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ORIGINAL PAPER

Pistachio (*Pistacia vera* L.) Hull as a Potential Source of Phenolic Compounds: Evaluation of Ethanol–Water Binary Solvent Extraction on Antioxidant Activity and Phenolic Content of Pistachio Hull Extracts

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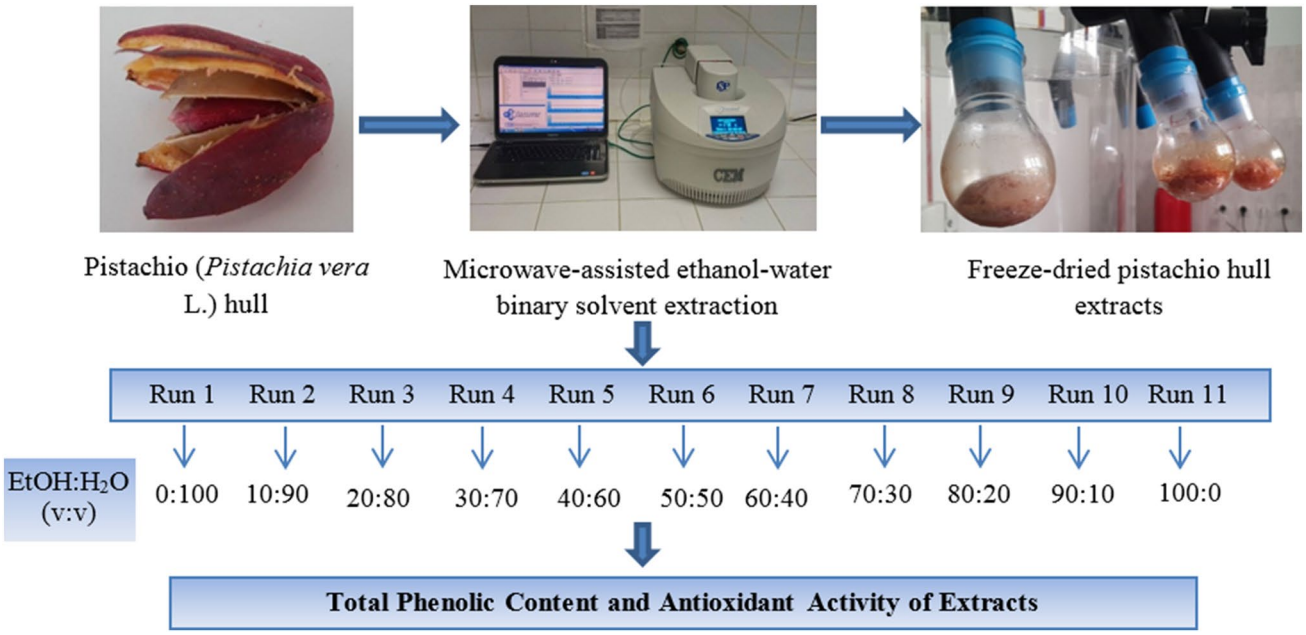
Abstract
Pistachio (*Pistacia vera* L.) hull is the largest by-product of pistachio industry which is accumulated every year at harvest time and can lead to environmental pollution. This study investigated the microwave-assisted ethanol–water binary solvent extraction of phenolic compounds from pistachio hull. The effect of different ethanol/water ratios was investigated on the changes in extraction yield, total phenolic content and antioxidant activity. The obtained results indicated relationships between the tested parameters, i.e. ethanol concentration and extraction yield. The maximum yield was obtained with 50% ethanol (32.9 g dry extract/100 g dry matter). Total phenolic content of the extracts was found in the range of 21.3–39.3 mg/g extract as gallic acid equivalent. Antioxidant activity was determined by using three different tests. Using the DPPH test, the best antioxidant activity with the lowest IC_{50} value (0.70 mg/mL) was obtained for 40% ethanol and the least antioxidant activity was obtained for 100% ethanol with the highest IC_{50} value of 2.73 mg/mL. Using the β-carotene bleaching assay, the pistachio hull extracts showed antioxidant activity at 5000 mg/L in the range of 44–97%. The ORAC test showed that 50% ethanol extract had the highest antioxidant activity (260.9 µmol Trolox equivalents/g extract). Using HPLC assay, the gallic acid was the main phenolic compound of hull extracts which ranged between 1.18 and 19.83 mg/g crude extract. These findings propose that pistachio hull extracts can be a valuable source of bioactive compounds.

