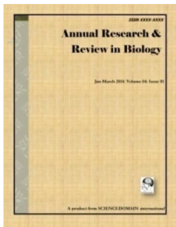

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Extraction of Phenolic Compounds and Tannins from Pistachio By-products

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study was conducted to evaluate the effects of solvent, particle size, extraction time and ultrasound on extraction and quantification of phenolic compounds and tannins in pistachio by-products (PB).
Study Design: Factorial experiment based on completely randomized design.
Methodology: Four solvents (70% aqueous acetone, 50% aqueous methanol, 50% aqueous ethanol and water), 2 particle size (fine vs coarse) and 2 extraction time (12 vs 24h) were used to extract total phenolics (TP) and total tannins (TT) of sun-dried PB (93.5% DM). Folin-Ciocalteu reagent was used for phenolics and tannins quantification. In another experiment, ultrasound-assisted extraction (UAE) was used for extracting phenolic compounds.
Results: Using 70% aqueous acetone resulted in more TP (13.86% of DM as tannic acid equivalent) compared to other solvents and the lowest TP content was measured in water (9.87%). Particle size and time of extraction had no effect on TP content. Tannin concentration were not affected by particle size but decreased by increasing time from 12 to 24h (7.34 vs 6.81%). Higher tannin was extracted by 50% aqueous ethanol compared to aqueous methanol and/or water (7.82%). However, no differences were observed between 70% aqueous acetone and 50% aqueous ethanol and/or methanol. The interactions between main effects were not statistically significant. In another experiment, TP and TT

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were extracted by ultrasound-assisted extraction (UAE) and results were compared to 12h extraction (without ultrasonic). No difference was observed between two methods in terms of TP and TT.

Conclusion: It can be concluded that using 70% aqueous acetone and 50% aqueous ethanol are more convenient solvent for extraction of phenolics and tannins in PB, respectively.

Keywords: Phenolics; pistachio by-products; tannins; ultrasonic assisted extraction.

1. INTRODUCTION

Plants may contain phenolic compounds ranging from simple molecules such as phenolic acids to highly polymerized molecules such as tannins [1]. Tannins are a complex group of polyphenolic compounds [2] and capable of binding and/or precipitating water soluble proteins [3].

There is an interest in quantification of phenolics and polyphenols in plant tissues because of their impact on human and animal health and performance. For example, the phenolic compounds contained in pistachio nuts (anthocyanins, flavan-3-ols, proanthocyanidins, flavonols, isoflavons, flavanones, stilbenes and phenolic acids) and skins [4] are known for their antioxidant [4,5], anti-inflammatory [6] and antimicrobial [5] activities.

Choosing inappropriate methods for tannin assay may lead to erroneous conclusions and flaw the investigations of nutritional significance of tannins [3]. Over the years, several assay methods, solvents and sample preparation techniques have been adopted for the quantification of polyphenolics [7] but there is no universal extraction procedure suitable for extraction of all plant phenolics. The method of sample preparation (air or oven drying and freeze-drying) and the choice of solvent have been shown to strongly influence polyphenol extractability in plant materials [7]. Extraction methods (microwave, ultrasound-assisted extractions and techniques based on use of compressed fluids as extracting agents, such as subcritical water extraction, supercritical fluid extraction, pressurized fluid extraction, or accelerated solvent extraction), particle size (coarsely ground versus finely ground), time of extraction and temperature may also affect the extractability of polyphenols [8].

Up to 400,000 tones/year pistachio by-products (PB) obtained after de-hulling of pistachio (Pistacia Vera L.) in Iran and it can be used as a convenient source of feed for ruminants [9]. However, the use of these by-products by ruminants might be restricted due to tannin content through toxicity [10] or interaction with protein, carbohydrates, minerals and/or rumen microorganisms [11].

Given the considerable variation resulting from sample preparation techniques and solvents, it appears necessary to optimize these parameters for quantification of phenolics and tannins in PB. Therefore, the aim of this experiment was to evaluate the effects of solvents, particle size and time of extraction and ultrasound assisted extraction method on total phenolics and total tannins of PB.

