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Original Research

Comparative Analysis of Chemical Profiles and Antioxidant Activities Obtained from Irrigated and Rainfed Olive Trees (*Olea europaea* L.) in Southeastern Tunisia

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Abstract

Olive tree cultivation is a vital activity in central and southern Tunisia's semi-arid and arid regions due to their nutritional value and adaptability to water-stressed conditions. In this study, the phytochemical profile, including the content of α-tocopherol, minerals, polyphenols, flavonoids, and tannins, was identified from the leaves of two oleaster trees from the "Chemlali" variety in southern Tunisia. Two olive sites, rainfed and irrigated, were used for comparison. Antioxidant activities were assessed using the 2,2-diphenylpicrylhydrazyl (DPPH) assay. HPLC/MS analysis of ethyl acetate leaf extracts identified 15 organic compounds. The main phenolic compounds were quinic acid (569.11±1.49 mg/100 g DW and 560.95±0.80 mg/100 g DW in rainfed and irrigated leaves, respectively) and luteolin-7-O-glucoside (127.36±4.61 and 141.64±0.45 mg/100 g DW, respectively). The α-tocopherol, sugar, and mineral contents were also evaluated. DPPH scavenging capacity was slightly higher in rainfed leaves (90%) than in irrigated ones (88%). A significant decline in total phenolics and sugar content was observed in leaves from irrigated trees compared to those from the rainfed site. The sugar content ranged between 8.4 g/kg in the irrigated trees and 7.54 g/kg in the rainfed ones. The minerals K, Mg, Na, and Ca were detected in notably high quantities in the extracts of both sites. Overall, the effects of irrigation were very pronounced, mainly in terms of sugar content and total phenolic compounds. Olive

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tree leaves consistently serve as an outstanding source of high-value natural compounds with significant biological activities, even though irrigation directly influences leaf composition and quality.

Keywords: *Olea europaea*, phenolic compounds, HPLC/MS, mineral contents, antioxidant activity, irrigation

Introduction

The olive tree (*Olea europaea* L.) is one of the most important native species in the Mediterranean Basin's semi-arid and arid areas, owing to its nutritional value in addition to great adaptability to water-deficient conditions [1]. It is highly long-lived, often planted in nutrient-poor, rocky hillside soils where extended drought periods during the summer are common [2] because it has developed adaptive biochemical, physiological, and anatomical mechanisms to resist drought stress [3, 4]. Olive cultivation is one of the most economically important resources of the local population, and olive oil constitutes a strategic export product in Tunisia, a North African and Mediterranean country, ranked globally third as an olive oil producer and second as an exporter [4, 5]. The ancient and intangible heritage of the olive tree positions it not merely as an economic asset but as the centerpiece of a vast network of social, cultural, and environmental practices. These practices are inextricably linked to local identities as well as to a broader trans-Mediterranean cultural identity rooted in the culture of the olive tree [6]. The Mediterranean Basin is home to 97% of the world's olive orchards. In Tunisia, olive cultivation spans approximately 1.6 million hectares, which represents about one-third of the country's arable land [7].

The Tunisian climate is characterized by low and irregular rainfall, with periods of both drought and heavy rain. It also experiences high temperatures, intense evapotranspiration, and significant variability in meteorological events. These conditions can lead to severe drought [8, 9]. The water resources of this country are decreasing alarmingly due to the excessive exploitation of freshwater resources [10]. To ensure sustainability, agricultural management must adapt to these conditions [9]. Enhancing the economic and environmental sustainability of irrigated agriculture poses a significant challenge for the Mediterranean crop production sector [11].

