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Original Article

Efficacy of Ultrasonic Waves in Extraction Ascorbic acid and Bioactive Compounds from Ajowan seeds *(Carum copticum* L.) and Comparison with Conventional Soxhlet Extraction

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ABSTRACT

The main objective of this study was to compare the performance of ultrasound assisted extraction (UAE) with the soxhlet extraction method. In this research, the extraction of ascorbic acid from ajowan seeds (*Carum copticum* L.) was studied and compared with the soxhlet method, based on the Response Surface Methodology (RSM). The experiments consisted of four factors with three levels. A Central Composite Design (CCD) experiment was employed at powers of 100, 200, and 300 W; temperatures of 40, 50, and 60 °C; total time of 10, 20, and 30 min and pulsed time of 0, 2, and 4 s, including 30 experimental runs. The soxhlet method was considered as a control with a run time of 240 min and a boiling temperature of 85 °C. Ascorbic acid, Total Soluble Solids (TSS), and DPPH radical scavenging assay of ajowan seeds extracts were determined, and the results were analyzed by RSM. The amount of ascorbic acid, in the ultrasound method was 0.30 mg/ml more than the soxhlet method and the free radical scavenging in the ultrasound method increased by 19.86 % compared to the soxhlet method. The results of the present study demonstrate that the ultrasound assisted extraction method is an alternative affordable for yield extraction compared to the soxhlet method.Optimum extraction conditions (power 172.59 W, temperature 59.94 °C, sonication duration 21.77 min, and pulsed time 2.10 s) were obtained for extraction TSS (23.76 °Bx), DPPH (63.18 %) and ascorbic acid (0.73 mg/ml).

INTRODUCTION

With the introduction of chemical and biological drugs, the role and importance of herbal medicines in human health have been forgotten. However, over time, there has been considerable growth in the use of herbal medicines [1-4]. It's important to acknowledge that herbal medicines comprise 10% of the world's total medicinal products, a percentage that is on the rise. People's tendency to use herbal medicine and herbal products has increased in recent years, and perhaps one of the most important causes of people turning to herbal medicine is the numerous side effects of chemical drugs [5-7]. The herbal medicines native to a country represent a valuable natural resource that serves as a significant asset for the inhabitants of the society whether for personal use or commercial purposes [8]. As herbs are natural products, they are generally free from side effects, comparatively safe, eco-friendly, and locally available [9]. Traditionally, there were a lot of herbs used for ailments related to different seasons and there is a need to promote these herbs to save human lives [10]. These herbal products are today the symbol of safety in contrast to synthetic drugs, which are regarded as unsafe to human beings and the environment [11-13]. According to the World Health Organization, today more than 80% of people worldwide still use herbal remedies to treat illnesses. Almost a quarter of the world's medicines are of plant origin [14].

Ajowan seeds (*Carum copticuml*.) is an annual herbaceous essential oil-bearing plant belonging to the Apiaceae family, which grows in India, Iran, and Egypt [15-17]. Ajowan fruit oil has diuretic, carminative, analgesic, anti-dyspnoea, and anti-inflammatory compounds, in addition, it is one of the

significant medicinal plants containing blood cholesterol-lowering properties [18]. The substance also has an impact on the function of digestive enzymes in the pancreas and small intestine, as well as its effectiveness in treating fungal infections, acting as a blood flow cleanser, providing sedative effects, and alleviating kidney pain [18, 19]. Ajowan products are marketed as powder, ointment, lotion, and solution and are used as antispasmodics, lung disease treatment, rheumatoid arthritis, anti-cold, anti-cough anti-fever, and anti-constipation [20]. The medicinal part of this plant is the fruit. The aqueous extract of ajowan is commonly utilized as a traditional remedy to alleviate flu symptoms in children. Also, the methanol extract of this plant has been shown to have antibacterial activity against multi-drug-resistant *Salmonella typhi* [20-22]. The ripening seeds of this plant contain 2 - 4% essential oil that is rich in monoterpenes like thymol and is mainly used as an antiseptic agent as well as a drug component in medicine. In one study, the components of *C. copticum* essential oil were analyzed and examined for their inhibitory activities against some pathogenic and food-borne bacteria [23].

Traditional methods of obtaining natural plant compounds, such as water or steam distillation and organic solvent extraction, have many disadvantages including dissolved volatiles, low yields, long extraction time, degradation of unsaturated compounds, and persistence of toxic solvents [24]. The growing demand for greener alternatives and natural substances that do not contain toxic compounds, have fewer side effects, and do not endanger the environment, has attracted the attention of industries through non-toxic and reliable extraction methods [20]. New methods of herbal extraction include ultrasonic extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) [25]. Due to the time-consuming nature of conventional methods as well as the difficulty of extracting some compounds and the need for high use of solvents, there is a demand for new extraction methods [26]. The benefits of new extraction methods can be listed as shorter time, less organic solvent consumption, and less pollution [27].

Today, the use of ultrasound is increasing due to its effectiveness in food storage and processing. The mechanical effects of the ultrasound and the cavitation phenomenon caused by these waves increase the permeability of the solvent into the plant cells [28] and also increase the mass transfer, consequently increasing the extraction efficiency at lower temperatures [29]. Ultrasound extraction is one of the most important methods of extracting valuable compounds from plant sources. In this method, waves with frequencies above 20 kHz penetrate the material, causing continuous stretching and shrinkage, which results in cavities within the plant material [30]. These cavities are asymmetrically interconnected and cause the material to exit rapidly from the cells. Besides, these waves can damage the cell wall and facilitate the exit of materials [25]. The three most important benefits of the ultrasound method are, low-temperature extraction that prevents damage to heat-sensitive compounds such as phenolic compounds, high yields, and low extraction time [31].

