DOI:10.25092/baunfbed. 1330843 *J. BAUN Inst. Sci. Technol., 26(1), 279-292, (2024)*

Optimization of ultrasound-assisted extraction of phenolic compounds from *Ziziphus jujube Mill*. leaves using response surface methodology

Elif SAVAŞ*

Balıkesir University, Faculty of Engineering, Department of Food Engineering, 10145, Balıkesir, Turkey,

Geliş Tarihi (Received Date): 21.07.2023
Kabul Tarihi (Accepted Date): 27.10.2023

Abstract:

The phenolic compounds in jujube (Ziziphus jujuba Mill) leaf were extracted using ultrasound assisted extraction (UAE) for potential of antioxidant food additive in this study. The extraction factors such as methanol concentration, temperature and time were optimized using response surface methodology (RSM) to maximize of the total phenolic content (TPC), 2,2 -diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and phenolic profile in jujube leaf extracts (JE). The best possible range for methanol concentration (25-50%), duration of ultrasound (20-40 min), ultrasonic temperature (40–60 °C) were obtained using the Box-Behnken design (BBD). The optimum extraction parameters were obtained with 25% methanol concentration, 20 min duration of ultrasound and 49.89 °C ultrasonic temperature. Ellagic, caffeic, rosmarinic acid, and rutin were determined as major phenolics in JE under optimal extraction parameters. The results revealed that UAE is an effective pretreatment for extracting bioactive ingredients from JE as potential functional food additive. Furthermore, RSM is an effective method for optimizing the UAE factors.

Keywords: *Ultrasound assisted extraction, response surface methodology, zizyphus zizyphus, antioxidant capacity, phenolic compounds.*

* Elif SAVAŞ, e-savas@balikesir.edu.tr, http://orcid.org/0000-0002-4878-0013

Yanıt yüzey yöntemi kullanılarak *Ziziphus jujube Mill* yapraklarından fenolik bileşenlerin ultrason destekli ekstraksiyonunun optimizasyonu

Özet

Bu çalışmada potansiyel gıda katkısı olabilecek hünnap (Ziziphus jujuba Mill) yaprağı fenolik bileşenleri ultrason destekli olarak ekstrakte edilmiştir. Hünnap yaprağı ekstraktlarında (JE) toplam fenolik madde miktarı (TPC), 2,2 -diphenyl-1-picrylhydrazyl (DPPH) giderme aktivitesi ve fenolik profil maksimize edilmek üzere, yanıt yüzey yöntemi (YYY) kullanılarak metanol konsantrasyonu, sıcaklık ve süre gibi ekstraksiyon faktörleri optimize edilmiştir. Metanol konsantrasyonu (% 25-50), ultrason süresi (20-40 dakika) ve sıcaklık (40–60 °C) için muhtemel en iyi aralık Box-Behnken deneme deseni (BBD) kullanılarak saptanmıştır. Optimum ekstraksiyon parametreleri %25 metanol, 20 dakika ve 49.89 °C olarak belirlenmiştir. Optimum ekstraksiyon parametreleri ile elde edilen hünnap yaprağı ekstraktında ellajik, kafeik, rosmarinik asitler ve rutin ana fenolik bileşikler olarak belirlenmiştir. Bulgular, potansiyel gıda katkısı olarak hünnap yapraklarına ait bioaktif bileşenlerin ekstraksiyonunda ultrason destekli ekstraksiyonun etkili bir yöntem olduğunu göstermiştir. Bununla birlikte yanıt yüzey yöntemi utrason destekli ekstraksiyon faktörlerinin optimizasyonu için etkili bir yöntem olarak belirlenmiştir.

Keywords: *Ultrason destekli ekstraksiyon, yanıt yüzey yöntemi, zizyphus zizyphus, antioksidan kapasite, fenolik bileşenler.*

1. Introduction

Zizyphus species (*Rhamanceae*) are widely used in Asian countries, especially Taiwan and China, as a medicine for the treatment of allergies, constipation, urinary problems, depression, chronic bronchitis, insomnia and liver diseases [1]. It is known for health-promoting effects such as anti-inflammatory [2], antimicrobial [3], antiproliferative, and apoptotic effects. 135-170 species of *Zizypus* are reported [4]. In addition to the cultivated ones, there are many *Ziziphus* species reported from Turkey that show great diversity in terms of plant and fruit characteristics. *Z. sativa Gaertn., Z. vulgaris L., Z. soporifera (Lour) Stokes, Z. tomentosa Poir., Z. trinervia Roth, Z. orthacantha DC., Z. rotundata DC., Z. poirretti G. don, Z. mairei (H. Lev.) Browicz and Lauener, Z. zizyphus (L.) Meikle, and Z. lotus (L.) Lam* are among the species grown in Anatolia [5]. *Ziziphus jujuba Mill* (synonyms *Rhamnus zizyphus* L. and *Rhamnus jujuba* L.) is the most cultivated jujube species whose fruit is called "hünnap" [6]. Fruits are widely consumed for its potential high nutritional value. Studies on the nutritional content of fruit have reported that they have besides high protein (14.13%), K (0.12%), Ca (10.21%), P (0.12%), Mg (0.07%), N (2.26%) [7] and high vitamin C content [8] in dry weight (DW). Benammar et al. [9], reported that the fruit pulp of jujube have a higher vitamin A and C which responsible for human cell T-prolifiration than the other parts of plant. Fruit pulp has also been reported as a rich source of phenolic compounds [10] responsible for the antioxidant properties to prevent permanency of some diseases. Many antioxidant compounds in the group of