The *O. europaea* species includes several varieties that show a wide diversity in their morphology and phenology. Tunisian olive orchards are distributed over all agricultural land from North to South and East to West, covering nearly 1.89 million hectares, and count about 105 million trees [12]. “Chemlali” in the South and Center, together with “Chetoui” in the North, represent 95% of the olive germplasm and more than 90% of the national olive oil production [13, 14]. The primary reason for cultivating *O. europaea* is the production of olive oil and table olives. However, the utilization of other byproducts, such as olive leaves, is becoming increasingly important due to their therapeutic value, biological properties, and organoleptic characteristics [15]. These leaves are recovered from olive industries, as they constitute 10% of the total weight of olives collected, and they accumulate during the pruning of olive trees [16]. They are a cheap raw material that can be used as a valuable source of high-added-value products such as natural phenolic antioxidants, secoiridoids, and flavonoids, which have protective effects against oil oxidation [17] and have a positive effect on human health by reducing oxidative stress and providing anti-inflammatory, antimicrobial activity, including antiviral, antifungal, and antibacterial effects against various pathogenic microorganisms [18, 19]. Thus, nowadays, many homeopathic remedies are sold as capsules containing the powder or the extract of dried olive leaves [20]. Given the growing interest in olive leaves, and their chemical composition allowing them several applications in the pharmaceutical and food industries, and knowing that irrigation can have a significant impact on vegetation, production, the development of fruits, and oil quality [21, 22], it is important to consider these factors.

This research aims to investigate the effect of irrigation on the biochemical and mineral composition of Chemlali olive leaves. It examines how irrigation influences the phenolic profile, including total polyphenol and flavonoid content, as well as specific compounds such as quercetin. The antioxidant activity of the leaves under different irrigation regimes is also evaluated, along with variations in mineral composition, sugar content, and α-tocopherol levels. By addressing these aspects, the study aims to enhance our understanding of how water management affects the quality of olive leaf by-products. This knowledge could support the development of more sustainable olive cultivation practices that reduce water consumption, promote the valorization of agricultural residues, and contribute to local economic development and environmental protection in the Euro-Mediterranean Region.

Materials and Methods

Sampling Site, Irrigation Water Characterization, and Plant Material Preparation

“Chemlali” olive leaves were collected in February 2017 from two sampling sites in the Tataouine governorate, southeastern Tunisia, an area characterized by an arid climate with both mountainous and flat

Table 1. Chemical characteristics of irrigation water.

Parameters	Value water irrigation	Irrigation water (Bedbabis et al., 2016)
pH	8.5	6.5-8.5
EC m*S*/cm	2.45	7
K^+ mg/L	30.92	50
Na^+ mg/L	176.22	300
Ca^{2+} mg/L	402.60	-
Mg^{2+} mg/L	41.72	-
Cl^- mg/L	497	600
SO_4^{2-} mg/L	992.57	1000
N mg/L	0.7	30
CO_3^{2-} mg/L	24	-

terrain. The first site, Ksar Ouled Dabbab (10°22'00" E, 32°51'01" N; 314 m a.s.l.), contains irrigated olive trees, while the second site, Douiret (10°17'36" E, 32°50'05" N; 437 m a.s.l.), includes rainfed trees. Irrigation is carried out continuously on a daily basis using a well-water drip irrigation system at a rate of 4 L/h to ensure good adipogenesis, particularly during flowering, fruiting, and fall. The olive trees are all of the same age and are planted densely: 20×20 m for non-irrigated trees and 10×10 m for irrigated ones.

The chemical characteristics of the irrigation water met the guidelines for fruit tree irrigation (Table 1). It had electrical conductivity, indicating a low level of salinity, and the concentrations of chloride (Cl^-), sulfate (SO_4^{2-}), and nitrogen (N) were below the threshold values shown in the guidelines for olive irrigation [23]. The leaves collected were stored at −20°C. Subsequently, oven-drying (80°C, 72 h) was carried out. The dried samples were then ground into powder.

Mineral Content

Mineral analysis was conducted using the method described by Al-Showiman [24]. One g of powdered plant material was ignited and incinerated in a muffle furnace at 530°C for 5 h. Then, 5 mL of hydrochloric acid (20%) was used to dissolve the obtained ash. The solution was transferred to a 50 mL volumetric flask. The final volume was adjusted with distilled water. A separate analysis for each mineral element was performed using an atomic absorption spectrophotometer (Shimadzu AA-6800, Kyoto, Japan).

Sugar Content

A sample of 6 g of leaves was refluxed with 100 mL ultrapure water–ethanol (80/20, v/v) for 2 h. Under reduced pressure, the remaining filtrate was evaporated to dryness. Then, each sample was diluted and adjusted with ultrapure water to 50 mL. The obtained solutions were subjected to centrifugation at 6000 rpm for 20 min and filtration over a 0.45 µm membrane [25]. HPLC analysis at room temperature was used to examine sugar composition. A Eurospher NH2 column was used (pore size: 100 Å, particle size: 7 µm, I.D.: 250×4.6 mm) (Knauer, Germany). Before use, the solvents were filtered through a 0.45 µm membrane filter and degassed in an ultrasonic bath (Cleaner Model SM 25E-MT, Branson Ultrasonics Corporation, USA) for 15 min. The mobile phase was a mixture of acetonitrile and ultrapure water (80/20, v/v). The liquid chromatograph was connected to a refractive index detector (K-2301, Knauer, Germany). The flow rate and the injection volume during the experiment were 1.0 and 2.0 mL min^{-1}, respectively.

