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Optimization of Bioactive Compounds Extraction from *Platanus Orientalis* Leaves using Microwave-Assisted Technique

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ABSTRACT

Background: The increasing consumers′ demands for natural bioactive compounds can be attributed to the adverse effects of synthetic antioxidants. While *Platanus orientalis* leaves are considered waste material, they are a rich source of bioactive compounds. Conventional extraction techniques, however, have different drawbacks for the recovery of bioactive compounds. The current study aimed to optimize and evaluate the impact of microwave-assisted extraction (MAE) as an independent variable on bioactive compounds recovery from *P. orientalis* leaves.
Methods: Response surface methodology was employed to optimize the extraction conditions and maximize the quantitative yield of bioactive compounds. Moreover, the influence of the MAE process on antioxidant activity and phenolic compound profile was investigated using spectroscopy and chromatography techniques.
Results: The optimal MAE conditions were determined to be 300 W microwave power, 17 min irradiation time, and a solvent-to-sample ratio of 31 *v/w*. The extract obtained under these optimized conditions showed a higher concentration of valuable phenolic compounds, including rutin, gallic acid, myricetin, and catechin.
Conclusion: The leaves of *P. orientalis* have the potential to be a source of bioactive compounds with significant anti-radical capacity. MAE is a potential modern technique to separate bioactive compounds from *P. orientalis* leaves, which could find further applications in the food and pharmaceutical industries.

1. Introduction

The recent dramatic growth of knowledge about free radicals in biology has led to important changes in the food and pharmaceutical industries, promising a new era of health and disease management. Oxygen, an essential substance for human life can have harmful effects on humans under certain conditions. These potentially harmful effects are primarily caused by a group of chemical compounds known as reactive oxygen species (ROS), which are highly active and contain at least one oxygen atom and one or more unpaired electrons. Consequently, it has become common to discuss disease mechanisms in terms of free radicals and antioxidants (Jakubczyk et al., 2020). In the last few decades, food scientists have focused on the valorization of agricultural waste to determine potential sources of valuable bioactive compounds. Furthermore, in light of the adverse health effects of synthetic antioxidants, the consumer demand for natural bioactive compounds has increased dramatically. Food fortification with valuable bioactive compounds is recommended for enhancing the immune system by ensuring adequate daily intake. Extraction is an important stage of the recovery process that should be considered for the successful separation of valuable bioactive compounds from natural sources (Roshani Neshat et al., 2020). Generally, extraction methods can be divided into major groups of conventional and modern techniques. Conventional Soxhlet extraction (CSE) which has been

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applied widely in the food and medicine industries has been explained in the literature (Wang & Weller, 2006). In another study, the applications and aspects of the CSE compared with modern extraction methods such as supercritical fluid, microwave-assisted, and ultrasound-assisted extractions (Luque de Castro & Garcia-Ayuso, 1998). However, some disadvantages associated with CSE make its application limited and uneconomical due to the excessive consumption of processing time, energy, and polluting solvents. Furthermore, the application of high temperatures during long extraction time is a serious limiting step for applying CSE for thermo-sensible compound extraction. To overcome such limitations, research has been started to establish and develop new and green techniques to extract bioactives from a wide range of plant matrices and their by-products (Naduvilthara et al., 2023). MAE offers several benefits over traditional techniques, including shorter extraction time, less solvent consumption, higher extraction speed, and lower cost. An electromagnetic wave with a frequency between 300 MHz and 300 GHz is considered a microwave. As part of the electromagnetic spectrum, microwaves are located between radio-frequency ranges at lower frequencies and infrared ranges at higher frequencies. During microwave heating, the weak hydrogen bond disruption is enhanced by the rotation of the polar molecules, and there is the migration of dissolved ions, which improves the penetration of the solvent into the cell structure, facilitating the extraction of the target compounds (Tomasi et al., 2023; López-Salazar et al., 2023). Process variables such as microwave power, irradiation time, sample particle size, and solid-to-solvent ratio are considered critical parameters that should be investigated to achieve a successful MAE process. The MAE technique has been extensively applied to recover valuable bioactive compounds from natural sources (Rahmi et al., 2020; Elyemni et al., 2019; Tomasi et al., 2023). Considering the effect of various variables on the process efficiency process optimization is a critical and major step to achieve a successful. The response surface methodology (RSM) is a set of statistical and mathematical methods for constructing empirical models. RSM is beneficial for enhancing and optimizing processes where achieving a goal is affected by multiple parameters (Chatzimitakos et al., 2023). *Platanus orientalis* is a tree native to Asia and found abundantly in the Himalayas. This tree is widely found in different regions of Iran. Its bark is first yellowish-gray and smooth and then becomes light brown and flakes. Leaves are almost maple-like and large. The tree is valuable for providing shade. Its wood is incredibly tough, dense, and hard, making it a popular choice for furniture. The tree produces springtime flowers that are followed by fruits, which grow in groups of three to six on stalks. Annually, a large amount of *P. orientalis* leaves are produced as agro-waste, which can be considered a valuable source of various beneficial compounds. Nowadays, attention has been paid to the adverse effects of synthetic antioxidant compounds on human health, and their replacement by the effective natural antioxidants obtained from plant sources. Different plants contain various valuable bioactive compounds such as essential oils, extracts, phytochemicals, and pigments (Mirsadraee et al., 2018). Therefore, the current study aimed to evaluate the effect of MAE independent variable on bioactive compounds recovery from *P. orientalis* leaves. The process optimization was performed using RSM to maximize the quantitative raw yield (QRY) of bioactive compounds. Furthermore, the effect of the MAE process on the free radical scavenging capacity and major phenolic compound contents was investigated.

