

Original Article

OPTIMIZATION OF DIFFERENT PARAMETERS FOR THE EXTRACTION OF POLYPHENOLS AND PROANTHOCYANIDINS FROM *PISTACIA VERA* HULLS

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ABSTRACT

Objective: Pistachio (*Pistacia vera* L.) hull are usually discarded as waste, which can lead to environmental pollution even a significant portion of polyphenols are often present in high concentrations in the outer parts of fruits. In this way, using a pistachio hull as a source of bioactive compounds will increase the value of pistachio production and offer valorization for a useless by-product.

Methods: Different ethanol concentrations (5, 10, 25, 50, 75 and 95 %) were investigated and the extraction efficiency at extraction temperature from 20 to 90 °C and extraction times from 5 to 45 min was studied. The extraction yield of total polyphenols and proanthocyanidins from the pistachio hull and the antioxidant activity of the extract were evaluated.

Results: The obtained results indicated relationships between the tested parameters and extraction yield. The maximum yield for total polyphenols and proanthocyanidins was obtained with 25% ethanol at 60 °C for 15 min (13.91±0.72 and 5.86±0.45 g/100g dry weight, respectively). DPPH radical scavenging activities of extracts were proved to have a linear relationship with the polyphenols yield in the extracts ($R^2=0.9907$) with a maximum DPPH radical scavenging activity of 60.25%.

Conclusion: These findings propose that pistachio hull extracts can be a valuable source of bioactive compounds.

Keywords: *Pistacia vera*, Hulls, Polyphenols, Proanthocyanidins, Extraction, Solvent, Temperature, Time, DPPH

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INTRODUCTION

Fruit and vegetable processing residues and wastes are considered as an environmental problem. Phenolic compounds, secondary metabolites and biologically active molecules found in plants, are often present in high concentrations in these processing residues which caught up the interest of researchers [1]. Over the last decade, the interest for these natural components has increased because of their role in nutrition and human health [2-5]. These phenolic compounds can also be used as natural antioxidants and functional food ingredients to replace their synthetic equivalents and for the preservation of food due to their protective impacts against microorganisms [6-8].

Pistachio (*Pistacia vera* L.), is a plant member of the Anacardiaceae family and native to Asia [9]. Different parts of *Pistacia* species including leaves, kernels, hulls and gum have caught up the interest of researchers due to their biological activities such as antioxidant potential, antimicrobial and anti-inflammatory activities [10-12]. It has been proved that *Pistacia* species are rich sources of phenolic compounds including flavonols, flavanones, isoflavons, flavan-3-ols, proanthocyanidins and anthocyanin [13]. Pistachio hull is the largest by-product of pistachio industry, which is accumulated every year at harvest time and can lead to environmental pollution. Considering the high phenolic content of the outer parts of fruits, pistachio hull has caught up the interest of researchers which had proved that pistachio hull extracts have antioxidant, antimicrobial and antimutagenicity activities [12, 14, 15].

The extraction of compounds from plant materials is the first step in the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, food ingredients, pharmaceutical, and cosmetic products [16]. Polyphenols are generally more hydrophilic than lipophilic owing to their phenolic nature. Therefore, free polyphenols along with oligomers such as proanthocyanidins can be readily extracted by solvents such as methanol, ethanol, acetonitrile and acetone, or by their mixtures with water [17].

Extraction time and temperature, as well as the concentration of the extraction solvent, are the crucial parameters affecting the extraction yield. Therefore, the aim of the present study was to evaluate the effect of different parameters on the extraction yield, polyphenol and proanthocyanidins content in pistachio hull extracts and their antioxidant activity.

MATERIALS AND METHODS

Chemical and reagents

Folin-Ciocalteu reagent 2N (Sigma-Aldrich, Switzerland), gallic acid (Sigma-Aldrich, China), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA). Ethanol and methanol were obtained from Sharlau (Spain). 1-butanol and hydrochloric acid were obtained from Surechem (England)

Equipment

Micropipette 100-1000 µl (Iso lab, Germany), moisture analyzer balance (Precisa, Switzerland), centrifuge (Shanghai surgical instruments factory, China), spectrophotometer (Jasco V-530, USA).

