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Original Research

Comparison of Supercritical Fluid Extraction and Ultrasound-Assisted Extraction of *Corchorus Olitorius* L. and Evaluation of Their Antioxidant Properties and Identification of Compounds Using LC-ESI-MS

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Abstract

Corchorus olitorius L. is a medicinal herb with anti-inflammatory, laxative, and tonic properties, which is effective on the treatment of cancer and tumors. In this study, the extraction efficiency of supercritical fluid extraction (SFE) and ultrasound-assisted extraction (UAE) of *C. olitorius* as well as their antioxidant activities were investigated by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical. For this purpose, central composite block cube star and central composite design were employed to determine the optimal conditions. Thereafter, the qualification analysis of the effective ingredients of *C. olitorius* was done by LC-ESI-MS in negative mode. The results of LC-MS showed that *C. olitorius* includes phenolic and flavonoid compounds such as caffeic acid, p-coumaric acid, trans-ferulic acid, quercetin, naringenin, cirsiliol, cirsilineol, quercetrin, and naringin. Moreover, the SFE efficiency of extraction and IC50 value was obtained 4.25% and 1208.99 µg/mL, respectively. Also, the UAE efficiency of extraction and IC_{50} of antioxidant activity was obtained as 15.29% and 994.284 µg/mL, respectively. These results confirmed that the efficiencies of extraction and antioxidant activity of UAE were higher than that of SFE; however, the SFE is an environmentally friendly method and consumed less organic solvent.

Keywords: *Corchorus olitorius*; Supercritical fluid; Ultrasound-assisted extraction; Liquid chromatography-mass spectroscopy (LC-MS); Antioxidant; 2,2-diphenyl-1-picrylhydrazyl (DPPH); Optimization; Central composite design

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Introduction

During history, many diseases have been treated with herbal medicines [1]. The chemical composition of plants mostly determines the amount and type of their therapeutic function in the body. In recent years, medicinal plants and traditional therapies due to their lower cost, fewer side effects, and more impact on the human body, have gained much attention [2,3]. There are various dosage forms of herbs such as pills, teas, extracts, ointments, and essential oils for therapeutic use [4].

Plants can be widely used due to antioxidants and can replace synthetic chemical compounds [5]. Accordingly, antioxidants can inhibit the activity of free radicals, regenerate damaged cells, and improve body function [6].

Corchorus olitorius L. grows in the tropical region and can be found in southwestern Asia, and Iran. In addition, it is an agricultural plant, various parts of which can be consumed. Of note, it is called "Azivash" in Iran. This plant is used for the treatment of some diseases such as tumors, cancer, inflammation of the intestines, and bladder [7]. Moreover, *C. olitorius* is effective on protecting the digestive system, strengthening the immune system, and enhancing memory [8]. For determining phenolic and flavonoid compounds from *C. olitorius*, numerous methods were proposed [7,9].

The classic extraction method used is traditional solvent extraction, and various solvents are used in this method such as hexane, ethanol, and ethanol-water mixture that was used for herbal extract [10].

Supercritical fluid extraction (SFE) due to having several advantages such as less environmental pollution (due to requiring less organic solvent, it is called green method), feasibility, short extraction time, exhaustive extraction, selectivity, ability to extract heat-sensitive compounds, and a preconcentration effect, has received much attention [11,12]. Various solvents can be used as SFE solvent, and in this regard, carbon dioxide is popularly used. Correspondingly, carbon dioxide is nontoxic, noncorrosive, with critical pressure equal to 74 bar, and temperature 31 °C reachable, which evaporates quickly upon extraction [13,14]. Moreover, it is a nonpolar solvent and unable to extract polar compounds. Using this method, the addition of modifier (a few microliters organic solvent) can improve the extraction yield [15].

Another method is ultrasound-assisted extraction (UAE). In this method, ultrasound waves with high intensity and frequency are used by cavitation mechanism that consequently lead to cell-wall destruction [10].

UAE enables the generated free radicals by increasing energy to more than 20 kHz. Ethanolic and methanolic media also inhibit the production of free radicals [16].

This study aimed to optimize the effective parameters on the extraction efficiency and antioxidant activity. In this study, SFE and UAE extracts of *C. olitorius* were obtained, and their antioxidant properties were then investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. Finally, the active ingredients of *C. olitorius* were determined using the LC-MS method.

Material and methods

Chemicals

Analytical grades of Methanol, Ethanol, Acetonitrile, HPLC grade water, and DPPH were purchased from Merck. Carbon dioxide (CO_2) was put in a cylinder with an educator tube, which was obtained from Roham Co (Tehran, Iran) with a purity of 99.99%.

Plant material

Fresh green leaves of *Corchorus olitorius* were obtained from Golestan province, Iran. These leaves were air-dried without any direct sunlight, afterward, they were powdered in the blender and then stored in a glass container at room temperature.