phenolic acids and flavonoids act as protective against tissue damage and inflammation caused by reactive oxygen species in plant, human and food tissues. Mostly, it is associated with reactive oxygen damage, resulting in a decrease of nutritional value with the formation of free radicals. The oxidative stress causes many diseases including inflammation, cataract, cancer, arteriosclerosis, autoimmune, Parkinson and neurodegenerative syndromes in human. Further, oxidative variations cause changes of color, aroma, texture and flavor in foods. The antioxidants inhibit oxidative damage mechanism and for scavenging free radicals like peroxide, hydroperoxide of lipid hydroxyl. It is a well-known that flavonoids and phenolic acids play an active role in preventing quality losses in foods due to the effect of light and oxygen [10].

Plant parts except for fruit such as leaves, seeds and roots are known as high antioxidant sources [11]. In vivo studies have been reported that plant leaves and roots demonstrated anti-spasmodic, anti-inflammatory and analgesic activity in rats and rodents [12,13]. Besides the health benefits, these plant parts are added as antioxidant food additives to increase the shelf life instead of synthetic antioxidants for adverse effects. Plant leaves can be used directly or as an extract for food additive. In case of use as an extract, its effectiveness varies depending on the extraction method and conditions. Solvent extraction is the most common extraction method [14]. The efficiency of the method depends on the extraction parameters such as solvent concentration, solvent type, temperature, stirring speed (rpm). The method requires high temperature and long processing time [15]. High temperatures applied to achieve high extraction efficiency may damage some phenolic compounds and excessive use of solvents increases the amount of waste which harmful for the environment. Green energy extraction methods are preferred over conventional methods as they provide better recovery of bioactive compounds in plant tissues without loss and with high antioxidant activities. For example, ultrasound-assisted extraction (UAE) is an ecofriendly method offering a high recovery of bioactive compounds [16, 17]. The effect occurs with the collapse of bubbles growing gradually with the pressure created by ultrasound waves moving on a solid phase wall in the liquid phase. A high extraction efficiency can be obtained, especially for phenolic compounds with antioxidant properties [18] and lossless recovery by preventing the chemical degradation of bioactive compounds. The extraction yield is affected by several factors, including solvent, sample size, pH, temperature, pressure, particle size, and UAE time [19]. The antioxidant effect of extracted compounds from several plant tissues has been determined by assessing the total phenolic content (TPC), DPPH scavenging activity, and total flavonoid content [20-22].

Many of the experiments on the genus *Zizyphus jujube* demonstrates the biological potential of its bioactive components, mainly polyphenols, proteins and polysaccharides, for all possible pharmaceutical and nutraceutical applications. The variety of these studies focuses on the presence of bioactive components in jujube fruit and seeds. No data has reported the effect of ultrasound on phenolic compounds extracted *from Ziziphus jujuba Mill* leaves (JLs) and evaluation of its antioxidant properties. Here, we studied the linear and quadratic effects of different UAE parameters such as time, temperature, and solvent concentration on the phenolic and antioxidant compounds of *Z. jujube* extracts obtained under optimized UAE conditions using response surface methodology (RSM). Furthermore, in the future projection, general aim is to evaluate the possibilities of use with its high antioxidant effect in the food and pharmaceutical industry with its possible nutraceutical and nutritional effects. The general objective for the future projection is to

develop its use as a new functional and nutritional food and pharmaceutical industry additive in terms of high nutritional value.

2. Materials and methods

2.1.Plant materials

Hand-picked Z. zizyphus leaves from the tree in Balıkesir, Turkey were washed and dried on a clean filter paper in the shade and at room temperature for approximately 24 h. The dried leaves were powdered using a Waring blender (Clarkson 8011S, USA). All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2.Process design

Optimum UAE conditions were designed for combinations of variables by RSM, using Box–Behnken design. The maximum antioxidant activity and TPC values in Jujube leave extracts were determined using the Minitab® statistical software (Pennsylvania, USA). Independent variables were temperature (X_1: 40, 50, and 60 °C), time (X_2: 20, 30, and 40 min), and methanol concentration (X_3: 25, 37.5, and 50%). Dependent variables were TPC and the scavenging activity of DPPH in the process (Table 1). The values of triplicate result responses were placed into the model (Eq. 1).

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta11X1X1 + \beta22X2X2 + \beta33X3X3 + \beta12X1X2 + \beta13X1X3 + \beta23X2X3 \quad (1)$$

The constant is β_0, whereas TPC and DPPH from JE are expressed as Y. β_1, β_2, and β_3 are symbolized in the equation as linear regression coefficients, and β_{11}, β_{22}, and β_{33} are used as quadratic coefficients; β_{12} and β_{23} are used as interaction coefficients. X_1 refers to temperature, X_2 refers to time, and X_3 refers to methanol concentration.

