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Research Article

Parrotia persica as a Potential Source of Bioactive Phenolic Compounds: Optimization of Extraction Parameters and Biological Activity Assay

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Parrotia persica is one of the endemic plants in Iran and belongs to the Hamamelidaceae family. A wide range of biological activities of this plant have been attributed to several phenolic compounds. In this study, the phenolic bioactive compounds extraction from *P. persica* leaves was performed using conventional and ultrasonic-assisted extraction (UAE) techniques through the response surface methodology (RSM) to optimize the best extraction conditions and reach the maximum amount of phenolic compounds. The central composite design (CCD) was conducted for the optimization of four extraction parameters, including extraction time, alcohol concentration, solvent-to-solid ratio, and temperature. The coding of parameters was performed as an independent variable at five levels. Quantitative and qualitative assessments were achieved by using HPLC, LC-MS, and UV-Vis spectrophotometry. According to the results, UAE was chosen as the best method with the optimal extraction values: 15 min as an extraction time, 40% alcohol portion in solvent, solvent-to-solid ratio equal to 30 : 1, and 25°C for temperature. In this regard, it was found that the similarity of experimental to predicted findings was 98.7% for the phenolic content and 94.9% for the mass extract in the UAE method. The findings showed a good similarity between the experimental and predicted values, and no significant changes were observed between the real and theoretical results. In addition, our finding revealed that the optimum extraction yield was 28.5% for the mass extract in the UAE optimization process. Furthermore, the antioxidant activity by DPPH assay indicates that the extract which was obtained using UAE in optimum condition, proposed remarkable antioxidant activity (IC_{50} 29.86 $\mu g \cdot ml^{-1}$). Moreover, the cytotoxic assay was performed against PC-3 cancer cells, and it was found that the optimized extract using UAE has a promising cytotoxic activity (IC_{50} 10.4 $\mu g \cdot ml^{-1}$) without toxicity toward normal cells. Since there is a possibility to use *P. persica* as one of the commercial herbal sources, the optimized extraction models could be utilized for scaling up the phenolic compound extraction from *P. persica* leaf.

1. Introduction

Parrotia persica C.A.Mey. or Persian ironwood, is an endemic plant that is restricted to a region of northern Iran. This plant is a deciduous tree of the Hamamelidaceae family, which contains more than 140 species in 31 genera and has a wide geographic distribution [1]. Ethnobotanical studies have referred to the traditional use of this plant in the treatment of various fevers and respiratory infections [2].

Alkaloids, terpenoids (such as steroidal saponins), and different types of phenolic compounds, such as tannins, flavonoids, and phenolic acids, have been reported as the main principles in *P. persica* [3]. Moreover, a number of attempts have been made to demonstrate that *P. persica*

possesses various biological activities such as wound healing effects, antifungal activities, antidiabetic, anticancer, antioxidant, and antimicrobial activities [4–8].

P. persica due to the presence of tannins has an astringent effect which leads to antiperspirant activity. In addition, *in vitro* analysis of the two abundant phenolic compounds belonging to *P. persica* including chlorogenic acid and myricetin-3-O-β-rhamnoside demonstrated the effective promotion of wound closure and capillary tube formation. More recently, the antiproliferative activity of *P. persica* against different cancer cell lines was attributed to gallotannins [2].

Phenolic compounds, as one of the main active constituents in *P. persica*, have been shown to have a variety of pharmacological activities such as antioxidant, antimutagenic, antiallergic, anti-inflammatory, and antimicrobial effects, and therefore are nowadays widely considered by the fields of biology and medicine [9–13].

Thus, the selection of the best method to extract phenolic compounds with the highest yield is necessary. Among the different methods, maceration and UAE were preferred. These two techniques are economically viable and are widely used in plant extraction due to their simplicity and availability [14, 15]. Besides, UAE is one of the quick, simple, and effective extraction techniques extensively used to extract bioactive compounds from natural sources [16–19].

However, based on our knowledge, there is no research on optimizing the phenolic compound extraction process from *P. persica.* Therefore, this study is planned to enhance the phenolic compound extraction rate from *P. persica* using a different method through response surface methodology (RSM).

RSM is an efficient statistical technique for optimizing different processes. RSM was applied to obtain a large number of data points that are needed to evaluate several factors and their interactions using fewer experiments [20–23]. Besides, it is widely used to optimize the extraction process of some natural compounds, such as alkaloids, saponins, and phenolic compounds, from different sources [24, 25].

Although there are a number of studies about the biological activities of *P. persica*, so far no research has been carried out to extract the active constituents from *P. persica* using RSM, so the current study tends to focus on obtaining the highest amount of phenolic compounds by RSM and optimizing the parameters for the extraction process. Therefore, the main objective of this study was to optimize the extraction parameters of two extraction methods (maceration and UAE) using RSM in the case of phenolic compound extraction from *P. persica* to obtain the optimal extraction condition. Moreover, all the biological activities of the optimized extract were studied.

2. Materials and Methods

2.1. Plant Materials and Chemicals. P. persica leaves were collected from Guilan province and vouchered (No. 8551) at Guilan University Herbal Bank. The leaves were dried at room temperature and powdered using a mill. Folin–Ciocalteu phenol reagent and sodium carbonate (Na_2CO_3) were prepared from Merck Co. (Darmstadt, Germany). HPLC-grade ethanol, 2-2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were provided by Sigma Aldrich (USA). Distilled deionized water (dd. H2O) was prepared by the Ultrapure TM water purification system from Lotun Co. Ltd., (Taipei, Taiwan). The PC-3 human prostate cancer cell line was provided by the Pasteur Institute of Iran. The cells were cultured in RPMI-1640 medium (RPMI-1640, Gibco, Waltham, MA, USA). All culture media were supplemented with 10% (v/v) fetal bovine serum (FBS) (PAN-Biotech) and 1% antibiotics (100 U/mL penicillin, 100 mg/L streptomycin) (PAN-Biotech). The incubation of cells was performed at 37°C in a humidified atmosphere containing 5% CO_2, and passaged twice a week. The MTT dye was purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Phenolic Compounds Extraction

2.2.1. Conventional Maceration. One gram of powdered *P. persica* leaves was extracted through the conventional maceration method by changing the effective factors such as extraction time (8–36 hours), ethanol concentration (50–100%), and solvent-to-solid ratio ($5:1–30:1\,g{\cdot}ml^{-1}$). The factor levels for the optimization process were designed based on some in-house experiments. The levels of conventional maceration extraction parameters were designed using the Design-Expert 11.0 (DOE) software® (Stat-Ease). The samples were shaken at different durations, and the obtained extracts were kept at 4°C for further investigation. All the experiments were doubled.

