Songklanakarin J. Sci. Technol.
42 (1), 8-14, Jan. - Feb. 2020

http://www.sjst.psu.ac.th

*Original Article*

# Optimized ultrasound-assisted extraction of antioxidant and bioactive compounds from *Ferulago angulata* by response surface methodology

Abdoreza Aghajani[1], Seyed Ali Mortazavi[2*], Farideh Tabatabaei Yazdi[2], Masoud Shafafi Zenozian[1], and Mohammad Reza Saeidi Asl[1]

[1] *Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, 9618956878 Iran*

[2] *Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, 9177948978 Iran*

Received: 13 April 2018; Revised: 7 August 2018; Accepted: 29 August 2018

## Abstract

In this study, response surface methodology (RSM) was used to optimize the conditions for the extraction of total flavonoid content (TFC) and determine the free radical scavenging activity ($IC_{50}$ value) from *Ferulago angulata* extract by ultrasound bath at constant temperature. The investigated factors in this method were the solvent ratio (ethanol/water at 0 to 100%) and extraction time (4–8 min). Experiments were carried out based on a central composite design. The results showed that the extraction conditions had a significant effect on the $IC_{50}$ value and TFC extraction. The optimal conditions for TFC (0.56 mgRE/g) included ethanol content and extraction time and were determined to be 49.49% (v/v) and 7 min and 39 sec, respectively. Furthermore, the $IC_{50}$ value (1.13 mg/g) was 98.27% (v/v) and 8 min. It was also found that RSM in optimizing extraction conditions, especially in predicting TFC and $IC_{50}$ values, is an efficient method. These optimized conditions allow the rapid and maximum extraction of bioactive compounds from *F. angulata*.

**Keywords:** antioxidant activity, *Ferulago angulata*, bioactive compounds, response surface methodology

## 1. Introduction

As a result of consumer demand great attention has focused on replacement of synthetic additives by natural ones (Bimakr *et al.*, 2013). Antioxidants control rancidity development, retard the formation of toxic oxidation products, maintain nutritional quality, and extend the shelf-life of products (Yashin, Yashin, Xia, & Nemzer, 2017). Polyphenols are a large family of compounds found in fruits and vegetables which exhibit strong antioxidant activity (AA) (Ayoub, de Camargo, & Fereidoon, 2016., Tuyen *et al.*, 2017) by scavenging different groups of reactive oxygen species (Aras, Silinsin, Bingol, & Bursal, 2017). *Ferula* is a genus of perennial herbs belonging to the Apiaceae family. This genus is comprised of about 170 species distributed in central Asia, Mediterranean region, and Northern Africa (Akbarian, Rahimmalek, & Sabzalian, 2017). *Ferulago angulata (Schlecht.) Boiss*. (Chevil or Chavir in Persian) is an important medicinal and aromatic plant (Ghasemi Pirbalouti, 2010) which possesses strong antioxidant properties and can be used instead of synthetic antioxidants (Nassa, Bohlouli, & Ghanbari, 2018).

Extraction is a crucial stage in the isolation, identification, and use of bioactive compounds (Lapornik, Prosek, & Wondra, 2005). Traditionally, the extraction of antioxidants and phenols has been accomplished by percolation, soaking (Vinatoru, 2001), maceration and soxhlet extraction (Hamdaoui & Naffrechoux, 2007) which are very time-consuming and require relatively large quantities of toxic organic solvents (Majd, Rajaei, Bashi, Mortazavi, & Bolourian, 2014). Therefore, various novel extraction techniques have been introduced and investigated, most of