The interaction between dependent and independent variables is shown using the polynomial equation and three-dimensional (3D) graphics. The optimized conditions of UAE were calculated as regression coefficients (β) of linear, interaction, and quadratic using analysis of variance (ANOVA) for TPC and antioxidant activity. The regression coefficient (R^2) was used (95%, $p < 0.05$) to estimate the suitability of responses of the polynomial equation using RSM. The validity of the model was determined by comparing it with predicted values.

2.3.Ultrasound-assisted extraction of phenolic compounds from jujube leaves

The phenolic compounds of jujube *(Z. zizyphus)* leaves were extracted using a 3.3 L ultrasonic bath (Daihan Scientific, Korea) at 40 kHz and 172 W. The independent variables are temperature, time and solvent concentration (Table 1). 0.5 g of dried powdered leaves were used for the trials that met the conditions specified in the trial fractions modeled according to Table 1, and the extraction solution was obtained by completing it with 50 ml extraction solution. Afterward, ultrasonic extraction was applied. Process factors (temperature, time) were observed using the control panel. After the application, mixture was cooled to room temperature and filtered. The TPC and antioxidant properties of JE were analyzed.

2.4.Phenolic composition of JE

The profile of phenolic compounds was analyzed at optimized conditions by HPLC [23]. The flow rate of mobil phase (A: 3% of formic acid, B: Methanol) was set at 0.8 ml/min and was monitored using Diode-Array Detector (DAD, SPD-M20A) at 260 nm. A Zorbax C18 column (250*4.6 mm, 5 micron) was used for separation by isocratic elution at 25ºC. The results of trials were expressed as means ±SD (standard deviation).

2.5.Total phenolic content

A modified Folin–Ciocalteu method (Mohamed Ahmed et al. 2020) was used to spectrophotometrically (T80+ UV–Visible; PG Instruments, UK) determine the TPC of JE [24]. 900 µL of distilled water was added into the100 µL of JE. After 5 mL of Folin-Ciocalteu reagent (0.2 N) was added, the blend was kept during three minutes. 4 mL of Na2CO3 solution (% 20; w/v) was added on to the blend and kept in the dark at room temperature for 90 minutes. The results were expressed as milligrams of gallic acid equivalents per gram (g GAE/g DW). Absorbance was measured at 765 nm against blank and different gallic acid concentration as a substrat. The results expressed as miligrams of gallic acid equivalents per gram (g GAE/g DW).

2.6.*DPPH*

The total antioxidant capacity (TAC) of JE was assessed using the modified DPPH method [25]. The scavenging activity of DPPH (% inhibition) was calculated by measuring the change in the absorbance of the DPPH solution at 517 nm. The calibration curve (R2 = 0.9972) was plotted using different amounts of Trolox (10–100 µmol/L) (Eq. 2).

$$Inhibition\ (\%) = \frac{Ac-As}{Ac} x100 \qquad (2)$$

A_c = absorbance of the control
A_s = absorbance of the sample

3. Results and discussion

The Box–Behnken design was used to determine the interaction of factors and optimize UAE conditions (temperature, time, and solvent concentration). The uncoded values of independent variables are indicated in Table 1. The TPC in JE ranged from 2486.44 to 4438.52 mg GAE/g DW, and the percentage inhibition of DPPH ranged from 09.76 to 50.50 % as dependent variables affected by extraction parameters. The temperature was highly effective on the TPC value. The maximum DPPH inhibition was determined at the highest temperature and methanol concentration. Different studies indicated that the methanol concentration and temperature were effective UAE factors on TPC and antioxidant activity for oil palm (Elaeis guineensis Jacq.) leaf [26] and Cassia auriculata leaves [27].

Table 1. Optimization of extraction parameters using the Box–Behnken experimental design used in TPC (mg GAE g^{-1} DW) and DPPH (% inhibition) of JE using UAE

Run	X_1	X_2	X_3	TPC	DPPH
1	60.00	40.00	37.50	2782.21 ±0.24	36.02 ±0.02
2	40.00	40.00	37.50	3048.40 ±0.32	9.76 ±0.01
3	50.00	30.00	37.50	2959.67 ±0.00	27.27 ±0.22
4	60.00	30.00	25.00	2880.80 ±0.89	43.09 ±0.10
5	50.00	40.00	25.00	4290.64 ±0.87	13.46 ±0.09
6	40.00	30.00	50.00	2486.44 ±0.02	35.69 ±0.32
7	60.00	20.00	37.50	2821.65 ±0.18	50.50 ±0.46
8	50.00	30.00	37.50	3156.85 ±0.22	35.01 ±0.25
9	50.00	30.00	37.50	3285.02 ±0.24	10.43 ±0.14
10	50.00	30.00	37.50	2703.34 ±0.45	19.19 ±0.02
11	50.00	20.00	25.00	4438.52 ±0.52	12.79 ±0.01
12	50.00	30.00	37.50	2723.06 ±0.28	24.24 ±0.00
13	50.00	20.00	50.00	3137.13 ±0.16	25.58 ±0.18
14	40.00	20.00	37.50	2555.45 ±0.36	23.56 ±0.44
15	40.00	30.00	25.00	2831.50 ±0.06	11.44 ±0.12
16	50.00	40.00	50.00	4044.16 ±0.42	41.07 ±0.08
17	60.00	30.00	50.00	3827.27 ±0.18	50.16 ±0.12

X_1: temperature (°C); *X_2*: time (min); *X_3*: methanol concentration (%, v/v)

The interaction between dependent and independent variables was modeled using the quadratic polynomial equation (Eq. 1). The regression coefficients calculated from the model for TPC and DPPH were control respectively. The overall variability of the response was determined to be more than 80.7% (Eq. 1).