Ascorbic acid, also known as Vitamin C, is a water-soluble natural antioxidant with potential benefits for age-related diseases such as atherosclerosis, cancer, neurodegenerative, and ocular diseases. It is believed that ascorbic acid may counteract reactive oxygen and nitrogen species, thereby mitigating oxidative damage to vital biological macromolecules such as DNA, lipids, and proteins [32].

So far, there is no report on UAE from Ajowan seeds (*C. copticum* L.). The objective of this research is to compare ultrasound and conventional (soxhlet) methods by evaluating the efficiency of ascorbic acid and antioxidant properties of the extractions, which are extracted by these methods, and optimization of the ultrasonic extraction method using Response Surface Methodology (RSM).

MATERIALS AND METHODS

Sample Preparation

In the first stage, seeds were prepared to carry out the research. Samples were kept in a dry and cool environment after preparation by Pakan Seed Company of Isfahan, Iran until they reached 14% moisture content. Before extraction, each sample was milled for 30 s by a grinder (Moulinex MC 300132, Germany). Each seed sample was milled before testing to extract easily the aromatic compounds. Extraction from the seed of ajowan (*C. copticum* L.) was performed using ultrasound. This research was carried out at the College Aburaihan of the University of Tehran in 2019. All the needed chemicals,

including metaphosphoric acid, Sodium bicarbonate, 2, 6-dichloroenophenol, Hydrogen Peroxide, 1,1 diphenyl-2-picrylhydrazine (DPPH), and Methanol were purchased from Merck, Germany.

Methods of Extraction

Prevalent soxhlet and ultrasound approaches were used to extract from the seeds powder of ajowan. The solvent used in both methods was 70% ethanol which was prepared by mixing 97% alcohol and distilled water at a ratio of 73:27 ml (volumetric-volumetric). Alcoholic solvents are generally used to extract ascorbic acid from natural sources. Alcohol-water mixtures increase the extraction rate of compounds by several times compared to the pure state of the solvents. The solvent used in this study was 70% ethanol, and for all treatments, the ratio of 1:10 (w/v) was considered for powder to solvent, 70% ethanol.

Ultrasound-Assisted Extraction (UAE)

Ultrasonic extraction was first performed using a cylindrical glass container with two plastic lids (to prevent solvent evaporation). One of the doors has a circular hole to accommodate the thermometer to increase the solvent temperature to the desired temperature and the second door contains two circular holes to accommodate the thermometer and probe when bathing. In the ultrasonic test, a mercury thermometer was used to control the temperature of the extract inside the glass container. In the first step, ultrasonic tests were performed for each sample of 10 g of material with a precision of 0.001 and then by blade grinding for 30 seconds. In the second step, 100 ml of 70% ethanol was poured into a designed glass container and placed on a heater to reach the desired temperature according to the experimental treatment. Immediately, 10 grams of ajowan powder was added to the solvent, after the solvent reached the desired temperature then, it was tested in a water bath with a pre-set temperature. Then, the ultrasonic cylindrical probe with a 2 cm diameter was inserted into the container designed with a lid containing two circular-shaped holes to a height of 1 cm below the liquid surface. The sample then received ultrasonic waves with a constant frequency of 20 ± 0.5 kHz, according to the treatment. After ultrasonic extraction, the extract was poured into 6 ml of 50 ml of the solution, and then centrifuged at 12,000 rpm for 10 min, and then streamed into the lid. It was transferred into a vacuum rotary evaporator balloon to remove the solvent at vacuum pressure, 40 °C, and 200 rpm for 30 min using a rotary evaporator. To completely remove the residual solvent after rotary treatment, the extract was poured into plates and placed at 40 °C for 60 min, then the extract and solvent were completely dried. Then plated the lids, wrapped them in paraffin, covered them with aluminum foil to prevent light penetration, and stored them at -18 °C until the analysis.

Conventional Soxhlet Extraction

In this method, 30 g dried seeds with an accuracy of 0.001 were weighed by a weighing balance after grinding into a filter paper. The filter paper was placed in the soxhlet extraction portion, and then 300 ml 70% ethanol solvent was added to the soxhlet balloon. Then, the soxhlet apparatus was mounted on the heater until reaching 240 °C. After extraction, the extract was transferred into a vacuum rotary evaporator balloon, and similar steps were performed by sonication.

