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Research Article

Novel Green Method for Enhancing the Extraction Efficiency of Bioactive Compounds: *Falcaria vulgaris* Extract

Zeinab Hassanloofard,[1] Mohsen Zandi,[2] Mehdi Gharekhani,[1] Ali Ganjloo,[2] and Leila Roufegarinejad[1]

[1]*Department of Food Science and Technology, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
[2]*Department of Food Science and Engineering, Faculty of Agriculture, University of Zanjan, Zanjan 45371-38791, Iran*

Correspondence should be addressed to Mohsen Zandi; zandi@znu.ac.ir

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The aim of the present research is to investigate the effect of ohmic-assisted extraction (OAE) parameters on the *Falcaria vulgaris* extract (FVE) properties. Various parameters, including ethanol-to-water ratio (0%–100%), voltage gradient (10–30 V/cm), temperature (30°C–60°C), and extraction time (10–30 min), were applied as independent factors. Physicochemical process, antimicrobial properties, and energy analysis were accomplished to achieve OAE optimization. Results indicated that extraction yield (EY) and energy consumption (EC) significantly increased with increasing the voltage gradient and extraction time ($p < 0.05$). Also, EY significantly increased with increasing of process temperature and the ethanol concentration in the solvent ($p < 0.05$). The findings demonstrated that as the voltage gradient, process duration, temperature, and ethanol concentration in the solvent were increased, there was a significant initial increase followed by a decrease in both the total phenolic content (TPC) and the DPPH radical scavenging activity of FVE ($p < 0.05$). The optimization results indicated that the extraction process with an ethanol-to-water ratio of 65.5%, voltage gradient of 20.3 V/cm, temperature of 56.6°C, and extraction time of 22.1 min results in maximum extract TPC, DPPH, and EY, and minimum extraction EC. The OAE method showed greater EY and less EC and CO_2 production compared to the hydrodistillation (HD) method. In general, OAE caused improved antioxidant and antimicrobial properties of the FVE.

Keywords: antimicrobial properties; extraction yield; *Falcaria vulgaris*; ohmic-assisted extraction; TPC

1. Introduction

Falcaria vulgaris (FV) belong to the Apiaceae family, called Paghazeh in Iran which grows as a weed in fields and mountains in the spring. This medicinal herb is consumed fresh, dry, and cooked, and is mainly used as a vegetable and spice in foods. FV has smooth and hairless stems, long leaves, and fleshy and sweet roots with white flowers. The aerial part of this herb contains many bioactive compounds, including spatolnol, carvacrol, alphafin, anthraquinone, alkaloids, phenol, tannin, saponin, steroids, and flavonoids, that have medicinal, antioxidant, and antimicrobial properties. The presence of alkaloid, carotene, saponin, and tannin in FV plant caused this herb to treat skin and digestive lesions such as stomach ulcers, liver diseases, kidney stones, and bladder [1]. The use of the *Falcaria vulgaris* extract (FVE) as antimicrobial, antioxidant, and preservative agents in food and drug has been reported by some researchers [2]. Extraction is the first step to separate and purify many bioactive components such as phenolic compounds from medicinal herbs [3–5].

Extraction method, solvent type, and solvent polarity could affect the quality and quantity of extracted compounds. Traditional extraction methods such as a hydrodistillation (HD) have been used for many decades. These extraction methods are time-consuming and mainly have

low efficiency and high energy and solvent consumption. On the other hand, traditional extraction methods could cause thermal decomposition of bioactive compounds, which affected the antioxidant, medicinal, and antimicrobial properties of the extract [6–8]. In recent years, novel extraction techniques with short process time, less energy and solvent consumption, and high efficiency were developed. These green extraction methods could improve the antioxidant, medicinal, and antimicrobial properties of the extract. Ultrasound-assisted extraction, microwave-assisted extraction, and ohmic-assisted extraction (OAE) are some eco-friendly novel extraction methods [3, 9, 10].

Ohmic heating, also called Joule heating, is the volumetric heating method in which electric current passed from food. In the Ohmic heating method, heat generation is strongly dependent on the food resistance. Heat is produced internally, and approximately 100% electric current is converted to heat (efficiency near to 100%). The performance of the Ohmic heating system is influenced by various factors such as electric field strength, electrode type, electrical conductivity and electrical characteristics of the food, amount of input energy, electrode distance, and applied frequency [11–13]. Recently, OH system has been used in the extraction of herbal essential oil. OAE improved the quality and quantity of essential oil compared to HD. Nonthermal effect in the OAE as a result of the passing electric current caused plant wall cell distortion, which increased the extraction yield (EY) and speed. Some researchers reported the application of ohmic heating for essential oil extraction as an ohmic-assisted HD system [11, 12, 14–16].

However, the use of ohmic heating for the extraction of plant extract was only reported in our previous work, in which the effect of process parameters on the extraction of radish (*Raphanus sativus* L.) leaf extract was evaluated. The aim of the present study is to optimize the OAE of FVE. For this purpose, the effect of process parameters (including voltage gradient, temperature, and time) and solvent polarity on the quality and quantity of FVE was evaluated. Finally, optimum condition of OAE was compared with the traditional extraction method (i.e. HD).

2. Materials and Methods

2.1. Materials. After the approval of a plant taxonomist, aerial part of FV was purchased from local markets in Zanjan, Iran. Ethanol, Folin-Ciocalteu reagent, DPPH, gallic acid, anhydrous sodium acetate, and iron (III) chloride were procured from Merck (Germany). Lyophilized strains of microorganisms were obtained from the Persian Type Culture Collection (PTCC, Iran).

2.2. Extraction of Bioactive Compounds From FV Leaves. In the OAE process, 5 g of FV powder was mixed with 50 mL of solvent (1:10 w/v), then transferred to the OAE glass balloon. After placing the electrodes, the OAE process was carried out in the considered voltage gradient (10–30 V/cm) and extraction temperature (30°C–60°C) for 10–30 min (Table 1).

Then, the mixture was filtered by using a vacuum filtration system with filter paper (Whatman® Qualitative Filter Paper), and the solution was transferred to a vacuum rotary evaporator (at 40°C and 95 rpm) for solvent removal. Finally, FVE was kept in a dark glass vial in the refrigerator for other experiments. For the HD method, 5 g of FV powder was mixed with 50 mL of solvent (ethanol-to-water ratio of 65.5% (v/v)) and kept at 57°C in water bath for 8 h. Then, the mixture was filtered and the solvent was removed. Finally, FVE was kept in a dark glass vial in the refrigerator for other experiments [6].

