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ORIGINAL ARTICLE

Optimizing the Extraction of Phenolic Compounds from Pistachio Hulls

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A R T I C L E I N F O

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A B S T R A C T

Pistachio hull, as the dominant component of pistachio processing wastes, is a source of phenolics with antioxidant properties. Extracting these compounds from pistachio wastes has recently attracted a lot of attention. The current study aimed to identify the optimal conditions for extracting phenolics from pistachio hulls. Firstly, some parameters such as different sample to solvent ratios (1:10, 1:20, and 1:40), extraction temperatures (25, 45, and 65°C), and duration times (1, 2, and 3 h) were evaluated under extracting solvent of water. The content of total phenolics and antioxidant activity were determined using spectrophotometric methods. The results indicated that the highest phenolics (~35 mg g^{-1}) were extracted at 2 h extraction time, 65 °C, and the sample to solvent ratio of 1:10 (optimum condition by the solvent of water). Secondly, other organic solvents of ethanol, methanol, and acetone 50% were evaluated to identify the best solvent under optimal conditions. All of these organic solvents were more efficient than water in extracting total phenolics (with antioxidant property) from pistachio hulls. However, the highest amount of total phenolics (~47 mg g^{-1}) was extracted by ethanol. Optimizing the extraction of phenolic compounds from pistachio hulls would be important in terms of reducing human and environmental risks caused by the waste accumulation, as well as producing naturally beneficial compounds and using them instead of synthetic antioxidants in the human diet.

Introduction

Pistachio (*Pistacia vera* L.) is originated from Central Asia and then introduced throughout the Middle East and the Mediterranean area (Hosseini *et al*., 2022; Nazoori *et al*. 2022). The history of pistachio cultivation in Iran dates back to 3-4 thousand years ago (Abrishami *et al*., 2019) when this plant was well adapted to arid

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and semi-arid regions of Iran (Norozi *et al*., 2019). With the start of pistachio export about 70 years ago, it attracted special economic and commercial importance and Iran was recognized as the first and the most important producer in the world. Since then, the area under cultivation of this crop has expanded to about 10-12 thousand hectares annually. Abiotic stresses hiders plants from survival in a specific environment by imposing adverse effects on plant performance (Babaei *et al*., 2021) The other important factor involved in the expansion of pistachio cultivation is its resistance to drought and salinity conditions (Abrishami *et al.*, 2019). Its cultivated area is estimated at 459000 hectares, with an average annual production of 240000 tons of dried pistachio (Hokmabadi, 2018).

Hulling of pistachio product after harvesting annually produces about 400000 tons of wastes (byproducts) in Iran (Bagheripour *et al.*, 2008). Pistachio wastes are a set of materials that remains in the hulling machine by separating pistachio seeds. They are composed of colored hulls (exo- and mesocarp), clusters, and leaves (Aminian and Shakerardekani, 2009). It has experimentally shown that 1 kg pistachio is obtained from every 3 kg product transported from gardens to processing terminals. The rest (2kg) are wastes, which mainly include pistachio hulls (Erşan *et al.*, 2016). Because of high moisture content, these compounds have a perishable nature and after a few days (sometimes 1 or 2 days) become black, rotten, and moldy (Shakerardekani and Molaei, 2020). In addition, the infestation of annoying insects can be seen around them. Such compounds are also a suitable substrate for overwintering of *Aspergillus* spores, which makes it difficult to prevent the spread of aflatoxin contamination (Foroogh-Ameri, 1997). Some gardeners unfortunately use these wastes as green manure, which in turn introduce aflatoxin to their orchards (Shakerardekani and Molaei, 2020). As a result, pistachio wastes cause widespread environmental risks. On the other hand, significant amounts of agricultural products accrue having low or no commercial value, which must be disposed of at the processor's expense (Erşan *et al.*, 2016).

