The Journal of Medicinal Plants and By-products

Original Article

Screening and Optimization for *Smyrnium Cordifolium Boiss*: Extraction, Isolation, and Identification of Bioactive Components

Pardis Allahyari and Marjan Nouri*

Department of Food Science and Technology, Ro.C., Islamic Azad University, Roudehen, Iran

*Corresponding Author: Email: Marjan.nouri@iau.ac.ir

Article History: Received 12 January 2026/Accepted in revised form 30 April 2026
© 2012 Iranian Society of Medicinal Plants. All rights reserved

ABSTRACT

Smyrnium cordifolium boiss is considered a medicinal plant of Apiaceae family, which has a bright future in the identification of bioactive components and the fields of medicine. The present research aimed to optimize the extraction of *Smyrnium cordifolium boiss* leaves using ultrasound-assisted extraction and determine phytochemical constituents, total phenolic content and antioxidant attributes. Initially, the effective factors on extraction were screened in eight examined assays through the Taguchi design approach. The best conditions were as follows in the present research: 80% ethanol concentration, 20 °C temperature, 200 W ultrasound power, 30 min period, young leaves and ratio of extract to solvent 1:60. The most suitable responses were hexanoic acid (8.96 mg/g), 2-methylhexanoic acid (8.22 mg/g), 8-quinolinol-2-butyl (9.99 mg/g), octadecanoic acid (10.22 mg/g) and phytol (34.3 mg/g) using the high-performance liquid chromatography. Furthermore, TPC (48.25 mg gallic acid/g) and antioxidant traits (73.26%) were calculated for the optimized sample. The ultrasound power, solvent concentration, and leaf age were screened as the most effective factors for optimization by the central composite design (CCD). The levels of 8.46, 6.78, 10.15 and 11.24 mg/g for hexanoic acid, 2-methylhexanoic acid, 8-quinolinol-2-butyl and octadecanoic acid were predicted based on CCD in twenty samples and conditions of 43.7% ethanol, 97.99 W ultrasound power, and 56 days old leaves, respectively. The TPC (43.97 mg gallic acid/g) content and antioxidant activity (63.68%) were reported for the optimized treatment. According to the obtained results, *Smyrnium cordifolium boiss* extract has suitable phytochemical components that possess the necessary potential to play a functional role.

Keywords: Antioxidant, *Smyrnium cordifolium boiss,* Optimization, Taguchi, Uultrasound

INTRODUCTION

Smyrnium genus has only endemic and biennial species with scientific name *Smyrnium cordifolium boiss*, which is known as avandol, widely distributed in Zagros mountain and creates flavor in food products, and is caused by furanoscoterpenoids, including isofuranodine [1]. The researchers reported that essential oil obtained from leaves and roots of mentioned plant had antibacterial effects and more sesquiterpene hydrocarbons such as corzinone and also germacrone [2,3]. Phytochemical investigation illustrated that flavonoids, phenolic acids, and sesquiterpene lactones were the main components of different *Smyrnium* species [4]. *Smyrnium cordifolium* Boiss. is increasingly recognized as a promising medicinal species due to its rich phytochemical profile and its traditional use in regional herbal preparations. The recent interest in this plant stems from its potential to serve as a natural source of bioactive compounds with relevance to nutraceutical and pharmaceutical applications [1].

Ultrasound has fundamental advantages over analytical techniques, which are employs to control food processing operations [5]. The useful applications of ultrasound waves are checking texture, viscosity, and concentration for some solid and also liquid foods, fruits, vegetables, meat, dairy products, thickness, surface and temperature [6]. Ultrasound-assisted extraction was selected because acoustic cavitation enhances cell wall disruption and solvent penetration, leading to improved recovery of bioactive compounds. Compared with conventional methods, UAE reduces extraction time, solvent consumption, and thermal degradation, making it particularly suitable for heat-sensitive phenolics and antioxidants [3].

The Taguchi design was first applied as a screening method to identify the most influential extraction parameters and determine their optimal preliminary levels with a limited number of experiments. This approach helps reduce experimental runs while evaluating [7]. Taguchi design was used to screen the important influential factors affecting the conversion of isoeugenol to vanillin by *Psychrobacter spp*., and RSM was applied to optimize the selected factors [7]. In past studies, the optimization of essential oil extraction for *Smyrnium cordifolium boiss* had been done through separation using the hydro-distillation method and a Clevenger-type apparatus from ten ecotypes [3], supercritical carbon

Accepted Article Publish

[8] and isolation of aqueous and also methanol extracts according to antioxidant activity, total phenolic (TPC) and flavonoid (TFC) contents [9]. However, the effect of various extraction factors of this plant on phytochemical components from qualitative, quantitative, and statistical dimensions has not been performed so far.

As a result, this plant was extracted with ultrasound-assisted extraction; in the next step, the factors affecting extraction were monitored through Taguchi designs in eight assays and three variables selected and optimized using central composite design (CCD) that had the greatest impact on phytochemical substances, TPC, and antioxidant activity as responses.

MATERIALS AND METHODS

Plant Material

The leaf sample of *Smyrnium cordifolium boiss* collected in spring 2025 over time. The obtained sample was botanically identified in the herbarium section, and the plant was thoroughly washed with distilled water, dried in the shade at room temperature, and then powdered using a laboratory mill.

Extraction by the Ultrasound Technique

A specific ratio of powdered *Smyrnium cordifolium boiss* leaf (young or mature) to solvent based on samples with solvent (10 and 80%), temperature (20 and 70 °C), ultrasound (0 and 200 W), time (5 and 30 min), leaf age (young and mature) and extract/solvent ratio (E: S)% (1:10 and 1:60) and obtained from statistical method (Table 1) was poured into a beaker and an ultrasonic device (Bandelin, Germany) containing water and ice (to control temperature), which ultrasonic extraction process was carried out using a frequency of 24 kHz [1].

Analysis and Evaluation

High-performance Liquid Chromatography (HPLC) Assessment of Phytochemical Content

The HPLC was applied to determine the area of the *Smyrnium cordifolium boiss* extract compared to the standard. The chromatographic system, including a UV detector at 230 nm wavelength (C18 column 4, 60 A, Nova-pack, micrometer, 4.6 mm x 150 mm) was employed, and all outputs were recorded and analyzed using software. The mobile phase consisted of acetone and ethanol solvents, and the volume for all injections was equal to 1 µL. After each process, the column was calibrated and equilibrated for 15 min, and also solutions with 5 standards were created at 1 µmol concentration in a final volume of 10 mL ethanol. In order to draw a calibration curve, solutions with combined standards in 2.5, 25, 50, 1.12, and 3.6 µmol concentrations were produced by diluting in volumetric flasks of mobile phase, and then standards were injected into the chromatography machine (Fig. 1) [8].

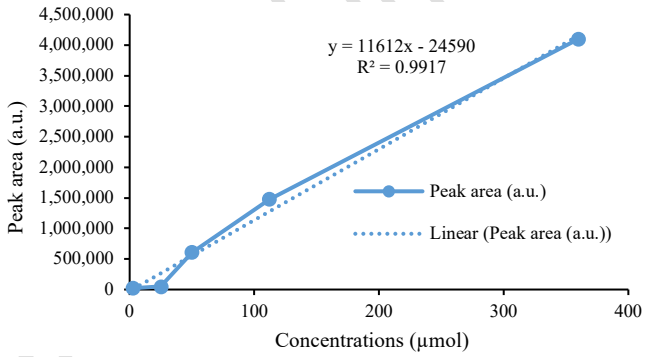


Fig. 1 Calibration curve of the mixed standards used for quantitative HPLC analysis, showing the linear relationship between peak area and concentration (2.5–360 µmol) at 230 nm.

TPC Determination

The TPC was determined using the Folin-Ciocalteu method in the extract of *Smyrnium cordifolium boiss*. Briefly, the *Smyrnium cordifolium boiss* extract was diluted with 100 µL of Folin-Ciocalteu reagent and mixed for about 5 min, followed by adding 1 mL of 5% sodium carbonate in a 5 mL volumetric flask. The absorbance of the reaction mixture was recorded at 750 nm using a spectrophotometer (Zeiss, Jena, Germany) after incubation at room temperature for 60 min, and TPC was expressed as mg gallic acid/g extract (Fig. 2) [9].