2.3. Process Evaluation

2.3.1. EY. EY (%) was calculated by using the initial weight of FV (m_{FV}) and the final weight of FVE (m_{FVE}), via equation:

$$EY\,(\%) = \frac{m_{FV}}{m_{FVE}} \times 100. \tag{1}$$

2.3.2. Energy Consumption (EC). The EC (kW·h/g) calculated by using the amount of heat produced or energy consumed (Q_{ohmic}) (equations (2) and (3)) [11, 12]:

$$Q_{ohmic} = \int V.I.\,dt, \tag{2}$$

$$EC\left(\frac{kWh}{g}\right) = \frac{Q_{ohmic}}{m_{FVE}} \times \frac{1\,h}{3600\,s}, \tag{3}$$

where V, I and t are the voltage (v), field intensity (A), and process time (s), respectively.

2.4. FVE Evaluation

2.4.1. Total Phenolic Content (TPC). TPC was determined by colorimetric method using Folin-Ciocalteu reagent. For this purpose, 1 g of FVE was mixed with 3 mL of methanol–water solution (ratio of 90:10), and stirred for 4 min; then centrifuged at 3000 rpm for 5 min. 20 μL of the upper phase was mixed with 2.8 mL of water and 0.5 mL of Folin reagent. After 5 min, 1 mL of 10% (W/V) sodium carbonate was added to the mixture and put in a dark place for 1 h at room temperature. Finally, the solution absorbance was measured at 765 nm. TPC was reported as mg gallic acid/g FVE [17].

2.4.2. DPPH Free Radical Scavenging. For antioxidant activity measurement, 1 mL of 0.1 mM DPPH methanolic solution was added to 1 mL of the FVE. Then, the solution was shaken well, and was put in a dark place at room temperature for 15 min. Finally, the solution absorbance was measured at 517 nm [6]:

$$DPPH\,(\%) = \frac{A_S - A_C}{A_C} \times 100, \tag{4}$$

where A_S and A_C represent the absorbance of the sample and control, respectively. IC_{50}, as the amount of FVE required to reduce the initial DPPH concentration to 50%, was estimated using an exponential curve.

Table 1: OAE extraction treatments.

Run	Voltage gradient (V/cm)	Temperature (°C)	Time (min)	Ethanol (%)
1	20	45	20	50
2	20	45	20	50
3	30	45	20	50
4	25	37.5	15	75
5	15	37.5	25	75
6	15	52.5	15	75
7	25	52.5	15	25
8	20	45	20	0
9	20	45	20	50
10	20	45	20	100
11	20	30	20	50
12	25	52.5	15	75
13	25	37.5	25	75
14	15	37.5	25	25
15	25	52.5	25	75
16	25	37.5	25	25
17	20	45	20	50
18	20	45	20	50
19	20	45	10	50
20	10	45	20	50
21	20	60	20	50
22	20	45	20	50
23	25	37.5	15	25
24	15	52.5	25	25
25	15	37.5	15	75
26	15	52.5	15	25
27	15	37.5	15	25
28	20	45	30	50
29	25	52.5	25	25
30	15	52.5	25	75

2.5. Optimization of OAE Process Extraction and Comparison With the HD Method. OAE extraction optimization was conducted by defining the voltage gradient (10–30 V/cm), extraction temperature (30°C–60°C), extraction time (10–30 min), and ethanol-to-water ratio (0%–100%) as an independent parameter. A central composite design (CCD) was used to create an optimization matrix via Design-Expert software (V 12, USA). Finally, maximum EY, TPC, and DPPH free radical scavenging and minimum EC were defined as criteria. Treatment with maximum desirability was selected as an optimum. In the optimization process, the weight of all four responses including EY, EC, TPC, and DPPH free radical scavenging was defined the same in software. Comparison of FVE extracted by OAE and HD methods was carried out via TPC, antimicrobial, and DPPH free radical scavenging analyses. The amount of CO_2 produced was measured per kW/h of EC [7]. Also, process parameters including EC, EY, and CO_2 production of OAE and HD methods were compared. A scanning electron microscope (SEM) was used to evaluate the morphological microstructure of FV aerial parts before and after OAE and HD methods.

2.5.1. Antimicrobial Properties. Agar well diffusion method was used to measure the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of FVE [3]. For this purpose, *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Candida albicans* (yeast), and *Aspergillus niger* (mold) microbial suspensions were diluted via Ringer's solution. For MIC measurement, 100 μL serial dilution of FIVE (0.0975–100 mg/mL) with 100 and 5 μL of the BHI culture medium and bacterial suspension were poured into an agar well and incubated for 24 h at 37°C for bacteria and for 72 h at 25°C for mold and yeast. FVE with culture medium, and microbial suspension and culture medium with 0.5 μL of dimethyl sulfoxide (DMSO) were used as negative and positive controls, respectively. Finally, the lowest FVE concentration without any turbidity and sediment in an agar well was selected as the MIC. Müller–Hinton agar medium and sabrodextrose agar were used for MBC determination for bacteria and fungi, respectively.

2.5.2. SEM. SEM images were used for observation of the morphology of FV glands in the plant before and after the OAE and HD methods, for investigating the extent of damage. For SEM analysis, dried plant was coated with gold (6–11 nm, 10 mA, 40 s) and placed in the SEM chamber. Electron acceleration voltage and working distance were 15 kV and 5.7–5.9 mm, respectively.

2.6. Statistical Analysis. The response surface methodology (RSM) with CCD was used to evaluate the effect of voltage gradient (V/cm), temperature (°C), process time (s), and

ethanol-to-water ratio (%) on the EY, TPC, DPPH free radical scavenging, and EC of FVE via Design Expert software (V 12, USA). One-way analysis of variance (ANOVA) was applied for comparing the OAE and HD methods via SPSS (V 26, USA) software. All measurements were conducted at least in 3 replications. Duncan's multiple-range tests were conducted for comparing the mean values at $\alpha = 0.05$.

3. Results and Discussion

3.1. EY of the OAE Method. In this study, EY of the FVE in the OAE method was in the range of 9.11%–10.65%. Table 2 presents the ANOVA results of the EY, EC, TPC, and DPPH responses as a function of voltage gradient, extraction time and temperature, and ethanol-to-water ratio. According to the result, the quadratic model could able to predict experimental results of EY with high accuracy ($R^2 = 0.9897$). As seen in Table 2, nonstatistically significant lack of fit error ($p > 0.05$) along with statistically significant model ($p < 0.05$) indicated that the model was able to well fit the experimental values. ANOVA result indicated that all main variables have a significant effect on the EY ($p < 0.05$). However, among the interaction effects, the interaction effect of the voltage gradient with the extraction time and the interaction effect of the voltage gradient with the ethanol-to-water ratio were statistically significant ($p < 0.05$). Also, quadratic effects of temperature and ethanol-to-water ratio on the EY were significant ($p < 0.05$) (Table 1).