Sample preparation

Mature healthy pistachio nuts were harvested from a village nearby Aleppo city, Syria during September 2018. A voucher specimen is deposited in the herbarium (number 1384) of the Laboratory of Pharmacognosy, Al-Andalus University for Medical Sciences, Syria. The hulls of the harvested nuts were removed, dried, 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 5% (w/w %).

Extraction procedure

One gram of accurately weighed milled-sieved powder was mixed with 50 ml of ethanol at different concentrations (5, 10, 25, 50, 75 and 95 %) and stirred at 400 rpm for different time periods ranging from 5 to 45 min at a wide range of temperatures from 20 to 90 °C.

Subsequently, the extracts were filtered through a series of filter papers, followed by collecting filtrates in reagent bottles covered with aluminum foil to avoid light exposure. Extraction by each solvent for different temperatures and time periods was performed in three triplicates. The total polyphenols and proanthocyanidins concentrations, as well as the radical scavenging activity, were measured.

Total polyphenol content

The total phenolic content was determined using a modified version of the Folin-Ciocalteu colorimetric method [18]. To a mixture of 1 ml extract and 4.5 ml distilled water, 0.5 ml of Folin-Ciocalteu reagent was added. Then, 4 ml of Na_2CO_3 (7.5%) was added and the mixture was vortexed. The sample was incubated for 30 min at 40 °C and absorbance were measured at 734 nm. Gallic acid was used for the calibration curve.

Proanthocyanidins content

The proanthocyanidin content in the extract was determined by the acid-butanol assay according to the method of Porter *et al.* [19]. A sample of 200 μl extracts diluted with 300 μl of acetone 70%. 3.0 ml of butanol-HCL reagent and 0.1 ml of 2% ferric acid were added. The mixture was vortexed and put in the heating block at 97 to 100 °C for 60 min. The tube was then allowed to cool and absorbance was recorded at 550 nm.

DPPH radical-scavenging activity

DPPH radical-scavenging activity was performed according to the method proposed by Brand-Williams *et al.*, with certain modifications [20]. Aliquots of 200 μl of samples were mixed with 2 ml of 100 μM DPPH methanolic solution. The reaction mixture was vortexed and then incubated for 30 min at room temperature. The

absorbance was read at 520 nm against a blank. The ability to scavenge the DPPH radical was calculated using the following equation [21]:

$$\text{DPPH scavenging effect (\%)} = \left[\frac{(A_0 - A_1)}{A_0} \right] * 100$$

RESULTS AND DISCUSSION

Influence of solvents

Solvents, such as water, ethanol, methanol, propanol, acetone, ethyl acetate, and their mixtures have been used for the extraction of polyphenols, often with different proportions of water [22]. Alcohols are known as the most suitable solvents for polyphenols extraction because of their suitable physical properties. Additionally, polyphenols are polar compounds, which usually are extracted with polar solvents such as ethanol and methanol [23].

The total polyphenols and proanthocyanidins yield in pistachio hull extracts obtained using ethanol at different concentrations show significant differences. As seen in fig. 1, ethanol 25% show the highest total polyphenols and proanthocyanidins yield (11.69 ± 0.67 and 5.55 ± 0.37 g/100g dry weight). This can be explained by the low solubility of oxygen in 25% ethanol samples, which play an important role in preventing the degradation reactions of polyphenols [24]. Additionally, the increase in solubility of polyphenols upon the addition of water to-alcoholic solvents could be due to the weakening of the hydrogen bonds between polyphenols and proteins, normally present in plant cells in aqueous solutions [25]. It could also be due to the increase of ionization of the polyphenols in such solutions. Özbek *et al.* previously proved that the maximum yield of polyphenols from pistachio hull could be obtained using 50% ethanol (32.9 g dry extract/100 g dry matter) which is in accordance with our results [1].

