Sustainable utilization of olive leaves and pomace as sources of fatty acids and bioactive compounds

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Abstract: Olive leaves and pomace are being wasted in Pakistan and remain unexplored concerning their bioactivity and fatty acids. The objective of the present work is to study fatty acids and antioxidant potentials of olive leaves and fruit pomace indigenous to Pakistan. The antioxidant potential of both water extract (WE) and ethanol extract (EE) of olive leaves and fruit pomace was also investigated. Results showed that olive leaves exhibited the highest oleic acid content (54.60%±3.40%), followed by palmitic acid (15.20%±1.20%). For olive pomace, oleic acid was the most abundant fatty acid (59.30%±2.40%), while palmitic acid and linoleic acid were recorded at 6.30%±0.56% and 5.2%±0.61%, respectively. However, oleic acid (59.30%±2.40%) was present in the highest quantities in olive pomace, followed by palmitic acid (6.30%±0.56%) and linoleic acid (5.20%±0.61%). The EE of olive leaves had higher total phenolics (1902.00±189.00 mg GAE/100 g) than WE (1216.00±143.00 mg GAE/100 g). The WE of olive pomace had higher total phenolics (292.40±18.00 mg GAE/100 g) than EE (201.80±15.00 mg GAE/100 g). The scavenging activity of DPPH of ethanol-soluble extracts (EE) (60.60±3.50 μM TE/g) of olive leaves was also greater than their respective water-soluble extracts (WE) (51.20±3.00 μM TE/g). Similarly, scavenging activity of ABTS of EE (9.88±0.90 μM TE/g) of olive leaves was greater as compared to WE (3.24±0.40 μM TE/g) of leaves. The DPPH radical scavenging activity of EE (29.44±1.10 μM TE/g) of olive pomace was higher than its respective WE (14.24±1.40 μM TE/g). However, the ABTS scavenging activity of EE (6.05±0.90 μM TE/g) of olive pomace did not vary significantly (p<0.05) with regard to its respective WE (5.99±0.45 μM TE/g). Results concluded that olive leaves and olive pomace are good sources of fatty acids and antioxidants, which are recommended in food application according to a sustainable approach strategy.

Keywords: olive leaves, olive pomace, fatty acid profile, antioxidant potential, sustainability
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1 Introduction

Olive oil is typically obtained from olive (*Oleaeuropaea* L.) fruit via solvent extraction as well as physical methods. Linoleic acid, palmitic acid, and oleic acid are present in olive oil in appreciable quantities[1]. A number of antioxidant molecules (cafeic, *p*-coumaric, syringic, vanillic, luteolin, apigenin-7-*O*-glucoside, quercetin-3-rutinoside (rutin), cyanidin-3-rutinoside, α-Tocopherol, triterpenic acids, phytosterols) in olives have been reported[2].

Pakistan meets 75% of its demand for olive oil through imports. The total production of olive in Pakistan is approximately 20 tons in comparison to 0.5 million tons of oils from other seed crops[3]. It is thus imperative to enhance the cultivation and production of oil seeds, preferably olive, owing to its health-promoting and revenue potential. Pakistan has the potential to produce a significant volume of olives and to extract olive oil for human consumption. This would not only support local consumption but also result in estimated annual export revenue of approximately $6 billion[4].

The recent increase in livestock feed prices is due to the increasing cost of grains triggered by high input prices (irrigation, fertilizers, etc.), irregular availability, and a shortage of conventional feed ingredients. As a result, there is an urgent need to provide less costly, nonconventional alternatives that would be available throughout the year. For example, various agro-industrial by-products are being used as non-conventional feed ingredients in the livestock feed industry[5]. By-products from olive oil may be used in the ruminant's feed in order to enhance growth performance as well as meat and milk quality[6]. Olive pomace (OP) is considered a by-product from olive fruits during oil extraction[7], and is used as animal feed, organic fertilizer, and a source of energy[8,9]. OP contains phenolic compounds (vanillic acid, oleuropein, hydroxytyrosol, tyrosol, *p*-coumaric acid)[10,11] as well as flavonoids (hesperidin, esculetin, quercetin, luteolin, quercetin-3-*O*-arabinoglucoside)[12]. Compounds responsible for the increased antioxidant activities in samples can be separated and identified by Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectra[13].

To date, the chemical characteristics concerning the fatty acids profile and chemical functional groups present in olive pomace and leaves from various countries other than Pakistan have been published. However, to our knowledge, no study is reported concerning the aforementioned characteristics of olive pomace and leaves from indigenous cultivars grown in Pakistan. As olive leaves and pomace are usually wasted in our country, they therefore can be used as fodder for animals. The characterization of olive leaves and pomace is also necessary for exploring their positive impacts on the health of animals. Thus, the aim of the current research is to examine the chemical characterization of indigenous olive leaves and olive pomace with regard to their fatty acid profile, antioxidant potential, and FTIR spectra.

2 Materials and methods

2.1 Materials

2,2-diphenyl-1-picrylhydrazyl (DPPH), Gallic acid, 2,2′-azino-bis (3ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent, and Trolox were from Merck (KGaA, Darmstadt, Germany). Olive pomace and leaves were procured from the Centre of Excellence for Olive Research and Training (CEORT), Barani Agriculture Research Institute (BARI), Chakwal, Pakistan. The olive pomace and olive leaves belonged to the cultivar "Arbequina" which is usually cultivated in Pakistan. The extraction of oil is usually done by using fully ripened olive fruit which is usually harvested in November. Both olive pomace and olive leaves were procured during November.

