





## Optimization of Ultrasound-Assisted Acidic-Solvent Extraction of Colchicine from *Colchicum kurdicum* (Bornm.) Stef. Using Response Surface Methodology

Mohammad Azadbakht<sup>1</sup> , Elnaz Khoshvishkaie<sup>2</sup>, Ali Davoodi<sup>1\*</sup> , Seyed Jalal Hosseinimehr<sup>3</sup>, Masoud Azadbakht<sup>4</sup>, Saeed Emami<sup>5</sup>, Hossein Bakhshi Jouybari<sup>1</sup>, Fatemeh Mirzaee<sup>1</sup>, Kiana Ghadiri<sup>6</sup>

<sup>1</sup>Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

<sup>2</sup>Pharmaceutical Cares Department, Ayatollah Khamenei Hospital, Mazandaran University of Medical Sciences, Abbas Abad, Iran.

<sup>3</sup>Department of Radiopharmacy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

<sup>4</sup>Department of Plant Systematics, High Educational of Sanna Institute, Sari, Iran.

<sup>5</sup>Department of Medicinal Chemistry and Pharmaceutical Sciences Research Centre, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

<sup>6</sup>Active Pharmaceutical Research Centre (APIRC), Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

---

### Abstract

**Background and objectives:** *Colchicum kurdicum* (Bornm.) Stef. is a flowering perennial monocotyledon plant that has many important bioactive compounds especially colchicine and colchicine derivatives. In this study, the ultrasound-assisted acidic-solvent extraction method coupled with response-surface method was presented as the successful method for large scale extraction of colchicine as an alkaloid compound. Moreover, *Colchicum kurdicum* was introduced as an important endemic plant for extraction of colchicine. **Methods:** According to the literatures, methanol/deionized water (70:30) solvent system was selected for the extraction. In addition, the response-surface method was used for analysis and optimization of colchicine extraction by ultrasonic-assisted acidic-solvent extraction method. Subsequently, colchicine was extracted using this method and the effects of solvent pH, extraction time, solvent/plant ratio, power, and temperature were evaluated. **Results:** After all analysis procedures, 0.99 mg colchicine/g dried corms was achieved with the following conditions: solvent pH 4, Extraction time 120 minutes, solvent/plant ratio 20 mL/g, power 100 W, and temperature 60 °C. **Conclusion:** According to this study, ultrasonic-assisted acidic-solvent extraction was found an effective method for extraction of colchicine from *Colchicum kurdicum* (Bornm.) Stef. compared to other extraction methods.

**Keywords:** colchicine; *Colchicum kurdicum*; extraction; HPLC; ultrasound

**Citation:** Azadbakht M, Khoshvishkaie E, Davoodi A, Hosseinimehr SJ, Azadbakht M, Emami S, Bakhshi Jouybari H, Mirzaee F, Ghadiri K. Optimization of ultrasound-assisted acidic-solvent extraction of colchicine from *Colchicum kurdicum* (Bornm.) Stef. using response surface methodology. Res J Pharmacogn. 2020; 7(3): 5-13.

---

\* Corresponding author: ali.davoodi@mazums.ac.ir, adavoodi.pharm@gmail.com

## Introduction

*Colchicum kurdicum* (Bornm.) Stef. is a flowering perennial monocotyledon plant that is indigenous in Iran, Turkey and Europe; it has the bulb-like corms and underground ovaries as individual morphological characteristics [1,2]. All organs of this plant especially the corm and seeds have been used for inflammations, rheumatoid arthritis, gout, joint pains and cancer in ethnomedicines and traditional medicines [1,3,4].

Some bioactive compounds have been isolated from *Colchicum kurdicum* including tropolone and isoquinoline alkaloids, carbohydrates, flavonoids and other phenolic compounds, which have induced different biological effects [1,2].

Tropolone alkaloids are the main bioactive compounds from *C. kurdicum*, which are potent tubulin polymerization inhibitors, anti-mitotic agents and P-glycoprotein inhibitors. Moreover, colchicine has been used for treatment of gout and showed to be effective in some diseases such as Behcet's syndrome and Mediterranean fever [1,5].

Anticholinesterase isoquinoline alkaloids, phenolic acids such as coumaric acid, ferulic acid, caffeic acid, vanillic acid, 2-hydroxybenzoic acid have been isolated from different species of *Colchicum* [1, 5].