Supercritical fluid extraction (SFE)

Supercritical fluid extraction is an environmentally friendly (called green) method that was performed with the Suprex MPS/225 apparatus manufactured pits burg co (Virginia USA) in this study. Thereafter, 0.5 g of *C. olitorius* powder was weighed and then added to glass beads (1 mm diameter). Next, a stainless steel with 3 mL volume cell was filled with this mixture and the value of ethanol was appropriately added to it as a modifier. The temperature and static time were adjusted at 40 °C and 10 min, respectively. By passing 10 min (static time) from the start of the dynamic time, the outlet valve was opened and the carbon dioxide flow rate was then adjusted to 0.2 ± 0.05 mL/min. Next, the herbal extract was collected in a vial containing ethanol as solvent. After the solvent evaporation, the extraction efficiency was calculated.

Ultrasound-assisted extraction

The Ultrasound-assisted extraction at this stage led to the destruction of the cell wall, and the effective compounds released. The Ultrasound assisted extraction was performed using ultrasonic apparatus (Tecno-Gaz Ultra 6-Italy), internal dimension 300 × 150 × 150 mm, and with the power of 550 W at the fixed frequency of 50 KHz.

The first step for performing the ultrasonic extraction was to select the appropriate solvent. For this purpose, water, methanol, ethanol, and acetonitrile were tested as the extraction solvents subjected to the similar operating conditions in terms of temperature, volume, and time extraction [17]. The results demonstrated that the ethanol extract of *C. olitorius* has a higher extraction yield; therefore, ethanol was finally selected as the extraction solvent.

About 0.5 g of the plant powder was weighed and mixed with an adequate amount of ethanol. Thereafter, the tube was immersed into an ultrasonic water bath with temperature and time-controlled, and ultrasonic ethanol extract was then centrifuged (3500 rpm, for 20 min). Eventually, the solvent was evaporated, and the mass extract was determined. Next, ultrasonic extraction yield was obtained using the equation 1.

Equation 1.

% Efficiency = (extract's weight)/(dry mass plant powder) × 100

Antioxidant activity

Antioxidant scavenging activity with DPPH radical was measured using the method proposed by Sethi, Joshl et al [18]. Briefly, SFE and UAE methanolic extracts were provided. As well, 250 mL of a fresh methanolic solution of DPPH (10^{-4} M) was prepared. About 0.1 mL of the sample solution (SFE, UAE) was added to 3.9 ml of the DPPH purple solution in a dark vessel, afterward the mixture was stored at temperature room for 30 min. The absorbance rate was assessed at 517 nm using a UV-Vis spectrophotometer (UV-2100 Shimadzu) apparatus. The inhibition percentage was calculated using the equation 2.

Equation 2.

$$\% \text{ Inhibition} = (A_{control}-A_{sample})/(A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of DPPH solution, and A_{sample} is the absorbance of sample solution. The plotted inhibition percentage versus concentration curve and IC_{50} was measured as well. IC50 is a concentration of the sample scavenging 50% of DPPH radical.

Liquid chromatography-mass spectrometry analysis

LC-ESI-MS (Liquid Chromatography-Electrospray ionization equipped with mass spectrometry) analysis was performed in terms of the method by Yakoub, Abdehedi [9].

LC-MS analysis was conducted using an Agilent1 200 series LC-MS system (Agilent Technologies; Waldhorn, Germany). Thereafter, the active ingredients of *C. olitorius* were detected using 6410 triple quadrupole tandem mass spectrometer that was equipped with an electrospray ionization and then operated in the negative ionization mode. For LC-MS analysis, Aquasil C18 column with dimension 150 × 3mm and 5µm stationary phase, and Aquasil C18 guard column (10 × 3 mm, 3 µm) were used.

The mobile phase consisted of 0.1% formic acid in H_2O v/v (A), 0.1 % formic acid in methanol v/v (B) linear gradient elution for 0-45 min, 10-100% B, and 45-55 min 100% B. In this regard, the Flow rate mobile phase and the injection volume were obtained as 0.4 mL/min and 5 µL, respectively. Of note, the temperature of the column was set at 40. A nebulizer gas flow was 1.5 L/min and the flow rate of dry gas was 12 L/min. The mass spectrophotometer adjusted in negative ion mode and capillary voltage was -3.5 V and the detector voltage was 1.2 V. The spectra were monitored in SIM (single ion monitoring) and then recorded full scan from 50 to 2000 Da.

Statistical analysis

The effective parameters on the extraction efficiency and antioxidant activity were optimized using STATGRAPHICS18 software.

