Journal of Medicinal Plants and By-Products (2024) 3: 527 – 538
https://doi.org/10.22034/jmpb.2024.364420.1646

Original Article

Phytochemical Composition and Metabolomic Variation in Olive Leaves (*Olea europaea*) Across Different Cultivated Varieties in Iran

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Article History

Received: 07 January 2024
Accepted: 28 February 2024

Keywords
Olive leaf
Antioxidant activity
Oleuropein
Fatty acids
Total phenol

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ABSTRACT

The utilization of olive tree leaves for medicinal purposes has been prevalent for centuries, and recent research has unveiled their significant properties, including antibacterial, antiviral, anticancer, and particularly antioxidant effects. This study aimed to investigate the content of phenolic acids and flavonoids, as well as the antioxidant activities, oleuropein concentration, and fatty acids profile in the leaves of 30 *Olea europaea* L. varieties sourced from various locations in the Fadak Agricultural Research Garden, Qom province, Iran. The leaves from these 30 Olive varieties were collected, dried, and ground. Subsequently, an ultrasound-assisted extraction was performed using an ethanol-water (70:30) solution. The total flavonoid content, total phenol content, oleuropein concentration, and fatty acid composition in the extract were determined using the aluminum chloride colorimetric method, Folin-Ciocolteau method, high-performance liquid chromatography (HPLC), and gas chromatography (GC-FID), respectively. The antioxidant activity was evaluated through DPPH radical scavenging activity. Various cultivars of olive trees were thus analyzed, providing valuable insights into their chemical composition and potential benefits for medicinal uses.

INTRODUCTION

The olive tree (*Olea europaea* L. subsp. europaea) is an extensively cultivated and highly significant agricultural crop in the Mediterranean region. It has a rich history and is grown in various countries such as Italy, Spain, Greece, France, Tunisia, Morocco, Turkey [1], and even in northern Iran [2]. The olive tree has garnered attention due to its medicinal and nutritional properties, which contribute to the maintenance and promotion of good health. These properties include the ability to lower blood pressure and blood sugar levels, as well as exhibiting hypouricemic, antimicrobial, and antioxidant activities [3]. As a result, olive leaf extract has been utilized for many centuries [4] as a means to harness these beneficial effects.

Plant species are known to produce various molecules that have the ability to scavenge free radicals, and among them are phenolic compounds, nitrogen compounds, terpenoids, and more. These compounds have the potential to act as antioxidants, protecting against oxidative stress [5]. Phenolic compounds, in particular, are highly versatile and exhibit a wide range of bioactive properties. These include antioxidant effects, anti-inflammatory properties, antimicrobial activity, inhibition of cell proliferation, anti-arrhythmic effects, prevention of platelet aggregation, and vasodilatory effects [6]. The diverse bioactive properties of phenolic compounds make them valuable in various fields, including medicine, nutrition, and natural product research.

Due to their antioxidant properties, there is an increasing interest in utilizing these phytochemicals in the food industry to enhance the nutritional value of commonly consumed foods. Olive leaves have been found to contain a variety of phenolic compounds, such as verbascoside, hydroxytyrosol, luteolin-7-glucoside, oleuropein, oleuropein aglycone, rutin, and ligstroside, as demonstrated by several studies [7,8].

Multiple studies have provided evidence for the beneficial effects of these components on human health, primarily due to their antioxidant properties

[9-12]. Oleuropein, which is the most abundant biophenol found in olive leaves [13-15], is particularly recognized for its strong antioxidant activity. Numerous studies have demonstrated that oleuropein and its derivatives possess a wide range of biological activities, including antiatherogenic, antihypertensive, anti-inflammatory, antifungal, hypoglycemic, antioxidant, antiproliferative, and hypocholesterolemic properties [16]. By inhibiting the oxidation of LDL, oleuropein and hydroxytyrosol have shown potential in the treatment of various diseases, such as osteoporosis, cancer, and coronary artery disease [17].

Indeed, due to the significant presence of phenolic compounds, olive leaves can be regarded as an affordable and easily accessible natural source for the aforementioned compounds. Numerous studies have explored the antioxidant activity of these compounds and have elucidated their mechanisms, including hypoglycemic, antihypertensive, antimicrobial, antiviral, and antiatherosclerotic effects [18].

The phenolic characteristics and fatty acid composition of olive leaves can be influenced by various factors, including the cultivar, agroecological conditions, geographical location, and particularly the seasons [19]. It is worth noting that there are approximately 2500 known olive cultivars, out of which 250 are recognized as commercial cultivars by the International Olive Oil Council [4]. It is possible that there are cultivars whose phenolic content has not yet been thoroughly investigated.