Recently, the valorization of pistachio wastes has attracted widespread interest, so far been hampered by lacking identification of valuable target compounds. Pistachio hull, as the predominant byproduct of commercial pistachio processing, is recognized as the main source of phenolic compounds with antioxidant and antimicrobial activities (Goli *et al.*, 2005; Rajaei *et al.*, 2010; Barreca *et al.*, 2016). Phenolic compounds are an integral part of the human diet and their benefits are related to antioxidant activity (Cheniany *et al.*, 2013; Khodadadi *et al.*, 2016; Gam *et al.*, 2020). In recent years, using natural phenolic compounds instead of synthetics, such as butylated hydroxyetholuene (BHT) and butylated hydroxyanisole (BHA), in the food industry has attracted much attention (Goli *et al.*, 2005; Taghvaei and Jafari, 2015; Habibie *et al.*, 2019; Liu and Mabury, 2020; Wang *et al.*, 2021). In addition to plant products (like rosemary, thyme, pepper, peach, tea, pistachio, walnut, etc.), some agricultural byproducts such as date, grape and pomegranate seeds, and pistachio and walnut hulls can also be important sources of phenolic compounds (Al-Farsi and Lee, 2008; Kabir *et al.*, 2015; Shamshiri and Hasani *et al.*, 2015; Mokrani and Madani, 2016; Ambigaipalan *et al.*, 2017; Erşan *et al.*, 2017; Habibie *et al.*, 2019; Sharifkhah *et al.*, 2020, Habibi *et al.*, 2021). HPLC assessment realized 66 phenolic compounds (as gallic acid, monogalloyl glucoside, monogalloyl quinic acid, penta-*O*-galloyl-*β*-D-glucose, hexagalloyl hexose, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-glucoronide, etc.) in pistachio exo- and mesocarp (red and green hulls) (Erşan *et al.*, 2016). So, pistachio hulls were recognized as a structurally diverse and potentially active source of phenolic compounds. They therefore would be a valuable byproduct of pistachio processing which have further application as raw materials for the recovery of nutritional, pharmaceutical, and chemical products.

Mokhtarpour *et al.* (2014) studied the effects of different solvents (70% acetone, 50% methanol, 50% ethanol and water), particle sizes of fine versus course, and extraction times of 12 and 24 h on the extraction of total phenolics of pistachio byproducts. Their results indicated that the extracted total phenolics were more by using 70% acetone (13.86%) compared to other solvents. The lowest phenolics content was extracted by water (9.87%). Parameters as particle size and extraction time had no significant effect on extracted total phenolics. In another part of this experiment, ultrasound-assisted extraction was used for phenolics extraction, and results were compared to 12 h extraction without ultrasonic. There was not any significant difference between the total phenolics content extracted by these two methods (Mokhtarpour *et al.*, 2014).

In another research, phytochemicals and free radicle scavenging activity of wild pistachio (*P. khinjuk*) hull and kernel were evaluated by using the ethanol percolation method (Mortazavi *et al.*, 2015). Phenolic content was detected in the hulls and kernels as 25.6 and 6.3 mg g^{-1} dry weight, respectively. The hull extract had a greater effect on the scavenging of free radicles (88%) compared to the kernels, which could be due to its higher amounts of phenolic compounds.

As mentioned above, various works have been performed on pistachio wastes and extracting phenolic compounds under different conditions. In the current research, it has been tried to evaluate and compare a set of different conditions (such as different extraction times, temperatures, sample to solvent ratios, and solvents) together, which were examined separately in previous studies. On the other hand, unlike some researches that were performed on pistachio green hull (Rajaei *et al.*, 2010), we focused more on the ripped and red hull at harvesting time to reduce the human and environmental risks caused by their accumulation at pistachio processing period and also to optimize the production of naturally beneficial compounds.