3.1.Effect of UAE factors on TPC

Several known effective variables such as solvent type [28], concentration [29], time, temperature [30], sample-to-solvent ratio [31], diameter, and shape of the extraction vessel [32] affected the extraction of bioactive compounds using UAE. Unlike other extraction methods, UAE increases the extraction performance by destroying the cell walls with the formation of acoustic cavitation and accelerates the mass transfer from the solid matrix [33]. The highest TPC content of Jujube leave extracts was recorded as 4438.52 ±0.52 in the 11th trial (X1: 50 °C, X2:20 min, and X3: 25%) in the UAE conditions, where 15 different factor combinations were applied ($p < 0.05$). The lowest TPC value of 2486.44 ±0.02 was recorded while X1: 40 °C, X2:30 min, and X3: 50%.

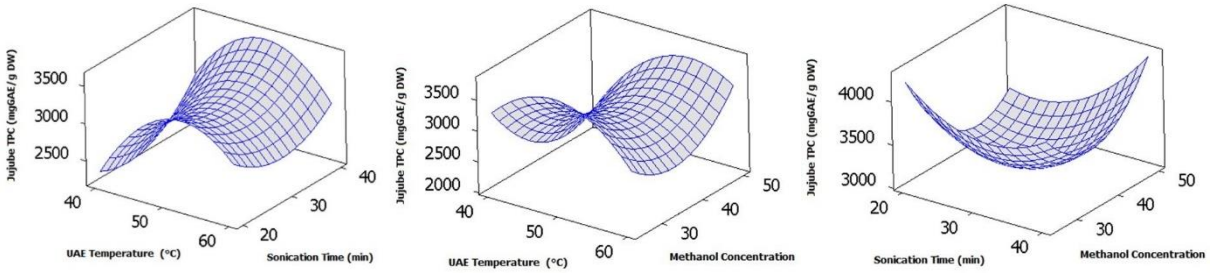
The 3D surface plots were generated for maximizing the TPC of Jujube leave extracts under optimum UAE conditions. Quadratic effect of temperature was significant (Table 2). The predictive equation is shown below that was calculated using regression coefficients of factors for TPC (Table 2).

Table 2. Modeled regression coefficients for dependent variables of JE using UAE

	TPC		**DPPH**	
	Coefficient estimate	**Standard error**	**Coefficient estimate**	**Standard error**
Intercept				
β0 Linear	–2.88[a]	0.79	11.6993	204.06
β1	1.35	236.82	–6.216	6.06
β2	–0.42	180.28	0.666	4.61
β3	–1.01[a]	144.22	0.556	3.69
Interaction				
β12	–1.33[a]	2.10	–0.002	0.05
β13	2.58 [a]	1.68	–0.034[a]	0.04
β23	2.11	1.68	0.03	0.04
Quadratic				
β11	–6.52 [a]	2.19	0.088	0.05
β22	3.20	2.19	–0.031	0.05
β33	3.35	1.40	0.013	0.03
R^2	0.99		0.81	
F value (model)	2.83		2.27	
F value (Lack of fit)	10.31		0.56	

[a]$p < 0.05$.

The estimated maximum level of temperature for TPC was 59 °C using the model (Figure 1a). Although quadratic effect of methanol concentration for TPC was insignificant, linear effect and interaction of temperature and methanol concentration were significant (Table 2). Depending on temperature and time, 25% of methanol concentration gave the best extraction environment for TPC (Figure 1a,1b,1c). The linear and quadratic effects of UAE time were insignificant. The TPC started to decrease after increasing slowly until 38.86 min. The interaction of UAE temperature and time was highly effective ($p < 0.05$). The increase in TPC was observed depending on the increase in UAE temperature, whereas the UAE time was constant. In addition, TPC did not change at a constant UAE temperature despite the increase in UAE time. Similar results were obtained by Mohamed et al. for Argel leaf extracts [24]. In contrast, the TP content increased depending on the methanol concentration (Figure 1c). It was reported that the TP content of the methanol (80%) extracts from jujubes ranged from 275.6 to 541.8 mg GAE/100 g FW under unoptimized extraction parameters (by an ultrasonic bath for 20 min) [34].

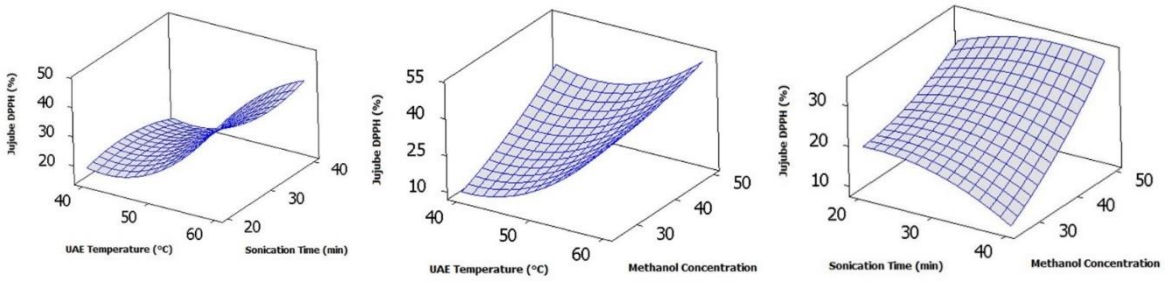


(a) (b) (c)

Figure 1. Response surface plot of TPC (mg GAE/g DW) of JE as a function of temperature, time and methanol concentration.

