



**Extraction of bioactive compounds of Persian Golnar (*punica pranatum*) using ohmic, ultrasound and percolation methods**

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ARTICLE INFO	ABSTRACT
<p><b>Article History:</b> Received: 2023/11/25 Accepted: 2024/1/13</p>	<p>Herbal bioactive compounds are secondary metabolites of plants that are produced in response to environmental stress and to protect the plant against harsh conditions. These compounds have both health effects on humans and also have preservative effects in food products. The conditions for extracting active compounds have a significant effect on their functional activities, and conventional and new methods have been used to extract bioactive compounds from plants. The aim of this study was to compare the extraction methods of bioactive compounds of Persian Golnar extract (PGE). In this research three methods of ohmic (temperatures of 45 °C and 60 °C for 40 and 60 min), ultrasonic (temperatures of 40 °C, 50 °C and 60 °C for 20 and 40 min) and percolation (for 24, 48 and 72 h) were used to prepare PGE, and the best extract was selected based on the extraction yield, total phenol content (TPC), total anthocyanin content (TAC) and antioxidant activity (DPPH radical scavenging). The results showed that there was no significant difference between the extraction yields of all three methods (<math>p &gt; 0.05</math>). The ohmic method had the same TPC as the ultrasonic method, but showed a higher TAC and antioxidant activity and was chosen as the best method. The optimal treatment in this stage included ohmic method, 60 °C and 40 min, and the extract obtained under these conditions contained 109.74 mg GAE/g of TPC, 373.26 mg/g of TAC and 81.11% antioxidant activity.</p>
<p><b>Keywords:</b> <i>Punica pranatum</i>, Extraction methods, Phenolic compounds, Antioxidant activity</p>	
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## 1. Introduction

In recent years, consumers have become more interested in food products that have sufficient amounts of bioactive components. The main reason is that many studies have proven that there is a relationship between the consumption of bioactive components and the prevention of various chronic diseases such as cardiovascular diseases [1]. Bioactives are compounds that exist naturally and are an essential or non-essential component of the food chain and have a beneficial effect on human health. Polyunsaturated fatty acids, polyphenol compounds, anthocyanins and vitamins are some of the bioactive compounds that help improve health [3, 2]. Pomegranate flowers are the flowers of deciduous shrubs or small trees of pomegranate and are a plant of the pomegranate genus [4]. Pomegranate is rich in phenols, tannins, sugars, pigments and trace elements, and polyphenols are its most abundant active compounds [5]. Pomegranate crude extract has good pharmacological effects such as antioxidant, anti-aging, lowering blood sugar, lowering blood pressure, lowering blood fat and anti-arteriosclerosis [6]. In early summer, the bell-shaped flowers that fail to set fruit are usually removed when the pomegranate flowers first appear in bud, and most of those flowers that don't bud properly during the next growth will drop off naturally. Therefore, pomegranate flowers are widely available. The polyphenols in pomegranate flowers have significant antioxidant activity [4] and functions such as lowering blood pressure and blood lipids [7], preventing arteriosclerosis and inhibiting the proliferation of cancer cells [8]. Many functions of pomegranate flowers are due to the presence of anthocyanins. Important

anthocyanins in pomegranate flowers include: pelargonidin-5,3-diglucoside and pelargonidin-3-glucoside [9].

Various methods have been used to extract bioactive compounds from plants. Traditional extraction methods include percolation and hot extraction by Soxhlet system, which usually have a long extraction time and their extraction yield is insufficient [10]. Therefore, unconventional and newer methods such as extraction methods using ultrasound, microwaves, and ohmic heating have been presented to overcome the disadvantages of traditional methods for extracting bioactive compounds from plant sources. These methods have a shorter operation time and have a higher capacity to recover active compounds. Studies have also shown the environmental problems of conventional methods due to the need for high volume of organic solvent [12, 11]. The use of ohmic heat due to the ionic movement caused by the heating process with an electric field in the medium range (more than 100 V/cm), increases the temperature and is a powerful tool for extracting active compounds with higher yield provides [13]. In the research conducted by Shahidi et al. (2020), they compared ohmic, ultrasonic, decoction, and maceration methods on the extraction of active compounds from flaxweed and found that the best method for extracting bioactive compounds from flaxweed was the ultrasonic method [14]. Moeini et al. (2022) in the study of extract extraction methods from *Agrimonia eupatoria* showed that the ultrasonic method was more effective in extracting active compounds compared to the microwave method [15]. Matini et al. (2020) reported that the ultrasonic method is more efficient than the conventional

maceration method in extracting the remaining bioactive compounds of Sardasht black grapes pomace [16]. In this research, the comparison of different extraction methods as well as operation conditions on the extraction of bioactive compounds from Persian golnar was discussed.

