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Research Article
Effect of Pretreatment of Olive Leaves on Phenolic Content and Antioxidant Activity

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Abstract

Background: Recent studies suggest that olive leaves are a significant source of bioactive phenolic compounds comparable to olive oil and fruits. Identifying pretreatment before extraction such as solar dried, oven dried and blanched is thus essential to increase the level of such bioactive components in olive leaves, which is considered an agricultural waste. One of these treatments was blanching on 90-95°C for 20 sec. **Objective:** So, the aim of the present study was undertaken in order to investigate the impact of appropriate treatment before drying and extraction on the total phenolic compounds quantity and antioxidant activity in olive leaves. **Methodology:** Olive leaves samples were divided into four parts, Fresh Leaves (FL), Solar Dried Leaves (SDL) and Oven Dried Leaves (ODL) and Blanched Dried Leaves (BDL) on (90-95°C) (1:4 w/v) for 20 sec. Total phenolic content, flavonoids and antioxidant activity of different treatments of olive leaves were estimated. **Results:** The blanching of olive leaves for 20 sec increased up to 593.00 μg GAE g^{-1} (61.70%). A linear relationship was observed between the potential antioxidant activity, total phenolic and flavonoid levels of the olive leaves extract. These results emphasized that olive leaves were contained significant amounts of phenolic content and flavonoids which crucial for their antioxidant capacity. Ethanol extract of the blanched-dried leaves showed the highest antioxidant activity (IC_{50} = 149.92 μg mL^{-1}) compared to methanol and water extract. **Conclusion:** In the present study, it can conclude that the bunching of olive leaves may improve the level of phenolic content and flavonoids and consequently antioxidant capacity has been enhanced.

Key words: Olive leaves, blanching, flavonoid, antioxidant, phenolics

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Olive (*Olea europaea* L.) leaves (OL) are agricultural by-products that can be considered a rich source of bioactive compounds, especially phenolic compounds[1]. *Olea europaea* L., is the most widespread and the characteristic species in the Mediterranean basin covering 9.6 million hectares in 2011[2]. Olive tree (*Olea europeaea*, Oleaceae) is an important crop in the Mediterranean area, which produces 98% of the world total olive leaves by-products resulting from the pruning. The industry of olive leaves appear to be essential components and may be partially responsible for health promoting properties observed among the Mediterranean population, due to its antioxidant and anti-inflammatory effects[3]. Olive leaves can be found in high amount in the olive oil industry by-product and also accumulate during pruning of the olive trees[4,5]. Natural foods and their antioxidants such as phenolic compound and vitamins have received considerable attention, because they are known to function as preventive agents against oxidative damages[6-8]. Many researchers have been targeted at the identification of alternative novel antioxidants from natural sources that have similar properties[9,10]. The olive tree and its products (leaves, olive fruit and its beneficial oil) have a rich history of nutritional, medicinal and commercial purposes[11]. For instance, in the olive oil industry, one of the most promising sources of bioactive are olive leaves obtained as biomass after pruning of olive trees[12]. Olive Leaves Extract (OLE) contains a high level of oleuropein and hydroxytyrosol that makes OLE a promising ingredient for functional food[13]. The chemical composition of olive leaves varies according to many factors such as olive variety, climatic conditions, tree age, wood proportion, agricultural practices, temperature and extraction procedures[14]. The extensive quantitative and qualitative changes in phenolic compounds are also dependent on the biological cycle of the olive tree[15,16]. Drying of olive leaves at room temperature or in the shade causes no detrimental impact on its nutritional value. Different types of dryers have been used over the years for the dehydration purposes of olive leaves including the pilot scale heat pumps conveyor dryer, tray dryer, thin layer dryer, convective laboratory solar dryer and freeze dryer. However, freeze-drying of olive leaves lowers its antioxidant potential[16]. Drying is an essential operation in different processing industries such as agricultural, biotechnology, food and pharmaceutical. Drying of agricultural products is needed for easy handling, safe preservation, longer storage and reduction in the cost of transportation. This process involves removal of water by application of heat. Improper drying may lead to irreversible damages to product quality, high-energy and time consumption, unseasonable charges[17]. So, the present study focused on the investigation of the impact of appropriate treatment before drying and extraction, on the level of total phenolic content and antioxidant capacity of olive leaves extract.