Colchicine is a tropolone alkaloid with  $C_{22}H_{25}NO_6$  molecular formula and 399.437 g/mol molecular weight. The availability of this bioactive compound is very important to use in relevant diseases and the optimized methods are needed to extract colchicine from natural sources. Colchicine as an alkaloid compound has a specific extraction method which is dependent to the nitrogen atom in the chemical structure. The main method for extraction of colchicine is sequential acidifying-basifying method with high yielding value. Other methods such as supercritical fluid extraction, preparative thin layer chromatography, percolation and Soxhlet extraction have been used for high scale extraction of colchicine [1,6,7].

One of the main effective methods for extraction of the bioactive compounds with high yielding value and low chemical structure damages is ultrasonic-assisted extraction method [8,9].

In the ultrasound-assisted extraction method, the plant cell wall disrupts by ultrasound energy and the bioactive compounds are extracted effectively by the solvent; moreover, in the acidic-solvent modifying method, the specific extraction of

alkaloid compounds could be optimized [8,10]. This ultrasound-assisted acidic-solvent extraction method has several parameters for optimization of alkaloid extraction including solvent pH, time of extraction, solvent/plant ratio, power of apparatus and temperature.

The response surface method is a statistical-mathematical method used for optimization of effective parameters on experimental processes such as extraction, drug formulation and secondary metabolite production [11].

In the present study, ultrasonic-assisted extraction method coupled with response surface method was used for optimization of extraction of colchicine from *Colchicum kurdicum* (Bornm.) Stef. In addition, Box-Benken method and polynomial model were used for reducing the number of tests and identifying the interaction between the parameters, respectively. The main purpose of this study was optimization of colchicine extraction from the corms of *C. kurdicum*. Moreover, the best ratio of extraction parameters including solvent pH, extraction time, solvent/plant ratio power of apparatus and temperature were obtained.

## Material and Methods

### Ethical considerations

Ethics Committee of Mazandaran University of Medical Sciences approved the study protocol (IR.MAZUMS.REC1398.113).

### Plant materials

The plant specimen was collected from 1500 to 2500 m heights of Tang-e-Rah area of the Golestan National Park (37.366335, 55.780033), Golestan province during October to April 2018. The taxonomic identification of plant was identified by Dr. Masoud Azadbakht as taxonomist, and representative voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy of Mazandaran University of Medical Science (E1-11312). Figure 1 shows the natural and herbarium view of plant specimen. The dried corms of *Colchicum kurdicum* (Bornm.) Stef. Were powdered to 200 mesh size by mill. The optimized conditions of colchicine extraction from the corms of *C. kurdicum* were obtained by response surface methodology using analysis of different effective parameters on the extraction yield.



**Figure 1.** *Colchicum kurdicum* (Adapted from <https://www.biolib.cz/>)

### Experimental design and optimization of the extraction process

In this study, solvent pH, time of extraction, solvent/plant ratio, power of apparatus and temperature were considered the main parameters for extraction of colchicine by ultrasound-assisted extraction method [8]. Moreover, the response surface analysis was applied by quadratic Box-Benhken method and linear and Box-Cox models. Briefly, the minimum and maximum levels of the five parameters (A: solvent pH, B: time of extraction, C: solvent/plant ratio, D: power of apparatus, E: temperature) were used for determination of the number and conditions of the tests. The Box-Benhken method was completed using the Design-Expert software 7.0.0 (Stat-Ease Inc., Minneapolis, USA) with 46 experiments (with 3 replicates of factorial points) [9,10].

### Ultrasound-assisted extraction system

The extraction of colchicine was conducted in the  $5 \times 15$  cm glass beaker by ultrasonic bath (Elmasonic P 60 Hz, Germany) at different solvent pH (1-7), time of extraction (60-129 min), solvent/plant ratio (5-20 mL/g), power of apparatus (5-100 W), and temperatures (30-60 °C) using methanol/water solvent system (30:70). All extraction processes were carried out at the constant ultrasonic frequency 37 KHz. After the sonication of samples, the extracts were concentrated by rotary evaporator in 40 °C and freeze-dried. Finally, the obtained extracts were analysed using HPLC method [9,10].