The objective of this study was to examine the phytochemical properties, total phenolic contents, total flavonoid contents, oleuropein content, and fatty acid profile in the leaves of 30 varieties of O. europaea. These varieties were collected from various origins within the olive Fadak Agricultural Research Garden, located in Qom province, Iran.

MATERIALS AND METHODS

Chemicals

Gallic acid and Folin standards were obtained from Sigma Aldrich, while all solvents used were of analytical reagent grade. Potassium hydroxide, sodium nitrite, sodium hydroxide, sodium carbonate, and aluminum chloride were purchased from Merck. The Folin Reagent was prepared by diluting 1 ml of the Folin standard with 10 ml of distilled water.

Plant Sample and Extraction Chemical

The leaves of 30 different varieties of O. europaea, including Zard, Derag, Dezfoul, Rowghani, Rashid, Dakal, Mari, Fishomi, Gorgan, and Kazerooni (Iran), Dorsalaee, Khaziri, Mahzam, Dan, and Hamed (Syria), Leccino, Coratina, Nochara, Pendolino, and Carolea (Italy), Conservolea, Koroneiki, Mastoidis, Kalamata, and Kardolia (Greece), Sevillana, Verdial de Jaén, Manzanilla, Manzanilla Cacerena, and Arbequina (Spain), were collected from various origins at the olive Fadak Agricultural Research Garden in Qom province, Iran, in October 2022.

The leaf samples were obtained from various sections of the trees and combined to create a single cultivar sample. Subsequently, the leaves were dried at room temperature and ground into a fine powder. In the static ultrasound-assisted extraction method, 10 mL of a solvent mixture consisting of 70% ethanol and 30% deionized water was added to 5 g of the milled olive leaves. The mixture was then subjected to ultrasound waves for a duration of 20 minutes using an ultrasonic water bath. This process was repeated three times with a 20-minute interval between each extraction. The resulting solutions were filtered, combined, and stored at 4 °C until further analysis.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) in the extract was determined using the aluminum chloride colorimetric method [20]. In this method, 25 µl of the sample, 100 µl of distilled water, and 7.5 µl of 5% sodium nitrite were mixed in a plate well. After 6 minutes, 7.5 µl of 10% aluminum chloride, 100 µl of 4% sodium hydroxide, and 10 µl of distilled water were added. The same procedure was repeated for the control well, using methanol instead of the sample. After 15 minutes, the absorbance was measured at 510 nm using a microplate plate reader. The total flavonoid content was calculated using a calibration curve and expressed as Rutin equivalent per gram (mg RE/g dried extract).

DPPH Radical Scavenging Activity

The antioxidant activity of the extract was assessed using the DPPH radical scavenging activity method

[21]. Various concentrations of each sample were added to a methanol solution containing DPPH. The absorbance was then measured at 517 nm (λmax). The percentage of radical scavenging activity of the extracts was determined using the following formula:

$$\text{DPPH scavenging effect} = \frac{\text{Control OD Absorbance} - \text{Sample ODAbsorbance}}{\text{Control ODAbsorbance}} \times 100$$

In the equation, Control OD represents the absorbance of the blank, and Sample OD represents the absorbance of the sample. The IC50 (Half maximal Inhibitory Concentration) values, which indicate the concentration of the extract capable of scavenging 50% of DPPH free radicals, were calculated by plotting the scavenging percentage against the extract concentration on a graph, as described in reference [22].

Determination of Total Phenolic Compounds in the Extracts

The measurement of the total phenol content was carried out using the spectrophotometric Folin-Ciocalteu method [23]. This method relies on the regeneration of Folin reagent by phenolic compounds in an alkaline environment, leading to the formation of a blue complex with absorbance at a wavelength of 760 nm. In brief, 25 µl of the sample (1000 ppm), 100 µl of sodium carbonate (7.5% w/v), and 125 µl of Folin reagent were combined. Subsequently, the mixture was covered and allowed to stand for 2 hours in the dark at room temperature, following which the absorbance was measured at 760 nm using a microplate plate reader. Gallic acid served as the standard for constructing the calibration curve, and the total phenol content was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g dried extract) [24].