The design for SFE with three parameters, including pressure, dynamic time, and modifier volume was done with the central composite blocked cube star. This design included 2^k, 2k and C_0 as are factorial point, star point (k is the number of parameters), and central point [6], respectively. Subsequently, 20 tests were performed for SFE on different days. The num-

ber of the experiments was obtained using $N=2^k+2k+C_0$. As well, the Design for UAE with three parameters, including time, solvent volume, and temperature was accomplished by a Central composite design (CCD) along with performing 17 tests. The number of the experiments was obtained using $N= 2^k+2k+C_0$. Accordingly, this design contained factorial point (2^k), 2k (star point), and C_0 (the central point (3; for this design)), where k is the number of parameters.

Results and Discussion

Evaluation of extraction and optimization of condition

Supercritical fluid extraction

The results show that three parameters, including pressure, modifier volume, and dynamic, affected the extraction efficiency.

The pressure Effect: Pareto chart shows that the effect of pressure was positive (Figure 1a). The results confirmed that, as the pressure increases, the density of the fluid and solubility of the sample increase as well, which consequently enhance the extraction efficiency.

According to figure 1b, when pressure is more than a certain value (about 250 atm), due to the disruption of the interaction between the solvent and soluble by the compacted fluid, the efficiency decreases. In this regard, the optimal value of pressure was reported as 248.533 atm.

The dynamic time effect: Pareto chart demonstrates that Dynamic time had a negative effect (Figure 1a). As shown in figure 1b, because the sample was in contact with the fluid for a longer time, the extraction efficiency enhanced with increasing the dynamic time. Moreover, in longer times (about 22 min), efficiency reduced, which may possibly be due to the evaporation of the valuable volatile compounds. The optimal value of the dynamic time was reported as 19.8284 min.

The modifier volume effect: figure 1a (Pareto chart) shows that the modifier volume effect was significantly negative. As shown in the figure 1b, it is known that as polar modifier volume increases, Polar compounds are extracted at higher modifier volumes (about 175 µL), and the polarity conditions became unfavorable for nonpolar compounds. Consequently, the extraction efficiency decreases. Of note, an optimal modifier volume was obtained as 150.306 µL.

The interaction between two parameters is visualized using response surface plots in figure 1c, 1d, 1e. It was observed that the interaction between pressure and modifier volume has a positive effect on efficiency (Figure 1d), pressure with dynamic time has a negative, and time with modifier volume has a positive one (Figure 1c and 1e, respectively).

Optimization: Central composite block cube star design was used to achieve the optimum condition for SFE. In this design, three parameters, including dynamic time (x_1; 15-25 min), pressure (x_2; 200-300 atm), and modifier volume (x_3; 100-250 µL) were adjusted to perform the experiments (Table1). The results of ANOVA test showed that R^2 and R^2 were adjusted at 0.98 and 0.97, respectively. According to the central composite block cube star design,

the second-order polynomial model was also achieved by STATGRAPHICS software for SFE yield using the equation 3.

Equation 3.

$$Yield = -2.9876 + 0.303899\ x_1 + 0.0292874 x_2 + 0.00791441 x_3 - 0.00496289\ x_1^2 - 0.0004496\ x_1x_2 + 0.0000310667\ x_1x_3 - 0.0000487122\ x_2^2 + 0.00002556\ x_2x_3 - 0.0000495164\ x_3^2$$

Where x_1, x_2, and x_3 are the independent variable parameters denoting dynamic time, pressure, and modifier volume, respectively. The optimum supercritical fluid extraction yield was achieved as 4.25%. The optimum condition of time (19.82 min), pressure (248.53 atm), and modifier volume (150.30 µL) was obtained as well.

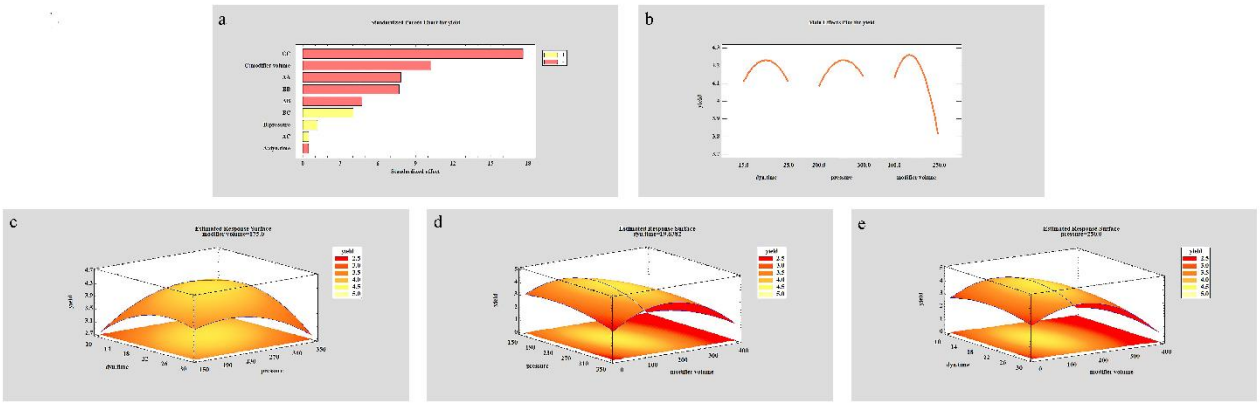

Figure 1. Supercritical fluid extraction yield (a) Pareto chart, (b) the main effect plot, Response surface plots: (c) interaction between dynamic time and pressure, (d) interaction between pressure and modifier volume, and (e) interaction between dynamic time and modifier volume