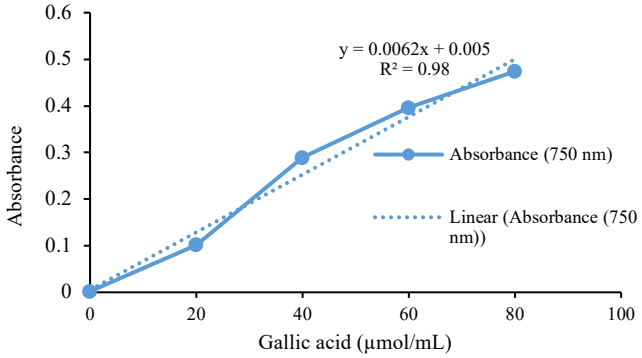


Fig. 2 Calibration curve of gallic acid for determination of total phenolic content using the Folin–Ciocalteu method at 750 nm.

Antioxidant Feature

The inhibition potential of the extract was measured by a modified quantitative method of Brand-Williams. The 2.9 mL antioxidant free radical reduction solution (DPPH, 100 µM) prepared in 70% aqueous ethanol was added to 100-µL extract. The mixture was shaken vigorously and allowed to stand at 25 °C in the dark for 30 min. The absorbance reduction for the resulting solution at 517 nm was measured with 100 µl of 70% aqueous ethanol and 2.9 mL DPPH solution. The inhibition percentages of DPPH radical were calculated for all examined samples [10].

Statistical Analysis

A two-level Taguchi L8 orthogonal array was applied using Minitab Statistical Software (Version 7) to screen significant factors. The eight treatments (ES1 to ES8) for six variables (concentration, temperature, ultrasound power, time, leaf age, and ratio of extract to solvent) were obtained according to Table 1. The most effective factors influencing the responses in Taguchi tests were selected using CCD for optimization. In the design, three factors (ultrasound power, leaf age and solvent concentration) were optimized at levels and central points in twenty treatments (ESC1 to ESC20).

Taguchi and CCD were applied to evaluate the effect of independent variables on physicochemical responses in the extract. All computations and graphics were performed using the statistical software Minitab version 20 and Microsoft Excel 2021. The measurements were repeated at least twice using freshly prepared samples, and value were expressed as means ± SD.

RESULTS

HPLC Analysis of Phytochemical Content

HPLC was performed to identify the main peaks for phytochemicals of *Smyrnium cordifolium boiss*. There were five main peaks of this plant, including phytol (6.87 mg/g), octadecanoic acid (7.56 mg/g), 8-quinolinol-2-butyl (3.15 mg/g), 2-methylhexanoic acid (2.32 mg/g) and hexanoic acid (5.33 mg/g), as outlined in figure 3. This analysis was carried out in one step on *Smyrnium cordifolium boiss* extract obtained under operating conditions of solvent concentration 74%, temperature 20 °C, ultrasound power 0.5 W, time 17 min, young leaves, and ratio of extract to solvent 1:60.

Accepted to Online Publish

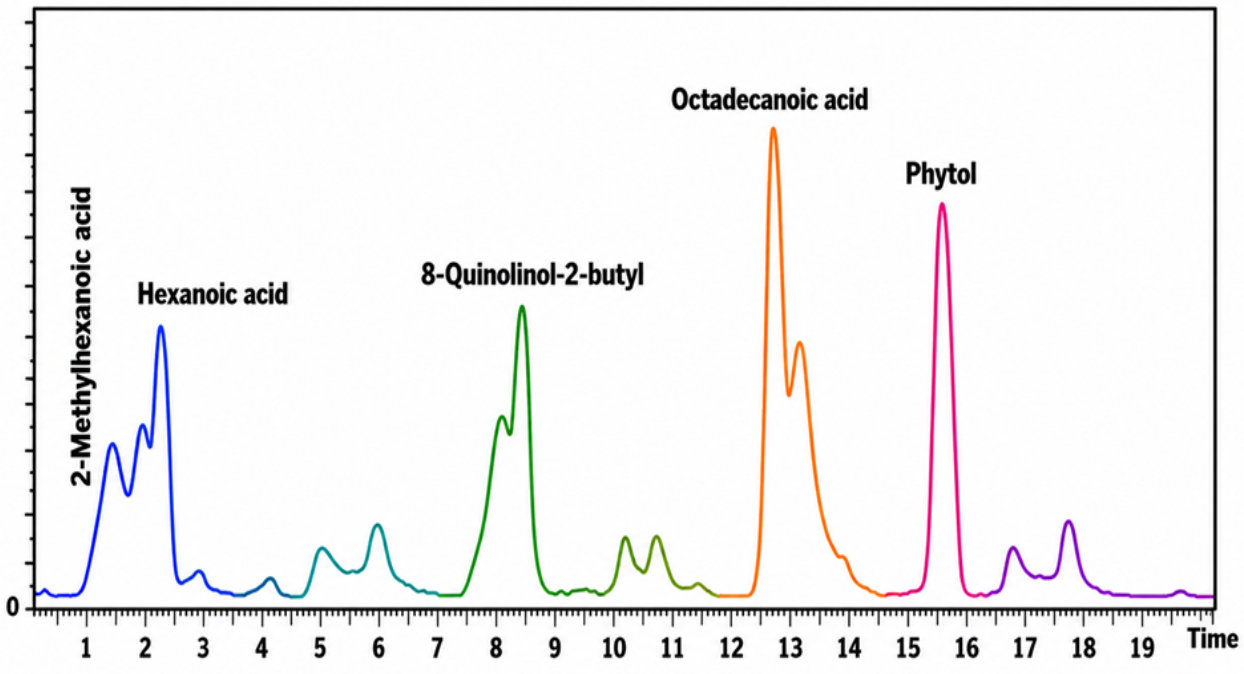


Fig. 3 Chromatogram spectrum related to the main phytochemical compounds of *Smyrnium cordifolium boiss* leaf extract

Taguchi Design Approach

Based on the obtained answers, ES6 (*Smyrnium cordifolium boiss* extract) treatment indicated the best results; therefore, the highest phytochemical substances, polyphenols and antioxidant function were achieved by 80% solvent concentration, 20 °C temperature, 200 W ultrasound power, 30 min, young leaves and a 1:60 ratio of extract to solvent. In ES6, the best sample, the largest volumes of phytol (12.34 mg/g), octadecanoic acid (10.22 mg/g), 8-quinolinol-2-butyl (9.99 mg/g), hexanoic acid (8.96 mg/g) and 2-methylhexanoic acid (8.22 mg/g), were reported (Table 1). The phenolic components and maximum antioxidant capability obtained 48.25 mg gallic acid/g and 73.26% for ES6 treatment, respectively; as demonstrated in Table 1.

Based on graphs obtained from Taguchi's table (Fig. 4), the most favorable conditions to reach the highest phytochemical constituents and important influencing factors were determined. In Figure. 4a outlines hexanoic acid results and the most favorable conditions to reach the maximum amount is 10% solvent concentration, 20 °C temperature, 200 W ultrasound power, 5 min, young leaf, and 1:60 extract to solvent ratio. The most important factors regarding to hexanoic acid production include ultrasound power, solvent concentration, temperature, extract to solvent ratio, leaf age, and time, with the highest constituents observed in ES6 treatment. In the graph related to 2-methylhexanoic acid (Fig. 4b), the optimal levels to reach the maximum amount include 80% solvent concentration, 70 °C temperature, 200 W ultrasound power, 5 min time, young leaves, and 1:60 extract to solvent ratio. The most prominent impacting factors were ultrasound power, solvent concentration, leaf age, extract to solvent ratio, temperature and time, respectively. In the values obtained from these experiments, which reported for the effective substance such as 2-methylhexanoic acid, the highest volume detected in ES6 treatment (22.8 mg/g). In graph related to 8-quinolinol-2-butyl (Fig. 4c), appropriate levels to reach the maximum volume of this ingredient were 10% solvent concentration, 70 °C temperature, 200 W ultrasound power, 30 min, young leaf and extract to solvent ratio of 1:10. The most important suitable factors identified as the highest ultrasound power, leaf age, time, extract to solvent ratio, temperature and solvent concentration, respectively and also the maximum level (9.99 mg/g) was observed in ES6 treatment. The Figure. 3d illustrates octadecanoic acid according to graph, the optimal levels to reach the maximum levels are 10% solvent concentration, 20 °C temperature, 200 W ultrasound power, 5 min time, young leaf age and extract to solvent ratio of 1:10. According to Taguchi analysis, the most important factors in further volume of this substance were ultrasound power, leaf age, solvent concentration, temperature, extract to solvent ratio and time, respectively and the largest volume (10.22 mg/g) was in ES6 treatment. In Figure. 3e related to fifth graph about phytol, the most favorable conditions to reach the maximum phytol are 80% solvent concentration, 70 °C temperature, 200 W ultrasound power, 5 min time, young leaf age and extract to solvent ratio of 1:10. The most prominent agents in phytol production were ultrasound power and followed by leave age, extract to solvent ratio, solvent concentration, time and temperature; therefore, the largest volume (12.34 mg/g) observed in ES6 treatment. Generally, the most important factors are ultrasound power, leaf age and solvent concentration, respectively and also the next three factors, including extract to solvent ratio, time and temperature, are less prominent in all graphs.

