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Optimization of Microwave-Assisted Extraction of Bioactive Compounds from *Veronica Persica* Using Response Surface Methodology

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A B S T R A C T

Background: The current study aimed to optimize microwave-assisted extraction (MAE) for the recovery of bioactive compounds from *Veronica Persica* and to enhance extraction yield through ultrasonic pretreatment as an emerging technique.
Methods: The MAE was optimized in terms of microwave power, solid-to-solvent ratio, and irradiation time through response surface methodology (RSM) for maximum extraction yield. The extraction yield was further enhanced by using ultrasonic pretreatment with different levels of ultrasound power, temperature, and sonication time. The total phenolic content and radical scavenging activity were determined through the Folin-Ciocalteu method and DPPH and OH free radicals scavenging activity assays, respectively.
Results: The optimal conditions for maximizing extraction yield were 211 W microwave power, 14 min irradiation time, and 1:33.5 g/mL solid-to-solvent ratio. Under the optimal conditions, the predicted extraction yield was 12.12%. The results confirmed that ultrasonic pretreatment effectively enhanced the release of bioactive compounds from aerial parts of *V. persica*. Moreover, scanning electron microscopy images indicated a positive effect of ultrasonic pretreatment on enhancing bioactive compound recovery through damage to the cell structure of the treated samples.
Conclusion: The results indicated that using ultrasonic and microwave energy can speed up the extraction process and increase the quality of the extracted biomass.

1. Introduction

Over the past decades, researchers have shown great interest in plant-derived biomolecules from different parts of plants due to their significant antioxidant properties. The antioxidant properties of the plant-derived biomolecules make them an attractive alternative to synthetic antioxidants in the food and pharmacy industries. The adverse effects of synthetic antioxidants and growing consumer demand for healthier products have led to a search for antioxidants recovered from the plant matrix (Roshani Neshat et al., 2022). *Veronica persica* which is commonly referred to as bird-eye speedwell known as Sizaab in Iran. *V. persica* is a flowering plant belonging to the family Scrophulariaceae, widely distributed in Europe and Asia (Shim et al., 2022). It is an annual or perennial plant that can be found in fields, road margins, and gardens as wild flora, and is characterized by its small sky-blue flowers. Various species of *V. persica* are traditionally utilized worldwide, with the stem and leaves serving as a food source in certain regions. Previous studies demonstrated various biological activities of *V. persica* such as antioxidant, antimicrobial, antifungal, and anti-inflammatory effects (Harput et al., 2002) which could be due to its content of secondary metabolites such as iridoids, phenolic acids, and flavones (Fierascu et al., 2018). The extraction of valuable bioactive compounds from natural sources such as *V. persica* could be one of the most important steps for their further research and usage in food or pharmaceuticals as it plays a critical role in the extraction yield, and bioactivity of extracts (Salehi et

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al., 2019; Sharifi-Rad et al., 2018). Conventional extraction techniques have several drawbacks including long extraction times, large quantities of solvents, low extraction yield, and the degradation of heat-sensitive compounds. Consequently, emerging extraction techniques such as ultrasound-assisted, pressurized water, infrared-assisted, pulsed electric field, and enzyme-assisted extractions have been applied to overcome the above-mentioned limitations (Ajami et al., 2023; Alara et al., 2023). In recent years, MAE has been successfully applied for the recovery of bioactive-rich extracts due to its higher extraction yield, lower energy and solvent consumption, and shorter processing time when compared with conventional extraction techniques (Ajami et al., 2023; Saini & Keum, 2018). It should be noted that the bioactive composition and extraction yield are strongly affected by operational variables such as solid-to-solvent ratio, microwave power, irradiation time, solvent concentration, and temperature. Therefore, the determination of optimal extraction conditions to achieve a successful process in terms of the quantity and quality of extracted bioactive compounds is essential. In this regard, RSM has been widely applied to explore the influence of process parameters on the response(s) and optimization of MAE conditions of bioactive compounds from various natural resources such as *Careya sphaerica* Roxb. flowers (Sai-Ut et al., 2024), *Gossampinus malabarica* flowers (Sai-Ut et al., 2023), chestnut processing waste (Tomasi et al., 2023), and black bean waste (Mali & Kumar, 2023). Moreover, the intensification of bioactive compound release using efficient methods is of great interest to researchers in various disciplines. The application of ultrasound as a pretreatment for intensifying bioactive compound extraction is one such method. Ultrasound is a cost-effective approach since cavitation can increase the penetration of the solvent into plant cells, leading to an increase in the release of bioactive compounds (Yadav et al., 2023). Several researchers have demonstrated that using ultrasonic and microwave energy can speed up the extraction process and increase the quantity and quality of the bioactive compounds from *Zataria multiflora* (Karimi et al., 2020), *Lavandula coronopifolia* Poir (Sharifzadeh et al., 2022), tuberose flowers (Yadav et al., 2023), roses (Patrascu & Radoiu, 2016), and jasmine (Sommano et al., 2015). The optimization of the MAE approach and its intensification through ultrasonic pretreatment for recovery of bioactive compounds from *V. persica* has not been reported till now. Therefore, the objective of the current study was to optimize the MAE conditions in terms of microwave power, irradiation time, and solid-to-solvent ratio through RSM based on a Box-Behnken Design (RSM-BBD) to achieve highest extraction yield of valuable bioactive compounds from the aerial parts of *V. persica*. Additionally, ultrasonic pretreatment with various sonication power (100 and 200 W), temperature (30 and 40 °C), and sonication time (20 and 40 min) was utilized under optimum MAE conditions to intensify the release of bioactive compounds.

