 400 ppm GAE and BHT at 200 ppm were individually added to a free antioxidant sunflower oil. The tested oil samples (2.5 g) were placed in rancimat tubes (Metrohm's 743, Switzerland), which were exposed to air flow rate of 20 L/h and elevated temperature of 110°C. The organic acids, which were produced as a result of thermal decomposition of oil, were absorbed in deionized water filling the measuring vessel. Conductivity measuring cell was used to continuously monitor the formed organic acids. The induction periods of different samples were used to calculate the protection factor using Equation (3)

$$\text{Protection factor} = \frac{\text{induction period of sample with antioxidant}}{\text{induction period of sample without antioxidant (control)}} \quad (3)$$

3 | RESULTS AND DISCUSSION

3.1 | Preliminary extraction experiments

Preliminary extraction experiments were conducted to investigate the individual effect of studied factors (ET and EtOH%) on the yield of polyphenols and its corresponding antioxidant activity. Thus, through all preliminary experiments only one factor was changed while the other extraction conditions were kept constant. The obtained results are outlined in Figure 1.

3.1.1 | Effect of extraction time (ET)

Different ET (10–60 min) were used to extract polyphenols from dried plum leaves powder at EtOH% of 50% (Figure 1a). The highest and lowest yields of TPC were 119.40 ± 1.50 and 94.63 ± 4.72 mg GAE/g powder which were obtained at ET of 60 and 10 min, respectively. In general, it could be noted that extending ET increased TPC yield until it reached equilibrium at 50 min and there was no change in TPC yield after that. Similar behavior was observed during extraction polyphenols from olive leaves (Mkaouar, Gelicus, Bahloul, Allaf, & Kechaou, 2016). The obtained results are consistent with previously reported data for TPC of quince and cranberry leaves, which were found to be varied from 89.57 to 175.36 and from 89.81 to 127.64 mg GAE/g dry matter, respectively (Teleszko & Wojdyło, 2015). Also, TPC of black tea was ranged between 80.5 to 134.9 mg GAE/g dry matter (Khokhar & Magnusdottir, 2002). Moreover, Gougoulias (2015) found that TPC of plum leaves was 9.381 mg GAE/g dry matter, which is lower than our results. These variations in TPC could be attributed to several factors, such as plum leaves variety, climate, cultivation conditions, harvesting time, extraction methods, and solvent type (Brahmi, Mechri, Dhibi, & Hammami, 2013; Nashwa & Abdel-Aziz, 2014).

Several analytical methods have been adopted to evaluate the antioxidant activity of different materials. Among these analytical methods, DPPH method is extensively used to evaluate in vitro scavenging ability of plant extracts against free DPPH radicals due to its efficiency, simplicity, and cheapness (Kandi & Charles, 2019). DPPH IN% was increased from 60.07 ± 0.26 to 80.51 ± 0.89% as ET increased from 10 to 40 min, respectively (Figure 1b); however, further increase in ET significantly decreased DPPH IN% with low decrement to 77.01 ± 1.41%. The correlation between TPC and DPPH IN% of the tested extracts was significant ($p < .001$) and positive ($r = 0.7853$). Tohidi, Rahimmalek, and Arzani (2017) reported that polyphenols have a capability to scavenge reactive oxygen intermediate compounds without any further support of oxidative reactions.

3.1.2 | Effect of ethanol percentage (EtOH%)

At ET of 50 min, Data illustrated in Figure 1c show that increasing EtOH% to 50% significantly ($p < .05$) increased TPC yield to 119.12 ± 1.75 mg GAE/g powder; however, further increase in EtOH% insignificantly ($p > .05$) decreased the yield of TPC to 116.23 ± 1.53 mg GAE/g powder. Similar trend was observed during extraction polyphenols from *Myrtuscommunis* L. leaves using different EtOH% (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015). The reason behind this decrement in TPC yield at higher EtOH% could be attributed to the binding affinity of polyphenols for protein (Papadopoulou & Frazier, 2004). Higher EtOH% induce protein denaturation which in turn decreased the dissolution of polyphenols leading to low TPC yield (Dahmoune et al., 2015). Furthermore, the low TPC yield at high EtOH% might be ascribed to the effect of ethanol on barrier properties of the plant cell membrane (Frontuto et al., 2019).