TPC was measured in eight treatments, and different values were obtained in each test (Table 2). In figure 4f, about TPC according to the graph, solvent concentration 80%, temperature 70 °C, and ultrasound power 200 W, time 5 min, young leaf and ratio of extract to solvent 1:10 are the best conditions to reach the maximum amount. The most important factors in this combination were ultrasound power, leaf age, extract

Accepted to Online

to solvent ratio, time, temperature and solvent concentration, respectively. The highest TPC measured in this research which was related to ES6 treatment with 48.25 mg gallic acid/g.

The optimal levels of factors, including solvent concentration, temperature, ultrasound power, time, leaf age and extract to solvent ratio, were 80%, 70 °C, 200 W, 30 min, young, and 1:10 to achieve the highest antioxidant activity, respectively (Table 2). The most important factors were ultrasound power, leaf age, extract to solvent ratio, solvent concentration, and temperature and time (Fig. 4g). The highest antioxidant potential was distinguished at 73.26% for ES6 treatment in the present research. According to the obtained results in figure 4g, the antioxidant traits such as TPC improved using ultrasound and leaf age has an effect on this feature, so that young leaves, compared to older leaves, have higher levels. In an antioxidant investigation and rosmarinic acid amount in ethanolic extracts of medicinal plants extracted with ultrasound, it was found that milk thistle and sage exhibited the highest, 92.7 and 94.7%, respectively.

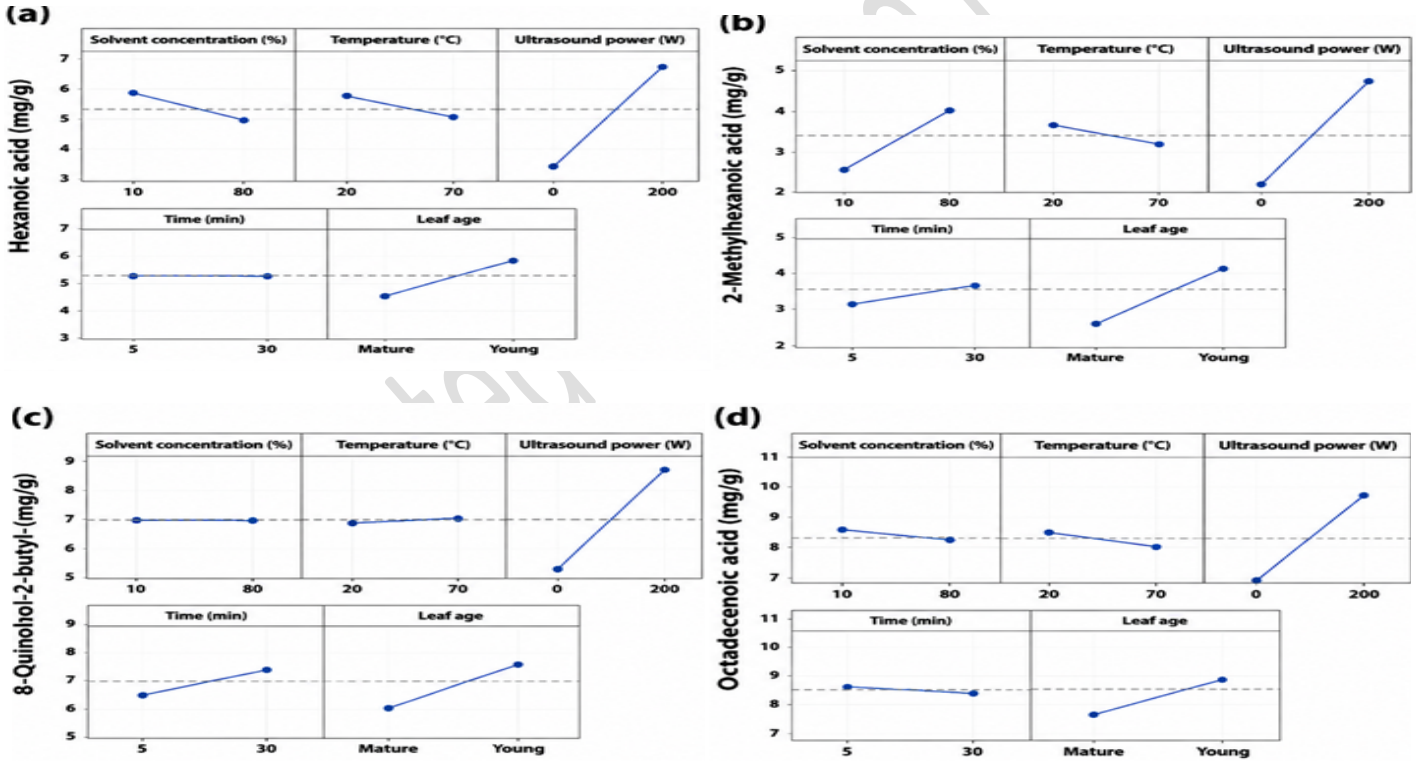
Table 1 Identified compounds, retention times, and relative amounts (%) in Taguchi extracts of *Smyrnium cordifolium*

Sample	Compound name	Retention time (min)	Amount (%)
ES1	2-Methylhexanoic acid	6.2	1.85
	Hexanoic acid	7.8	4.70
	8-Quinolinol-2-butyl	14.5	5.78
	Octadecanoic acid	18.9	8.95
	Phytol	21.3	9.99
ES2	2-Methylhexanoic acid	6.2	1.29
	Hexanoic acid	7.8	3.83
	8-Quinolinol-2-butyl	14.5	4.48
	Octadecanoic acid	18.9	6.31
	Phytol	21.3	4.35
ES3	2-Methylhexanoic acid	6.2	4.47
	Hexanoic acid	7.8	8.40
	8-Quinolinol-2-butyl	14.5	8.92
	Octadecanoic acid	18.9	10.01
	Phytol	21.3	11.47
ES4	2-Methylhexanoic acid	6.2	3.05
	Hexanoic acid	7.8	6.67
	8-Quinolinol-2-butyl	14.5	8.63
	Octadecanoic acid	18.9	9.54
	Phytol	21.3	10.21
ES5	2-Methylhexanoic acid	6.2	3.35
	Hexanoic acid	7.8	5.49
	8-Quinolinol-2-butyl	14.5	7.22
	Octadecanoic acid	18.9	9.31
	Phytol	21.3	10.10
ES6	2-Methylhexanoic acid	6.2	8.22
	Hexanoic acid	7.8	8.96
	8-Quinolinol-2-butyl	14.5	9.99
	Octadecanoic acid	18.9	10.22
	Phytol	21.3	12.34
ES7	2-Methylhexanoic acid	6.2	3.00
	Hexanoic acid	7.8	2.99
	8-Quinolinol-2-butyl	14.5	4.17
	Octadecanoic acid	18.9	5.52
	Phytol	21.3	7.41
ES8	2-Methylhexanoic acid	6.2	2.25
	Hexanoic acid	7.8	2.21
	8-Quinolinol-2-butyl	14.5	6.34
	Octadecanoic acid	18.9	6.52
	Phytol	21.3	9.44

Accepted to Online Publish

Table 2 Taguchi design and the results of phytochemicals, polyphenol and antioxidant activity for the factor screening experiments