2. Materials and Methods

2.1 Materials

The *P. orientalis* leaves were obtained from Zanjan, Iran. After washing with tap water and removing excess water, the leaves were dried in a shaded area until they reached a constant weight. The dried leaves were powdered by a laboratory mill and passed through stainless steel sieves with a mesh size of 18. The obtained sample was stored in polyethylene bags impermeable to air and moisture for further experiments. Ethanol was acquired from Dr. Mojallali Co., Iran. 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$), phenolic compound standards, and potassium persulphate were obtained from Sigma-Aldrich, Germany.

2.2 Microwave-assisted extraction

To carry out the microwave-assisted extraction (MAE) process, a certain amount of sample and ethanol 70% *v/v* as solvent is transferred to the extraction container and placed in the microwave-assisted extractor equipment. The process-independent variables were microwave power (200-400 W), solvent-to-sample ratio (15-45 *v/w*), and microwave irradiation time (6-18 min). As the process is finished, the sample is passed through Whatman filter paper, and the solvent is eliminated using a rotary evaporator under vacuum (0.08 MPa and 40 °C) (Bodea et al., 2022). Furthermore, a visual summary of the experimental order on *P. orientalis* leaves was presented in Figure 1.

2.3 Determination of quantitative raw yield of bioactive compounds

The QRY of bioactive compounds extracted from the *P. orientalis* leaves was calculated gravimetrically using a digital balance (WTC2000, Radwag, Poland) according to Eq. 1:

$$\text{QRY}\,(\%) = \frac{m_e}{m_s} \times 100 \qquad \text{(Eq. 1)}$$

where m_e and m_s were the crude extract mass (g) and the sample mass (g), respectively (Zandi et al., 2023).

2.4 Assessment of radical scavenging activity

2.4.1 Determination of DPPH free radical scavenging activity

DPPH free radical scavenging activity of bioactive compounds extracted from the *P. orientalis* leaves was measured according to the method explained by Zandi *et al.* (2023). The amount of color removal from DPPH solution by anti-radical compounds shows the ability to inhibit DPPH free radicals. For this purpose, 2 mL of the extract diluted with ethanol (concentrations of 200 µg/mL) were mixed with 2 mL of DPPH solution (0.1 mM), and the mixture was kept in the dark for 30 min. Then the absorption of the mixture was measured by a Uv-VIS spectrophotometer (Specord 250, Analytik Jena, Germany) at a wavelength of 517 nm. The inhibition percentage of DPPH free radicals (%$DPPH_{sc}$) was calculated using the Eq. 2:

$$\%DPPH_{sc} = \frac{A_c - A_s}{A_c} \times 100 \quad \text{(Eq. 2)}$$

Where, A_c and A_s are the absorbance of the control and sample, respectively (Zandi et al., 2023).