2. Materials and Methods

2.1 Plant material and preparation

The aerial parts of the *V. persica* plant were harvested from Khorgam (Roudbar, Guilan, Iran) and identified by a medicinal plant expert in the Modern Biological Techniques Research Center at the University of Zanjan, Iran. The samples were washed with distilled water and then dried at an ambient temperature of 25 °C. Subsequently, the dried samples were ground into powder using a grinder (GSC-911, China) and sieved through the 18-mesh sieve. The powder was collected and stored at 4 °C until further use.

2.2 Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, and sodium carbonate were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Absolute EtOH (analytical grade), and hydrogen peroxide were purchased from Dr. Mojallali Chemical Complex Co. (Tehran, Iran).

2.3 Soxhlet extraction

Soxhlet procedure as a reference method was used for the extraction of bioactive compounds according to the method of Roshani Neshat *et al.* (2020). In brief, 5 g of the dried powder sample was placed into a thimble and then inserted into the Soxhlet extractor. The extractor was placed on top of a round-bottomed flask containing 200 mL of 96% ethanol, and the recovery process was carried out for 6 h. The solvent was then removed using a rotary evaporator (RV10, IKA, Germany) under vacuum, and stored at -18 °C for further analysis.

2.4 Microwave-assisted extraction (MAE)

A microwave-assisted apparatus (MR249, KIAN TAJHIZ GD, Iran) was used to extract bioactive compounds from aerial parts of *V. persica*. Briefly, 5 g of dried powder sample was mixed with the solvent at the solid-to-solvent ratio of 1:15-1:45 g/mL and extracted for 9-15 min with 100-300 W microwave power level. The extracts were filtered and the solvent was removed using a rotary evaporator (RV10, IKA, Germany) under a vacuum, and stored at -18 °C for further use (Roshani Neshat et al. 2022).

2.5 Ultrasonic pretreatment (USP)

The ultrasonic pretreatment was conducted using an L09 ultrasonic bath (VCLEAN, Backer Co., Iran) for the duration of 20 and 40 min under ultrasonic power of 100 and 200 W at the processing temperature of 30 and 40 °C to intensify the release of bioactive compounds from aerial parts of *V. persica* under the optimized MAE conditions. Following the ultrasonic pretreatments, the bioactive extracts were recovered as reported in Section 2.4.

2.6 Determination of extraction yield

The extraction yield of bioactive compounds was determined as follows:

$$Extraction\,yield(\%) = \frac{W_1}{W_0} \times 100$$

Where W_1 (g) is the mass of crude *V. persica* extract (g) and W_0 is the mass of dried powder of *V. persica* (g).

2.7 Determination of total phenolic content (TPC)

The Folin-Ciocalteu method was used to determine the TPC of the bioactive extract. To carry out the assay, 0.5 mL of the extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and left at room temperature for 5 min. Then, 2 mL of sodium carbonate (7.5% w/v) was added to the mixture and mixed thoroughly. The reaction mixture was kept in the dark at room temperature for 90 min, and then the absorbance value was measured at 765 nm (Singleton et al., 1999). The TPC of the extract was calculated as gallic acid equivalents (mg GAE/g).

2.8 Determination of free radical scavenging activity

2.8.1 DPPH free radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) method was utilized to assess the free radical scavenging activity. Briefly, 0.1 mL of the extract was mixed with 2.9 mL of ethanolic DPPH solution (0.1 mM), and the mixture was then placed in the dark at room temperature for 30 min. Subsequently, the absorbance was measured at 517 nm. The DPPH free radical scavenging activity (%) was determined as follows (Zengin et al., 2010):

$$DPPH\ free\ radical\ scavenging\ activity\ (\%) = \frac{A_b - A_s}{A_b} \times 100$$

Where A_b represents the absorbance value of the blank (DPPH without sample), and A_s represents the absorbance of the sample with DPPH.