Samples	Variables						Responses (mg/g)						
	Solvent (%)	Temperature (°C)	Ultrasound (W)	Time (min)	Leaf age	Extract/Solvent ratio (E:S) (%)	Hexanoic acid	2-Methylhexanoic acid	8-Quinolinol-2-butyl-	Octadecanoic acid	Phytol	Polyphenol (mg GA/g)	Antioxidant activity (%)
ES1	10	20	0	5	Young	1:10	4.70	1.85	5.78	8.95	9.99	38.48	52.41
ES2	10	20	0	30	Mature	1:60	3.83	1.29	4.48	6.31	4.35	22.30	39.80
ES3	10	70	200	5	Young	1:60	8.40	4.47	8.92	10.01	11.47	46.72	70.11
ES4	10	70	200	30	Mature	1:10	6.67	3.05	8.63	9.54	10.21	43.00	70.02
ES5	80	20	200	5	Mature	1:10	5.49	3.35	7.22	9.31	10.10	41.50	68.18
ES6	80	20	200	30	Young	1:60	8.96	8.22	9.99	10.22	12.34	48.25	73.26
ES7	80	70	0	5	Mature	1:60	2.99	3.00	4.17	5.52	7.41	25.31	43.11
ES8	80	70	0	30	Young	1:10	2.21	2.25	6.34	6.52	9.44	36.74	54.98



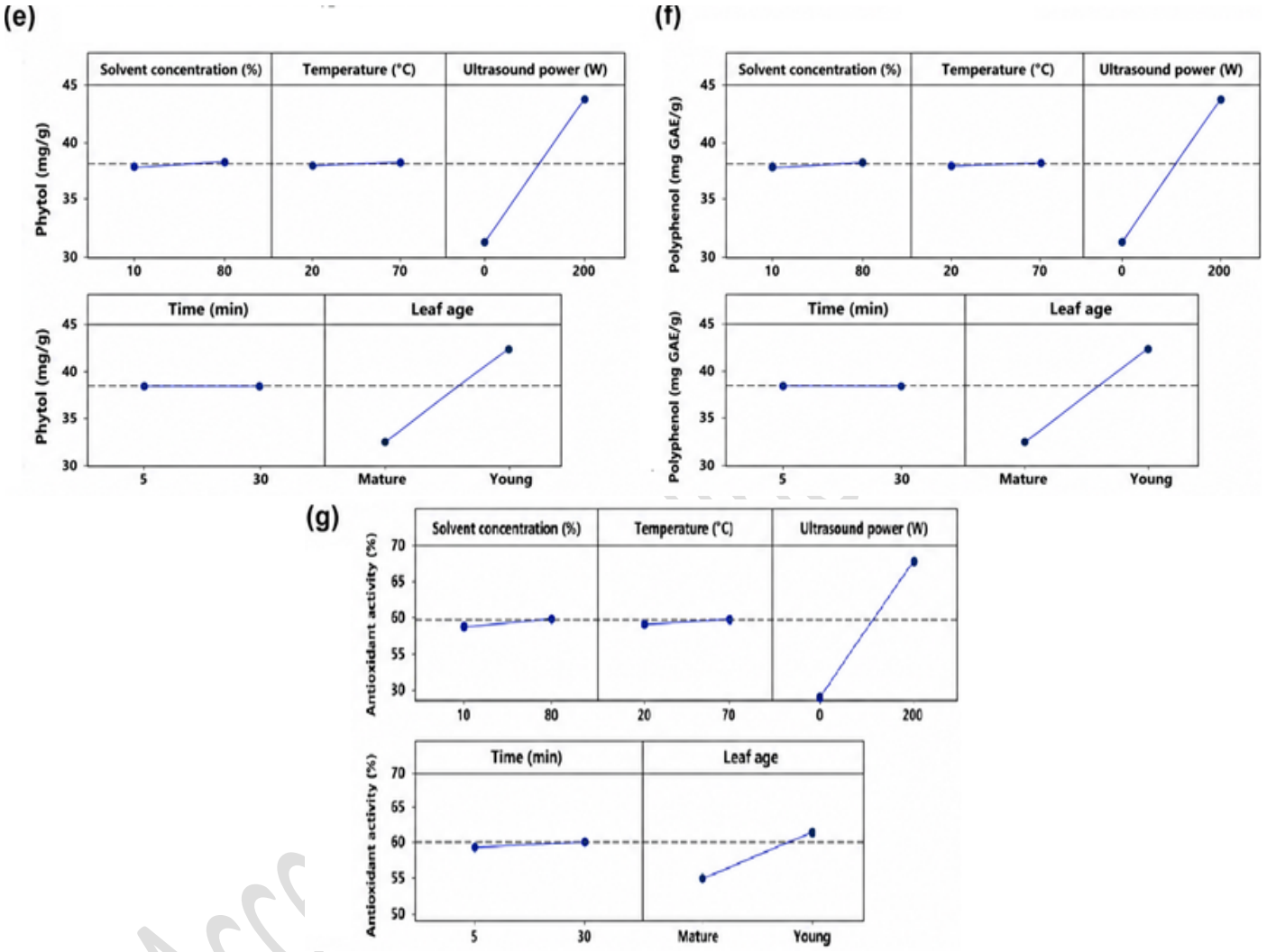


Fig. 4 Graphs of Taguchi test results of *Smyrnium cordifolium boiss* leaf extract in different conditions on effective substances (mg/g) (a-e), polyphenol content (mg GA/g) (f), and antioxidant activity (%) (g)

CCD Response Results

Statistical evaluation and optimization of independent and dependent variables are done using CCD obtained from RSM, and twenty samples with solvent (10, 45, and 80%), ultrasound (0, 110, and 220 W) and age of leaf (20, 100 and 180 days) are presented in Table 3. Ranges of 4.23 to 11.84, 1.97 to 9.56, 5.04 to 14.19, 8.41 to 14.84 and 9.89 to 14.89 mg/g were calculated by HPLC for hexanoic acid, 2-methylhexanoic acid, 8-qinolinol-2-butyl-, octadecanoic acid and phytol, respectively. The range of 35.45 to 61.81 mg gallic acid/g and 52.12 to 24.80% for polyphenols and antioxidant activity were obtained, respectively. The minimum and maximum responses of all variables were calculated for ESC5 and ESC4, respectively, which reported the highest phytochemical, polyphenol and antioxidant activity in phytol. The dependent and independent variables fitted into the second-order polynomial equations and all seven answers after removing non-significant variables were obtained as follows in 1 to 7 formulations:

$Y_{\text{Hexanoic acid (1)}} = 8.65 + 1.36\ A + 1.57\ B - 0.637\ C + 0.832\ AB - 1.30\ B^2$

$Y_{\text{2-Methylhexanoic acid (2)}} = + 6.96 + 1.55\ A + 1.56\ B - 0.739\ C + 0.6312\ AB$

$Y_{\text{8-Quinolinol-2-butyl (3)}} = + 10.33 + 2.05\ A + 1.71\ B - 1.11\ C - 0.936\ AB - 0.468\ AC - 0.413\ BC$

$Y_{\text{Octadecanoic acid (4)}} = + 11.31 + 1.06\ A + 0.844\ B - 0.923\ C - 0.562\ AC - 0.360\ BC$

$Y_{\text{Phytol (5)}} = + 11.36 + 0.936\ A + 1.03\ B - 0.397\ C + 0.350\ AB - 0.210\ AC - 0.175\ BC + 0.335\ A^2 + 0.475\ B^2 - 0.490\ C^2$

$Y_{\text{Polyphenol (6)}} = + 44.79 + 4.44\ A + 4.63\ B - 2.42\ C + 1.55\ AB - 1.67\ AC - 1.79\ BC$

$Y_{\text{Antioxidant activity (7)}} = + 64.21 + 4.79\ A + 5.10\ B - 2.56\ C + 1.79\ AB - 1.45\ AC - 1.62\ BC - 4.08\ C^2$

A, B, and C indicated the solvent concentration, ultrasound power, and leaf age, respectively.

