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RESEARCH ARTICLE

Parameters Influencing Phenolic compounds extraction from *Pistacia palaestina* fruits during different stages of Maturity

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ABSTRACT:

Pistacia species are used in food industry and as medicinal for the remedy for various diseases. Chemical studies on *Pistacia* genus have led to discovering diverse secondary metabolites in addition to high level of vitamins and minerals. The present study aimed to investigate the effects of experimental variables, such as solvent composition, time and temperature on total phenolic content and DPPH activity of *P. palaestina* fruits cultivated in Syria at three maturity stages. Antioxidant activities and total phenolic content values were estimated using (2,2'-diphenyl-1-picrylhydrazyl) DPPH reagent and Folin Ciocalteu reagent methods respectively. The highest total phenolic compounds and antioxidant activity were found in unripe fruits with 6g/100g and 85.67% respectively. The optimum extraction solvents for phenolic recovery and antioxidant capacity were methanol 60% and acetone 60% respectively. Changes in both temperature (25, 40, 60, 80°C) and time (15, 30, 45, 60 min) had no significant influence on TPC and AA% of *Pistacia* extracts. Therefore, phenolic compounds could be extracted in a short time and low temperature and could be used as antioxidant agents.

KEYWORDS: *Pistacia palaestina*; maturity; total phenolic compounds; DPPH.

1. INTRODUCTION:

The genus *Pistacia* belongs to the Anacardiaceae, a cosmopolitan family that comprises about 70 genera and over 600 species. *Pistacia lentiscus* L., *P. atlantica* Desf., *P. terebinthus* L., *P. vera* L., and *P. khinjuk* stocks are distributed from the Mediterranean basin to central Asia[1]. This plant is rich in tannin and resinous substances and has been known for its aromatic propertie [2]. *Pistacia* species are used in food industry as food additives, snack foods, coffee-like drinks and food colorants[1]. For centuries, medicinal plants have been used as a remedy for various diseases[3]. The essential oil of *Pistacia* species has been shown to have antibacterial, anti-fungal, insecticides, and antioxidants effects[4]. The decoction of its leaves is used as a stomachic, while its fruits are used in the treatment internally for gastralgia and externally for rheumatism and coughs; and as a stimulant, diuretic and antitussive[5].

In a study conducted by Özca, it was stated that terebinth oil extracted with diethyl ether included 0.1% lauric, 0.1% myristic, 21.3% palmitic, 3.4% palmitoleic, 2% stearic, 52.3% oleic, 19.7% linoleic, 0.6% linolenic, 0.1% eicosanoic and 0.2% Eicosenoic acid. In another study done by Matthäus and Özcan in the same field, similar data regarding to fatty acid composition were reported[6]. Chemical studies on *Pistacia* genus have led to discovering diverse secondary metabolites in addition to high level of vitamins and minerals[1]. The extraction of bioactive 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 [7,8]. Phenolics can be extracted from fresh, frozen or dried plant samples. Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency, and wide applicability[9]. A number of methods have been developed in recent years such as microwave, ultrasound-assisted extractions, and techniques based on use of compressed fluids as extracting agents, such as subcritical water extraction (SWE), supercritical fluid extraction (SFE), pressurized fluid extraction (PFE) or accelerated solvent extraction (ASE) were also applied in the extraction of phenolic compounds from plant

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materials[7,8,10,11] . Fresh fruits of *Pistachio mutica* were extracted with methanol or ethanol in ultrasonic bath. The content of individual phenolics in extracts was analyzed by HPLC system and methanol was found to be more effective in phenol extraction than with ethanol. Similar effects of the solvents were observed in the case of (+)-catechin, gallic, protocatechuic and chlorogenic acid but this is not the case for all the individual phenols, such as ellagic and sinapic acid, where higher contents were gained with ethanol[12]. In a study conducted on ripe fruits of *Pistacia lentiscus* L. for the extraction of antioxidants, different solvents were used (methanol, ethanol, acetone, butanol)[13]. The antioxidant activity of butanolic extracts was the lowest among the four studied extracts, whereas, the highest antioxidant activity was measured in the methanol extract. Due to the increasing interest toward the use of natural substances and the influence of extraction method on total extracted compounds, we aim in this study to determine the optimal solvent, time and temperature for the extraction of total phenolic compounds from *Pistacia palaestina* cultivated from the countryside of Tartous city (Syria) in three ripening periods and their antioxidant activity (AA) was also evaluated.