Statistical Analysis

This essay was implemented by the Response Surface Method (RSM) with Design Expert 11 software. The experiment consisted of four factors with three levels. Central Composite Design (CCD) experiments were employed at 100, 200, and 300 W power; temperatures of 40, 50, and 60 °C; total time of 10, 20, and 30 min, and pulsed time of 0, 2, and 4 s (Table 1), including 30 experimental runs. The soxhlet method was considered as control, with 240 min extraction time and a boiling temperature of 85 °C. The results were analyzed by RSM, which consists of the generation and performance of experimental design, followed by fitting a regression model to the responses that were generated. This model was statistically validated by ANOVA and F-test and used to build the surface graphs, which can be contour or tri-dimensional plots. Further, the selection of optimum conditions and their validations were performed, based on the CCD data. For each response variable, second-order polynomial (Eq. 1) was determined:

$$Yn = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{i^2} + \sum_{i=1}^{k} \sum_{j=i+1}^{k-1} \beta_{ij} X_i X_j \qquad (1)$$

Where, Yn is the response variable, β_0 is intercept with Yn, β_i is the linear regression coefficient for *i*th factor, β_{ii} for quadric, and β_{ij} for the cross-product term. X_i and X_j are independent variables. k is the number of tested variables. The three-dimensional response surface plots were generated for each response by keeping one response variable at its optimal value and plotting the other two independent factors. The experimental data were fitted to the second-order polynomial model to obtain the regression coefficients (β), which determine the extent of the effect of an individual factor over response [33, 34].

Total Soluble Solids (TSS)

A refractometer was used to measure TSS content in the extract. Brix is a unit that expresses the number of solid particles in a solution and is primarily dependent on the concentration and viscosity. For each sample, the refractometer prism was washed and dried with distilled water, and a few drops of extract were poured onto the apparatus and the refractive index was read in degrees Brix (°Bx). When light enters a liquid it redirects, this process is called light failure. The refractometer measures the amount of light redirection, which is called the angle of refraction of the light. The refractometer measures the angle of the refraction of the light and relates it to the refractive index values. These values can be used to determine the concentration of solutions. Sizes can be read as soon as the sample is placed on the chart. With a low-concentration solution, the refractive index of the prism is much larger than the sample solution, resulting in a large failure angle and a small read number. The opposite is the case with a high-concentration liquid. Based on the refractometer's work, the light is conditioned and directed to the desired solution and passed through because the two environments differ. The light is then broken, and it can be obtained with a limited refractive index. The rate of light failure such as a fingerprint is unique for solutions of the same concentration and at equal temperatures. Inside the refractometer is an optical line created by different prisms and lenses. This light line is visible to the user with the help of a camera lens. TSS was measured using a refractometer (ATAGO R500, Japan). The refractive index was recorded and converted to °Bx by using a conversion table. Measurements were performed at 25±0.5 °C.

DPPH Radical Scavenging Assay

In this test, the ability of the hydrogen atom or electron was measured by different compounds and extracts with the amount of achromatized DPPH solution in methanol. The antioxidant virtues of the extract were measured. According to this method, 0.1 ml extract sample was mixed with 0.9 ml of distilled water when 0.1 ml of the final solution was mixed with 3.9 ml of a methanolic solution of DPPH (25 mg L^{-1}). The mixture was strongly shaken and left to stand at room temperature for 40 min in the darkness. For creating the control sample, 0.1 ml of methanol was mixed with 3.9 ml of methanolic solution of DPPH. The attraction was measured using the UV-Vis spectrophotometer at 515 nm. The radical-scavenging activity was presented as a percentage of inhibition based on (Eq. 2) [35, 36].

$$\% \text{ Inhibition of DPPH radical Inhibition } (\%) = 100 \times [(Abs_{control} - Abs_{sample})/(Abs_{control})] \quad (2)$$

Ascorbic Acid

Ascorbic acid was determined based on the method of Klein and Perry (1982) with some modifications. Extracts samples (1.5 ml) were extracted with metaphosphoric acid (1%, 10 ml) for 45 minutes at room temperature. 1 ml of the resulting solution was mixed with 2.6-dichloroindophenol 9 ml, and the absorbance was measured within 30 min at 515 nm against a blank.

Table 1 Independent variables and their coded and actual values used for optimization

Independent variable	Units	Code levels		
		-1	0	+1
Power	W	100	200	300
Temperature	°C	40	50	60
Total Time	min	10	20	30
Pulsed Time	s	0	2	4

The content of ascorbic acid was calculated based on the calibration curve of authentic L-ascorbic acid (0.025-0.1 mg.ml-1; $y = 4.4283x - 0.0066$; $R2 = 0.9995$), and the results were expressed as l mg of ascorbic acid per 1 ml of the extracts.

RESULTS AND DISCUSSION

In this research, the relation between the response operations and the process variables was identified by a four-factor contained central composite design. The extraction conditions of TSS, DPPH, and ascorbic acid were optimized using design expert software. The results of the three responses TSS, DPPH, and ascorbic acid, based on the experiment design to extract by ultrasound along with the control test are presented in Table 2.

Total Soluble Solids (TSS)

The ANOVA (Table 3) Model F-value of 5.44 implies the model is significant. In this case, the CD is a significant model term. The 'Lack of Fit F-value' of 3.25 implies there is a 9.68 % chance such a 'Lack of Fit F-value' could occur due to the noise. According to (Table 3) only the relationship between the two factors of C and D are influential. Fig. 1 shows the interaction of the two factors pulsed time and total time with the amount of TSS to the °Bx. The amount of TSS varies from 23.00 to 24.50. In the highest pulsed time and the lowest total time, the TSS content is maximum also in the low pulsed time and high total time.