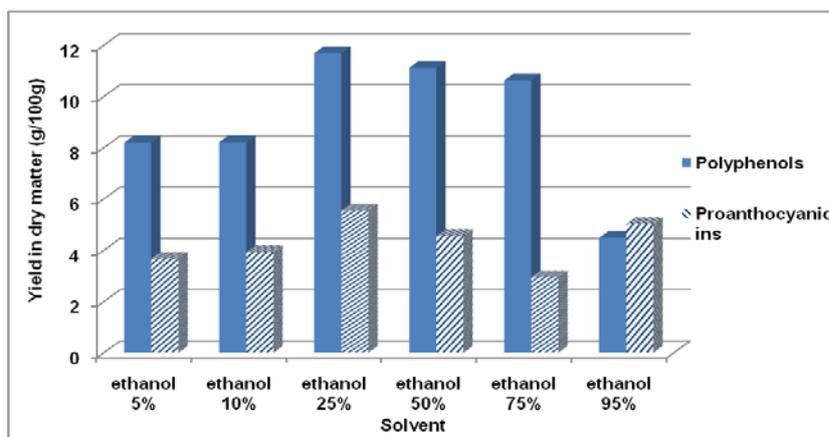


Fig. 1: Effect of different ethanol at different concentrations on total polyphenols and proanthocyanidins yield, As shown in fig. (2) the DPPH radical scavenging activity of extracts has a linear relationship with the polyphenols yield in the extracts ($R^2=0.9907$)

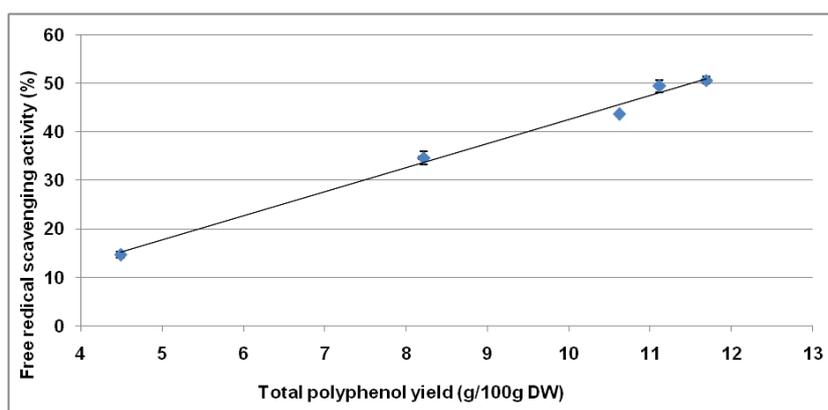


Fig. 2: The relationship between total polyphenols yield and DPPH radical scavenging activity

Influence of extraction temperature

Besides the selection of the suitable solvent, extraction temperature is an important factor that affects solute extraction yield. In order to investigate temperature influence on total polyphenols and proanthocyanidins content, 25% aqueous ethanol was used. Temperatures over the range of 20 to 90 °C were used. According to the results presented in fig. 3, the highest extractability was observed at the temperature of 60 °C and the lowest at the temperature of 20 °C. However, no significant difference ($P < 0.05$) was observed between 60 and 80 °C. Therefore, a fixed maximum temperature of 60 °C was adopted. Similar results were previously obtained during the

extraction of phenolic compounds from grape seeds using 50% ethanol [26]. Sulaiman *et al.* proved that optimal temperature for the extraction of phenolic compounds from *Clinacanthus nutans* Lindau was 60 °C using water: ethanol solvent ratio of 90:10 v/v% [27]. Theoretically, an increase in temperature increases the efficiency of the extraction since plant tissues are softened and the weak interactions affect the cell membranes. Additionally, high temperatures decrease the viscosity of the solvent, thus facilitating its passage through the solid substrate mass. As a result, phenolic compounds can be easily extracted into the solvent [28]. However, very high temperatures could cause oxidation and degradation of the desired compounds which would decrease the extraction yield [29].

