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Optimization of Conventional and Superheated Solvent Extraction Methods for Extracting Polyphenolic Compounds from *Salvia leriifolia* Leaves

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Abstract

The efficiency of an extraction procedure in recovering bioactive phenolic compounds is highly dependent on its mechanism and operating conditions. Polyphenols were extracted from *Salvia leriifolia* leaves using conventional solvent extraction and also superheated extraction techniques. The faced central composite experimental design of response surface methodology (RSM) applied to evaluate the effects of the solvent ratio, temperature, and time on both the process yield and total phenolic content. Optimization revealed that the modification in the extraction technology greatly affected the procedure. According to the results, the superheated solvent method was significantly more efficient compared to the conventional solvent extraction, and the highest yield of 53.29 % and total phenolic content of 737.21 mg GAE/g E were obtained under the optimal conditions (25.17 % ethanolic solvent at the extraction temperature of 160 °C for 28.97 min). In contrast, the optimization of conventional solvent extraction method revealed a maximum extraction yield and total phenolics of 30.96 % and 693.23 mg GAE/g E, respectively (using 50 % ethanolic solvent at the extraction temperature of 82.57 °C for 103.31 min). This is the first report on optimizing the superheated solvent extraction of phenolic compounds from *Salvia leriifolia* leaves.

Keywords: Conventional extraction, Polyphenols, Response surface methodology (RSM), *Salvia leriifolia*, Superheated solvent extraction



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Introduction

The *Salvia* genus is part of the Nepetoideae subfamily within the Mentheae tribe of the Lamiaceae family. Various *Salvia* species are recognized for their high polyphenol levels and medicinal uses (Jia *et al.*, 2019; Loizzo *et al.*, 2010; Tepe *et al.*, 2004). *Salvia leriifolia* is an endemic perennial herbaceous plant native to the south-tropical areas of Khorasan and Semnan provinces in Iran (Hosseinzadeh, Sadeghnia, Imenshahidi, & Fazly Bazzaz, 2009; Shahi, Abrishamchi, Radjabian, & Salami, 2025). The plant was included in *Flora Iranica* in 1982 and is known by several local names (Nouroozak, Jobleh, etc.) (Modarres *et al.*, 2014). Recently, many pharmacological and preservative properties of this plant—including hypoglycemic, analgesic, anti-inflammatory, anticonvulsant, anti-ulcer, antioxidant, and antibacterial effects—have been successfully assessed (Farhoush, Rahimzadeh, Pourazarang, & Seyedi, 2004; Hosseinzadeh *et al.*, 2009; Loizzo *et al.*, 2010; Modarres & Yazdi, 2021; Shahi *et al.*, 2025). Plant extraction is a complex procedure designed to isolate high-quality active compounds from plant tissues. Nowadays, enzymatic, mechanical, and thermal extraction methods have been improved or customized based on the chemical nature and amount of bioactive compounds (such as polyphenols) and also available facilities (Patel, Dave, & Patel, 2021). Conventional extraction methods primarily rely on solvent-based extraction techniques (Alara, Abdurahman, & Ukaegbu, 2021; Adamez, Samino, Sanchez, & Gonzalez-Gomez, 2012; Hayrapetyan, Hazeleger, & Beumer, 2012; Sagdic, Ozturk, & Kisi, 2012). In this method, the crude powder is soaked in a specific solvent and agitated frequently for a set period to dissolve the soluble bioactive material (Patel *et al.*, 2021). Despite being simple and widely applied, conventional extraction methods require long processing times, high solvent volumes, and may co-extract unwanted constituents. These drawbacks have led to the adoption of modern instrumentation techniques, resulting in

remarkable progress in making the extraction process more efficient and precise (Da Porto, Porretto, & Decorti, 2013; Wijngaard, Ballay, & Brunton, 2012). Superheated solvent extraction is an eco-friendly technique that may serve as an alternative to traditional solvent extraction. In this method, an aqueous or organic solvent is heated and pressurized below its critical point, making it suitable for extracting a broad range of analytes in both polar and non-polar forms. The basis of the superheated solvent extraction method is the application of high pressure (between 4 and 20 MPa) to keep the solvent in a liquid state at temperatures above its normal boiling point. Under these conditions, the solvent penetration in the solid matrix, the diffusivity of analytes to the outer space, and the solubility of the analytes in the solvent are enhanced, which facilitates the extraction process. The use of a combination of temperature and pressure allows the extraction to be carried out quickly and in a short time. (Azmir *et al.*, 2013; Morales-Munoz, Luque-Garcia, & de Castro, 2003; Wijngaard *et al.*, 2012). Based on the literature review, superheated extraction overcomes several drawbacks of conventional solvent extraction, thereby improving both selectivity and extraction efficiency. By lowering solvent viscosity and surface tension, this technique enhances extraction rate and decreases solvent consumption (Alupului, Calinescu, & Lavric, 2012). Performing the process without exposure to air or light substantially reduces oxidation and degradation of phenolic compounds (Oubannin *et al.*, 2024; Japon-Lujan & de Castro, 2006; Luque-Rodríguez, de Castro, & Pérez-Juan, 2007). Also, the elevated temperature in a relatively short time employed in such extraction method enhances the solubility of certain fractions and mass transfer rates, while weakening and disrupting the matrix-analyte interactions (Wong, Khoiroh, Gan, & Croft, 2025). This extraction method has previously been employed to recover phenolic bio actives from numerous plant and fruit sources (e.g., red grape stems (Wenzel *et al.*, 2015), citrus

fruits (Ledesma-Escobar, Priego-Capote, & de Castro, 2015), olive leaves and pomace (Cea Pavez *et al.*, 2019; Japon-Lujan & de Castro, 2006), pomegranate peel (Rahnemoon, Sarabi Jamab, Javanmard Dakheli, Bostan, & Safari, 2018)). Based on our knowledge, this is the first application of this technique for extracting phenols from *Salvia leriifolia* leaves. This study focused on optimizing superheated solvent extraction for isolating phenolic compounds from *Salvia leriifolia* leaves. The effects of extraction condition (solvent ratio, time and temperature) upon the process yield and total phenolic content were determined and compared with those obtained using the conventional extraction method. As this species is endemic to the Khorasan region of Iran, optimization of the extraction conditions offers considerable potential for providing a variety of polyphenolic compounds.