HPLC Analysis of the Oleuropein

The quantification of oleuropein was conducted using an HPLC system (Agilent; Agilent Technologies, Germany) equipped with a C18 column (4.6mm i.d., 250 mm Length). The separation conditions for oleuropein were as follows: column temperature, 25 °C; injection volume, 10 µl; and flow rate, 1 ml/min. The separation was performed in gradient mode with the modified method using mobile phases of acetonitrile and water containing 0.1% phosphoric acid [25]. All mobile phases were filtered using a 0.2µm PTFE membrane filter. Chromatograms were recorded at 280 nm. The concentrations of oleuropein in the olive leaf extracts were determined using calibration curves prepared from standard solutions of oleuropein (ranging from 15 to 300 mg L^{-1}). All standard and sample solutions were analyzed in triplicate. The oleuropein content of the olive leaf extract is expressed as micrograms of oleuropein per gram of olive leaf extract.

Determination of Fatty Acids Composition

The derivatization of fatty acid methyl esters involved the utilization of a hexane extract of dried leaves and methanolic potassium hydroxide (2 M). The samples were subjected to a hot water bath at 60 °C for 1 hour. Subsequently, 0.5 ml of distilled water was added to the samples, followed by centrifugation. The upper layer was dissolved in hexane and subjected to analysis using GC-MS (Gas Chromatography-Mass Spectrometry) [26]. The GC-MS procedure was conducted using an Agilent HP-5MS 5% Phenyl Methyl Silox capillary column (30 m × 250 µm ID, 0.25 µm). Helium was employed as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program initiated at 100 °C for 1 minute, then ramped up to 205 °C at a rate of 12 °C/min and held for 2 minutes. Subsequently, the temperature was increased to 209.5 °C at a rate of 4 °C/min and maintained for 4 minutes. Finally, the oven temperature was adjusted to 310 °C at a rate of 7 °C/min for 5 minutes. The inlet temperature was set at 250 °C, while the ion source and quadrupole temperature were maintained at 230 °C and 150 °C, respectively. The results were reported as the percentage of the total fatty acids with three replicates.

RESULTS

Total Phenol Content

The evaluation of thirty samples of collected olive leaves to assess the total phenolic content, oleuropein content, total flavonoid contents, and antioxidant activity through proposed methods provides valuable insights into the chemical composition and defensive capabilities of these trees. The observed variations in the quantitative and qualitative characteristics of metabolites emphasize the impact of various factors such as cultivar, climate, leaf maturity, phenological stage, cultivation area, and plant health status on the

composition of secondary metabolites. Studying these aspects contributes to a deeper understanding of the adaptability and biochemistry of olive trees in response to their surroundings. The collection of olive leaves from 30 cultivars grown in similar pedoclimatic conditions provides a valuable platform to investigate the diversity in phenolic composition and antioxidant activity. The hypothesis that attributes the observed differences mainly to the genetic profile of the olive cultivars or the season of sample collection is intriguing, and it aligns with the known influence of genetic makeup and environmental factors on plant metabolite production. This study offers a pivotal opportunity to explore the underlying mechanisms driving the variations in phenolic composition and antioxidant activity, shedding light on the interplay between genetic factors and environmental cues in shaping the chemical profile of olive leaves.

Phenolic compounds derived from phenylalanine and tyrosine are present in a wide range of plant species. These compounds are of great importance in the food industry due to their ability to slow down the oxidative degradation of lipids, thereby improving the nutritional quality and value of food products. One of key properties of phenolic compounds is their redox activity, which enables them to act as antioxidants. This ability is facilitated by the presence of hydroxyl groups in the phenolic structure. As a result, the evaluation of total phenol content can serve as an indicator of antioxidant activity.

The diversity of phenolic compounds in plants offers a vast array of antioxidant options for the food industry. By incorporating plant-based ingredients rich in phenols, food manufacturers can enhance the shelf life and stability of their products, while also providing additional health benefits to consumers [27].

The total phenol contents in the leaves harvested from the olive's cultivars were measured using the Folin-Ciocalteu colorimetric method. The results were expressed as mg of gallic acid equivalent per g of extract (mg GAE/g) and were obtained in triplicate. The data is presented as mean ± SD. The total phenol contents in the leaves harvested from the olive cultivars were measured using the Folin-Ciocalteu colorimetric method and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g). The data were collected in triplicate and are presented as the mean ± standard deviation (SD). The results demonstrate a wide variation in total phenolic contents, ranging from 84.65 mg GAE/g extract for the Kardolia extract to 168.65 mg GAE/g extract for the Dezfoul extract. Among the cultivars, Dezfoul, Rowghani, and Derag exhibited the highest concentrations of total phenolic compounds, with values of 168.65 mg GAE/g, 158.87 mg GAE/g, and 156.25 mg GAE/g, respectively. The lowest phenolic compound levels were found in the cultivars Kardolia, Mahzam, and Kazerooni, with total phenolic contents of 85.75, 86.36, and 87.53 mg GAE/g, respectively.