Table 1. Condition and results of the central composite block cube design for SFE optimization yield and IC_{50}

Run	Dynamic time	Pressure	Modifier volume	Yield	IC_{50}
	min	atm	µL	%	µg/mL
1	25	200	250	3.25	6004
2	20	250	175	3.94	1886
3	25	300	250	3.30	2868
4	15	200	250	3.00	6006
5	15	300	100	3.64	1335
6	20	250	175	4.04	2178
7	15	200	100	3.54	3332
8	25	300	100	3.40	1876
9	15	300	250	3.45	3985
10	25	200	100	3.80	4201
11	20	250	175	4.05	2036
12	20	340	175	4.14	1879
13	20	250	175	4.40	2666

14	20	250	175	4.43	2790
15	20	250	175	4.51	2789
16	20	250	310	3.30	7889
17	29	250	175	4.06	4392
18	20	159	175	4.10	6939
19	11	250	175	4.20	4006
20	20	250	39	3.90	2889

Ultrasonic-assisted extraction and optimization

The results indicated the effects of three parameters, including time, temperature, and solvent volume on the extraction efficiency.

The effect of solvent volume: When a high solvent volume is employed, the solvent's capacity is augmented, and its saturation with sample occurs later. Moreover, solvation emission and mass transfer increase, which consequently improve efficiency.

According to figure 2b, in case of any excessive increase of the solvent volume (about 19 mL), efficiency began to downturn. Accordingly, this may possibly be due to the production of free radicals during the cavitation process, and oxidation of phenols and antioxidants. In this regard, Pareto chart confirms that the effect of solvent volume was positive and significant (Figure 2a).

The effect of extraction time: The Pareto chart shows that the effect of extraction time was significant and positive (Figure 2a). At long times, the solvent was observed to have more opportunity to penetrate the tissue of the plant. Hence, the extraction efficiency increased. Figure 2b confirms that for a longer period of time, as about 45 min, the extraction efficiency began to fall because heat-sensitive compounds were degraded due to the exposure to heat for a long time.

The effect of temperature: Temperature was observed to affect the surface tension, viscosity, and solubility. Figure 2b indicates the effect of temperature on the extraction efficiency. Increasing temperature until a certain value broke the bond between the solute and plant matrix. Moreover, this made desorption and dissolving the compound in the solvent easier.

High temperature (about 50 ºC) not only led the solvent and volatile compounds to evaporate, but also reduced the solvent volume as well. Consequently, the extraction efficiency decreased. Pareto chart indicates that the effect of temperature was positive and significant (Figure 2a). The interaction between these two effects is shown in figures 2c, 2d, and 2e. Additionally, the interactions between time and temperature, time and solvent, and solvent volume and temperature were found to be positive.

Optimization: Central composite cube star (CCD) design was used to achieve the optimum condition for UAE. Time between 25 and 45 min (x_1), solvent volume 8-18 mL (x_2), and temperature 30-55 °C (x_3) were adjusted to perform the experiments (Table 2). In this study, the results of ANOVA test showed that R^2 (0.98) and R^2 were adjusted (0.97). Based on the Central composite design, the second-order polynomial model was obtained for UAE yield from software using the equation 4.