2.8.2 HO free radical scavenging activity

The HO free radical scavenging activity was determined based on the method of Boulekbache-Makhlouf *et al.* (2013). In brief, 1 mL of the extract was mixed with 1 mL of 30% hydrogen peroxide (H_2O_2) solution. The mixture was then placed in a water bath at 37 °C for 1 h. Subsequently, the absorbance was measured at 520 nm. The HO free radical scavenging activity (%) was calculated as follows:

$$HO\ free\ radical\ scavenging\ activity\ (\%) = \frac{A_b - A_s}{A_b} \times 100$$

Where, A_s and A_b were the absorbance of the sample and blank (without H_2O_2), respectively.

2.9 Scanning electron microscopy (SEM)

SEM images were acquired for untreated, dried residues of MAE, and USP + MAE to investigate the effect of extraction methods on the morphology of *V. persica* aerial parts samples. The obtained residues after MAE and USP + MAE were dried at 25 °C until a constant weight. Then, the samples were sputter-coated with a thin layer of gold at room temperature and imaged using a scanning electron microscope (VEGA II TESCAN, Czech) at 15 kV and a magnification of 100-500.

2.10 Experimental design and statistical analysis

In the present study, RSM based on a Box-Behnken Design (BBD) was employed to investigate the main, interaction, and quadratic effects of MAE operating parameters including microwave power (100-300 W), solid-to-solvent ratio (1:15-1:45 g/mL), and irradiation time (9-15 min) on extraction yield and to determine the optimum MAE conditions to attain the maximum extraction yield. A total of 17 experiments with five central points were performed. The second-order polynomial was fitted to the experimental data as follows:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j$$

Where Y is the response variable, β_0 is the constant coefficient; β_i, β_{ii}, and β_{ij} are the coefficients of the linear, quadratic, and interactive effects, respectively; X_i and X_j are the independent variables, and k equals the number of independent variables (k = 3). The adequacy of the fitted model was assessed through the lack-of-fit test, the coefficient of determination (R^2), and the adjusted coefficient of determination (R^2-adj). To determine the optimal conditions, numerical optimization was used to maximize the extraction yield. Design Expert Version 7.1.3 software (Stat-Ease, Inc., Minneapolis, MN) was used for experimental design, data analysis, and three-dimensional response surface plots generation and optimization. Furthermore, other data were analyzed through one-way ANOVA, followed by the comparison of means using Tukey's test at 95% confidence level using Minitab V16.0 software (State College, PA, USA). The results were represented as mean ± standard deviation.

3. Results and Discussion

3.1 Statistical analysis and fitting of the model using RSM-BBD

In the present study, the effects of microwave power (A), solid-to-solvent ratio (B), and irradiation time (C) as independent variables of MAE on the extraction yield of bioactive compounds from the aerial parts of *V. persica* were investigated. The predicted and experimental extraction yield of bioactive compounds from the aerial parts of *V. persica* under each of 17 sets of treatment conditions is shown in Table 1. The extraction yield of bioactive compounds ranged from 9.88 to 12%. The results of ANOVA, lack-of-fit, R^2, Adj-R^2, and coefficient of variation of the model, as well as the statistical significance of the regression model terms were shown in Table 2. According to the Table 2, the proposed model was significant (*F*-value = 280.85, *p* <

0.0001). The fitted model in terms of coded factors was determined as follows:

$$Extraction\ yield = 11.85 + 0.29A + 0.22B - 0.26C - 0.54AB - 0.055AC - 0.065BC - 0.46A^2 - 0.43B^2 - 0.15C^2$$

In the above-mentioned equation, A, B, and C represented microwave power, solid-to-solvent ratio, and irradiation time, respectively. According to Table 2, R^2 of the proposed second-order polynomial model was 0.9972 showing the fitted model adequacy. The predicted R^2 was in very well agreement with the adjusted R^2. The high value of adjusted-R^2 (0.9937), predicted-R^2 (0.9642), and the low value of C.V. (%) proved a high correlation degree between experimental and predicted values and the precision and reliability of the fitted model, respectively. The lack-of-fit test was not significant ($p > 0.0773$), indicating a non-significant relationship with the pure error. The linear, quadratic, and interaction terms of operating parameters were all significant ($p < 0.05$). Therefore, the fitted second-order polynomial is suitable for predicting the extraction yield. The perturbation plot (Figure 1) for the extraction yield of bioactive compounds from *V. persica* was plotted by varying one factor over its range and keeping constant the other factors to make a comparison between operating parameters at a selected point in the considered design space. According to Figure 1, the most effective operating parameters on the extraction yield of bioactive compounds from *V. persica* were microwave power, solid-to-solvent ratio, and irradiation time, respectively.