2.2 Chemical characteristics of olive pomace and leaves

The moisture (%), crude fat (%), crude protein (%), and fiber (%) contents of olive pomace and olive leaves were determined by using AOAC[14] methods. These chemical characteristics were done on a wet basis.

2.3 Determination of fatty acids of olive pomace and leaves

Fatty Acid Methyl Esters (FAMEs) were produced by following the method with some modifications of El Riachy et al.[15]. About 500 mg of pomace oil was taken and refluxed with 0.5 N KOH for 3-5 min. Further, 15 mL of prepared reagent (2 g NH_4Cl, 3 mL concentrated H_2SO_4 dissolved in 60 mL methanol) was added and refluxed for 15 min. The mixture was refluxed again after swirling. The upper phase (1 mL) containing FAME was separated and transferred to vial (1.5 mL) with 25 μL of external standard (nonadecanoate methyl ester of 1000 ppm).

The FAMEs were separated with a Shimadzu gas chromatograph (GC-17) having a flame ionization detector. The capillary column (DE; 30 mm length×0.25 mm i.d. and 0.25 μm of film thickness) of silica was utilized. Nitrogen (mobile phase) was a 30 mL/min flow rate. The initial oven temperature was 140°C for 5 min, which is subsequently raised to 240°C (at 4°C/min) for 30 min. Hydrogen and compressed air were used as flame detector. The volume of injected sample was 2 μL, and the results of identified fatty acids were calculated as % of total fatty acids.

2.4 Preparation of water-soluble extracts (WE) and ethanol-soluble extracts (EE) of olive pomace and leaves

The method given in Gupta et al.[16] was adopted for the preparation of water extracts (WE) and ethanol extracts (EE) of olive pomace and leaves. The purity of the ethanol used was 99.8%. The mixture of ethanol and water (70:30) was prepared for the preparation of ethanol extracts. 5 g of dried olive leaves or pomace was mixed with 100 mL of the aforementioned solvents and stirred (50 r/min) for 5 h at 25°C. The centrifugation of mixture was done at 6000 r/min for 10 min (4°C). The filtered extracts were frozen at –20°C immediately after making aliquots in Eppendorf tubes (Figure 1).

2.5 Total phenolic contents (TP)

The TP of WE and EE of olive leaves and pomace were done using the procedure as described by Reis et al.[17] and Qureshi et al.[18] The reagent (Folin-Ciocalteu, 5%) of 1 mL was added into WE and EE (each 500 μL) of olive leaves and pomace. Then, sodium carbonate (5%, 1 mL) solution was added. The above mixture was then kept at 25°C for 1 h. Absorbance was measured at 760 nm using a UV/VIS spectrophotometer (T80, PG Instruments). Total phenolic contents were expressed as mg Gallic acid equivalent (GAE) per 100 g of olive leaves or pomace.

2.6 Total flavonoid contents (TF)

The flavonoids of WE and EE of olive leaves and pomace were measured using the procedure of Jia et al.[19] with some minor modifications. Both WE and EE of olive leaves and pomace (each 500 μL) were mixed with sodium nitrite (75 μL, 5%) solution. After that, aluminum chloride (150 μL, 10%) was mixed with the above mixture. Finally, after adding sodium hydroxide (500 μL, 1 mol/L), the absorbance was taken at 510 nm. The results were calculated as mg Quercetin equivalent (QE) per 100 g of olive leaves or pomace.

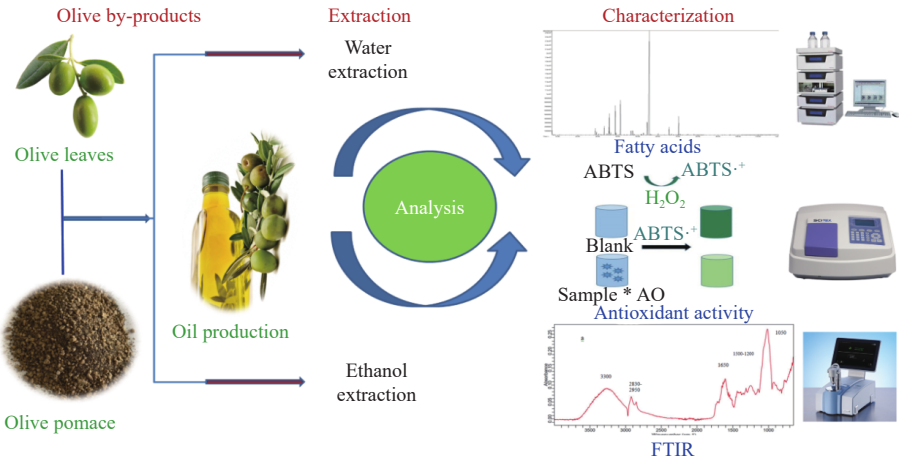


Figure 1 Experimental protocol of olive leaves and pomace by-products

2.7 Ferric reducing antioxidant power (FRAP) assay

The WE and EE of olive leaves and pomace were also assessed for their FRAP according to Qureshi et al.[19]. Each sample (500 μL) was mixed with 500 μL potassium phosphate buffer (0.2 mol/L, pH 6.6). Then potassium ferricyanide ($K_3Fe(CN_6)$) was added into the above mixture. 500 μL of trichloro acetic acid (%) was added after placing the mixture at 50°C for 20 min. Then centrifugation (HermleLabortechnik GmbH Siemensstr-25D-78 564 Wehingen, Germany) of sample mixture was done at 7000 r/min (10 min, 4°C). Then, 200 μL of ferric chloride (0.2%) was added to the clear supernatant of the above mixture. The absorbance was taken at 700 nm. The FRAP was calculated as μmol/L Trolox equivalent (TE)/g of olive leaves or pomace using standard curve of Trolox.