Table 2 Central composite design with Experimental planning and results obtained in the seed extracts

Run	Power (A) (W)	Temperature (B) (°C)	Total Time (C) (min)	Pulsed Time (D) (s)	Response TSS (Y_1) (°Bx)	Response DPPH (Y_2) (%)	Response Ascorbic acid (Y_3) (mg/ml)
1	200	50	20	0	24.20	46.55	0.47
2	200	50	20	2	23.70	30.13	0.40
3	100	40	30	4	23.31	25.86	0.05
4	200	50	20	2	23.72	46.89	0.41
5	200	50	20	4	23.00	32.75	0.39
6	200	50	20	2	23.73	46.72	0.48
7	300	60	10	0	24.31	63.08	0.66
8	200	50	10	2	23.80	51.72	0.34
9	200	50	20	2	23.75	46.73	0.41
10	100	40	30	0	24.00	10.34	0.10
11	300	40	30	4	23.60	29.58	0.19
12	200	50	20	2	24.22	46.89	0.41
13	300	60	30	0	23.41	50.00	0.70
14	200	50	20	2	23.63	46.86	0.40
15	100	60	10	0	23.30	34.48	0.58
16	200	60	20	2	23.61	58.62	0.71
17	100	50	20	2	24.42	12.06	0.26
18	300	40	10	0	23.40	60.34	0.15
19	100	60	10	4	24.11	56.89	0.55
20	300	60	30	4	23.52	36.20	0.68
21	200	50	30	2	24.30	15.51	0.49
22	300	50	20	2	23.51	37.93	0.31
23	100	60	30	4	23.00	55.17	0.61
24	300	40	30	0	23.90	7.00	0.21
25	300	60	10	4	23.71	17.24	0.65
26	100	40	10	4	24.35	27.58	0.01
27	200	40	20	2	23.90	56.89	0.23
28	100	40	10	0	23.84	24.13	0.03
29	100	60	30	0	23.70	51.72	0.63
30	300	40	10	4	24.50	5.00	0.00
control (Soxhlet)	0	85	240	0	32.86	43.10	0.11

Table 3 ANOVA for Response Surface Reduced 2FI model

	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	0.72	1	0.72	5.44	0.0271	significant
CD	0.72	1	0.72	5.44	0.0271	significant
Residual	3.72	28	0.13			
Lack of Fit	3.49	23	0.15	3.25	0.0968	not significant
Pure Error	0.23	5	0.04			
Cor Total	4.44	29				
Std.Dev =0.36			Mean=23.77			C.V.%=1.53

Table 4 ANOVA for Response Surface Reduced Quadratic model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	4921.36	5	984.27	6.44	0.0006	significant
B-Temperature	1733.92	1	1733.92	11.34	0.0026	significant
AD	1176.96	1	1176.96	7.70	0.0105	significant
CD	664.08	1	664.08	4.34	0.0480	significant
B^2	1336.60	1	1336.60	8.74	0.0069	significant
Residual	3668.91	24	152.87			
Lack of Fit	3436.63	19	180.88	3.89	0.0690	not significant
Pure Error	232.27	5	46.45			
Cor Total	8590.27	29				
Std. Dev.= 12.36			Mean = 37.70		C.V. % = 32.80	

Final Equation in Terms of Actual Factors

The (Eq. 3) in terms of actual factors can be used to make predictions about the response for given levels of each factor.

$$Y_{TSS} = 23.91 - 3.68\times10^{-3}CD \quad (3)$$

Radical Scavenging Activity (DPPH)

In the analysis of variance (Table 4), the Model F-value of 6.44 implies that the model is significant. In this case, B, AD, CD, and B2 are significant model terms. The 'Lack of Fit F-value' of 3.89 implies that there is a 6.90 % probability that such 'Lack of Fit F-value' occurs due to the noise.

Fig (2a) shows as DPPH increases with increasing power and time pulsed, this trend continues up to 200 W. However, from this point (200 W) towards higher power and higher time pulsed, DPPH decreases. At the constant pulsed time, DPPH increases with increasing power but decreases at power 200 W and higher [37]. The highest DPPH at the lowest total time and the lowest pulsed time is shown in Fig (2b). The same amount of DPPH was obtained at the tallest total time and highest pulsed time. A study on the extraction of phenolic and flavonoid compounds from blackberry juice was used for sonication probes and found that in both methods the percentage of free radical scavenging increased with time and the highest percentage of free radical scavenging [38].

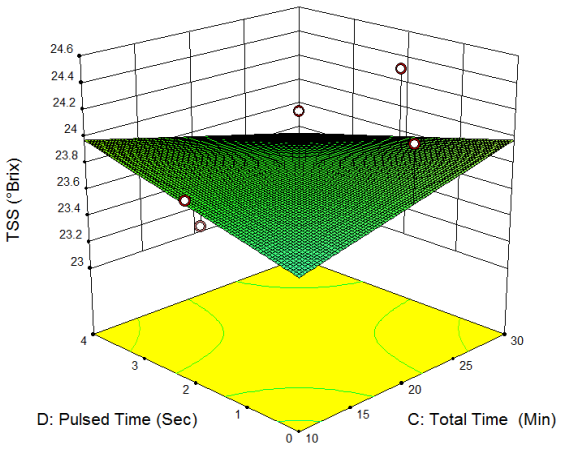

Fig. 1 3D Surface plot for the effect of extraction total time and pulsed time on TSS.

Fig (2c) shows the effect of temperature on the DPPH content at low temperatures up to 50 °C which has a decreasing trend but with increasing temperature from 50 °C the trend increased, and the DPPH value

increased with increasing temperature. A nearly similar trend was observed between phenolic compounds and free radical scavenging of DPPH.