The amount of total phenol in our study was much higher than the similar cultivar in previous studies. For example, in our study, the amount of total phenolic content in cultivars of Manzanilla, Conservolea, Arbequina, Coratina, Yellow, Mary, Leccino, Sevillenca and Fishomi were 93.11, 96.51, 128.07, 142.58, 146.33, 150.00, 90.36, 146.65, 142.87, respectively, while in another study [28], these values were 134.5, 92.35, 42.35, 155.91,73.85, 62.24, 59.23, 83.63 and 109.98, respectively [28]. However, it is worth mentioning that the phenol content for the Coratina and Conservolea cultivars was similar in both studies.

Total Flavonoid Content

Flavonoids, such as those found in olive leaves are a type of phenolic compound that have antioxidant activity. This is because they contain free OH groups [29,30]. Flavonoids are naturally occurring compounds in plants and have positive effects on human health. They are effective in scavenging oxidizing molecules, including free radicals and singlet oxygen, which are linked to various diseases. Therefore, consuming foods rich in flavonoids, like olive leaves, can have health benefits [31]. The study evaluated the flavonoid components of different cultivars of olive trees and their correlation with antioxidant activity. The total flavonoid contents in the leaves of all cultivars were measured using the aluminum chloride colorimetric method. The results, presented in Table 1, showed a wide variation in flavonoid content among the different cultivars. The Pendolino extract had a flavonoid content of 122.2 mg RE/g extract, while the Dezfoul extract had the highest flavonoid content at 408.5 mg RE/g extract. The cultivars Dezfoul, Derag, Mari, and Dorsalaee had the highest concentration of total flavonoid compounds among all the

cultivars mentioned. Their total flavonoid contents were 438.5, 405.3, 404.5, and 399.1 mg RE/g, respectively. On the other hand, the cultivars Pendolino, Nochara, and Carolea had the lowest flavonoid compounds, with total flavonoid contents of 122.2, 124.5, and 130.2 mg RE/g, respectively. Overall, all cultivars contained a high amount of flavonoids, but the highest concentrations were observed in Dezfoul, Derag, Mari, and Dorsalaee.

DPPH Assay

The results of the DPPH assay for different olive cultivar extracts are shown in Table 1. The values obtained ranged from 59.48 μg/ml for the Derag extract to 96.97 μg/ml for the Pendolino extract. These values indicate the ability of the extracts to scavenge DPPH radicals, with lower values indicating stronger antioxidant activity. The correlation between the phenolic contents and IC_{50} values in the DPPH radical scavenging activities was found to be high, suggesting that phenolic compounds play a significant role in the antioxidant activity of olive tree parts. Based on the given information, the cultivars Pendolino, Nochara, and Manzanilla Cacerena have the highest IC_{50} values, indicating a lower antioxidant activity. Their respective IC_{50} values are 96.97, 95.32, and 94.78 μg/ml. On the other hand, the cultivars Derag, Mari, and Dezfoul have the lowest IC50 values, indicating higher antioxidant activity. Their respective IC_{50} values are 39.48, 36.90, and 33.38 μg/ml. The results of this study are consistent with Ghasemi's previous research, which also found a relationship between radical scavenging activities and phenolic contents. In Ghasemi's study, the correlation coefficient between phenolic contents and the Ferric Reducing Antioxidant Power assay (FRAP) was low [28]. Similarly, in this current study, the DPPH radical scavenging activities of olive leaves across the 30 cultivars were found to be in line with their total phenolic contents. These findings are similar to other studies conducted on Italian varieties, as mentioned [27].

Oleuropein Content

Oleuropein is indeed one of the most abundant phenolic compounds found in olive leaves [32,33]. It is known for its antioxidant properties, which can help protect cells from damage caused by free radicals. Due to its medicinal properties, oleuropein is also available as a food supplement in Mediterranean countries. Oleuropein, a compound found in olive leaves, has been widely studied for its antioxidant properties [34]. Its ability to scavenge free radicals helps protect cells and tissues from oxidative damage [35-37]. The presence of hydroxyl groups in its chemical structure allows oleuropein to donate hydrogen, which helps prevent oxidation and lipid oxidation, improving lipid metabolism. This antioxidant activity makes oleuropein valuable in maintaining cellular health and overall well-being [38].