Moreover, the highest TPC yield at EtOH% of 50% could be returned to the polarity of ethanol water mixture. The polarity of ethanol is lower than that of water. Thus, adding water to ethanol increases its polarity regarding the proportion of added water. Polyphenols are polar compounds, which are more soluble in ethanol concentration of 50% (high polarity) other than high ethanol concentration of 75% (low polarity) according to the principle of "like dissolve like" (Dahmoune et al., 2015; Zhang et al., 2007).

Similar to TPC recovery pattern, the DPPH IN% of the obtained extracts was significantly ($p < .05$) increased as EtOH% increased to

50% and significantly decreased after that. The correlation between TPC and DPPH IN% of the tested extracts was significant ($p < .001$) and positive ($r = 0.9638$).

3.2 | Full factorial design

Three level FFD was found to be more adequate to fit experimental data comparing with other experimental designs (Rakić, Kasagić-Vujanović, Jovanović, Jančić-Stojanović, & Ivanović, 2014). Therefore, in the present study three level FFD was chosen. Mean values of the actual and predicated values of TPC yield of plum leaves extracts and their DPPH IN% are listed in Table 1. Probability values of Brown–Forsythe's test ($p \geq .2486$) and one-way ANOVA ($p < .0001$) indicate that all dependent variables are homoscedastic and significantly differed. Implementing the preceding tests are essential for performing FFD (Granato, de Araújo Calado, & Jarvis, 2014).

TPC yield of various extracts were significantly ($p < .0001$) varied between 66.50 ± 0.10 mg GAE/g powder (run 1) and 143.57 ± 2.52 mg GAE/g powder (run 9), while the DPPH IN% were significantly ($p < .0001$) varied between 55.74 ± 0.77% (run 4) and 80.51 ± 0.90% (run 2). The correlation between TPC and DPPH IN% under applied extraction conditions was significant ($p < .0001$) and positive ($r = 0.7493$).

Results of fitting experimental data using various models are outlined in Table 2. Despite the cubic model which was confounding or aliased, the reduced cubic model was the most suitable model to fit experimental data. As, the reduced cubic model was significant ($p < .0001$), lack of fit was insignificant ($p \geq .0800$) and it has the highest values of R^2, adjusted R^2 and predicted R^2.

In terms of coded factors, reduced cubic models (Equations 4 and 5) were adopted to the following forms.

$$Y_{TPC(mg\ GAE/g\ powder)} = 119.10 + 2.32X_1 + 15.52X_2 + 6.25X_1X_2 - 1.98X_1^2 - 18.35X_2^2 + 11.68X_1^2X_2 + 9.02X_1X_2^2 \quad (4)$$

$$Y_{DPPHIN\%} = 75.95 - 1.75X_1 + 6.27X_2 + 0.524X_1X_2 + 3.34X_1^2 - 13.40X_2^2 - 0.563X_1^2X_2 + 1.63X_1X_2^2 \quad (5)$$

Multiple regression analysis in addition to analysis of variance (ANOVA) was implemented to assess the adequacy of the obtained models to represent the variability of response variables (Table 2). Low probability values ($p < .0001$) of the obtained models indicate their high significances. Despite, regression coefficients of X_1X_2, $X_1^2X_2$ and $X_1X_2^2$ for DPPH IN% which were insignificant ($p > .05$), all other regression coefficients were significant ($p \leq .0320$). Adjusted R^2 values of the obtained models were 0.9921 and 0.9647 for TPC and DPPH IN% and their lack of fit were insignificant ($p = .9368$ and 0.0800, respectively), which show the ability of the obtained models to explain 99.21% and 96.47% of the variance, respectively (Pedro, Granato, & Rosso, 2016). According to Maran, Priya, and Manikandan (2014), the desirable value of signal-to-noise ratio (adequate precision) is greater than 4. In the present study, adequate precision values were greater than 28 which indicate the adequacy of the signals. CV% values of the obtained models were lower than 2.3 which indicates high degree of experimental values precisions and adequately of the obtained models (Maran et al., 2014).