Materials and Methods

Plant material extraction

Pistachio (*P. vera*) crop was harvested at the maturity phase. Their hulls, which often become red after ripening, were physically separated, dried, and grounded using a laboratory mill. Dried materials were subjected to phenolic compounds extraction using a magnetic stirrer plate. To determine the optimal extraction condition of phenolics, firstly some parameters as a different sample to solvent ratios (1:10, 1:20, and 1:40), extraction temperatures (25, 45, and 65°C) and times (1, 2 and 3 h) were applied under extracting solvent of water. The supernatant was separated by centrifugation (15 min at 10000 rpm) and filter paper of Whatman No. 4.

Total phenolics

Total phenolic compounds of pistachio hull extracts were measured according to the modified method of Folin-Ciocalteu (Yoo *et al.*, 2004). About 200 µl of hull extract was mixed with 1 ml of Folin-Ciocalteu reagent and 0.8 ml of 7.5% sodium bicarbonate solution (w/v). After 30 min at room temperature, the absorbance was read at 765 nm. Total phenolic content was expressed as mg gallic acid equivalents per g sample dry weight using the calibration curve.

Antioxidant activity

The other factor evaluated in this research was antioxidants activity. Its measuring was based on the reduction of the radical form of DPPH (1,1-diphenyl1-2picrylhydrazyl) alcoholic solution in the presence of hydrogen-giving antioxidants, especially phenolic compounds. As 0.1 ml of extract was mixed with 0.9 ml of methanolic solution of DPPH (0.04 mg ml^{-1}). After 20 min at room temperature and darkness, the absorbance was read at 517 nm (Mokrani and Madani, 2016).

Extraction of some organic solvents

According to the content of total phenolics extracted in the previous step (by water solvent), the optimal conditions for extraction were determined. Then other organic solvents (ethanol, methanol, and acetone 50%) were evaluated to identify the best solvent under optimal conditions. Factors studied in this part of the research include total phenolics as well as antioxidant activity.

Statistical analysis

In this research, a factorial experiment in a completely randomized design was employed to evaluate the effects of independent variables (3 extraction times of 1, 2 and 3h; 3 temperatures of 25, 45 and 65°C; 3 samples to solvent ratios of 1:10, 1:20 and 1:40; and 4 solvents of water, ethanol, methanol, and acetone 50%) on dependent variables of total phenolics content and antioxidant activity, with 3 replications for each treatment. Statistical analysis of data was performed using SPSS (version 16) and Duncan's test ($P<0.05$) was applied to compare the means.

Results

The results of variance analysis indicated the effects of different sample to solvent ratios (1:10, 1:20 and 1:40), extraction times (1, 2 and 3h), temperatures (25, 45 and 65°C), and solvents (water, ethanol, methanol, and acetone 50%) on phenolic compounds and antioxidant activity of pistachio hull extract (Table 1).

Table 1. The variance analysis indicated the effects of different factors, as a sample to the solvent ratio (1:10, 1:20, and 1:40), extraction time (1, 2 and 3h) and temperature (25, 45, and 65°C), on phenolic compounds and antioxidant activity of pistachio hull extract.

source of variation	Phenolic compounds		Antioxidant activity	
	df	Mean Square	df	Mean Square
Sample to solvent ratio	2	428.861*	2	1146.978*
Extraction time	2	2033.966*	2	903.588*
Temperature	2	1480.091*	2	2990.821*
Sample to solvent ratio*extraction time	4	16.617	4	3.197
Sample to solvent ratio*temperature	4	22.752	4	24.854
Extraction time* temperature	4	109.053*	4	3281.887*
Sample to solvent ratio*extraction time*temperature	8	5.567	8	12.391
Error	216		216	
Total	243		243	
Solvents	3	118.865*	3	267.064*
Error	8		8	
Total	12		12	