That's interesting! It appears that the HPLC analysis was conducted to determine the amount of oleuropein, a phenolic compound, in different extracts. The results, as shown in Table 1, and figure 1 indicate a wide range of variation in the concentration of oleuropein among the extracts. The Fishomi extract had the lowest concentration at 66 μg/g of dried extract, while the Mari extract had the highest concentration at 595 μg/g of dried extract. The Mari, Derag, and Dezfoul cultivars have the highest oleuropein content, with contents of 595, 588, and 566 μg/g of dried extract, respectively. On the other hand, Fishomi, Pendolino, and Nochara cultivars have the lowest oleuropein contents, with contents of 66, 126, and 131 μg/g of dried extract, respectively. This study highlights the diversity in oleuropein levels among different olive cultivars.

Fatty Acid Profile

The main fatty acids present were palmitic acid (C16:0) with a range of 39.1% to 73.14% and oleic acid (C18:1) with a range of 6.11% to 48.15%. The diversity and quantity of fatty acids in olive leaves among different varieties were found to be similar with slight variations.

Specifically, the difference in the amount of oleic acid among Iranian varieties was only 0.37%. These findings (Table 2 and figure 1) suggest that myristic acid (C14), palmitic acid (C16), and stearic acid (C18) are common components in all varieties of the studied species. Oleic acid (C18:1) is present in all species except the Spanish variety. Linoleic acid (C18:2) is found only in Spanish and Greek varieties. Additionally, linolenic acid (C18:3) is exclusively detected in Spanish varieties. Linoleic acid, which is an omega-6 fatty acid, has gained popularity in the cosmetic industry due to its various beneficial properties for the skin. Studies have shown that when applied topically, linoleic acid exhibits anti-inflammatory properties, helping to calm and reduce inflammation in the skin.

Table 1 Total phenol contents, Total Flavonoid Content, antioxidant activities and Oleuropein content of different cultivars of olive leaves extracts

No	Cultivar	TPC (mg GAE/g dried extract)	TFC (mg RE/g dried extract)	DPPH IC_{50} (µg/ml)	Oleuropein (µg/g of dried extract)
1	Zard	146.33 ± 0.025	307.2 ± 0.078	66.96 ± 0.061	457 ± 0.021
2	Derag	156.25 ± 0.046	405.3 ± 0.049	39.48 ± 0.031	588 ± 0.025
3	Dezfoul	168.65 ± 0.025	438.5 ± 0.050	36.90 ± 0.049	566 ± 0.018
4	Rowghani	158.87 ± 0.027	347.5 ± 0.052	49.24 ± 0.041	484 ± 0.029
5	Rashid	128.42 ± 0.017	245.5 ± 0.058	60.08 ± 0.053	363 ± 0.030
6	Dakal	107.09 ± 0.097	173.3 ± 0.068	60.60 ± 0.028	300 ± 0.054
7	Mari	150.00 ± 0.021	404.5 ± 0.076	33.38 ± 0.042	595 ± 0.046
8	Fishomi	142.87 ± 0.041	146.5 ± 0.068	94.78 ± 0.021	66 ± 0.036
9	Gorgan	109.91 ± 0.031	189.5 ± 0.073	64.02 ± 0.050	306 ± 0.028
10	Kazerooni	87.53 ± 0.048	157.5 ± 0.026	77.32 ± 0.025	251 ± 0.041
11	Dorsalaee	150.25 ± 0.021	399.1 ± 0.042	47.59 ± 0.052	504 ± 0.015
12	Khaziri	142.76 ± 0.030	329.5 ± 0.020	52.48 ± 0.030	468 ± 0.062
13	Mahzam	86.36 ± 0.032	128.5 ± 0.033	89.78 ± 0.046	193 ± 0.047
14	Dan	149.42 ± 0.041	282.5 ± 0.063	65.30 ± 0.037	464 ± 0.019
15	Hamed	101.49 ± 0.028	217.5 ± 0.013	65.75 ± 0.032	316 ± 0.038
16	Leccino	90.36 ± 0.036	132.6 ± 0.027	87.98 ± 0.037	165 ± 0.067
17	Coratina	142.58 ± 0.015	270.5 ± 0.030	69.07 ± 0.025	269 ± 0.039
18	Nochara	93.56 ± 0.017	124.5 ± 0.030	95.32 ± 0.041	131 ± 0.022
19	Pendolino	94.65 ± 0.021	122.2 ± 0.059	96.97 ± 0.058	126 ± 0.035
20	Carolea	91.89 ± 0.040	130.2 ± 0.009	89.94 ± 0.015	167 ± 0.038
21	Conservolea	96.51 ± 0.020	143.4 ± 0.021	86.14 ± 0.022	191 ± 0.044
22	Koroneiki	123.82 ± 0.015	246.5 ± 0.041	77.77 ± 0.031	270 ± 0.048
23	Mastoidis	128.55 ± 0.026	242.1 ± 0.041	71.33 ± 0.040	272 ± 0.033
24	Kalamata	145.67 ± 0.023	243.2 ± 0.019	70.95 ± 0.016	224 ± 0.058
25	kardolia	85.75 ± 0.035	167.3 ± 0.033	76.37 ± 0.035	197 ± 0.012
26	Sevillana	146.65 ± 0.018	281.3 ± 0.028	75.32 ± 0.024	236 ± 0.043
27	Verdial de Jaén	141.42 ± 0.012	335.1 ± 0.037	65.05 ± 0.072	266 ± 0.056
28	Manzanilla	93.11 ± 0.008	136.5 ± 0.054	78.02 ± 0.047	184 ± 0.064
29	Manzanilla Cacerena	91.84 ± 0.004	130.8 ± 0.033	94.78 ± 0.034	143 ± 0.017
30	Arbequina	128.07 ± 0.044	214.4 ± 0.019	64.51 ± 0.021	317 ± 0.039