*Corresponding author
Email address: mortazavi_s.a@yahoo.com

which claimed to be better in terms of efficiency, solvent consumption, and extraction time. The novel techniques available are microwave-assisted extraction, ultrasound-assisted extraction (UAE), supercritical fluid extraction, and pressurized solvent extraction (Pederen & Olsson, 2003). UAE is based on the principle of acoustic cavitation which is capable of damaging the cell walls of the plant matrix and thereby favoring the release of bioactive compounds (Medina-Torres *et al*., 2017). UAE has been found to be a more effective and environmentally friendly way of extracting natural antioxidants from plant materials (Rosello-Soto *et al*., 2015). For these reasons, UAE from plant materials has been widely used lately to facilitate extractions of phenolic compounds from *Acer truncatum* Leaves (Yang *et al*, 2018), *Chrysanthemum morifolium* flower-head material (Yuan *et al*., 2015), *Jatropha integerrima* (Li *et al*., 2014), and other value-added compounds from various natural resources. Considering the maximization of extraction yield or target compound(s), with respect to the extracts quality, optimization of the solvent ratio and extraction time and other potential operating parameters are usually performed by response surface methodology (RSM) (Bas & Boyaci, 2007). This methodology is used to optimize the extraction processes of polyphenols from various plant materials through conventional and non-conventional extraction techniques (Kuo *et al*., 2013). The objective of this study was the optimization of extraction conditions (extraction time and solvent ratio) needed to extract the flavonoids and perform an antioxidant assay in *F. angulata* by RSM.

## 2. Materials and Methods

Absolute methanol and absolute ethanol were obtained from Merck Company (Germany), and Folin-Ciocalteu reagent, and 2-2-diphenyl-1-pycril hydrazine (DPPH) were purchased from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). All other chemicals were of analytical grade.

### 2.1 Preparation of the samples

The plant was confirmed to be *F. angulata* by the Herbarium group of the Research Institute of Forests and Rangelands of Iran (Alborz Province, Karaj). After drying in the shade, the *F. angulata* aerial parts were milled and transferred to the extraction section at the Laboratory of Science and Technology Park of Tehran University, Karaj. The drying time and temperature were 24 h at 60 °C, respectively. The aerial parts of the *F. angulata* were ground and passed through a 40 mesh screen that resulted in particle sizes of about 400 µm. Extraction was performed with water and ethanol. The dried aerial parts of the plant were mixed with solvent at the specified ratio (Table 1). Then, the mixture was filtered through Whatman filter paper No. 2. The residue was rinsed with the same solvent and the extract dried under vacuum. The yield of each extract was calculated (Table 4).

### 2.2 UAE of TFC and $IC_{50}$ value of *F. angulata*

UAE of the *F. angulata* was performed in a digitally-controlled ultrasonic bath (FS-60, Fisher Scientific, Germany). The extraction variables were selected according to Zhou *et al.* (2013). A sample of 3 g was placed into a flask (250 mL), soaked with ethanol at the given concentration in a specified ratio of liquid to solid, and then placed in an ultrasonic cleaning bath at 40 kHz for 4, 6, and 8 min at a constant temperature. Extracts were filtered through a filter paper under vacuum and the residue was extracted again three times with the same volume of fresh solvent. The filtrates were then combined and concentrated using a rotary evaporator at 50 °C under vacuum. Finally, the filtrate was prepared to a constant volume of 150 mL using 60% ethanol for the estimation of total flavonoid content (TFC) and antioxidant measurements through various chemical assays.

### 2.3 Total flavonoid content (TFC)

The TFC was measured by colorimetry of aluminum chloride. An extract in the amount of 0.5 mL, 0.1 mL of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 mL of distilled water were mixed. After 30 min at room temperature, the absorbance was read at 4.5 nm. Rutin was used as the standard for drawing the calibration curve where $y=0.0057x+0.0195$ ($R^2=0.9862$) (Chen, Xie, & Gong, 2007). The flavonoid content is expressed in milligram rutin equivalents per gram of extract (mgRE/g extract).

### 2.4 Antioxidant Assay: DPPH radical-scavenging activity

DPPH is a lipophilic radical showing peak absorbance at 517 nm. The hydroxyl groups of antioxidant compounds reduce the DPPH molecule by donating hydrogen to the free radical DPPH that is demonstrated by a change of color in the reaction solution from dark purple to bright yellow; therefore, absorbance at 517 nm decreases (Sousa *et al*., 2007). In this study, 40 µL of *F. angulata* extract were transferred to a test tube to which 1 mL of 0.2 mmol of DPPH was added. The decline in the absorbance at 517 nm after 30 min was read. The absorbance of DPPH without extract was the control. Radical scavenging was calculated by equation 1:

$$\text{DPPH scavenging (\%)} = ((A_0-A_1)/A_0) \times 100 \qquad (1)$$

where $A_0$ is the absorbance without extract (blank) and $A_1$ is the absorbance of the antioxidant extract. It should be noted that the blank was prepared as a sample but 40 µL distilled water was used instead of extract.