Extended author information available on the last page of the article

Graphical Abstract



Keywords Pistachio hull · *Pistacia vera* L. · Microwave-assisted extraction · Total phenolic content · Antioxidant activity

Introduction

Phenolic compounds are secondary metabolites and biologically active molecules found in plants. Over the last decade, the interest for these natural components has increased because of their role in nutrition and human health [1, 2]. Some phenolic compounds available in natural products have high antioxidative and anticarcinogenic activities as a result of their major role in protecting organisms against oxidative stress induced by free radicals [3]. These phenolic compounds can also be used in foods as a preservative due to their protective impacts against microorganisms [4].

The extraction of phenolic compounds from solid samples is generally carried out by using traditional methods such as maceration and Soxhlet with organic solvents [5, 6]. However, these extraction methods take long time, which can raise the possible degradations of the bioactive ingredients. In recent years, different faster and more automatic extraction methods have been replacing traditional methods such as extraction by pressurised liquids (PLE), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE). These techniques provide shorter extraction times in a more selective way and offer better control over the extraction conditions [2, 7]. MAE is a technique which uses the microwave energy to heat the solvent so that the substances can be easily partitioned from the sample to the solvent [8]. The principal advantage of MAE is decrease in both extraction time and solvent consumption [9, 10].

Fruit and vegetable processing residues which are considered as an environmental problem are increasingly used as sources of high-phenolic products. Phenolic compounds from wastes deriving from agro-industrial production can be used as natural antioxidants and functional food ingredients to replace their synthetic equivalents [11–13]. The pistachio nut (*Pistacia vera* L.) is one of the principal tree nuts of the world. The main regions where it is cultivated are Mediterranean countries, saline, dry and hot areas of the Middle East, and the United States [14]. Turkey (85,000 tons) comes after United States (233,147 tons) and Iran (230,000 tons) in annual pistachio production [15]. During the industrial processing of pistachios, their reddish purple hulls are removed as a major waste of pistachio industry [16, 17]. Pistachio hull is often mixed with soil as land amendment and less commonly used for feedstuff in local livestock farmer [18]. If not processed further, this by-product can lead to environmental pollution.

Pistachio hull has caught up the interest of researchers by considering its natural phenolics and antioxidants compounds. It has been shown that pistachio hull extracts have antioxidant, antimicrobial and antimutagenicity activities [16, 19, 20]. Investigations have also showed that the antioxidant effect of pistachio hull extract was not different from the synthetic antioxidants BHA and BHT which can make

it a good substitute for synthetic equivalents. Due to its high phenolic content and antioxidant activity, pistachio hull can be used as an alternative source of biologically active compounds [21]. In this way, using pistachio hull as a source of bioactive compounds will increase the value of pistachio production and offer valorisation for a useless by-product [22].

The aim of the present study was to evaluate the effect of different ethanol /water ratios on the extraction yield, phenolic content in pistachio hull extracts and their antioxidant activity by using MAE technique.

Materials and Methods

Materials and Chemicals

Folin–Ciocalteau phenol reagent, gallic acid, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), ascorbic acid, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), sodium fluorescein, methanol and other solvents were purchased from Sigma Aldrich (St. Louis, MO, USA). All solvents and reagents were analytical or chromatographic grade.

Sample Preparation

Mature healthy pistachio nuts were harvested from a village nearby Gaziantep, Turkey during September 2015. The hulls of the harvested nuts were removed and dried in an alpha 1–4 LD plus freeze drier (Christ, Osterode am Harz, Germany). The hulls were ground and the fraction was sieved using a 250-mesh sieve and stored in a freezer at − 20 °C until being used. The final moisture content of the hull was less than 3% (w/w on wet basis).

Microwave Assisted Extraction (MAE) of Phenolic Compounds

Amounts of 1.5 g of dried pistachio hull was mixed with 15 mL (1:10 w:v) solvents in a 35 mL vessel and subjected to MAE at 125 W irradiation power for 3 min. Ethanol and water at different ratios was used as extraction solvents. Eleven different mixtures [100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100 (v:v)] of ethanol and water were used to test their effect on the extraction yield under defined experimental conditions. The mixture was stirred at high level under a closed system, using a Discover SP-D microwave reactor (CEM Corporation, Matthews, USA). After extraction process, all samples were centrifuged at 6000 rpm for 20 min and the upper phase was collected. Solvent was evaporated at 40 °C under vacuum using a rotary evaporator. Extracts were dried in an alpha 1–4 LD plus freeze drier (Christ, Osterode am Harz, Germany) and stored at − 20 °C prior to further analysis.