*Significant at $P<0.05$

The effect of different extraction times (1, 2, and 3 h), temperatures (25, 45, and 65°C), and sample to solvent ratios (1:10, 1:20, and 1:40) on the concentration of total phenolics extracted from pistachio hulls were indicated in Fig. 1. As the results of 1 h extraction show, the content of total phenolics decreased significantly by changing the sample to solvent ratio from 1:10 to 1:20 and 1:40 at 25 and 45°C. There was not any significant difference between different samples to solvent ratios at 65°C. At 2 h extraction time, total phenolic reduced significantly by changing sample to solvent ratio from 1:10 to 1:40 at 25 and 45°C. Total phenolic compounds reached the highest amount of ~35 mg g^{-1} at 2 h extraction time, 65°C, and the sample to solvent ratio of 1:10 (optimum phenolic extraction condition by water). There was not any significant difference between different samples to solvent ratios at this condition. By increasing extraction time to 3 h, the content of total phenolics reduced significantly. As the lowest content (~13 mg g^{-1}) of total phenolics was detected at 3h extraction time and 1:40 sample to the solvent ratio (Fig. 1).

Fig. 2 shows that the radicle-scavenging activity of pistachio hull extracts was enhanced by changing sample to solvent ratios from 1:10 to 1:40. This relationship was somewhat the same in different extraction times and temperatures (Fig. 2).

In the other part of current research (Fig. 3), total phenolics extracted by different organic solvents (ethanol, methanol, and acetone 50%) were compared to those extracted by water at optimal condition (2 h time, temperature of 65°C, and the sample to solvent ratio of 1:10). All three organic solvents of ethanol, methanol and acetone 50% were significantly more efficient than water in extracting total phenolic compounds from pistachio hulls. The highest amount of total phenolics (~47 mg g^{-1}) was extracted by ethanol and there was not any significant difference between methanol and acetone 50% (Fig. 3).

The effect of different solvents of ethanol, methanol, and acetone 50% on antioxidant activity (RSA %) of pistachio hulls extraction was compared to water in Fig. 4. As the results show, all organic solvents (ethanol, methanol, and acetone 50%) increased RSA % of the hull extract, compared to water. While there was not any significant difference between them (Fig. 4).

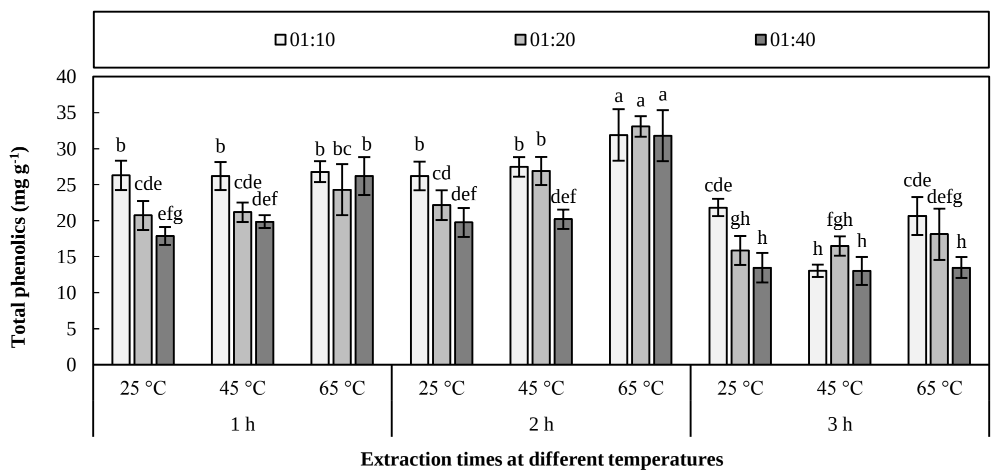

Fig. 1. The effect of different times (1, 2, and 3 h), temperatures (25, 45, and 65°C), and sample to the solvent ratio (1:10, 1:20, and 1:40) on the extraction efficiency of total phenolics from pistachio hulls. The columns with common letters are not significantly different according to Duncan test ($P<0.05$).