According to the variance table for all answers, the p-value of the model is less than 0.0001 ($p < 0.0001$), which indicates significance. The lack of fit is greater than 0.05 ($p > 0.05$), which illustrates its non-significance and indicates that it adequately fits the experimental data for all answers. According to analysis of variance (Table 4), the effect for the factors A, B and C is significant in linear mode on all answers. In $Y_{\text{Hexanoic acid}}$, the AB interaction and the quadratic term of B had a significant effect on factors; however, AB and C had a considerable impact on the production of $Y_{\text{2-Methylhexanoic acid}}$. The interaction effect for AB, AC and BC and the quadratic mode of A had a significant effect on 8-quinolinol-2-butyl production. In $Y_{\text{Octadecanoic acid}}$, the interaction of AC and BC, AB, AC with BC, phytol and also A, B and C for quadratic mode had a significant effect on response. The AB, AC and BC interaction had a significant effect on phenolic compounds and antioxidant activity of treatments. The R^2 (0.93 to 0.97) and R^2_{adj} (0.86 to 0.96) of models indicated that all models adequately agreed with experimental results.

Figure. 5 depicts the 3D graphs of intensity obtained from CCD, which 5a, b, c, h, k, and n demonstrate the interaction of solvent concentration and ultrasound power (AB) variables on hexanoic acid, 2-methylhexanoic acid, 8-quinolinol-2-butyl, phytol, polyphenols and antioxidant activity, which increase simultaneously, respectively. In figure 5d, f, l, o, higher solvent concentration (A) and I, and also lower leaf age (C) cause an enhancement of products, so that the maximum phytochemical compounds (8-quinolinol-2-butyl, octadecanoic acid, and phytol), polyphenol and antioxidant activity are obtained at the highest solvent concentration and leaf age. According to figure 5e, g, h, j, and p, the highest phytochemical compounds (8-quinolinol-2-butyl, octadecanoic acid and phytol), polyphenols and antioxidant activity are obtained at the maximum ultrasound power (B) and the minimum leaf age (C), respectively.

Accepted to Online Publish

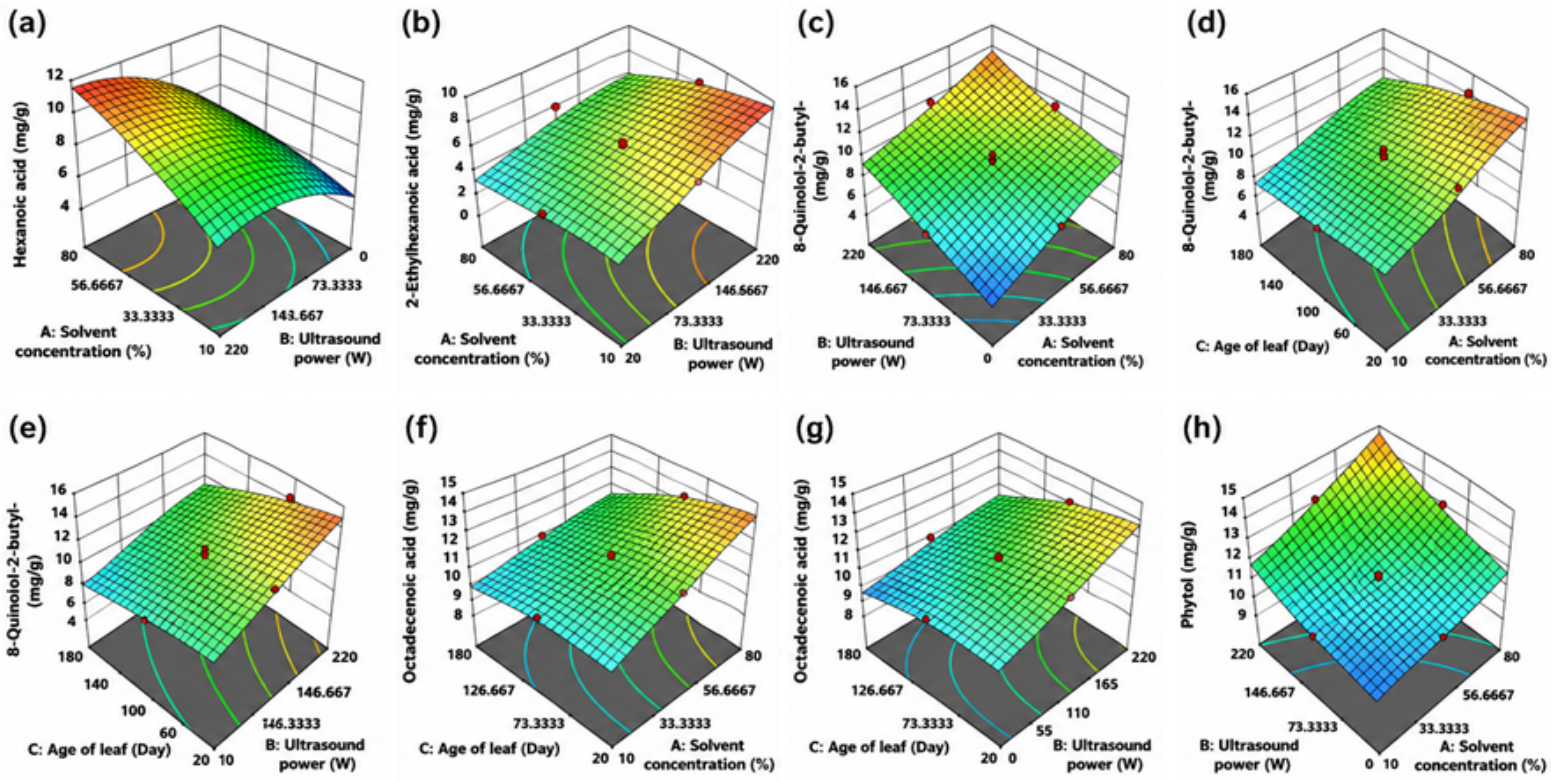
Table 3 The central composite design with experimental values of responses (hexanoic acid, 2-methylhexanoic acid, 8-quinolinol-2-butyl, octadecanoic acid, and phytol) based on independent variables (ethanol concentration, temperature, ultrasound power, period time, young leaves, and ratio of extract to solvent).

Run	Variables			Responses						
	Solvent concentration (%)	Ultrasound power (W)	Age of leaf (Day)	Hexanoic acid (mg/g)	2-Ethylhexanoic acid (mg/g)	8-Quinolinol-2-butyl- (mg/g)	Octadecanoic acid (mg/g)	Phytol (mg/g)	Polyphenol (mg GA/g)	Antioxidant activity (%)
ESC1	10	0	20	4.91	2.85	5.12	9.01	10.15	36.12	53.11
ESC2	80	0	20	6.12	4.98	11.87	11.86	11.45	43.59	60.42
ESC3	10	220	20	5.81	5.11	11.32	10.78	11.53	44.12	61.18
ESC4	80	220	20	11.84	9.56	14.19	14.48	14.89	61.81	80.24
ESC5	10	0	180	4.23	1.97	5.04	8.41	9.89	35.45	52.12
ESC6	80	0	180	5.78	4.11	9.78	10.28	11.01	41.26	58.23
ESC7	10	220	180	5.83	3.56	9.45	10.01	11.23	41.31	58.31
ESC8	80	220	180	9.22	8.43	10.58	10.19	13.09	49.34	66.97
ESC9	10	110	100	7.68	5.98	7.13	10.32	10.93	43.54	61.07
ESC10	80	110	100	9.12	7.87	12.17	12.33	12.65	48.98	67.87
ESC11	45	0	100	5.49	5.29	8.37	9.99	10.88	43.06	62.78
ESC12	45	220	100	9.51	8.15	11.78	12.53	12.98	49.23	70.98
ESC13	45	110	20	9.98	8.09	12.04	12.03	11.55	46.09	64.05
ESC14	45	110	180	7.23	5.13	8.56	10.04	10.38	42.13	57.76
ESC15	45	110	100	8.12	6.98	10.15	11.56	11.47	44.34	65.53
ESC16	45	110	100	8.96	7.14	10.45	11.12	11.29	43.98	63.89
ESC17	45	110	100	8.85	6.54	10.98	10.84	11.34	44.4	63.12
ESC18	45	110	100	7.93	6.12	10.68	11.29	11.35	44.98	63.42
ESC19	45	110	100	8.74	6.98	10.47	11.38	11.09	43.76	64.23
ESC20	45	110	100	8.71	6.42	9.61	11.22	11.23	41.78	62.01

Table 4 The obtained analysis of variance and regression results for treated samples in linear mode on all answers