α-Tocopherol Content

Samples were prepared as described by Baccouri et al. [26]. A quantity of 1 g of each plant material was weighed, mixed with 10 mL of methanol, and shaken vigorously. Then, solutions were manually injected into the HPLC (JASCO, Japan). The separation was carried out on a LiChrospher Si column (250×4.6 mm, particle size 5 µm) by a mobile phase composed of an isocratic mixture of hexane/isopropanol (99.5:0.5 v/v) at a flow rate of 1 mL/min. The fluorescence detector was set to an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The chromatographic peak was identified using an α-tocopherol standard (Sigma-Aldrich Co., St. Louis, MO, USA) and quantified using a calibration curve.

Phenolic Compounds Analysis

Sample Preparation

Phenolic compounds were extracted using three different solvents (90% v/v) at room temperature: chloroform, ethyl acetate, and methanol. A 2 g quantity of dry leaves was ground and dissolved in 20 mL of each solvent. The mixture was stirred and then placed in a 60°C water bath for 20 min with constant stirring; the obtained extract was centrifuged at 25°C for 20 min. The extracts were stored at 4°C and protected from light for further analysis.

Total Polyphenol Content

The concentration of total phenolic compounds was determined using Folin–Ciocalteu reagent, as adopted by Önder et al. [27]. 100 µL of the methanolic extract was assayed with 750 µL of the Folin–Ciocalteu reagent. After 5 min, 750 µL of 6% (w/v) sodium carbonate was added. After incubation for 90 min at room temperature in the dark, the absorbance was determined at 765 nm wavelength. Three measurements were performed on

each sample. The results were expressed as mg gallic acid equivalents per g dry weight (mg GAE g^{-1} DW).

Total Flavonoid Content

The total flavonoid content was measured by the colorimetric assay described by Antunes et al. [28]. 1 mL of appropriately diluted samples or standard were supplemented with 1 mL of a fresh aluminium chloride solution ($AlCl_3$, 2%). After 10 min of incubation at room temperature, the absorbance of the mixture was determined at 430 nm wavelength and compared to the control tube. The total flavonoid content of the different extracts was expressed as mg catechin equivalents per g dry weight (mg CE g^{-1} DW).

Condensed Tannin Content

In the reaction tube, 2 mL of freshly prepared vanillin solution in methanol (1%, v/v) was added to 2 mL sulfuric acid solution in methanol (25%, v/v). A volume of 0.5 mL of suitably diluted samples (polyphenol extract corresponding to 1 g of dry plant material) was added to each tube. The mixture was incubated in the dark, and after exactly 15 min, the absorbance was measured at 500 nm wavelength in a spectrophotometer (Single Beam LI-295, IndiaMart) compared to the control tube [29]. The condensed tannin content of the different extracts was expressed as mg catechin equivalents per g dry weight (mg CE g^{-1} DW).