A 3D surface chart of the EY change versus variables is shown in Figure 1. Results indicated that the EY of FVE was significantly increased with the increase of voltage gradient ($p < 0.05$). The increase of voltage gradient has two thermal and nonthermal effects on the gland containing biocomponent. Heating rate increases with increasing voltage gradient, which accelerated the increase of gland internal vapor pressure, causing intensification of gland wall damage. On the other hand, the rate of electrical current passing increases with increasing voltage gradient, which causes an acceleration of the rate of the gland wall breakdown. Eventually, both thermal and nonthermal effects intensify the electroporation of gland wall, which increases the EY of FVE [3, 11, 18–20]. Similar results have been reported by Al-Hilphy, Al-Musafer, and Gavahian[18]. In addition, an increase in extraction time significantly increased EY ($p < 0.05$), which is due to providing sufficient time for a biomaterial outflow from the glands. As shown in Table 1, EY significantly increases with increase in extraction temperature ($p < 0.05$), which is probably due to the decrease of solvent viscosity and therefore the facility of extract exit from glands. Similar results have been reported by other researchers [18, 19]. It was found that the increase in the ratio of ethanol to water led to significant increases in the EY ($p < 0.05$). This is probably due to the high penetration coefficient of ethanol compared to water, which indicates the more influential effect of extraction with ethanol solvent in the extraction of effective and bioactive compounds from FV.

Equation (5) shows the quadratic model derived from experimental EY modeling as a function of the independent process variable.

$$\begin{aligned} EY = {} & +9.73 + 0.1612A + 0.0637B + 0.266C + 0.1354D \\ & + 0.0324B^2 + 0.0386D^2 + 0.1381AC + 0.0394AD, \end{aligned} \tag{5}$$

where A, B, C, and D are the main effects of the voltage gradient, extraction temperature, extraction time, and ethanol-to-water ratio, respectively; B^2 and D^2 are the quadratic effects of extraction temperature and ethanol-to-water ratio, respectively; and AC and AD are the interaction effects of voltage gradient with extraction time and voltage gradient with ethanol-to-water ratio, respectively. According to the results, it can be said that the extraction time had the greatest effect on the EY, followed by voltage gradient. The main effect of FVE extraction time has the highest coefficient, which refers to the high sensitivity of EY to extraction time change. This result is confirmed by the perturbation plot (Figure 2). As seen, the highest slope in the perturbation plot is related to the FVE extraction time. The lowest model coefficient has the main effects related to the extraction temperature main effect (B), which indicates that the EY sensitivity to the change in the extraction temperature is low. The plus and minus sign before the main, interaction, and second-degree effects indicates that this effect is directly and indirectly correlated with the response (EY), respectively. As shown in Figure 1 and equation (5), the voltage gradient and the extraction time have a synergistic effect, which caused the EY increase sharply.

3.2. EC. As shown in Table 2, the quadratic model was able to predict experimental results of EC with a high coefficient of determination ($R^2 = 0.9993$). Nonsignificant lack of fit error ($p > 0.05$) and significant model ($p < 0.05$) indicated the proper function of quadratic model for EC estimation. The statistical analysis results indicated that the main effects of the voltage gradient and extraction time on the EC were significant ($p < 0.05$). Among the interaction effects of independent variables, only the interaction effects of the voltage gradient with the extraction time were statistically significant ($p < 0.05$). Moreover, the second-order effect of extraction time on the EC was significant between the quadratic effects ($p < 0.05$). Karami, Zandi, and Ganjloo [3] inferred that during the yarrow (*Achillea millefolium* L.) essential oil extraction via ohmic-assisted HD, all main effect including voltage gradient and extraction time and the quadratic effect of extraction time on the EC was significant ($p < 0.05$) [3]. Figure 3 shows the 3D surface plot of the change in EC as a function of voltage gradient, extraction time and temperature, and ethanol-to-water ratio. As seen, the EC significantly increases with increases of voltage gradient and extraction time ($p < 0.05$). Furthermore, the voltage gradient and extraction time have a synergistic effect, which caused the significant increase in EC ($p < 0.05$).

Table 2: Analysis of variance (ANOVA) of EY, EC, TPC, and DPPH.

Source	Energy consumption (EC)		Extraction yield (EY)		Total phenolic content (TPC)		DPPH	
	SS	*p* value	SS	*p* value	SS	*p* value	SS	*p* value
Model	0.2331	< 0.0001	3.28	< 0.0001	441.98	< 0.0001	103.28	< 0.0001
A: voltage gradient	0.1741	< 0.0001	0.6240	< 0.0001	138.67	< 0.0001	32.55	< 0.0001
B: temperature	6.667E – 07	0.8115	0.0975	< 0.0001	1.02	0.0985	0.1488	0.2154
C: time	0.0484	< 0.0001	1.70	< 0.0001	28.58	< 0.0001	1.59	0.0007
D: ethanol-to-water ratio	0.0000	0.0886	0.4401	< 0.0001	12.31	< 0.0001	7.34	< 0.0001
AB	2.500E – 07	0.8838	0.0018	0.3880	0.6931	0.1672	0.0495	0.4672
AC	0.0070	< 0.0001	0.3053	< 0.0001	2.85	0.0101	0.4865	0.0336
AD	0.0000	1.0000	0.0248	0.0049	0.6202	0.1899	0.1914	0.1631
BC	2.500E – 07	0.8838	0.0001	0.8774	0.0248	0.7873	0.0014	0.9016
BD	1.000E – 06	0.7703	0.0008	0.5736	0.0248	0.7873	0.0856	0.3423
CD	1.000E – 06	0.7703	0.0014	0.4450	0.0856	0.6175	0.1425	0.2249
A^2	0.0035	< 0.0001	0.0053	0.1498	121.28	< 0.0001	54.01	< 0.0001
B^2	1.714E – 06	0.7025	0.0288	0.0029	9.72	< 0.0001	0.1372	0.2333
C^2	4.286E – 07	0.8483	0.0053	0.1498	74.93	< 0.0001	3.18	< 0.0001
D^2	0.0000	0.1403	0.0410	0.0007	131.89	< 0.0001	11.22	< 0.0001
Residual	0.0002		0.0343		4.93		1.33	
Lack of fit	0.0001	0.2940	0.0284	0.1715	2.69	0.7719	1.28	0.0756
Pure error	0.0000		0.0059		2.25		0.0495	
Cor total	0.2333		3.32		446.91		104.61	
R^2	0.9993		0.9897		0.9890		0.9872	

Abbreviation: SS = sum of squares.