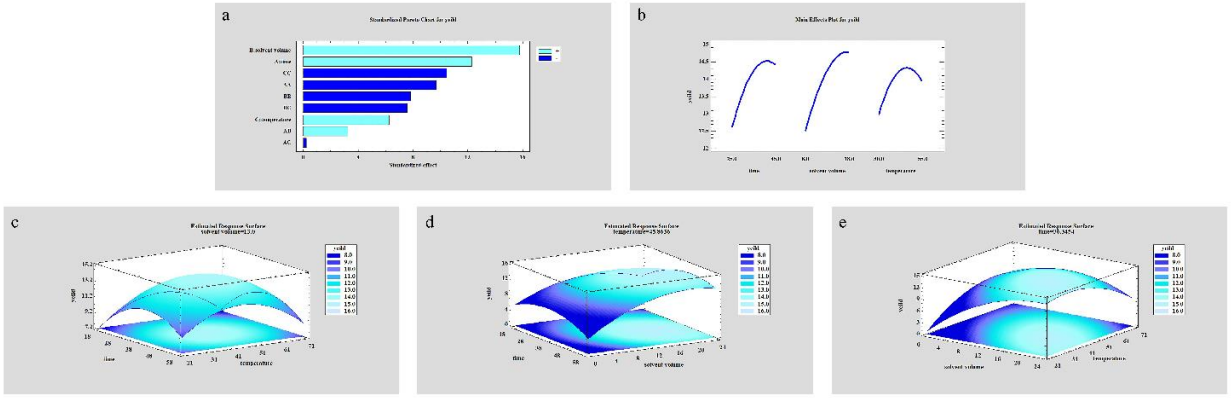

Figure 2. Ultrasonic assisted extraction yield (a) Pareto chart, (b) the main effect plot, Response surface plot: (c) interaction between time and temperature, (d) interaction between time and solvent volume, and (e) interaction between solvent volume and temperature

Table 2. Condition and results of the Central composite design for UAE optimization yield and IC_{50}

Run	time	Solvent volume	Temperature	Yield	IC_{50}
	min	mL	℃	%	µg/mL
1	35	13	42.5	14.25	1289
2	35	13	42.5	14.28	1345
3	35	13	42.5	14.32	1285
4	25	18	30	12.10	1689
5	25	8	55	11.47	3458
6	35	13	21	11.08	1912
7	25	8	30	9.46	2700
8	35	21	42.5	14.35	1354
9	35	5	42.5	10.42	2871
10	45	8	55	12.98	2381
11	35	13	63	12.52	2268
12	45	18	55	14.01	1100
13	25	18	55	12.02	1432
14	45	18	30	14.92	1397
15	18	13	42.5	10.54	2370
16	45	8	30	10.32	1798
17	51	13	42.5	13.40	1300

Equation 4.

$$Yield = -20.0601 + 0.550793\ x_1 + 1.1353\ x_2 + 0.637398\ x_3 - 0.00763785\ x_1^2 + 0.0061\ x_1x_2 - 0.00018\ x_1x_3 - 0.0246823\ x_2^2 - 0.01132\ x_2x_3 - 0.00527288\ x_3^2$$

Where x_1, x_2, and x_3 are the independent variable parameters as time, solvent volume, and temperature, respectively. The optimum ultrasonic-assisted extraction yield was reported as 15.29%. The optimum point was reported at 43.36 min, 19.45 µL, and 38.81ºC for time, solvent volume, and temperature, respectively.

The evaluation of Antioxidant activity and optimization of condition

Antioxidant activities of *C. olitorius* extracts (UAE and SFE) were evaluated using DPPH radical scavenging activity.

DPPH is a stable radical with a non-established additional electron in all molecules; hence, unlike other radicals, it does not dimmer easily. Plants contain high amounts of phenols and flavonoids. The IC_{50} value was assessed as the concentration of the sample required to inhibit 50% of free radicals. The lower the value of IC_{50}, the higher the antioxidant activity of the extract.

Antioxidant activities of supercritical fluid extracts

The effects of modifier volume, pressure, and dynamic time on oxidant activity of *C. olitorius* supercritical fluid extract were evaluated. Figure 3b shows the minimum point for all the parameters. As stated earlier, increasing the pressure led to a high density of fluid; hence, more compounds were extracted. Pareto chart confirms that the significant effect of pressure was negative (Figure 3a). Moreover, the IC_{50} value decreased, which consequently increased the antioxidant activity. According to figure 3b, IC_{50} value raised by increasing the modifier volume a significant positive effect (Figure 3a). Correspondingly, it means that the decrement in antioxidant activities due to the enhanced extracted compounds was nonpolar. Therefore, the high volume of polar modifiers led to a downturn in both the efficiency and antioxidant activity.

Figure 3a shows that the dynamic time effect was positive. In a long time, the IC_{50} value increased; however, the antioxidant activity decreased due to the antioxidant compound's degradation and the valuable volatile compound's evaporation.

Response surface plots show the Interaction between the two effects (Figure 3c, 3d, and 3e). According to figure. 3a, the interaction between pressure and modifier had a negative effect on IC_{50}. In addition, the interactions between time and pressure (AB) and time and modifier volume (AC) were negative.

Optimization: the optimum conditions for antioxidant activities of SFE extracts were obtained from the Central composite block cube star design. The values of the three parameters (including dynamic time x_1, pressure x_2, and modifier volume x_3) to perform the experiments were equivalent to the optimization of table1. The results were obtained using the ANOVA test. According to the central composite block cube star design, the second-order polynomial model was achieved for IC_{50} using the equation 5.