MATERIALS AND METHODS

Plant material: The samples of olive leaves were collected from "Experimental farm of the City of Scientific Research and Technological Applications" fine-quality olive leaves were harvested during the pruning of trees of 'Kalamata' olive (*Olea europaea* L.) stored at -80°C until use. All collected leaves were of six years old plants.

Sample preparation

Olive leaves samples were divided into four parts:

Part 1: Fresh olive leaves were washed with distilled water and minced into small pieces for extraction as Fresh Leaves (FL)

Part 2: Leaves dried by solar drying until constant weight as Solar Dried Leaves (SDL)

Part 3: Other leaves oven-dried at 40°C for three days or until constant weight (ODL)

Part 4: Leaves were blanched on (90-95°C) (1:4 w/v) in stainless steel cooker on different periods (10, 15, 20, 25 and 30 sec) before drying. Then oven dried at 40°C for 3 days or until constant weight (BDL). All leaves were powdered and stored in a dry, dark place until next use

Extraction preparation

Water extract: The four samples boiled with distilled water (1:10 w/v) at 100°C for 10 min then centrifuged at 3000×g for 10 min at 20°C and filtered through Whatman No. 1 filter paper. The extract was lyophilized by (Vacuum freeze dryer model: FDF 0350, Korea)[18].

Ethanol extract: Ethanol extraction was carried out according to Vongsak *et al.*[18] and Abaza *et al.*[19]. Samples were macerated with 70% ethanol (1:10 w/v) for 72 h at room temperature (28±2°C) with occasional shaking. The extract centrifuged at 3000×g for 10 min at 20°C, then filtered through Whatman No. 1 filter paper. Ethanol was evaporated at 45°C, the extract was dried by (Vacuum freeze dryer model: FDF 0350, Korea).

Methanol extract: Methanol extraction was carried out according to Vongsak *et al.*[18] and Abaza *et al.*[19]. Samples were macerated with 70% methanol (1:10 w/v) for 72 h at room temperature (28±2°C) with occasional shaking. The extract centrifuged at 3000×g for 10 min at 20°C, then filtered through Whatman No. 1 filter paper. Methanol was evaporated at 45°C, the extract residue was dried by (Vacuum freeze dryer model: FDF 0350; Korea).

Chemicals and reagents: Solvents, chemicals and reagents were obtained from El-Gomhouria Company, Alexandria, Egypt and Sigma-Aldrich (Steinheim, Germany).

Proximate analysis: Moisture content, crude fiber, ash, protein and fat contents were determined according to the method of AOAC[20]. Total carbohydrates were calculated by difference.

Determination of phenolic content of olive leaves extract: Total phenolic content of the olive leaves extract was determined by the Folin-Ciocalteu micro method Arabshahi-Delouee and Urooj[21]. Twenty microliters aliquot of extract solution was mixed with 1.16 mL of distilled water and 100 µL of Folin-Ciocalteu reagent. And then 300 µL of Na_2Co_3 solution (20%) was added. Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance was measured at 760 nm against gallic acid as a standard for the calibration curve. Total phenolic content expressed as gallic acid equivalent (GAE g^{-1} dry weight sample).

Determination of total flavonoid content of olive leaves extract: Flavonoid content of olive leaves extract was measured according to Dewanto *et al.*[22]. Two hundred and fifty microliters ethanolic extract were mixed with 75 µL (5% $NaNO_2$). After 6 min, 150 µL of 10% $AlCl_3$ and 500 µL of 1 M NaOH were added to the mixture. Finally, the mixture was adjusted to 2.50 mL with distilled water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid content was expressed as microgram equivalents per gram of dry weight (µg CE g^{-1}) through the calibration curve with catechol.

Antioxidant activity: Free radical scavenging activity was determined by a DPPH radical assay, performed according to the method described by Cheung *et al.*[23]. A DPPH radical solution was dissolved thoroughly in anhydrous ethanol. One milliliter of 0.2 mM DPPH radical solution was added to a 200 mL aliquot of sample and standard solution (5:1, v/v). After incubation in the dark at room temperature for 30 min, changes in the absorbance of the samples were analyzed at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the tested compound) and A_{sample} is the absorbance of the samples.