Materials and Methods

Materials

Salvia leriifolia leaves were collected from Mashhad surrounding highlands, Iran; and the samples botanical type were confirmed. The gallic acid, CaCl_2 , Na_2CO_3 , and Folin–Ciocalteu reagent were prepared from Sigma–Aldrich (USA). Deionized water, methanol, Ethanol, and all other chemicals applied in this research were of analytical grade.

Preparation of Fresh *Salvia leriifolia* Leaves for Extraction

In brief, fresh *Salvia leriifolia* leaves were cleaned and air dried to the moisture content of 9.94 ± 0.05 % (based on dry matter content) at room temperature. The dried leaves were then powdered and sieved using a 35-mesh sieve to obtain a fine powder. Then they were placed in sealed jars and stored in a dry and dark place until use.

Conventional Solvent Extraction

For conventional extraction of phenols, ethanol and water were utilized as solvents. The ratio of leave powder to the solvent was

10% w/v. Different levels of ethanol: water ratios (50:50, 40:60, and 30:70), temperatures (70, 80, and 90 °C), and times (30, 75, and 120 min) were examined to modify the extraction procedure. The solvents and the process conditions were chosen based on pre-experiments. After extraction, the mixture was filtered and centrifuged ($3000 \times g$ / 10 min); then dried at 40 °C under the pressure of 0.8 bar in a vacuum oven (Gallenkamp, UK) for 5–8 h (based on the solvent ratios). In our study, the dry matter content of the powder obtained after extractions was 95%, so 5% of the solvent still remained in the powder (Hayrapetyan *et al.*, 2012).

Superheated Solvent Extraction

Based on pre-treatments, the leaves powder was extracted with a mixture of water and ethanol in the ratios of 60:40, 80:20 and 100:0. Three extraction times (10, 20 and 30 min) were used at three different temperatures (130, 145 and 160 °C), and the extraction solid: liquid ratio (leaves powder to the solvent) was 10% w/v. The procedure was carried out using a laboratory-built extraction apparatus (RIFST, Iran) equipped with a sample cell and a high-pressure pump. The schematic of superheated solvent extraction device is shown in Fig. 1. The design of the extractor is such that it is capable enough to withstand high pressures, helps to maintain the solvent in a liquid state at a high temperature. In the heater, the solvent is heated to the desired temperature and introduced to the extractor contacting takes place between the substance and the solvent to dissolve the constituents in the fluid. The system pressure was maintained at a range of 5 to 15 bar during the process given by a nitrogen tank (based on the other extraction parameters). The fluid leaving the extractor is then cooled in a cooler. Finally, fresh extract is filtered, centrifuged and then dried (40 °C/0.8 bar (Aliakbarian, Fathi, Perego, & Dehghani, 2012; Garcia-Marino, Rivas-Gonzalo, Ibanez, & Garcia-Moreno, 2006)).

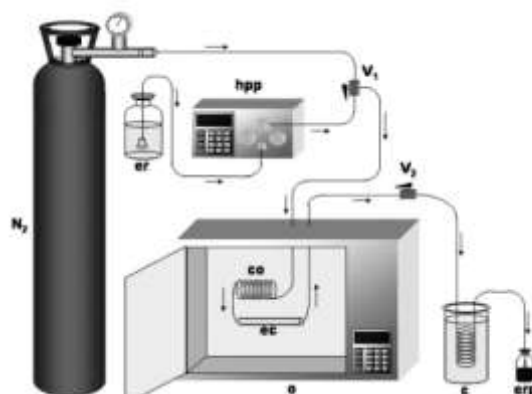


Fig. 1. Schematic of superheated solvent extraction device (Rodriguez et al, 2007).

er: solvent vessel; hpp: high pressure pump; co: heating coil; ec: extraction cell; o: oven; c: chiller, erp: extract collection vessel, v1: selection valve, v2: restrictor valve.

The Extraction Yield

The yield of extractions was calculated using following equation:

$$\text{Yield (g/100 g)} = (W_1/W_2) \times 100 \quad (\text{Equation 1})$$

Where W_1 is the weight of dried extract after the solvent removal and W_2 is the weight of *Salvia leriifolia* leaves powder.

Total Phenolic Content

Modified Folin–Ciocalteu method was used to determine the total phenols (Singleton & Stikeleather, 1999). According to the method, 1 g of sample powder was dissolved in 10 ml solvent (water/ethanol), and diluted in 1:10 ratio. Then the obtained filtered solution (100 μ l), and aqueous Folin-Ciocalteu solution (10% v/v / 1 ml) were mixed in a volumetric flask. The mixture final volume was adjusted to 10 ml using distilled water. After 3 min, 1 ml of NaHCO_3 solution (7.5% w/v) was added to the mixture and the sample incubated in the dark (25 $^\circ\text{C}$ / 1 h). Finally, the absorbance was measured at 760 nm by UV-Vis spectrophotometer (Cecil CE 2040, cecil instruments, Cambridge, England). The calibration curve was created by measuring the light absorption of the solutions at various known concentrations of gallic acid (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 ml of the standard gallic acid stock solution (1000 g/ml of gallic acid)). The perfect fit established with

the R^2 of 0.995 in a linear regression model. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g E).