2.2.2. Ultrasonic Extraction. The extraction of phenolic compounds by the ultrasonic-assisted method was carried out in an ultrasonic generator (Pars Nahand Engg. Co, Tehran, Iran) under different extraction parameters such as extraction time (0–60 minutes), ethanol concentration (20–100%), solvent-to-solid ratio ranging from 7.5 : 1 to $37.5:1\,g{\cdot}ml^{-1}$, and temperature (7.5–77.5°C). For the extraction, the *P. persica* leaves (1.00 g) were placed into a 20-mL tube, and then added to different concentrations of solvent, and the sonication was accomplished at different temperatures for various periods of time. Sonicated working power was fixed at 600 W. After extraction, the extract was separated from the solid part by centrifugation at 4000 rpm for 15 min, and then the aqueous phase was transferred into a microtube to remove the solvent using a concentrator (Eppendorf concentrator 5301) at 30°C. Finally, the weight of the dry extract was recorded.

2.3. Total Phenolic Content (TPC) Determination. The assay of total phenolic content (TPC) was conducted using the procedure illustrated by Sookjitsumran et al. [26] and Nickel et al. [27] with little changes. In brief, 90 μL of the extract (concentration 600 $\mu g{\cdot}ml^{-1}$) was mixed with 40 μL of 10% (W/V) Folin–Ciocalteu reagent in a microplate. After 8 min,

300 μL of 10% Na_2CO_3 solution was added to the mixture and incubated for 2 h at the same conditions. The absorbance of the mixture was assessed using an ELISA reader (EPOCH2C) at a wavelength of 760 nm against ethanol (blank). The standard curve (5–50 mg·ml^{-1}) was drawn from gallic acid to determine the TPC. The calculated concentration was characterized as a milligram of gallic acid equivalent per gram of the extract.

2.4. DPPH Assay. The potential of plant extracts' scavenging of 2-2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was evaluated. In brief, different concentrations of samples were prepared in methanol. Then, 200 μL of DPPH 200 μM dissolved in methanol was added to 50 μM of each sample [28]. The absorbance was monitored at 517 nm after shaking for 30 min at room temperature in the darkness. Ascorbic acid was considered a positive control, and the negative control was DPPH without a sample.

$$\text{The equation } Y = \left(\frac{A0 - A1}{A0}\right) \times 100 \text{ was used to estimate the \% inhibition,} \tag{1}$$

where A_0 is the absorbance of the control (without extract) and A_1 is the absorbance in the presence of the extract. Prism software was used to determine the concentration that inhibits DPPH by 50% (IC_{50}).

2.5. MTT Assay. The determination of cell viability was accomplished by a colorimetric assay using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye (Sigma) with the catalogue number 475989. PC-3 cells were seeded at 104 cells per well into a 96-well plate. The *P. persica* extract was dissolved in DMSO at the nontoxic concentration for cells and diluted to the desired concentrations (10, 50, 100, 150, and 200 μg·ml^{-1} and 62, 125, 250, 500, and 1000 μg·ml^{-1}) in the medium. In this regard, the final DMSO concentration did not exceed 0.5% (v/v). Then, the cells were treated at each concentration in triplicate as per the experimental design. After 24 hours of incubation, the medium was removed, the cells were washed twice with phosphate buffer, and 50 μl of MTT (0.5 mg·ml^{-1}) solution was added to each well. The plate was incubated for 3 h at 37°C and then DMSO was added to the wells to dissolve the formazan crystals. The optical density of the formazan solution was measured at 570 nm using an ELISA reader (BioTek) [29]. The IC_{50} value was calculated by constructing semilogarithmic dose-response plots using GraphPad Prism 8 software.

2.6. LC-Mass Spectrometry Analysis. A Triple Quadrupole 1260 HPLC Agilent Co. (USA) Mass Spectrometer (Millipore, USA) was used for the LC-MS study. The Agilent 1260 Infinity HPLC system (Millipore, USA) has an automated injector for the RP-HPLC. A binary pump, a 20-L injection loop, a photodiode array detector tuned at 218 and 280 nm, ChemStation software for chromatography data analysis, a binary pump, and a 20-μL injection loop were used. C18 column (Beckman, USA, 150 × 4.6 mm, 5 μm, 100 A°) and loop 20 μL were used for analytical purposes, and the mobile phase used was as follows: A: methanol with 0.02% trifluoroacetic acid and B: Milli-Q water containing 0.02% trifluoroacetic acid. A gradient elution in LC was set as follows: for 5 min at 10% methanol (90% water), followed by a linear gradient to 100% methanol, and hold for 16 min at 100% methanol, with a flow rate of 0.4 mL·min^{-1}. The injection volume was 20 μl. The multiple reaction monitoring (MRM) mode of the electrospray ionization mass spectrometer analyzer was selected. The operating settings were as follows: the capillary voltage was −3200 V, the nebulizer gas (N_2) pressure was set at 25 psi, the temperature was 350°C, and the flow rate was 10.0 Lmin^{-1}. To get full-scan MS and MS/MS spectra, we scanned throughout the mass ranges of 100–1500 amu.

2.7. Experimental Design for Extraction. The optimal conditions of extraction were determined using RSM, with face-centered central composite design (FCCCD) at three independent factors for conventional maceration, which include solvent-to-feed ratio (X_1; 5 : 1–30 : 1 mL·g^{-1}), extraction time (X_2; 8–36 h), and ethanol concentration (X_3; 50–100%, v/v (ethanol/water), and four independent factors in UAE, including solvent-to-feed ratio (X_1; 7.5 : 1–37.5 : 1 mL·g^{-1}), extraction time (X_2; 0–60 min), ethanol concentration (X_3; 20–100%, v/v (ethanol/water), and temperature (X_4; 7.5–77.5°C)) [30–32]. Extraction yield (Y) and TPC (mg·g^{-1}) were taken as the responses of the designed experiments. The different levels of independent variables, center points, and code values are shown in Table 1. In addition, for the assessment of the sum of square errors, six center points with a total number of 20 experimental runs for the conventional method and 30 experimental runs for the UAE were used.

2.8. Model Verification and Statistical Analysis. All experiments were performed in triplicate. Design-Expert software version 11 (Stat-Ease) was employed for the regression analysis and optimization. GraphPad Prism 9.3.1.471 software was used to determine the concentration that inhibits DPPH up to 50% (IC_{50}).