2.8 ABTS activity assay

The WE and EE of olive leaves and pomace were also assessed for ABTS scavenging activity by following the method of Zeghad et al.[20]. The mixture of 7 mmol/L ABTS solution and 2.5 mmol/L potassium persulfate (1:1) was kept for 20 h. After dilution of the above mixture, the absorbance was adjusted at 0.70±0.04 at 734 nm. Sample solutions (25 μL, 50 μL, and 100 μL) were individually mixed with 3 mL of the prepared ABTS solution for reaction. The absorbance of the above mixture was taken at 734 nm and the values were expressed as μmol/L Trolox equivalent (TE)/g of olive leaves or pomace. The ABTS inhibition (%) and IC_{50} were also calculated. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH inhibition}(\%) = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} 100$$

The IC_{50} (half-maximal inhibitory concentration) of DPPH was calculated by determining the sample concentration required to reduce the initial DPPH radical concentration by 50%. Inhibition rate (%) was plotted versus sample concentration (mg/mL), and the target concentration was calculated using the linear regression equation ($y=mx+c$).

2.9 DPPH activity assay

The investigated samples (WE and EE) of olive leaves and pomace were monitored for DPPH scavenging activity by the method as described by Yi et al.[21]. 2 mL of DPPH solution (60 μmol/L) was mixed with WE and EE (each 1 mL) of olive leaves and pomace. After incubating the mixture (for 30 min), the absorbance was taken at 517 nm. This activity was expressed as μmol/L Trolox equivalent (TE)/g of olive leaves or pomace. DPPH inhibition (%) and IC_{50} were also calculated.

2.10 ATR-FTIR spectroscopic analysis

The FTIR spectra analysis was carried out via the method of Agatonovic-Kustrin et al.[13]. The FTIR spectra were measured via Agilent Cary 630 (Agilent Technologies, Mulgrave, Australia) FTIR spectrometer. For sampling, an ATR accessory attached with a crystal was used. The absorbance spectra were recorded from 4000 to 650 cm^{-1} (middle infrared region). For the spectra of the sample, a small quantity of sample was put on the ATR crystal surface.

2.11 Data analysis

Minitab software was used to statistically analyze the data (Minitab Inc., State College, PA, USA, version 16). An independent *t*-test was used for the comparison (at $p<0.05$) between the results of water-soluble (WE) and ethanol-soluble (EE) extracts of olive leaves and pomace.

3 Results

3.1 Composition of olive leaves and pomace

Data concerning composition of olive leaves and pomace is given in Table 1.

Table 1 Chemical characteristics of olive pomace and leaves (Means±SD)

Properties	Olive pomace/%	Olive leaves/%
Moisture	68.07±1.40	49.07±0.83
Dry matter	31.93±1.40	50.93±0.83
Crude protein	4.11±0.37	7.76±0.09
Crude fat	2.62±0.18	6.60±0.14
Crude fiber	19.00±0.49	18.51±0.70
Crude ash	1.22±0.12	5.40±0.07
Carbohydrates	6.20±1.94	12.66±1.57

3.2 Fatty acids of olive leaves and pomace

Figures 2 and 3 show the fatty acids of both olive leaves and pomace. The leaves were found to have the maximum concentration of oleic acid (54.6%±3.4%), followed by palmitic acid (15.2%±1.2%). The leaves also contained small quantities of linoleic acid, arachidonic acid, and gadoleic acid. Among the fatty acids, oleic acid (59.3%±2.4%) was present in the highest quantities in olive pomace, whereas palmitic acid (6.3%±0.56%) and linoleic acid (5.2%±0.61%) were present in appreciable quantities. Some other fatty acids, i.e. stearic acid, arachidic acid, and gadoleic acid, were present in very small quantities in olive pomace.

3.3 Phytochemicals in olive leaves and pomace

The water-soluble extracts (WE) as well as ethanol-soluble extracts (EE) of olive pomace and leaves were investigated for their phytochemicals. Figure 4 shows the total phytochemicals present in both olive pomace and leaves. It was observed that significant variations ($p<0.05$) were present between the phenolic contents of

olive leaves and olive pomace. Moreover, a significant (p<0.05) impact of extraction solvent (water and ethanol) was also observed regarding phenolic contents. The EE of olive leaves had higher total phenolics (1902.01±189.00 mg GAE/100 g) than WE (1216.02±143.00 mg GAE/100 g). The WE of olive pomace had higher total phenolics (292.40±18.00 mg GAE/100 g) than EE (201.80±15.00 mg GAE/100 g). Higher contents (4915.03±433.00 mg QE/100 g) of total flavonoids were found in WE of olive leaves than their EE (4065.02±365.00 mg QE/100 g) in the present study. Similarly, higher contents (1306.03±35.00 mg QE/100 g) of TF were found in WE of olive pomace than their EE (577.04±21.00 mg QE/100 g).