2. MATERIALS AND METHODS:

2.1 Sample Collection:

P. palaestina fruits were collected during three growth and development periods. Unripe, semi-ripe and ripe fruits were harvested from the countryside of Tartous city (Syria) at the beginning of August, in mid-August and at the beginning of September, respectively.

2.2 Sample Preparation:

Pistacia's fruits were washed and dried by three technical drying methods (in microwave, under vacuum, and in the dark for 8 days). Drying in a microwave oven was conducted at 900W for 2, 3 and 5 minutes at maximum power. Whereas, drying under vacuum was performed at -0.5 bar and 55℃ for 24 h.

Pistacia fruits dried were grinded by blender and their moisture content was evaluated by infrared moisture content measurement device. The scavenging activity was also estimated by DPPH reagent method after the extraction of 5g dried samples using ethanol 80% according to Rhouma *et al.* and Kavak *et al.*[14,15]. The scavenging activity and moisture content were estimated in order to evaluate the best technical drying method.

2.3 Chemicals:

All solvents and reagents were of the highest purity needed for each application. Folin-Ciocalteu (2N) reagent (Sigma-Aldrich, Switzerland), 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich, USA), extra pure methanol, ethanol, acetone were obtained from Sharlau (Spain); sodium carbonate (Himedia, India), Gallic acid (Sigma-Aldrich, China).

2.4 Extraction Procedures

Three concentrations (40, 60, 80%) of three different solvents (ethanol, methanol, and acetone) were used for extracting phenolic compounds from *P. palaestina* fruits.1g of fine powder were mixed with 50 ml of each solvents in a glass flask, and kept in a water bath at various temperatures (25, 40, 60, 80°C) for various times (15, 30, 45, 60) min. All samples were extracted in duplicates and the total phenolic concentrations as well as the radical scavenging activity were measured.

2.5 Determination of total phenolic content (Folin–Ciocalteu assay)

The total phenolic content (TPC) of the fruits extract was determined by Folin-Ciocalteu assay as reported in Skerget *et al* with little modifications[16]. Briefly, 4.5 ml of distilled water and 0.5 ml of Folin–Ciocalteu reagent were added to 200µl of the diluted extract. The solution was mixed and incubated at room temperature for 5 min. After incubation, 4ml of 7.5% sodium carbonate solution was added. After incubation at 40 °C for 30 min the absorbance was measured at 734 nm and results were expressed as g of Gallic acid equivalents per 100 g of dry matter[17].

2.6 Radical Scavenging activity assay (DPPH assay)

Free radical-scavenging activity of the sample extracts was evaluated with the modified DPPH (1,1-diphenyl-2-picrylhydrazil radical) assay as described by Brand-Williams *et al.*[18], which is based on the measurement of the reducing ability of antioxidants toward the DPPH radicals. 200 µm of the diluted extract was added to 2ml of a DPPH solution ($1*10^{-4}$ M in methanol). The mixture was then shaken vigorously and left to stand in the dark at room temperature for 60 min. The absorbance was measured at 520 nm[19].

The ability to scavenge DPPH radicals was calculated by the following equation[20]:

DPPH radical scavenging activity (%) = [(Abs control - Abs sample)]/ (Abs control)] x 100.

3 RESULTS AND DISCUSSION:

3.1 Effect of drying methods on the moisture content and the AA% of Pistacia atlantica fruits.

The effect of heat drying on moisture level and antioxidant activity of *P. palaestina* fruits using three different drying methods (in microwave, under vacuum, and in the dark for 8 days) was evaluated. According to results shown in **Table 1**, the moisture content was best reduced by microwave oven drying method for 3 minutes. Our results are in accordance to Saadatian *et al.*

[21]who found that the lowest moisture content was recorded in the case of microwave drying method for hawthorn. According to results presented in table 1, the highest antioxidant activity was observed in dried fruits with microwave (3 minutes) and under vacuum with a DPPH scavenging effect of 96.07% and 95.75%, respectively.