3.2.1 | Effect of extraction process variables

Equations (4) and (5) were used to draw 3D plots (Figure 2c,d) which were used in addition to perturbation plots (Figure 2a,b) to illustrate the interactive and individual effects of studied factors on response variables. Perturbation plots were drawn at midpoint of studied

TABLE 1 Full factorial design (FFD), experimental means, and predicted values of plum leaves extract total polyphenols content (TPC) and their radical scavenging activity against DPPH

Run	Extraction time (min) X_1	Ethanol conc. (%) X_2	TPC yield (mg GAE/gm powder)		DPPH inhibition %	
			Experimental	Predicted	Experimental	Predicted
1	40	25	$66.50^{f} \pm 0.10$	66.48	$61.09^{c} \pm 0.69$	60.82
2	40	50	$114.77^{b} \pm 5.01$	114.80	$80.51^{a} \pm 0.90$	81.04
3	40	75	$108.40^{c} \pm 1.50$	108.38	$71.46^{b} \pm 0.38$	71.19
4	50	25	$85.20^{d} \pm 0.60$	85.23	$55.74^{d} \pm 0.77$	56.28
5	50	50	$119.17^{b} \pm 1.75$	119.10	$77.01^{a} \pm 2.52$	75.95
6	50	75	$116.23^{b} \pm 1.53$	116.27	$68.29^{b} \pm 1.22$	68.82
7	60	25	$76.67^{e} \pm 0.42$	76.65	$59.80^{cd} \pm 2.40$	59.54
8	60	50	$119.40^{b} \pm 1.50$	119.43	$77.01^{a} \pm 1.41$	77.54
9	60	75	$143.57^{a} \pm 2.52$	143.55	$72.27^{b} \pm 1.53$	72.01
	P (Browne–Forsythe)		0.2486		0.9298	
	P (ANOVA)		<0.0001		<0.0001	

Note: Values are expressed as means ± standard deviations of three replicates.
Different letters in the same column indicate significant differences at $p < .05$.

TABLE 2 Adequacy of the tested models and ANOVA analysis of the reduced cubic-order models in addition to their statistical parameters

Model	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Prob < F	Prob (Lack of Fit)
TPC							
Linear	12.02	0.7607	0.7408	0.6997	4,351.53	<0.0001	<0.0001
2FI	11.42	0.7931	0.7661	0.7257	3,975.40	0.0706	<0.0001
Quadratic	6.74	0.9341	0.9184	0.8931	1549.95	<0.0001	<0.0001
Reduced Cubic	**2.10**	**0.9942**	**0.9921**	**0.9884**	**168.81**	**<0.0001**	**0.9368**
Cubic*	2.10	0.9942	0.9921	0.9884	168.81	<0.0001	0.9368
DPPH IN%							
Linear	7.09	0.3443	0.2896	0.2056	1,462.62	0.0063	<0.0001
2FI	7.24	0.3461	0.2608	0.1848	1,500.86	0.8041	<0.0001
Quadratic	1.68	0.9677	0.9601	0.9470	97.53	<0.0001	0.0611
Reduced Cubic	**1.58**	**0.9742**	**0.9647**	**0.9507**	**90.76**	**<0.0001**	**0.0800**
Cubic*	1.58	0.9742	0.9647	0.9507	90.76	0.1194	0.0800

ANOVA analysis and statistical parameters of reduced cubic-order models

Source	TPC			DPPH IN%		
	RC	SS	p value	RC	S	p value
Model	119.10	14,408.90	<.0001	75.95	1793.63	<.0001
X_1	2.32	32.20	.0141	−1.75	18.36	.0139
X_2	15.52	1,444.60	<.0001	6.27	236.07	<.0001
X_1X_2	6.25	468.75	<.0001	0.5241	3.30	.2651
X_1^2	−1.98	23.60	.0320	3.34	67.05	<.0001
X_2^2	−18.35	2020.34	<.0001	−13.40	1,077.53	<.0001
$X_1^2X_2$	11.68	546.00	<.0001	−0.5632	1.27	.4848
$X_1X_2^2$	9.02	325.20	<.0001	1.63	10.64	.0530
Residual		83.68			47.48	
Lack of fit		0.0300	.9368		7.62	.0800
Pure error		83.65			39.86	
Cor total		14,492.59			1841.11	
Mean	105.54			69.24		
C.V.%	1.99			2.28		
Adeq precision	67.4622			28.7802		

*Aliased model.

variables (coded 0.0 (50 min, 50%)) to compare the influences of these variables on different responses.