3. Results and Discussion

3.1. Extraction Techniques

3.1.1. Statistical Analysis for Extraction Techniques. Tables 2 and 3 display the outcomes of a CCD of traditional maceration and UAE extraction. According to the statistical analysis of the models for mass extract and total phenol, the

Table 1: The operational parameters affecting the extraction of *P. persica* and their levels.

Extraction method	X_j	Factor levels				
		$-\alpha$	-1	0	1	$+\alpha$
Conventional	Solvent-to-feed ratio (X_1; ml·g^{-1})	5 : 1	10 : 1	17.5 : 1	25 : 1	30 : 1
	Time (X_2; min)	8	14	22	30	36
	Ethanol concentration (X_3; %, v/v (ethanol/water))	50	65	75	85	100
UAE	Feed-to-solvent ratio (X_1; ml·g^{-1})	7.5 : 1	15 : 1	22.5 : 1	30 : 1	37.5 : 1
	Time (X_2; min)	0	15	30	45	60
	Ethanol concentration (X_3; %, v/v (ethanol/water))	20	40	60	80	100
	Temperature (X_4; °C)	7.5	25	42.5	60	77.5

quadratic model was the best for conventional maceration (Table S1), with a p value of 0.2644, a lack of fit of 0.7824, coefficients of determination (R^2) of 0.9833 and 0.9661, and adjusted R^2s of 0.9682 and 0.9356, respectively, and with a p value of 0.3227, a lack of fit of 0.4779, coefficients of determination (R^2) of 0.9323 and 0.9231, and an adjusted R^2 of 0.8691 and 0.8513 for mass extract and total phenol, respectively, for the UAE extraction technique.

Based on the results of the variance analysis, the F-values for the mass extract and total phenol regression models were significant at the 5% level (65.27 and 31.66, respectively) for conventional maceration (Table S1) and 14.76 for mass extract and 12.86 for total phenol for the UAE method (Table S2). Also, the lack of fit was not significant (1.82 and 0.48) for conventional maceration and (1.82 and 0.48) for the UAE method, respectively, which means that the second-order model is very good. Adequate precision measures the signal-to-noise ratio, and a ratio greater than 4 is required. In the case of conventional maceration, a ratio of 25.90 for mass extract and 20.54 for total phenol was obtained. Also, a ratio of 14.72 for mass extract and 13.28 for total phenol achieved in the UAE method, shows that the model has a suitable signal and can be used to navigate the design space. Figure S1 (mass extract, Figure S1(a), and total phenol, Figure S1(b)) for the conventional maceration method and Figure S2 (mass extract, Figure S2(a), and total phenol, Figure S2(b)) for the UAE method show how well the predicted values of recoveries match up with what was found in experiments.

In addition, the impacts of the factors and also some interactions between factors were interpreted. The mass extract (equations (2) and (4)) and total phenol (equations (3) and (5)) for conventional maceration (Table S3) and UAE (Table S4), respectively, were shown as functions of solvent-to-feed ratio (X_1), time (X_2), solvent percent (X_3), and temperature (X_4), using the coded units as follows:

$$Y = 249.95 + 39.65X_1 - 9.44X_2 - 11.59X_3 + 28.68X_2X_3 - 11.93X_1^2 - 13.97X_2^2 - 36.04X_3^2, \quad (2)$$

$$Z = 94.73 + 15.95X_1 - 9.49X_2 - 8.95X_1X_2 + 12.52X_2X_3 - 7.01X_1^2 - 13.01X_3^2, \quad (3)$$

$$Y = 354.43 + 13.18X_1 + 12.71X_2 - 37.29X_3 - 14.41X_1^2 - 21.53X_2^2 - 39.57X_3^2 - 26.59X_4^2, \quad (4)$$

$$Z = 172.64 + 6.76X_1 + 4.53X_2 - 16.75X_3 + 2.38X_1X_4 + 4.64X_2X_3 - 9.07X_1^2 - 12.85X_2^2 - 19.87X_3^2 - 14.61X_4^2. \quad (5)$$

From Figure S3, it can be seen that raising the solvent-to-feed ratio considerably increased responses in conventional extraction, including mass extract and total phenol. The response quantity, on the other hand, decreases as extraction time and solvent percentage are increased (Figures S3(a) and S3(b)). In addition, a linear relationship between total phenol content and extraction time can be seen (Figure S3(b)). In the UAE method, according to Figure S4(a) as well, the mass extract increases gradually while the solvent-to-feed ratio (X_1) and extraction time (X_2) rise and become insignificant at higher concentrations. Lower mass extracts result from raising the temperature (X_4) and solvent percent (X_3), respectively. Solvent percent (X_3) is important at higher quantities for the total phenol response (Figure S4(b)), and total phenol somewhat reduces as the other 3 parameters are increased.

3.1.2. Effect of the Ratio of Raw Material to Aqueous Ethanol on Extraction Yield of Phenolic Compounds. Solvent-to-feed (X_1) ratio is one of the crucial extraction factors. Maximizing extraction recovery yield and minimizing extraction solvent usage are critical in industrial operations. In this study, the recoveries of mass extract and total phenol from *P. persica* leaf extract increased from 154.1 mg·g^{-1} and 52.6 mg·g^{-1} to 282 mg·g^{-1} and 97.5 mg·g^{-1}, respectively, as the solvent-to-feed (X_1) ratio increased from 5 : 1 to 30 : 1 ml·g^{-1} in conventional maceration techniques (Table 2). Better wettability during the extraction was made possible by an increase in the ratio. However, if the ratio is increased further, the extraction medium will contain more ethanol and water.

As the solvent-to-feed ratio rose from 7.5 : 1 to 22.5 : 1 mL·g^{-1} in the case of the UAE technique, the recoveries of mass extract and total phenol from *P. persica* leaf extract

Table 2: CCD matrix and experimental (actual) values of conventional maceration extraction of *P. persica*.