3.4 FRAP of olive leaves and pomace

The FRAP of both olive leaves and pomace is presented in Figure 5. It was found that significant variations (p<0.05) were present between the FRAP of olive leaves and olive pomace. Moreover, a significant (p<0.05) effect of extraction solvent (water and ethanol) was also observed regarding FRAP. The FRAP of EE (36.42±2.27 μmol/L TE/g) of olive leaves was higher than their respective WE (31.07±2.13 μmol/L TE/g). Similarly, the ferric-reducing power of EE (9.35±1.19 μmol/L TE/g) of olive pomace was higher than its respective WE (2.88±0.67 μmol/L TE/g).

3.5 DPPH/ABTS radical scavenging activity of olive leaves and pomace

Figure 6 shows ABTS and DPPH scavenging capacity of investigated samples. It was found that significant (p<0.05) variations were present between DPPH/ABTS scavenging capacity of olive leaves and olive pomace. Moreover, a significant (p<0.05) impact of extraction solvent (water and ethanol) was also observed regarding DPPH and ABTS activity. The DPPH scavenging activity of EE (60.60±3.50 μmol/L TE/g) of olive leaves was higher than their respective WE (51.20±3.00 μmol/L TE/g). In the same way, ABTS scavenging activity of EE (9.88±0.90 μmol/L TE/g) of olive leaves was higher than their respective WE (3.24±0.40 μmol/L TE/g). The DPPH scavenging activity of EE (29.44±1.10 μmol/L TE/g) of olive pomace was higher than their respective WE (14.24±1.40 μmol/L TE/g). But, ABTS radical scavenging activity of EE (6.05±0.90 μmol/L TE/g) of olive pomace was more or less similar to its respective WE (5.99±0.45 μmol/L TE/g).

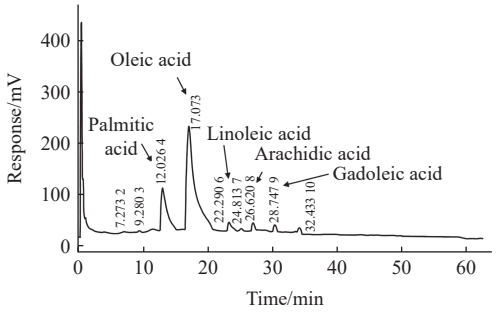


Figure 2 Fatty acid profile of olive leaves

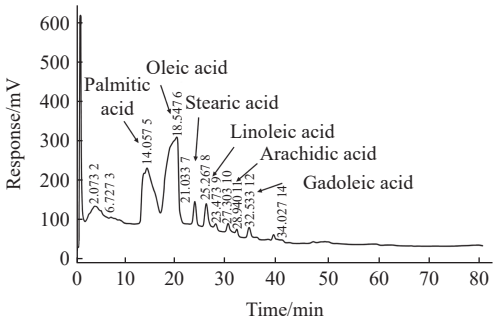
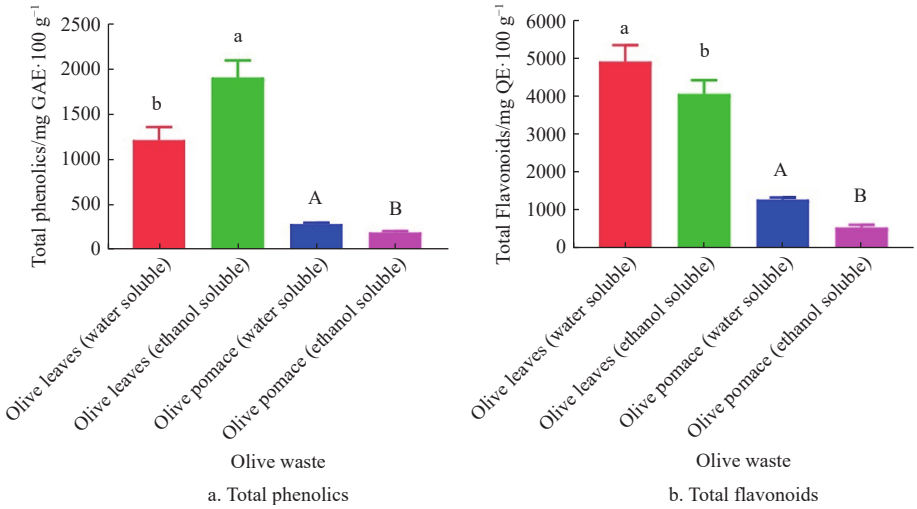


Figure 3 Fatty acid profile of olive pomace



Note: Lowercase letters (a-b) indicate statistical differences between water-soluble and ethanol-soluble extracts of olive leaves; same lowercase letters in each figure indicate no significant difference between paired means (p≥0.05). Uppercase letters (A-B) represent statistical differences between water-soluble and ethanol-soluble extracts of olive pomace; same uppercase letters in each figure indicate no significant difference between paired means (p≥0.05). The same as below.