3.2.Effect of UAE factors on DPPH scavenging activity

The radical scavenging activity of DPPH from extracts is shown in Table 1. The highest DPPH radical scavenging activity (% inhibition) was recorded (50.16%) in the 15th trial at 60 °C (X_1), 20 min (X_2), and a 37.5% (X_3) under the UAE conditions, where 15 different factor combinations were applied. The lowest % inhibition was determined in the second trial (9.76%) at 40 °C of temperature, 40 min of time, and 37.5% of methanol. The DPPH radical scavenging activity of extracts was influenced significantly ($p < 0.05$) by the interaction between X_3 and X_1 according to the data of the multiple regression. Methanol–water mixture is classified as the most suitable extraction solvent due to its high dielectric constant/polarity [35]. In addition, it reduces phenolic degradation by inhibiting the activity of polyphenol oxidases [36].



(a) (b) (c)

Figure 2.Response surface plot of DPPH radical scavenging activity (%) of JE as a function of temperature, time and methanol concentration

Each of the extraction factors were increased to inhibition of DPPH quadratically ($p \leq 0.05$) (Table 2). However, linearity of each factors were found effective on DPPH significantly ($p \leq 0.05$). 3D plots have showed that the interaction relationship between independent variables and DPPH data. The increase in temperature and time caused an increase of DPPH (Figure 2a). However, the combined effect of temperature and methanol concentration was more effective on DPPH (Figure 2b). Although sonication time with methanol concentration increased DPPH inhibition, it was clearly seen that the main effect depends on the methanol concentration (Figure 2c). Previous studies have reported that a high extraction temperature increased the substance solubility, mass transfer, and solvent diffusion [37]. The increase in methanol concentration elevated the radical scavenging of DPPH (Figure 2c).

3.3.Optimization of the extraction parameters

The response variables of TPC and radical scavenging activity of DPPH under optimum conditions are given in Table 3. The maximum value was 4208.16 mg GAE/g DW for TPC at 49.89 °C, 20 min, and 50% of methanol concentration under predicted conditions and 52.51% for the maximum DPPH inhibition at 60 °C, 33.33 min of time, and 50% of methanol concentration under predicted UAE conditions.

Table 3. Optimum conditions and validation of predicted and experimental values obtained under the same conditions

Response variable	Optimum Extraction parameters			Maximum values	
	X_1	X_2	X_3	Predicted	Experimental
Individual responses					
TPC (mg GAE/g DW)	49.89	20.00	25.00	4208.16	3423.05
DPPH (% inhibition)	60.00	33.33	50.00	52.51	51.51
Combined responses					
TPC (mg GAE/g DW)	58.98	20.00	25.00	3670.36	3482.20
DPPH (% inhibition)				41.67	34.14

The combined experimental and predicted results for each response of TPC and radical scavenging of DPPH from extracts under optimum extraction parameters were compared. The experimental data for DPPH (34.14% inhibition) were lower than the predicted value (41.67% inhibition), whereas the predicted result (3670.36 mg GAE/g DW) obtained using the RSM model was close to the experimental result (3482.20 mg GAE/g DW) for TPC of JE. The value of total desirability as 0.95 was recorded in experiments at 58.98 °C and 25% methanol concentration for 20 min. The designed model fitted well for extracting phenolics from JE under optimum UAE conditions and predicted optimum extraction parameters. High TPC (6098.14 mg GAE/100 g DM) and antioxidant activity (4010.63 mg ascorbic acid equivalent/100 g DM) under optimal conditions (64.20% methanol, 73.60% ultrasound intensity, and 13.27 min) have been reported for jujube leaves [38]. On the other hand UAE has been reported as an effective method for extracting phenolic compounds from Moringa oleifera L. leaves. But that effect has no significantly related the ratio of liquid to solid and ultrasonic time. In the same study it has been reported that the optimal conditions for maximization of the TPC/TFC and antioxidant activities were 37% water content, 144 W ultrasonic power, and 40 °C temperature [39].

3.4.Phenolic compounds in Jujube leave extracts

The phenolic compounds obtained under optimum conditions from Jujube leave extracts using UAE were listed in Table 4. Phenolic acids (24307.9 mg/kg Jujube leave extracts DW) predominated among the 13 different phenolic compounds reported. Ellagic acid (13127.200 mg/kg Jujube leave extracts DW) and caffeic acid (7761.7 mg/kg Jujube leave extracts DW) were phenolics with the highest detectable amount, followed by rutin (3285 mg/kg Jujube leave extracts DW) and rosmarinic acid (467.6 mg/kg Jujube leave extracts DW). Other phenolics were detected in low amounts.