Results are expressed as mean ± standard deviation of three replecates

It also has acne-reducing effects, as it can help regulate sebum production and prevent clogged pores.

Additionally, linoleic acid is known for its skin-lightening properties, making it useful in treating hyperpigmentation or dark spots. Moreover, it is a moisture retentive agent, helping to keep the skin hydrated and improving its barrier function [39]. cholesterol. However, it is important to note that individual responses to linolenic acid intake may vary, and consulting with a healthcare professional or nutritionist is recommended for personalized dietary advice [42].

Linoleic acid and linolenic acid are both essential fatty acids that cannot be synthesized by the human body and must be obtained from dietary sources. Sevillana and Manzanilla Cacerena varieties of olives are known to have particularly high levels of these fatty acids, even more so than hemp, walnut, canola, and soy. These fatty acids play important roles in various bodily functions, including hormone production, cell membrane formation, and inflammation regulation [43,44].

The Folin-Ciocalteu method is widely used to estimate the total phenolic content in a sample. However, it is important to note that different phenolic compounds can exhibit various responses in this assay due to variations in their chemical structures.

Linolenic acid, which is an omega-3 fatty acid, has been associated with reduced risk of cardiovascular disease and fatal coronary heart disease. Some studies have shown that consuming linolenic acid can improve lipid profiles by reducing triglyceride levels, total cholesterol, and low-density lipoprotein (LDL) cholesterol, commonly known as the "bad" cholesterol [40,41]. Additionally, it may also decrease high-density lipoprotein (HDL) cholesterol, often referred to as the "good"

Table 2 Fatty acids composition of different cultivars of olive leaves.