## 2. Materials and methods

### 2.1. Materials

Persian golnar (*punica pranatum*) was purchased from medicinal plant shop in Tehran (Iran) and all chemicals used in this research were obtained from Merck Company (Germany).

### 2.2. Preparation of Persian golnar extract by ohmic method

The extraction process was carried out in an ohmic heating system that was bath type with a voltage of 2-8 V/cm and 25 kHz. The extractor consisted of a 500 ml round glass flask equipped with two titanium electrodes and a transformer control source. Two temperatures of 45 °C and 60 °C and two operation times of 40 and 60 min were used for this extraction. A condenser was attached to the extraction chamber to minimize solvent evaporation from the flask. In each experiment, golnar powder was mixed with 70% ethanol solvent at a ratio of 1:10, and 0.5 g of sodium chloride was added to the ohmic chamber. Salt was added to achieve proper conductivity. The salt addition was repeated in three other cases to ensure the stability of operation [17].

### 2.3. Preparation of Persian golnar extract by ultrasonic method

To extract the golnar extract by ultrasonic method, the plant powder was placed in a

50 ml centrifuge tube and 70% ethanol solvent was added to it in a ratio of 1:10. The extraction was done at three temperatures of 40, 50 and 60 °C and two times of 20 and 40 min. After extraction, the resulting solutions were filtered and then their solvent was evaporated in a rotary evaporator at a temperature of 45°C [4].

### 2.4. Preparation of Persian Golnar extract by percolation method

In percolation method, 10 g of plant powder was mixed with 100 ml of 70% ethanol and poured into a glass, and a magnet was placed inside it, and the glass was placed on a heater, and the extraction of the extract was carried out in three times of 24, 48 and 72 h. The resulting extracts were filtered and their solvent was evaporated in a rotary evaporator at a temperature of 45 °C [18].

### 2.5. Extraction yield

In order to determine the extraction yield of the extracts, the extract (2 ml) was placed in an oven at a temperature of 60 °C until the solvent evaporates, and the extraction yield of the extracts was calculated using equation (1) [19]:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Initial weight of sample}} \times 100$$

### 2.6. Total phenol content

To determine the total phenolic content of the extracts of Persian golnar, 30 µl of each extract was mixed with deionized water (3 ml) and Folin-Ciocalteu reagent (200 µl) and the resulting mixture was kept at room temperature for 10 min. After that, 600 µl of sodium carbonate solution (20%) was added to the mixture and the resulting mixture was kept in a hot water bath at 40°C for 20 min and then cooled. The color

of the resulting mixture was read by a UV/VIS spectrophotometer at a wavelength of 760 nm, and through the standard curve of gallic acid, the total phenol content of the extracts was obtained and reported in terms of mg GAE/100g dw [20].

## 2.7. Anthocyanin content

The total anthocyanin content of the extracts of Persian golnar was determined using differential pH method. First, the extract was dissolved in a pH 1 buffer solution (0.25 M potassium chloride and hydrochloric acid) and a pH 4.5 buffer solution (0.4 M sodium acetate). The absorbance of the solution in each of the buffers was measured at both wavelengths of 510 and 700 nm. The anthocyanin concentration of the extract was calculated through the following equation, in which: Mw molecular weight of anthocyanin for cyanidin-3-glucoside (449.2); DF dilution factor; L tube length;  $\epsilon$  absorption coefficient (26900); A is the absorbance of the sample obtained through equation (2) [21]:

$$\text{Anthocyanin} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{A \cdot \text{MW} \cdot \text{DF} \cdot 1000}{\epsilon \cdot L}$$