### 2.5 Experiment design by RSM

The independent variables of the process included the ethanol content (or ethanol-water ratio) (%:$X_1$) and extraction time (min:$X_2$). In other words, the sum of the ethanol and water as solvent was considered to be 100% in the experiments and for the calculations by RSM. The treatments were determined in the form of ethanol content (0–100%v/v) and extraction time (4–8 min) by Minitab software (Table 2). The model used in the RSM is generally a quadratic equation. For each dependent variable, a model is defined to determine the critical and interaction effects of factors on each individual variable. The multivariate model is given in equation 2, where Y is the predicted response, $_0\beta$ is the constant coefficient, $\beta_1$ and $\beta_2$ are linear coefficients, $\beta_{11}$ and $\beta_{22}$ are square effects

and $\beta_{12}$ and $\beta_{21}$ are interactions.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_1^2 X_1 X_2 + \beta_1^2 X_1^2 + \beta_2^2 X_2^2 \quad (2)$$

In this study, the central composite design was selected to optimize the process variables in 2 levels with 13 components, including 6 replicate at the central point for the estimation of the experimental error. The ranges and levels of independent variables are presented in Table 1. Experimental data were processed with a quadratic polynomial model, indicating the TF efficiency and DPPH radical scavenging activity as a function of independent variables .The statistical significance test was based on total error with 95 % confidence level ($P<0.05$).

### 2.6 Statistical analysis

In this study, Microsoft Office 2010 software was used to draw tables and charts and software Minitab version 16 was used to analyze the data and draw optimization charts. The data are in the form of a mean±standard deviation of three repetitions. The levels of the independent variables are presented in actual and coded form in Table 2. All experiments were run in triplicate. The deviation from the mean at the 95% confidence level was employed to determine the differences in results.

Table 2. Independent variable values of the process and their corresponding levels.

| +1 | 0 | –1 | Symbol | Independent variable |
|---|---|---|---|---|
| 100 | 50 | 0 | $X_1$ | Ethanol (%,v/v) |
| 8 | 6 | 4 | $X_2$ | Time (min) |

## 3. Results and Discussion

### 3.1 Selection of an appropriate model

The best results for TFC and DPPH were obtained under conditions of ethanol at 0–100% v/v and 4–8 min. In this experiment, the TFC (0.32–0.60 mgRE/g) and free radical scavenging activity (1.09–1.81 mg/g) were within the range. Data matching and analysis of variance (ANOVA) showed that the quadratic model is the most appropriate model. Statistical models that provide TFC and $IC_{50}$ values as a function of dependent variables within the studied range are presented in equations in Table 3 in which $X_1$ is the ethanol content and $X_2$ is the extraction time. The model accuracy with two $R^2$ and $R^2$. The adjusted parameters are also reported in ANOVA tables. The models were checked using a numerical method including the coefficient of determination ($R^2$). $R^2$ provided a measure of how well future outcomes are likely to be predicted by the model. In the models, $X_1$, $X_2$, $X_1^2$, $X_2^2$, and $X_1X_2$ were ethanol content (%), time (min), square ethanol (%), square time (min) and ethanol (%) × time (min) respectively. In general, lack of fit test for the model describes the variation in the data around the fitted model (Trinh & Kang, 2010).

### 3.2 Analysis of response surface the the TFC

The TFC obtained from the experiment and the predicted values by the software show that different extraction conditions have a significant effect on the flavonoid contents (Table 4). The TFC of the *F. angulata* extract varied from 0.32 to 0.60 (mg/g). The ANOVA results of the RSM in optimizing the flavonoids of *F. angulata* were identified and the ANOVA results of this quadratic polynomial model showed that the predicted model was significant ($P<0.05$) (Table 5). The $R^2$=82.08% and adj.$R^2$=89.28% of this model were obtained which indicated the goodness of fit of the model to the experimental data (Table 3). The time linear effects and second-order effects of ethanol ($X_1$) on TFC were significant ($P<0.05$).