2. MATERIALS AND METHODS

2.1 Plant Material and Chemicals

Fresh pistachio by-products containing hulls (53.5%), twigs (27.7%), leaves (9.5%), hard shells (5.3%) and green kernels (4%) were collected from pistachio de-hulling factories in Feizabad (Khorasan Razavi Province, Iran) which is located on the north east part of Iran at 35º01′N latitude and 58º78′E longitude. All chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany) or Sigma (Taufkirchen, Germany).

2.2 Sample Preparation and Extraction

Pistachio by-products were completely dried in sun and were ground to pass a 2 mm sieve by cyclic mill which is defined as coarse particle size. For achieving fine particles, dried samples were ground to pass a 2 mm sieve and then 0.5 mm sieve. Four Solvents included: 70% aqueous acetone, 50% aqueous methanol, 50% aqueous ethanol and water and two extraction time were 12h versus 24h.

2.3 Laboratory Analysis

For total phenolics (TP) measurement, exactly 200 mg samples were extracted in 10 ml of solvents (as mentioned above) with four replicates overnight at room temperature. Contents were centrifuged (3000 × g at 4ºC) for 10 min and the supernatant collected and kept in refrigerator (4ºC).

Non-tannin phenolics (NTP) were determined following absorption of tannins in total phenolic extract to insoluble polyvinylpyrrolidone (PVPP). After addition of 100 mg PVPP to 100 mm × 12 mm test tubes, 1ml distilled water and 1 ml tannin containing extract were added and vortexed. The tubes were kept at 4ºC for 15 min, vortexed again. After centrifugation (3000 × g, 4ºC, 10 min), supernatant collected and the phenolic content of this supernatant was defined as the NTP. Total tannins (TT) were calculated as the difference between TP and NTP. Total phenolics and Non-tannin phenolics were determined by Folin-Ciocalteu reagent using tannic acid (Merck GmbH, Darmstadt, Germany) as a standard and absorbance was recorded at 725 nm. Results were expressed as tannic acid equivalent [12].

2.4 Ultrasonic Extraction

The ultrasound-assisted extraction procedure (UAE) was used for the extraction of TP and TT of PB. Thus, 10 ml of each solvents (as mentioned above) was added to 200 mg of finely ground PB, the mixture was sonicated in an ultrasonic bath for 20 min.

2.5 Statistical Analysis

All data were statistically analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc.) [13]. For experiment 1, the model included the effects of particle size (coarse vs fine), extraction time (12h vs 24h), solvent (aqueous acetone, methanol, ethanol and water) and the interactions between particle size × extraction time, particle size × solvent, extraction time × solvent and particle size × extraction time × solvent. For experiment 2, the model included the effects of ultrasonic level (with or without), solvent (aqueous acetone, methanol, ethanol and water) and interaction between ultrasonic × solvent.

If a value of $P < .05$ was detected, differences among means and variables interactions were tested with least squares means procedure (LSMEANS).

3. RESULTS AND DISCUSSION

Effect of particle size, extraction time and solvents are given in Table 1. Interaction effects were not statistically significant, however, finely ground PB extracted in aqueous ethanol and water tended to more TT content than coarsely ground materials ($P = .08$) but a trend for more TT was observed in coarsely ground PB extracted in aqueous acetone than finely ground ones ($P = .1$).

Means of total phenolics and total tannins from main effects are shown in Table 2. Total phenolics concentration was not affected by particle size and extraction time ($P > .05$). Similar results were observed by Karsheva et al. [14]. They reported that TP of mandarin peels was not affected by particle size ($P > .05$). In contrast, increasing particle size from 0.5 to 2.5 mm and time of extraction from 1 to 5h decreased polyphenolic compounds in *Chineese Sumac* [15]. Surya Prakash et al. [16] showed that extraction for 24h caused more TP content in *Terminalia Chebulla* compared to 48, 72 and 96h.