Effect of extraction process variables on TPC yield

Data in Figure 2a illustrate that TPC yield was more sensitive to the change in EtOH% than the change in ET. As, the line (B) that represent the change of TPC yield with changing EtOH% at constant ET is highly curved, while the line (A) that represent the change of TPC yield with changing ET at constant EtOH% is approximately flat line. Furthermore, regression coefficients of linear terms for ET and EtOH% were 2.32 and 15.52 (Table 2), respectively, which show greater effect of EtOH% than ET on TPC yield.

The results in Figure 2c show that the highest TPC yield was observed at the highest levels of ET and EtOH%. All regression coefficients for TPC yield were statistically significant ($p \leq .0320$) which result in curvilinear change of TPC yield for all investigated factors. Two distinct effects of EtOH% on TPC yield were observed. At low ET, it could be noted that increasing EtOH% increased the yield of TPC till maximum which was observed at the range of 55%–65% and further increase in EtOH% decreased the yield of TPC. At high ET, increase EtOH% resulted in progressive increment of TPC yield.

Effect of extraction process variables on DPPH IN%

Data in Figure 2b indicate that DPPH IN% was sensitive to the change of both EtOH% and ET. However, its sensitivity against the change in

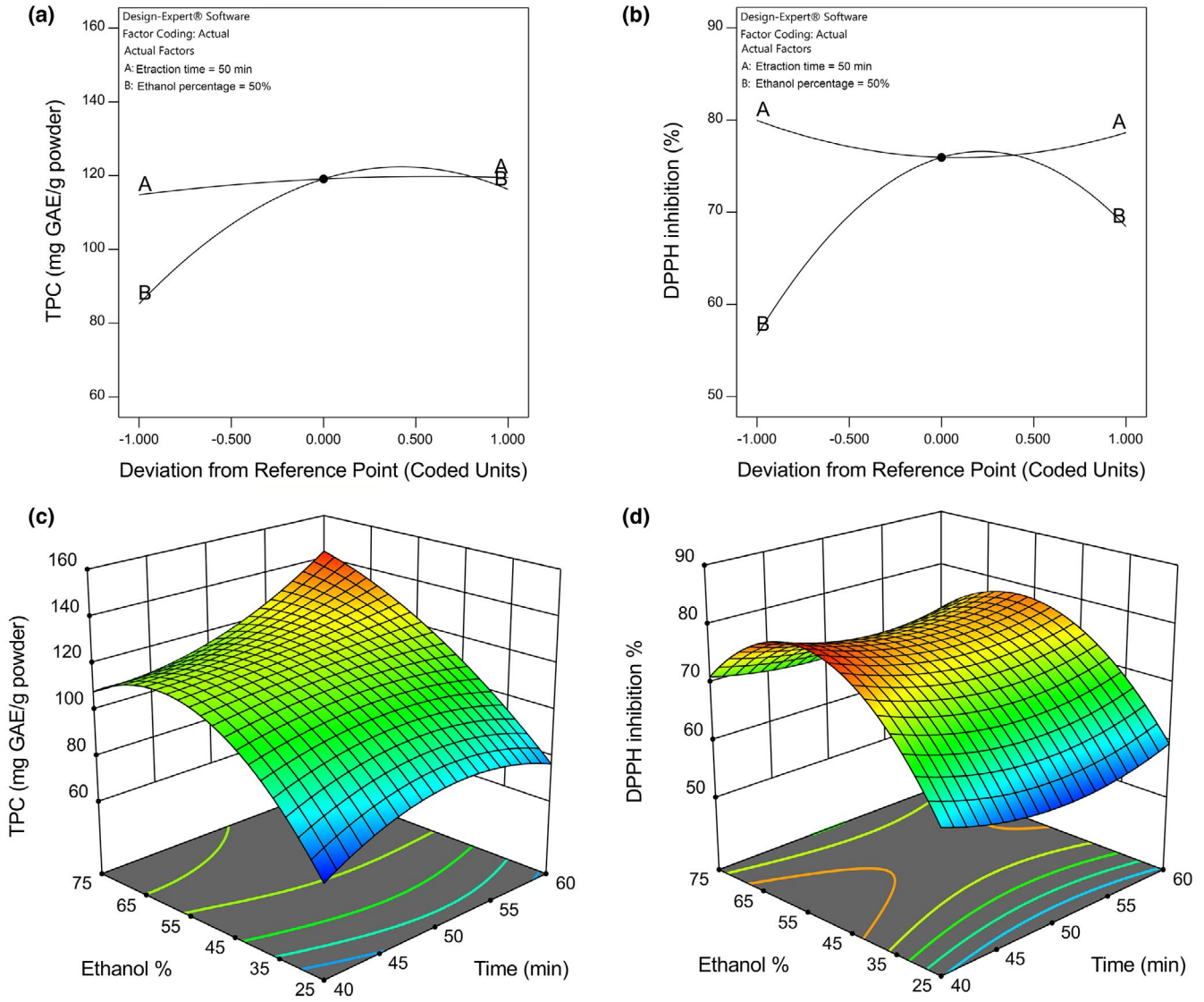

FIGURE 2 Perturbation and response surface plots of total polyphenols content [TPC (mg GAE/ g dried powder] of plum leaves (a & c) and its associated DPPH radical scavenging activity [DPPH I%] (b & d)