Table 1. Box-Behnken experimental design matrix and actual and predicted values of extraction yield

Run	Microwave power (W)	Solid-to-solvent ratio (g/mL)	Irradiation time (min)	Extraction yield (%)	
				Actual value	Predicted value
1	100.00	15.00	12.00	9.88	9.92
2	300.00	15.00	12.00	11.60	11.57
3	100.00	45.00	12.00	11.40	11.43
4	300.00	45.00	12.00	10.97	10.93
5	100.00	30.00	9.00	10.98	10.94
6	300.00	30.00	9.00	11.61	11.62
7	100.00	30.00	15.00	11.60	11.58
8	300.00	30.00	15.00	12.00	12.04
9	200.00	15.00	9.00	11.02	11.03
10	200.00	45.00	9.00	11.58	11.59
11	200.00	15.00	15.00	11.70	11.69
12	200.00	45.00	15.00	12.00	11.99
13	200.00	30.00	12.00	11.88	11.85
14	200.00	30.00	12.00	11.85	11.85
15	200.00	30.00	12.00	11.84	11.85
16	200.00	30.00	12.00	11.81	11.85
17	200.00	30.00	12.00	11.86	11.85

3.2 Effect of MAE operating parameters on extraction yield

The simultaneous effect of studied operating parameters on the extraction yield was investigated through three-dimensional response surface plots (Figure 2 a-c). Three-dimensional response surface plots illustrated the main and interactive effects of any two operating parameters on the extraction yield of bioactive compounds from aerial parts of *V. persica* while the other operating parameters were kept constant at the center point value.

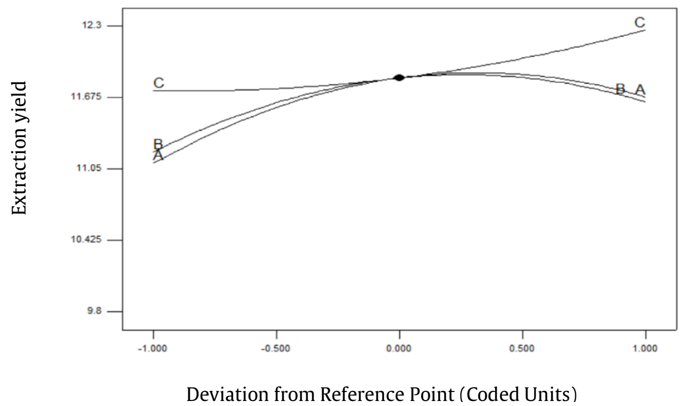

Figure 1. Perturbation plot for extraction yield of bioactive compounds from aerial parts of *V. persica* (A = microwave power, B = solid-to-solvent ratio, and C = irradiation time)

Table 2. Analysis of variance (ANOVA) for second-order polynomial model obtained from experimental results

Source	df	Sum of squares	Mean squares	*F-value*	*p-value*
Model	9	4.59	0.51	280.85	< 0.0001
A	1	0.67	0.67	367.50	< 0.0001
B	1	0.38	0.38	210.92	< 0.0001
C	1	0.56	0.56	309.53	< 0.0001
AB	1	1.16	1.16	636.71	< 0.0001
AC	1	0.012	0.012	6.67	0.0364
BC	1	0.017	0.017	9.31	0.0185
A^2	1	0.88	0.88	486.09	< 0.0001
B^2	1	0.77	0.77	424.46	< 0.0001
C^2	1	0.10	0.10	55.55	0.0001
Residual	7	0.013	1.815E-003		-
Lack-of-Fit	3	0.010	3.342E-003		0.0773
Pure Error	4	2.680E-003	6.700E-004		-
Cor Total	16	4.60			
C.V.%		0.37	Adj-R^2		0.9937
R^2		0.9972	Pred-R^2		0.9642