Statistical analysis: The results were reported as the Mean±Standard Deviation (SD) (n = 3). The average contents of total phenolics, total flavonoids and IC_{50} of the extracts prepared by the different extraction methods were statistically investigated using one-way analysis of variance (ANOVA) with Duncan by SPSS for Windows 16.0. A statistical probability (p-value) less than 0.05 indicated a statistically significant difference between groups[24].

RESULTS AND DISCUSSION

The chemical composition of olive leaves varies according to the origin, the proportion of branches present in the extract, storage conditions, weather conditions, moisture content and degree of soil contamination[25,26]. The composition of the extract from olive leaves strongly influenced by processing (drying and extraction)[27]. Table 1 showed the chemical composition of olive leaves collected during the

Table 1: Effect of different treatments on olive leaves proximate chemical composition

	Treatments			
Components (%)	Solar dried	Oven dried	Blanched oven dried	Significant
Moisture	33.19±0.00[b]	42.39±0.56[a]	42.39±0.56[a]	0.000
Fat	6.79±0.61	6.83±0.79	5.69±0.01	0.088
Protein	4.95±0.09	4.95±0.09	4.95±0.09	1.000
Ash	5.77±0.58	4.58±0.38	5.80±0.82	0.083
Fiber	3.32±0.10[b]	4.71±0.59[a]	3.68±0.24[b]	0.009
Carbohydrate	45.96±0.92[a]	36.75±0.00[b]	37.66±0.00[b]	0.000

Each reported value is the Mean±SD of three replicates, means in the same row followed by different letters are significantly different (p<0.05)

Table 2: Effect of different treatment method and solvent on extracted yield percentage of olive leaves

	Treatments				
Solvent	Fresh leaves	Solar dried	Oven dried	Blanched oven dried	Significant
Ethanol (70%)	23.17±0.81^{bA}	32.03±0.85^{aA}	22.36±0.10^{bA}	23.66±0.81^{bB}	0.000
Methanol (70%)	20.03±1.50^{bB}	32.91±2.23^{aA}	22.03±0.90^{bA}	28.83±4.00^{aA}	0.001
Water	17.96±0.06^{bC}	18.75±0.00^{bB}	13.21±0.58^{cB}	23.17±0.81^{aB}	0.000
Significant	0.002	0.000	0.000	0.050	

Each reported value is the Mean±SD of three replicates, means in the same row followed by different lower case letters are significantly different ($p<0.05$), means in the same column followed by different upper case letters are significantly different ($p<0.05$)

pruning with different treatments. Data revealed that the moisture content was significantly different between the treatments. Solar dried (SDL) showed lower moisture content (33.19±0.00%) than the Oven Dried Leaves (ODL) (42.39±0.56%) and Blanched Dried Leaves (BDL) (42.39±0.56%). The ODL showed the highest fat content (6.83±0.79%), while the BDL showed the lowest fat content (5.69±0.01%) as compared to the other treatments; however, there is no significant differences were found among all treatments ($p>0.05$). In the same respect, protein content was (4.95±0.09%) for all treatments. No significant differences were observed among the different treatments. The BDL showed the highest ash content (5.80±0.82%) while the ODL showed the lowest content (4.58±0.38%) as compared to the other olive leaves treatments. These obtained results represent the positive effect of blanching that increased the ash content from 4.58-5.80%. On the other hand, oven dried (ODL) showed the highest fiber contents (4.71±0.59%). While, there are no significant differences were observed between BDL and SDL. The SDL had the highest carbohydrate content as compared to other treatment (45.96%), while the ODL was (36.75%). These results agreed well with that obtained by Boudhrioua *et al.*[28] who studied the proximate composition of four olive leaves cultivars in Tunisia (chemlali, chemchali, zarrazi and chetoui) and found that the fresh olive leaves were intermediate moisture products. The moisture content was varied from 46.24% (zarrazi) to 49.75% (chemlali). Protein and fat contents of the leaves varied, from 2.86% (zarraazi) to 4.45% (chemlali). Accordingly, carbohydrates contents ranged from 37.14% (chemlali) to 42.58% (chetoui). Abdel-Nabey *et al.*[29] showed that significant differences between sun-dried leaves and dehydrated ones concerning their content of crude ether extract and carbohydrates that (8.19 and 6.21%) to sun dried and dehydrated, respectively. Carbohydrates were (60.12 and 58.35%) to sun dried and dehydrated, respectively.