In contrast, the linear effects of ethanol ($X_1$), second-order effects of time ($X_2^2$) and the interaction between ethanol-time ($X_1X_2$) were not significant ($P>0.05$). By increasing the extraction time to more than 7 min and an ethanol content from 45% to 50% (55% to 50% water), the TPC increased significantly ($P<0.05$) (Figure 1). Optimum conditions for extraction of flavonoids of *F. angulata* were 7 min and 39 sec and the ethanol content was 49.49% (Figure 1). The predicted results were confirmed by repeated trials and there were no significant differences between predicted and actual values (Table 4).

Table 1. Experimental central composite design for the optimization of extraction of *F. angulata* extract.

| $IC_{50}$(mg/g) | TFC (mg/g) | $X_2$ | $X_1$ | Treatments |
|---|---|---|---|---|
| 1.186 | 0.580 | 6 | 50 | 1 |
| 1.222 | 0.492 | 6 | 50 | 2 |
| 1.235 | 0.578 | 6 | 50 | 3 |
| 1.272 | 0.602 | 6 | 50 | 4 |
| 1.098 | 0.600 | 8 | 50 | 5 |
| 1.394 | 0.397 | 4.58 | 85.355 | 6 |
| 1.198 | 0.451 | 6 | 50 | 7 |
| 1.351 | 0.418 | 7.414 | 14.645 | 8 |
| 1.159 | 0.388 | 4 | 50 | 9 |
| 1.717 | 0.323 | 6 | 100 | 10 |
| 1.548 | 0.337 | 4.585 | 14.645 | 11 |
| 1.510 | 0.342 | 6 | 0 | 12 |
| 1.811 | 0.439 | 7.414 | 85.355 | 13 |

$X_1$: ethanol content (%., v/v)., $X_2$: time (min)

Table 3. Analysis of variance (ANOVA) response surface model models.

| Adj.$R^2$ | $R^2$ | Model | Source |
|---|---|---|---|
| 89.28 | 82.08 | $Y_2$= 0.540877-0.006676($X_1$)-0.052877($X_2$)-0.009649($X_1X_2$)-0.107719($X_1^2$)+0.027018($X_2^2$) | Total flavonoids (mgRE/g) |
| 81.60 | 89.27 | $Y_3$= 1.22307-0.07505($X_1$)-0.01675($X_2$)-0.15346($X_1X_2$)-0.23436($X_1^2$)-0.00834($X_2^2$) | $IC_{50}$ (mg/g) |

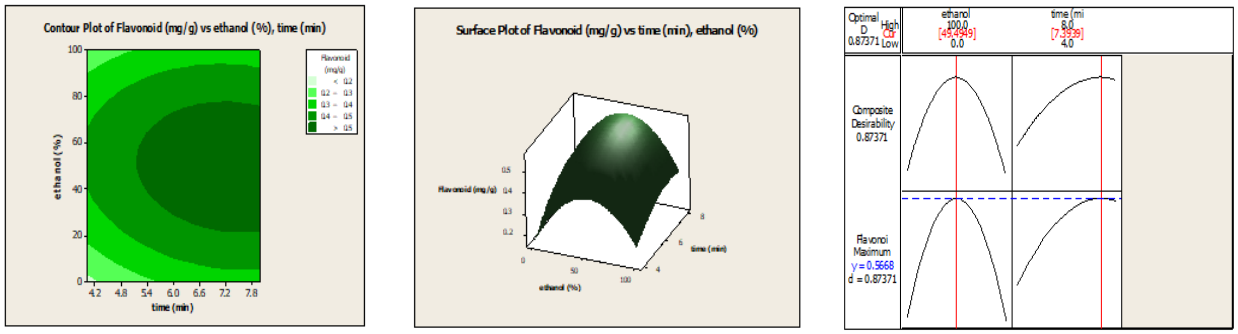
Table 4. TFC, $IC_{50}$ and extract yield of *F. angulata* in different conditions.