HPLC Analysis of Phenolic Compounds

Phenolic compounds were extracted and analyzed according to the IOC [30]. An aliquot of oil was mixed with 5 mL of the methanol–water (80/20, v/v) extraction solution. The mixture was homogenized by shaking on an agitator for 1 min and subsequently extracted in an ultrasonic bath for 15 min at room temperature. The suspension was centrifuged at a speed of 5000 rpm for 25 min, and then an aliquot of the supernatant was filtered through a 0.45 mm PVDF syringe filter into a vial and injected into the LC–MS/MS system. The extracts were analyzed using a Sciex Applied Biosystems API 4000 Q-Trap mass spectrometric system. The instrument was operated in negative ion mode using multiple reaction monitoring (MRM) tandem mass spectrometric acquisitions. The ion spray voltage was 4500 V, the curtain gas was 20 psi, the temperature was 400°C, and the ion source gas pressures were 35 and 45 psi. Individual collision gas thickness medium, entrance potential, declustering potential, entrance collision energy, and exit collision energy were optimized for each MRM transition. The separation was performed using an Eclipse XDB-C8-A HPLC column (5 mm particle size, 50 mm length, and 4.6 mm i.d.) (Agilent Technologies, Canada) with a mobile phase flow rate of 350 mL/min and an injection volume of 10 mL. The mobile phases consisted of 0.1% aqueous formic acid and methanol, with a gradient increasing from 10% to 100% in 20 min. Compounds were identified by comparing their retention time with those of the standards and by monitoring (MRM) transitions. Reference standards were bought from Extrasynthese (Genay, France) and Sigma-Aldrich/Riedel-de Haën (Sofia, Bulgaria). Methanol and formic acid were LC/MS grade (VWR International, USA). All aqueous solutions were prepared using ultrapure water, with a resistivity of 18.2 MΩ cm, and produced from a Milli-Q Plus system (Millipore, USA).

DPPH Radical Scavenging Activity

The leaf antioxidant activities of olive trees were measured in terms of hydrogen-donating or radical scavenging ability using the DPPH method [31]. The samples were diluted in methanol at different concentrations (5, 10, 20, and 50 µg/mL). 1 mL of each diluted extract was added to 250 µL of a DPPH methanolic solution (0.2 nmol). After stirring the mixture, the solution was placed in the dark for 30 min at room temperature. Then, the absorbance of the mixture was determined at 517 nm wavelength and compared to the control (DPPH methanolic solution). The DPPH scavenging effect of the samples was calculated according to the following Equation:

$$\text{DPPH radical scavenged (\%)} = [(A0-A1)/A0] \times 100 \quad (1)$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the tested extract sample.

Statistical Analyses

Statistical analyses were performed using ANOVA with SPSS 26 (IBM Corp., 2019**)**. All experimental assays were carried out in triplicate. Duncan's test was used to compare significant differences with the significance level at $p<0.05$. The mean values and standard deviations were calculated for all analyzed compounds and antioxidant activities, and the data were expressed as mean±SD.

Results and Discussion

The quality and quantity of leaves produced by the olive tree are influenced by several factors, such as water stress. In this section, we aimed to evaluate the effect of irrigation on the quality of olive leaves. We identified the phytochemical profile, including α-tocopherol content, minerals, polyphenols, flavonoids, and tannins, from the leaves of two olive trees of the "Chemlali" variety in southern Tunisia. We compared leaves from two sites: rainfed and irrigated.

Table 2. Mineral composition of olive leaves (mg/g of dry weight).

Site	Na	Fe	Mg	Mn	K	Zn	Ca	Cu
Rainfed	0.856±0.01^{a}	0.371±1.01^{a}	1.974±0.01^{b}	0.025±0.01^{b}	3.679±1.01^{b}	0.008±0.01^{a}	18.021±0.02^{a}	0.0026±0.01^{a}
Irrigated	0.27±0.02^{b}	0.20±0.98^{b}	2.393±0.00^{a}	0.037±0.01^{a}	5.904±1.01^{a}	0.007±0.02^{a}	16.08±0.02^{b}	0.0028±0.03^{a}

Note: The data are presented as mean values ± standard deviation (n=3); a, b, c, superscript letters indicate homogenous sub-classes.

Mineral Content

Eight mineral elements were determined in the leaves of rainfed and irrigated olive trees; calcium (Ca) was the predominant mineral, followed by potassium (K), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) (Table 2). Oliveira [32] confirmed that the major mineral components present in the leaves of different olive cultivars are Ca and K, in addition to phosphorus, Mg, and silicon. Ca, Na, and Fe were detected in significantly higher amounts in the leaves from the rainfed site than in those from the irrigated one. For Mg and K, the concentrations in the leaves of the rainfed site were lower than those of the irrigated trees. The mineral amounts found in this study show that the leaves of olive trees constitute a good source of mineral elements. They are comparable with those presented by Hannachi [33] for K (5.85 mg/g) in "Chemlali" olive leaves. Similarly, for the other minerals, the concentrations detected are consistent with those found by Magdich [34] for Ca (18.31 mg/g) and Mg (1.54 mg/g) and Mechri [35] for Fe (0.101 mg/g) and Mn (0.033 mg/g) in leaves of irrigated olive trees. The use of irrigation influences mineral concentrations. Generally, as mentioned in several studies, irrigation decreases most of the mineral elements in olive leaves [36]. This difference is mainly due to the salinity of the irrigation water and the nature of the chemical composition of the considerable mineral concentrations, such as Na, K, Ca, and Mg. Therefore, olive leaves have great potential to provide variable secondary metabolites and minerals that can enhance the healing process of diseases [37]. These findings provide a quantitative evaluation of the phytochemicals and mineral elements, providing essential insights into their pharmacological and toxicological actions.