Determination of Total Phenolic Content

Total phenolic contents of extracts were determined according to the Folin–Ciocalteu colorimetric method as described previously by Fernández-Agulló et al. [23] with some modifications. Briefly, the pistachio hull extracts (50 mg) were dissolved in 10 mL dimethyl sulfoxide (DMSO) to give 5000 mg/L solution. Samples of 0.5 mL extract or phenolic standards were pipetted in a 10 mL test tube and 1 mL of Folin–Ciocalteau phenol reagent (2N) was added. After 3 min incubation, 1.5 mL sodium carbonate solution (20%, w/v) was added to the reaction mixture. The mixture was left to stand in the dark for 30 min and then diluted with water to 10 mL at the end of the incubation period. The absorbance of the solution was measured against a blank sample at 725 nm using an Evolution 260 Bio UV–Visible Spectrophotometer (Thermo Fisher Scientific Inc., MA, USA). The results were expressed as gallic acid equivalents per gram extract (mg/g extract).

Antioxidant Activity

DPPH Radical Scavenging Activity

The antioxidant activity of the pistachio hull extracts were analyzed using the DPPH radical as described previously [24]. Briefly, 0.1 mL of pistachio hull extracts prepared in methanol at different concentrations (500–2000 mg/L) were pipetted in test tubes and 3.9 mL 50 ppm DPPH solution in methanol was added to each tube. The mixtures were shaken vigorously and incubated in the dark at 37 °C for 30 min. At the end of the incubation period, the absorbance value of the solutions was taken against a blank (methanol) at 517 nm. The control consisted of methanol instead of the hull extract. Ascorbic acid was used for comparison. The scavenging activity (%) was calculated using the formula:

$$Scavenging\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The scavenging ability of the different extracts was presented as IC_{50}, which is the effective concentration at which DPPH radicals were scavenged by 50%. The scavenging ability of the extracts was also defined as ascorbic acid equivalents (mg/g extract).

β-Carotene Bleaching (BCB) Assay

This test was applied as previously described by Martorana et al. [25] with some modifications. Briefly: An emulsion of β-carotene-linoleic acid was prepared as follows: 5 mg of β-carotene was dissolved in 50 mL chloroform (0.1 mg/mL). Then, 40 µL linoleic acid and 400 µL Tween 20 were added to 5 mL of β-carotene solution. Chloroform was removed under vacuum at 40 °C using a rotary evaporator and 100 mL of distilled water was slowly added with continuous shaking to the mixture to form emulsion. 200 µL of samples in DMSO at concentrations of 1250, 2500 and 5000 mg/L were mixed with 5 mL emulsion in different test tubes. As control samples, 200 µL of DMSO were used. The absorbance of the samples were immediately taken (t = 0) at 470 nm using spectrophotometer against a blank. Blank consisted of emulsion without β-carotene. Then, the tubes were incubated for 60 min at 50 °C and the absorbance values of the samples were measured at 470 nm. Tert-butylhydroquinone (THBQ 1250 mg/L) was used as a reference standard. The antioxidant activity was calculated using the following formula:

$$Antioxidant\ activity\ (\%) = \left[\frac{A_{sample(t)} - A_{control(t)}}{A_{control(0)} - A_{control(t)}}\right] \times 100$$

where $A_{sample(t)}$ and $A_{control(t)}$ are the absorbance of the sample and control at t, respectively, and $A_{control(0)}$ is absorbance of the control at t = 0 min.

Oxygen Radical Absorbance Capacity (ORAC) Assay

For ORAC assay, the procedure described by Azaizeh et al. [24] was used. Briefly: different concentrations of Trolox (20–0.625 µM) and pistachio hull extracts (5, 10, 20 and 40 mg/L) were prepared in 10 mM phosphate buffer (pH 7.4). The following mixtures were pipetted in triplicates in every working well of microplates: 140 µL fluorescein (1000 nM) solution in all wells, 20 µL of extract dilutions in sample wells. The control well contained of 20 µL of Trolox dilutions and blank well contained 20 µL of phosphate buffer. The background signal was determined, where fluorescence was monitored (excitation 485 nm, emission 535 nm) every 120 s. 40 µL (240 mM) AAPH solution was added to each well after 3 cycles, and fluorescent measurements were taken every 120 s up to 120 min.