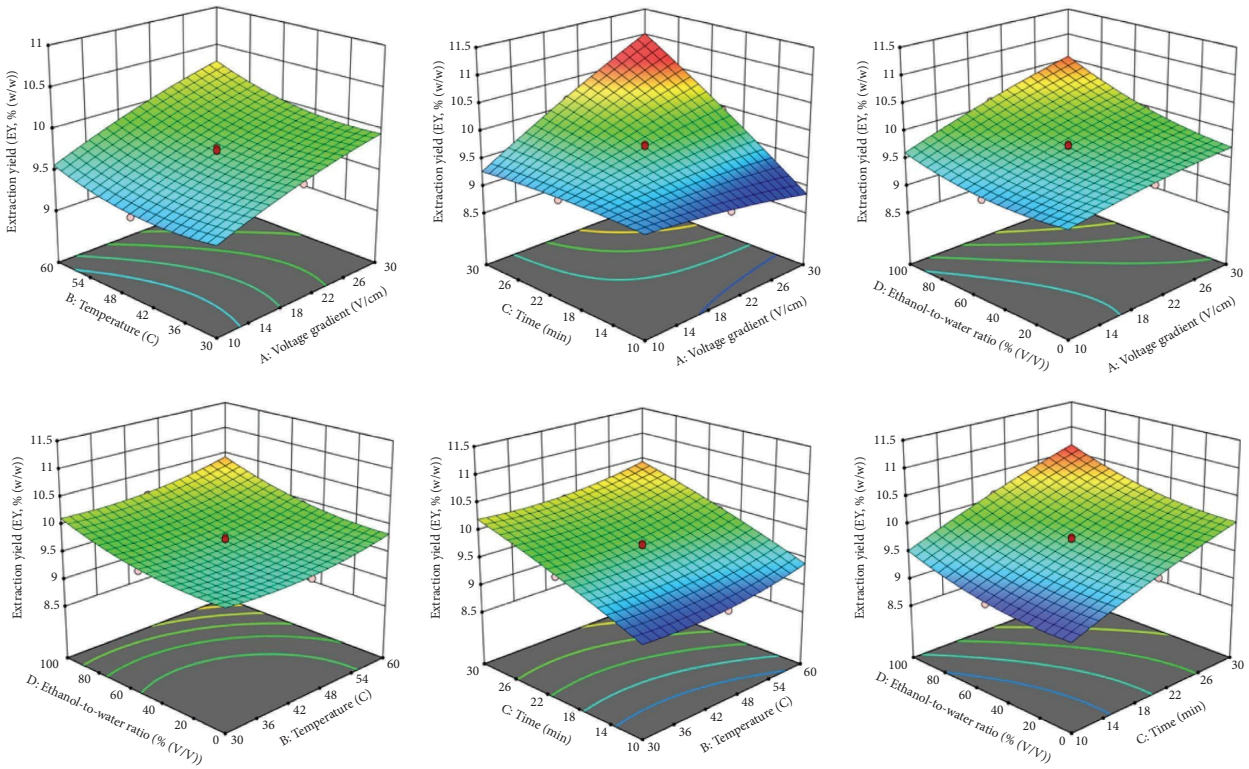


Figure 1: The 3D surface chart of the EY change versus voltage gradient, extraction time, extraction temperature, and ethanol-to-water ratio.

The electrical EC increased with increasing voltage gradient and extraction time, which significantly increases EC ($p < 0.05$). Similar result was reported by Tunç and Koca [11, 12] and Jafari et al. EC results indicated that the experimental EC-derived quadratic model (equation (6)) was capable of predicting changes in EC in the treatment of OAE, which shows the high accuracy of predicted values.

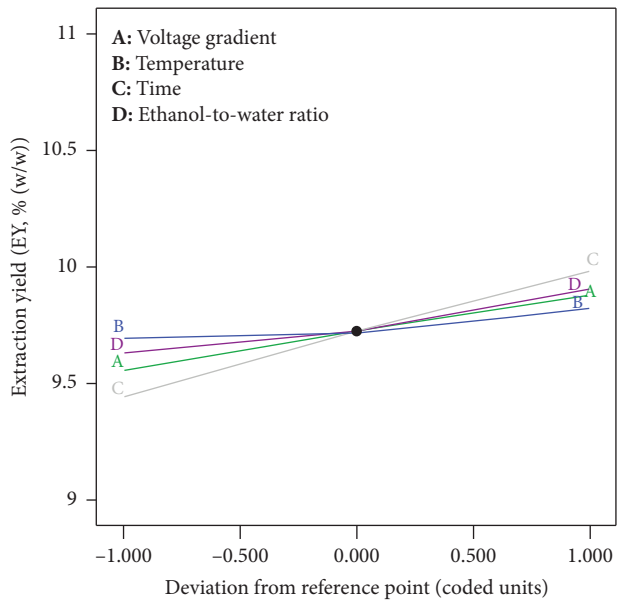


Figure 2: Perturbation plot of the extraction yield in ohmic-assisted extraction.

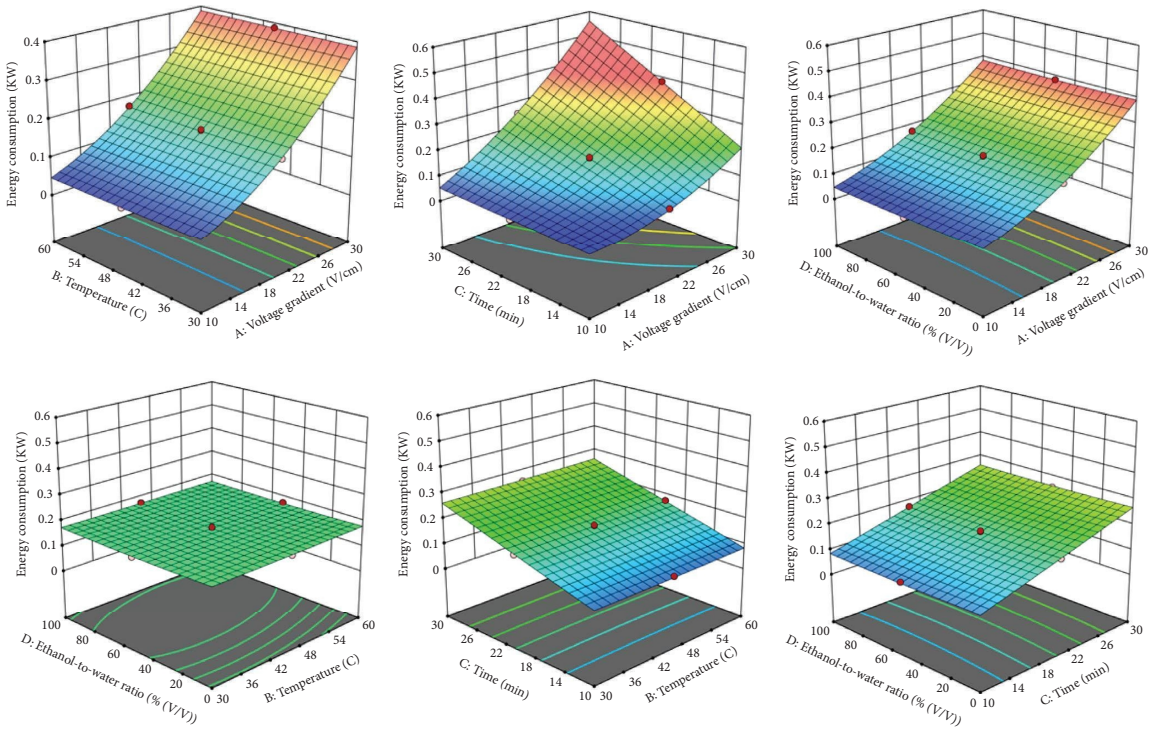


Figure 3: The 3D surface diagram of the change in energy consumption (EC) as a function of voltage gradient, extraction time, extraction temperature, and ethanol-to-water ratio.