### HPLC analysis

The amount of colchicine in corm extracts of *C. kurdicum* was determined by HPLC coupled with UV spectrophotometry. The separation, detection and assay of colchicine was performed by a HPLC Smartline Manager 5000 (Knauer, Germany) with Smartline pump 1000 and EC

Nucleodur C<sub>18</sub> column (4.6 mm × 250 mm, 5 μm particle size) at 25 °C with UV detector 2500 basic model in 245 nm. Data acquisition was performed by EZchrom Elite 3.2.0 software. The confidence of the accuracy of the individuality of the peaks was obtained by standard addition method. Briefly, the extracts (1 mg/mL HPLC graded methanol) and the HPLC grade solutions of colchicine (125, 250, 500, 1000 and 2000 ppm in HPLC graded methanol) as standard were injected into the HPLC with 20 μL volume and 1 mL/min flow rate. The mobile phases were HPLC grade acetonitrile (ACN) and deionized water. The gradient condition was: 0-5 min, ACN 10%; 5-20 min, ACN 100%; 20-25 min, ACN 10%; 25-30 min, ACN 10%. Finally, the calibration curve of colchicine was obtained by plotting the peak area against the concentration and the amount of colchicine was calculated by the obtained formula [1,10,11].

### Statistical analysis

The Analysis of variance was applied to determine the regression coefficient and the suitable mathematical model. F-value, p-value, R<sup>2</sup>, R<sup>2</sup><sub>Adjusted</sub>, and R<sup>2</sup><sub>Predicted</sub> were predicted [10].

### Results and Discussion

Table 1 shows the parameter conditions and the actual and coded levels of the variables of this study.

The Box-Cox plot of power transforms has been demonstrated in figure 2. This plot represents the Lambda value +0.86 with low confidence interval -0.52 and high confidence interval +2.31. Moreover, a natural log was used instead the predicted values. Table 2 shows the extraction conditions of colchicine based on Box-Benhken analysis method. A linear model was used for data normalization. Moreover, tables 2 and 3 show the result of HPLC analysis and ANOVA. Table 4 shows the calculated regression coefficients indicated, which demonstrated that the predicted values were close to the actual values. Figure 3 represents the predicted values versus actual values of study. The value of adequate precision was 53.85, indicating an adequate signal.

The effects of all parameters on colchicine extraction yield were analysed. Response surface and contour plots of the study parameters were presented in figure 4. Figure 4A shows the effects of solvent pH and extraction time on colchicine

extraction; figure 4B shows the effects of extraction time and solvent/plant ratio on colchicine extraction while figure 4C shows the effects of solvent/plant ratio and power on the

colchicine extraction and figure 4D the effects of power and temperature of colchicine extraction. Figure 3E shows the effects of temperature and solvent pH on the colchicine extraction.

**Table 1.** The actual and coded levels of independents variables of this study

Variables	Levels					
	Low Actual	Median Actual	High Actual	Low Coded	High Coded	Standard Deviation
<b>A: Solvent pH</b>	1	4	7	-1	+1	1.8
<b>B: Extraction Time (min)</b>	60	120	180	-1	+1	35.4
<b>C: Solvent/Plant Ratio</b>	5	12.5	20	-1	+1	4.4
<b>D: Power (W)</b>	5	52.5	100	-1	+1	28.1
<b>E: Temperature (°C)</b>	30	45	60	-1	+1	8.8