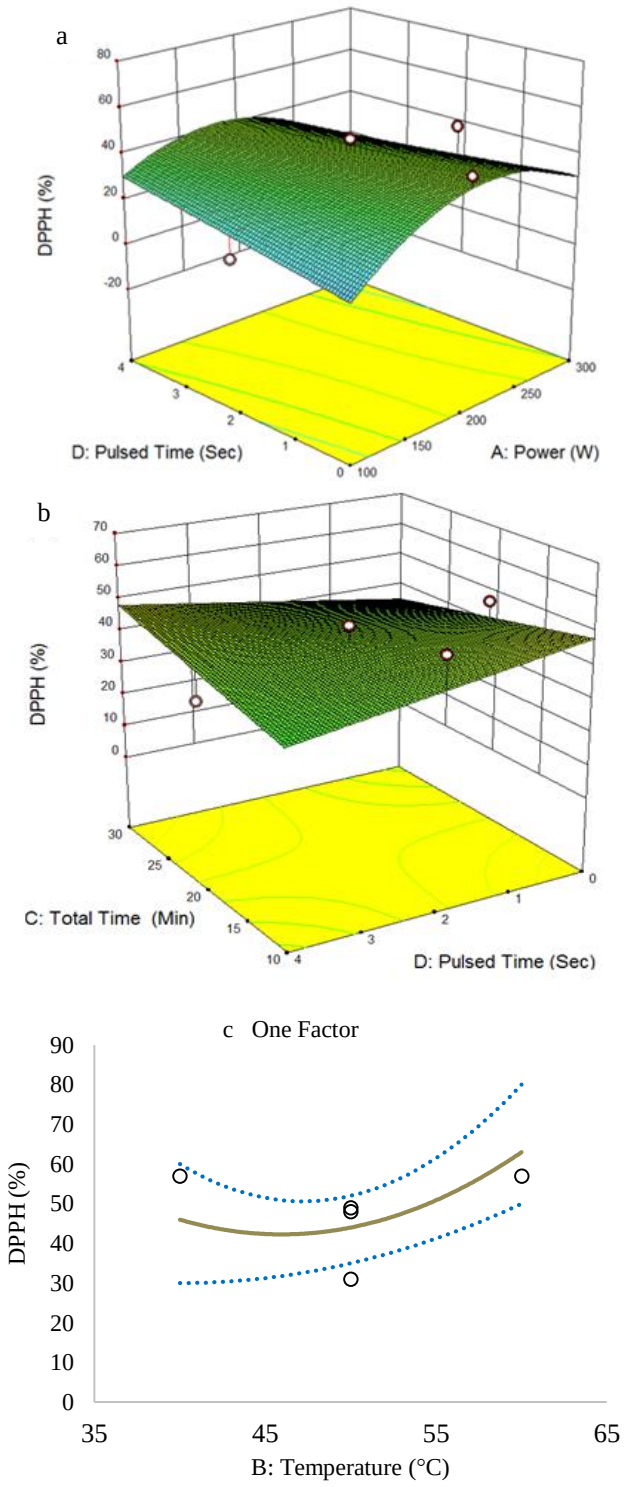

Fig. 2 (a) 3D Surface plot for the effect of extraction power and pulsed time on the DPPH; (b) 3D Surface plot for the effect of extraction total time and pulsed time on the DPPH; (c) Model graph one factor for the effect of extraction temperature on the DPPH.

Results of a study about the effect of ultrasound on the DPPH found that there was a positive correlation between polyphenol content and antioxidant activity in Ajowan essential oil and extract, also the effect of time on the free radical scavenging ability of DPPH in sonication to be very significant and reported that with increasing the extraction time, a certain degree of inhibitory power was increased [25].

Final Equation in Terms of Actual Factors

The (Eq 4), in terms of actual factors, can be used to make predictions about the response for given levels of each factor.

$$Y_{DPPH} = +41.68 + 9.81B - 8.58AD + 6.44CD + 13.06B^2 \quad (4)$$

Ascorbic Acid

In the analysis of variance, the Model with an F-value of 226.71 implies that the model is significant (Table 5). In this case, A, B, C, D, A^2, and B^2 are significant model terms. The Lack of Fit with an F-value of 1.13 implies that the Lack of Fit is not significant relative to the pure error. There is a 49.10 % chance that a Lack of Fit F-value this large could occur due to the noise. Fig (3a) shows the relation of a power factor to the amount of ascorbic acid. Ascorbic acid increased with increasing power, however, it was reduced at the power of 200 W and higher. Ascorbic acid degradation in sonication can be attributed to the oxidation and interaction reactions with free radicals formed during sonication. Mainly due to chemical reactions and physical conditions that occur during ultrasonication, the formation of hydrogen ions (H+), free radicals (O^-, OH^-, $HO2^-$, and hydrogen peroxide from the water molecules in the sample). It has been identified that during ultrasonication [39], the hydroxyl radicals produced by cavitation can be effective in the degradation of ascorbic acid. Fig (3b) shows the independent relation of temperature with ascorbic acid. Ascorbic acid is lower at low temperatures, and ascorbic acid content increases with increasing temperature. In a study on the amount of ascorbic acid in orange juice [40], the juice was processed by ultrasound at different levels of power (24.4 to 61 µm) and temperature (5 to 30 °C). The results of that study showed that in ultrasonic processing, the lowest amount of ascorbic acid was obtained at the highest amplitude (61 µm) and temperature (30 °C) and ascorbic acid increased with increasing temperature and sonication range. They argued that as temperature rises, the number of bubbles decreases due to cavitation increases, and the activity of the cavities, and the bubbles themselves act as a barrier and reduce the energy of the ultrasound [41]. In sonication, the ascorbic acid