Std	Run	Factor 1: S/F (ml·g^{-1})	Factor 2: time (h)	Factor 3: solvent percent (%)	Response 1: mass extract (mg)	Response 2: total phenol (mg)
1	16	10.1	13.7	60.1	198	66.18
2	2	24.9	13.7	60.1	277.2	132.33
3	9	10.1	30.3	60.1	97.3	44.64
4	15	24.9	30.3	60.1	209.3	69.06
5	11	10.1	13.7	89.9	132.04	44.52
6	6	24.9	13.7	89.9	193.4	85.34
7	3	10.1	30.3	89.9	156.16	67.14
8	13	24.9	30.3	89.9	230.1	78.1
9	12	5.0	22.0	75.0	154.14	52.66
10	20	30.0	22.0	75.0	282	97.5
11	5	17.5	8.0	75.0	218.6	109.44
12	14	17.5	36.0	75.0	206	73.64
13	7	17.5	22.0	50.0	176.1	62.26
14	8	17.5	22.0	100.0	123.64	53.92
15	1	17.5	22.0	75.0	258.3	99.8
16	17	17.5	22.0	75.0	244.7	83.74
17	18	17.5	22.0	75.0	236.54	88.66
18	19	17.5	22.0	75.0	253.1	97.96
19	4	17.5	22.0	75.0	255.6	100.42
20	10	17.5	22.0	75.0	250.8	97.76

Table 3: CCD matrix and experimental (actual) values of UAE of *P. persica*.

Std	Run	Factor 1: S/F (ml·g^{-1})	Factor 2: time (min)	Factor 3: solvent percent (%)	Factor 4: temperature (°C)	Response 1: mass extract (mg)	Response 2: total phenol (mg)
1	12	15	15	40	25	266.45	128.52
2	13	30	15	40	25	328.56	152.09
3	11	15	45	40	25	299.74	131.51
4	16	30	45	40	25	296.15	130.13
5	18	15	15	80	25	206.4	92.42
6	9	30	15	80	25	221.65	101.69
7	22	15	45	80	25	248	118.88
8	20	30	45	80	25	233.3	105.82
9	26	15	15	40	60	260	127.24
10	1	30	15	40	60	294.35	136.08
11	17	15	45	40	60	304.2	129.88
12	10	30	45	40	60	308.45	131.3
13	15	15	15	80	60	205.7	87.22
14	29	30	15	80	60	210.4	95.82
15	23	15	45	80	60	195.05	84.04
16	19	30	45	80	60	230.42	121.58
17	6	7.5	30	60	42.5	243.15	112.7
18	2	37.5	30	60	42.5	332.5	156.37
19	25	22.5	0	60	42.5	213.5	100.27
20	3	22.5	60	60	42.5	305.18	138.6
21	28	22.5	30	20	42.5	259.18	127.05
22	14	22.5	30	100	42.5	115.2	55.64
23	8	22.5	30	60	7.5	233.45	106.32
24	24	22.5	30	60	77.5	244.8	118.46
25	7	22.5	30	60	42.5	354.98	175.1
26	27	22.5	30	60	42.5	381.58	188.44
27	30	22.5	30	60	42.5	333.16	159.72
28	5	22.5	30	60	42.5	356	177.82
29	21	22.5	30	60	42.5	367.63	176.43
30	4	22.5	30	60	42.5	333.24	158.32

increased from 243.1 mg·g^{-1} and 112.7 mg·g^{-1} to 381.58 mg·g^{-1} and 188.44 mg·g^{-1}, respectively (Table 3). The ratio was raised to 22.5 : 1 mL·g^{-1}, which improved the plant matrix's wettability and ultrasonic adsorption during the extraction. The recoveries of mass extract and TPC were simultaneously decreased to 332.5 mg·g^{-1} and 156.3 mg·g^{-1}, respectively, by further increasing the solvent-to-feed ratio to 37.5 : 1 mL·g^{-1} (Table 3).

3.1.3. Effect of Extraction Time on Extraction Yield of Phenolic Compounds. The conventional extraction process was carried out using extraction times ranging from 8 to 36 hours (Table 1). As shown in Table 2 when extraction time increases, the variance of extraction yield is relatively rapid and reaches a maximum at 22 hours. Likewise, ultrasonic extraction was performed using extraction times ranging from 0 to 60 minutes. Figure S4 depicts how the extraction time affects the amount of phenolic compounds extracted from *P. persica* leaves. When the extraction time increases, the variance of extraction yield is relatively rapid and reaches a maximum at 30 min (Table 3). Due to the structural degradation and disintegration of polyphenols brought on by the longer extraction period, the extraction yield was reduced in both the extraction processes [33]. Therefore, the optimal extraction times for phenolic compounds are 22 hours for the conventional extraction technique and 30 minutes for the UAE.

3.1.4. Effect of Ethanol Concentration on Extraction Yield of Phenolic Compounds. Ethanol concentration (X_3), one of the main parameters in the extraction process, was changed during phenolic compound extraction to evaluate the extraction yield. As a result of its low toxicity, ethanol has been widely employed to extract physiologically active chemicals from a variety of plants, thereby increasing the extraction efficiency. Ethanol concentration (X_3) is typically utilized in water at various concentrations to improve the extraction efficiency. The high dielectric property of water made it a good cosolvent for extraction. The effect of 50–100% ethanol on the recoveries of mass extract and TPC is presented in Table 2. As seen, the recoveries of mass extract and total phenol of these bioactive compounds at 50% ethanol were 176.1 mg·g^{-1} and 62.2 mg·g^{-1}, respectively, in the conventional maceration technique. A further increase in ethanol concentration to 75% enhanced the yields of recoveries to 258.3 mg·g^{-1} and 100.4 mg·g^{-1} (Table 2). However, when the ethanol concentration reached 100% in the extraction medium, the yields declined to 123.6 mg·g^{-1} and 53.9 mg·g^{-1}, respectively (Table 2).

Also, in the case of the UAE technique, the effects of 20–100% ethanol on the recoveries of mass extract and TPC are shown in Table 3. As seen, the recoveries of mass extract and total phenol of these bioactive compounds at 60% ethanol were 381.5 mg and 188.4 mg·g^{-1}. Lower recovery yields (at 115.2 mg·g^{-1} and 55.6 mg·g^{-1}), as well as the usage of the conventional maceration method, were obtained when the ethanol content was increased further, up to 100% (Table 3).

Ethanol alone is not capable of extracting more bioactive compounds as most of the bioactive components such as saponins, phenolics, and flavonoids are high in polarity. Thus, a polar solvent is required to enhance the recovery yield. On the other hand, more than 45% of the water in ethanol can decline the recovery of TPC. Therefore, the yields of TPC increased by increasing the concentration of ethanol to 75%. As the concentration of ethanol exceeded 90%, significant declination was observed in the TPC of *P. persica* leaves because of decreasing the water portion in the extraction solvent.