Soluble Sugar Composition

Soluble sugars are essential for the normal functioning of plant organs. The main soluble sugars that accumulate are glucose, fructose, and sucrose. They protect the process of enzymatic synthesis, leading to better plant drought tolerance. Changes in sugar content can also help identify the resistance mechanisms of different plant types. This trait can be a potential selection criterion for drought-tolerant cultivars [38]. Analysis of soluble sugars in the investigated cultivars revealed that only fructose and glucose were present (Fig. 1). Glucose is more abundant than fructose in the analyzed samples. Glucose values ranged from 7.616 g/kg in irrigated sites and 6.224 g/kg in rainfed sites, while fructose levels ranged from 0.784 g/kg to 1.32 g/kg. These results exceed those reported by Boussadia et al. [39], where the glucose content varied between 1.36 g/kg and 2.42 g/kg for the Meski and Koroneiki varieties, respectively, and a fructose content of 0.03 g/kg for both varieties. However, they are significantly lower than

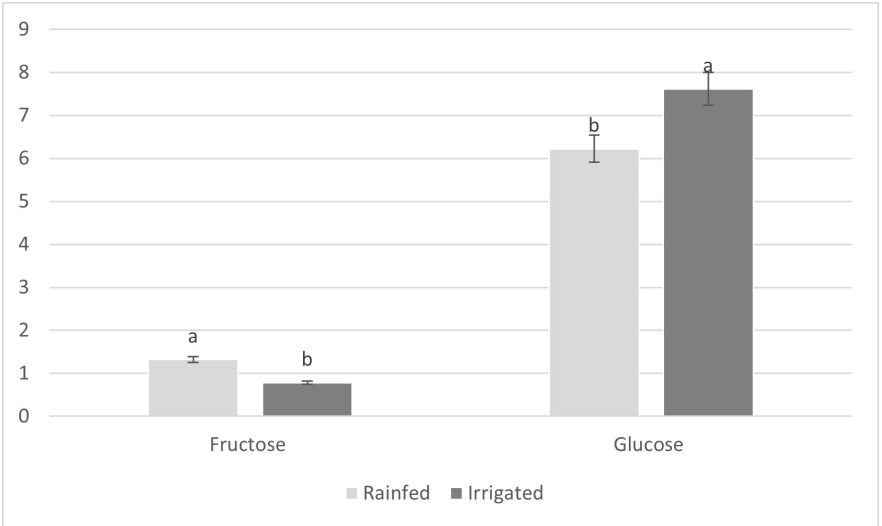

Fig. 1. Sugar composition of "Chemlali" olive leaves (g/kg). (a, b) Superscript letters indicate homogeneous subclasses among organs according to ANOVA ($p<0.05$) and Duncan's new multiple range test.

Table 3. α-tocopherol content of olive leaves on a dry weight basis expressed as µg/g dry weight.

	Extract	Rainfed	Irrigated
α- tocopherol	Chloroform	33.60 ±0.006$^{C\,a}$	31.62±0.002$^{C\,b}$
	Ethyl acetate	132.24±0.005$^{A\,b}$	148.85±0.001^{Aa}
	Methanol	106.72±0.001$^{B\,b}$	147.30±0.001$^{B\,a}$

Note: The data are presented as mean values± standard deviation (n=3); superscript lowercase (a, b, c, d) and capital letters (A, B, C) indicate homogeneous sub-classes as a result of ANOVA ($p<0.05$). Duncan's new multiple range test) among irrigation regimes and extract solvents, respectively.

those noted by Bustan et al. [40], who reported glucose levels of 20.8 g/kg and fructose levels of 7.3 g/kg for the Barnea variety fertilized with NPK. On the one hand, Oueslati et al. [29] also recorded glucose and fructose contents of approximately 46.73 g/kg dry matter (DM) and 32.65 g/kg DM, respectively, while working on the "Chemlali" olive tree irrigated with poultry wastewater (PWW) in Mahdia. On the other hand, our results were comparable to those obtained by Mechri et al. [35] for a local variety in Ouled Jaballah (olive mill wastewater (OMW)) in Northern Tunisia, where glucose values ranged from 2.55 g/kg to 3.9 g/kg, and fructose values ranged from 0.7 g/kg to 1 g/kg.