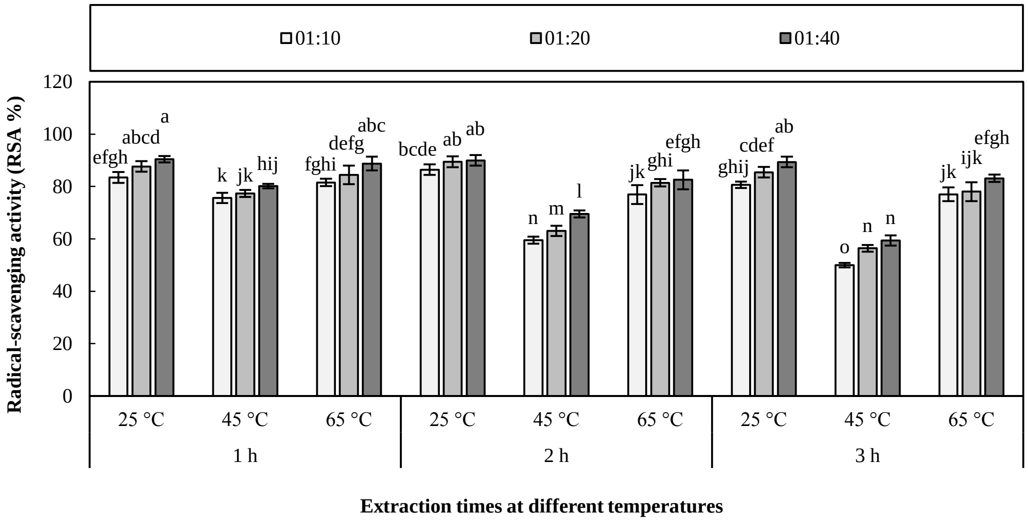

Fig. 2. The effect of different times (1, 2, and 3 h), temperatures (25, 45, and 65°C), and sample to the solvent ratio (1:10, 1:20, and 1:40) on radical-scavenging activity percent (RSA%) of pistachio hulls extraction. The columns with common leters are not significantly different according to Duncan test (P<0.05).

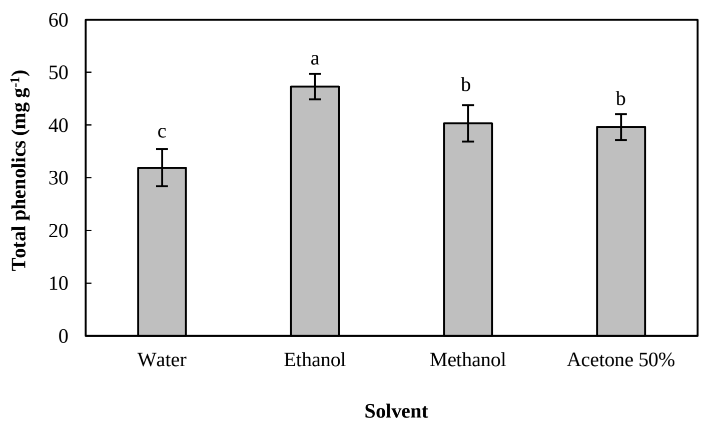

Fig. 3. The effect of solvent on the extraction efficiency of total phenolics from pistachio hulls under optimal conditions (2 h extraction time, temperature of 65 °C, and sample to solvent ratio of 1:10). The columns with common leters are not significantly different according to Duncan test (P<0.05).