| Extract yield (%,w/w) | Predicted $IC_{50}$ value (mg/g) | $IC_{50}$ value (mg/g) | Predicted TFC (mg/g) | TFC (mg/g) | Treatments |
|---|---|---|---|---|---|
| 12.74 | 1.223 | 1.186 | 0.541 | 0.580 | 1 |
| 12.27 | 1.223 | 1.222 | 0.541 | 0.492 | 2 |
| 12.93 | 1.223 | 1.236 | 0.541 | 0.578 | 3 |
| 12.61 | 1.223 | 1.272 | 0.541 | 0.603 | 4 |
| 13.31 | 1.230 | 1.098 | 0.562 | 0.601 | 5 |
| 11.16 | 1.354 | 1.395 | 0.370 | 0.397 | 6 |
| 12.4 | 1.223 | 1.199 | 0.541 | 0.452 | 7 |
| 14.67 | 1.237 | 1.351 | 0.462 | 0.418 | 8 |
| 11.34 | 1.193 | 1.159 | 0.412 | 0.389 | 9 |
| 10.55 | 1.798 | 1.718 | 0.335 | 0.324 | 10 |
| 13.75 | 1.511 | 1.548 | 0.337 | 0.338 | 11 |
| 14.42 | 1.586 | 1.511 | 0.316 | 0.343 | 12 |
| 15.3 | 1.694 | 1.812 | 0.456 | 0.439 | 13 |

Table 5. Analysis of variance (ANOVA) response surface model of TFC and $IC_{50}$ values.

| $IC_{50}$ value | | TFC | | | |
|---|---|---|---|---|---|
| P value (Prob>F) | F value | P value (Prob>F) | F value | DF | Source |
| 0.003 | 11.64 | 0.015 | 6.41 | 5 | Regression |
| 0.145 | 2.58 | 0.090 | 3.47 | 2 | Linear |
| 0.062 | 4.91 | 0.751 | 0.11 | 1 | Ethanol (%,v/v)($X_1$) |
| 0.636 | 0.24 | 0.035 | 6.83 | 1 | Time (min)($X_2$) |
| 0.001 | 21.40 | 0.005 | 12.50 | 2 | Square |
| 0.001 | 41.64 | 0.002 | 24.65 | 1 | Ethanol(%,v/v)×ethanol (%,v/v) ($X_1^2$) |
| 0.825 | 0.05 | 0.253 | 1.55 | 1 | Time (min)×time (min)($X_2^2$) |
| 0.015 | 10.27 | 0.746 | 0.11 | 1 | Interaction |
| 0.015 | 10.27 | 0.746 | 0.11 | 1 | Ethanol(%,v/v)×time (min)($X_1X_2$) |

*Significant effect (P<0.05).




a b c

Figure 1. The contour (a) and surface (b) plot and optimal conditions (c) for extraction of TFC (mg/g) vs time (min), ethanol (%).

Ethanol and water mixtures are commonly used for the extraction of bioactive compounds from plant materials because a wide range of phenols can dissolve in aqueous ethanol mixtures which are acceptable for human consumption models (Alothman, Rajeev, & Karim, 2009). It has been reported that polar extracts have higher AA than non-polar species due to their phenolic acids and flavonoids (Kamkar,

Jebelli Javan, Asadi, & Kamalinejad, 2010). Therefore, the role of ethanol in extraction cannot be ignored.

### 3.3 The analysis of response surface of $IC_{50}$ value (DPPH method)

The $IC_{50}$ values obtained from the experiment and the predicted values by the software are presented in Table 4 which shows that different extraction conditions have significant effects on the $IC_{50}$ values.