Figure 4 Total phenolic contents (a) and flavonoid contents (b) (mg GAE/100 g) (Means±SD) of water-soluble and ethanol-soluble extracts in olive leaves and olive pomace

Figure 7 shows DPPH radical inhibition (%) and IC_{50} values of olive leaves and pomace concerning DPPH activity. It was found that significant variations (p<0.05) were present between DPPH radical inhibition (%) as well as IC_{50} values of olive leaves and olive pomace. Moreover, a significant (p<0.05) effect of extraction solvent (water and ethanol) was also observed regarding DPPH radical inhibition (%) as well as IC_{50} values. Even though ethanol-soluble extracts (76.20%±4.00%) of olive leaves showed slightly higher DPPH radical inhibition (%) than their respective water-soluble extracts (75.00%±3.00%), that variation was non-significant (p>0.05). However, the ethanol-soluble extracts (86.00%±5.00%) of olive pomace showed higher (p<0.05) DPPH radical inhibition (%) than their respective water-soluble extracts (65.00%±4.50%).

Regarding IC_{50} values, ethanol-soluble extracts (2.32±0.43 mg/mL) of olive leaves showed very slight variation (non-significant) with their respective water-soluble extracts (2.30±0.44 mg/mL). The ethanol-soluble extracts (2.18±0.48 mg/mL) of olive pomace showed lower (p<0.05) IC_{50} values than their respective water-soluble extracts (10.68±0.67 mg/mL).

Figure 8 shows ABTS radical inhibition (%) and IC_{50} values of ABTS scavenging activity of olive pomace and leaves. It was observed that significant variations (p<0.05) were present between ABTS radical inhibition (%) as well as IC_{50} values of olive leaves and olive pomace. Moreover, a significant (p<0.05) impact of extraction solvent (water and ethanol) was also observed regarding ABTS radical inhibition (%) as well as IC_{50} values. The ethanol-soluble extracts (88.00±4.50%) of olive leaves showed significantly higher ABTS radical inhibition (%) than their respective water-soluble extracts (71.80±5.50%). But the ethanol- and water-soluble extracts of olive pomace showed non-significant variation regarding ABTS radical inhibition (%). Concerning IC_{50} values, ethanol-soluble extracts (1.48±0.19 mg/mL) of olive leaves showed lower (p<0.05) values than their respective water-soluble extracts (2.82±0.23 mg/mL). Both ethanol- and water-soluble extracts of olive pomace showed non-significant variations concerning their IC_{50} values.

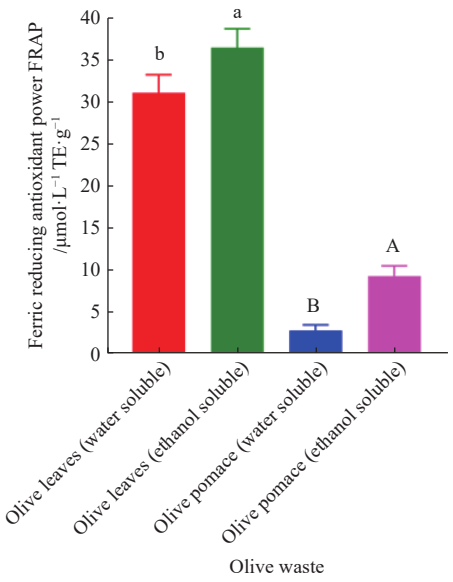


Figure 5 Ferric reducing antioxidant power (FRAP) (μmol/L TE/g) (Means±SD) of water-soluble and ethanol-soluble extracts from olive leaves and olive pomace

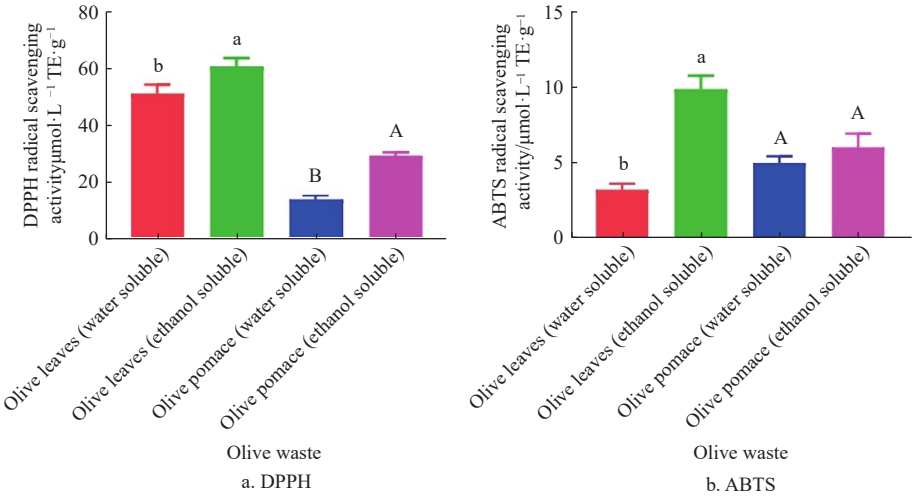


Figure 6 DPPH and ABTS radical scavenging activity (μmol/L TE/g) (Means±SD) of water-soluble and ethanol-soluble extracts of olive leaves and olive pomace