$$EC = +0.1712 + 0.0852A + 0.0449C + 0.0001C^2 + 0.0209AC, \quad (6)$$

where A, C, AC, and C^2 are the main effect of the voltage gradient, main effect of extraction time, interaction effects of voltage gradient with extraction temperature, and quadratic effects of extraction time, respectively. As discussed, the plus sign before the main, interaction, and the quadratic effects indicates that increases of each effect resulted in an increase in EC values. By investigation of the model coefficients in equation (6), it is clear that the highest coefficient is for voltage gradient, which indicates the maximum sensitivity of the EC to the change in the voltage gradient. This result is confirmed by the perturbation plot (Figure 4). As seen, the highest slope in the perturbation plot is related to the voltage gradient. The lowest model coefficient in the main effects

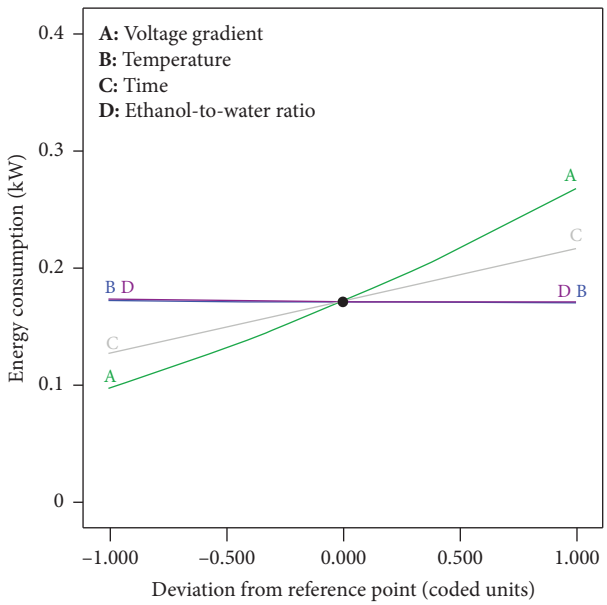


Figure 4: Perturbation plot of the energy consumption in ohmic-assisted extraction.

related to the extraction time main effect (C) indicates that the EY sensitivity to the change in the extraction temperature is low. A horizontal line with zero slope in the perturbation plot of the extraction temperature and ethanol-to-water ratio indicates that the EC is independent of these parameters. This result is confirmed by ANOVA.

3.3. TPC. Phenolic compounds are an important group of secondary plant metabolites whose antioxidant capacity is caused by the presence of hydroxyl groups in their structure. The use of natural phenol in the pharmaceutical and food industry is increasing. By slowing down the oxidative breakdown of lipids, these compounds enhance the quality and nutritional value of food [10, 21]. In this study, TPC of the FVE in the OAE method was approximately 53.64–67.32 mg GAE/g extract. The statistical analysis indicated that the quadratic polynomial model was able to model experimental TPC of the FVE results with high accuracy ($R^2 = 0.9890$). The lack of fit error and model were nonsignificant ($p > 0.05$) and significant ($p < 0.05$), respectively, which indicated that the model was able to well fit the experimental values (Table 2).

As shown in Table 2, all main effects of the variables except the extraction temperature have a significant effect on the TPC of FVE ($p < 0.05$). Moreover, quadratic effects of all independent parameters on the TPC of FVE were significant ($p < 0.05$). However, among the interaction effects, the interaction effect of the voltage gradient with the extraction time was statistically significant ($p < 0.05$). Figure 3 shows the 3D surface plot of the change in EC as a function of voltage gradient, extraction time and temperature, and ethanol-to-water ratio. Results indicated that with the increase of voltage gradient, TPC of FVE was significantly increased and then decreased ($p < 0.05$). The initial increase of TPC with increasing voltage gradient is probably due to nonthermal effect of the voltage gradient, which caused the destruction of the gland wall and therefore led to the release of phenolic compounds. With more voltage gradient increases, the thermal effect of this independent variable leads to temperature increase, which caused the thermal decomposition of the phenolic compounds and TPC decreases in the FVE [3]. In addition, an increase in the extraction time led to the initial increase and then decrease of the TPC of FVE ($p < 0.05$). Up to 20 min, the phenolic component release rate is greater than the decomposition rate; after this time, the TPC decreases could be attributed to the thermal decomposition of phenolic component and increases of heavy compound extraction [22, 23]. As shown in Figure 5, the voltage gradient and the extraction time have a synergistic effect, which caused the intensification of the phenolic component decomposition. Ma et al. [25] reported the increasing voltage gradient and extraction time during the OAE of active compounds from tomatoes, with TPC increasing and then decreasing [24]. Equation (7) shows the quadratic model derived from experimental TPC of FVE modeling as a function of voltage gradient, extraction time, extraction temperature, and ethanol-to-water ratio.

$$\begin{aligned} \mathrm{TPC} = {} & +66.38 + 2.40\mathrm{A} + 1.09\mathrm{C} + 0.7162\mathrm{D} - 2.10\mathrm{A}^2 \\ & - 0.5953\mathrm{B}^2 - 1.65\mathrm{C}^2 - 2.02\mathrm{D}^2 - 0.4219\mathrm{AC}, \end{aligned} \quad (7)$$

where A, B, C, and D are the main effects of the voltage gradient, extraction temperature, extraction time, and ethanol-to-water ratio, respectively; A^2, B^2, C^2, and D^2 are the quadratic effects of voltage gradient, extraction temperature, extraction time, and ethanol-to-water ratio, respectively; and AC is the interaction effect of voltage gradient with extraction time. As seen, the main effect and the quadratic effect of voltage gradient have the greatest effect on the TPC response. The slope and concavity of the voltage gradient curve in the perturbation plot show the sensitivity of TPC to the change in the voltage gradient (Figure 6). The lowest model coefficient in the main effects is related to the ethanol-to-water ratio main effect (D), which indicate that the TPC sensitivity to the change in the ethanol-to-water ratio is low. The significant ($p < 0.05$) quadratic effects of the independent variable are confirmed by the concavity of the voltage gradient, extraction temperature, extraction time, and ethanol-to-water ratio curves in the perturbation plot.