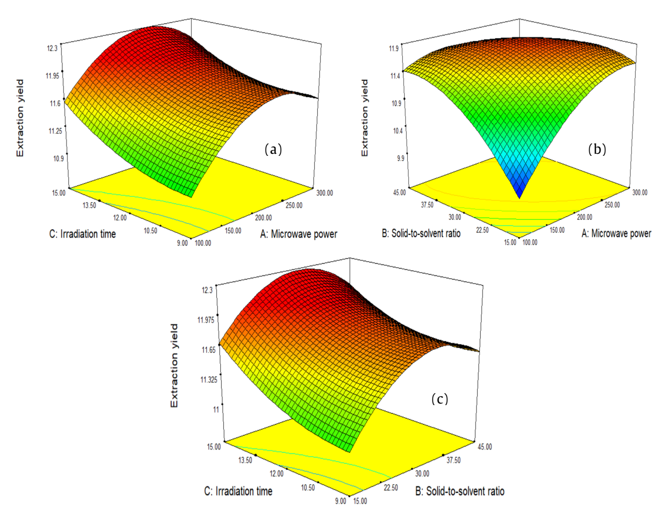

Figure 2. Three-dimensional response surface plots for extraction yield (%) as a function of (a) microwave power (W) and irradiation time (min), (b) microwave power (W) and solid-to-solvent ratio (g/mL), (c) solid-to-solvent ratio (g/mL) and irradiation time (min)

Generally, the increase in microwave power up to approximately 250 W led to a rise in the extraction yield of bioactive compounds up to a certain point, beyond which no further change was observed (Figure 2 a-b). Microwave power is a crucial parameter in MAE, as it also causes a temperature increase in the system due to the alignment of polar molecules with the oscillating electromagnetic field. The increase in temperature due to microwave power is caused by the random motion of polar molecules, which experience inertia and are unable to follow the field. This phenomenon leads to heat production and ultimately increases the extraction yield of bioactive compounds. Additionally, exposure to microwave radiation causes the moisture in the sample to evaporate, creating pressure inside the cell and damaging the cell wall, which releases the compounds into the solvent. These interactions have been observed to significantly impact the extraction efficiency of bioactive compounds (Liu et al., 2023). When the temperature increases, the viscosity of the solvent decreases, which can lead to changes in the cell wall structure and enhance the transfer of compounds to the solvent (Shen et al., 2023). An increase in temperature can cause plant tissue to soften, leading to enhanced mass transfer rates and increased solubility of certain compounds by facilitating the breaking of chemical bonds (Kumar et al., 2023). It is necessary to explain that the use of high levels of microwave power has limitations due to the thermal instability of bioactive compounds, therefore, the use of high-power levels can reduce the biological activity of the extracted compounds (Ozdemir & Karagoz, 2023). Also, due to the rapid rupture of the cell wall that occurs at a higher temperature, more impurities are extracted from the plant matrix with bioactive compounds (Saravana et al., 2023). Therefore, to extract bioactive compounds efficiently and minimize energy consumption, it is crucial to choose the appropriate microwave power level. In this regard, Feki *et al.* (2021) optimized the extraction of simmondsins and polyphenols from Jojoba (*Simmondsia chinensis*) seed cake using MAE. The results showed that increasing the microwave power up to 500 W led to a 23.35% increase in the extraction yield of the target compounds (Feki et al., 2021). In another study, the extraction of parthenolide from the stems of *Tarconanthus camphoratus* using MAE was optimized. The results revealed that increasing the microwave power from 50 to 200 W led to a rise in the extraction yield of the target compounds. However, beyond 200 W, no significant increase in the extraction yield was observed. The optimal power level was found to be 211 W (Alam et al., 2021). The solid-to-solvent ratio is another critical factor that influences the extraction yield of bioactive compounds from various plant sources. The extraction yield of bioactive compounds from the aerial parts of *V. persica* increased up to a certain level of solid-to-solvent ratio to about 1:37 g/mL as shown in Figure 2 (b-c). Beyond that point, no significant change was observed. It is important to note that increases in solvent volume often result in less absorption of microwave energy by the solvent. From the industrial point of view, minimizing solvent consumption and maximizing yield are crucial factors. However, there must be a sufficient volume of solvent to ensure complete immersion of the sample during the extraction process (Gala et al., 2022; Nazal et al., 2023). Dahmoune *et al.* (2015) investigated the extraction of polyphenols from *Myrtus communis* L. leaves using MAE. They explored the effect of the solid-to-solvent ratio within the range of 1:20 to 1:40 g/mL and found that the optimal ratio for maximum extraction yield was 1:30 g/mL resulting in an extraction yield of 162.49 ± 16.95 mg gallic acid equivalent/g dry weight (Dahmoune et al., 2015). Özbek *et al.* (2020) applied MAE to extract bioactive compounds from the pistachio (*Pistacia vera* L.) hull. The researchers investigated the effect of the solid-to-solvent ratio on the extraction yield and found that increasing the ratio from 1:8 to 1:20 g/mL increased the extraction yield. However, beyond this ratio, the amount of total phenolic compounds (TPC) was decreased (Özbek et al., 2020). Figure 3 (a-c) illustrates that the extraction yield of bioactive compounds increased with the irradiation time up to 15 min. The irradiation time is another important operating variable in the MAE approach. By increasing the irradiation time, the sample is exposed to microwave radiation for a longer duration which results in more damage to the sample tissue as well as plant cell wall. This leads to an increase in the release of bioactive compounds into the solvent, ultimately increasing the extraction yield (Imtiaz et al., 2023; Xiaokang et al., 2020). However, long irradiation time is limited due to the thermal instability of bioactive compounds (Walayat et al., 2023). In this regard, Dairi *et al.* (2021) optimized the extraction of phenolic compounds from red onion using the MAE method (Dairi et al., 2021). The results showed an increase in phenolic content (10.50 mg GAE/g) when the irradiation time was increased from 55 to 80 sec. However, long extraction times with strong microwave power led to lower efficiency. This is likely due to the destruction of target compounds by microwave heating, which aligns with the findings of the present study. In another study, Saifullah *et al.* (2021) investigated the extraction of polyphenols from lemon myrtle using MAE. The results revealed that the extraction yield increased as the irradiation time was increased from 2 to 6 min. The optimal irradiation time was found to be 6 min (Saifullah et al., 2021).