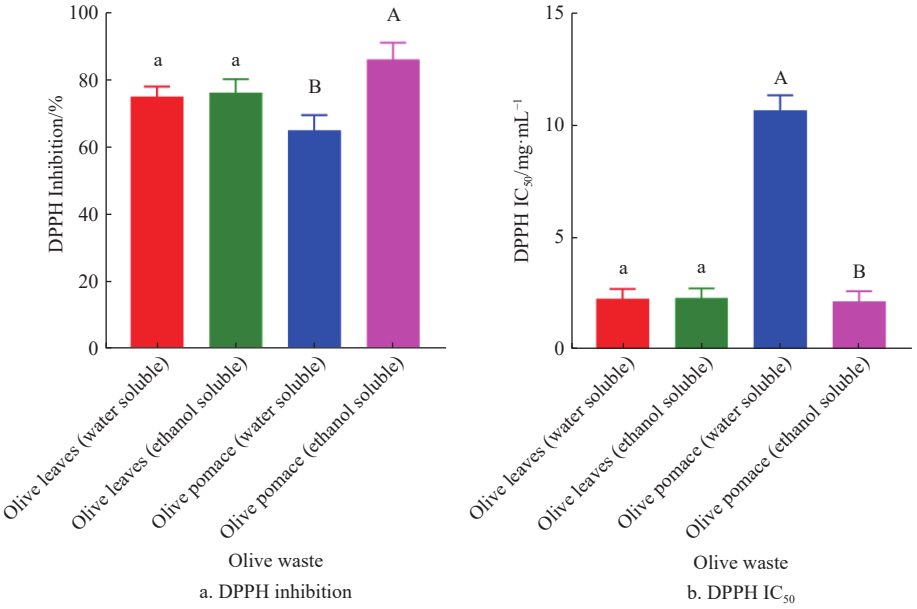


Figure 7 DPPH inhibition and DPPH IC_{50} of water-soluble and ethanol-soluble extracts of olive leaves and olive pomace

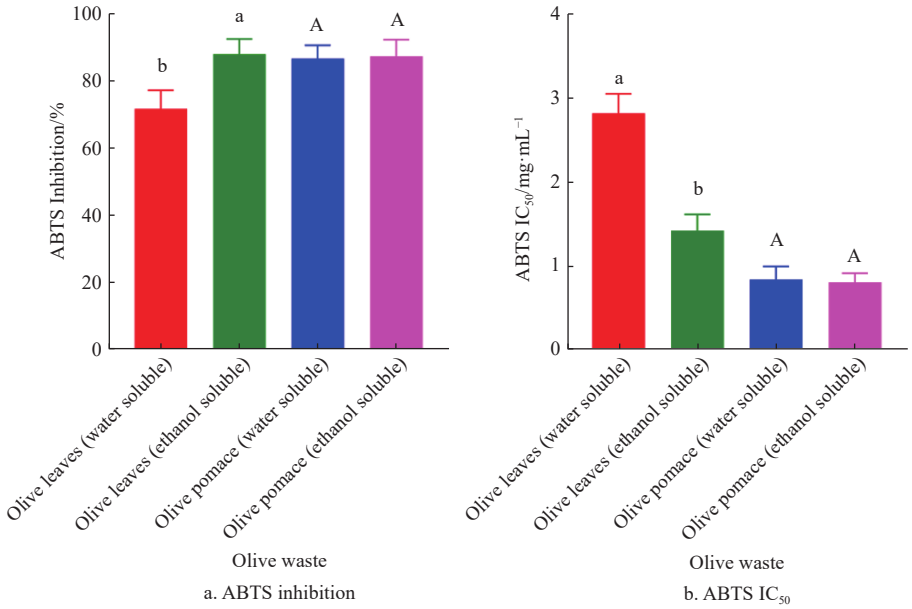


Figure 8 ABTS inhibition and ABTS IC_{50} of water-soluble and ethanol-soluble extracts of olive leaves and olive pomace

3.6 FTIR spectra of olive pomace and olive leaves

The FTIR spectra showed the main functional groups that are found in olive pomace (Figure 9a). These include esters, phenols, alcohols, lignin, cellulose, and hemi-cellulose structures. The broad peak of 3300 cm^{-1} displayed a stretched vibration of O–H, showing the presence of alcohols and phenols. The peak at 2830-2950 cm^{-1} is equivalent to the C-H stretching vibration, which indicates the presence of lignin structure. There is a peak at ~1650 cm^{-1} due to the presence of conjugate C=C stretching in polyphenols and unsaturated compounds. Furthermore, peaks near about ~1200 cm^{-1} may be due to the existence of C-O-C in a cellulose biopolymer chain. Some very small peaks in the range 1500-1200 cm^{-1} were also observed in the spectrum, which indicates the presence of lignin structures. The strong intensity peak at about 1050 cm^{-1} is possibly because cellulose and hemicellulose have C-O-H stretching vibrations, or because alcohol or C-O-R esters are present.

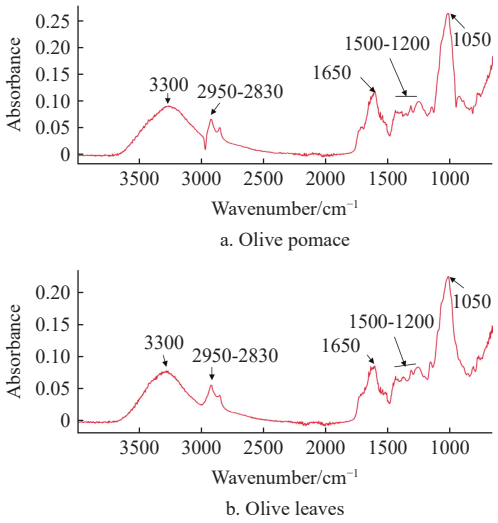


Figure 9 FTIR spectra of olive pomace (a) and leaves (b)