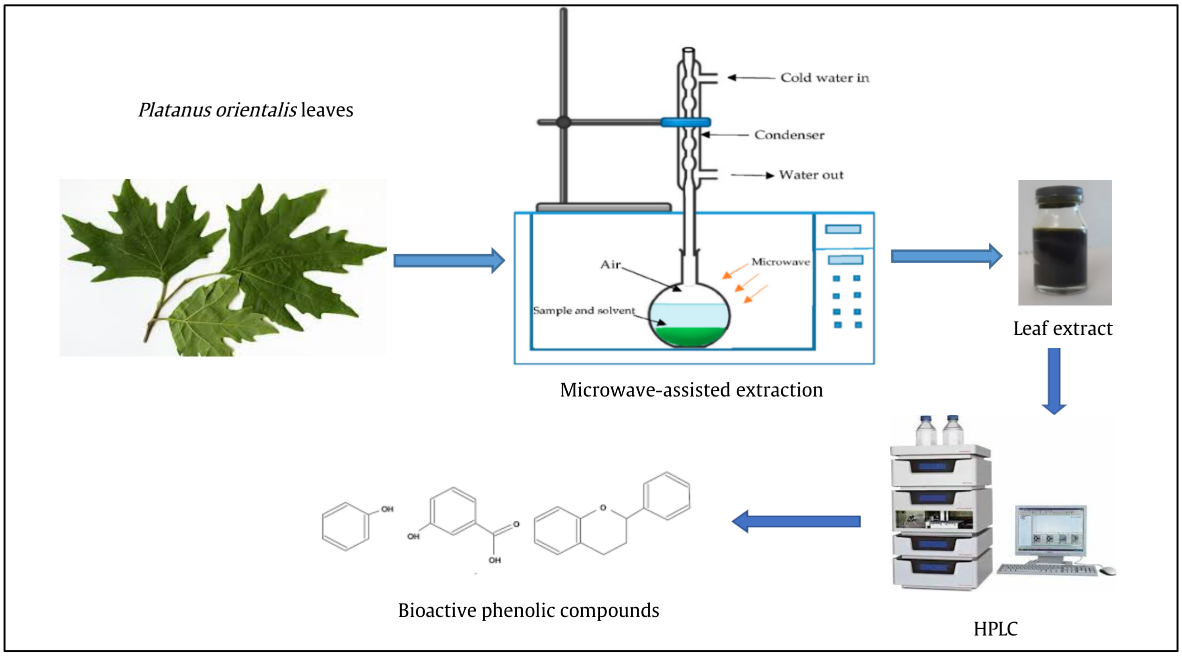

Figure 1. A visual summary of the experimental order on *P. orientalis* leaves

2.4.2 Determination of hydroxyl free radical scavenging activity

Hydroxyl free radical scavenging activity of bioactive compounds extracted from the *P. orientalis* leaves was measured according to the method explained by Mojarradi *et al.* (2021). Firstly, 1.5 mL of extract is mixed with hydrogen peroxide solution (30%). Then, the absorbance is measured at a wavelength of 530 nm using a UV-Vis spectrophotometer (Specord 250, Analytik Jena, Germany). The inhibition percent of scavenged HO free radicals (%HO_{sc}) was calculated using Eq. 3:

$$\%HO_{sc} = \frac{A_c - A_s}{A_c} \times 100 \quad \text{(Eq. 3)}$$

where, A_c and A_s are the absorbances of the control and sample, respectively (Mojarradi et al., 2021).

2.5 Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

The major phenolic compounds of the extract obtained under different extraction conditions of MAE were measured using a reversed-phase high-performance liquid chromatography (RP-HPLC) device equipped with a UV-Vis detector. The mobile phase consisting solvent A (TFA, 2.5 pH) and Solvent B (pure methanol). The temperature and solvent flow rate were set at ambient temperature and 1.0 mL/min, respectively. The injection volume was 20 µL. Before injection, the solutions were filtered through a 0.45 µm filter. All major phenolic compounds were identified according to their retention time against the standard compounds and their amount was calculated according to the linear calibration curve prepared from the standard compound (Noroozi et al., 2021).