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$$

## 2.8. Measurement of antioxidant activity of extracts

DPPH radical scavenging method was used to measure the antioxidant activity of Persian golnar extracts. For this purpose, the extract (5 ml) was first mixed with 100  $\mu\text{M}$  DPPH methanolic solution (1 ml) and after vigorous stirring, it was kept for 1 h in the dark at 30 °C. Finally, the absorption of the resulting mixture was recorded by the spectrophotometer at 517 nm. The standard solution consisted of 5 ml of ascorbic acid mixed with 1 ml of DPPH solution. Finally, DPPH radical scavenging values of the

samples were obtained using equation (3) and reported as percentage. In the following relationship:  $A_c$  and  $A_{hs}$  were the absorption of the control and the absorption of the extract sample, respectively [22].

$$\text{DPPH (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

## 2.9. Statistical analysis

Analysis of the data obtained from three repetitions of tests related to the extracts was performed using one-way analysis of variance (ANOVA) and SPSS 22.0 software. To compare the significance of means at the 5% level, Duncan's multiple range test was used and the corresponding graphs were drawn with Excel.

## 3. Results and Discussion

### 3.1. Extraction yield

The values of yield extraction percentage of extracts of Persian golnar prepared by different methods and operating conditions are shown in Table 1. In general, the extract extraction method didn't have a significant effect on the extraction yield of extract. In ohmic and ultrasonic methods, with increasing temperature and extraction time, an increase in the extraction yield of the resulting extracts was observed, and in the percolation method, increasing the operation time increased the extraction yield of Persian golnar extracts. Increasing the extraction temperature by softening the plant cell wall and reducing the viscosity of the solvent increases the permeability and releases more active plant compounds. As the extraction time increases, more destruction occurs in the plant cell structure, which increases the extraction yield of the extracts. In general, the highest extraction yield was related to the extract prepared by the ultrasound method at a temperature of 60°C and a time of 40 min

(21.65%), and the extract prepared by an ultrasound method at a temperature of 40°C and a time of 20 min had the lowest extraction yield (20.38%), however, there was no significant difference between this treatment and the extract prepared by the ohmic method at a temperature of 45 °C and a time of 40 min (20.52%), as well as the extract prepared by the percolation method at a time of 24 h (20.45%). Shahidi et al. (2020) investigated the effects of different techniques (maceration, ultrasound, ohmic and boiling) on the extraction performance of the flixweed seeds extracts and observed that the ohmic and ultrasound extraction obtained the highest (10%) and lowest (2%) extraction yield, respectively [14]. Gahruie et al. (2020) also found that increasing the extraction time with the help of ultrasonic waves and ohmic heating increased the extraction yield of saffron extract. These researchers reported the extraction yield of the obtained extracts by the ultrasonic method higher than the ohmic method [23]. Perreira et al. (2016) also showed a direct relationship between the extraction time and the extraction yield of active compounds from potato [24]. Mohagheghi et al. (2010) showed that there was no statistically significant difference between the extraction yield of ramus potato skin extracts prepared by percolation and ultrasonic methods [25].

### 3.2. Total phenolic content

The results of the total phenolic content of extracts of Persian golnar are given in Table 1. In the extraction of plant extracts, temperature is both a stimulus for the extraction of active compounds and can cause the destruction of phenolic compounds. Increasing the temperature to the appropriate level makes the plant tissue softer and facilitates the release of phenolic

compounds from the plant [26]. Increasing the time under suitable conditions can also increase the temperature of the solvent and reduce the viscosity and surface tension of water, thereby increasing the rate of diffusion and mass transfer of phenolic compounds and improving the extraction of active compounds [27]. Since the temperatures used to extract the extract by the ohmic method were relatively low, increasing the temperature from 45 to 60 °C showed a positive effect on the extraction of phenolic compounds of golnar, and with increasing temperature, the total phenol content of the extracts increased. At a lower temperature (temperature of 45°C) as a result of increasing the operation time, due to sufficient time to extract active compounds, the content of total phenol increased, but at a higher temperature (temperature of 60°C), increasing the time caused a decrease in the content of total phenol of the extracts. Because at higher temperatures, as a result of prolonging the process, a part of the extracted phenols is destroyed. In the percolation extraction system, increasing the extraction time initially increased and then decreased the total phenol content of the extracts. In general, in shorter times, due to the higher concentration gradient of phenolic substances between the plant cell and the solvent, the extraction process occurs faster, but in longer times, the concentration gradient of substances decreases and the release rate of active compounds decreases. In general, the total phenolic content of the extracts obtained by different methods was obtained in the range of 120.53-85.36 mg GAE/g. The highest content of total phenol was related to the extract prepared by the ultrasonic method at a temperature of 50 °C and a time of 20 min, and the extract prepared by the percolation method for 72