The $IC_{50}$ values of the *F. angulata* extract varied from 1.09 to 1.86 (mg/g). The results of ANOVA on a quadratic polynomial model indicated that the predicted model was significant ($P<0.05$) (Table 5). The $R^2$=89.27% and adj.$R^2$=81.60% of this model were obtained (Table 3). The second-order effects of ethanol ($X_1^2$) and the interactions of ethanol × time ($X_1X_2$) on $IC_{50}$ value were significant ($P<0.05$) while the linear effects of variables ($X_1$,$X_2$) and the quadratic effect of time ($X_2^2$) were not significant ($P>0.05$) (Table 5).

Increasing the extraction time from 7 to 8 min and water content to more than 70% reduced the $IC_{50}$ value or the highest free radical scavenging activity (Figure 2). Optimum conditions for obtaining the highest free radical scavenging activity of *F. angulata* extract were 8 min and water content of 72.02%. The predicted results were confirmed by repeated trials and there were no significant differences between predicted and actual values (Table 4). The optimum conditions for obtaining the highest radical scavenging activity of *F. angulata* was 8 min and ethanol content equal to 27.98% (water content: 72.02%) (Figure 2). One of the important observations of this study is that AA was related to TFC and the extraction time. However, in many cases a lack of correlation between TFC and the antioxidant capacity may have many reasons. For example, the observed AA is not only due to flavonoids but probably also due to the presence of other phytochemicals such as ascorbic acid, tocopherols, and pigments which have a synergistic effect and aid the antioxidant capacity. The extract probably contains various types of phenolic compounds that have different antioxidant capacities. However, the $R^2$ value indicated a significant effect of TFC on AA.

The antioxidant activity of various extracts is often used as an $IC_{50}$ value. $IC_{50}$ refers to the concentration of extract in which 50% of the DPPH free radicals contained in the reaction are inhibited. Therefore, a lower concentration indicates greater antiradical activity of the extract (Xiao, Han, & Shi, 2008). Grujic *et al.* (2012) reported that the AA of mate tea extracts was higher in 40% ethanol extracts than in 50% and 60% ethanol content. In contrast, Mohammedi and Atik (2012) reported that the AA of *Tamarix aphylla* leaf extracts was higher in 70% ethanol extracts than water extracts. The antioxidant capacity has often been correlated with the flavonoids (Zahin, Aqil, & Ahmad, 2009), although some authors have reported that the AA was not correlated with the TFC (Ruanma *et al*., 2010). Although the relationship between the molecular structure and the AA was not investigated in this study, the AA of plant extracts is strongly dependent on the solvent due to different antioxidant potentials of compounds with different polarities.

## 4. Conclusions

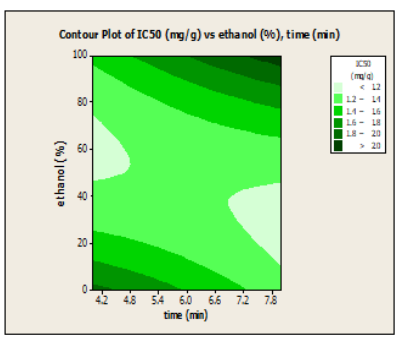
The RSM was successfully applied for optimization of the conditions for UAE of TFC and AA of *F. angulata* extracts. ANOVA showed that a second-order polynomial model provided adequate mathematical description of the UAE of TFC with high AA. To further verify the models obtained from RSM, flavonoids were extracted under the predicted optimal UAE conditions and the AA and TFC were evaluated and compared to the predicted maximum. For operational convenience, the optimal parameters were modified slightly in the verification experiment as follows: ethanol content 49.49% (v/v); extraction time 7 min and 39 sec (7′:39″) for the TFC (0.56 mgRE/g) and 27.98% (v/v); and 8 min for the $IC_{50}$ value (1.13 mg/g). No significant differences were observed between the predicted and experimental values ($P>0.05$) which indicated that the experimental results confirmed adequate fitness of the predicted model.

## Acknowledgements

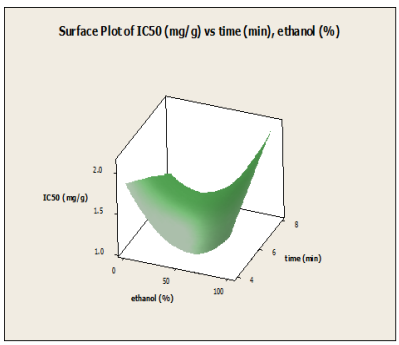
This work was supported by the Laboratory of Science and Technology Park of Tehran University (Karaj). The researcher gratefully thanks Dr. Pooneh Amini Geram for his assistance in the experimental work.