2.6 Statistical Analysis

In this research, RSM based on Box-Behnken design (BBD) was performed to investigate and optimize the MAE process for bioactive compounds recovery from the *P. orientalis* leaves. The process optimization was carried out considering three independent variables microwave power (200-400 W), solvent to sample ratio (15-45 *v/w*), and microwave irradiation time (6-18 min). To determine the optimal conditions, the numerical optimization aimed to maximize QRY. All experiments were performed at least three repetitions and data were reported as mean ± standard deviation (SD). Design Expert version 7 software was used to analyze data, draw graphs, and fit a second-order polynomial model as follows (Eq. 4):

$$Y = \beta_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_j^2 + \sum_{i}^{k-1} \sum_{j}^{k} B_{ij} X_i X_j \qquad \text{(Eq. 4)}$$

Where Y is the predicted response; β_0 is the constant coefficient; β_i is the linear coefficient; β_{ii} is the quadratic coefficient; β_{ij} is the cross-product coefficient; X_i and X_j are independent variables.

3. Results and Discussion

3.1 Fitting Model

RSM is a modeling method that is used to evaluate the relationship between predicted and experimental results. In the current research, this method is used to propose the appropriate model for optimization of the MAE process of bioactive compounds from *P. orientalis* leaves, considering three independent variables including microwave power (MP), solvent-to-sample ratio (SR), and irradiation-time (RT) at three levels. In Table 1, the independent variables and their levels were presented.

Table 1. Independent variables and their levels

Independent variables	Units	Symbol	Coded Levels		
			-1	0	+1
Microwave power (MP)	W	A	200	300	400
Solvent-to-sample ratio (SR)	*v/w*	B	15	30	45
Irradiation time (RT)	min	C	6	12	18

The experimental treatments determined based on the Box-Benken design are also presented in Table 2. The experiments include 17 treatments considering 5 replications at the central point. The values of experimental results under studied treatments were also presented in Table 2. From the results, the values of QRY of bioactive compounds ranged from 10.00 to 16.32%, which indicates the potential effect of the MAE technique to release target compounds from the cell matrix of *P. orientalis* leaves. The quadratic polynomial model resulting from the analysis of the experimental data is shown in Equation 5. This model was presented to predict the QRY of bioactive compounds obtained using the MAE as a function of three independent variables including microwave power (A), solvent-to-sample ratio (B), and irradiation time (C).

$$\text{QRY (\%)} = 16.12 + 1.08\,A + 0.87\,B + 1.99\,C + 0.12\,AB - 0.72\,AC - 0.13\,BC - 1.05\,A^2 - 1.19\,B^2 - 1.29\,C^2 \qquad \text{(Eq. 5)}$$

Where, A, B, and C were coded independent variables for microwave power, solvent-to-sample ratio, and irradiation time, respectively. The *p-value* of the proposed model was less than 0.05 indicating that the model was statistically significant. One-way analysis of variance (one-way ANOVA) results of the proposed model are presented in Table 3. From the results, the independent variables studied including microwave power (A), solvent-to-sample ratio (B), and irradiation time (C), their interactions including AB, AC, and BC, and also the second order of each of these variables, including A^2, B^2, and C^2, showed a significant effect ($p < 0.05$) on the QRY of bioactive compounds. The coefficient of variation (C.V.%) determined in this study was 0.66%, which is a standardized measure of dispersion of a probability distribution, and low values of this criteria are desirable (Piasecka et al., 2024). Also, the coefficients of determination of the proposed model were higher than 0.99, which being close to 1 means that the predicted data fit well with the actual data (Ramic et al., 2015).

Table 2. Experimental treatments and results of predicted and experimental QRY values of bioactive compounds from *P. orientalis* leaves

Independent MAE variables				QRY (%)		
Run	MP (W)	SR (*v/w*)	RT (min)	Experimental values	Predicted values	Residuals
1	300.00	45.00	6.00	12.67	13.96	0.043
2	300.00	15.00	18.00	14.85	13.54	-0.043
3	300.00	30.00	12.00	16.10	15.94	0.057
4	200.00	15.00	12.00	12.00	9.99	0.012
5	300.00	15.00	6.00	10.70	13.59	-0.087
6	300.00	45.00	18.00	16.32	15.41	0.087
7	300.00	30.00	12.00	16.20	16.11	-0.012
8	400.00	30.00	18.00	16.10	10.65	0.045
9	400.00	15.00	12.00	14.00	12.64	0.030
10	200.00	45.00	12.00	13.50	14.88	-0.030
11	300.00	30.00	12.00	16.22	16.36	-0.045
12	400.00	45.00	12.00	16.00	16.12	0.100
13	300.00	30.00	12.00	16.08	16.12	-0.12
14	200.00	30.00	18.00	15.50	16.12	-0.040
15	300.00	30.00	12.00	16.00	16.12	-0.020
16	400.00	30.00	6.00	13.50	16.12	0.080
17	200.00	30.00	6.00	10.00	12.06	-0.057