h had the lowest content of total phenol. The reason for the good efficiency of the ultrasonic system in extracting active compounds from plants compared to conventional methods is that the phenomenon of cavitation created by ultrasound waves creates high shear forces in the structure of the plant and breaks the cell wall and causes the penetration of the solvent into the plant material and allows the intracellular content to be released in the environment [23]. In the ohmic extraction system, volume heating occurs in the system, which increases the temperature much faster than traditional and unconventional extraction methods, and increasing the applied voltage increases the heating rate of the process. This sudden increase in temperature damages the cells, disrupts the structure, and releases the target molecule into the surrounding compartment, accelerating the extraction of active compounds [28]. In the present study, the content of phenolic compounds extracted by ohmic and ultrasonic methods was higher than the conventional percolation method. In the research of Kutlu et al. (2021), it was found that the amounts of phenolic compounds extracted from cranberries in the ohmic method were significantly higher than the conventional maceration method and the ultrasonic method [12]. Coelho et al. (2017) stated that due to the use of a high electric field

and rapid heating in the ohmic method, this method resulted in better preservation of the phenolic compounds of tomato by-products compared to conventional methods [29]. In the research of Safarzadeh Markhali et al. (2022), it was observed that by increasing the extraction temperature with the help of ohmic heating from 45 to 75 °C, the content of phenolic compounds in olive leaf extract increased slightly at first and then did not change [30]. In the research conducted by Saifullah et al. (2020), the conventional extraction method (vibrating water bath) with the ultrasonic extraction method on the extraction of phenolic compounds from the leaves of *Leptospermum petersonii* were compared, and observed that the ultrasonic extraction method has a higher yield for the extraction of phenolic compounds and also the activity showed a higher antioxidant compared to the conventional method. The optimal extraction conditions in this research included: extraction time of 60 min, extraction temperature of 50 °C and ultrasound power of 200 W [31]. Sadeghii et al. (2021) also found that by increasing the percolation extraction time up to 40.45 h, the total phenol content of the extracted Khandal extracts increased, but a further increase in the extraction time decreased the total phenol content, which was consistent with the results of the present study [32].

**Table 1.** Extraction yield and total phenol content of Persian golnar extracts

Extraction methods	Temperature (°C)	Time	Extraction yield (%)	TPC (mg GAE/g)
Ohmic	45	40 min	20.52 ± 0.09 <sup>fg</sup>	94.80 ± 1.27 <sup>e</sup>
		60 min	20.89 ± 0.07 <sup>d</sup>	102.15 ± 2.59 <sup>d</sup>
	60	40 min	20.83 ± 0.11 <sup>de</sup>	109.74 ± 0.53 <sup>b</sup>
		60 min	21.35 ± 0.09 <sup>bc</sup>	104.80 ± 2.87 <sup>cd</sup>
Ultrasonic	40	20 min	20.38 ± 0.07 <sup>g</sup>	107.04 ± 0.36 <sup>c</sup>
		40 min	20.65 ± 0.12 <sup>ef</sup>	106.66 ± 0.18 <sup>c</sup>
		20 min	20.97 ± 0.05 <sup>d</sup>	120.53 ± 0.10 <sup>a</sup>

Percolation	60	40 min	21.24 ± 0.08 <sup>c</sup>	103.33 ± 2.36 <sup>d</sup>
		20 min	21.47 ± 0.06 <sup>b</sup>	89.15 ± 0.21 <sup>f</sup>
-	-	40 min	21.65 ± 0.05 <sup>a</sup>	86.00 ± 0.17 <sup>h</sup>
		24 h	20.45 ± 0.10 <sup>fg</sup>	87.78 ± 0.54 <sup>g</sup>
		48 h	20.93 ± 0.08 <sup>d</sup>	106.69 ± 0.20 <sup>c</sup>
		72 h	21.38 ± 0.12 <sup>bc</sup>	85.36 ± 0.25 <sup>i</sup>

\*Values represent mean (n=3) ± SD. Different letters in each column represent statistical significant difference at 5% level. TPC: Total phenol content; GAE: Gallic acid equivalent.