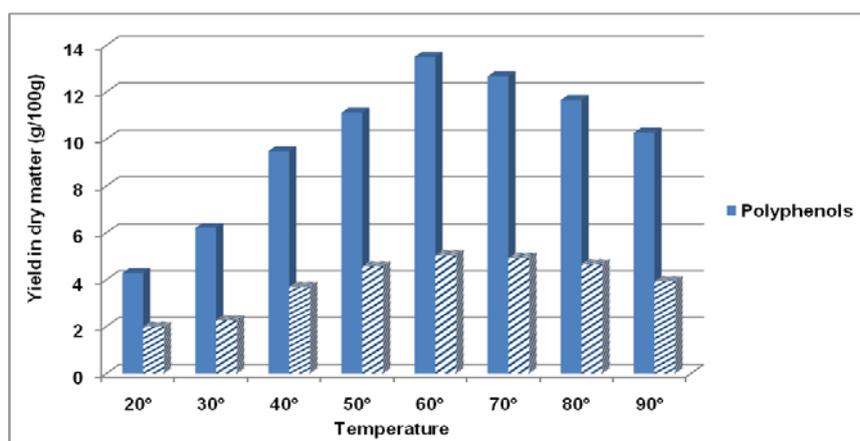


Fig. 3: Effect of extraction temperatures on total polyphenols and proanthocyanidins yield

Influence of extraction time

Several studies had proved that both high temperature and long exposure time can reduce the extraction yield, because of temperature sensitivity and enzymatic degradation and oxidation of polyphenols, as well as polymerization among insoluble constituents [30]. Additionally, a longer time of

extraction can damage extracted natural antioxidants and degrade extract quality [31]. As shown in fig. (4) as extraction time increased (5 to 15 min), the yield of polyphenols and proanthocyanidins also increased. Times longer than 30 min has been shown to cause rapid polyphenols and proanthocyanidins degradation. DPPH activity was evaluated for the optimal extract and was found to be 60.25%.

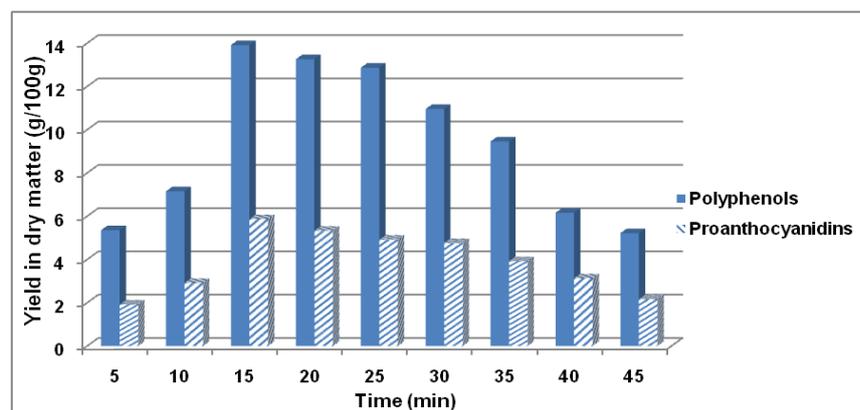


Fig. 4: Effect of extraction time on total polyphenols and proanthocyanidins yield

CONCLUSION

The yield of chemical extraction depends on the type of solvents with varying polarities, extraction time, temperature, and chemical composition and physical characteristics of the samples, which reflects the conflicting actions of solubilization and analyte degradation by oxidation. Therefore, it is of critical importance to select efficient extraction procedures and to maintain the stability of phytochemical compounds.

Results revealed that ethanol 25% is the most suitable solvent for the extraction of polyphenols and proanthocyanidins. A temperature

of 60 °C for 15 min was optimal for the extraction of these compounds with minimal degradation. A yield of 13.91 ± 0.72 and 5.86 ± 0.45 g/100g dry weight was obtained for polyphenols and proanthocyanidins, respectively. This yield was correlated with the best DPPH activity at 60.25%.

Our results indicated that pistachio hulls could represent a valuable source of bioactive compounds that could be successfully employed in food, cosmetic and pharmaceutical industries.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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