3.1.5. Effect of Extraction Temperature on Ultrasonic Extraction Yield of Phenolic Compounds. Ultrasonic extraction was performed in the temperature (X_4) range of 7.5–77.5°C (Table 1). In this study, the recoveries of mass extract and total phenol from *P. persica* leaf extract increased from 233.4 mg·g^{-1} and 106.3 mg·g^{-1} to 381.5 mg·g^{-1} and 188.4 mg·g^{-1}, respectively, as the temperature increased from 7.5 to 42.5°C. However, a further increment in the temperature to 77.5°C minimized the recoveries of mass extract and total phenol to 244.8 mg and 118.4 mg, respectively, at the same time (Table 3).

3.1.6. Effect of the Conventional Maceration Parameters and Their Interactions. As shown in Figure 1(a), when the volume ratio of solvent to feed (X_1) and extraction time (X_2) increases, extraction efficiency increases rapidly. This indicates that the effect of the solvent-to-feed ratio (X_1) and extraction time (X_2) on extraction efficiency is significant. Moreover, in Figure 1(b), it was found that in the ratio of 25 : 1 mL·g^{-1} and 65% ethanol, extraction efficiency is maximized. It can be seen that the interaction between the solvent-to-feed ratio (X_1) and ethanol concentration (X_3) is significant. It is clear from Figure 1(c) that as the extraction time (X_2) increases, the extraction efficiency increases slowly, and while increasing the ethanol concentration (X_3) from 65%, the extraction efficiency decreases. In the case of total phenol recoveries, as shown in Figure 1(a), when the solvent-to-feed ratio (X_1) and extraction time (X_2) range from 15 : 1 mL·g^{-1} to 25 : 1 mL·g^{-1} and 5 to 13.5 h, respectively, the total phenol content increases rapidly. Figure 1(b) shows that the interaction between the solvent-to-feed ratio (X_1) and ethanol concentration (X_3) for total phenol is significant. It is obvious from Figure 1(c) that when the extraction time increases (X_2), the total phenol decreases slowly.

3.1.7. The Impact of the UAE Parameters and Their Interactions. Figure 2 revealed the response surface plot showing the effect of UAE parameters on mass extract and total phenol. Each of these graphs is evaluated by assuming that the two factors that are not in the graph are fixed (solvent-to-feed ratio at 22.5 : 1 ml·g^{-1}, time at 30 min., percentage of ethanol at 60%, and temperature at 42.5°C). Results from Figures 2(a)–2(c) show that by increasing the solvent-to-feed ratio to 15 : 1 ml·g^{-1}, the mass extract reaches its maximum amount. Also, when extraction time increases (Figures 2(a), 2(d), and 2(e)), mass

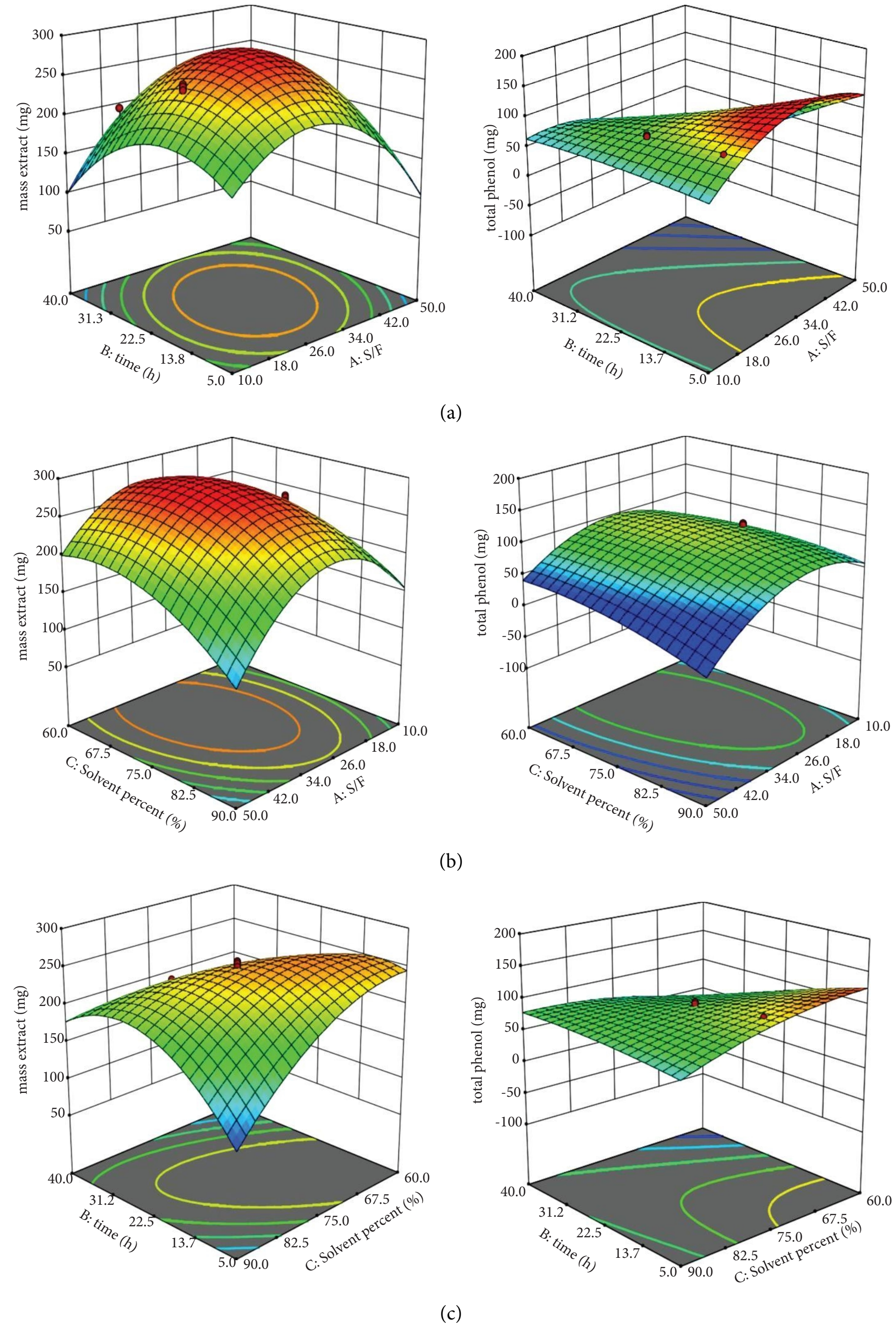

Figure 1: Response surface plot showing the effect of conventional maceration parameters on total phenol and mass extract: (a) solvent-to-feed ratio and time interaction, (b) solvent-to-feed and solvent percent interaction, and (c) time and solvent percent interaction.