HPLC Analysis of the Extract Phenolic Compounds

Three main phenolic compounds in *Salvia leriifolia* plant (rosmarinic acid, salvianolic acid, caffeic acid) were used as the respective reference compounds (Modarres, Esmailzadeh Bahabadi, & Taghavizadeh Yazdi, 2018; Vishwakarma, Singh, Shukla, Singh, & Singh, 2013). By a Smartline HPLC (Kenner, Germany), phenolic compounds were quantified. This instrument equipped with a pump (S2100) and a UV-Vis detector (UV (2489)). The selected wavelength was 330 nm and the samples were filtered (0.45 μ m) before injection to the system. For the analysis, C18 column (5 μ m $\times$ 150 mm $\times$ 4.6 mm) was used at room temperature (20 $^\circ\text{C}$) with phosphoric acid (1%) and methanol as the mobile phase (60:40 v/v). The flow rate was 1.8 mL/min and the injection volume was 20 μ L. The extract samples were injected three times to HPLC and for each phenolic compound, the determination was done using the corresponding calibration curve (1-100 μ g/ml).

Statistical Analysis

To determine the relationship between the variable factors and responses and also

optimize the extraction methods, faced central composite design (faced-CCD) with the six replicates of a central point were applied using Version 7 of Design-Expert software (Stat-Ease, Inc., Minneapolis, USA). Temperature (X_1), time (X_2) and the ratio of solvents (water to ethanol) (X_3) were chosen as three independent variables for both extraction methods (conventional extraction with solvent and superheated solvent extraction) with two dependent variables (responses) including yield as Y_1 and Y_3 and total phenolic content as Y_2 and Y_4 for conventional and superheated extraction respectively. There was a total of 20 runs for optimizing the parameters. The stepwise regression model was developed to optimize the favorable process factors and suitable equation was fitted for obtained response in each analysis; in which, β_0 was constant and β_i (β_1 , β_2 and β_3), β_{ii} (β_{11} , β_{22} and

β_{33}) and β_{ij} (β_{12} , β_{13} and β_{23}) were linear, quadratic and interaction coefficients, respectively. To evaluate the accuracy of the optimized model, P-value, Lack of fit, R^2 , Adjusted- R^2 , and CV were calculated.

Results and Discussion

Optimization of *Salvia leriifolia* Leaves Extraction

Conventional Solvent Extraction

The experimental design with encoded values and the responses for each analysis were presented in Table 1. To determine the optimal conditions of the three factors (temperature, time, and water: ethanol ratio) towards conventional solvent extraction method, three levels of time (30, 75 and 120 min), temperature (70, 80 and 90 °C) and water: ethanol ratio (50:50, 60:40 and 70:30) were chosen based on our pre-experiments (Table 1).

Table 1- Design matrix for three independent variables in conventional solvent extraction method

Variables				Variables			
Run	Temperature (°C)	Time (min)	Water: Ethanol ratio	Run	Temperature (°C)	Time (min)	Water: Ethanol ratio
1	80	30	60:40	11	80	75	60:40
2	80	75	60:40	12	70	30	50:50
3	70	120	50:50	13	70	120	70:30
4	90	120	50:50	14	80	120	60:40
5	90	30	50:50	15	70	30	70:30
6	70	75	60:40	16	80	75	60:40
7	80	75	50:50	17	80	75	70:30
8	90	75	60:40	18	80	75	60:40
9	80	75	60:40	19	90	30	70:30
10	90	120	70:30	20	80	75	60:40

According to the results (Table 2), the linear model was found to be a good fitting model for the efficiency of extraction; and the evaluation of the coefficients in various terms indicated that the changes in the levels of time and temperature had significant effects on EY % ($p < 0.05$).

The analysis of the data showed that the Lack of fit value was not significant, and the high amounts of R^2 (0.991), and Adj- R^2 (0.989) indicated that the linear model was properly accurate for predicting the efficiency of the yield of conventional extraction process. The obtained R^2 and Adj- R^2 values implied a

strong relationship, explaining about 99% of the variance in the dependent variable. The EY % was calculated using the following equation:

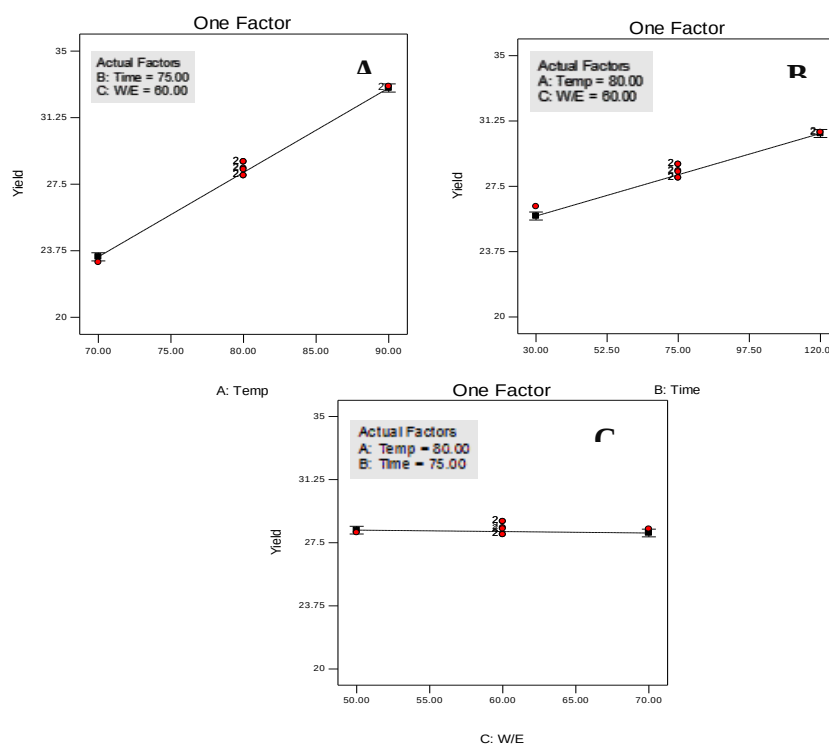
$$Y_1 = -13.327 + 0.475 X_1 + 0.053 X_2 - 0.008 X_3 \quad (\text{Equation 2})$$

Where Y_1 is EY % response and X_1 , X_2 and X_3 are temperature, time and water: ethanol ratio, respectively.