EtOH% is higher. The values of regression coefficient of linear terms of both ET and EtOH% sustained the preceding observation. As, their values were −1.75 and 6.27, respectively. Significances of linear and quadratic terms for DPPH IN% resulted in a curvilinear change of DPPH IN% for all investigated variables (Figure 2d). Two different trends were observed as a result of increasing EtOH%. For EtOH% lower than 62%, DPPH IN% was increased as EtOH% increased. However, for percentages higher than 62%, increasing EtOH% resulted in a decrease of DPPH IN%.

3.2.2 | Optimal extraction process conditions determination and model validation

Desirability function was implemented to maximize the yield of TPC and its associated radical scavenging activity. The optimum conditions for extracting TPC with the highest DPPH radical scavenging activity were 60 min and EtOH% of 64.37% with desirability of 0.856. To validate the obtained model, extraction of plum leaves powder using aqueous ethanol solution (65%) for 60 min were carried out. The predicted and validated values of TPC yield and DPPH IN% were 135.57 and 136.67 ± 2.52 mg GAE/g powder and 77.24 and 76.54 ± 0.86%, respectively. The predicted and validated values are very close indicating the adequacy of the obtained models to predict experimental data.

3.3 | HPLC analysis

Identification and quantification of phenolic components of the plum leaves extract obtained at optimal conditions were determined using HPLC (Figure 3 and Table 3). The total identified components were 18 compounds. The major identified phenolic compounds were o-Coumaric acid, Rosmarinic acid, Resveratrol, Quercetin,