**Table 2.** Experimental designs and the responses using ultrasonic-assisted extraction method

Run	Solvent pH	Time (min)	Solvent/Plant (mL/g)	Power (W)	Temperature (°C)	Colchicine/Plant (mg/g)
1	4.00	120.00	12.50	52.50	45.00	0.98
2	4.00	120.00	5.00	5.00	45.00	0.45
3	4.00	180.00	5.00	52.50	45.00	0.76
4	1.00	180.00	12.50	52.50	45.00	0.83
5	7.00	120.00	20.00	52.50	45.00	0.79
6	4.00	60.00	12.50	52.50	30.00	0.63
7	4.00	120.00	12.50	100.00	60.00	0.93
8	4.00	180.00	12.50	52.50	30.00	0.85
9	1.00	120.00	12.50	100.00	45.00	0.87
10	4.00	120.00	12.50	52.50	45.00	0.63
11	1.00	120.00	12.50	5.00	45.00	0.54
12	4.00	180.00	12.50	100.00	45.00	0.69
13	7.00	180.00	12.50	52.50	45.00	0.73
14	4.00	120.00	5.00	52.50	60.00	0.61
15	4.00	120.00	20.00	52.50	30.00	0.87
16	4.00	60.00	12.50	100.00	45.00	0.91
17	4.00	60.00	12.50	5.00	45.00	0.53
18	1.00	120.00	12.50	52.50	60.00	0.81
19	7.00	60.00	12.50	52.50	45.00	0.62
20	4.00	60.00	12.50	52.50	60.00	0.71
21	4.00	120.00	5.00	52.50	30.00	0.64
22	7.00	120.00	12.50	52.50	30.00	0.52
23	4.00	120.00	12.50	52.50	45.00	0.58
24	4.00	120.00	20.00	5.00	45.00	0.72
25	4.00	120.00	20.00	100.00	45.00	0.99
26	4.00	120.00	12.50	100.00	30.00	0.92
27	4.00	60.00	20.00	52.50	45.00	0.85
28	4.00	60.00	5.00	52.50	45.00	0.74
29	4.00	180.00	20.00	52.50	45.00	0.85
30	7.00	120.00	5.00	52.50	45.00	0.64
31	4.00	120.00	12.50	52.50	45.00	0.72
32	7.00	120.00	12.50	5.00	45.00	0.69
33	4.00	120.00	12.50	5.00	30.00	0.51
34	4.00	180.00	12.50	5.00	45.00	0.55
35	4.00	120.00	12.50	52.50	45.00	0.62
36	4.00	120.00	12.50	52.50	45.00	0.71
37	4.00	120.00	5.00	100.00	45.00	0.86
38	1.00	60.00	12.50	52.50	45.00	0.72
39	1.00	120.00	12.50	52.50	30.00	0.78
40	1.00	120.00	5.00	52.50	45.00	0.76
41	4.00	120.00	20.00	52.50	60.00	0.89
42	1.00	120.00	20.00	52.50	45.00	0.92
43	7.00	120.00	12.50	52.50	60.00	0.82
44	4.00	120.00	12.50	5.00	60.00	0.56
45	7.00	120.00	12.50	100.00	45.00	0.72
46	4.00	180.00	12.50	52.50	60.00	0.95

Figure 5 illustrated the effect of interaction of parameters on extraction yield of colchicine; figure 5A demonstrates the interaction between the solvent pH and extraction time; figure 5B displays the interaction between the extraction time and solvent/plant ratio; figure 5C shows the interaction between solvent/plant ratio and power; figure 5D displays the interaction between power and temperature and figure 5E demonstrates the interaction between temperature and solvent pH. In the present study, extraction of colchicine has been optimized by ultrasound-assisted acidic-solvent extraction method and statistical-mathematical response-surface methodology and five variables including solvent pH, time of extraction, solvent/plant ratio, power of apparatus, and temperature have been selected and analyzed using the Box-Benhken method and polynomial model.

According to this analysis, the Lambda value created the best power fit model and the studied

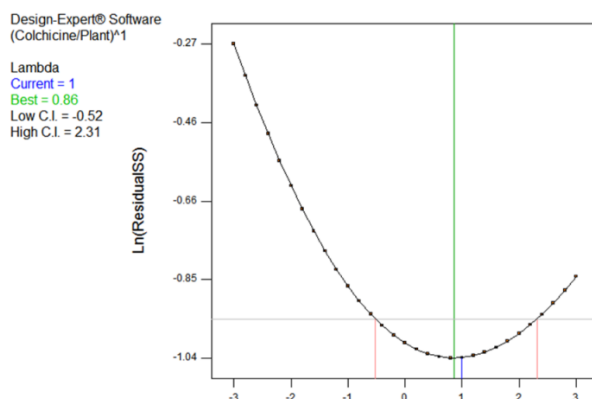
model showed high F-value and low p-value and was significant, which is suitable for the optimization of parameters of colchicine extraction.