Yield of olive leaves extract: Recent studies suggest that olive leaves are a significant source of bioactive phenolic compounds compared to oil and fruits. Thus identifying appropriate extraction method is a crucial step to increase the yield of such bioactive components from olive leaves[30]. The extraction yields are a measure of the solvent to extract specific compounds from the original material. The extraction method must be obtaining a maximum amount of interested bioactive compounds without any adverse effect on their chemical structure. The activity of natural extraction has been found to depend on the active components of the raw material, the type and polarity of extraction solvent and the extraction procedure[31]. The yield of crude extracts of different solvents is shown in Table 2. Ethanol gave the highest yield in Fresh Leaves (FL) (23.17±0.81%) followed by methanol (20.03±1.50%) while water extract gave the lowest yield (17.96±0.06%). On the other hand, methanol gave the highest yield with Solar Dried Leaves (SDL) (32.91±2.23%) followed by ethanol (32.03±0.85%), while water extract gave the lowest yield (17.96±0.06%). In Oven Dried Leaves (ODL), ethanol gave the highest yield (22.36±0.10%) followed by methanol (22.03±0.90%), while water gave the lowest yield (13.21±0.58%). Methanol gave the highest yield with Blanched Dried Leaves (BDL) (28.83±4.00%) followed by ethanol (23.66±0.81%), while water extract gave the lowest yield (23.17±0.81%). From the obtained results, methanol extracts gave the highest yield (32.91±2.23%) with solar dried samples among all different solvents and treatment. While the very interested remark that is the water extract (23.17±0.81%) in the blanched samples very similar to ethanol extract (23.66±0.81%). This statement encourages us to use the water extracts instead of ethanol in food application due to its safety for human use and more economical. These results agreed well with Al-Attar and Shawush[32] where the yields mean of the olive and rosemary water extracts were (18.7 and 20.6%, respectively).

Effect of blanching time on phenolic content: The blanching depends upon the time required for inactivation of enzymes and thus on the rate of heat transfer[33]. Blanching is the most helpful method to inactivate enzymes in the plant materials[34]. Exposure of polyphenol oxidase (PPO) to the temperature of 70-90°C destroys their catalytic activity, but the time required

Table 3: Effect of blanching time on polyphenol oxidase (PPO) activity and total phenolic content

Blanching time (sec)	PPO activity (%)	Total phenolic compounds (ug GAE g^{-1} extract)
10	31.30	329.00 ± 2.65^{C}
15	20.90	358.67 ± 1.15^{B}
20	0.00	532.15 ± 0.95^{A}
25	0.00	274.33 ± 1.15^{D}
30	0.00	263.67 ± 1.15^{E}
Significant (p)		0.000

Values are presented in the Mean±SD of three replicates, means in the same column followed by different letters are significantly different (p<0.05)

Table 4: Effect of treatment and solvent on total phenolic content (μg GAE g^{-1} extract) of olive leaves

	Treatments				
Solvent	Fresh leaves	Solar dried	Oven dried	Blanched oven dried	p-value
Ethanol (70%)	266.33 ± 14.29^{dC}	406.67 ± 10.79^{b}	325.67 ± 31.88^{cA}	593.00 ± 27.71^{a}	0.000
Methanol (70%)	317.33 ± 0.58^{dB}	418.33 ± 19.86^{b}	283.33 ± 12.50^{cB}	563.33 ± 16.77^{a}	0.000
Water	387.00 ± 11.79^{cA}	423.67 ± 25.66^{b}	224.33 ± 11.72^{dC}	549.33 ± 7.02^{a}	0.000
p-value	0.000	0.587	0.003	0.076	

Values are the Mean±SD of three replicates, means in the same row followed by different lower case letters are significantly different (p<0.05), means in the same column followed by different upper case letters are significantly different (p<0.05)

to inactivation depends on the products[35]. Data in a Table 3 showed that increasing the time to 20 sec lead to enhance inactivation of PPO up to (zero) and increase total phenolic content up to (532.15±0.95 μg GAE mg^{-1} extract). The least time for inhibition of PPO activity was 20 sec then the total phenolic decreased by increasing the time. It may be referred to partial degradation of the phenolic compound when blanching more than 20 sec.