Finally, using the regression equation between the Trolox concentration and the net area under the FL decay curve (AUC), each ORAC value was calculated. The results were expressed as µmole Trolox equivalents per gram dry extract.

HPLC Analysis of Phenolic Compounds

The different pistachio hull extracts were dissolved in methanol in order to identify and quantify the main phenolic compound and were analysed using HPLC-PAD techniques as described by Tafesh et al. [26]. The experiments were carried out by using reversed-phase HPLC, Thermo Scientific Finnigian Surveyor system with a PDA plus detector (220–340 nm). For the chromatographic separation, a Gemini C6-Phenyl column (5 µm, 110 Å, 250 × 4.6 mm) (Phenomenex, Torrance, CA, USA) was used. The main phenolic compounds were identified by comparison with pure standards of gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, (−)-epicatechin, syringic acid, *p*-coumaric acid, quercetin and caffeic acid (Sigma Aldrich).

Statistical Analysis

All extractions and assays were carried out three times and averaged. Results are represented as means ± SD. The effect of different ethanol:water ratios were statistically analysed by ANOVA and Tukey's post hoc test was used for comparison of mean values ($P < 0.05$), using SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA). Different letters were used in tables and figures to show the statistically significant differences. In addition, relationships between variables, i.e. ethanol:water ratio and total phenolic content or antioxidant activity (N = 6 and 5 for ethanol concentration from 0 to 50% and from 60 to 100%, respectively), were evaluated by linear regression analyses and Pearson's correlation coefficients (r) were calculated.

Results and Discussion

Effect of Ethanol–Water Ratio on the Extraction Yield

Extraction procedure was performed to determine the effect of ethanol–water ratio (v:v) on the extraction yield under defined experimental conditions between 0:100 and 100:0. The efficiency of the extraction procedure was expressed as the percentage extraction yield on the dry basis (%). Generally, 40–50% ethanol has a greater effectiveness for the extraction of polyphenolic compounds when compared to the pure ethanol [27]. The extraction yield increased as the volume of ethanol increased and reached a maximum value at 50:50 (v:v) which was probably due to the increased solubility of phenolic compounds, tannins, flavonoids and polysaccharides in the ethanol–water mixture. After this point, the extraction yield was decreased (Fig. 1). The results showed that 50:50 ethanol:water ratio with 32.90 ± 0.33% yield was the best solvent mixture while the 100% ethanol

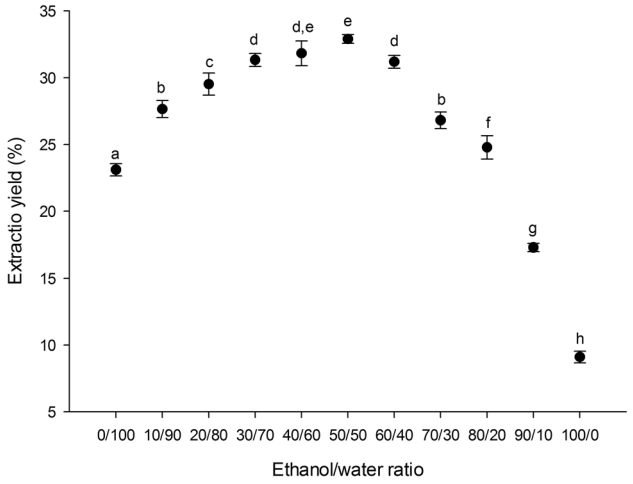


Fig. 1 Effect of ethanol–water ratio on the extraction yield. Each data point represents an average of three replicates. Different letters represent significant differences at ($P < 0.05$)

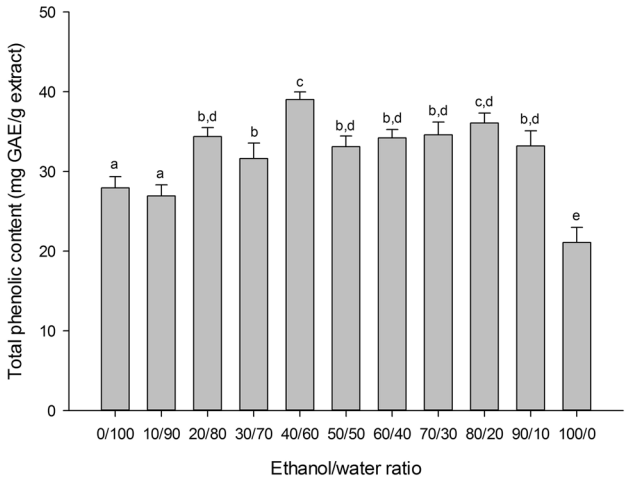


Fig. 2 Effect of ethanol–water ratio on the total phenolic content (TPC) of pistachio hull extracts equivalents as gallic acid. Each data point represents an average of three replicates. Different letters represent significant differences at ($P < 0.05$)