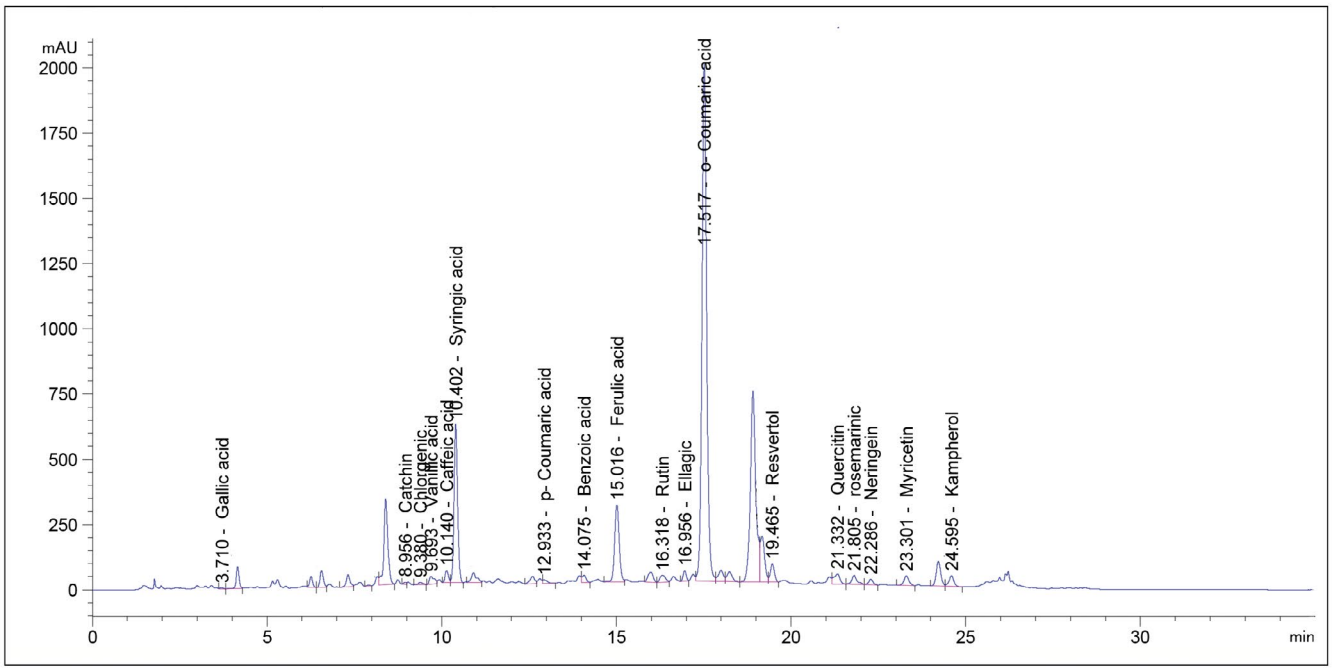

FIGURE 3 HPLC profile of phenolic compounds in plum leaves extract obtained at optimal conditions

TABLE 3 Phenolic compounds of plum leaves extract obtained at optimal conditions

Compounds	Retention time (min)	Concentration (μg/g leaf)
Gallic acid	3.71	24 ± 1.41
Catechin	8.956	18 ± 0.80
Chlorogenic acid	9.38	81 ± 1.50
Vanillic acid	9.693	499 ± 25.00
Caffeic acid	10.14	164 ± 10.00
Syringic acid	10.402	3,300 ± 80.00
p-Coumaric acid	12.933	61 ± 4.50
Benzoic acid	14.075	2,991 ± 225.00
Ferulic acid	15.016	1888 ± 112.01
Rutin	16.318	1,359 ± 51.33
Ellagic acid	16.956	264 ± 7.50
o-Coumaric acid	17.517	6,836 ± 570.01
Resveratrol	19.465	4,060 ± 152.00
Quercetin	21.332	3,839 ± 113.00
Rosmarinic acid	21.805	4,465 ± 51.99
Naringenin	22.286	1844 ± 66.00
Myricetin	23.301	3,180 ± 21.00
Kaempferol	24.595	2,678 ± 75.00

Note: Values are expressed as means ± standard deviations of two replicates.

Syringic acid, Myricetin, Benzoic acid, and Kaempferol. Several studies have been conducted to examine the antioxidant activity of identified phenolic compounds (Erkan, Ayranci, & Ayranci, 2008; Guitard, Paul, Nardello-Rataj, & Aubry, 2016; Gülçin, 2010; Lesjak et al., 2018; Peñalvo et al., 2016), which showed high scavenging activities against DPPH radicals. Thus, high antioxidant activity of the obtained extracts could be related to its content of phenolic compounds and its synergistic effect with other ingredients present in the same extract (Xu et al., 2017).