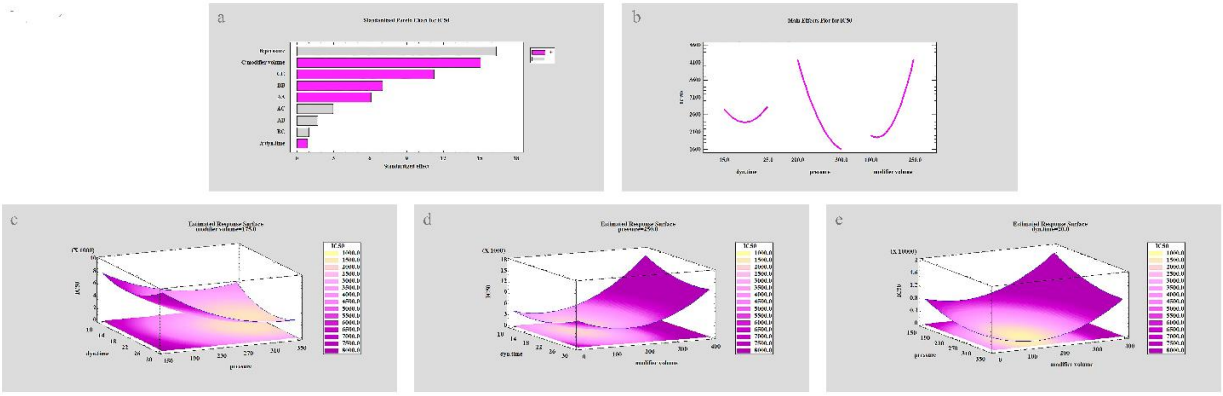

Figure 3. Antioxidant activity of SFE extracts, (a) Pareto chart, (b) the main effect, Response surface plot, (c) interaction between Dynamic time and pressure, (d) interaction between dynamic time and modifier volume, and (e) interaction between modifier volume and pressure

Equation 5.

$IC_{50} = 21491.0 - 344.491\,x_1 - 104.903\,x_2 - 9.75598\,x_3 + 17.1565x_1^2 - 0.722465x_1x_2 - 0.843037x_1x_3 + 0.197211x_2^2 - 0.0278563x_2x_3 + 0.140894x_3^2$

In this equation, x_1, x_2, and x_3 are dynamic time, pressure, and modifier volume, respectively.

The ANOVA test demonstrated that R^2 and R^2 adjusted were 0.98 and 0.97, respectively.

The optimum conditions for the antioxidant activity SFE extracts were also reported at Dynamic time 19.62 min, pressure 310.68 atm, and modifier volume of 124.61 µL and the optimum IC50 value for the SFE extract was achieved as 1208.94 µg/mL.

Antioxidant activities of the ultrasonic extraction extracts

The results of antioxidant activity characterized the effect of three significant parameters of temperature, time, and solvent volume (Figure 4a). Figure 4b indicates that reducing IC_{50} value was resulted from increasing time and solvent volume. Time and solvent volume were shown to have significant and negative effects (Figure 4a).

After a long time, and exposure to heat, the antioxidant compound degraded and then the antioxidant activity decreased. The effect of temperature on IC_{50} is shown in figure 4b. According to Pareto chart (Figure 4a), the temperature effect on IC_{50} was significant and positive. The temperature had two behaviors as follows: up to about 50 ºC, IC_{50} value decreased and antioxidant activity augmented, but the IC_{50} value increased after about 50 ºC due to solvent's evaporation, approaching solvent boiling temperature, the effective compound evaporation, and degradation of antioxidants.

Response surface plots shown in figures 4c, 4d, and 4e interpret the interactions among time, solvent volume, and temperature.

The interaction between time and solvent volume had a positive effect. Moreover, the interaction between temperature and time as well as that with solvent volume were negative.

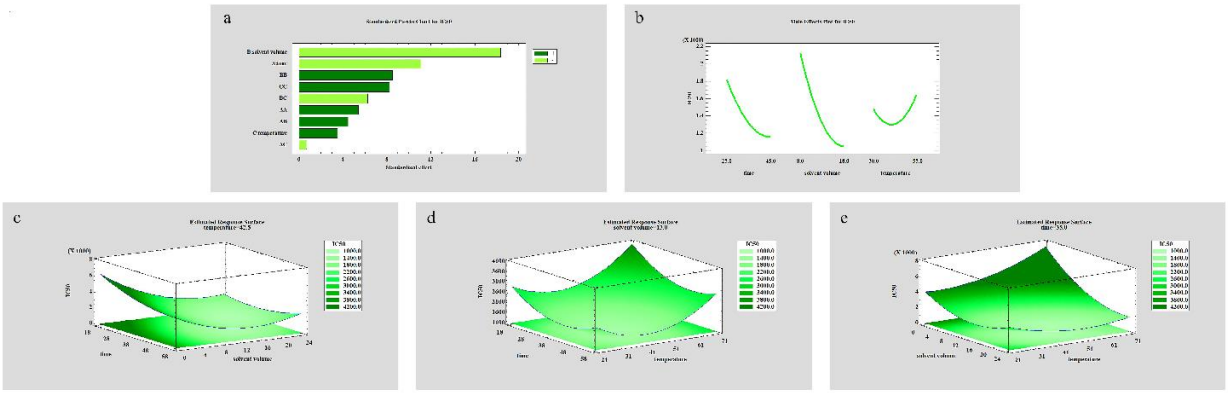

Figure 4. Antioxidant activity of UAE extract, (a) pareto chart, (b) the main effect, Response plot: (c) interaction between time and solvent volume, (d) interaction between time and temperature, and (e) interaction between solvent volume and temperature.