3.2 Verification of the Proposed Model

To verify the proposed polynomial model (Eq. 4), the experimental values of the response were compared with the predicted values by the fitted model (Figure 2). According to the results, there is a high statistical correlation between the experimental and predicted values. Therefore, the response surface polynomial model obtained in this study can determine the optimal operating conditions for the bioactive compounds recovery from the *P. orientalis* leaves under the studied conditions.

3.3 Effect of MAE on the QRY of Bioactive Compounds

3.3.1 Effect of Microwave Power

In the present study, MAE technology was used to recover bioactive compounds from the *P. orientalis* leaves. The effect of independent variables including microwave power, solvent-to-sample ratio, and irradiation time was investigated to determine the optimal conditions for bioactive recovery. Three-dimensional diagrams were drawn to evaluate the simultaneous effect of the independent variables studied on the QRY (Figure 3 a-c). According to the results, microwave power is one of the most important independent variables of MAE technology to separate bioactive compounds from the *P. orientalis* leaves. It has been documented that with a change in microwave power, it is possible to change the wave amplitude and consequently the energy applied to convert into heat and activate water molecules (Cavalluzzi et al., 2022). The simultaneous effect of microwave power and solvent-to-sample ratio, as well as microwave power and irradiation time on the QRY of bioactive compounds, were shown in Figure 3 (a) and (b), respectively. It is obvious that by increasing the microwave power up to a certain level the QRY increased, thereafter equilibrium phase was observed. Further increment led to a decline in the recovery process of the target compounds. In general, microwave power is considered one of the important and effective variables of MAE technology. Sufficient microwave power is required to generate the heat and power required for the destruction of the plant cell matrix to dissolve secondary metabolites in the solvent (Norouzi et al., 2021).

Table 3. ANOVA results of the proposed model

Quantitative CY of bioactive compounds					
Source	Sum of Squares	df	Regression coefficients	F-Value	*p-value* Prob > F
Model	68.84	9	16.12	830.11	< 0.0001
A	9.25	1	1.08	1003.33	< 0.0001
B	6.02	1	0.87	653.38	< 0.0001
C	31.60	1	1.99	3429.59	< 0.0001
AB	0.063	1	0.12	6.78	0.0352
AC	2.10	1	-0.72	228.18	< 0.0001
BC	0.063	1	-0.13	6.78	0.0352
A^2	4.66	1	-1.05	506.20	< 0.0001
B^2	5.99	1	-1.19	649.82	< 0.0001
C^2	7.03	1	-1.29	763.37	< 0.0001
Residual	0.65	7			
Lack of Fit	0.032	3		1.29	<0.3926
Pure Error	0.033	4			
Cor Total	68.90	16	C.V.%	0.66	
R^2	0.9991	Adj R^2	0.9979	Pred R^2	0.9919

As mentioned, the application of microwave causes an increase in temperature, which can be caused by the fact that polar molecules try to align themselves with the electromagnetic field facing an oscillating electromagnetic field. This action leads to the movement of molecules and ultimately heat production. On the other hand, as the sample is exposed to microwave irradiation, the moisture in the sample evaporates, which causes increased pressure inside the cell matrix and destroys the cell wall. Therefore, increasing the microwave power may reduce the time of the recovery process and enhance the extraction efficiency (Naduvilthara et al., 2023). On the other hand, applying higher levels of microwave power led to a decline in the response due to the destruction of heat-sensitive bioactive compounds. Generally, the extraction efficiency shows a proportional increase with higher microwave power, reaching a point beyond which no significant increment was observed. Therefore, it is very important to determine the appropriate level of microwave power to achieve a successful MAE process. Feki *et al.* (2021) in a study investigated and optimized the recovery of polyphenolic compounds from jojoba seed (*Simmondsia chinensis*) using MAE technology. According to their results, increasing the microwave power up to 500 W led to a quantitative increase in the recovery performance of the target compounds up to 23.35%, and after that, no considerable change was observed in the recovery of bioactive compounds (Feki et al., 2021). The same results have been reported in literature (Samanta & Ghosh, 2023; Kadi et al., 2022).