Particle size had no effect on TT content of PB (Table 2). However, extraction for 12h increased TT content compared to 24h (7.34 vs 6.81%). Increasing length of extraction, would lead to oxidation of polyphenolic and polymerization of oxidative products into insoluble compounds [17] and consequently decrease extraction efficiency.

The highest and the lowest concentration of TP were observed in 70% aqueous acetone and water, respectively (13.86 vs 9.87%). However, there was no difference between 50% aqueous ethanol and aqueous methanol (Table 2). Surya Prakash et al. [16] stated that more TP from *Terminalia Chebulla* was extracted by 25% aqueous ethanol than aqueous methanol and water. This difference might be attributed to different materials and different material's solubility used in those experiments. In another study [15] no difference was found in polyphenols *of Chineese Sumac* by using 20, 40, 60, 80 and 100% ethanol.

Selecting the right solvent affects the amount and rate of polyphenols extracted [18]. Solvents, such as acetone, methanol, ethanol and their combination with water have been often used for the extraction of phenolics from plants [1] and aqueous acetone or methanol, with water contents between 30 and 50% (v/v) are the most common solvents for extraction of polyphenols. It is generally accepted that aqueous acetone is more efficient than aqueous methanol, but it does not always give greater recovery with all plant materials [7].

Using 70% aqueous acetone and 50% aqueous ethanol resulted in higher TT compared to other treatments (Table 2). Mokhtarpour et al. [9] reported that TP and TT concentration of PB silage were 10% and 5.2% of DM as tannic acid equivalent when it was extracted in 70% aqueous acetone for 12h. Ghasemi et al. [19] showed that TP and TT content of sun-dried PB were 7.85 and 3.16%, respectively. No difference in TP of pistachio hulls was observed by Goli et al. [20] when they used water and/or methanol with or without ultrasonic bath ($P > .05$).

The yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample to solvent ratio and chemical composition and physical characteristics of the samples [1]. Phenolics include wide range of compounds with different structure and are often polar, however, due to non-polar molecules linkage, they may be better extracted in low polarity solvents.

Extraction of phenolic compounds of PB decreased by increasing solvent polarity. According to Markom et al. [21], Snyder's polarity indexes for 70% aqueous acetone, 50% aqueous ethanol, 50% aqueous methanol and water were 6.5, 7.1, 7.8 and 9, respectively. Thus, PB phenolic compounds are often polar with some non-polar groups, hence, the highest TP and TT was observed in 70% aqueous acetone and 50% aqueous ethanol.

The ratio of extracted TP of PB in water to 70% aqueous acetone was 71.02% and the ratio of TT content in water to 70% aqueous acetone and 50% aqueous ethanol was 74.02 and 72.51%, respectively. This shows that, most of phenolics and tannins compounds in PB are water soluble or hydrophilic. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight compounds are better extracted with aqueous acetone [22]. Therefore, it seems that PB tannins have moderate molecular weight. Chromatography techniques should be used for determination of tannin molecular weight following tannin purification.

Total phenolics and total tannin content of PB were similar in both extraction methods (with or without UAE) (Table 3). More TP was extracted in 50% aqueous ethanol and/or 70% acetone than 50% aqueous methanol and/or water. However, using 50% aqueous ethanol yielded more tannins compared to other treatments (Table 3).

Bagheripour et al. [23] reported that TP and TT of sun-dried PB with UAE method was 15.22 and 8.99%, respectively. In another study, it was reported that TP and TT of PB silage and sun-dried PB were 14.54%, 10.08% and 13.71% and 9.99%, respectively [24]. Recently, ultrasound assisted extraction has been widely used in the extraction of various phenolic compounds from different parts of plants [25]. A comparison study showed that UAE caused less degradation of phenolics and was a much faster extraction process in extraction of phenolic compounds from strawberries compared with other extraction methods including solid-liquid, subcritical water and microwave-assisted method [26]. Ultrasound-assisted extraction is a useful technology as it does not require complex instruments and is relatively low-cost and its mechanism involves the sheer force created by implosion of cavitations bubbles upon the propagation of the acoustic waves in the kHz range [27].