3.3 Numerical optimization of MAE conditions and practical validation

The numerical optimization was conducted to determine the optimal levels of independent variables to maximize the extraction yield of bioactive compounds from the aerial parts of *V. persica* using the MAE approach. The optimal conditions were microwave power of 211 W, irradiation time of 14 min, and a solid-to-solvent ratio of 1:33.5 g/mL. The predicted maximum extraction yield of bioactive compounds was 12.12% under the above-mentioned conditions. The validation experiment was repeated five times under the optimal conditions. There was no significant difference between the experimental value (12.05% ± 0.12) and the predicted one. Furthermore, the Soxhlet method has been widely used as a traditional and reference method for extracting target compounds from different plant sources (Abbas et al., 2021; Latiff et al., 2021). In the current study, the extraction efficiency of the MAE technique under the optimized conditions was compared to the Soxhlet method. The extraction yield of bioactive compounds by the Soxhlet method was 19.35% ± 0.20 which was 59.60% higher than MAE.

3.4 Effect of ultrasonic pretreatment on extraction yield under the optimal MAE conditions

In the present study, ultrasonic pretreatment (USP) was used to enhance the release of bioactive compounds from the aerial parts of *V. persica* under optimized MAE conditions. The results are presented in Table 3. The application of USP resulted in an extraction yield ranging from 13.50% ± 0.15 to 17.92% ± 0.19, confirming that USP is a favorable and effective method for intensifying the release of bioactive compounds. According to the results, when the temperature and sonication time are held constant, increasing the power of ultrasonic waves from 100 to 200 W resulted in a higher extraction yield. For instance, at a temperature of 30 °C and a sonication time of 20 min, when increasing the power from 100 to 200 W the extraction yield rose from 13.50% ± 0.15 to 14.60% ± 0.14. The power of ultrasound is one of the most important factors affecting cell structure through the cavitation phenomenon (Walayat et al., 2023). The collapse of cavitation bubbles can cause various phenomena such as pore formation, increased absorption, and swelling index in the cellular matrix of plants. This effect is one of the most important factors contributing to the increase in the extraction yield of bioactive compounds (Kumar et al., 2023; Nguyen et al., 2023). It is important to note that the impact of ultrasound on different plant samples can vary depending on the tissue and cell structure, as well as the type of system used (Poodi et al., 2018). The results indicated that increasing the sonication time at a constant level of wave intensity (especially low level) and temperature resulted in a favorable increase in extraction yield. For example, at the ultrasonic power of 100 W and a temperature of 40 °C, the extraction yield increased from 14.20% ± 0.18 to 15.93% ± 0.21 with the increase in the sonication time up to 40 min. Increasing the sonication time contributed to an increase in the number of microjets produced by the collapse of a cavitation bubble. However, the destructive effects of ultrasound on bioactive compounds limit the increase in sonication time to higher levels (Ogura et al., 2023). It is worth noting that the possibility of oxidation and degradation of bioactive compounds increases with a longer sonication time (Maier et al., 2023). As shown in Table 3, the extraction yield increased with an increase in temperature, indicating a positive effect of temperature on changes in cell structure. Temperature affects the properties of the solvent by reducing its viscosity and surface tension, leading to an increase in the formation of bubbles during the cavitation phenomenon. Consequently, solvent permeability rises, resulting in enhanced mass transfer and higher extraction yields. However, excessive temperatures can lead to the denaturation or breakdown of bioactive compounds, posing a risk to the extraction process and the integrity of the compounds (Nanzai et al., 2023). Pongmalai *et al.* (2015) investigated the extraction of bioactive compounds from cabbage outer leaves using MAE and ultrasonic pretreatment. The results demonstrated that the use of ultrasonic-assisted extraction (UAE) + MAE resulted in a higher content of extractable bioactive compounds compared to UAE or MAE alone. This suggests that the combination of UAE and MAE is more effective for extracting bioactive compounds from cabbage outer leaves than either method alone (Pongmalai et al., 2015). Garcia-Vaquero *et al.* (2020) found that the combined use of UAE and MAE resulted in a higher extraction yield of bioactive compounds and antioxidants from brown macroalgae compared to the UAE or MAE methods separately. Based on the findings, it was observed that if USP is applied under conditions of 200 W of ultrasonic power, temperature of 40 °C and sonication time of 20 min, the extraction yield of bioactive compounds under optimal conditions of MAE is increased by about 47.80% (Garcia-Vaquero et al., 2020).