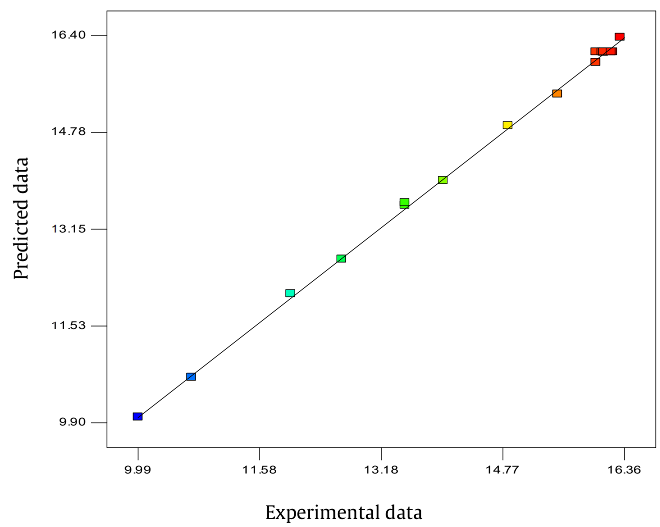

Figure 2. Verification of the proposed second-order polynomial model

3.3.2 Effect of Solvent-to-Sample Ratio

Figure 3 (a and c) showed the simultaneous effect of solvent-to-sample ratio with other independent variables studied, including microwave power and irradiation time on the QRY of bioactive compounds from *P. orientalis* leaves. As can be seen, by increasing the levels of the solvent-to-sample ratio up to a certain value, the QRY of bioactive compounds was increased. According to Figure 3 (b), the solvent-to-sample ratio of about 35 *v/w* showed the highest quantitative performance. A proper solvent-to-sample ratio determination must be considered to obtain adequate heating. The more solvent consumption, the more heat generated by the microwave will be affected due to the absorption of microwave irradiation by the surrounding solvent. Excessive absorption of microwaves by the higher volume of solvent may prevent sufficient pass of microwaves through the solvent environment to reach the plant matrix. Consequently, simultaneous heating of the plant matrix, which plays an important role in breaking the cell wall and releasing the desired analytes, may not occur efficiently. On the other hand, if there is an insufficient amount of solvent, it reduces the movement of secondary metabolites in the cell matrix and thus reduces mass transfer (Chaari et al., 2024). It is necessary to use the right amount of solvent to fully immerse the plant material in the solvent during microwave irradiation. However, increasing the solvent-to-sample ratio does not lead to improved extraction efficiency, as it can lead to unfavorable distribution and insufficient microwave exposure. In another study, similar results were reported by Özbek *et al.* (2019) regarding the effect of sample to solvent ratio. They recovered bioactive compounds from pistachio skin by MAE technology. They reported that by using a sample-to-solvent ratio of 1:8 to 1:20 *w/v*, the yield increased slightly with increasing sample-to-solvent ratio and then gradually decreased (Özbek et al., 2019).