Table 1. Effect of different drying methods on the moisture content and antioxidant activity of *P. palaestina* fruits

Drying operation type of fruits	DPPH %	Time	Moisture %
	-----	2 minutes	8.35
Microwave	96.07	3 minutes	4.03
Under vacuum	95.75	At 0.5 bar, 55 °C, for 24h	9.49
In the dark	89.02	For 8 days	8.67

3.2 Effect of maturity of fruits on TPC and AA

Fruit ripening is a biologically complex process, typically involving changes in chemical composition, pigmentation, texture, color, flavor, and other organoleptic characteristics[22]. *P. palaestina* fruits at three different stages of maturity were extracted using ethanol 80% according to Rhouma *et al.* and Kavak *et al.* [14, 15]. As reported in **Table 2**, the highest TPC and AA were found in unripe fruits, whereas ripe fruits contain the lowest TPC correlated with low antioxidant activity. Our results are in accordance with Nadernejad *et al.*[23] who reported that the ripening characteristics of the fruits in pistachio cultivars affect the total phenolic contents, which decrease as maturity progresses associated with a decrease in antioxidant activity [23]. Ballistreri *et al.*[24] also proved that the TPC decreases in the methanolic extract of unripe *Pistacia vera* L fruits as maturity progresses. This decrease in TPC and AA could be due to the oxidation of phenolic compounds by polyphenol-oxidase that characterizes these stages of maturity[25]. It could also be explained by the loss of flavonoids, trans-resveratrol and tocopherols during ripening of pistachios[24].

Jon Anders Stavang *et al.*[26] found that the concentrations of organic acids decrease and so does titratable acidity whereas the concentrations of anthocyanins increase while the Raspberry fruit color develops. Similar results were found by Olaniyi Amos Fawole *et al.* [27] on pomegranate fruit arils.

Thus, unripe fruits were selected for further experiments in order to determine the influence of the type of solvent, temperature and time on TPC and AA.

Table 2. TPC and AA of *P. palaestina* fruits hydro alcoholic extract at different stages of maturity.

Degree of fruits' maturity	TPC (g/100g)	DPPH %
Ripe	3.06	55.70
Semi-ripe	5.10	59.21
unripe	6.00	85.67

3.3 Effect of extraction solvent on TPC and AA:

Many researchers have previously reported that the extraction yield of phenols is greatly depending on the solvent polarity, and they found that the amount of total phenolic compounds extracted from pistachio were higher in methanol compared to ethanol extracts[12]. Botsaris *et al.*[13] also indicated that methanol was the better extraction solvent for polyphenol antioxidant compounds from *P. lentiscus* fruits compared to ethanol extracts. Rajaei *et al.*[28] revealed that water is the most suitable solvent for the extraction of phenolic compounds from Pistachio (*Pistachia vera*).

Table 3. Effect of solvents on tpc and aa of p. Palaestina unripe fruits extracts.

Solvent type	Solvent concentration %	Total Phenolic (g/100g)	DPPH%
Ethanol	40	6.52	86.92
	60	6.42	90.69
	80	6.31	87.26
Methanol	40	6.96	90.40
	60	8.23	92.20
	80	7.05	90.50
Acetone	40	5.07	85.44
	60	5.59	94.12
	80	2.67	79.98

Solubility of phenolics is governed by their chemical nature in the plant, which may vary from simple to very highly polymerised. Plant materials may contain varying quantities of phenolic acids, phenylpropanoids, anthocyanins, and tannins, among others[29]. There is a possibility of interaction of phenolics with other plant components such as carbohydrates and proteins that may lead to the formation of complexes that may be quite insoluble. Likewise, the solubility of phenolics is affected by the polarity of solvent used[30,31]. Data presented in **Table 3** show that the total phenolic content of *P. palaestina* unripe fruits extracts varies in response to solvents types and concentrations due to differences in polarities, which probably influence the solubility of various compounds present in *P. palaestina* fruits. TPC varied from 2.67g/100g to 8.23g/100g in 80% acetone and 60% methanol extracts respectively. It was found that the lowest total phenolic content was found in acetone extracts which could be due to its non-polar entity which make it not suitable in extracting polar compounds like phenols[32]. Whereas alcoholic solvents tend to increase cell wall permeability, facilitating the efficient extraction of large amounts of polar and medium- to low-polarity constituents[33].

It should be noticed that the absolute alcoholic solvents decrease extraction yield, so application of water combined with alcoholic solvents makes it a moderately polar medium ensuring the optimal conditions for extraction of polyphenols[34].

Results presented in **Table 3** also show that the ability of *P. palaestina* fruits extracts to scavenge the DPPH radical varied significantly from 79.98% to 94.12% in both acetone 80% and acetone 60% extracts respectively. This radical scavenging activity was not found to be correlated with the total phenolic content which could be due to the variation in types of phenolic compounds with different antioxidant activities depending on their structure, therefore the extraction processes seem to recover different phenolic compounds with different antioxidant capacities[35].