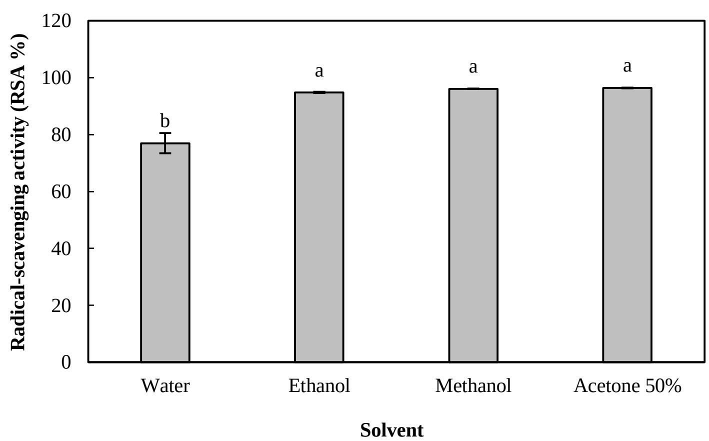

Fig. 4. The effect of solvent on radical-scavenging activity percent (RSA%) of pistachio hulls extraction under optimal conditions (2 h extraction time, temperature of 65°C, and sample to solvent ratio of 1:10). The columns with common leters are not significantly different according to Duncan test (P<0.05).

Discussion

Phenolic compounds are a group of secondary metabolites with antioxidant activity that are widely used in the food industry. Extracting natural phenolic compounds from agricultural products as well as byproducts and using them instead of synthetic compounds, has attracted scientists attention recently (Fallahi *et al.*, 2016).

In the first part of the current study, it was indicated that total phenolic compounds reached the highest amount of ~35 mg g^{-1} at 2 h extraction time, 65 °C, and the sample to solvent ratio of 1:10 (optimum phenolic extraction condition by water).

Rajaei *et al*. (2010) studied the effect of liquid to solid ratio (8-20 times), temperature (25-65°C), and time (5-45 min) on total phenolic compounds of pistachio green hull. They found that the optimal conditions for extraction of phenolic compounds through ME (Maceration Extraction) and MAE (Microwave-Assisted Extraction) methods were 20 (v/w), 65 °C, and 45 min. Their results in the case of extracting temperature and time are somehow in agreement with the current study (on ripped and mostly red pistachio hull).

According to current work, Pinelo *et al.* (2005) indicated that temperature and solvent to solid ratio have a critical role in the extraction efficacy of phenolic compounds and their corresponding antiradical activity from grape byproducts. Their results showed that temperature of 50°C (between 25 and 50°C) and solvent to solid ratio of 1:1 (between 1:1 and 5:1) maximized the antiradical activity of phenolic extracts.

In the second part of this study, organic solvents of ethanol, methanol, and acetone 50% were recognized more efficient than water in extracting total phenolic compounds (with antioxidant property) from pistachio hulls. However, the highest amount of total phenolics (~47 mg g^{-1}) was extracted by ethanol.

Riciputi *et al.* (2018) also found that the optimum phenolic extraction condition from industrial potato byproducts was obtained with ethanol/water 55/45 (v/v) and 1:10 sample to solvent ratio.

In agreement with these results, Pinelo *et al.* (2005) indicated that the order of decreasing capacity to extract phenolic compounds from grape byproducts was ethanol > methanol > water, and ethanol was the most efficient solvent.

Al-Farsi and Lee (2008) conducted a study for optimizing phenolic extractions from date seed. They evaluated the effect of solvent type (water, ethanol, methanol, acetone, 50% ethanol, 50% methanol, 50% acetone, butanol, and butanone) on the efficiency of phenolic extraction. In disagreement with current results, they found that acetone 50% and butanone were the most efficient solvents for phenolics purification and extraction.

Variety of results obtained from different studies may be related to various conditions of the experiment, plant species/cultivar, harvesting time, and other factors.

Finally, it can be concluded that different extraction parameters as solvent types, time of extraction, temperature, sample to solvent ratio, etc. have important impacts on the content of phenolics and corresponding antioxidant activity of pistachio hull extract.

According to the results obtained in this and other studies (Özbek *et al.*, 2018), the pistachio hull as the largest byproduct of the pistachio industry is a very rich source of phenolic compounds with remarkable antioxidant activity. In addition, the potential demonstrated by the extracts can lead to the valorization of a significant byproduct of pistachio processing which is inadequately used today and may reduce the environmental risks of these wastes.

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Conflict of interest

The authors declare no conflict of interest.

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