with 9.10 ± 0.44% yield was the least solvent by considering the extraction yield (Fig. 1). From Fig. 1, it is shown that different ethanol:water ratios have statistically significant effects on extraction yield ($P < 0.05$). Similar trends were reported in previous studies [27, 28]. Prasad et al. [27] who have studied the extraction of longan fruit pericarp, demonstrated that extraction yield increased increasing ethanol concentration from 25 to 50% and decreased for concentration higher than 75%. Spigno et al. [28] studied the effect of ethanol concentration on the extraction yield for the extraction of phenolics from grape marc and they have reported higher extraction yield when 50% ethanol was used. Our results are also comparable to the results reported in previous studies for pistachio hull [21, 29]. Grace et al. [29] have reported 31% extraction yield by using 80% acidified methanol and Kilic et al. [21] have obtained 36.11% yield by using methanol from the pistachio hull.

Extraction of phenolic compounds is an important process that is affected by various parameters. Solvent characteristics are one of the critical parameters that affect the extraction efficiency due to the different chemical characteristics and the polarities of phenolic compounds [30]. The possibility of mixing ethanol with water in any ratio, different polarity of both solvents and their acceptability for human consumption make ethanol–water mixtures to be the most suitable solvents for obtaining extracts from plants since solvents such as acetone and methanol may leave toxic residues [31, 32].

Effect of Ethanol–Water Ratio on the Total Phenolic Content (TPC) of Pistachio Hull Extracts

The content of total phenolic compounds of the hull extracts was determined to evaluate the effect of ethanol–water ratio on the phenolic content and was calculated as gallic acid equivalents. TPCs of the extracts were found in the range of 21.07–39.03 mg/g extract as gallic acid equivalent (Fig. 2). These results were similar with the results obtained by Kilic et al. [21] who have reported that the methanol extracts of mature pistachio hull have the total phenolics of 33.65 mg gallic acid equivalent/g extract. Results revealed that 40% ethanol in water were the best solvent mixture to extract phenolic compounds. The lowest phenolic content was obtained with 100% ethanol. The extraction solvent was significantly ($P < 0.05$) effective on the TPC value of hull extracts. The obtained results for total phenolic content were in accordance with previous reports suggesting that binary solvent systems were more effective for the extraction of phenolic compounds from plant materials as compared to mono-solvent systems (water or pure ethanol) [23, 33]. Water addition to organic solvents such as ethanol, methanol, and acetone forms a more polar medium that simplifies the phenolic compound extraction [28]. However, using pure water as an extraction solvent is not effective for extraction of phenols because these components are generally more soluble in organic solvents which have polarity lower than water [34, 35]. The results obtained in this study were in agreement with the literature findings reported by Fernández-Agulló et al. [23] and Fontes-Candia [36]. Fernández-Agulló et al. [23] have reported the highest total phenols content with 50% ethanol, followed very closely by 50% methanol and the lowest value with water for the walnut green husk extracts. In addition, Fontes-Candia [36] have reported 50% ethanol as the best solvent for the highest TPC while the extract obtained with 100% ethanol had the lowest TPC for oat extracts.

Effect of Ethanol–Water Ratio on the Antioxidant Activity

It is recommended to use at least two methods to confirm the antioxidant activity of samples because the methods are based on different reaction mechanisms [30]. For this reason, the antioxidant activity of hull extracts were determined using three different tests; DPPH assay, β-carotene bleaching (BCB) assay and the oxygen radical absorbance capacity (ORAC) assay. Significant effect of ethanol/water ratio on the antioxidant activities of hull extracts was obtained based on statistical analysis ($P < 0.05$). The radical scavenging activity of the extracts was determined by DPPH assay. The IC_{50} values of the hull extracts decreased with the increase in ethanol concentration up to 40%. Increase from 70 to 100% in ethanol concentration dramatically lowered the radical scavenging activity of the extracts. The results showed that 40% ethanol extract has the best antioxidant activity with the lowest IC_{50} value (0.70 ± 0.04 mg/mL). Using the DPPH assay, the least antioxidant activity was obtained for 100% ethanol with the highest IC_{50} value (2.73 ± 0.07 mg/mL) (Fig. 3). Extracts with higher total phenolic content showed the strongest radical scavenging activity (lower IC_{50} values). However, all the extracts had lower antioxidant activity compare to ascorbic acid (IC_{50} value for ascorbic acid was 0.19 mg/mL). When calculated as the ascorbic acid equivalent (AAE), the antioxidant activities of the extracts were in the range of 347.97–39.56 mg AAE/g extract.