**Table 3.** Analysis of variance for the response surface polynomial model

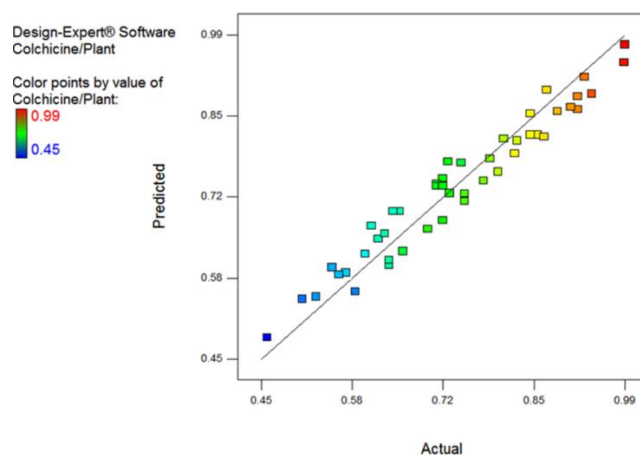
Source	df <sup>b</sup>	F-Value	P-Value	Significance
<b>Model</b>	5	0.45	< 0.0001	Yes
<b>A</b>	1	0.10	0.1098	Yes
<b>B</b>	1	0.06	0.2322	Yes
<b>C</b>	1	0.47	0.0011	Yes
<b>D</b>	1	1.53	< 0.0001	Yes
<b>E</b>	1	0.09	0.1357	Yes
<b>Residual</b>	40	-	-	-
<b>Lack of fit</b>	35	0.56	0.8589	No
<b>Pure error</b>	5	-	-	-
<b>Total</b>	45	-	-	-

**Table 4.** Statistical parameters for the polynomial model

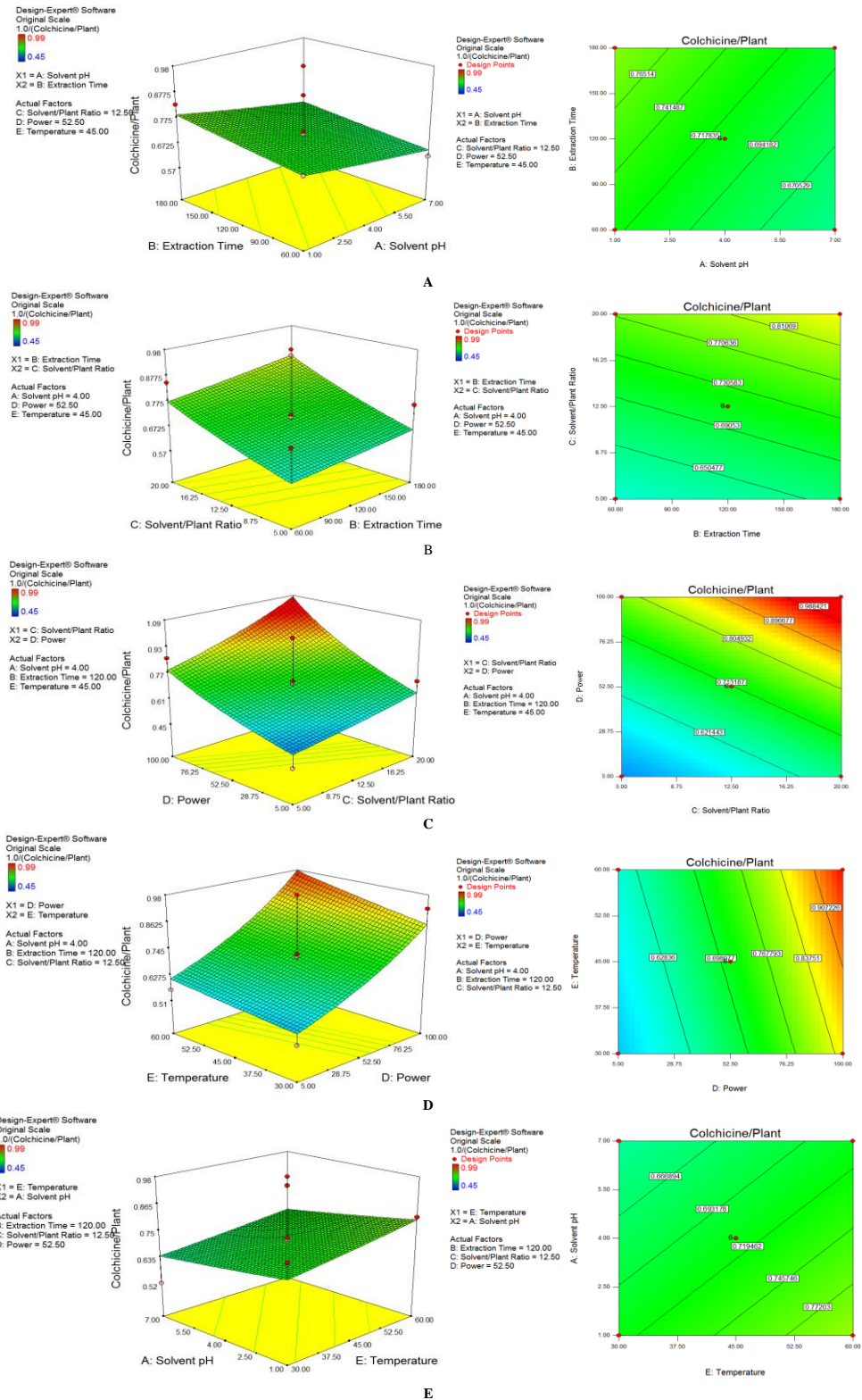
<b>Std. Dev.</b>	0.094	<b>R-Squared</b>	0.91
<b>Mean</b>	0.74	<b>R<sub>Adj</sub>-Squard</b>	0.95
<b>C.V. %</b>	12.72	<b>R<sub>Pred</sub>-Squard</b>	0.99
<b>PRESS</b>	0.45	<b>Adeq Precision</b>	53.85