Source	Hexanoic acid (mg/g)		2-Methylhexanoic acid (mg/g)		8-Quinolinol-2-butyl- (mg/g)		Octadecanoic acid (mg/g)		Phytol (mg/g)		Polyphenol (mg GA/g)		Antioxidant activity (%)	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Model	14.92	<0.0001	16.89	< 0.0001	39.24	< 0.0001	21.07	< 0.0001	54.84	< 0.0001	17.59	< 0.0001	24.30	< 0.0001
A-Solvent concentration	36.49	0.0001	52.84	< 0.0001	154.73	< 0.0001	66.25	< 0.0001	179.65	< 0.0001	57.41	< 0.0001	73.70	< 0.0001
B-Ultrasound power	48.36	< 0.0001	53.73	< 0.0001	107.85	< 0.0001	41.92	< 0.0001	219.24	< 0.0001	62.40	< 0.0001	83.48	< 0.0001
C-Age of leaf	7.98	0.0180	12.04	0.0060	45.48	< 0.0001	50.14	< 0.0001	32.32	0.0002	17.08	0.0020	21.03	0.0010
AB	10.91	0.0080	7.03	0.0243	25.74	0.0005	0.5191	0.4877	20.10	0.0012	5.62	0.0392	8.20	0.0169
AC	1.30	0.2807	0.0510	0.8259	6.45	0.0293	14.90	0.0032	7.23	0.0227	6.45	0.0294	5.39	0.0426
BC	0.6138	0.4515	0.2384	0.6359	5.03	0.0488	6.10	0.0331	5.02	0.0489	7.41	0.0215	6.73	0.0267
A^2	0.8675	0.3736	1.08	0.3224	3.47	0.0923	0.1618	0.6959	6.33	0.0306	0.2948	0.5991	0.2335	0.6394
B^2	9.15	0.0128	2.39	0.1532	0.2614	0.6203	0.4406	0.5218	12.72	0.0051	0.1937	0.6692	3.17	0.1054
C^2	0.2066	0.6591	3.30	0.0993	0.0415	0.8427	2.46	0.1477	13.54	0.0042	1.90	0.1979	14.68	0.0033
Residual														
Lack-of-Fit	4.72	0.0569	4.74	0.0565	1.46	0.3446	4.68	0.0578	4.95	0.0519	5.12	0.0520	3.50	0.0979
R^2	0.93		0.93		0.97		0.94		0.98		0.94		95	

Accepted to Online Publish


(a)
Hexanoic acid (mg/g)
A: Solvent concentration (%)
B: Ultrasound power (W)
(b)
2-Ethylhexanoic acid (mg/g)
A: Solvent concentration (%)
B: Ultrasound power (W)
(c)
8-Quinolol-2-butyl- (mg/g)
B: Ultrasound power (W)
A: Solvent concentration (%)
(d)
8-Quinolol-2-butyl- (mg/g)
C: Age of leaf (Day)
A: Solvent concentration (%)
(e)
8-Quinolol-2-butyl- (mg/g)
C: Age of leaf (Day)
B: Ultrasound power (W)
(f)
Octadecenoic acid (mg/g)
C: Age of leaf (Day)
A: Solvent concentration (%)
(g)
Octadecenoic acid (mg/g)
C: Age of leaf (Day)
B: Ultrasound power (W)
(h)
Phytol (mg/g)
B: Ultrasound power (W)
A: Solvent concentration (%)

Accepted

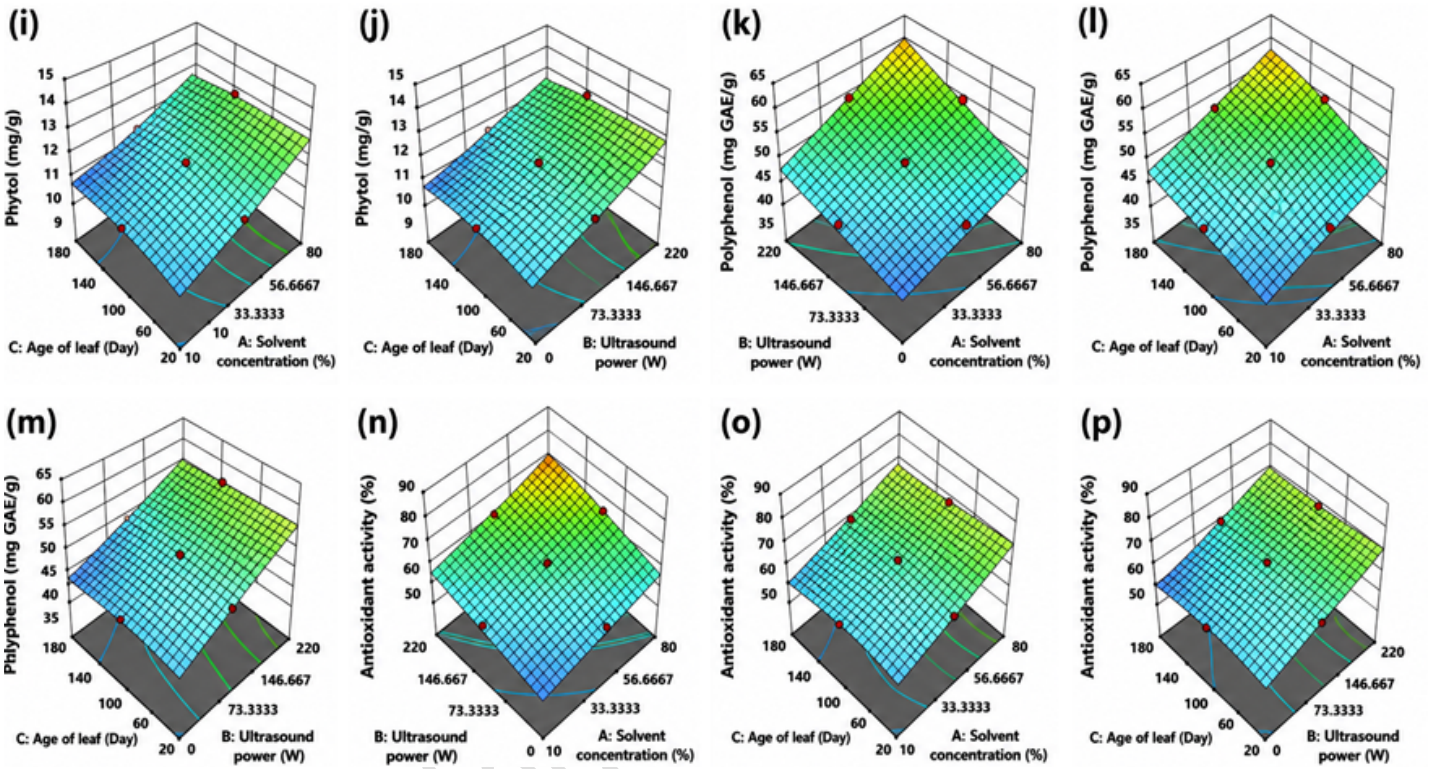


Fig. 5 Response surface plots for the effects of ethanol concentration (A), ultrasound power (B) and age of leaf (C) on different responses. (a) Hexanoic acid; (b) 2-Methylhexanoic acid; (c, d, and e) 8-Quinolinol-2-butyl; (f and g) Octadecanoic acid; (h, I, and j) Phytol; (k, l, and m) Polyphenol; (n, o, and p) Antioxidant activity.

Accepted

Response Optimization

The optimal point in the design space can be the maximum and minimum values or a region where the response is constant throughout the variable ranges. In this plan, the goal is to achieve the maximum amount of hexanoic acid, 2-methylhexanoic acid, 8-quinolinol-2-butyl, octadecanoic acid, phytol, polyphenols, and antioxidant activity in the best operating conditions. Based on CCD, the amounts of 8.46, 6.78, 10.15, and 11.24 mg/g were predicted for hexanoic acid, 2-methylhexanoic acid, 8-quinolinol-2-butyl, octadecanoic acid and phytol in the condition of 43.7% ethanol, 97.99, W and also 56 days old leaves, respectively. Phenolic substances and antioxidant features were reported 43.97 mg gallic acid/g and 63.68% with desirability equal to one, respectively.