3.4. DPPH Free Radical Scavenging. The determination of the ability to inhibit DPPH free radicals is widely used as a reliable test to assess the antioxidant activity of plant extracts. In addition, the high sensitivity of the DPPH radical measurement also provides the possibility of determining the activity of low concentrations of antioxidant compounds [22, 24]. As shown in Table 2, the quadratic model was able to predict experimental results of DPPH free radical scavenging of FVE with a high coefficient of determination ($R^2 = 0.9872$). Nonstatistically significant lack of fit error ($p > 0.05$) along with the statistically significant model ($p < 0.05$) showed that the model was able to adequately fit to the experimental values. The results of the statistical analysis

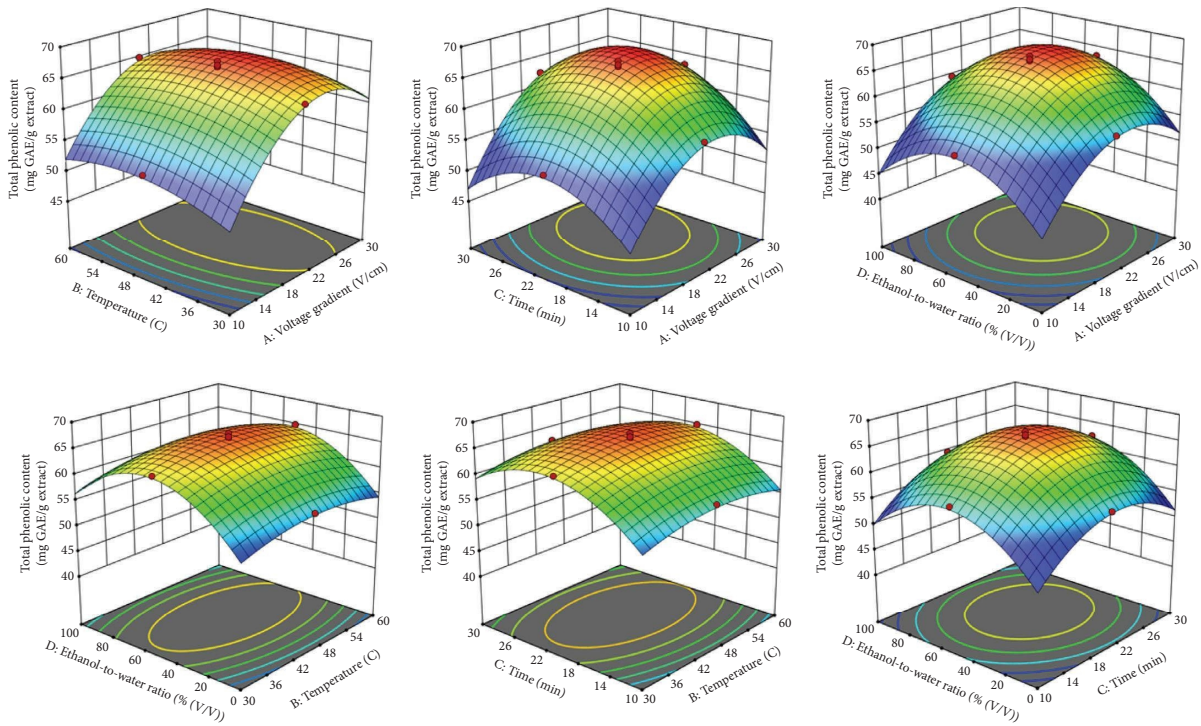


Figure 5: The 3D surface diagram of the change in total phenolic content (TPC) of *Falcaria vulgaris* extract (FVE) as a function of voltage gradient, extraction time, extraction temperature, and ethanol-to-water ratio.

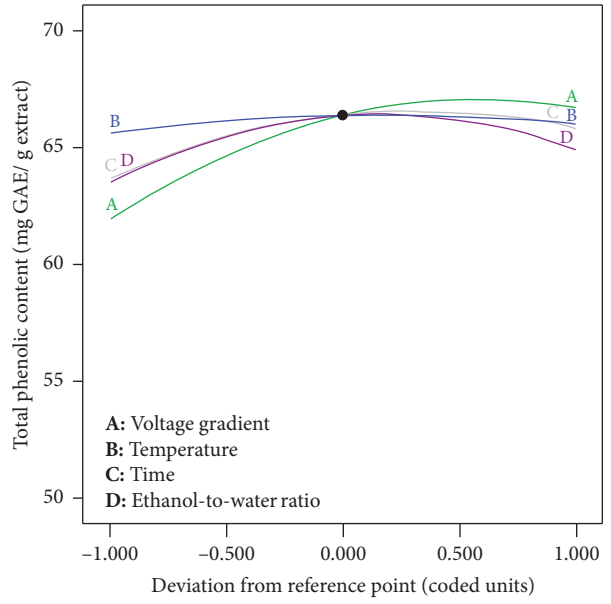


Figure 6: Perturbation plot of the total phenolic content (TPC) of *Falcaria vulgaris* extract (FVE) in ohmic-assisted extraction.

showed that all the main effects of the variables, with the exception of extraction temperature, were significant ($p < 0.05$); among the interaction effects, however, only the interaction effect of voltage gradient and extraction time was significant ($p < 0.05$). As shown in Table 2, all quadratic effects, with the exception of the quadratic effect of extraction temperature on the ability to inhibit DPPH free radicals, were significant ($p < 0.05$). The 3D surface chart of the change in DPPH free radical scavenging of FVE versus variables is shown in Figure 7. The results showed that as the voltage gradient increased, the ability to inhibit DPPH free radicals statistically increased and decreased ($p < 0.05$). However, with increasing extraction time, the ability to inhibit DPPH free radicals has increased slightly ($p < 0.05$) (Figure 7). Probably, the increase in the ability to inhibit free radicals of DPPH may be attributed to the release of more