α-Tocopherol Content

Tocopherols are fat-soluble monophenolic antioxidants, which catalyze vitamin E activity in the diet [41]. This activity is associated with the production of prostaglandins and the inhibition of platelet aggregation [42]. Table 3 shows the α-tocopherol content of various olive leaf extracts.

Results indicated that leaf α-tocopherol content was significantly influenced by both the extraction solvent and the type of irrigation ($p<0.05$). Ethyl acetate extracts showed the highest α-tocopherol content: 132.24 and 148.85 µg/g from the rainfed and irrigated sites, respectively. Chloroform extracts showed the lowest α-tocopherol content (33.60 and 31.62 µg/g). In a previous study on the same olive trees, oil samples presented α-tocopherol content of 415.237 µg/g and 335.513 µg/g in rainfed and irrigated olive trees, respectively [43]. These values are closer to the value announced by Botsoglou et al. [44] (284.6 µg/g) regarding α-tocopherol leaf content than those found in the present study. Previous research has confirmed that the extracted amounts of α-tocopherols from olive leaves depend on the solvent used for extraction. Indeed, Tarchoune et al. [45] showed that hexane extraction yielded a high concentration of α-tocopherol: 82.37 µg/g from the Neb Jmel cultivar and 10.12 µg/g from the Oueslati cultivar. Olive leaves are considered an alternative source of α-tocopherol [46, 47]. The obtained olive oils with the addition of less than 10% leaves were classified as Extra Virgin Olive Oil (EVOO). Polyphenols and bitterness index were the highest for treatments with the addition of less than 10% leaves [48].

Table 4. Phytochemical composition of olive leaves on a dry weight basis expressed as mg/g dry weight.

	Extract	Rainfed	Irrigated
Flavonoids	Chloroform	23.24±1.02^{aB}	14.85±1.95^{Bb}
	Ethylacetate	157.65±2.05^{aA}	144.95±0.05^{aA}
	Methanol	155.23±1.01 ^{aA}	135.01±1.01^{Aa}
Tannins	Chloroform	78.01±4.8 ^{aA}	50.001±2.805^{Ba}
	Ethyl acetate	77.20±10.32^{aA}	60±8.46^{aA}
	Methanol	45.20± 5.6^{aB}	25.46±4.38^{Bb}
Polyphenols	Chloroform	180.49±2,01^{aB}	153.28±0.98^{Ab}
	Ethylacetate	241.34±0,01^{aA}	233.69±0.01^{aA}
	Methanol	155.64±0,02^{aB}	135.62±0.01^{Ab}

Note: The data are presented as mean values± standard deviation (n=3); superscript lowercase (a, b, c, d) and capital letters (A, B, C) indicate homogeneous sub-classes as a result of ANOVA ($p<0.05$). Duncan's new multiple range test) among irrigation regimes and extract solvents, respectively.

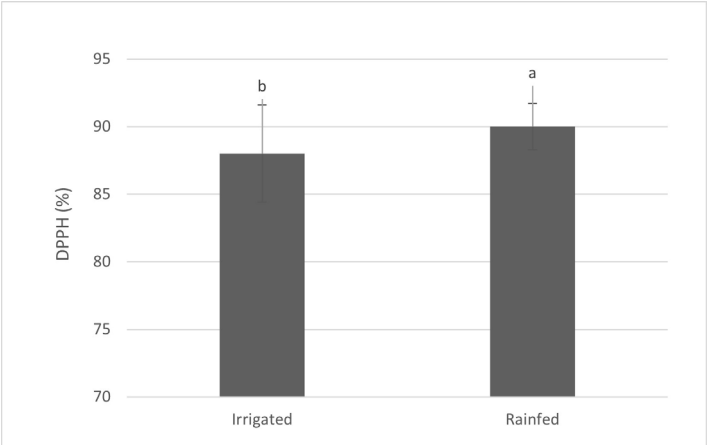

Fig. 2. DPPH% free radical scavenging activity of two sites using methanol extraction. (a, b) indicate homogeneous subclasses among the sites according to ANOVA ($p<0.05$) and Student's new multiple range test.