extracts rise. These findings show that the best total phenol is obtained at a ratio of about 30 : 1 ml·g^{-1} (Figures 2(a)–2(c)) and at a temperature of 40°C (Figures 2(c), 2(e), and 2(f)), also in the time range of 25 min–40 min (Figures 2(a), 2(d), and 2(e)), and in the range of 40–60% ethanol concentration (Figures 2(b), 2(d), and 2(f)), which is the best range to achieve the maximum amount of total phenol of the plant. The temperature range of 25°C–50°C is the best temperature for extraction, and further increases in temperature reduce the total extract, which may be due to the destruction of the plant structure at higher temperatures. The amount of total phenol in the percentage of ethanol between 40% and 60% and the temperature between 25°C and 50°C (Figure 2(f)), reaches its maximum. Due to the lack of destruction of the structure of phenolic compounds and also for saving energy in a large-scale plan, the room temperature (25°C) is more suitable for extraction.

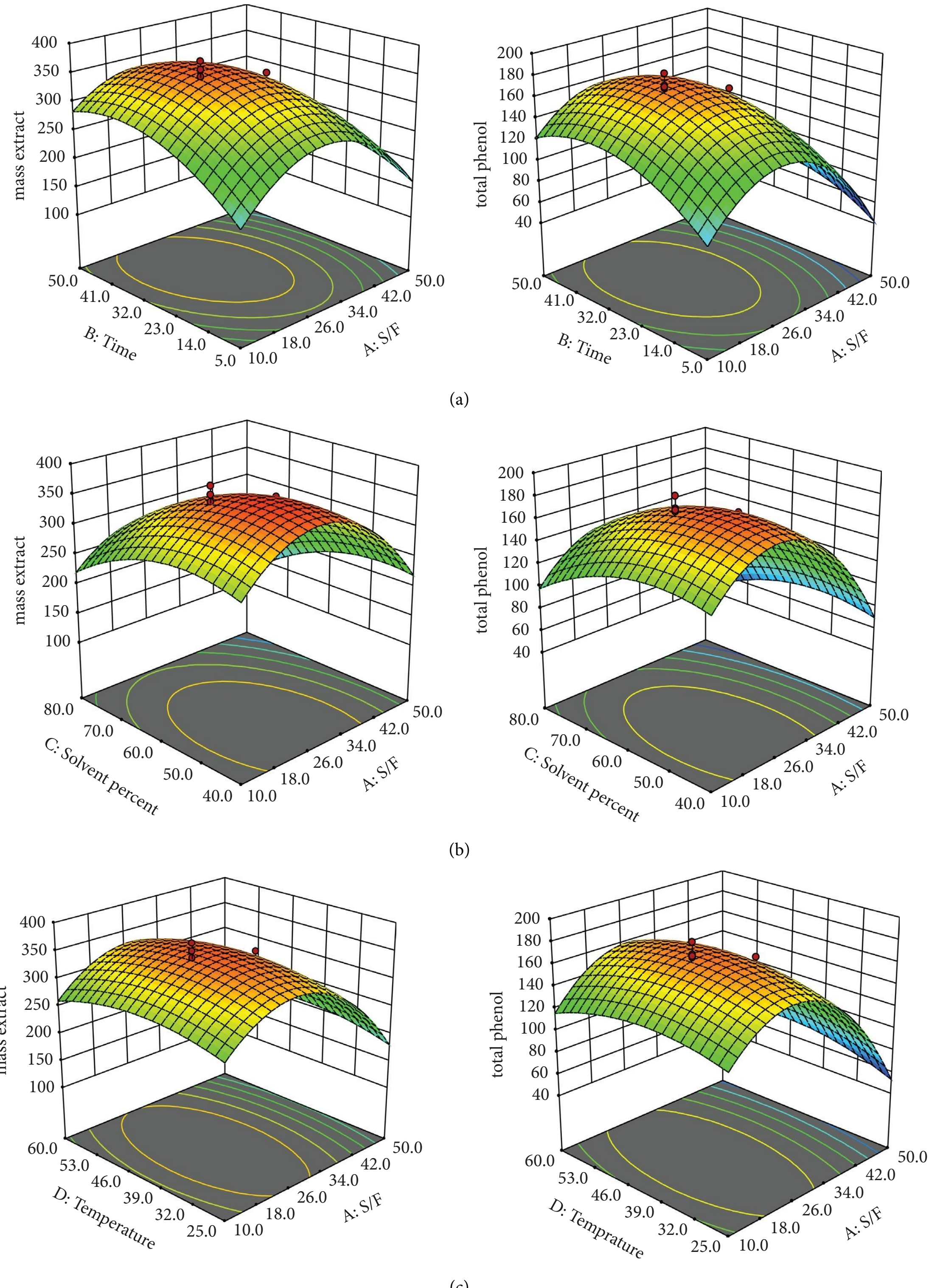

Figure 2: Continued.