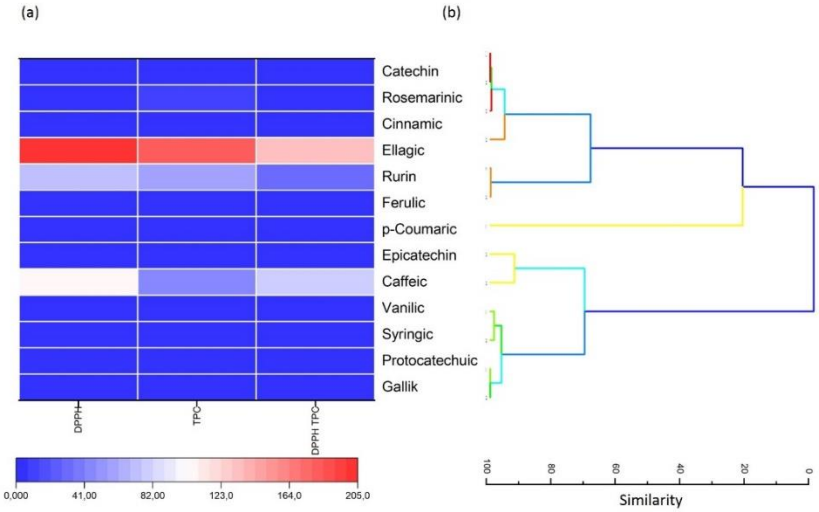


Figure 3.Heatmap (a) and cluster analysis (b) of phenolic compounds (mg/kg DW) in methanolic extracts of *Zyzypus jujube* leaf obtained under optimum extraction parameters for DPPH, TPC and combined response DPPH with TPC.

Table 4. Phenolic compounds of JE under optimized UAE conditions

Phenolic compound	Jujube leaf extract	
	Retention time (min)	Amount (mg kg^{-1} DW)
Catechin	15.2	133.1
Caffeic acid	22.7	7761.7
Cinnamic acid	71.1	6.800
Ferulic acid	30.1	85.600
Vanillic acid	19.2	8.200
Ellagic acid	47.7	13127.200
p-Coumaric acid	26.1	ND
Gallic acid	6.8	24.400
Rosmarinic acid	61.9	467.600
Protocatechuic	10.7	ND
acid	21.3	126.800
Epicatechin	45.7	3285.000
Rutin	15.7	9.000
Syringic acid		

Hydroxybenzoic acid, syringic acid, vanillic acid, and caffeic acid constitute the major polyphenolic antioxidant profile in Argel [24], mulberry *Salvia officinalis* L., *Rosmarinus officinalis* L., *Olea europaea* L., and *Punica granatum* L., *Ruta graveolens* L., *Mentha piperita* L., and *Petroselinum crispum* [40], and blackberry leaves [41]. Contrary to several conventional methods [42], UAE was used safely for effective recovery of phenolic compounds with less solvent and time requirement, as reported in previous studies [43]. The altered cell wall permeability by the cavitation effect of ultrasound accelerates the transition from the solid matrix to the liquid matrix [44].

4. Conclusion

In the present study, phenolic compounds were extracted from jujube leaves using ultrasound and their antioxidant properties were investigated. Extraction parameters such as temperature, time, and methanol concentration were optimized using RSM. UAE temperature and methanol concentration played a significant role in the extraction of jujube phenolics under optimized extraction parameters. Significant similarities were found between the experimental and predicted values of DPPH and TPC. Significant amounts of rutin, ellagic acid, rosmarinic acid, and caffeic acid were found in JE. These results demonstrated that JE can be added to foods as a good source of antioxidants

References

[1] Hammi, K.M., Jdey, A., Abdelly, C., Majdoub, H. and Ksouri, R., Optimization of ultrasound-assisted extraction of antioxidant compounds from Tunisian *Zizyphus lotus* fruits using response surface methodology, **Food Chemistry**, 184, 80-89, (2015).

[2] Al-Reza, S.M., Yoon, J.I., Kim, H.J., Kim, J.S. and Kang, S.C., Anti-inflammatory activity of seed essential oil from *Zizyphus jujube***, Food and Chemical Toxicology**, 48, 2, 639–43, (2010).

[3] Vahedi, F., Fathi Najafi, M. and Bozari, K., Evaluation of inhibitory effect and apoptosis induction of *Ziziphus Jujube* on tumor cell lines, an in vitro preliminary study, **Cytotechnology**, 56, 105–111, (2008).

[4] Maraghni, M., Gorai, M., and Neffati, M., Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of *Zizyphus lotus*, **South African Journal of Botany**, 76, 453–459, (2010).

[5] Kamiloğlu, Ö., Ercisli, S., Şengül, M., Toplu, C. and Serçe, S., Total phenolics and antioxidant activity of jujube (*Zizyphus jujube Mill*.) genotypes selected from Turkey, **African Journal of Biotechnology,** 8, 303–307, (2009).

[6] Serçe, S. and Özgen, M., Physical, Chemical, and Antioxidant Properties of Jujube Fruits from Turkey. **Chinese Dates A Traditional Functional Food**, Taylor & Francis Group, LLC, Section II, 199-204, (2016).