As shown in Fig. 2, temperature changes, compared to the other independent variables, had the greatest effect on the EY %. With an increase in level of this factor, from 70 to 90 °C, EY % increased by 10 %.

Table 2- Results of regression analysis for extraction yield and total phenolic content in conventional solvent extraction

Source	df	Extraction yield			df	Total phenolic content		
		Equation coefficient	Sum of squares	p-value		Equation coefficient	Sum of squares	p-value
Linear model	3	-13.327	282.53	0.000	9	-17648.632	707600.5	0.000
X ₁	1	0.475	226.29	0.000	1	468.810	474.86	0.000
X ₂	1	0.053	56.17	0.000	1	19.627	125000.5	0.000
X ₃	1	-0.008	0.074	0.501	1	-44.155	471.14	0.767
Quadratic model								
X ₁ X ₁					1	-2.751	208200.5	0.000
X ₂ X ₂					1	-0.067	50539.38	0.010
X ₃ X ₃					1	0.620	10536.82	0.183
Interaction model								
X ₁ X ₂					1	-0.076	9314.08	0.209
X ₁ X ₃					1	-0.037	10890.67	0.177
X ₂ X ₃					1	0.017	481.90	0.766
Residual	16		2.50		10		51651.15	
Lack of Fit	11		1.90	0.364	5		40728.16	0.087
Total	19		285.04		19		759300.5	
R ²		0.991				0.932		
Adj-R ²		0.989				0.871		
CV		1.40				9.30		

**Fig. 2. The Scatter plot of extraction yield (EY) vs. predicted level of Temperature (A), Time (B) and water:ethanol ratio (C) in conventional extraction method**

*Temp= temperature; W/E= water:ethanol ratio.

Design and data of optimal experiments (Table 2) showed that the time factor at the linear level as well as the temperature factor at both linear and quadratic levels affect significantly ($p < 0.05$) on the amount of total phenolic content.

The coefficients of the independent variables are displayed in an equation as follows:

$$Y_2 = -17648.632 + 468.810 X_1 + 19.627 X_2 - 44.155 X_3 + 0.076 X_1 X_2 - 0.037 X_2 X_3 + 0.017 X_2 X_3 - 2.751 X_1^2 - 0.067 X_2^2 + 0.620 X_3^2 \quad (\text{Equation 3})$$

where Y_2 is the total phenol (mg GAE/g extract) and X_1 , X_2 and X_3 are temperature, time and water: ethanol ratio, respectively.

The 3D response surface graphs of the combined effects of time, temperature and the solvent ratio on the extracted phenolic compounds are shown in Fig. 3. Based on the results, increasing the extraction time to 120 min had a positive effect on the total phenolic content, with a greater effect in the range of 30-75 min compared to 75-120 min (Fig. 3-A and 3-C). The figure illustrated that as the temperature increased from 70 to 80 °C, the total phenols increased along with the temperature; whereas in the range of 80 to 90 °C, the extract total phenols decreased (Fig. 3-A and 3-B). As the data showed, the extraction solvent ratio did not have a significant effect ($p \geq 0.05$) on the total phenols (Fig. 3-B and 3-C).

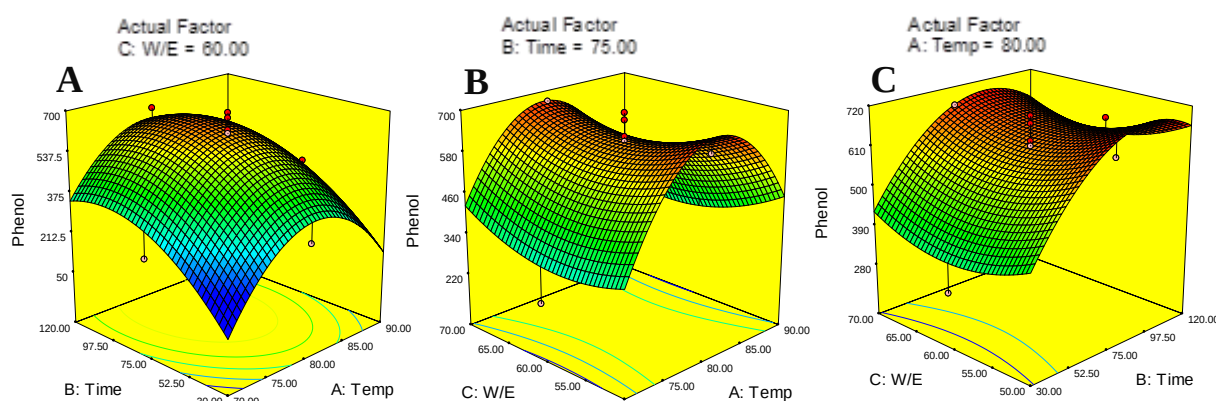


Fig. 3. Response surface for the effect of independent variables including temperature to time (A), temperature to the solvents ratio (B) and time to the solvents ratio (C) on the total phenolic content in Conventional extraction method

*Temp= temperature; W/E= water:ethanol ratio.