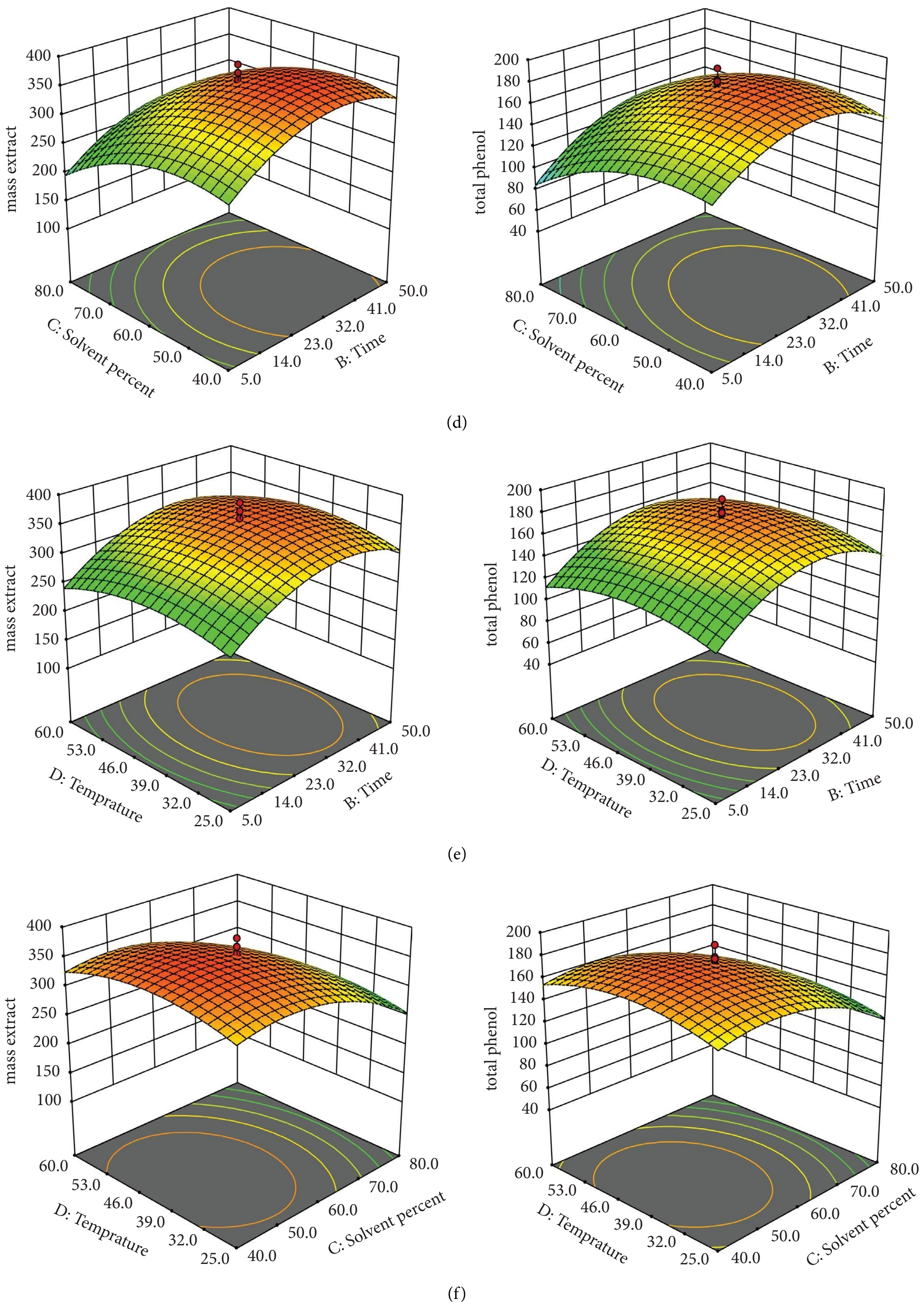

Figure 2: Response surface plot showing the effect of UAE parameters on mass extract and total phenol: (a) solvent-to-feed ratio and time interaction, (b) solvent-to-feed and solvent percent interaction, (c) time and solvent percent interaction, (d) time and solvent percent interaction, (e) time and temperature ratio, and (f) solvent percent and temperature interaction.

Table 4: Validation of conventional maceration and UAE extraction.

Extraction method	Extraction parameters				Total phenol (mg·g^{-1})		Mass extract (mg·g^{-1})	
	Solvent-to-feed ratio (ml·g^{-1})	Time (h)	Solvent percent	Temperature (°C)	Experimental	Predicted	Experimental	Predicted
Conventional maceration	24.9 : 1	13.67	60.13	—	114.18	129.032	299.6	278.688
UAE	30 : 1	0.25	40	25	137.36	139.234	284.22	299.525

3.2. Optimization of Extraction Parameters and Validation of the Obtained Model. To find the optimal extraction conditions for conventional maceration with the highest extraction rate (mass extract and total phenol), numerical optimization was performed using DX software, as shown in Figure S5. The predicted optimal conditions are a solvent-to-feed ratio of 24.93 : 1 (X_1) ml·g^{-1}, an extraction time (X_2) of 13.67 h, and an ethanol concentration (X_3) of 60.13%, as shown in Table 4. The validity of the model was performed to predict the optimized responses using the selected optimal conditions. It was found that the ratio of experimental to predicted results which determined the similarity of experimental to predicted findings was 88.5% for the total phenolic content and approximately 100% for the mass extract. However, with respect to performance in actual production, the optimal conditions are modified as follows: solvent-to-feed ratio (X_1) of 25 : 1, extraction time (X_2) of 14 h, and ethanol concentration (X_3) of 60%, which has a low error rate and thus indicates that the model is accurate.

The optimum UAE conditions as predicted by DX software are a solvent-to-feed ratio of 30 : 1 mL·g^{-1}, a time of 15 minutes, an ethanol concentration of 40%, and a temperature of 25°C (Figure S6). These parameters are based on equations (4) and (5) of the mathematical model. Validation of the models was performed based on the optimized UAE extraction conditions. The results showed a good similarity between the predicted and experimental values, and no significant changes were observed between the real and predicted values (Table 4). The similarity of the results was 98.7% for the phenolic content and 94.9% for the mass extract in the ultrasonic method. Therefore, it has been proven that the proposed conditions were suitable for predicting the optimal conditions and sufficient for the study.

Using the traditional approach under the ideal circumstances, a solvent-to-feed ratio of 25 : 1, an extraction duration of 14 hours, and a 60% concentration of ethanol resulted in a maximum phenolic compound extraction of 114.18 mg·g^{-1}. 137.36 mg·g^{-1} of phenolic compound recovery was achieved by using the ultrasonic aided extraction technique at a solvent-to-feed ratio of 30 : 1, an extraction period of 15 minutes (0.25 h), a concentration of 40% ethanol, and a temperature of 25°C. As a result, this study shows that UAE has the potential to outperform the maceration approach in terms of yield and total phenols of *P. persica* leaf extract due to its quick extraction time and the 25°C temperature that is ideal for the ultrasonic method.

In comparison, some research studies were conducted to optimize the extraction of phenolic compounds by the UAE method from *Deverra scoparia* Coss. & Durieu flowers using RSM, and it was discovered that the optimal conditions are at 50°C, 70 : 1 ml·g^{-1} of solvent-to-feed ratio, and 55 minutes with 10.78% as the final yield [34], Also, in the optimization of the ultrasound extraction of phenolic compounds from the aerial parts of the Moroccan *Lavandula stoechas* L. using the RSM method, an optimal yield of 31.88% of the mass extract was obtained, with the following parameters: ethanol concentration of 40%, the ratio of solvent to substance of 30 : 1 ml·g^{-1}, and a time processing of 32.62 minutes [35].