### 2.3. Total anthocyanin content

Anthocyanins are natural pigments responsible for blue, red and purple colors in flowers, vegetables and fruits. These pigments are red in acidic pH, colorless in neutral pH and blue and purple in alkaline pH. Anthocyanins obtained from different plant sources have also shown significant antioxidant activities [33]. The results of the analysis of the total anthocyanin content of the extracts of Persian golnar (Table 2) showed that in the ohmic system, increasing the temperature and time increased the content of anthocyanin extracted from Persian golnar. In the ultrasonic system, in a shorter time, with the increase in temperature from 40 to 50 °C, the amount of anthocyanin increased and then with the increase in temperature from 50 to 60 °C, the amount of anthocyanin decreased significantly. Increasing the extraction time with sonication decreased the anthocyanin content of the extracts. In the extraction by percolation, as the extraction time increased, the content of anthocyanins decreased due to degradation. Degradation of anthocyanins due to the increase in ultrasonic extraction time was also observed in the research conducted by Tiwari et al. [34]. The mechanism of destruction of anthocyanins and active compounds due to the increase in the extraction time with sonication, due to the formation of free radicals due to the hydrolysis of water, the effect of heat and

isomerization, has been stated [35]. In general, the content of anthocyanins extracted by the ohmic extraction method was higher than the other two methods, and the ultrasonic method extracted the content of anthocyanins better compared to the percolation method. In general, the extract prepared by the ohmic method at a temperature of 60°C and a time of 60 min had the highest total anthocyanin (426.42 mg/g) and the lowest amount of these active compounds was related to the extract prepared by the percolation method at a time of 72 h (152.65 mg/g).

In the research conducted by Zhang et al. (2011), two anthocyanins including pelargonidin 3,5-diglucoside and pelargonidin 3-glucoside were identified in pomegranate flower extract, which had strong anti-radical activities [9]. In the research of Perreira et al. (2016) it was also observed that increasing the ohmic temperature from 30 to 90 °C increased the extraction efficiency of anthocyanins and increasing the extraction time from 0 to 10 min also increased the content of anthocyanins in the extract. [24]. Belwal et al. (2019) found that increasing the ultrasonic time up to 21 min increased the content of anthocyanins in pear peel extract, but the use of times higher than 21 min resulted in a decrease in the content of anthocyanins in the extract. Increasing the ultrasonic temperature to 55 °C also increased the anthocyanin content of the extract [36]. Kutlu et al. (2021) reported

that the content of anthocyanins extracted from cranberry was higher in the ohmic method than other methods, followed by the ultrasonic method, and the lowest amount was related to the common maceration method, which was consistent with the results of the present study [12]. In the research of Gahruie et al. (2020), the content of total anthocyanins in saffron extracted by the ohmic method was higher than the ultrasonic method, which was consistent with the results of the present study [23].

### 3.4. Antioxidant activity

In Table 2, the results of the investigation of the antioxidant activity of extracts of Persian golnar in the common DPPH radical inhibition method are shown. Although the total phenol content is almost equal to the extracts obtained by ohmic and ultrasonic methods, the antioxidant activity of the extracts obtained by the ohmic method was higher, which can be due to two reasons: the first reason is the higher content of anthocyanins in the extract in the ohmic method compared to the ultrasonic method, and due to the antioxidant activity of anthocyanins, the presence of their higher amounts causes higher antioxidant activity. On the other hand, the position and number of hydroxyl groups of the extracts have a significant effect on their antioxidant activity, and research has shown that the extraction method with the help of ohmic heating has a hydroxylation effect and increases the number of hydroxyl groups [28], which can be a reason for higher DPPH radical inhibitory activity of extracts obtained by ohmic heating compared to ultrasonic and percolation methods. In the ohmic method, at a lower temperature, the antioxidant activity of the extracts increased with an increase in the extraction