In a study, Tan, Tan, & Ho (2013) optimized the conditions used for extracting total phenolic compounds from the Henna plant. They reported that prolonging the extraction time increased significantly the total phenolic yield up to 270 min, after which it gradually decreased and reached its lowest level at 360 min. They suggested that after some time, an equilibrium may form between the solute level in the plant matrix and the solvent that slows down the extraction yield. Moreover, the extended extraction periods may enhance the degradation or oxidation of

phenolic compounds because of prolonged exposure to environmental elements such as light. Wang *et al.* (2011) examined how variations in solvent type and temperature affect the extraction of total phenolics from pomegranate peel. In mentioned research, by boosting the temperature from 25 to 95 °C, the amount of phenolic compounds was increased. Tomsik *et al.* (2017) noted that elevating the temperature alters the physicochemical behavior of water, making its characteristics more like organic solvents and thereby improving extraction yield. During the

extraction procedure, polyphenols and other bioactive compounds in the matrix will be dissolved by the solvent (based on their polarities). The extraction solvent can affect the release of the target compound into the solution. The present study used ethanol and water, both of which are polar, food-grade solvents suitable for extracting phenolic compounds. Our results indicated a significant interaction between the extraction parameters and the amount of phenolic compounds obtained from *Salvia leriifolia* leaves. Different levels of the extraction yield were typically obtained due to the differences in the solvent polarity and the process parameters, which affect the solubility of the sample's chemical constituents and their extraction (Butsat & Siriamornpun, 2016). Throughout the process, the changes in the process parameters, such as temperature, time, type of solvent, etc., affect the phenolic extraction by changing both the diffusion coefficient and solubility (Al-Farsi & Lee, 2008; Chew, Ng, Thoo, & Khoo, 2011; Li, Wong, Cheng, & Chen, 2008). However, temperature exerts a stronger influence on polyphenol extraction yield compared to other variables (solvents ratio and time). This is primarily because elevated temperatures simultaneously reduce

solvent viscosity and surface tension, enhance solute solubility, accelerate mass transfer and diffusion rates, and improve penetration into the plant matrix as well as desorption kinetics. While solvent ratio mainly affects equilibrium partitioning and time influences diffusion duration (after a certain point), temperature directly impacts multiple physicochemical processes simultaneously, leading to more pronounced yield increases. This dominant role of temperature is well-supported in the literature on various extraction techniques, including ultrasound-assisted, microwave-assisted, and pressurized liquid extraction methods (Lopez-Salas, Exposito-Almellon, Borrás-Linares, Lozano-Sanchez, & Segura-Carretero, 2024; Osorio-Tobón, 2020; Tomasi, Santos, Boaventura, & Botelho, 2023).

Superheated Solvent Extraction

Three variables of extraction: temperature (130, 145 and 160 °C), time (10, 20 and 30 min) and water: ethanol ratio (60:40, 80:20 and 100:0), were chosen (Table 3) to determine the optimal conditions for the extraction efficiency and total phenolic content towards superheated solvent extraction method.

Table 3- Design matrix for three independent variables in superheated solvent extraction method

Run	Variables			Run	Variables		
	Temperature (°C)	Time (min)	Water: Ethanol ratio		Temperature (°C)	Time (min)	Water: Ethanol ratio
1	160	10	60:40	11	145	30	80:20
2	145	20	80:20	12	130	10	60:40
3	130	20	80:20	13	145	20	80:20
4	145	20	80:20	14	145	20	80:20
5	130	30	100:0	15	160	30	100:0
6	160	30	60:40	16	145	20	80:20
7	145	20	100:0	17	130	30	60:40
8	145	20	60:40	18	160	20	80:20
9	145	20	80:20	19	145	10	80:20
10	160	10	100:0	20	130	10	100:0

Table 4 shows the analysis of variance for extraction efficiency and total phenolic content in superheated solvent extraction technique. Considering the EY % and phenolic compounds experimental results, the non-

linear quadratic multivariate model was the predictor model for independent variables, and their interaction and quadratic effects (equation 4 and equation 5).

The calculated R^2 and CV values were 0.980 and 1.47 for the EY % and 0.991 and 5.40 for the total phenolic content, respectively. The results for each of the response factor were described by the following non-linear equations:

$$Y_3 = 135.127 - 1.788 X_1 - 0.0323 X_2 + 0.438 X_3 - 1.867 X_1 X_2 - 1.308 X_1 X_3 + 7.610 X_1^2 + 0.013 X_2^2 - 1.594 X_3^2 \quad (\text{Equation 4})$$

Where Y_3 is EY % the response for three independent variables (X_1 = temperature, X_2 = time and X_3 = water: ethanol ratio).

$$Y_4 = 11158.175 - 184.060 X_1 + 20.238 X_2 + 16.265 X_3 - 0.043 X_1 X_2 - 0.031 X_1 X_3 + 0.014 X_2 X_3 + 0.664 X_1^2 - 0.307 X_2^2 - 0.078 X_3^2 \quad (\text{Equation 5})$$

Where Y_4 is the total phenolic content response and X_1 , X_2 and X_3 are the temperature, time and water: ethanol ratio, respectively.