The FTIR spectra of olive leaves showed many functional groups such as phenols, alcohols, esters, lignin, etc. (Figure 9b). The broad peak at ~3300 cm^{-1} showed O-H stretching, such as phenols and alcohols. The peak near 2929 cm^{-1} indicates C-H stretching due to the presence of C–H indicating presence of lignin structure. Furthermore, the peak at 1650 cm^{-1} could be explained by the carboxyl's C–O stretching attached to the amide bond in amide I. Some very small peaks in the range 1500-1200 cm^{-1} were also observed in the spectrum, which indicates the presence of phenols and lignin structures. The strong intensity peak at about 1050 cm^{-1} is possibly due to C-O-H stretching vibrations owing to the presence of hemicellulose and cellulose or C-O-R esters or alcohol. The weak peak at 1190 cm^{-1} showed C–O stretching, as olive leaves contain esters, phenolic alcohols, etc.

4 Discussion

Hannachi et al.[22] found ash contents ranging from 6%-11% in the leaves from different olive trees, whereas Bahloul et al.[23] observed ash contents ranging from 6.60%-9.82% in the leaves from some other different olive varieties, which were more or less consistent with the findings of the current study. Ibrahim et al.[24] observed 14.5% crude fibers and 10.6% crude protein in olive leaves, which were slightly lower as compared to the observed values of this study. Another study reported ash contents and protein contents of olive leaves in the range of 4.37%-6.00% and 10.50%-13.10%, respectively[25], which were slightly higher than the results of the current study. They also reported that the proximate composition of olive leaves was also affected by different cultivars.

Nunes et al.[26] reported 61.1% moisture contents in olive pomace, which was less than the moisture contents observed in the present study. Wedyan et al.[27] reported that olive pomace from Jordan had a protein content of up to 4%, which was slightly lower than that observed in the present study. This study's results for crude fat content were lower than those reported by Ribeiro et al.[28], who found 15.61% crude fat (on dry weight basis) in crude olive pomace from Portugal. The variation was due to determination on dry basis. Cheng et al.[29] observed variations in the moisture contents and oil contents of different cultivars belonging to olive fruits from various origins, for example China, Italy, Spain, Greece, and Israel. Such variations might be expected in olive leaves as well as olive pomace on the basis of origin and cultivars.

Cavalheiro et al.[25] observed palmitate, stearate, and oleic acid from the leaves of different varieties of olive trees in appreciable quantities. The variations in fatty acids content might be due to the

cultivars from different geographical regions[27,30].

It has been reported that palmitate and oleic acid in olive pomace of mixed variety were present in considerable quantities[31]. Nunes et al.[32] also observed palmitate and oleic acid in olive pomace in abundant quantities. The palmitate content in olive pomace obtained in this study was consistent with the findings of Nasopoulou et al.[31] and Nunes et al.[32]; however, the oleic acid content was lower than those reported in their studies.

A study conducted in Brazil also confirmed that olive oil from the Arbequina cultivar was found to have palmitic acid, linoleic acid, arachidic acid, oleic acid, and gadoleic acid[33]. This study's results concerning the presence of oleic acid, palmitic acid, linoleic acid, arachidic acid, and gadoleic acid in the oils of olive leaves and olive pomace were also concurrent with the findings of aforementioned studies.

Total phenolics in the water-soluble extract of olive leaves from Egypt was investigated by Ibrahim et al.[24] and found TP ranging from 3680-3720 mg GAE/100 g dry basis, whereas another study reported TP as 38.14 mg/100 g of leaves[11]. Kiritsakis et al.[34] reported TP ranging from 2300-2400 mg/100 g of methanolic extracts of olive leaves from Greece. It has also been reported that olive leaves from various cultivars contained TP in the range 391.92-831.44 mg/100 g of olive leaves[22]. The TP contents reported in the aforementioned studies were higher than those found in the current study. Khelouf et al.[35] observed variations in the polyphenols in different extracts of olive leaves belonging to various cultivars. Similarly, variations in total phenolic contents of olive leaves from different geographical origins were found by Bilgin and Sahin[36]. Taamalli et al.[37] studied leaves from the Tunisian cultivar "Chemlali" from nine different regions and observed significant variations in the phenolic contents of samples from those regions. The variations in phenolic contents between this study's samples and other researchers might be explained by the aforementioned studies.

A study conducted in Italy showed that ethanol extracts of olive pomace contained total phenolics in the range of 57-98 GAE mg/100 g of pomace[7]. These findings were in accordance with the findings in the current study. Cioffi et al.[11] found phenolics in olive pomace ranged from 20.74 to 21.00 mg/100 g. Ribeiro et al.[28] reported a phenolic content of approximately 2000 mg GAE/100 g in olive pomace, which was considerably higher than the value obtained in the present study. Nunes et al.[32] also observed phenolics in olive pomace in the range from 3100-3800 mg GAE/100 g, which were higher than this study.

Nunes et al.[32] also observed flavonoids in olive pomace ranging from 1960-3170 mg CE/100g, which was higher than this study. Various concentrations of phenolic compounds in the leaves collected from various locations in the north of Tunisia were found by Zakraoui et al.[38]. Khelouf et al.[35] also observed variations in the flavonoids in different extracts of olive leaves belonging to various cultivars. The results of the present study also observed variations in the values concerning flavonoid contents of different extracts of olive leaves and pomace.