Phenolic content of olive leaves: Phenolic compounds are a major class of plant secondary metabolites with bioactive potential attributed to antioxidant activity[36], mentioned that the concentration of polyphenol compounds in olive leaves changed depending on the quality, origin and variety of the plant material. Table 4 showed that the phenolic content in the leaves extracts expressed as micrograms of gallic acid equivalents per gram of extract (μg GAE g^{-1} extract). In fresh leaves the order of different solvent in the extraction of total phenolic was (water>methanol 70%>ethanol 70%, respectively). The highest phenolic content was registered with water extract of fresh samples (387±11.79 μg GAE g^{-1}) compared to methanol and ethanol (317.33±0.58 and 266.33±14.29 μg GAE g^{-1}, respectively). Also, SDL showed the same trend of the ability of different solvents in the extraction of phenolics (water>methanol 70%>ethanol 70%, respectively). Water extract showed the highest phenolic content with solar dried (SDL) (423.67±25.66 μg GAE g^{-1}) compared to methanol and ethanol (418.33±19.86 and 406.67±10.79 μg GAE g^{-1}, respectively). On the other hand in the ODL the order of different solvent in the extraction of phenolic compounds was (ethanol 70%>methanol 70%>water, respectively). Ethanol extract registered the highest phenolic content with Oven Dried Leaves (ODL) (325.67±31.88 μg GAE g^{-1} extract) compared to methanol and water (283.33±12.50 and 224.33±11.72 μg GAE g^{-1} extract, respectively). In the Blanched Dried Leaves (BDL), the order of different solvent in of the extraction of phenolic compounds was (ethanol 70%>methanol 70%>water, respectively), the highest phenolic content was registered with ethanol extract (593.00±27.7 μg GAE g^{-1} extract) followed by methanol and water extract (563.33±16 and 549.33±7.02 μg GAE g^{-1} extract, respectively). The highest phenolic content was registered with ethanol, methanol and water extract of blanched leaves compared to other treatments and also showed significant differences among the samples. These results agreed with Boudhrioua *et al.*[28] who showed that drying leaves by blanching and infrared drying method increased total phenolic content as compared to the fresh ones. Suggesting the preservation of olives leaves before their use in food or cosmetic applications and also with one study showed that the dehydration by oven drying without blanching reduced the nutritional value of the extract[25].

Flavonoids content of olive leaves: Flavonoids are widespread groups of natural compounds, probably the most natural phenolics[37]. The extracts in the present study were found to have various levels of flavonoids. Table 5 showed the content of flavonoids in different extracts of the leaves that are significantly different. Therefore, these levels vary considerably, from 41.75±0.50 to 190.33±1.66 μg g^{-1}. Ethanol, methanol and water of fresh leaves had total flavonoids (62.92±0.58, 86.00±3.44 and 141.17±0.14 μg g^{-1}, respectively). Water extract had the highest flavonoids content (141.17±0.14 μg g^{-1}), while ethanol extract showed the

Table 5: Effect of different treatment and solvent on total flavonoids (μg g^{-1} extract) of olive leaves

	Treatments				
Solvent	Fresh leaves	Solar dried	Oven dried	Blanched oven dried	p-value
Ethanol (70%)	62.92 ± 0.58^{cC}	147.42 ± 5.53^{bA}	46.67 ± 0.95^{d}	162.42 ± 2.02^{aC}	0.000
Methanol (70%)	86.00 ± 3.44^{bB}	87.33 ± 1.42^{bB}	41.75 ± 0.50^{c}	188.00 ± 6.06^{aA}	0.000
Water	141.17 ± 0.14^{cA}	154.33 ± 5.77^{bA}	43.08 ± 5.59^{d}	190.33 ± 1.66^{aA}	0.000
p-value	0.000	0.000	0.245	0.000	

Values are the Mean±SD of three replicates, values in the same row followed by different lower case letters are significantly different ($p<0.05$), values in the same column followed by different upper case letters are significantly different ($p<0.05$)