Table 4- Results of regression analysis for extraction yield and total phenolic content in superheated solvent extraction method

Source	df	Extraction yield			df	Total phenolic content		
		Equation coefficient	Sum of squares	p-value		Equation coefficient	Sum of squares	p-value
Linear model	8	135.127	274.05	0.000	9	-11158.184	574869	0.000
X_1	1	-1.788	172.39	0.000	1	-175.060	466923	0.000
X_2	1	-0.032	45.80	0.000	1	20.238	7678	0.003
X_3	1	0.438	0.18	0.535	1	16.265	602	0.304
Quadratic model								
$X_1 X_1$	1	7.610	8.06	0.001	1	0.664	63141	0.000
$X_2 X_2$	1	0.013	4.59	0.008	1	-0.307	5774	0.048
$X_3 X_3$	1	-1.595	1.12	0.144	1	-0.078	2680	0.045
Interaction model								
$X_1 X_2$	1	-1.867	0.63	0.263	1	-0.043	335	0.438
$X_1 X_3$	1	-1.308	1.23	0.127	1	-0.031	685	0.278
$X_2 X_3$					1	0.014	60	0.739
Residual	11		4.98		10		5128	
Lack of Fit	6		4.25	0.051	5		3982	0.099
Total	19		252.03		19		552997	
R^2		0.980				0.991		
Adj- R^2		0.966				0.982		
CV		1.47				5.40		

As shown in Table 4, superheated solvent extraction revealed significant effects of the linear and quadratic terms of time and temperature on the extraction yield ($p < 0.05$). For total phenolic content, the linear and quadratic terms of time and temperature, along with the quadratic term of the water: ethanol ratio, were significant ($p < 0.05$).

Fig. 4 (4-A and 4-B) illustrates the effects of process time and temperature on the extraction yield. The change in temperature from 130 °C to 160 °C caused a sharp increase in the value of EY %. The changes in the

extraction time (from 10 to 30 min) had similar effects on the EY %, although the rate of increase was lower (Fig. 4-A).

The effects of temperature and solvent ratio at the fixed extraction time (20 min) were shown in Fig. 4-B. As the temperature increased, the level of EY % also increased, while increasing the water: ethanol ratio had no significant effect ($p \geq 0.05$) on EY %. Observing the effects of various levels of water: ethanol ratio, the highest yield was obtained in the 80:20 ratio of water to ethanol.

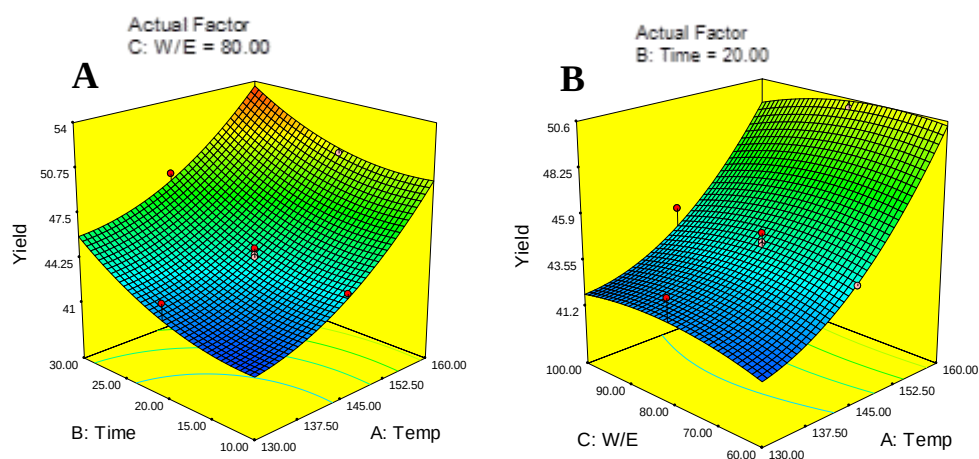


Fig. 4. Response surface for the effect of independent variables including temperature to time (A), temperature to the solvents ratio (B) on the extraction yield (EY %) in superheated solvent extraction method
*Temp= temperature; W/E= water:ethanol ratio.

Fig. 5 (5-A, 5-B and 5-C) illustrates the effects of three independent variables on the total phenolic content. The maximum level of phenols was found when the temperature increased to 160 °C. Treatments with water to solvent ratio ranging from 60 % to 80 %, increased total phenolic content ($p < 0.05$), whereas the higher ratios (ranging from 80 % to 100 %) decreased the level of total phenols. As a result, it could be concluded that increasing the water concentration in the solvent (to an optimum level) enhanced the phenolic extraction, and the combination of organic solvent and water portions can

improve the extraction of both components that are soluble in water or organic solvent (Cavalcanti *et al.*, 2021; Oubannin *et al.*, 2024). As illustrated in Fig. 5-A and 5-C, increasing the process time from 10 min to 20 min led to a significant increase ($p < 0.05$) in the total phenolic content. This result is consistent with existing literature on plant extraction optimization, which have demonstrated that the extraction time significantly affects the total phenolic content of the extract (Hamad & Hartanti, 2023; Malenica, Kass, & Bhat, 2025).