Optimization: Optimum condition for the antioxidant activity of UAE extracts was obtained by Central composite design. Thereafter, time, solvent volume, and temperature were adjusted to perform the experiments according to table 2. The results were obtained using the ANOVA test. Next, the second-order polynomial model was obtained for IC50 by software by the equation 6.

Equation 6.

$$IC_{50}=9610.92-188.511x_1-346.268x_2-78.4579x_3+1.73386x_1^2+3.38687x_1x_2-0.21475x_1x_3+10.8598x_2^2-3.7905x_2x_3+1.68667x_3^2$$

In this equation, x_1, x_2, and x_3 stand for time, solvent volume, and temperature, respectively. R^2, and R^2 adjusted were obtained from the ANOVA test (as 0.98, and 0.97, respectively). The optimum value of IC_{50} for UAE was reported as 994.284 µg/mL. In addition, the optimum conditions for antioxidant activity of UAE were obtained at 39.91 min (time), 17.69 mL (solvent volume), and 45.67 ºC (temperature).

LC-MS analysis for Corchorus olitorius

The identification of *C. olitorius* compounds with LC-MS is reported in table 3 and figure 5. The identified caffeic acid was found to have antioxidant properties, the ability to regulate the immune systems, and anti-inflammatory properties [19]. Quercetin, quercitrin, naringenin, and naringin are among the flavonoid compounds, which have antioxidant, antitumor, and anticancer properties [20-23].

P-coumaric acid and trans-ferulic acid were also identified as hydroxycinnamic acids with anti-inflammatory and antioxidant properties [24-26]. Correspondingly, *p*-coumaric acid can be used in the treatment of cardiovascular diseases, neuroinflammatory diseases, different types of cancer, and liver disease [27]. Cirsiliol and cirsilineol belong to the group of flavone, which were previously reported to have hypnotic and inhibitory effects on cancer cells, respectively [28,29].

It is notable that the antioxidant properties of *C. olitorius*, which were evaluated with DPPH free radicals, are due to these phenolic and flavonoid compounds.