3.4 | Rancimat test

Physical and chemical characteristics of sunflower oil are listed in Table 4. Refractive index value, acid value, peroxide value, and fatty acids composition of the tested oil sample are in accordance with those specified values for sunflower oil (Codex Alimentarius Commission, 2005) which indicates the authenticity of the oil sample. Also, data in Table 4 and Figure 4 indicates that the sunflower oil is rich with polyunsaturated fatty acids (greater than 88%) especially essential fatty acids (ω6 and ω3) (Delplanque, 2000).

The influence of optimal plum leaves extract on oxidative stability of sunflower oil was investigated using rancimat method and the obtained results are listed in Table 5. Adding antioxidants to sunflower oil significantly ($p < .05$) increased its induction period. The induction period of sunflower oil containing plum leaves extract at 200 ppm GAE was 4.730 ± 0.007 hr, which was close to the induction period of sunflower oil containing BHT at the same concentration. Moreover, data in Table 5 reveal that increasing plum extract concentration from 100 to 400 (ppm GAE) had insignificant ($p > .05$) effect on extending the induction period of sunflower. Despite the highest protection factor was recorded for BHT (1.187 ± 0.012), it was insignificantly ($p > .05$) differed than that

TABLE 4 Physical and chemical properties of sunflower oil

Parameter	Sunflower oil
Refractive index at 20°C/20°C	1.4750 ± 0.00
Acid value (mg KOH/g oil)	0.27 ± 0.01
Peroxide value (m. equiv./kg oil)	0.67 ± 0.02
Fatty acids	**Relative area percentage**
Myristic acid	0.072 ± 0.005
Palmitic acid	6.506 ± 0.425
Palmitoleic acid	0.106 ± 0.003
Stearic acid	3.574 ± 0.162
Oleic acid	28.617 ± 1.723
Linoleic acid	59.527 ± 3.124
Alpha-linolenic acid	0.291 ± 0.012
Arachidic acid	0.271 ± 0.015
Behenic acid	0.729 ± 0.041
Total unsaturated fatty acids	88.541
Total saturated fatty acids	11.152
Unknowns	0.307

Note: Values are expressed as means ± standard deviations of two replicates.