The BCB assay is based on the loss of the yellow colour of β-carotene because of its reaction with free radical caused by linoleic acid oxidation. The β-carotene bleaching rate can be slowed down in the presence of different antioxidants [20, 37]. Figure 4 shows the total antioxidant activity measured

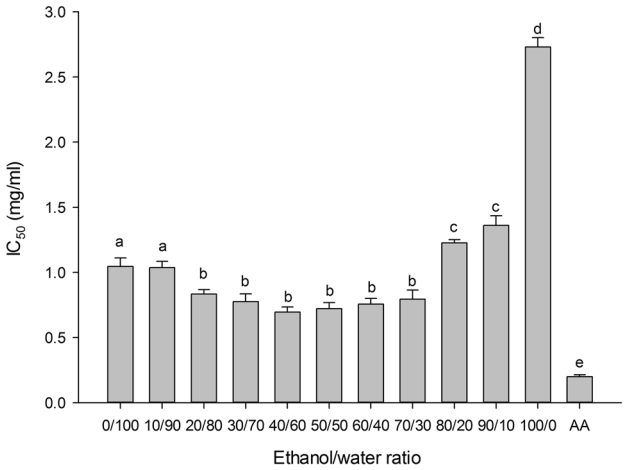


Fig. 3 Effect of ethanol–water ratio on radical scavenging (IC_{50}) of extracts compared to ascorbic acid (AA) as positive control using DPPH test. Each data point represents an average of three replicates. Different letters represent significant differences at ($P < 0.05$)

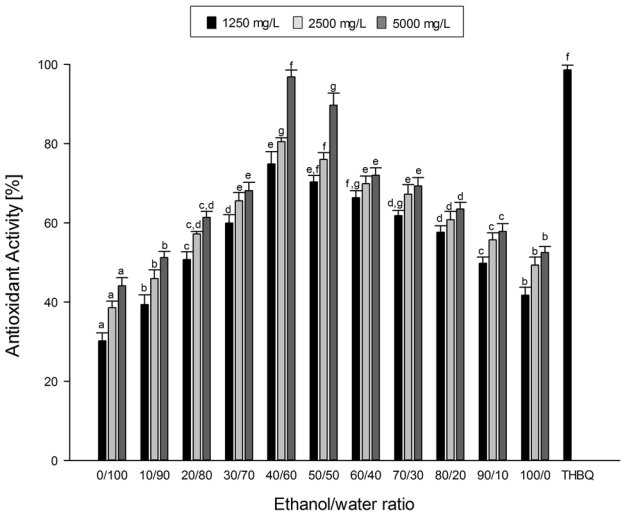


Fig. 4 Effect of ethanol–water ratio on antioxidant activity (%) of different concentrations of extracts as assessed with β-carotene bleaching test compared with THBQ positive control. Each data point represents an average of three replicates. Different letters represent significant differences at ($P < 0.05$)

by the BCB method for the different extracts. Antioxidant activity of hull extracts increased with the increase in ethanol concentration up to 40%. The activity of 40% ethanol extract at 5000 mg/L (96.88 ± 1.74%) was nearly equivalent to the THBQ at 1250 mg/L. Further increase in ethanol concentration caused a decrease in the antioxidant capacity of the extracts. The antioxidant activity for both tested extracts was dependent on their concentrations. This finding is in agreement with the results obtained by Rajaei et al.'s study [20]. The antioxidant capacities of both extracts were also evaluated using ORAC method. The antioxidant capacities of the different extracts as Trolox equivalents (TE) using ORAC test showed that the antioxidant activity values of the extracts were ranging from 51.80 ± 3.16 to 260 ± 6.01 µmol TE/g extract. The extract obtained by 50% ethanol had the highest antioxidant activity (260.86 ± 6.01 µmol TE/g extract) (Fig. 5). The lowest ORAC value was obtained from 100% ethanol (51.80 ± 3.16 µmole TE/g extract) (Fig. 5). The extraction solvent was significantly ($P < 0.05$) effective on the ORAC value of hull extracts.