Table 3. Intensification of bioactive compounds released through USP-MAE

Ultrasonic power (W)	Sonication time (min)	Temperature (°C)	Extraction yield (%)
100	20	30	13.50±0.15[D]
100	40	30	14.45±0.11[C]
100	20	40	14.20±0.18[C]
100	40	40	15.93±0.21[B]
200	20	30	14.60±0.14[C]
200	40	30	15.70±0.15[B]
200	20	40	17.80±0.16[A]
200	40	40	17.92±0.21[A]

Results were presented as mean ± SD (Standard Deviation, n = 3). Different letters described the significant difference between the values ($p < 0.05$).

3.5 Effect of extraction methods on TPC and free radical scavenging activity

In the current study, the effect of different extraction methods on TPC and free radical scavenging activity of bioactive extracts was investigated and the findings were presented in Table 4. The lowest values of antiradical capacity were observed in the sample obtained by the Soxhlet method (14.17% ± 0.11 and 11.00% ± 0.13) whereas the utilization of MAE and USP-MAE methods resulted in extracts with higher antiradical capacity. This outcome could be attributed to the more pronounced destruction of the cell structure, leading to a more efficient transfer of compounds from the cell structure into the solvent. Additionally, the thermal degradation of bioactive compounds in the Soxhlet method contributed to a reduction in the antiradical activity of the extracted compounds. The results revealed a clear correlation between the TPC and the antiradical capacity of the extracts. This finding is consistent with the results of Kumar *et al.* (2023). Trujillo-Mayol *et al.* (2019) conducted a study on the extraction of polyphenols from avocado peel using ultrasound and microwave methods (Trujillo-Mayol et al., 2019). It was found that the TPC was higher in the ultrasound-assisted extraction method compared to MAE which is consistent with the findings of the current study. Furthermore, Alara *et al.* (2018) explored the extraction of flavonoids and antioxidants from *Vernonia amygdalina* leaf using MAE (Alara et al., 2018). The results showed that the extract obtained by MAE had a higher TPC and antiradical capacity compared to the Soxhlet method, which is consistent with the findings of the present study. Sharma *et al.* (2021) investigated the extraction of betacyanin and betaxanthin from *Amaranthus tricolour* leaves using MAE, ultrasonic-assisted extraction, and Soxhlet methods (Sharma et al., 2021). The results showed that the Soxhlet method had a lower ability to inhibit DPPH free radicals compared to MAE and USP, which is consistent with the findings of the present study.

Table 4. Comparison of TPC content and free radical scavenging activity of *V. persica* extracts extracted using different extraction methods

Method	TPC (mg GAE/g)	$\%HO_{sc}$	$\%DPPH_{sc}$
Soxhlet	17.17 ± 0.20^{A}	11.00 ± 0.13^{A}	14.17 ± 0.11^{A}
Optimized MAE	79.28 ± 0.14^{B}	46.77 ± 0.13^{B}	53.45 ± 0.10^{B}
USP-MAE	94.63 ± 0.12^{C}	61.19 ± 0.14^{C}	69.55 ± 0.15^{C}

Results were presented as mean ± SD (Standard Deviation, n = 3). Different letters in each column described the significant difference between the values ($p < 0.05$).