Habibi et al.[30] reported ferric-reducing power ranging from 4.71-7.52 μmol/L TE/g in olive leaves from various cultivars, which were concurrent with this study. The FRAP values depict the reducing power of any sample. Higher FRAP values manifest the presence of more potent antioxidants. These antioxidants include phenolics and flavonoids, which reduce $FeCl_3$ in the reaction mixture during the carrying out of the FRAP assay. Ethanol-soluble extracts of olive leaves showed higher quantities of phenolics, and hence depicted the highest reducing power. On the other hand, water-soluble extracts of olive leaves and pomace showed higher phenolics and flavonoids but lower reducing power than ethanol-soluble extracts. This might be due to the presence of more powerful phytochemicals in ethanol-soluble extracts showing more reducing power. Polarity of solvent as well as its composition play an important role in the extraction of phenolic and flavonoid compounds from olive leaves[39]. Phenolic compounds in olive leaves may be highly polar (e.g., hydroxytyrosol or simple phenolic acids) and moderately polar glycosides (oleuropein and flavonoid glucosides)[40]. In general, water alone is regarded as a poor solvent for extracting most phenolic compounds, despite its ability to swell plant tissues. Nevertheless, aqueous ethanol mixtures can improve extraction yields by disrupting phenolic–matrix interactions and optimizing solvent polarity[39]. It has also been reported that ethanol is considered as the most effective co-solvent in increasing the solubility of phenolic compounds[41].

Radical scavenging activity in olive leaves from various cultivars belonging to Tunisia were reported in the range 354-1362 μmol/L TE/g and 226.81-316.98 μM TE/g, respectively[22,30]. The DPPH scavenging activity of the abovementioned studies was higher than the values of this study. Habibi et al.[30] observed ABTS scavenging activity of olive leaves (from various cultivars belonging to Tunisia) ranging from 103.02-176.78 μmol/L TE/g. Such variations in ABTS and DPPH scavenging activities might be due to the leaves collected from various locations[38]. Similarly, variations in antioxidant potential of olive leaves in different cultivars may also be observed[42].

Nunes et al.[32] reported that the DPPH radical scavenging activity of olive pomace ranged from 0.60 to 0.96 g Trolox equivalent (TE)/100 g, which was consistent with the results obtained in the present study. They also reported that the ABTS radical scavenging activity of olive pomace ranged from 0.80 to 1.13 g Trolox equivalent (TE)/100 g (15.53–21.94 μmol/L TE/g). The findings of the aforementioned studies were generally consistent with the results obtained in the present study. Similarly, Kiritsakis et al.[34] recorded a DPPH radical inhibition rate of approximately 90% for methanolic extracts from Greek olive leaves. Bruno et al.[7] reported 70% DPPH inhibition (%) in ethanol extracts of olive pomace, which was in accordance with this study.

Lower IC_{50} values of extracts (water- and ethanol-soluble) of olive leaves and ethanol-soluble extracts of olive pomace showed the presence of potent antioxidants, while higher IC_{50} values of water-soluble extracts of olive pomace manifested the presence of a lesser amount of potent antioxidants.

Dorado et al.[43] reported the FTIR spectra of olive pomace and found various peaks at 3350 cm^{-1}, 2950 cm^{-1}, 1440-1380 cm^{-1}, and 1050 cm^{-1}, which were concurrent with the findings of the current study. They reported that olive pomace had esters, alcohols, phenols, cellulose, lignin, and hemi-cellulose structures. Agatonovic-Kustrin et al.[13] recorded the FTIR spectra of olive leaf extract and observed characteristic absorption peaks at approximately 3257 cm^{-1}, 2929 cm^{-1}, 1508-1260 cm^{-1}, and 1072-1018 cm^{-1}, which are consistent with the results obtained in this study. In addition, Nasir et al.[44] analyzed olive leaf extract and identified various peaks at approximately 3383 cm^{-1}, 2935 cm^{-1}, 1396-1284 cm^{-1}, and 1076 cm^{-1}. They reported that olive leaves contain multiple functional groups, i.e., alcohols, phenols, lignin, esters, etc.

The FTIR spectra of olive oils showed its purity[45,46]. The FTIR spectra of olive pomace showed the presence of esters, phenols, alcohols, lignin, cellulose, and hemi-cellulose structures. The FTIR

spectra of olive leaves showed many functional groups such as phenols, alcohols, esters, lignin, etc. The peaks obtained in the current study were also observed by other studies[13,43].

5 Conclusions

The olive leaves and pomace had the maximum concentration of oleic acid, i.e., 54.6% and 59.3%, respectively. The extracts of olive leaves resulted in higher FRAP and DPPH scavenging activity than olive pomace. The biomass of olive pomace may include esters, phenols, alcohols, lignin, cellulose, and hemi-cellulose structures, whereas the biomass of olive leaves may include functional groups such as phenols, alcohols, esters, lignin, etc. Thus, it may be concluded that olive leaves and olive pomace indigenous to Pakistan contain fatty acids and possess antioxidant activities. Olive pomace and leaves can be used on an industrial scale as well as at the farmer's level through their incorporation into feed ration for both ruminants and non-ruminants. Moreover, olive leaves may be used for the preparation of olive extracts which can be used for the preservation of olive fruits.

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