The antioxidant potential of pistachio hull extract obtained using different solvents and methods have been studied by other researchers. Rajaei et al. [20] have reported that the water extract showed a strong DPPH antioxidant activity when was compared to THBQ and BHT standards. Kilic et al. [21] have also reported that the DPPH antioxidant activity of pistachio hull extract was 137.16 ± 3.50 mg TE/g extract. The antioxidant capacities of ethanol and methanol extracts of hull using ORAC assay were found as 1.79 ± 0.16 and 3.48 ± 0.31 µmoles TE per 100 g fresh

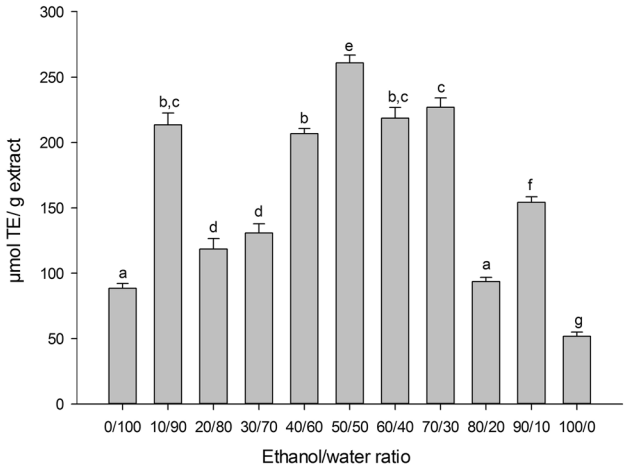


Fig. 5 Effect of ethanol–water ratio on antioxidant activity of extracts as Trolox equivalents using ORAC test. Each data point represents an average of three replicates. Different letters represent significant differences at ($P < 0.05$)

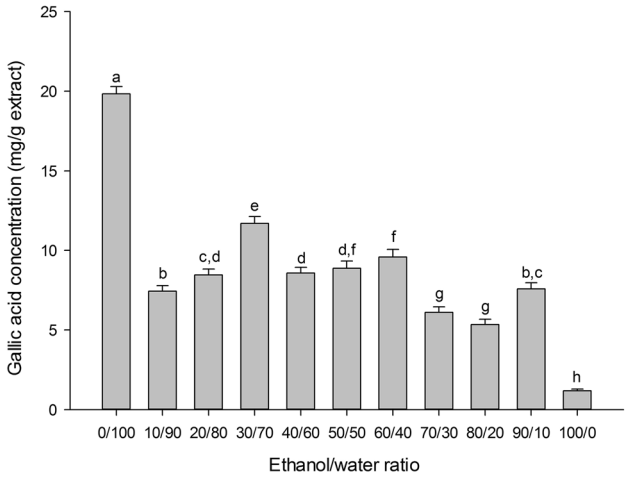


Fig. 6 Gallic acid concentration (mg/g extract) detected in extracts with various ethanol/water ratios determined using HPLC analysis

weight, respectively [38]. In all these studies, maceration method was used as an extraction technique. However, no previous study has been found on the microwave-assisted ethanol–water binary solvent extraction of phenolic compounds from pistachio hull and their antioxidant activities. Therefore, the current presented data could be assumed as the first report in the literature using the MAE method for extraction of phenolic compounds from these hulls.

Effect of Ethanol–Water Ratio on the Main Phenolic Compounds

The main phenolic compounds in the extracts were determined by HPLC-DAD. HPLC results showed that the main phenolic compounds in 50:50 ethanol:water extract were gallic acid (9340 ± 341 µg/g extract), *p*-hydroxybenzoic acid (6250 ± 143 µg/g extract), protocatechuic acid (958 ± 42 µg/g extract), (−)-epicatechin (873 ± 27 µg/g extract), syringic acid (58 ± 3 µg/g extract), *p*-coumaric acid (52 ± 2 µg/g extract), quercetin (41 ± 1 µg/g extract) and caffeic acid (36 ± 2 µg/g extract) and their content varied as a function of solvent composition. HPLC results also indicated that the main phenolic compound in the hull extracts was gallic acid and statistical analysis showed that the variation in its concentration as a function of solvent composition was significant ($P < 0.05$). The gallic acid content (GAC) in the extracts ranged between 1.18 ± 0.12 and 19.83 ± 0.46 mg/g extract. GAC was the highest in the 100% water and the least GAC was obtained from the 100% ethanol (Fig. 6). This experimental result was in accordance with previous report suggesting that the most suitable solvent was the polar protic water molecule for gallic acid which contains the aromatic ring surrounded by three hydroxyl groups and one carboxyl group [39]. On the other hand, increase in ethanol concentration increased the recovery of other phenolics that resulted increased extract yield. Using pure water as an extraction solvent is not effective for extraction of phenolics since these components are generally more soluble in organic solvents less polar than water [35]. Because the solubility of *p*-coumaric acid and syringic acid is higher in ethanol when compared to the water, recovery of these phenolics increased by increasing ethanol concentration.