content in the extract was increased by increasing the temperature from 40 to 60 °C due to improved mass transfer, increased solubility, and possibly removal of antioxidant compounds from the plant (which increased at 60 °C). Ascorbic acid degradation during the sonication process was dominant at this temperature and increased the amount of vitamin A in the extract at a temperature of 40 °C. Fig, (3c) illustrates the effect of a total time factor on the amount of ascorbic acid, in low total time the amount of ascorbic acid is low with the increase of the total time diagram, and the amount of ascorbic acid is increased slightly, but this upward trend is not noticeable. In another study on the effect of ultrasound on ascorbic acid in kiwifruit juice, researchers found that ascorbic acid increased at a constant power with increasing time and decreased at a constant time [42].

Fig (3d) shows the effect of pulse timing independent factor on the amount of ascorbic acid obtained. As can be seen at 0 pulse time, the amount of ascorbic acid is maximal, but as the pulse time increases, the slope curve decreases, and the resulting ascorbic acid decreases. The result is greater than when the waveform is discrete.

Final Equation in Terms of Actual Factors

The (Eq 5), in terms of actual factors, can be used to make predictions about the response for given levels of each factor.

$$Y_{\text{Ascorbic acid}} = 0.07 + 5.23\times10^{-3}A - 0.03B + 3.8\times10^{-3}C - 0.01D - 1.2\times10^{-5}A^2 + 6.41\times10^{-4}B^2 \quad (5)$$

Comparison of Ultrasound with Soxhlet Method

To compare the ultrasound method with the soxhlet method, two ultrasound treatments were selected and analyzed using a completely randomized design with three replications. Dennett's test was used for comparison of the sample (control) to others with SAS software. The results of the analysis of the experiments are shown in Table 6.

Comparison of TSS of Ultrasound Method with Soxhlet Method

The diagram of the amount of TSS in the various methods of ultrasound (24.26 °Bx) and soxhlet (32.73) extraction is displayed in Fig (4).

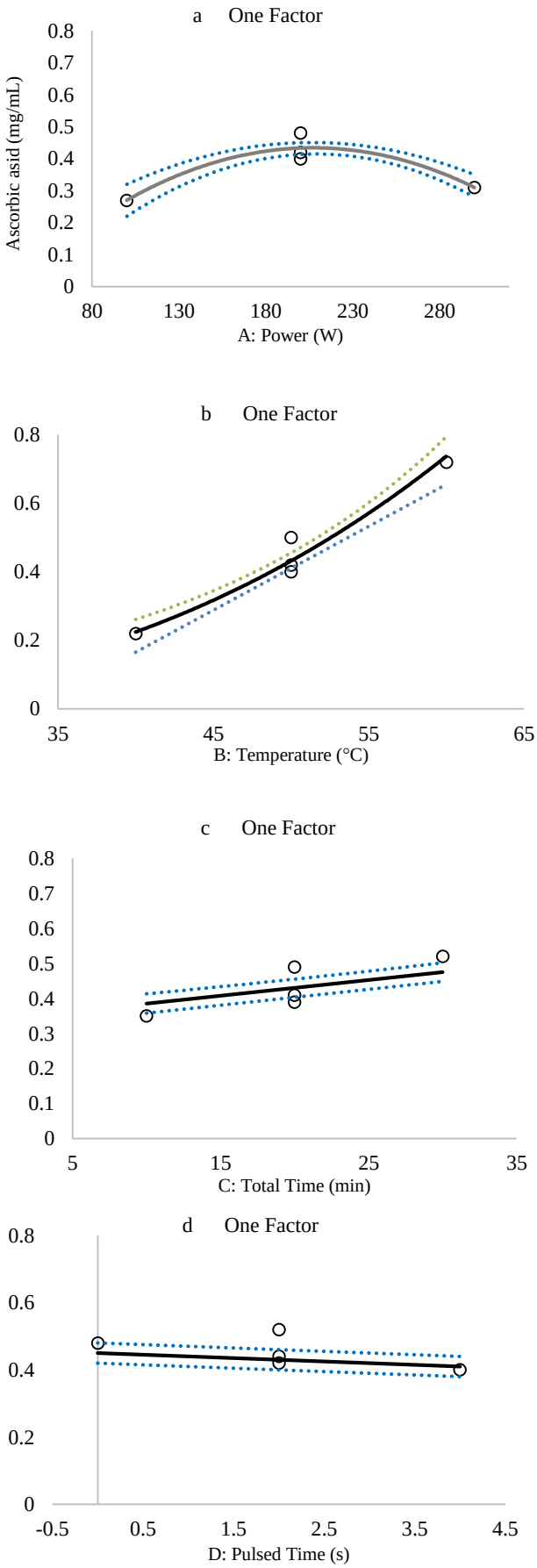

Fig. 3 (a) Model graph one factor for the effect of extraction power on the ascorbic acid; (b) Model graph one factor for the effect of extraction temperature on the ascorbic acid; (c) Model graph one factor for the effect of

extraction total time on the ascorbic acid; (d) Model graph one factor for the effect of extraction pulsed time on the ascorbic acid.