[7] Akbolat, D., Ertekin, C., Menges, H.O., Ekinci, K. and Erdal, I., Physical and nutritional properties of jujube (*Zizyphus jujuba Mill*.) growing in Turkey. **Chemistry: An Asian Journal**, 20, 757–766, (2008).

[8] San, B., Yildirim, A.N. (2010). Phenolic, alpha-tocopherol, beta-carotene and fatty acid composition of four promising jujube (*Ziziphus jujuba Miller*) selections. **Journal of Food Composition and Analysis**. 23, 706-710.

[9] Benammar, C., Hichami, A., Yessoufou, A., Simonin, A.M., Belarbi, M., Allali, H., Khan, N.A., *Zizyphus lotus L*. (Desf.) modulates antioxidant activity and human T-cell proliferation, **BMC Complement Alternative Medicine**, 24, 10–54, (2010).

[10] Hammi, K.M., Hammami, M., Rihouey, C., Le Cerf, D., Ksouri, R. and Majdoub, H., Optimization extraction of polysaccharide from Tunisian Zizyphus lotus fruit by response surface methodology: Composition and antioxidant activity, **Food Chemistry**, 212, 476–484, (2016).

[11] Arceusz, A., Wesolowski, M. and Konieczynski, P., Methods for Extraction and Determination of Phenolic Acids in Medicinal Plants: A Review, Natural Product **Communications**, 8, 1821 – 1829, (2013).

[12] Borgi, W., Ghedira, K. and Chouchane, N., Anti-inflammatory and analgesic activities of *Zizyphus lotus* root barks, **Fitoterapia**, 78, 16–19, (2007).

[13] Borgi, W. and Chouchane, N., Anti-spasmodic effects of Zizyphus lotus (L.) Desf. extracts on isolated rat duodenum. **Journal of Ethnopharmacology**, 126, 571–573, (2009).

[14] Şahin, S., Demir, C. and Malyer, H. Determination of phenolic compounds in Prunella L. by liquid chromatography-diode array detection. **Journal of Pharmaceutical and Biomedical Analysis**, 55, 1227–1230, (2011).

[15] Heleno, S.A., Prieto M.A., Barros, L, Rodrigues, A., Barreiro, M.F., Ferreira I.C.F.R., Optimization of microwave-assisted extraction of ergosterol from Agaricus bisporus L. by-products using response surface methodology, **Food and Bioproducts Processing**, 100, 25–35, (2016).

[16] Pawar, N., Pai, S., Nimbalkar, M., and Dixit, G., RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from different ginger cultivars. **Food Chemistry**, 126, 1330–1336, (2011).

[17] Crupi, P., Dipalmo, T., Clodoveo, M.L., Toci, A.T. and Coletta, A., Seedless table grape residues as a source of polyphenols: Comparison and optimization of non-conventional extraction techniques. **European Food Research and Technology**, 244, 1091–1100, (2018).

[18] Li, H.Z., Zhang, Z.J., Xue, J., Cui, L.X., Hou, T.Y., Li, X.J., Chen, T. Optimization of ultrasound-assisted extraction of phenolic compounds. antioxidants and rosmarinic acid from perilla leaves using response surface methodology, **Food Science and Technology Campinas**, 36, 686–693, (2016).

[19] Kagan, I.A., Effects of pH, sample size, and solvent partitioning on recovery of soluble phenolic acids and isoflavonoids in leaves and steams of red clover (*Trifolium pratense* cv. Kenland), **Natural Product Communications**, 6, 1657–1660, (2011).

[20] Wang, R., Ding, S., Zhao, D., Wang, Z., Wu, J. and Hu, X. Effect of dehydration methods on antioxidant activities, phenolic contents, cyclic nucleotides, and volatiles of jujube fruits, **Food Science and Biotechnology**, 25, 137–143, (2016).

[21] Dzah, C.S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G. and Ma, H., The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review, **Food Bioscience**, 35, 100547, (2020).

[22] Yu, L., Jiang, B.P., Luo, D., Shen, X.C., Guo, S., Duan, J.A. and Tang, Y.P. Bioactive components in the fruits of Ziziphus jujuba Mill. against the inflammatory irritant action of Euphorbia plants, **Phytomedicine**, 19, 239–244, (2012).

[23] Gomes, T., Caponio, F. and Allogio, V. Phenolic compounds of virgin olive oil: influence of paste preparation techniques, **Food Chemistry**, 64, 203-209, (1999).

[24] Mohamed Ahmed, I.A., Al-Juhaimi, F., Adisa, A.R. Adiamo, O.Q., Babiker, E.E., Osman, M.A., Gassem, M.A., Ghafoor, K., Alqah, H.A.S. and Elkareem, M.A., Optimization of ultrasound-assisted extraction of phenolic compounds and antioxidant activity from Argel (*Solenostemma argel* Hayne) leaves using response surface methodology (RSM), **Journal of Food Science and Technology**, 57, 3071–3080, (2020).

[25] Kedare, S.B. and Singh R.P., Genesis and development of DPPH method of antioxidant assay, **Journal of Food Science and Technology**, 48, 412–422, (2011).