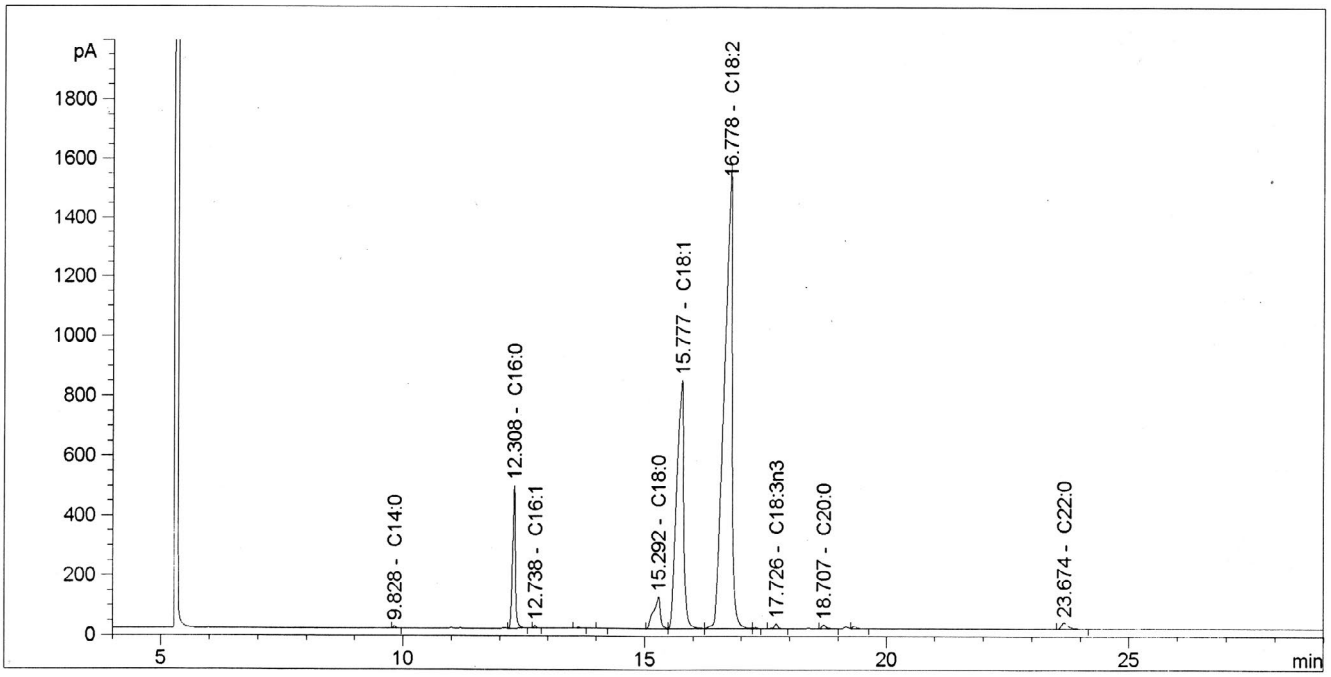

FIGURE 4 Typical GC chromatogram of sunflower fatty acid methyl esters

for plum extract (200 ppm GAE). Ghosh, Upadhyay, Mahato, and Mishra (2019) found that the induction periods of antioxidant free sunflower oil under the same air flow rate were 6.13 and 2.74 hr at 100 and 110°C, respectively. Kobus et al. (2009) found that adding ethanolic Ginkgo leaves extract at concentration of 200 ppm to stripped triacylglycerols of rape seed oil increased its induction period from 8.14 hr for antioxidant free sample to 10.04 hr. They ascribed the oxidative inhibition effect of Ginkgo leaves extract to bioactive components of the extract and its interactions. Additionally, Beddows et al. (2000) found that induction period of sunflower oil at 105°C was increased from 8.3 to 9.9 and 11.4 hr as a result of adding thyme and turmeric extract to the oil, respectively. They also found that adding mixture of both extracts to the oil increased its induction period to 13.8 hr.

TABLE 5 Effect of the concentration of plum leaves extract on oxidative stability of sun flower oil according to Rancimat analysis

Sample	Induction period (hours)	Protection factor
Control	$4.230^{c} \pm 0.078$	–
BHT (200 ppm)	$5.020^{a} \pm 0.042$	$1.187^{a} \pm 0.012$
Plum extract (100 ppm GAE)	$4.650^{b} \pm 0.007$	$1.099^{b} \pm 0.022$
Plum extract (200 ppm GAE)	$4.730^{b} \pm 0.007$	$1.118^{ab} \pm 0.022$
Plum extract (400 ppm GAE)	$4.720^{b} \pm 0.051$	$1.116^{b} \pm 0.009$

Note: Values are expressed as means ± standard deviations of two replicates.

Different letters in the same column indicate significant differences at $p < .05$.

4 | CONCLUSION

The results of the present work showed that the antioxidant activity of ethanolic plum leaves extracts was highly dependent on their phenolic compounds content. TPC yield and DPPH IN% of the obtained extracts were more sensitive to EtOH% change than changing ET. Reduced cubic model was found to be the best model to represent experimental data. RSM using 3^2 FFD successively optimized the extraction parameters (64.37% EtOH%, 60 min) and TPC yield and DPPH IN% of optimal extract were 136.67 ± 2.52 mg GEA/g powder and 76.54 ± 0.86%, respectively. The major identified phenolic components were o-Coumaric acid, Rosmarinic acid, Resveratrol, Quercetin, Syringic acid, Myricetin, Benzoic acid, and Kaempferol. Optimal plum leaves extract showed superior inhibitory characteristics for sunflower oxidation process. At concentration of 200 ppm GAE, the protection factor of optimal plum leaves extract was non statistically differed than that of BHT.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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How to cite this article: Elsayed N, Hammad KSM, Abd El-Salam EAE-S. Plum (*Prunus domestica* L.) leaves extract as a natural antioxidant: Extraction process optimization and sunflower oil oxidative stability evaluation. *J Food Process Preserv*. 2020;00:e14813. https://doi.org/10.1111/jfpp.14813