According to the diagram, the TSS contents were almost equal for the ultrasound treatments, but the TSS content in the soxhlet method was higher than the ultrasound in the extraction. The reason for the increased performance of the soxhlet method over ultrasonic was the high temperature and longer time of the soxhlet methods and that all seed tissues are generally disintegrated and degraded, resulting in increased TSS content.

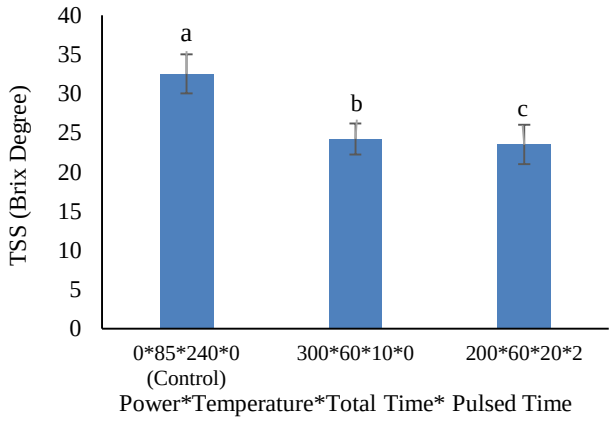

Fig. 4 TSS chart for ultrasound method and soxhlet method.

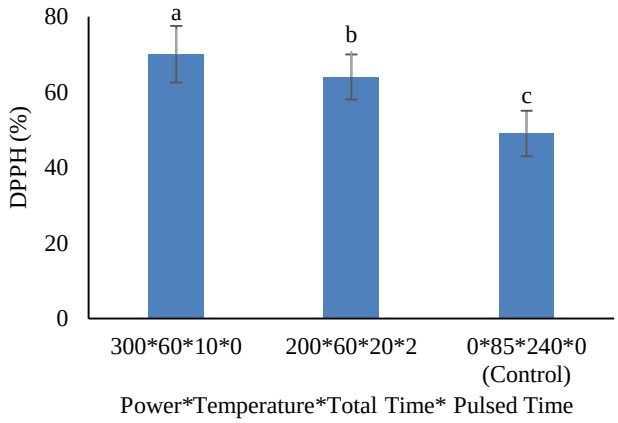

Fig. 5 DPPH chart for ultrasound method and soxhlet method.

Comparison of DPPH of Ultrasound Method with Soxhlet Method

Fig (5) demonstrates the comparison of DPPH free radical scavenging percentages for different methods of ultrasound and soxhlet extraction. According to the diagram, it is found that the treatment with the power of 300 W, temperature of 60 °C, total time of 10 min, and 0 s off time (continuous waving), obtained a DPPH value more than the other ultrasound and soxhlet treatments. The Soxhlet method (42.67 %) obtained less DPPH than ultrasound (62.53 %) content, which is reported by Chemat et al., (2017) due to high temperature and longer Soxhlet method which resulted in the decomposition of antioxidant compounds and consequently reduced DPPH free radical scavenging. Ultrasonic treatment with 300 W power, 60 °C temperature, 10 min total time, and 0s pulsed time (continuous sonication) was the highest free radical treatment, which was selected and reported.

Comparison of Ascorbic Acid of the Ultrasound with Soxhlet Method

The diagram of ascorbic acid content in the various methods of ultrasonic (0.714 mg/ml) extraction and soxhlet (0.415 mg/ml) is shown in Fig (6). According to the diagram, it can be said that the treatment with the power of 200 W, 60°C temperature, 20 min total time and 2s pulsed time, resulted in discrete ripple and more ascorbic acid than ultrasound and soxhlet methods. This is also due to the low-temperature sonication that prevents damage to ascorbic acid-sensitive compounds, but the high temperature of the soxhlet method destroys ascorbic acid-sensitive compounds. Numerous studies have also shown that non-thermal process technologies such as high pressure, electric field pulses, and sonication maintain a higher level of ascorbic acid than thermal processes [43]. There was a significant difference between the two ultrasound treatments, but the treatment with the 200W power, 60 °C temperature, 20 min total time, and 2s pulsed time was reported as a superior treatment in ascorbic acid performance.

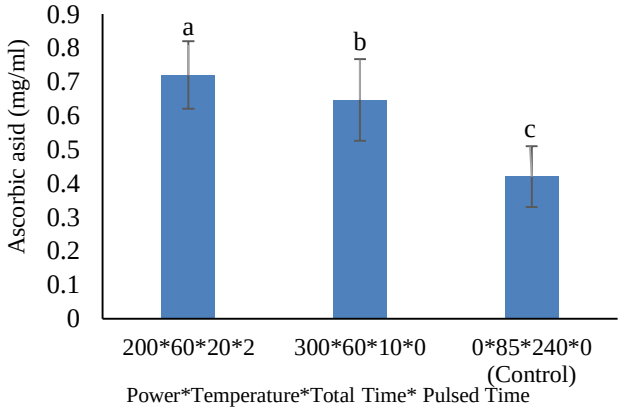

Fig. 6 Ascorbic acid chart for ultrasound method and soxhlet method.