[26] Che Zain, M.S., Jakariah, N.A., Yeoh, J.X., Lee, S.Y., and Shaari, K., Ultrasound-Assisted Extraction of Polyphenolic Contents and Acid Hydrolysis of Flavonoid Glycosides from Oil Palm (*Elaeis guineensis Jacq*.) Leaf: Optimization and Correlation with Free Radical Scavenging Activity, **Processes**, 8, 1540–1562, (2020).

[27] Sharmila, G., Nikitha, V.S., Ilaiyarasi, S, Dhivya K., Rajasekar, V., Manoj Kumar, N., Muthukumaran, K. and Muthukumaran, C., Ultrasound assisted extraction of total phenolics from Cassia auriculata leaves and evaluation of its antioxidant activities, **Industrial Crops and Products**, 84, 13–21, (2016).

[28] Singh, B., Singh, N., Thakur, S. and Kaur, A., Ultrasound assisted extraction of polyphenols and their distribution in whole mung bean hull and cotyledon, **Journal of Food Science and Technology**, 54, 921–932, (2017).

[29] Bayar, N., Bouallegue, T., Achour, M., Kriaa, M., Bougatef, A., Kammoun, R., Ultrasonic extraction of pectin from Opuntia ficus indica cladodes after mucilage removal: Optimization of experimental conditions and evaluation of chemical and functional properties, **Food Chemistry**, 235, 275–282, (2017).

[30] Kumar, K., Srivastav, S., Sharanagat, V.S., Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review, **Ultrasonic Sonochemistry**, 70, 105325, (2021).

[31] Ikhtiarini A.N., Setyaningsih, W., Rafi, M., Aminah, N.S., Insanu, M., Irnawati, I. and Rohman, A., Optimization of ultrasound-assisted extraction and the antioxidant activities of Sidaguri (*Sida rhombifolia*), **Journal of Applied Pharmaceutical Science**, 11, 070–076, (2021).

[32] Corrales, M., Garcı´a, A.F., Butz, P. and Tauscher, B. Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure, **Journal of Food Engineering**, 90, 415–421, (2009).

[33] Chanioti, S., Katsouli, M. and Tzia, C., Novel Processes for the Extraction of Phenolic Compounds from Olive Pomace and Their Protection by Encapsulation. **Molecules**, 26, 1781–1799, (2021).

[34] Gao, Q.H., Wu, C.S., Yu, J.G., Wang, M., Ma, Y.J., Li, C.L., Textural Characteristic, Antioxidant Activity, Sugar, Organic Acid, and Phenolic Profiles of 10 Promising Jujube (Ziziphus jujuba Mill.). Selections, **Journal of Food Science**, 77, 1218–1225, (2012).

[35] Majeed, M., Hussain, A.I., Chatha, S.A.S., Khosaa, M.K.K., Kamal, G.M., Kamal, M.A., Zhang, X., Liu, M., Optimization protocol for the extraction of antioxidant components from Origanum vulgare leaves using response surface methodology, **Saudi Journal of Biological Sciences**, 23:389–396, (2016).

[36] Ghoreishi, S.M. and Heidari, E., Extraction of Epigallocatechin-3-gallate from green tea via supercritical fluid technology: Neural network modeling and response surface optimization. **The Journal of Supercritical Fluids**, 74, 128–136, (2013).

[37] Getachew, A.T., Holdt, S.L., Meyer, A.S., Jacobsen, C., Effect of Extraction Temperature on Pressurized Liquid Extraction of Bioactive Compounds from Fucus vesiculosus. **Marine Drugs**, 20, 4, 263–279, (2022).

[38] Alioui, S.Z., Bey, M.B., Kurt, B.Z., Sonmez, F. and Louaileche, H., Optimization of ultrasound-assisted extraction of total phenolic contents and antioxidant activity using response surface methodology from jujube leaves (Ziziphus jujuba) and evaluation of anticholinesterase inhibitory activity, **Journal of Food Measurement and Characterization**, 13, 321–329, (2019).

[39] Wu, L., Li, L., Chen, S., Wang, L. and Lin, X., Deep eutectic solvent-based ultrasonic-assisted extraction of phenolic compounds from Moringa oleifera L. leaves: Optimization, comparison and antioxidant activity, **Separation and Purification Technology**, 247, 117014, (2020).

[40] Yu, M., Gouvinhas, I., Rocha, J., Barros, A.I.R.N.A., Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. **Scientific Reports**, 11, 10041–10055, (2021).

[41] Aybastıer Ö, Işık E, Şahin, S., and Demir, C., Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology, **Industrial Crops and Products**, 44, 558–565, (2013).

[42] Glisic, S.B., Ristic, M. and Skala, D.U., The combined extraction of sage (*salvia officinalis* L.) ultrasound followed by supercritical CO_2 extraction. **Ultrasonics Sonochemistry**, 18,318–326, (2011).

[43] Tao, Y., Wu, D., Zhang, Q.A. and Sun, D.W., Ultrasound-assisted extraction of phenolics from wine lees: Modeling, optimization and stability of extracts during storage, **Ultrasonics Sonochemistry**, 21, 706–715, (2014).

[44] Shirsath, S.R., Sonawane, S.H. and Gogate, P.R., Intensification of extraction of natural products using ultrasonic irradiations—A review of current status. Chemical Engineering and Processing, **Process Intensification**, 53: 10–23, (2012)n.