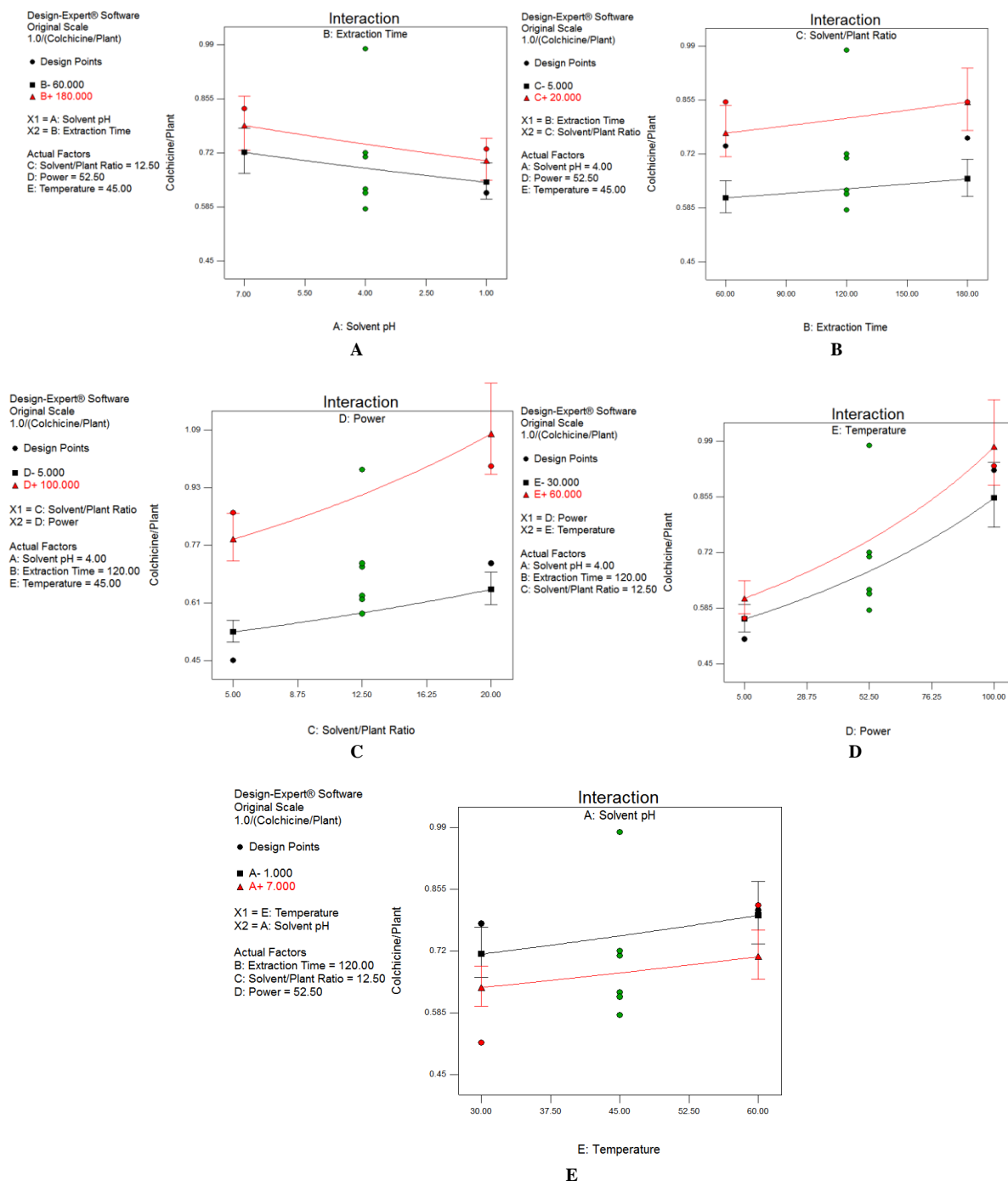
**Figure 2.** Box-Cox plot for power transforms