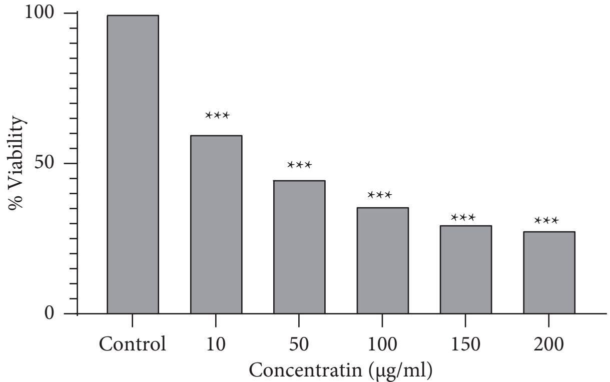

Figure 3: MTT assay for cytotoxicity (%) of *P. persica* in human prostate cancer PC-3 cells: bar graph showing the cell viability (%) of cells treated with different concentrations of *P. persica*.

3.3. Biological Activities

3.3.1. Antioxidant Activity of P. persica. The effect of antioxidants on the inhibition of DPPH radicals is attributed to their ability to donate hydrogen. The decreased absorption of DPPH radicals is determined by antioxidants at 517 nm. In investigating the inhibitory effect and concentration of phenolic compounds, R^2 is 0.97 ($p < 0.01$). These results show that the extract with the optimum condition has a significant effect on the elimination of DPPH-free radicals (Figure S7).

The results showed that sample 1 (extracted with UAE in optimum condition) with an $IC_{50} = 29.86\,\mu g \cdot mL^{-1}$ has a promising antioxidant activity (Table S5) in comparison to reference standard antioxidant (ascorbic acid, $IC_{50} = 24.15$ $\mu g \cdot mL^{-1}$).

3.3.2. Cytotoxic Activity of Phenolic Compounds from P. persica. The antiproliferative effect of *P. persica* extracts against the PC-3 cell line was determined by the MTT assay. The *P. persica* extract showed dose-dependent inhibition effects on the proliferation of carcinogenic cells. The IC_{50}

Table 5: List of compounds isolated and/or detected in the ethanolic extract of *P. persica*.

Peak	t_R (min)	Compound	m/z [M-H]-
1	15.96	Galloyl glucose	331.0000
2	16.68	1,2,4,6-Tetragalloyl glucose	787.0000
3	12.69	Pentagalloyl glucose	939.0000
4	8.33	Quercetin-3-(3,4,6 trigalloyl glucose)	919.0000

value for *P. persica* was 10.4 μg·ml^{-1}, which shows that it has considerable cytotoxic activity against cancer cells (Figure 3). Moreover, the present finding demonstrated selective toxicity in the PC-3 cell line, while the viability of normal cells (L929 cell line) was close to 100% (Figure S8). Recently, Rezadoost and coworkers provided an in-depth study regarding the antiproliferation activity of phenolic compounds isolated from *P. persica* and they observed that gallotannin derivatives are responsible for showing cytotoxic activity against KB, Hela, G292, A431, and MCF-7 cancer cells [2].

3.4. Phenolic Compound Profile of P. persica. The phytochemical analysis of an ethanolic extract of *P. persica* using HPLC coupled with electrospray ionization mass spectrometry (Figure S9) revealed the presence of four phenolic base compounds: galloyl glucose, 1, 2, 4, 6-tetragalloyl glucose, pentagalloyl glucose, and quercetin-3-(3,4,6 trigalloyl glucose) (Table 5).

4. Conclusion

This study aimed to determine the optimal mass extract and total phenolic content (TPC) from *P. persica* leaf. The extraction conditions were determined using RSM and Design-Expert software, and the quadratic model was found to be the best for both conventional and UAE extraction techniques. The maximum phenolic compound extraction was 114.18 mg.g^{-1} using the conventional method under optimum conditions, with a solvent-to-feed ratio of 25 : 1, extraction time of 14 hours, and 60% ethanol concentration. In the UAE method, with a solvent-to-feed ratio of 30 : 1, extraction time of 15 minutes (0.25 h), ethanol concentration of 40%, and temperature of 25°C, showed 137.36 mg·g^{-1} of phenolic compound recovery. Our results revealed a good similarity between the actual and predicted values, and no significant changes were observed between the real and theoretical findings. The UAE method was found to be more efficient due to its short extraction time and the optimal conditions for the ultrasonic method at 25°C. The conventional extract had less antioxidant activity, while the ultrasonic extract showed 29.86 μg·mL^{-1} antioxidant activity, indicating the importance of optimizing both mass extract and total phenolic content in the plant to exert antioxidant effects. The study also established the presence of phenolic compounds such as galloyl glucose, 1,2,4,6-tetragalloyl glucose, pentagalloyl glucose, and quercetin-3-(3,4,6 trigalloyl glucose) using LC-MS analysis. In addition, *P. persica's* cytotoxic activity showed selectivity against cancer cells with an IC_{50} value of 10.4 μg·mL^{-1}.

The evidence from this study suggests that changing of parameters in the optimization process leads to maximizing the recovery and biological activity. Further research should therefore concentrate on the quantification of each phenolic compound and to find which compounds vary significantly in optimized extract in comparison with nonoptimized one.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Figure S1: plot of predicted versus actual values for (a) mass extract and (b) total phenol of conventional maceration extraction. Figure S2: plot of predicted versus actual values for (a) mass extract and (b) total phenol of UAE. Figure S3: the effect of the main parameters (solvent-to-feed ratio (A), time (B), and solvent percent (C)) on conventional maceration extraction recovery for (a) mass extract and (b) total phenol. Figure S4: the effect of the main parameters (solvent-to-feed ratio (A), time (B), solvent percent (C), and temperature (D) on UAE extraction recovery for (a) mass extract and (b) total phenol. Figure S5: the proposed levels of parameters to maximize the recovery of conventional maceration extraction. Figure S6: the proposed levels of parameters to maximize the recovery of UAE. Figure S7: DPPH radical scavenging activity (%) for (a) ascorbic acid, (b) sample 1, and (c) sample 2. Figure S8: cell viability (%) of normal L929 cells treated with different concentrations of *P. persica.* Figure S9: phytochemical analysis of an ethanolic extract of *P. persica* performed using LC-MS. Table S1: statistical analysis of models for modelling the conventional extraction of *P. persica*. Table S2: statistical analysis of models for modelling the UAE of *P. persica*. Table S3: analysis of variance (ANOVA) of the response surface quadratic model to predict conventional extraction recovery. Table S4: analysis of variance (ANOVA) of the response surface quadratic model to predict UAE recovery. Table S5: IC_{50} amounts for standard and sample in DPPH assay. (*Supplementary Materials*)

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