Ultrasound Power, Solvent Concentration, and Leaf Age Effects

This method was widely used in the extraction of carotenoids, lignans, coumarins, alkaloids, glycosides, flavonoids, proanthocyanidins, polysaccharides, and seed oils. The percentage of organic solvent in water affects the overall performance as well as the performance for specific compounds. The extracts obtained from mature leaves had fewer phytochemicals and significantly lower effective substances compared to young parts.

DISCUSSION

In a study, HPLC was used to identify the main and important peaks in *Smyrnium cordifolium boiss* extract obtained through supercritical carbon dioxide and optimization of the response level was done to investigate factors influencing the components [8].

According to the conducted study, *Smyrnium ulosatrum* L., as a species of *Smyrnium cordifolium boiss* had two main peaks including isofuranodine and carzerne [3].

Similar to the present results, important compounds included n-hexanoic acid, linolenyl alcohol, phytol, octadecanoic acid, 8-quinolinol-2-butyl, 2-methylhexanoic acid, and hexanoic acid [8].

Various enzymes are effective in TPC and TFC (responsible for color, smell, and taste) synthesis, which are elevated in the initial growth of plant cells and before full structure, but after maturation and ripening, so component productions declines [11]. Food phenolics have antioxidant activity mainly due to their role as reducing agents, hydrogen donors and singlet oxygen quenchers, and also some phenols indicate the ability to bind metal ions, which act as catalysts in oxidation reactions [12].

The effects of high-intensity ultrasound on procyanidin breakdown in enriched grape seed extract and antioxidant evaluation caused a significant enhancement of up to 49, 41, and 35% for catechin, oligomeric, and polymeric purified extraction, respectively [11], which was similar to the present research. In previous research, ultrasound-assisted extraction of *Undaria pinnatifida sporophyll* at 960 W, temperature 30 °C, and time 3 h elevated efficiency [10]. Consistent with the present results, the effect investigation of ultrasound and microwave powers on phenolic acid, which has antioxidant activities for yellow soybean extract under 20 kHz and time from 2 to 15 min, exhibited that these factors significantly elevated and also ultrasound contributed to more efficient extraction [13]. In another study, ultrasound altered the spatial structure and improved the antioxidant capacity of yellow tea polysaccharide [1].

The TPC and antioxidant activities in aqueous and methanolic extracts of *Smyrnium cordifolium boiss* and *Sinapis arvensis* were investigated, and the highest TPC was observed in *Smyrnium cordifolium boiss* leaves, 31.51 mg gallic acid/g, followed by flowers and leaves of *Sinapis aronensis* 15 and 9.47 mg gallic acid/g [9]. Similarly, among all treatments with different strengths, a longer ultrasound extraction time from 10 to 20 min significantly improved TPC [14].

The antioxidant activities of aqueous and methanolic extracts of *Smyrnium cordifolium boiss* and *Sinapis arvensis* were determined; leaves had the highest activity 78.25% and *Sinapis aronensis* stems indicated the lowest 21.87% [13]. Antioxidant behavior of Moringa oleifera seed was increased after 3 h with ultrasound, while using 5 h of extraction with ultrasound-assisted tended to illustrate less activity [14]. After 5 h extraction with ultrasound, sulfated polysaccharide content in the plant could be degraded and lead to a decrease in antioxidant capability [10]. Phenolic compounds are secondary metabolites of plants that have significant antioxidant and chelating properties.[14] Their tendency to inhibit events caused by free radicals is controlled by the chemical structure and substitution pattern of their hydroxyl groups [15]. Among the identified compounds, the phenolic and flavonoid constituents are considered the most important contributors to antioxidant activity, since the manuscript associates higher antioxidant activity with greater TPC and phytochemical richness [10].

Ultrasound power factor is one of the most influential and important factors on extraction; hence, it is clear that optimization is prominent for each plant sample [16]. Similarly, higher ultrasonic power and intensity cause larger cavity bubbles; furthermore, pulsation and collapse occur more rapidly, releasing greater free radicals into the extract solution at higher potencies [17]. The extraction time is reduced to several minutes, while 2 to 20 h were needed in traditional methods [13]. Extraction with ultrasound waves was a tool to save time and work and is environmentally friendly, which can be used in commercial production [16].

Compared to others, ultrasound-assisted extraction results in the use of less solvent, shorter extraction time, higher extraction efficiency and minimal destruction of heat-sensitive compounds and is generally considered a green technique [13]. Ultrasound has been applied to extract polyphenolic compounds from grapes, fresh wheatgrass (*Triticum aestivum* L.), and peaches, respectively [15,18]. Plant flavonoids are polyphenols with a benzo-y-pyrone structure that are widely found in vegetables and fruits and are also mainly responsible for color, taste, lipid oxidation prevention, enzyme protection, anti-stress physiological, antioxidant, anti-inflammatory and antimicrobial activities [19].

Ethanol 43% was used as the proper concentration for the high total phenol and antioxidant activity in *Smyrnium cordifolium boiss* leaf extract, which contains the most hexanoic acid, 2-methylhexanoic acid, 8-quinolinol-2-butyl, octadecanoic acid, phytol, total phenol content and antioxidant activities [5]. Many solvents have been applied to extract polyphenols from different plants and solvent efficiency mainly depends on the ability to dissolve specific phenolics [19]. The solvent can affect plant cell permeability by a chemical or biophysical change, such that

Accepted to Online Publish

ethanol increases cell permeability by influencing the phospholipid bilayer in the membrane [20]. Sequential extraction with solvents of different polarity can provide fractions with distinct polyphenol groups; for example, methanol or ethanol mixtures with water have been successfully used [5]. As ethanol concentration in aqueous solution improved from 0 to 50%, the yield of polyphenols from grapevine shoots increased but then decreased [21, 22]. In contrast, 70% v/v ethanol in water was necessary for the quantitative recovery of total polyphenols from black currant, which differences can be attributed not only to the matrix but also to specific phenolic groups present in the plant [19]. Water is a good solvent for phenolic acids and their glucosides and can provide higher extraction yields of these compounds than organic solvents such as methanol, ethanol, or butanol, especially under ultrasonic treatment [5]. Aqueous ethanol solutions are better solvents than pure alcohols for the extraction of hydroxycinnamic acids and the best results for salvianolic acid were obtained with 60 to 70% ethanol in water [19]. Ethanol solutions in water (50%) were more effective in extracting phenolic acids and flavonoid glucosides from flaxseed than pure water [20].

Antioxidant activity and phytochemical contents of *Moringa oleifera* L. leaves were determined as a function of age and extraction solvent, and the results showed that methanolic and ethanolic extracts of 45-day parts indicated the best antioxidant potential [23]. Higher total phenolic content and antioxidant activity with improving age can be caused by active biosynthesis and their accumulation in cells during plant growth [19]. The effect of age had been reported that increase in total phenolic and flavonoid content is related to age for plant leaves [23-26]. It has been shown that the total concentration of catechins and polyphenols in different stages of growth decreases with increasing leaf age [26, 27].

CONCLUSION

This study provides the first systematic optimization of ultrasound-assisted extraction conditions for *Smyrnium cordifolium* Boiss. using a combined Taguchi design and CCD. The findings identify ultrasound power, leaf age, and solvent concentration as the key factors governing the extraction efficiency of phytochemicals, phenolic compounds, and antioxidant activity. Under the optimized conditions, the plant yielded an extract with significantly enhanced phenolic content and antioxidant capacity, highlighting its value as a rich natural source of bioactive compounds. The optimized extract shows promising potential for food applications (as a natural antioxidant or stabilizing agent) and nutraceutical development, although further validation is required before industrial adoption. Future research should focus on compound-level identification, bioactivity assays, toxicological evaluation, and stability studies under processing and storage conditions to fully clarify the functional and commercial applicability of *Smyrnium cordifolium* extracts.

Author Contribution

Pardis Allahyari conducted the experiments, collected, analyzed and interpreted the data, and prepared the initial draft of the manuscript. Marjan Nouri conceived and designed the study, supervised the research, contributed to data interpretation, critically revised the manuscript for important intellectual content, and approved the final version of the manuscript for publication.

Acknowledgments

The authors express their gratitude towards Islamic Azad University, Roudehen Branch, for providing the necessary facilities and support to carry out this research.

Conflict of Interest Statement

The authors have not declared any conflict of interests.

Consent for Publication

Not Applicable.

Funding

The authors acknowledge the financial support provided by Islamic Azad University, Roudehen Branch for conducting this research.