Table 6: DPPH free radical-scavenging activity of olive leaves with different treatments

	Concentration (μg mL^{-1})							
	Inhibition (%)							
Samples with different treatments and solvent	100	150	200	250	300	350	400	p-value
ODLM	2.23 ± 0.0^{g}	15.15 ± 0.15^{f}	25.93 ± 0.12^{e}	35.05 ± 0.13^{d}	38.28 ± 0.03^{c}	46.88 ± 0.13^{b}	47.67 ± 0.06^{a}	0
ODLE	10.40 ± 0.10^{f}	10.17 ± 0.15^{f}	16.30 ± 0.26^{e}	27.13 ± 0.23^{d}	35.50 ± 0.10^{c}	46.12 ± 0.03^{b}	59.80 ± 0.10^{a}	0
ODLW	8.15 ± 0.01^{g}	11.35 ± 0.13^{f}	16.33 ± 0.15^{e}	25.27 ± 0.21^{d}	35.03 ± 0.06^{c}	38.27 ± 0.07^{b}	40.37 ± 0.06^{a}	0
FLM	27.78 ± 0.03^{g}	39.60 ± 0.10^{f}	53.07 ± 0.12^{e}	64.87 ± 0.11^{d}	72.87 ± 0.11^{c}	81.80 ± 0.10^{b}	84.50 ± 0.10^{a}	0
FLE	6.60 ± 0.10^{g}	22.43 ± 0.12^{f}	34.39 ± 0.11^{e}	45.09 ± 0.16^{d}	62.08 ± 0.08^{c}	72.03 ± 0.15^{b}	82.10 ± 0.1^{a}	0
FLW	30.27 ± 0.01^{g}	33.10 ± 0.17^{f}	40.77 ± 0.25^{e}	42.12 ± 0.10^{d}	46.13 ± 0.06^{c}	86.60 ± 0.10^{b}	87.47 ± 0.06^{a}	0
SDLM	12.10 ± 0.10^{g}	25.20 ± 0.20^{f}	36.18 ± 0.16^{e}	47.63 ± 0.15^{d}	60.03 ± 0.06^{c}	65.87 ± 0.12^{b}	67.63 ± 0.03^{a}	0
SDLE	5.15 ± 0.26^{g}	8.03 ± 0.16^{f}	29.20 ± 0.20^{e}	43.57 ± 0.06^{d}	50.32 ± 0.03^{c}	60.47 ± 0.06^{b}	70.75 ± 0.05^{a}	0
SDLW	12.52 ± 0.23^{g}	21.25 ± 0.23^{f}	30.07 ± 0.12^{e}	38.40 ± 0.35^{d}	43.88 ± 0.13^{c}	53.83 ± 0.06^{b}	59.45 ± 0.05^{a}	0
BDLM	29.80 ± 0.20^{f}	50.25 ± 0.05^{e}	76.52 ± 0.50^{d}	76.15 ± 0.05^{d}	91.90 ± 0.10^{c}	94.08 ± 0.08^{b}	94.48 ± 0.03^{a}	0
BDLE	8.82 ± 0.18^{g}	21.35 ± 0.15^{f}	45.03 ± 0.06^{e}	55.75 ± 0.05^{d}	69.17 ± 0.03^{c}	81.93 ± 0.12^{b}	96.43 ± 0.03^{a}	0
BDLW	35.45 ± 0.05^{g}	43.78 ± 0.10^{f}	48.03 ± 0.06^{e}	54.57 ± 0.12^{d}	81.25 ± 0.50^{c}	$83.03\pm.06^{b}$	$89.65\pm.050^{a}$	0

Values are the Mean±SD of three replicates, values in the same row followed by different lower case letters are significantly different ($p<0.05$), ODLM: Methanol extract of oven dried leave, ODLE: Ethanol extract of oven dried leaves, ODLW: Water extract of oven dried leaves, FLM: Methanol extract of fresh leaves, FLE: Ethanol extract of fresh leaves, FLW: Water extract fresh leaves, SDLM: Methanol extract of solar dried leaves, SDLE: Ethanol extract of solar dried leaves, SDLW: Water extract of solar dried leaves, BDLM: Methanol extract of blanched leaves, BDLE: Ethanol extract of blanched leaves, BDLW: Water extract of blanched leaves