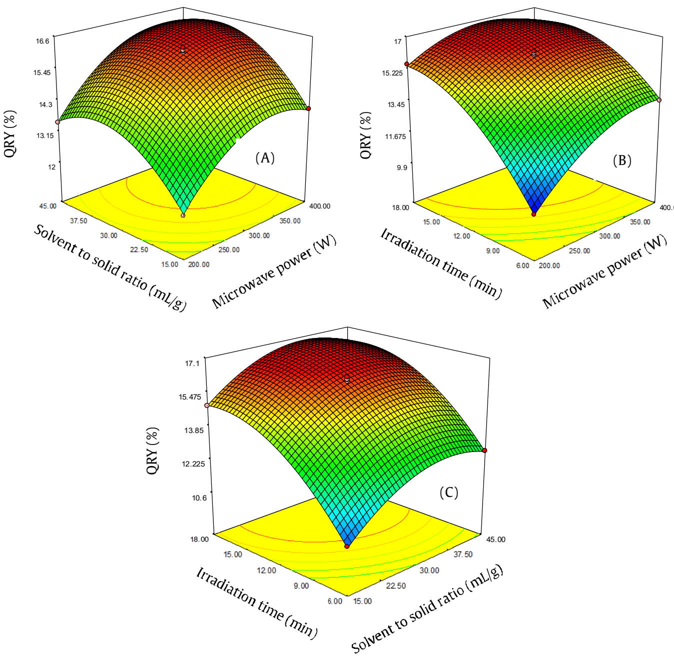

Figure 3. Three-dimensional graphs of the effect of MAE-independent variables on the QRY of bioactive compounds from the *P. orientalis* leaves. A: The interaction effect of microwave power (W) and the ratio of solvent-to-sample ratio (mL/g), (B): The interaction effect of the microwave power (W) and irradiation time (min), (C): The interaction effect of the microwave power (W) and solvent-to-solid ratio (mL/g)

3.3.3 Effect of Irradiation Time

Figure 3 (b and c) shows the simultaneous effect of microwave irradiation duration with other independent variables studied, including microwave power and solvent-to-sample ratio, on the QRY of the bioactive compounds from the *P. orientalis* leaves. From the results, by increasing the irradiation time up to a certain level, the response has been increased, and after that, no considerable change was observed. According to Figure 3 (b and c), applying the irradiation time of about 15 min the highest value of the QRY of the bioactive compounds was achieved, and then, a gradual equilibrium stage was observed. In general, by increasing the microwave irradiation time up to a certain level, the sample is exposed to irradiation for a longer time, which causes more severe damage to the cellular structure of the sample. On the other hand, to avoid the risk of thermal degradation and oxidation, the MAE time is an important factor that has to be controlled. In different studies, the microwave irradiation time usually ranges from minutes to seconds. The successful isolation of various plant-based compounds, including polysaccharides (Shen et al., 2022), phenolic compounds (Daliri Sosefi et al., 2024), and curcuminoids (Patil et al., 2023), through the application of microwave-assisted extraction (MAE) technology, has been reported in the literature.

3.4 Optimization of MAE Process

The numerical optimization was carried out to determine the optimal levels of the independent variables studied to maximize the QRY of the bioactive compounds from *P. orientalis* leaves by the MAE technique. The optimal MAE conditions were determined as 300 W microwave power, 17 min microwave irradiation time, and a solvent-to-sample ratio of 31 *v/w*. There was no significant difference between predicted (16.89%) and experimental (16.35 ± 0.12%) values of response under the optimized conditions. The results indicated successful optimization of MAE technology to obtain bioactive compounds from *P. orientalis* leaves using response surface methodology. To the best of the authors′ knowledge, there are no previously published results on the optimization of MAE for bioactive compounds recovery from *P. orientalis* leaves. However, the results were compared with those reported on the optimization of microwave-assisted extraction of bioactive compounds from *Veronica persica* using response surface methodology. The optimal MAE conditions for maximizing extraction yield were 211 W microwave power, 14 min irradiation time, and 1:33.5 g/mL sample-to-solvent ratio. Under the optimal conditions, the predicted extraction yield was 12.12% (Daliri Sosefi et al., 2024). According to the current results, higher extraction yield could be obtained using optimized conditions of MAE, which revealed the potential application of the MAE technique to obtain valuable bioactive compounds from *P. orientalis* leaves.

3.5 Determination of Radical Scavenging Activity

The free radical scavenging activity of the extract obtained under the optimized conditions of the MAE technique (300 W) microwave power, 17 min of microwave irradiation time, and the solvent-to-sample ratio of 31 *v/w* were determined by different methods using a spectrophotometer and results were presented in Table 4. Furthermore, a comparison was made with the results obtained using minimum and maximum levels of parameters studied. From the results, it is obvious that the radical scavenging activity (52.20 ± 0.19% of $DPPH_{sc}$ and 39.55 ± 0.13% of HO_{sc}) of extracts increased using the optimum conditions compared with the other MAE conditions. The radical scavenging activity of the extracts obtained under minimum and maximum levels of parameters studied was lower than those obtained using optimum conditions of MAE, which highlighted the importance of process optimization. The lower radical scavenging activity of the extracts obtained under the maximum levels of parameters studied could be due to the thermo-degradation of valuable bioactive compounds, which are involved in the radical scavenging activity (Roshani Neshat et al., 2022). The same results were reported in the literature (Aourach et al., 2021). Bioactive compounds are secondary plant metabolites with various biological activities, which highlighted the consumption of these compounds as nutraceuticals. Generally, there is a strong relationship between human diseases and the accumulation of free radicals. Bioactive compounds with antioxidant activity can scavenge free radicals and minimize their harmful effects. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer (Ribeiro et al., 2019; Kris-Etherton et al., 2002). According to the current findings, *P. orientalis* leaves are a potential source of bioactive compounds with a radical scavenging capacity.