ORCID

https://orcid.org/0000-0001-7028-8474

Data availability

Not Applicable.

REFERENCES

1. boonajmi M., Kakelahi A., Arabhosseini A., Sadat-Noori S.A. Efficacy of ultrasonic waves in extraction ascorbic acid and bioactive compounds from Ajowan seeds (Carum copticum L.) and comparison with conventional Soxhlet extraction. Journal of Medicinal Plants and By-products. 2024;13(4):1159-1165. doi:10.22034/jmpb.2024.363314.1596.
2. Doodman S., Saeidi K., Lorigooini Z., Kiani M. Chemical composition of essential oils from Smyrnium cordifolium Boiss. (Apiaceae) ecotypes. Biochemical Systematics and Ecology. 2023;110:104682. doi:10.1016/j.bse.2023.104682.
3. Taylor J.R., Graaff T., David J., Kamau D., Joshi D., Byinshi B., De Kock H.L. Vitamins are retained in fortified whole-grain maize meal when stored under tropical conditions when optimal storage practices are used. Cereal Chemistry. 2025;102(5):894-908. doi:10.1002/cche.70006.
4. Miri S.M. Influence of salicylic acid and citric acid on the growth, biochemical characteristics and essential oil content of thyme (Thymus vulgaris L.). Journal of Medicinal Plants and By-products. 2023;4(2):141-146. doi:10.1002/cche.10866.

Accepted & Online Publish

5. Oreopoulou A., Tsimogiannis D., Oreopoulou V. Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters. In: Polyphenols in Plants. 2019;1:243-259. doi:10.1016/B978-0-12-813768-0.00025-6.
6. Alkhamessi R.N., Abbas M.I., Bahedh S.B., Kadham M.J., Lahhob Q., Mudhafar M. Harnessing medicinal plants and their by-products to combat fungal pathogens in the face of climate change: implications for global food security. Journal of Medicinal Plants and By-products. 2025:1-10. doi:10.22034/JMPB.2025.369007.1934.
7. Ashengroph M., Nahvi I., Amini J. Application of Taguchi design and response surface methodology for improving conversion of isoeugenol into vanillin by resting cells of Psychrobacter sp. CSW4. Iranian Journal of Pharmaceutical Research. 2013;12(3):411-425. doi:10.1007/s12010-011-9397-6.
8. Sodeifian G.H., Azizi J., Ghoreishi S.M. Response surface optimization of Smyrnium cordifolium Boiss. (SCB) oil extraction via supercritical carbon dioxide. The Journal of Supercritical Fluids. 2014;95:1-7. doi:10.1016/j.supflu.2014.07.023.
9. Tabaraki R., Ghadiri F. In vitro antioxidant activities of aqueous and methanolic extracts of Smyrnium cordifolium Boiss and Sinapis arvensis L. International Food Research Journal. 2013;20(5):2111-2115.
10. Muñoz-Labrador A., Prodanov M., Villamiel M. Effects of high intensity ultrasound on disaggregation of a macromolecular procyanidin-rich fraction from Vitis vinifera L. seed extract and evaluation of its antioxidant activity. Ultrasonics Sonochemistry. 2019;50:74-81. doi:10.1016/j.ultsonch.2018.08.030.
11. Hęś M., Dziedzic K., Górecka D., Jędrusek-Golińska A., Gujska E. Aloe vera (L.) Webb.: natural sources of antioxidants—a review. Plant Foods for Human Nutrition. 2019;74(3):255-265. doi:10.1007/s11130-019-00747-5.
12. Eom S.J., Kim Y.E., Kim J.E., Park J., Kim Y.H., Song K.M., Lee N.H. Production of Undaria pinnatifida sporophyll extract using pilot-scale ultrasound-assisted extraction: extract characteristics and antioxidant and anti-inflammatory activities. Algal Research. 2020;51:102039. doi:10.1016/j.algal.2020.102039.
13. Đurović S., Nikolić B., Luković N., Jovanović J., Stefanović A., Šekuljica N., Knežević-Jugović Z. The impact of high-power ultrasound and microwave on the phenolic acid profile and antioxidant activity of the extract from yellow soybean seeds. Industrial Crops and Products. 2018;122:223-231. doi:10.1016/j.indcrop.2018.05.078.
14. Sharma P., Wichaphon J., Klangpetch W. Antimicrobial and antioxidant activities of defatted Moringa oleifera seed meal extract obtained by ultrasound-assisted extraction and application as a natural antimicrobial coating for raw chicken sausages. International Journal of Food Microbiology. 2020;332:108770. doi:10.1016/j.ijfoodmicro.2020.108770.
15. Savic I.M., Savic Gajic I.M. Optimization of ultrasound-assisted extraction of polyphenols from wheatgrass (Triticum aestivum L.). Journal of Food Science and Technology. 2020;57(8):2809-2818. doi:10.1007/s13197-020-04312-w.
16. Chen X.M., Ma Z., Kitts D.D. Effects of processing method and age of leaves on phytochemical profiles and bioactivity of coffee leaves. Food Chemistry. 2018;249:143-153. doi:10.1016/j.foodchem.2017.12.073.
17. Ghazy O.A., Fouad M.T., Saleh H.H., Kholif A.E., Morsy T.A. Ultrasound-assisted preparation of anise extract nanoemulsion and its bioactivity against different pathogenic bacteria. Food Chemistry. 2021;341:128259. doi:10.1016/j.foodchem.2020.128259.
18. Carrera C., Ruiz-Rodríguez A., Palma M., Barroso C.G. Ultrasound assisted extraction of phenolic compounds from grapes. Analytica Chimica Acta. 2012;732:100-104. doi:10.1016/j.aca.2011.11.032.
19. Dong R., Liu S., Xie J., Chen Y., Zheng Y., Zhang X., Yu Q. The recovery, catabolism and potential bioactivity of polyphenols from carrot subjected to in vitro simulated digestion and colonic fermentation. Food Research International. 2021;143:110263. doi:10.1016/j.foodres.2021.110263.
20. Boussetta N., Soichi E., Lanoisellé J.L., Vorobiev E. Valorization of oilseed residues: extraction of polyphenols from flaxseed hulls by pulsed electric fields. Industrial Crops and Products. 2014;52:347-353. doi:10.1016/j.indcrop.2013.10.048.
21. Rajha H.N., Boussetta N., Louka N., Maroun R.G., Vorobiev E. A comparative study of physical pretreatments for the extraction of polyphenols and proteins from vine shoots. Food Research International. 2014;65:462-468. doi:10.1016/j.foodres.2014.04.024.
22. Nouri M. Nanoemulsions of Trachyspermum copticum L. and Teucrium polium L. essential oils incorporated with coating to extend fillet chicken storage. Journal of Food Measurement and Characterization. 2024;18(6):4034-4046. doi:10.1007/s11694-024-02474-1.
23. Nobossé P., Fombang E.N., Mbofung C.M. Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh Moringa oleifera L. leaves. Food Science & Nutrition. 2018;6(8):2188-2198. doi:10.1002/fsn3.783.
24. Anand S., Sonar R.M., Jain K. Traditional knowledge research in India: a bibliometric-based review and thematic analysis. Indian Journal of Traditional Knowledge. 2025;24(3):275-287. doi:10.56042/ijtk.v24i3.9491.
25. Pandikumar P., Mutheeswaran S., Chelladurai V., Ignacimuthu S. Notes on traditional method of solidified Aloe vera exudate (musabhar) preparation in Tamil Nadu: preparation of musabhar. Indian Journal of Traditional Knowledge. 2025;24(8):796-798. doi:10.56042/ijtk.v24i8.15412.
26. Mamati G.E., Liang Y., Lu J. Expression of basic genes involved in tea polyphenol synthesis in relation to accumulation of catechins and total tea polyphenols. Journal of the Science of Food and Agriculture. 2006;86(3):459-464. doi:10.1002/jsfa.2368.
27. Semsarzadeh M., Sayfzadeh S., Darvishi Zeidabadi D., Zakerin H., Hadidi Masuleh I. The role of zeolite in mitigating physiological and biochemical stress responses and enhancing seed yield of coriander (Coriandrum sativum L.) under drought conditions. Journal of Medicinal Plants and By-products. 2025:1-9. doi:10.22034/JMPB.2025.369791.1996.

Accepted to Online Publish