**Figure 3.** Actual values versus the predicted values for the extraction of colchicine by ultrasonic-assisted acidic-solvent extraction method



**Figure 4.** Response surface and contour plots of the study parameters; A: effects of solvent pH and extraction time (min) on the response Y (mg colchicine/g corm); B: effects of extraction time (min) and solvent/plant ratio (mL/g) on the response Y (mg colchicine/g corm); C: effects of solvent/plant ratio (mL/g) and power (W) on the response Y (mg colchicine/g corm); D: effects of power (W) and temperature (°C) on the response Y (mg colchicine/g corm); E: effects of temperature (°C) and solvent pH on the response Y (mg colchicine/g corm)



**Figure 5.** Effect of interaction of parameters on the extraction yield of colchicine (mg/g); A: interaction of solvent pH and extraction time (min); B: interaction of extraction time (min) and solvent/plant ratio (mL/g); C: interaction of solvent/plant ratio (mL/g) and power (W); D: interaction of power (W) and temperature (°C); E: interaction of temperature (°C) and solvent pH

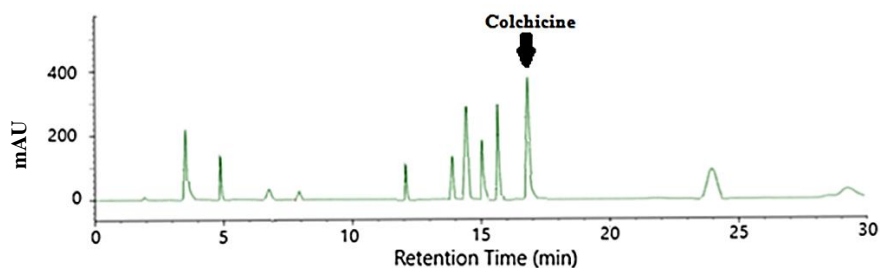


Figure 6. HPLC chromatogram of the optimized condition

The coefficients were statistically significant ( $p < 0.05$ ), which illustrated that this model, were suitable for the prediction of colchicine extraction yield within the ranges of variables.

According to figures 4 and 5, decreasing pH and increasing the extraction time, solvent/plant ration, power and temperature generally amplified the extraction yield of colchicine. On the other hand, with decreasing the pH, the optimum requirement for the extraction time decreased in order to obtain more colchicine extract. For other parameters, the optimum requirements increased with increasing any of the parameters.

After analysis of response surface, the amount of colchicine in all 46 conditions was determined using HPLC method. Figure 6 shows the HPLC chromatogram of the optimized condition. Table 2 shows the amount of extracted colchicine, which was obtained in conditions: solvent pH 4, extraction time 120 minutes, solvent/plant ratio 20 mL/g, power 100 W, and temperature 60 °C. Many researchers have optimized the extraction and isolation of important bioactive compounds using this studied method.

Amiri et al. studied the optimization of fatty acids extraction from *Aesculus hippocastanum*. In this study, response surface methodology was used for optimization. The results showed high efficacy of this method for optimization of fatty acid extraction [9]. Tahmasebi-Boldaji et al. evaluated the optimization conditions of hypericin extraction from *Hypericum perforatum* L. By ultrasonic-assisted extraction method coupled with response surface methodology. In this study, the amount of hypericin was 0.112 mg hypericin per gram dried extract in solvent/plant ratio 15, time 120 minutes and temperature 63.16 °C [8].

According to the present study, ultrasonic-assisted acidic-solvent extraction was found an effective method for extraction of colchicine

from *C. kurdicum* versus other extraction methods such as maceration, percolation and Soxhlet extraction. Ultrasound-assisted extraction methods have many advantages such as disrupting of the cell wall and increasing the cell components as well as bioactive compounds; however, these compounds should not be sensitive to ultrasound waves [8,9].