3.6 Determination of Major Phenolic Compounds

The major phenolic compounds of the extract obtained under optimum conditions of the MAE technique (300 W microwave power, 17 min of microwave irradiation time, and a solvent-to-sample ratio of 31 *v/w* were determined using RP-HPLC, and results were presented in Table 4. Furthermore, a comparison was made with the results obtained using minimum and maximum levels of parameters studied. According to the results, the extract obtained under optimized conditions had a higher concentration of valuable phenolic compounds compared with the other MAE conditions. These results showed the importance of MAE process optimization. The extract was a rich source of valuable bioactive compounds such as rutin, catechin, gallic acid, and myricetin. Polyphenols exhibited strong radical scavenging activities and have been utilized as food additives (Poodi et al., 2018). These valuable bioactive compounds showed significant protective effects against different diseases related to free radical formation such as cancer and cardiovascular diseases (Noroozi et al., 2021). Therefore, it is great of interest to determine natural sources rich in valuable phenolic compounds for further utilization in the food and pharmacy industries.

Table 4. Determination of RSA and major phenolic compounds

Extraction Mode	RSA		Major phenolic compounds			
	$\%DPPH_{sc}$	$\%HO_{sc}$	Gallic acid	Catechin	Rutin	Myrecitin
Type 1*	$20.12^{c} \pm 0.15$	$16.21^{c} \pm 0.20$	$1.67^{c} \pm 0.13$	$2.25^{c} \pm 0.13$	$1.40^{b} \pm 0.12$	$1.14^{b} \pm 0.14$
Type 2*	$52.20^{a} \pm 0.19$	$39.55^{a} \pm 0.13$	$3.45^{a} \pm 0.15$	$5.45^{a} \pm 0.15$	$3.35^{a} \pm 0.10$	$4.45^{a} \pm 0.13$
Type 3*	$41.18^{b} \pm 0.21$	$28.48^{b} \pm 0.18$	$3.10^{b} \pm 0.12$	$4.78^{b} \pm 0.16$	$3.10^{a} \pm 0.10$	$3.80^{a} \pm 0.12$

* Type 1: Minimum levels of parameters studied; * Type 2: Optimum levels of parameters studied; * Type 3: Maximum levels of parameters studied.
* Different lowercase letters in each column indicate a significant difference among different treatments ($P < 0.05$).

4. Conclusion

The potential application of the MAE technique as a promising and effective extraction process to obtain extracts from *P. orientalis* leaves with good QRY and containing valuable bioactive phenolic compounds was investigated. Furthermore, RSM based on BBD was an effective procedure for optimizing MAE. The second-order polynomial model was developed for predicting QRY. The optimal MAE conditions were found to be 300 W microwave power, 17 min of microwave irradiation time, and a solvent-to-sample ratio of 31 *v/w*. Under the optimal conditions, there was no significant difference between predicted (16.89%) and experimental (16.35 ± 0.12%) values of QRY indicating a high goodness of fit of the model proposed. Therefore, it can be stated that *P. orientalis* leaves as agro-waste are a potential source of valuable bioactive compounds and MAE is a potential tool to obtain these valuable compounds for further application in food and pharmacy industries.

Authors' Contributions

Seyedeh Tahereh Fazeli: Literature review; Performing laboratory tests and data collection; Writing. Mandana Bimakr: Developed the idea for research; Methodology; Resource; Supervision; Project administration; Visualization; Formal analysis; Writing; Writing-review & editing.

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Conflicts of Interest

The Authors declare that there is no conflict of interest.

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Ethical considerations

There were no ethical considerations to be considered in this research under Project number 162862552.

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