Cultivar	C14 (%)	C16 (%)	C18 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)
Zard	19.85 ± 0.14	70.71 ± 0.28	2.96 ± 0.12	6.48 ± 0.09	0	0
Derag	19.35 ± 0.22	71.45 ± 0.21	2.95 ± 0.23	6.25 ± 0.11	0	0
Dezfoul	20.75 ± 0.26	70.31 ± 0.35	2.83 ± 0.35	6.11 ± 0.14	0	0
Rowghani	19.45 ± 0.13	71.54 ± 0.12	2.88 ± 0.08	6.13 ± 0.16	0	0
Rashid	22.60 ± 0.25	68.35 ± 0.18	2.90 ± 0.22	6.15 ± 0.19	0	0
Dakal	20.83 ± 0.29	70.10 ± 0.16	2.90 ± 0.24	6.17 ± 0.18	0	0
Mari	18.42 ± 0.33	72.51 ± 0.39	2.74 ± 0.33	6.33 ± 0.22	0	0
Fishomi	18.95 ± 0.18	71.98 ± 0.26	2.77 ± 0.30	6.30 ± 0.07	0	0
Gorgan	18.22 ± 0.45	72.63 ± 0.24	3.01 ± 0.19	6.14 ± 0.02	0	0
Kazerooni	17.71 ± 0.35	73.14 ± 0.20	2.98 ± 0.25	6.17 ± 0.09	0	0
Dorsalaee	10.9 ± 0.24	71.3 ± 0.15	3.70 ± 0.28	14.10 ± 0.18	0	0
Khaziri	11.11 ± 0.21	70.97 ± 0.35	3.45 ± 0.31	14.47 ± 0.14	0	0
Mahzam	10.78 ± 0.19	71.31 ± 0.43	3.61 ± 0.36	14.30 ± 0.17	0	0
Dan	11.06 ± 0.28	71.49 ± 0.44	3.11 ± 0.37	14.34 ± 0.07	0	0
Hamed	11.18 ± 0.34	70.1 ± 0.32	4.56 ± 0.38	14.16 ± 0.09	0	0
Leccino	11.56 ± 0.49	68.84 ± 0.36	2.54 ± 0.32	17.06 ± 0.12	0	0
Coratina	11.81 ± 0.28	69.65 ± 0.23	2.47 ± 0.23	16.07 ± 0.13	0	0
Nochara	11.33 ± 0.42	68.08 ± 0.27	2.61 ± 0.11	17.98 ± 0.06	0	0
Pendolino	10.53 ± 0.26	63.81 ± 0.29	2.39 ± 0.32	23.27 ± 0.05	0	0
Carolea	10.71 ± 0.12	64.38 ± 0.31	2.32 ± 0.09	22.59 ± 0.17	0	0
Conservolea	4.08 ± 0.11	39.27 ± 0.33	2.53 ± 0.25	47.97 ± 0.14	6.15 ± 0.25	0
Koroneiki	4.09 ± 0.17	39.41 ± 0.16	2.60 ± 0.35	47.81 ± 0.12	6.09 ± 0.27	0
Mastoidis	4.06 ± 0.19	39.10 ± 0.32	2.46 ± 0.24	48.15 ± 0.05	6.23 ± 0.24	0
Kalamata	4.05 ± 0.22	40.34 ± 0.17	2.92 ± 0.31	46.32 ± 0.23	6.37 ± 0.22	0
Kardolia	4.07 ± 0.23	40.15 ± 0.22	1.92 ± 0.08	47.58 ± 0.16	6.28 ± 0.29	0
Sevillana	11.14 ± 0.29	47.83 ± 0.25	1.01 ± 0.37	0	12.16 ± 0.11	27.86 ± 0.06
Verdial de Jaén	18.45 ± 0.31	59.21 ± 0.29	2.08 ± 0.32	0	5.34 ± 0.28	14.92 ± 0.15
Manzanilla	19.06 ± 0.37	57.73 ± 0.34	2.13 ± 0.35	0	5.23 ± 0.22	15.85 ± 0.13
Manzanilla	11.87 ± 0.22	47.96 ± 0.31	1.06 ± 0.34	0	12.79 ± 0.14	26.32 ± 0.09
Arbequina	18.73 ± 0.19	58.04 ± 0.44	2.12 ± 0.28	0	5.28 ± 0.26	15.83 ± 0.17
SFA	45.62- 93.89	-	-	-	-	-
MUFA	6.11- 48.15	-	-	-	-	-
PUFA	6.09- 40.02	-	-	-	-	-
UFA	6.11- 54.38	-	-	-	-	-

SFA (Saturated fatty acids), MUSFA (Monounsaturated fatty acids), PUFA (Polyunsaturated fatty acids), UFA (Unsaturated fatty acids)

Similarly, the total flavonoid content can also be correlated with the antioxidant activity of cultivars. This suggests that the presence of higher levels of phenols and flavonoids in a sample might contribute to its antioxidant properties. The strong scavenging activity of phenolic compounds could be attributed to the techniques used during the experiment or the contribution of flavonoids which possess antioxidant properties [45,46]. Furthermore, it is worth noting that the different chemical structures of phenolic compounds can have a significant impact on their molecular antioxidant response [47].