Optimization

The numerical optimization technique was used to optimize the ultrasound process when the weight and importance value for both responses were considered equal. The degree of importance was chosen based on the DPPH (5), being the most important factor, and significance was assigned to the other factors (3) that were less important. The power, temperature, total time, and pulsed time were in range, which was found as the optimal conditions of the ultrasound process. The TSS is in range, the DPPH is maximized and the Ascorbic acid is maximized (Table 7). Table 8 shows the 10 top solutions that were selected for conditional optimization. These were acquired as the predicted results whose desirability values were equal to 1.00. The adequacy of the models for predicting the optimum response values was tested by extraction using the optimized conditions determined using RSM (temperature 59.97 °C, power 172.59 W, total time 21.77 min, and pulsed time 2.10 s). Predicted and mean experimental values for the extraction TSS (23.76 °Bx), DPPH (63.18 %), and ascorbic acid (0.73 mg/ml) indicated that the experimental values were very close to the predicted values and were not statistically different. These results indicate that the experimental values are in good agreement with the predicted ones, and also suggest that the models of TSS, DPPH, and ascorbic acid are satisfactory and accurate.

Table 5 ANOVA for Response Surface Reduced Quadratic model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	1.40	6	0.23	226.71	< 0.0001	significant
A-Power	0.03	1	0.03	28.78	< 0.0001	significant
B-Temperature	1.28	1	1.28	1244.50	< 0.0001	significant
C-Total Time	0.02	1	0.02	25.72	< 0.0001	Significant
D-Pulsed Time	8.88×10^{-3}	1	8.88×10^{-3}	8.64	0.0074	Significant
A^2	0.05	1	0.05	48.87	< 0.0001	Significant
B^2	0.01	1	0.01	13.80	0.0011	Significant
Residual	0.02	23	1.02×10^{-3}			
Lack of Fit	0.01	18	1.05×10^{-3}	1.13	0.4910	not significant
Pure Error	4.68×10^{-3}	5	9.36×10^{-4}			
Cor Total	1.42	29				
Std.Dev= 0.03			Mean=0.38			C.V.%=0.97

Table 6 Results of experiments in SAS software

Source	DF	Mean Square		
		TSS (°Bx)	DPPH%	Ascorbic acid (mg/ml)
Treat	2	77.44 **	325.02 **	0.07 **
Error	6	0.02	0.65	0.00
Total	8	-	-	-
C.V.%	-	0.64	1.19	1.16

Table 7 constraints imposed for Optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Power	is in range	100	300	1	1	3
B: Temperature	is in range	40	60	1	1	3
C: Total Time	is in range	10	30	1	1	3
D: Pulsed Time	is in range	0	4	1	1	3
TSS	is in range	23.00	24.50	1	1	3
DPPH	maximize	5.00	63.08	1	1	5
Ascorbic acid	maximize	0.00	0.71	1	1	3

Table 8 10 Top Solutions for Optimization

Number	Power	Temperature	Total Time	Pulsed Time	TSS	DPPH	Ascorbic acid	Desirability
1	172.59	59.97	21.77	2.10	23.76	63.18	0.73	1.00
2	195.25	59.95	21.48	1.15	23.78	63.78	0.76	1.00
3	189.08	59.77	12.25	1.10	23.69	65.32	0.71	1.00
4	199.54	59.99	20.35	0.67	23.77	64.35	0.76	1.00
5	209.67	59.89	23.10	1.26	23.79	63.55	0.76	1.00
6	211.33	59.80	11.28	0.07	23.59	69.95	0.73	1.00
7	212.98	58.94	17.64	0.07	23.72	63.11	0.72	1.00
8	200.00	60.00	20.00	2.00	23.77	64.55	0.74	1.00
9	216.18	59.99	29.05	3.97	23.58	68.42	0.76	1.00
10	170.28	59.75	28.47	3.63	23.62	68.47	0.73	1.00

CONCLUSIONS

In this study, TSS, DPPH, and ascorbic acid extract of ajowan seeds extracts obtained by ultrasound were compared to the soxhlet method. Ascorbic acid using the ultrasound method was 0.30 mg/ml more than the soxhlet method and the free radical scavenging was 19.86% higher compared to the soxhlet method. Ultrasonic treatment with the 300 W power, 60 °C temperature, 10 min total time, and 0 s pulsed time (continuous sonication) was identified as the superior treatment reducing the temperature and the extract time compared to the soxhlet method. The optimization of the ultrasound test showed 172.59 W power, 59.97 °C temperature, 21.77 min total time, 2.10 s pulsed time, and desirability of 1.00. Results showed that the ultrasound method provides the opportunity for enhanced extraction and improved retention of ascorbic acid in ajowan seeds at lower processing duration and temperature compared to thermal processing.

Abbreviations

ultrasound-assisted extraction (UAE)
Response Surface Methodology (RSM)
Central Composite Design (CCD)
Total Soluble Solids (TSS)
microwave-assisted extraction (MAE)
supercritical fluid extraction (SFE)
1,1 diphenyl-2-picrylhydrazine (DPPH)
degrees Brix (°Bx)

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Authors' contributions

A. Kakelahi and M. Aboonajmi designed and performed the experiments, and wrote the manuscript. S. A. Sadat-Noori analyzed the data. All authors read, edited, and approved the final manuscript.

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Availability of Data and Materials

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

Ethical Approval and Consent to Participate

No human subjects or vertebrate animals were used in this study.

Consent for Publication

All of the authors have been informed regarding submitting the manuscript to the journal of Journal of Medicinal Plants and By-Product.

Competing Interests

All authors declare no conflict of interest.

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