3.6 SEM analysis

The cell structure changes of untreated *V. persica* and the residues after extraction using MAE and USP-MAE were studied through scanning electron microscopy. The images are depicted in Figure 3 (a-c). The SEM images revealed that the cell structure of the untreated *V. persica* plant appeared intact and unwrinkled (Figure 3-a). Following MAE, the cell structure exhibited signs of swelling (Figure 3-b). This phenomenon is attributed to the impact of microwave irradiation on the elevation of the sample's temperature leading to increased internal pressure. Consequently, the elevated internal pressure causes the cell walls to rupture, facilitating greater solvent penetration into the cells and the subsequent release of more bioactive compounds (Shen et al., 2023). In Figure 3-c, the severe damage to the cell structure of the *V. persica* matrix treated by USP-MAE is obvious. The propagation of ultrasound waves in the solvent creates cycles of expansion and rarefaction, leading to the formation of bubbles within the solvent. The subsequent collapse of these bubbles generates local pressure and heat destroying the cell wall (Arya et al., 2023). Baltacıoğlu *et al.* (2021) conducted a study to optimize the extraction of phenolic compounds from tomatoes using MAE and compared it with conventional solvent extraction. The results of the morphological analysis showed that the recovery speed was related to the physical changes in the sample which could be observed through SEM analysis (Baltacıoğlu et al., 2021). Severe damage was not detected during conventional solvent extraction; however, cell damage was observed after MAE treatment. The MAE treatment caused cell wall rupture, and the structural changes during MAE allowed the solvent to enter the sample more easily, resulting in an increased extraction yield. Zhang *et al.* (2021) optimized the extraction of polysaccharides from perilla seed meal (PSM) using the ultrasound-assisted extraction method. The SEM results showed that the ultrasound created numerous holes on the surface of the sample, with uniform pore sizes and smooth edges. This suggests that the ultrasound treatment caused significant structural changes in the sample allowing for more efficient extraction of polysaccharides.

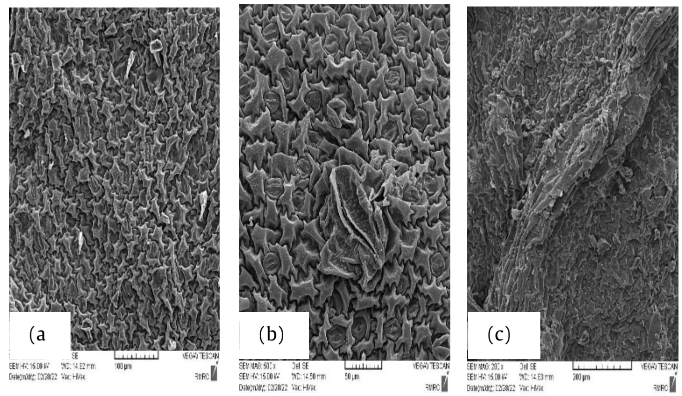

Figure 3. SEM images of *V. persica* samples: (a) untreated sample, (b) sample after MAE, and (c) sample after USP-MAE. MAE was performed under optimum conditions

4. Conclusion

This research experimentally extracted bioactive compounds from aerial parts of *V. persica* using a microwave-assisted extraction process. In the study, the microwave power (100-300 W), solid-to-solvent ratio (1:15-1:45 g/mL), and irradiation time (9-15 min) were first optimized for the maximum extraction yield. The optimal conditions for maximum extraction yield were 211 W microwave power, 14 min irradiation time, and 1:33.5 g/mL solid-to-solvent ratio. Under the optimized conditions, the predicted extraction yield was 12.12% which was lower than the Soxhlet method (19.35 ± 0.20%) as a conventional extraction approach. In this regard, ultrasonic pretreatment was applied to enhance the release of bioactive compounds from aerial parts of *V. persica*. The findings revealed that the ultrasonic pretreatment could result in a significant increase in the extraction yield by approximately 47.80%. The highest TPC and free radical scavenging activity were obtained by using the ultrasonic pretreatment-microwave-assisted extraction method. It is obvious from the results that the aerial parts of *V. persica* are a valuable source of bioactive compounds with potential applications in the food, pharmaceutical, and health industries. Furthermore, the findings confirmed that sequential ultrasound-microwave-assisted extraction is an efficient procedure for bioactive compound extraction (Zhang et al., 2021).

Authors' Contributions

Zohreh Daliri Sosefi: Literature review; Performing laboratory tests and data collection. Mandana Bimakr: Developed the idea for research; Methodology; Resource;

Supervision; Project administration; Visualization; Formal analysis; Writing; Writing-review & editing. Ali Ganjloo: Formal analysis; Methodology; Advisory; Writing-review & editing. All the authors read and approved the final manuscript.

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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.

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