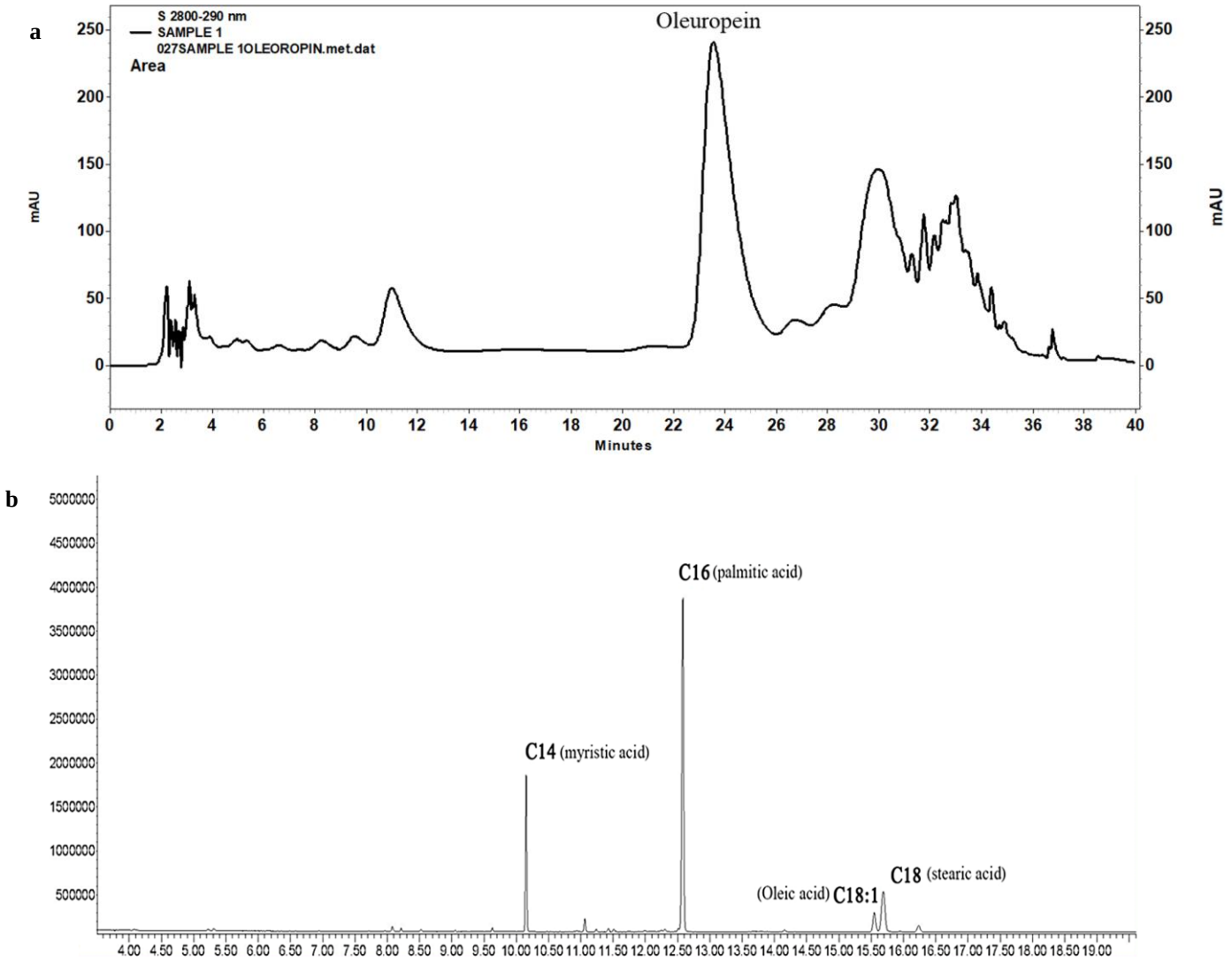

Fig. 1 a) HPLC-UV chromatogram of olive leaf extract recorded at 280 nm. b) GC-FID chromatogram of fatty acids obtained from olive leaves.

The presence of flavonoids in the extract has been found to be correlated with its antioxidant activity. Flavonoids are known for their potent antioxidant properties and they can contribute significantly to the overall antioxidant activity of the extract. However, it is important to consider other chemical components present in the extract as well, such as sugars or ascorbic acid, as they may also influence the antioxidant activity and need further study for a better understanding [48].

CONCLUSIONS

The analysis of bioactive components and antioxidant activity in 30 olive leaf cultivars from different origins but under the same growing conditions has highlighted significant variations. These variations were observed in total phenolic content, total flavonoid content, antioxidant activity, and oleuropein content among the different cultivars of olive leaves. This suggests that genetic factors or environmental conditions may influence the composition and properties of bioactive compounds in olive leaves. Further research could investigate the specific factors contributing to this variability and explore potential implications for health benefits or agricultural practices.

Among the cultivars studied, Mari, Derag, and Dezfoul showed the highest levels of oleuropein and flavonoids, known for their antioxidant properties. This suggests that these cultivars could be beneficial for human health due to their high antioxidant activity. Additionally, Spanish varieties were found to contain unsaturated fatty acids like linolenic acid and linoleic acid. Overall, this study highlights the potential of various olive leaf cultivars as a natural and cost-effective source of antioxidants with potential applications in promoting human health.

To obtain the maximum amount of useful compounds, further investigations are required on the cultivars evaluated in this study concerning the

specific season and climate conditions under which the samples were collected. These investigations would provide more insight into the influence of environmental factors on the production of the desired compounds.

ACKNOWLEDGMENTS

The authors would like to thank from Medicinal Plants and Drugs Research Institute, Shahid Beheshti University.

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