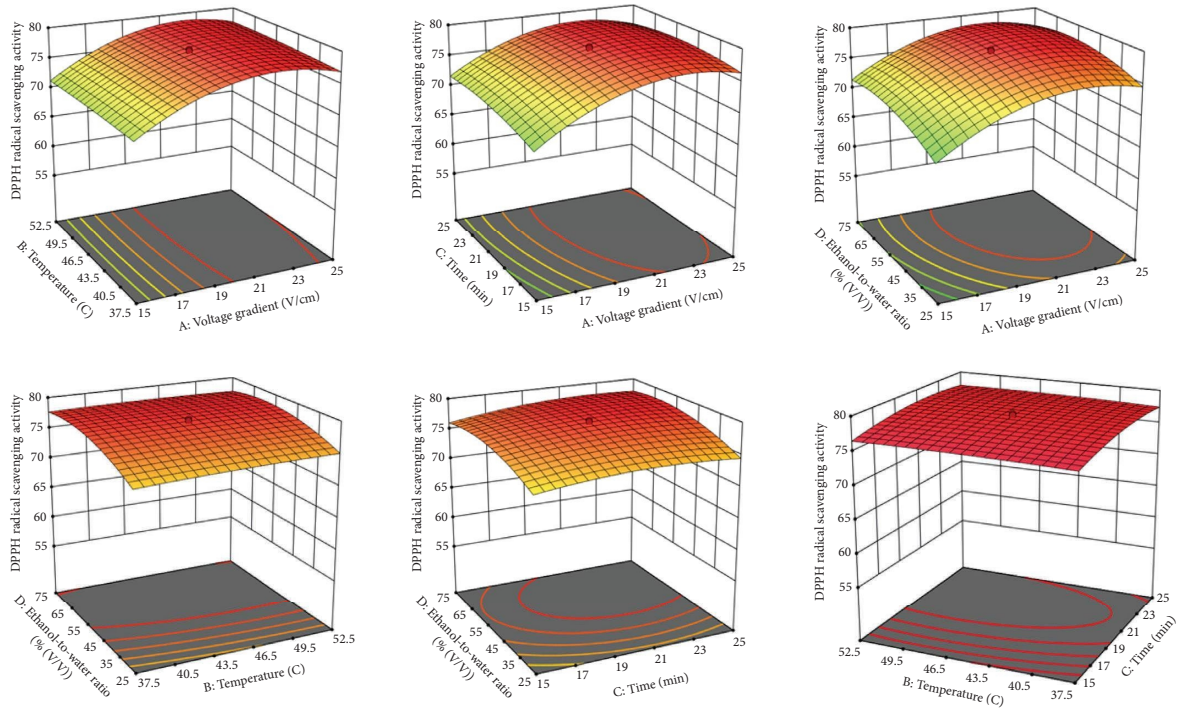


Figure 7: The 3D surface diagram of the change in DPPH free radical scavenging of *Falcaria vulgaris* extract (FVE) as a function of voltage gradient, extraction time, extraction temperature, and ethanol-to-water ratio.

antioxidant compounds such as tocopherols and phenolic compounds in the extract. As stated in the TPC results, the nonthermal effect of the voltage gradient caused the destruction of the gland wall and hence the release of phenolic compounds, leading to the initial increase of the inhibition DPPH free radicals of FVE [3].

The results also indicated that with the increase in the ethanol-to-water ratio, the DPPH free radical scavenging of FVE was significantly increased, and then it remains approximately constant ($p < 0.05$). DPPH free radical scavenging of FVE results indicated that the experimental DPPH free radical scavenging of FVE-derived quadratic model (equation (8)) was capable of predicting changes in the DPPH free radical scavenging of FVE in the treatment of OAE, which shows the high accuracy of predicted values.

$$\text{DPPH} = +78.05 + 3.03\text{A} + 0.6684\text{C} + 1.44\text{D} + 4.65\text{A}^2 - 0.8859\text{C}^2 - 1.66\text{D}^2 - 4.34\text{AC}, \quad (8)$$

where A, B, C, and D are the main effects of the voltage gradient, extraction temperature, extraction time, and ethanol-to-water ratio, respectively; A^2, B^2, C^2, and D^2 are the quadratic effects of voltage gradient, extraction temperature, extraction time, and ethanol-to-water ratio, respectively; and AC is the interaction effect of voltage gradient with extraction time. According to the equation, it can be said that the voltage gradient had the greatest effect on the DPPH free radical scavenging. This result is confirmed by the perturbation plot (Figure 8). The significant ($p < 0.05$) quadratic effects of the independent variable are confirmed by the concavity of the voltage gradient, extraction time, and ethanol-to-water ratio curves in the perturbation plot.

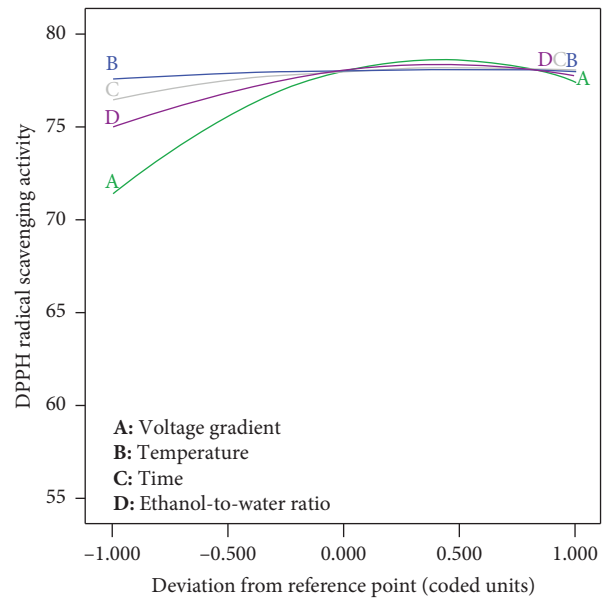


Figure 8: Perturbation plot of the DPPH free radical scavenging of *Falcaria vulgaris* extract (FVE) in ohmic-assisted extraction.

3.5. OAE Optimization. OAE numeric optimization was conducted by defining the voltage gradient (10–30 V/cm), extraction temperature (30°C–60°C), extraction time (10–30 min), and ethanol-to-water ratio (0%–100%) as an independent parameter. The optimization results indicated

Table 3: Comparison of experimental and predicted values for the optimum condition of ohmic-assisted extraction.

	Experimental value	Predict value
Extraction yield (%)	10.033 ± 0.165	10.142
Energy consumption (kWh)	0.1861 ± 0.0032	0.1882
Total phenolic content (mg GAE/g extract)	63.95 ± 0.68	64.66
DPPH (%, 50 μg/mL)	77.09 ± 1.21	78.05

Table 4: Comparison of the ohmic-assisted and hydrodistillation extraction.

	Ohmic-assisted extraction	Hydrodistillation extraction
Extraction yield (%)	10.033 ± 0.165^{a}	7.212 ± 0.134^{b}
Energy consumption (kWh)	0.1861 ± 0.0032^{a}	0.1805 ± 0.0026^{a}
Energy consumption (kWh/g extract)	0.0185 ± 0.0008^{a}	0.0264 ± 0.0016^{b}
CO_2 production	0.067 ± 0.004^{a}	0.071 ± 0.004^{a}
Total phenolic content (mg GAE/g extract)	63.95 ± 0.68^{a}	43.11 ± 0.62^{b}
DPPH (%, 50 μg/mL)	77.09 ± 1.21^{a}	68.98 ± 0.61^{b}
IC_{50} (μg/mL)	98.13 ± 3.58^{a}	115.41 ± 0.75^{b}

Note: The values with different superscript letters in a row are significantly different ($p < 0.05$).