time, but at a higher temperature, an increase in the time caused a decrease in the antioxidant activity of the extracts, because prolonged the extraction process at higher temperatures caused the destruction of some valuable active compounds extracted, such as phenolic compounds and anthocyanins, and operations that have shorter extraction times often show higher antioxidant activity [12]. In the extraction method with ultrasonic waves, increasing the extraction temperature up to 50 °C, due to the increase in the content of phenolic and anthocyanin compounds, increased the antioxidant activity of the extracts, however, increasing the temperature from 50 to 60 °C, due to the decrease in the content of bioactive compounds, reduced the DPPH radical scavenging of the extracts. Increasing the time of extraction with ultrasonic waves decreased the antioxidant activity of the extracts, and as expected, in this extraction method, there was a direct relationship between the content of phenolic compounds, anthocyanins and the antioxidant activity of the extracts of Persian golnar. In the percolation method, with the increase in the extraction time, the content of phenolic compounds increased at first, and therefore an increase in the antioxidant activity of the extracts was observed, but a further increase in the processing time caused a decrease in the antioxidant activity by decreasing the content of phenolic and anthocyanin compounds. In general, the highest amount of antioxidant activity was observed in the extract prepared by the ohmic method at a temperature of 60 °C and a time of 40 min (81.11%), and the lowest amount was related to the extract prepared by the percolation method and the operation time of 72 h (61.07%).



Abid et al. (2022) stated that hydroalcoholic extracts of pomegranate flowers show significant antioxidant activity [37]. The antioxidant activity of wheat bran extracts extracted by the ohmic method in the research of Al-Hilphy et al. (2015) was also higher than the extracts extracted by the conventional method, and these researchers also attributed the shorter extraction time by the ohmic method to the higher antioxidant activity of the extracts obtained by this method compared to the common method [38]. Rodrigues et al. (2021) stated that ultrasonic and ohmic methods, due to their operation at low temperatures, lead to better preservation of phenolic compounds in sugarcane juice [39]. Wu et al. (2021) stated that in all tests used to investigate antioxidant activity (FRAP, ABTS, DPPH and O<sub>2</sub>- radical scavenging), pomegranate flower extracts showed good antioxidant capacity [4]. Papoutsis et al. (2018) found that both the common hot water extraction method and the newer ultrasonic method destroy the cell wall, and the extracts obtained by both methods have high antioxidant capacity [40]. Some researchers stated that the content of phenolic compounds extracted

by different methods may be different, but in some materials, despite this difference in bioactive compounds, no significant difference has been observed between the antioxidant activity of extracted extracts, which is related to lack of antioxidant properties of some phenolic compounds in plants [41, 12]. In the study by Safarzadeh Markhali et al. (2022), it was observed that by increasing the extraction temperature with the help of ohmic heating from 45 to 75 °C, the content of the phenolic compounds of the olive leaf extract increased slightly, and then it didn't change, but the DPPH radical inhibition percentage of the extracts increased significantly. These researchers attributed their results to the presence of different phenolic compounds in this extract, which affected the relationship between total phenol content and antioxidant activity [30]. Sadeghii et al. (2021) found that by increasing the percolation extraction time up to 45.40 h, the antioxidant activity of the extracts increased, but a further increase in the extraction time caused a decrease in total phenol content and antioxidant activity, which was consistent with the results of the present study [32].

**Table 2.** Total anthocyanin content and antioxidant activity of Persian golnar extracts

Extraction method	Temperature (°C)	Time	TAC (mg/g)	Antioxidant activity (%)
Ohmic	45	40 min	175.54 ± 2.65 <sup>j</sup>	71.72 ± 0.46 <sup>d</sup>
		60 min	296.23 ± 0.52 <sup>f</sup>	76.69 ± 1.08 <sup>c</sup>
	60	40 min	373.26 ± 1.24 <sup>b</sup>	81.11 ± 0.96 <sup>a</sup>
		60 min	426.42 ± 1.54 <sup>a</sup>	78.59 ± 0.78 <sup>b</sup>
Ultrasonic	40	20 min	337.40 ± 1.48 <sup>d</sup>	75.42 ± 0.52 <sup>c</sup>
		40 min	309.42 ± 2.56 <sup>e</sup>	71.55 ± 0.72 <sup>de</sup>
	50	20 min	354.50 ± 1.94 <sup>c</sup>	78.98 ± 0.43 <sup>b</sup>
		40 min	297.56 ± 1.21 <sup>f</sup>	71.00 ± 1.83 <sup>de</sup>
Percolation	60	20 min	283.15 ± 0.98 <sup>g</sup>	75.63 ± 0.90 <sup>c</sup>
		40 min	250.54 ± 1.39 <sup>h</sup>	66.86 ± 1.05 <sup>f</sup>
	-	24 h	218.64 ± 1.12 <sup>i</sup>	65.02 ± 0.45 <sup>g</sup>
		48 h	159.72 ± 0.70 <sup>k</sup>	70.05 ± 0.84 <sup>e</sup>
		72 h	152.65 ± 3.09 <sup>l</sup>	61.07 ± 0.59 <sup>h</sup>