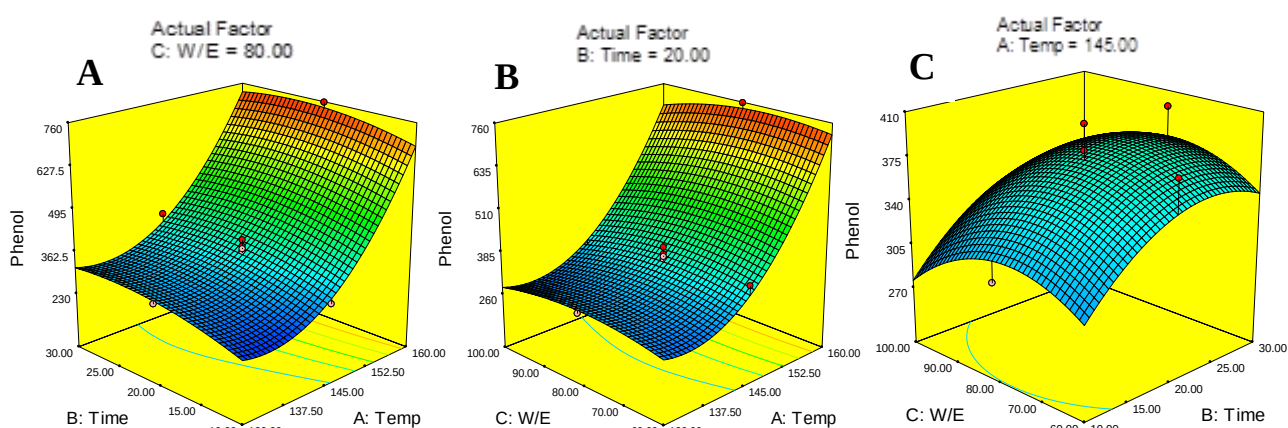


Fig. 5. Response surface for the effect of independent variables including temperature to time (A), temperature to the solvents ratio (B) and time to the solvents ratio (C) on the total phenolic content in the extract in superheated solvent extraction method
*Temp= temperature; W/E= water:ethanol ratio.

Application of instrumentation extraction techniques, such as superheated solvent, can reduce the extraction time and increase the final yield (Patel *et al.*, 2021). Longer extraction combined with higher temperatures can increase the possibility of undesirable reactions (hydrolysis, enzymatic oxidation of the phenolic compounds, etc.) and consequently affect the level of extracted polyphenols (Oubannin *et al.*, 2024; Biesaga & Pyrzynska, 2013). The high temperature and pressure used in the superheated solvent method lead to a change in the polarity of the solvent, which makes it possible to extract both polar and non-polar bioactive compounds from the plant tissue (Cavalcanti *et al.*, 2021; Shang, Kim, & Um, 2014). In addition, in such conditions, the solvent permeability into the plant cells increases during the extraction and the highest extraction output is achieved over a shorter period of time (Khan, Aslam, & Makroo, 2019).

The Optimal Parameters Calculated after Implementation of the Design

Evaluating the design information provides an efficient way to assess and optimize the process parameters in a minimum number of experimental runs (Nde & Foncha, 2020). Following the implementation of the experimental design, optimization studies were conducted to determine the main process parameters for both conventional and superheated solvent extraction techniques. The independent variables, specifically the extraction temperature, time, and water: ethanol ratio, were optimized to maximize the extraction yield and total phenolic content.

The optimized parameters, presented in Table 5, were derived from a comprehensive evaluation of the design information, enabling efficient process optimization with the minimal numbers of experimental runs. Considering the design points of each model, the outcome indicated the efficiency of the superheated method in *Salvia leriifolia* leaves extraction.

Table 5- Optimal predicted conditions of *Salvia leriifolia* leaves extraction using conventional and superheated solvent method

The type of extraction	Temperature (°C)	Time (min)	Water: Eethanol ratio	Yield	Total phenolic content	Desirability
Conventional method	82.57	103.31	50:50	30.96	693.23	0.949
Superheated solvent method	160.00	28.97	74.83:25.17	53.29	737.21	0.960

The optimal conditions for obtaining the highest phenolics and process yield were determined to be the water: ethanol ratio of 50:50 and 74.83:25.17, temperature of 82.57 and 160 °C, and time of 103.31 and 28.97 min for the conventional and superheated solvent extraction methods, respectively. In order to validate the optimal conditions represents by design-expert software, the extraction yield and the phenolic compounds content were measured on experimentally. Based on the optimal conditions of the conventional extraction method, the yield was 29.51% and the total phenols was 569.74 mg GAE/g E; While the yield and phenolic compounds

based on the optimal conditions of the extraction using the superheated solvent method were 52.38% and 730.65 mg GAE/g E, respectively. After doing the t-test, there was no significant difference between the data obtained from the software and experimental tests.

Based on our findings, increasing the extracted polyphenols content and the process yield can be achieved using superheated solvent extraction, which has the advantage of time/resources saving compared to the conventional method. This result showed a similarity with the investigations of Eikani, Golmohammad, & Homami (2012), Luque-

Rodriguez, Perez-Juan, & Luque De Castro (2006), Mijangos-Ricardez & Lopez-Luna (2013), and Rahnemoon *et al.* (2018), who found the superheated solvent extraction as a superior method with a high polyphenol extraction amount. Mijangos-Ricardez & López-Luna (2021) applied superheated liquid extraction to isolate phenolic compounds, specifically flavonoids and anthocyanins, from vinification lees. They optimized the extraction parameters (temperature, time, ethanol percent in the extractant and the extractant flow rate), and their findings indicated that superheated liquid extraction reduced the extraction time for flavonoids and anthocyanins compared to the conventional maceration method. Uzel (2018) used a mixture of water and alcohol in the extraction of olive leaves (at 180 °C) and showed that the superheated method has the highest recovery of bioactive organic compounds (phenol oleuropein). Rosales-Castro, Honorato-Salazar, Reyes-Navarrete, & Gonzalez-Laredo, (2015) studied *A. fraxinifolius* bark tree extracts (the ethanolic and hot water extraction) and the yields and total phenolic content were measured. Based on the results,

there was a significant direct correlation between the yield and total phenolic concentration, where it was higher in young tree barks compared to the extract from mature tree barks.

Analysis of Phenolic Compounds

As mentioned at section 2.5, rosmarinic acid, salvianolic acid and caffeic acid are the three main phenolic compounds in *Salvia leriifolia* plant (Hosseinpour Mohsen Abadi, Mahdavi, & Rezaei-Seresht, 2016; Modarres *et al.*, 2018), therefore, the respective reference compounds were used in this research. The evaluation of phenols in the optimized samples was performed by comparing the HPLC analysis and the retention time with those obtained from standard solutions and the data reported in the relevant literature. HPLC chromatograms of the extracts displayed two major peaks with retention times of approximately 4.28 and 8.74 min, which corresponded to caffeic acid and rosmarinic acid, respectively (Table 6). No salvianolic acid was detected in any of the extract samples.