3.4 Effect of extraction temperature and time on TPC and AA:

Temperature is a very essential parameter in studies related to food industry, because it intervenes in the chain of several manufacturing processes, extraction and conservation of the foodstuffs[36]. Optimal conditions for the extraction of phenolic compounds from Pistacia fruits depended on extraction time and extraction temperature. Rajaei *et al.*[28] studied the effect of temperature on TPC in *Pistacia vera* over the range of 25 to 85°C and they observed an increase in TPC as temperature increases with no significant difference observed between 65 and 85°C. Belyagoubi *et al.*[36] also found that the antioxidant activity of *Pistacia atlantica* fruits increases with temperature rise from 25°C to 100 °C for 30 min, this phenomenon can be explained by an appearance of new aglycone molecules endowed with a strong antioxidant activity formed after hydrolysis under the heat treatment.

In order to investigate the effect of temperature on extraction yield of phenolic compounds from *P. palaestina* unripe fruits and their radical scavenging activity, methanol 60% was used for the extraction over a range of temperature between 25 and 80°C. As noticed in **Table 4**, no significant difference was observed in both TPC and AA over the used range of extraction temperatures. In different literatures, it was observed that the extraction of phenolic compounds increased slightly when extraction temperature increased from 25 to 40°C. However, it should be noted that increasing the temperature beyond certain values may promoting possible concurrent decomposition of phenolic compounds which were already mobilized at lower temperature or even the break-down of phenolics that are still remained in the plant matrix, additionally, high temperature may encourage solvent loss through vaporization and increase the cost for extraction process from the industrialization point of view[37]. Normally, extraction efficiency increases at higher extraction temperatures, but the working temperature affects the stability of the phenolic compounds, which also depends on their chemical structure[29].

Andriyani *et al.*[38] concluded that temperature variables (50, 70 and 90°C) have no effect on total phenolic content of *Zingiber officinale* water extract. Diah Ratnaningrum *et al.*[39] have also reported that the total phenolic content of ginger ethanol extract (*Zingiber officinale* var. Rubrum) was not affected by either temperature (ambient temperature, 40, and 50°C) or time.

Table 4. Effect of extraction temperature on tpc and aa of p. Palaestina fruits extracts.

Temperature (°C)	Total Phenolic (g/100g)	DPPH%
25	8.19	96.28
40	8.47	95.62
60	8.24	96.05
80	8.18	95.98

Extraction time is crucial in minimizing energy and cost of the extraction process[40]. Chew *et al.* studied the effect of different time values ranged from 60 to 300min on TPC and AA of *Centella asiatica* 40% ethanolic extracts, they found that the maximum concentration of phenolic compounds was achieved at extraction time of 120min, after that point the TPC was decreased while the DPPH was decreased after reaching a maximum value at 180 min as they were observed[40]. Mokrani *et al.*[41] also studied the effect of time on phenolic content in peach extracts. Results showed that TPC increased when extraction time increased from 30min to 180min. After that, increasing the extraction time did not improve the recoveries. Chan *et al.*[37] explained that the prolonged extraction process might lead to phenolic oxidation due to light or oxygen exposure. According to our research and data presented in table 5, there is no significant effect of extraction time on TPC and antioxidant activity in both temperatures 25°C and 80°C.

Table 5. Effect of extraction temperature and time on TPC and AA of P. palaestina fruits extracts.

Temperature (°C)	25 °C		80 °C	
Time (min)	Total Phenolic (g/100g)	DPPH%	Total Phenolic (g/100g)	DPPH%
15 min	7.85	95.52	8.61	95.42
30 min	7.38	95.52	8.83	95.28
45 min	7.45	95.89	8.83	95.48
60 min	7.99	95.71	8.75	95.42

4 CONCLUSION:

The present study was conducted to optimize different conditions affecting the phenolic wield and antioxidant capacity of *P. palaestina* fruits at three stages of maturity. The nature and concentration of extraction solvent, temperature and time were studied. Results showed that unripe fruits contained more phenolic compounds compared with semi ripe and ripe fruits. Methanol 60% was selected as the most appropriate solvent for the extraction of phenolic compounds from *P. palaestina* fruits and acetone 60% use was correlated

with the highest antioxidant activity of the extract. This study confirmed that the extraction temperature and time didn't significantly affect the phenolic yield. Thus, *P. palaestina* fruits could be suggested as a potential natural source of antioxidants with a special focus on optimum extraction conditions at unripe stage. Add to that, the extract could be appropriate for use in nutritional and pharmaceutical fields.

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