\*Values represent mean (n=3) ± SD. Different letters in each column represent statistical significant difference at 5% level. TAC: Total anthocyanin content.

#### 4. Conclusion

The results showed that new extraction methods using ohmic heating and ultrasound waves compared to the conventional percolation method led to better extraction of active compounds from Persian golnar. Despite the lack of significant difference between total phenolic content of extracts prepared by ohmic heating and ultrasound methods, total anthocyanin content and antioxidant activity of extracts prepared by ohmic heating method were significantly higher than ultrasound method. The results of this research showed that among the extracts produced in this research, the extract prepared by ohmic method, temperature of 60 °C and extraction time of 40 min, was the optimal treatment, and the total phenol content, total anthocyanin, antioxidant activity and extraction yield of this extract were 109.74 mg GAE/g, 373.26 mg/g, 81.11% and 20.83% respectively. Due to the non-thermal and modern ohmic method, plant bioactive compounds are better extracted, so they can be used in the food industry as natural preservatives and in the pharmaceutical industry.

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## استخراج ترکیبات زیست فعال گلنار فارسی (*punica pranatum*) با استفاده از روش های اهمیتیک،

### فراصوت و پرکولاسیون

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اطلاعات مقاله	چکیده
<b>تاریخ های مقاله :</b>  تاریخ دریافت: ۱۴۰۲/۹/۴ تاریخ پذیرش: ۱۴۰۲/۱۰/۲۳	<b>چکیده:</b> ترکیبات زیست فعال گیاهی، دارای اثرات سلامت بخش برای انسان ها و دارای اثرات نگهدارندگی در محصولات غذایی می باشند. شرایط استخراج عصاره ها تأثیر قابل توجهی بر میزان ترکیبات زیست فعال آن ها دارد از روش های متداول و هم روش های جدیدتر برای استخراج ترکیبات زیست فعال از گیاهان استفاده می شود. هدف از این تحقیق، مقایسه روش های استخراج ترکیبات زیست فعال گلنار فارسی بود. در این تحقیق از سه روش اهمیتیک (دماهای ۴۵ و ۶۰ درجه سانتی گراد و زمان های ۴۰ و ۶۰ دقیقه)، اولتراسونیک (دماهای ۴۰، ۵۰ و ۶۰ درجه سانتی گراد و زمان های ۲۰ و ۴۰ دقیقه) و پرکولاسیون (زمان های ۲۴، ۴۸ و ۷۲ ساعت) برای استخراج عصاره گلنار فارسی استفاده شد و بر اساس بازده استخراج، محتوای فنول کل، آنتوسیانین کل و فعالیت آنتی اکسیدانی (مهار رادیکال DPPH) بهترین شرایط استخراج انتخاب گردید. نتایج نشان داد که بین بازده استخراج هر سه روش اختلاف معنی داری وجود نداشت ( $p > 0.05$ ). روش اهمیتیک دارای محتوای فنول مشابهی با روش اولتراسونیک بود، ولی محتوای آنتوسیانین کل و فعالیت آنتی اکسیدانی بالاتری نشان داد. تیمار بهینه شامل روش اهمیتیک، دمای ۶۰ درجه سانتی گراد و زمان استخراج ۴۰ دقیقه بود و عصاره حاصله تحت این شرایط حاوی $109.74 \text{ mg GAE/g}$ فنول کل، $373.26 \text{ mg/g}$ آنتوسیانین کل و $81.11$ درصد فعالیت آنتی اکسیدانی بود. از عصاره گلنار فارسی در صنایع غذایی و دارویی می توان استفاده کرد.
<b>کلمات کلیدی:</b> گلنار فارسی، روش های استخراج، ترکیبات فنولی، فعالیت آنتی اکسیدانی	
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