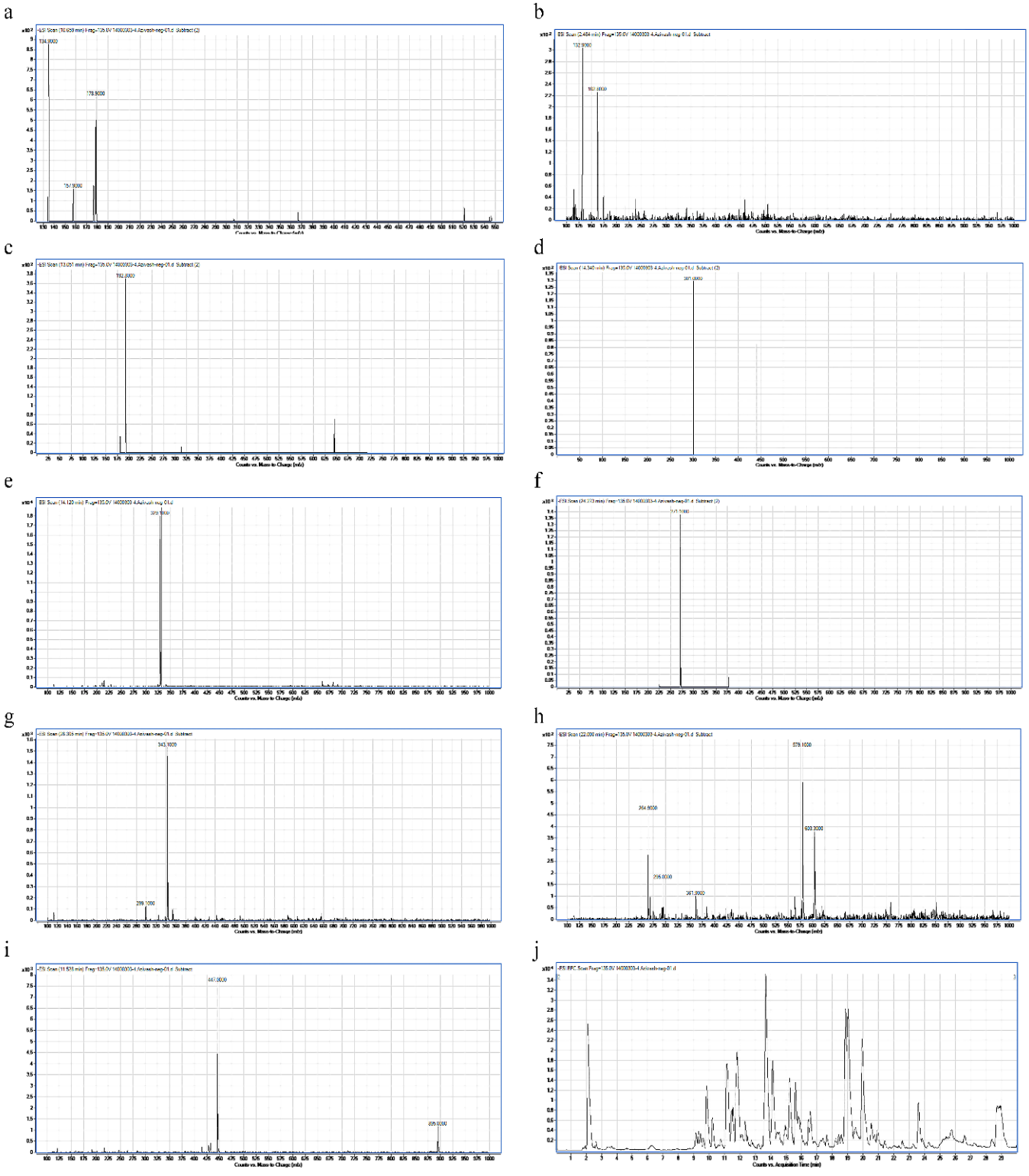

Figure 5. (a) mass spectrum of caffeic acid, (b) mass spectrum of *p*-coumaric acid, (c) mass spectrum of trans ferulic acid, (d) mass spectrum of Quercetin, (e) mass spectrum of Circiliol, (f) mass spectrum of Naringinin, (g) mass spectrum of Cirsilineol, (h) mass spectrum of Naringin, (i) mass spectrum of Quercetrin, and (j) HPLC chromatogram of *C. olitorius*.

Table 3. Compounds of *Corchorus olitorius* identified by LC-MS

Compounds	Chemical formula	Retention time (min)	m/z	Figure
Caffeic acid	$C_9H_8O_4$	10.66	179	5a
p-Coumaric acid	$C_9H_8O_3$	2.48	163	5b
Trans-ferulic acid	$C_{10}H_{10}O_4$	13.04	193	5c
Quercetin	$C_{15}H_{10}O_7$	14.34	301	5d
Naringenin	$C_{15}H_{12}O_5$	24.27	271	5f
Cirsiliol	$C_{18}H_{16}O_7$	14.12	329	5e
Cirsilineol	$C_{18}H_{16}O_7$	26.30	343	5g
Quercetrin	$C_{21}H_{20}O_{11}$	11.53	447	5i
Naringin	$C_{27}H_{32}O_{14}$	22.00	579	5h

Conclusion

The optimal conditions for SFE parameters were found as 19.82ºC for Dynamic time, 248.533 atm for pressure, and 150.306 µL for modifier volume. The optimized conditions of UAE were obtained at time, solvent volume, and temperature as 43.36 min, 19.45 mL, and 38.81ºC respectively.

In the current study, the identified phenolic and flavonoid compounds (including caffeic acid, p-coumaric acid, trans-ferulic acid, quercetin, naringenin, cirsiliol, cirsilineol, quercetrin, and naringin) using LC-MS confirmed the antioxidant activities of *C. olitorius.*

Since most of the compounds extracted using SFE usually are nonpolar and DPPH determined the polar antioxidant compounds, IC_{50} values of both UAE and SFE (obtained as 994.284 and 1208.94 µg/mL, respectively) confirmed that the antioxidant activity of UAE extract was higher than SFE extracts.

UAE consumed a high volume of organic solvent in comparison to SFE. Moreover, in SFE, SC-CO_2 evaporated convenience. Of note, the required sample was collected in a light volume of solvent into the vial. On the other hand, the UAE sample was diluted and needed pre-concentration.

In this study, SFE and UAE, as well as their antioxidant activities were compared. Based on the obtained experimental data, the ultrasound-assisted extraction efficiency (15.29%) is higher than supercritical fluid extraction efficiency (4.25%). Accordingly, this is due to the fact that ultrasound waves break the plant cell wall and also its access to the compounds in the plant is better for performing the extraction process. Using the SFE method, several compounds with varying polarities can be achieved by changing the temperature, pressure, and modifier volume. So, SFE can be known as a selective method for extracting compounds

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