It should also be noted that the temperature of pure water system is higher than the pure ethanol system because of the differences in their dielectric constants. So, the extraction efficiency of phenolic compounds has been affected in defined time of microwave process. The increased temperature of pure water system resulted in higher extraction efficiency of phenolic compounds which are more soluble in water (i.e. gallic acid). However, the addition of ethanol to this system caused little decrease in temperature of the system with increased solvency effect of ethanol for certain phenolic compounds (*p*-coumaric acid, syringic acid, *p*-hydroxybenzoic acid, caffeic acid, etc.). Further increase in ethanol concentration resulted in decreased temperature and decreased extraction efficiency.

Correlation Analysis Between the Ethanol Content, TPC, GAC, Antioxidant Activity and the Extraction Yield

The relationship between the changes in TPC, GAC, antioxidant activity (DPPH assay) and the extraction yield obtained at different ethanol–water ratios was also determined (Table 1). When the effect of solvent was analysed in the range of 0–50% of ethanol, the correlation between TPC, GAC, DPPH and the ethanol content was not statistically

Table 1 Correlation between the ethanol content, TPC, GAC, antioxidant activity and the extraction yield

Parameters	r[a]							
	Ethanol concentration (0–50%)				Ethanol concentration (60–100%)			
	Et-OH conc.	TPC	GAC	DPPH	Et-OH conc.	TPC	GAC	DPPH
TPC[b]	0.716^{NS}				-0.716^{NS}			
GAC[c]	-0.554^{NS}	-0.421^{NS}			-0.776^{NS}	0.787^{NS}		
DPPH[d]	0.377^{NS}	0.401^{NS}	-0.177^{NS}		−0.955**	0.847*	0.822*	
Ext. yield (%)	0.942**	0.690^{NS}	−0.739*	0.527^{NS}	−0.977**	0.837*	0.800^{NS}	0.968**

Linear regression: $y = ax + b$, $^{*}P < 0.05$, $^{**}P < 0.01$, NS the finding is not statistically significant ($P \geq 0.05$); Ethanol concentration 0–50% and ethanol concentration 60–100%—the analyses were performed for the ethanol concentration in extraction solvent ranging from 0 to 50% and from 60 to 100%, respectively

[a]Correlation coefficient

[b]Total phenolic content (mg GAE/g extract)

[c]Gallic acid content (mg/g extract)

[d]DPPH radical scavenging activity (mg AAE/g extract)

significant ($P \geq 0.05$); but significant correlation was obtained for the extraction yield ($P < 0.01$). The significant correlations between the ethanol content and the extraction yield were found when the effect of solvent was evaluated for both ranges of 0–50% and 60–100% of ethanol. The relationship analysis between the extraction yield and TPC or DPPH showed positive linear correlation but, it was statistically significant only when the effect of ethanol content was determined for the range of 60–100% (Table 1).

The relationship between the TPC, GAC and antioxidant activity measured by DPPH assay showed a positive significant correlation when the effect of ethanol content was evaluated for the range of 60–100%. These results indicated that the antioxidant activity of extracts was well correlated with TPC which has been also found in previous studies [33, 40]. However, no correlation was observed for the range of 0–50% of ethanol. Also, the results showed that the extraction of total phenolics and gallic acid was not affected by ethanol content ($P \geq 0.05$).

Conclusions

In this study, the effect of ethanol:water ratio on microwave-assisted extraction of phenolic compounds from pistachio hull was investigated. The results indicated strong relationships between the ethanol:water ratio and the phenolic content, together with the antioxidant activity of hull extracts. The ethanol ratio in the solvent mixture was critical for the efficient phenolic extraction.

According to the obtained results, pistachio hull, the largest by-product of pistachio industry is very rich source of natural phenolic compounds and shows remarkable antioxidant activity. Additionally, the potential showed by these extracts can lead to the valorisation of a significant by-product of pistachio industrial processing that nowadays has an inadequate use and may lead to the decrease of the environmental negative effects of this waste.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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