## References

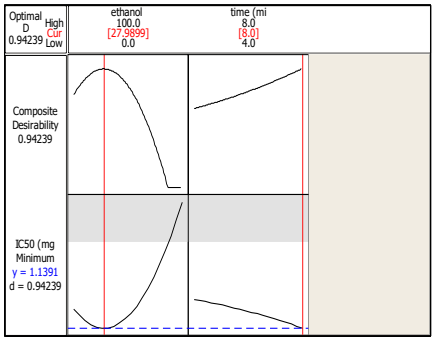
Akbarian, A., Rahimmalek, M, & Sabzalian, M. R. (2017). Variation in fruit morphological traits and bioactive compounds in different populations of Ferula assa-foetida, F. gummosa, and F. ovina Collected from Iran. *Journal of Agricultural Science and Technology*, *19*, 425-438.




a




b




c

Figure 2. The contour (a) and surface (b) plot and optimal conditions (c) for $IC_{50}$ values (mg/g) vs time (min), ethanol (%).


Alothman, M., Rajeev, B., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*, *115*, 785–788.

Aras, A., Silinsin, M., Bingol, M. N., & Bursal, E. (2017). Identification of bioactive polyphenolic compounds and assessment of antioxidant activity of *Origanum acutidens*. *International Letters of Natural Sciences*, *66*, 1-8.

Ayoub, M., de Camargo, A. C., & Fereidoon, S. (2016). Antioxidants and bioactivities of free, esterified and insoluble-bound phenolics from berry seed meals. *Food Chemistry*, *197*, 221–232.

Baş, D., & Boyaci, H. (2007). Modeling and optimization I: Usability of response surface methodology. *Journal of Food Engineering*, *78*, 836–845.

Bimakr, M., Rahman, R. A., Saleena Taip, F., Adzahan, N. M., Islam Sarker, Z., & Ganjloo, A. (2013). Ultrasound-assisted extraction of valuable compounds from winter melon (*Benincasa hispida*) seeds. *International Food Research Journal*, *20*(1), 331-338.

Chen, Y., Xie, M. Y., & Gong, X. F. (2007). Microwave-assisted extraction used for the isolation of total triterpenoid saponins from *Ganoderma atrum*. *Journal of Food Engineering*, *81*, 162–170.

Cujic, N., Savikin, K., Jankovic, T., Pljevljakusic, D., Zdunic, G., & Ibric, S. (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chemistry*, *194*, 135–142.

Ghasemi Pirbalouti, A. (2010). *Medicinal and aromatic plants* (Introduction and application, 3rd ed.). Shahrekord, Iran: IAU Press.

Hamdaoui, O., & Naffrechoux, E. (2007). An investigation of the mechanisms of ultrasonically enhanced desorption. *American Institute of Chemical Engineers*, *53*, 363–373.

Kamkar, A., Jebelli Javan, A., Asadi, F., & Kamalinejad, M. (2010). The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. *Food and Chemistry Toxicology*, *48*, 1796-1800.

Kuo, C. H., Hsiao, F. W., Chen, J. H., Hsieh, C. W., Liu, Y. C., & Shieh, C. J. (2013). Kinetic aspects of ultrasound-accelerated lipase catalyzed acetylation and optimal synthesis of 4'-cetoxyresveratrol. *Ultrasonics Sonochemistry*, *20*, 546–552.

Lapornik, B., Prosek, M., & Wondra, A. G. (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering*, *71*, 214–222.

Li, A. N., Li, S., Li, H. B., Xu, D. P., Xu, X. R., & Chen, F. (2014). Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. *Journal of Functional Foods*, *6*, 319–330.