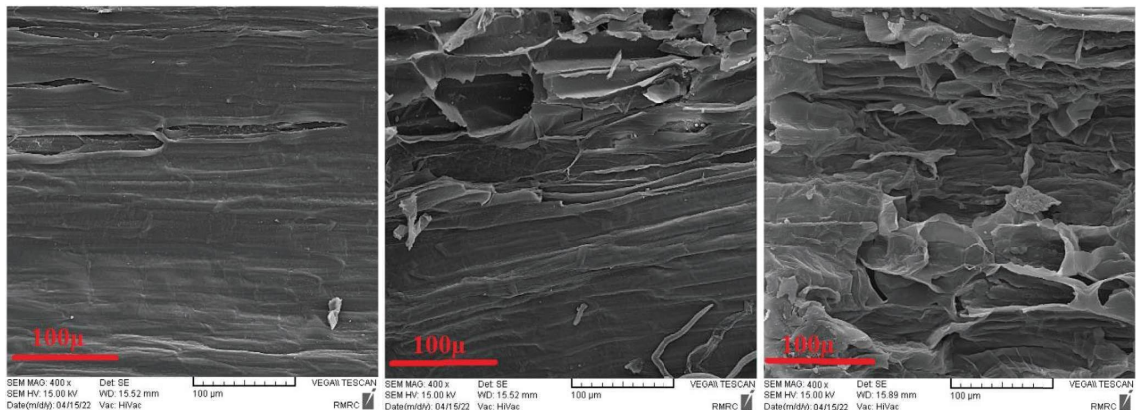


Figure 9: Scanning electron microscope (SEM) images of (a) untreated, (b) ohmic-assisted, and (c) HD-treated *Falcaria vulgaris* aerial part.

that the extraction process with an ethanol-to-water ratio of 65.5%, voltage gradient of 20.3 V/cm, temperature of 56.6°C, and extraction time of 22.1 min results in maximum extract TPC, DPPH, and EY, and minimum extraction EC. Table 3 shows the result of the comparison of experimental and predicted values for the optimal state of OAE. As seen, there are no significant differences between the experimental and predicted EC, EY, TPC, and DPPH free radical responses, which indicates that the model was able to fit the experimental values with high accuracy.

3.6. Comparison of the OAE and HD Methods. The results of the comparison of the OAE and HD methods are shown in Table 4. As can be seen, there were no significant differences between the amount of CO_2 produced by OAE and HD methods. The EY in the OAE method is about 39.11% greater than that in the HD method. This significant ($p < 0.05$) change in the OAE method is mainly due to the thermal and nonthermal effects of the OAE method. As mentioned previously, the thermal effect of the OAE process causes the accelerated increase of the gland internal vapor pressure, which causes intensification of gland wall damages. On the other hand, the passage of electric current causes the acceleration of the speed of rupture of the gland wall. Finally, the thermal and nonthermal effects intensify the electroporation of the gland wall, which increases the EY of FVE. This result is confirmed by the SEM image (Figure 9). As illustrated in Figure 9, in the untreated sample, the FV microstructure is intact. The destruction of the FV microstructure is a function of the extraction method. Although the microstructure of the HD-treated plant sample has been ruptured, its structure is not completely destroyed. However, the microstructure of the OAE-treated plant sample is completely disintegrated. This explains the higher EY of FVE in the OAE method.

Results indicated that although the difference in the EC in the OAE and HD methods was not significant ($p > 0.05$), the amount of the EC per gram of extract for the OAE method was significantly less compared to the HD method, which is related to the higher EY of FVE in the OAE method. Based on the statistical analysis, the use of the OAE

Table 5: Comparison of MIC and MBC (μg/mL) of ohmic-assisted and hydrodistillation extraction *Falcaria vulgaris* extract.

	Hydrodistillation extraction		Ohmic-assisted extraction	
	MIC	MBC	MIC	MBC
Escherichia coli	25	12.5	25	12.5
Staphylococcus aureus	50	25	50	25
Candida albicans	12.5	6.25	12.5	6.25
Aspergillus niger	25	12.5	12.5	6.25

technique improved the antioxidant properties of the FVE compared to the HD method ($p < 0.05$), which is probably related to the release of more antioxidant compounds such as tocopherols and phenolic compounds into the FVE. This result is confirmed by the TPC result. In other words, an increase in the extraction time led to TPC decreases, which could be attributed to the increases of heavy compound extraction. Khajehei et al., by evaluating the Yacon plant leaf extract obtained by OAE and conventional methods, reported that the DPPH free radical inhibition ability of the extract obtained by the OAE method was higher than that by the conventional method due to the more release of phenolic compounds [23].

The MIC, known as the MIC test, is the lowest concentration of an antibacterial that inhibits the visible growth of a microorganism after one day of incubation. After isolation of pure culture, MICs can be detected on solid culture media or agar or diluted liquid culture media [25]. The findings of the inhibitory and bactericidal activities of FVE extracted by the OAE and HD methods on *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Candida albicans* (yeast), and *Aspergillus niger* (mold) are shown in Table 5. The antimicrobial analysis of extract indicated that *Staphylococcus aureus* and *Candida albicans*, respectively, had the lowest and highest sensitivity to the FVE obtained by both OAE and HD. This result indicates that FVE has a greater inhibition and bactericidal effects on the yeast and mold compared to Gram-positive and Gram-negative bacteria. Previous studies have shown that there are bioactive compounds such as α-Terpiny acetate, α-Caryophyllene, and α-Pinene in the FV essential oil and extract that have antimicrobial activity [1, 2].

As it is shown, the extraction method of FVE had no significant difference ($p > 0.05$) in the MIC and MBC levels of all studied microorganisms except *Aspergillus niger*. The FVE obtained from the OAE method had significantly lower MIC and MBC against *Aspergillus niger* compared to the FVE from the HD method. Probably, the increase in the antifungal effect of the OAE extraction method is attributed to the release of more antimicrobial compounds such as α-Terpiny acetate, α-Caryophyllene, α-Pinene, tocopherols, and phenolic compounds in the extract.

4. Conclusion

In this study, the effects of independent extraction parameters on the quality and quantity of FVE were evaluated. The statistical results indicated that the quadratic model was able to predict experimental results of all responses with R^2 above 0.98. The results showed that by increasing the voltage gradient as well as the extraction time, the EY and EC significantly increased ($p < 0.05$). However, with the increase of the voltage gradient, the TPC value and DPPH free radical scavenging initially increase and then decrease. Ethanol-to-water ratio had a significant effect ($p < 0.05$) on all the responses except EC. However, extraction temperature has a significant effect only on the EY. The results of the comparison of the OAE and HD indicated that there were no significant differences between the EC and amount of CO_2 produced; however, the amount of the EC per gram of extract for the OAE method was significantly less compared to the HD method, which is related to the more EY of FVE in the OAE method. The microstructure of the OAE-treated plant sample is completely disintegrated. This explains the more EY of FVE in the OAE method. Result also indicated that the use of the OAE technique improved the antioxidant properties of the FVE compared to the HD method ($p < 0.05$). However, the extraction method of FVE had no significant difference ($p > 0.05$) in the MIC and MBC levels of all studied microorganisms except *Aspergillus niger*. Finally, it can be stated that the use of OAE is a suitable method for extraction of plant extract.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Zeinab Hassanloofard: conceptualization (lead), writing–original draft (lead), formal analysis (lead). **Mohsen Zandi:** software (lead), writing–original draft (supporting), writing–review and editing (equal), methodology (lead), conceptualization (lead). **Mehdi Gharekhani:** software (lead), writing–original draft (supporting), writing–review and editing (equal). **Ali Ganjloo:** review and editing (equal),

conceptualization (supporting). **Leila Roufegarinejad:** review and editing (equal), conceptualization (supporting).

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