The yield of colchicine extraction generally showed direct dependency with extraction time, solvent/plant ration, power and temperature and reverse dependency with solvent pH and 0.99 mg/g corms of colchicine was achieved in the experimental conditions: solvent pH 4, extraction time 120 minutes, solvent/plant ratio 20 ml/g, power 100 W, and temperature 60 °C. Studies such as the present study can be helpful to increase the extraction of important bioactive compounds with low cost and high facility as the main efficacies of this method.. In addition, this method has several advantages for the extraction of all compounds especially high yield, low extraction time and being held in lower temperatures.

### Acknowledgments

This article was a part of PhD thesis of Dr. Ali Davoodi, PharmD, supervised by Professor Mohammad Azadbakht. We thank all Pharmacy School personnel especially Pharmacognosy laboratory. This study was supported by a grant from Mazandaran University of Medical Sciences, Sari, Iran (Funding code: 1396-1102).

### Author contributions

Mohammad Azadbakht was the main study investigator and contributed to the collection of the data; Ali Davoodi was the study investigator, contributed to the collection of the data and critically revised the manuscript; Elnaz Khoshvishkaie, Seyed Jalal Hosseinimehr, Masoud Azadbakht, Saeed Emami, Hossein



Bakhshi Jouybari, Fatemeh Mirzaee and Kiana Ghadiri participated in data interpretation and revision of the manuscript.

### Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

### References

- [1] Kahraman A, Celep F. Anatomical properties of *Colchicum kurdicum* (Bornm.) Stef. (Colchicaceae). *Aust J Cr Sci*. 2010; 4(5): 369-371.
- [2] Fakhri M, Davoodi A, Parviz M, Sadeghi Ghadi Z, Mousavinasab SN, Farhadi R, Azadbakht M, Azadbakht M. Characterization and HPLC analysis of manna from some *Cotoneaster* species. *Int J Pharm Sci Res*. 2017; 8(12): 1000-1006.
- [3] Aghili Alavi Khorasani MH. Makhzan al-advieh. Tehran: Tehran University of Medical Sciences, 2009.
- [4] Choopani R, Ghourchian A, Hajimehdipoor H, Kamalinejad M. Scientific evaluation of pharmacological treatment of osteoarthritis in the Canon of medicine. *J Evid Based Complement Altern Med*. 2016; 21(3): 228-234.
- [5] Khan H, Tariq SA, Khan MA. Biological and phytochemical studies on corms of *Colchicum luteum* Baker. *J Med Plant Res*. 2011; 5(32): 7031-7035.
- [6] Gulsoy-Toplan G, Goger F, Yildiz-Pekoz A, Gibbons S, Sariyar G, Mat A. Chemical constituents of the different parts of *Colchicum micranthum* and *C. chalcedonicum* and their cytotoxic activities. *Nat Prod Commun*. 2018; 13(5): 535-538.
- [7] Davoodi A, Khoshvishkaie E, Azadbakht M. Plant cells technology as an effective biotechnological approach for high scale production of pharmaceutical natural compounds; a meta-analysis study. *Pharm Biomed Res*. 2019; 5(2): 1-9.
- [8] Tahmasebi-Boldaji R, Hatamipour MS, Khanahmadi M, Sadeh P, Najafipour I. Ultrasound-assisted packed-bed extraction of hypericin from *Hypericum perforatum* L. and optimization by response surface methodology. *Ultrason Sonochem*. 2019; 57(1): 89-97.
- [9] Amiri S, Shakeri A, Sohrabi MR, Khalajzadeh S, Ghasemi E. Optimization of ultrasonic assisted extraction of fatty acids from *Aesculus hippocastanum* fruit by response surface methodology. *Food Chem*. 2019; 271(1): 762-766.
- [10] Rambo DF, Biegelmeier R, Toson NSB, Dresch RR, Moreno PRH, Henriques AT. Box-Behnken experimental design for extraction optimization of alkaloids from *Erythrina verna* Vell. trunk barks and LC method validation. *Ind Crops Prod*. 2019; 133(1): 250-258.
- [11] Baş D, Boyacı IH. Modeling and optimization I: usability of response surface methodology. *J Food Eng*. 2007; 78(3): 836-845.

### Abbreviations

RSM: response surface methodology; HPLC: high performance liquid chromatography; ANOVA: analysis of variance