Majd, M. H., Rajaei, A., Bashi, D. S., Mortazavi, S. A., & Bolourian, S. (2014). Optimization of ultrasonic-assisted extraction of phenolic compounds from bovine pennyroyal (*Phlomidoschema parviflorum*) leaves using response surface methodology. *Industrial Crops and Products*, *57*, 195–202.

Medina-Torres, N., Ayora-Talavera, T., Espinosa-Andrews, H., Sanchez-Contreras, A., & Pacheco, N. (2017). Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. *Agronomy*, *7*(47), 1-19.

Mohammedi, Z., & Atik, F. (2011). Impact of solvent extraction type on total polyphenols content and biological activity from *Tamarix Aphylla (L.) karst*. I*nternational Journal of Pharma and BioSciences*, *2*, 609–615.

Nassab, G. R., Bohlouli, S., & Ghanbari, A. (2018). Therapeutic effect of *Ferulago angulata* extract on reproductive parameters and serum testosterone levels in Diabetic Male Rats. *Journal of Reports in Pharmaceutical Sciences*, *7*(1), 1-8.

Pedersen, J. R., & Olsson, J. O. (2003). Soxhlet extraction of acrylamide from potato chips. *Analyst*, *128*, 332–334.

Rosello-Soto, E., Galanakis, C. M., Brncic, M., Orlien, V., Trujillo, F. J., Mawson, R., . . . Barba, F. J. (2015). Clean recovery of antioxidant compounds from plant foods, by-products and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. *Trends in Food Science and Technology*, *42*, 134–149.

Ruanma, K., Shank, L., & Chairote, G. (2010). Phenolic content and antioxidant properties of green chilli paste and its ingredients. *Maejo International Journal of Science and Technology*, *4*, 193–200.

Sousa, C. M. M., Silva, G. M., Vieira Junior, M. C., Ayres, C. L. S., Costa, D. S., Araujo, L.C.D., & Chaves, M. H. (2007). Fenois totaise atividade antioxidante de cinco plantas medicinais. *Quimica Nova*, *30*, 351-355.

Trinh, T. K., & Kang, L. S. (2010). Application of response surface method as an experimental design to optimize coagulation test. *Environmental Engineering Research*, *15*, 63–70.

Tuyen, P. T., Xuan, T. D., Khang, D. T., Ahmad, A., Quan, N. V., Anh, T. T. T., . . . & Minh, T. N. (2017). Phenolic compositions and antioxidant properties in Bark, Flower, Inner Skin, Kernel and Leaf Extracts of Castanea crenata Sieb. et Zucc. *Antioxidants*, *6*(31), 1-14.

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*, *8*, 303−313.

Xiao, W., Han, N., & Shi, B. (2008). Microwave - assisted extraction of flavonoids from *Radix Astragali*. *Separation and Purification Technology*, *62*(3), 614-618.

Yang, L., Yin, P., Li, K., Fan, H., Xue, Q., Li, X., . . . Liu, Y. (2018). Seasonal dynamics of constitutive levels of phenolic components lead to alterations of antioxidant capacities in *Acer truncatum* leaves. *Arabian Journal of Chemistry*, *11*, 14-25.

Yashin, A., Yashin, Y., Xia, X., & Nemzer, B. (2017). Antioxidant activity of spices and their impact on human health: A review. *Antioxidants*, *6*(70), 1-18.

Yuan, J., Huang, J., Wu, G., Tong, J., Xie, G., Duan, J-A., & Qin, M. B. (2015). Multiple responses optimization of ultrasonic-assisted extraction by response surface methodology (RSM) for rapid analysis of bioactive compounds in the flower head of Chrysanthemum *morifolium Ramat*. *Industrial Crops and Products*, *74*, 192–199.

Zahin, M., Aqil, F., & Ahmad, I. (2009). The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. *International Journal of Pharmacy and Pharmaceutical Sciences*, *1*, 88–95.

Zhou, J., Zheng, X., Yang, Q., Liang, Z., Li, D., Yang, X., & Xu, J. (2013). Optimization of ultrasonic-assisted extraction and radical-scavenging capacity of phenols and flavonoids from *Clerodendrum cyrtophyllum turcz* leaves. *PLOS ONE, 8*(7), 1-8.