Table 7: Antioxidant activity (DPPH free radical-scavenging activity) of olive leaves with different treatments

	IC_{50} (μg mL^{-1})			
Solvent	Fresh leaves	Solar dried	Oven dried	Blanched oven dried
Ethanol (70%)	277.77	286.89	368.45	149.49
Methanol (70%)	192.90	262.27	427.00	212.67
Water	296.48	325.86	495.66	223.81

lowest (62.92 ± 0.58 μg g^{-1}). Ethanol, methanol and water extracts of Solar Dried Leaves (SDL) gave total flavonoids (147.42 ± 5.53, 87.33 ± 1.42 and 154.33 ± 5.77 μg g^{-1}, respectively). Water extract gave the highest flavonoids content (154.33 ± 5.77 μg g^{-1}), while methanol extract gave the lowest content (87.33 ± 1.42 μg g^{-1}). Ethanol, methanol and water of Oven Dried Leaves (ODL) gave total flavonoids content (46.67 ± 0.95, 41.75 ± 0.50 and 43.08 ± 5.59 μg g^{-1}, respectively). However, there was no a significant difference in the flavonoids content among the different solvents. Total flavonoids content of ethanol, methanol and water of Blanched Dried Leaves (BDL) were 162.42 ± 2.02, 188.50 ± 6.06 and 190.33 ± 1.66 μg g^{-1}, respectively. Water extract showed the highest flavonoids content (190.33 ± 1.66 μg g^{-1}) while ethanol extract showed the lowest (162.42 ± 2.02 μg g^{-1}). Generally, the highest flavonoids content was registered with ethanol, methanol and water extract of blanched-dried leaves compared to other treatments and also showed that the water extract was the highest flavonoids content for all treatment with significant differences among the samples. The obtained results agreed with Salah *et al.*[38] who reported that the total flavonoids content of olive leaves was ranged from (56.57 ± 6.0 to 125.64 ± 3.36 μg g^{-1}) and a significant difference was found in the content of total flavonoids of leaves among the samples.

Antioxidant capacity of olive leaves: The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants[39]. In order to evaluate antioxidant activity, DPPH radical scavenging capacity was measured for olive leaves extracts and results of percentage of inhibition are given in Table 6. A methanol extract showed the highest antioxidant capacity of fresh leaves (IC_{50} = 192.90 μg mL^{-1}), solar dried leaves (IC_{50} = 262.27 μg mL^{-1}) and oven dried leaves (IC_{50} = 427.00 μg mL^{-1}) (Table 7). On the other hand, ethanol extract showed the highest antioxidant activity in the case of

the blanched-dried leaves (IC_{50} = 149.92 μg mL^{-1}) compared to methanol and water extract. Generally, the highest antioxidant activity was registered with blanched-dried leaves compared to other treatments. Significant differences among the samples were expressed ($p<0.05$). The different antioxidant activities of the phenolic extracts can be attributed to the different ability of solvents to extract the phenolic compounds, as the antioxidant activity depends on the type and polarity of the extracting solvent, the isolation procedures, the purity of the active compounds, as well as the test system[40]. These results agreed with Talhaoui *et al.*[41] who showing IC_{50} values of 129.9±23.5 μg mL^{-1} 'Sikitita' and Luo[42] who also showed that 400 μg mL^{-1} of olive leaves extracts was required to achieve 50% DPPH radical scavenging activity, IC_{50} = 400 μg mL^{-1} which near from our results.

CONCLUSION

The present study provided evidences that significant quantitative and qualitative changes in phenolic compounds, flavonoids and antioxidant activity are dependent on pretreatment of olive leaves before extraction. The blanching of olive leaves in hot water (90°C) for 20 sec enhanced and increased the phenolic content level from 329.00-532.00 GAE g^{-1} (61.70%). While the oven-dried leaves had the lowest content of phenolic and flavonoid. Thus, it can be concluded that blanching of olive leaves before extract for 20 sec may reserve the phenolic compounds and their activities due to the stopping of the polyphenol oxidase activity which decreases the amount of bioactive compounds.

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