Table 6- HPLC analysis data for standard phenolic compounds detected in *Salvia leriifolia* leaves extract (conventional extraction and superheated solvent extraction method)

Extraction method	Salvianolic acid	Caffeic acid			Rosmarinic acid		
		Retention time(min)	Peak area (%)	Quantification (g/100 g dried extract)	Retention time(min)	Peak area (%)	Quantification (g/100 g dried extract)
Conventional extraction	Not detected	4.30	6.09	0.03	8.78	93.91	1.18
Superheated solvent extraction	Not detected	4.28	2.35	0.05	8.74	97.65	6.04

Based on our results, the content of caffeic acid was determined as 0.03 and 0.05 g/100 g dried extract using conventional and superheated solvent extraction techniques, respectively. Modarres *et al.* (2014) reported 0.15, 0.20, and 0.07 mg/g dry extract of caffeic acid in root, leaf, and callus extracts of *Salvia leriifolia*, respectively, using 60% aqueous ethanol as solvent. According to the literature,

there is only a limited number of studies on the presence of salvianolic acids in *Salvia* species, most of which report trace or very low levels (Hao *et al.*, 2012; Wang *et al.*, 2014; Wu, Zhu, Zhang, Yang, & Zhou, 2012). Furthermore, the plant age was shown to significantly affect phenolic content (Shahi *et al.*, 2025). In the present study, the highest rosmarinic acid level was observed in leaf extracts, with a value of

1.18 g/100 g dried extract using the conventional method and 6.04 g/100 g dried extract using the superheated solvent method. Modarres *et al.* (2014) identified rosmarinic acid as the primary phenolic constituent, with a content of 0.33, 4.65 and 6.39 mg/g dried extract in root, leaf, and callus of *Salvia leriifolia*, respectively. Shahi *et al.* (2025) and Vishwakarma *et al.* (2013) similarly concluded that rosmarinic acid constitutes a major portion of polyphenolic compounds in *Salvia* species and exhibits strong antioxidant properties.

Conclusion

In this research, RSM was employed to optimize the extraction of phenolic compounds from *Salvia leriifolia* leaves using both conventional and superheated solvent methods. Based on the analysis of variance (ANOVA) results and high correlations coefficients, the developed regression models proved highly effective in predicting key process variables (extraction time, temperature, solvent ratio) and maximizing total polyphenol yield and content. The superheated solvent technique demonstrated superior efficiency compared to the traditional approach. To achieve the highest extraction

yield and total phenolic content, the recommended condition for *Salvia leriifolia* leaves was superheated solvent extraction at 74.83: 25.17 (v/v) water:ethanol mixture and 160 °C for 28.97 minutes. The HPLC analysis of the optimized treatments revealed significantly higher levels of total phenolic content (mainly rosmarinic acid and caffeic acid) in the superheated solvent extracts than in those obtained by the conventional method. In addition to the above results, further studies should focus on exploring the scalability and industrial applicability of the superheated solvent extraction for utilization in food and pharmaceutical sectors.

Author Contributions

Mahboobe Sarabi-Jamab: Methodology, Investigation, Formal analysis, Project administration, Editing. **Elaheh Mansouri:** Conceptualization, Investigation, Writing. **Aram Bostan:** Methodology, Editing.

Founding Source

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مقاله پژوهشی

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بهینه‌سازی روش‌های استخراج متداول با حلال و فوق داغ جهت استخراج ترکیبات پلی‌فنلی از برگ گیاه نوروژک

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چکیده

کارایی روش استخراج در بازیابی ترکیبات فنلی زیست‌فعال به شدت به مکانیسم و شرایط عملیاتی آن وابسته است. پلی‌فنول‌ها از برگ‌های گیاه نوروژک با استفاده از روش‌های متداول استخراج با حلال و فوق داغ استخراج شدند. طرح آزمایشی مرکب مرکزی از روش آماری سطح پاسخ برای ارزیابی اثرات نسبت حلال، دما و زمان بر بازده فرآیند و محتوای فنلی کل به کار گرفته شد. بهینه‌سازی نشان داد که اصلاح در فرآیند استخراج تا حد زیادی بر راندمان روش استخراج تأثیر می‌گذارد. طبق نتایج، روش استخراج با حلال فوق داغ در مقایسه با روش استخراج متداول با حلال به‌طور قابل توجهی کارآمدتر بود و بالاترین بازده ۵۳/۲۹ درصد و محتوای فنلی کل ۷۳۷/۲۱ میلی‌گرم معادل اسید گالیک به گرم عصاره استخراج شده تحت شرایط بهینه (۲۵/۱۷ درصد حلال اتانولی در دمای استخراج ۱۶۰ درجه سانتی‌گراد به مدت ۲۸/۹۷ دقیقه) به دست آمد. در مقابل، بهینه‌سازی روش استخراج متداول با حلال، حداکثر بازده استخراج و فنول کل را به ترتیب به ۳۰/۹۶ درصد و ۶۹۳/۲۳ میلی‌گرم معادل اسید گالیک به گرم عصاره استخراج شده (با استفاده از ۵۰ درصد حلال اتانولی در دمای استخراج ۸۲/۵۷ درجه سانتی‌گراد به مدت ۱۰۳/۳۱ دقیقه) رساند. این اولین گزارش در مورد بهینه‌سازی استخراج ترکیبات فنولی با حلال فوق داغ از برگ گیاه نوروژک است.

واژه‌های کلیدی: استخراج با حلال فوق داغ، استخراج متداول، پلی‌فنول‌ها، روش سطح پاسخ، نوروژک

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