In regard to similar measuring methods for phenolic compounds, difference in TP and TT of PB among different studies might be due to sample preparation, PB components proportion (leaves, hulls, twigs, hard shells and green kernels), different varieties (nature of tannin) and weather conditions [23,28].

Table 1. Effect of experimental treatment on total phenolics and total tannins of PB (%DM)

Item	Treatments[1]																SEM[2]
	Coarse								Fine								
	12h				24h				12h				24h				
	AC	AM	AE	W	AC	AM	AE	W	AC	AM	AE	W	AC	AM	AE	W	
Total phenolics	14.09	13.37	13.24	9.06	13.5	11.58	12.79	9.87	13.26	11.68	13.5	9.8	14.57	11.85	12.21	10.76	0.256
Total tannins	7.97	7.61	7.74	5.73	7.91	6.69	7.26	4.97	7.61	7.38	8.67	6.04	7.13	7.01	7.6	5.92	0.156
Significance of effect[3]																	
	PS		ET	Solv	PS × ET		PS × Solv		ET × Solv		PS × ET × Solv						
Total phenolics	0.86		0.87	<0.01	0.1		0.23		0.11		0.33						
Total tannins	0.31		<0.01	<0.01	0.9		0.07		0.76		0.5						

[1]Two particle sizes (coarse vs fine), two extraction time (12h vs 24h) and four solvents (AC = 70% aqueous acetone, AM = 50% aqueous methanol, AE = 50% aqueous ethanol, W= water).
[2]SEM = standar error of the means.
[3]PS = particle size, ET = extraction time, Solv = solvent, PS × ET = interaction between particle size and extraction time, PS × Solv = interaction between particle size and Solvent, ET × Solv = interaction between extraction time and solvent, PS × ET × Solv = interaction between particle size, extraction time and solvent.

Table 2. Effect of particle size, extraction time and solvents on total phenols and tannins (%DM)

Item	Particle size			Extraction time			Solvent[1]				
	Coarse	Fine	SEM[2]	12	24	SEM	AC	AM	AE	W	SEM
Total phenolics	12.19	12.14	0.193	12.19	12.14	0.193	13.86[a]	12.12[b]	12.81[b]	9.87[c]	0.273
Total tannins	6.99	7.17	0.126	7.34[a]	6.81[b]	0.126	7.66[ab]	7.17[b]	7.82[a]	5.67[c]	0.178

[1]AC = 70% aqueous acetone, AM = 50% aqueous methanol, AE = 50% aqueous ethanol, W= water.
[2]SEM = standard error of the means.
[a, b, c]Means in a row without common superscript differ at $P < 0.05$.

Table 3. Total phenolics and tannin content of PB extracted by different methods (%DM)

Item	Extraction methods[1]											
	−ultrasonic				+ultrasonic				Significance of effect[3]			
	AC	AM	AE	W	AC	AM	AE	W	SEM[2]	Solv	Ultra	Solv × Ultra
Total	13.26	11.68	13.5	9.08	12.02	11.79	12.88	8.99	0.288	<0.01	0.10	0.17
Total	7.61	7.38	8.67	6.04	7.64	7.44	8.48	6.01	0.210	<0.01	0.72	0.99

[1]ultrasonic = without versus with ultrasonic bath, AC = 70% aqueous acetone, AM = 50% aqueous methanol, AE = 50% aqueous ethanol, W= water.
[2]SEM = standard error of the means.
[32]Solv = solvent, Ultra = ultrasonic, Solv × Ultra = interaction between Solv and Ultra.

4. CONCLUSION

It can be concluded that solvent has an important impact on concentration of PB phenols and tannins extraction. For extraction of phenolic compounds, 70% aqueous acetone can be used and it was suggested using 50% aqueous ethanol for total tannin. Due to similar results from phenolics and tannins content with or without ultrasonic bath, it is suggested using UAE for phenolics extraction. This study will provide bases for future studies in this area. To our knowledge, no data is reported about phenolic profile of pistachio (Pistacia vera L.) by-products. Therefore, it is recommended that other studies can be done for identification of PB phenolic compounds and their activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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