Total Polyphenols, Flavonoids, and Condensed Tannins

Total polyphenol, flavonoid, and condensed tannin contents of olive leaf extracts from irrigated and rainfed sites were tested, and results were presented in Table 4.

Results showed that the extraction solvent significantly affects the total polyphenols ($p<0.05$). Ethyl acetate extracts showed the highest polyphenol content (241.34 and 233.69 mg GAE/100 g of olive leaves from rainfed and irrigated sites, respectively). Methanol extracts showed the lowest polyphenol content (155.64

Table 5. Phenolic acids and flavonoids from olive leaf extract identified by HPLC (mg/100g).

	Rainfed			Irrigated	
		RT (min)	C (mg/100g)	RT (min)	C (mg/100g)
Flavonoids					
Epicatechin		16.143	0.454±0.02^{a}	16.129	0.375±0.021^{b}
Rutin		24.109	86.933±4.81^{a}	24.107	76.362±0.80^{a}
Luteolin-7-O-glucoside		24.798	127.363±4.61^{b}	24.802	141.643±0.45^{a}
Quercetrin (quercetin-O-rhamnoside)		27.163	119.676±4.08^{a}	27.125	134.531±0.76^{a}
Apigenin-7-O-glucoside		27.093	6.990±0.16^{a}	27.085	7.570±0.34^{a}
Naringin		26.191	9.669±0.41^{a}	26.197	7.631±0.33^{b}
Kaempferol		32.140	18.883±0.13^{b}	32.125	22.636±0.17^{a}
Quercetin		31.158	1.978± 0.02^{b}	32.154	2.776±0.34^{a}
Naringenin		34.039	0.404±0.11^{a}	34.045	0.494±0.005^{a}
Apigenin		34.709	1.080±0.078^{b}	34.702	1.282±0.03^{a}
Phenolic Acids					
Quinic acid		2.152	569.107±1.49^{a}	2.148	560.945±0.80^{a}
p-Coumaric acid		21.033	14.212±.0520^{b}	21.032	22.450±3.75^{a}
Caffeic acid		14.530	1.985±0.061^{b}	14.564	4.831±0.09^{a}
4-O-caffeoylquinic acid		11.775	0.472±0.011^{b}	11.759	1.317±0.04^{a}
Trans-ferulic acid		23.289	0.872±0.002^{b}	23.267	3.472±0.32^{a}

Note: The data are presented as mean values (n=3). Superscript lowercase letters (a, b, c) indicate homogeneous subclasses according to ANOVA ($p<0.05$) and Duncan's new multiple range test among irrigation schemes and extract solvents, respectively. CC: concentration; RT: retention time.

and 135.62 mg GAE/100 g of olive leaves from rainfed and irrigated sites, respectively). These findings were in perfect agreement with the literature. Thus, the studies by Jayaprakasha et al. [49] showed that the highest total polyphenol content was obtained using ethyl acetate as the extraction solvent, while the minimum content was found using methanol. Several authors reported that the content of total polyphenols in the olive tree depends on the extraction solvent used [50, 51]. The total polyphenol content of the leaves varies between 135 and 241 mg/g DM. These concentrations are higher than those obtained by Ben Salah et al. [52] in the leaves of eight Tunisian olive varieties (between 99.7 and 144.19 mg/g DM). However, these concentrations seem close to those obtained in the leaves of an Egyptian variety (222.1 mg EAG/g) [53].

Moreover, Table 4 shows that the flavonoid content depended, as well, on the solvent used. The highest content was obtained in ethyl acetate and methanol leaf extracts. Thus, the values of the flavonoid found for these two solvents oscillate between 135 and 157 mg CE/g DM. The flavonoid amounts found in this study are superior to those found in several studies. The concentration of flavonoid content in olive leaves is between 56.75 and 125.64 mg/g DM, as mentioned by Ben Salah et al. [52] 1.8 mg/g DW and 0.39 mg/g DW, respectively, for the methanol and chloroform solvents of the cultivar Neb Jmel [54], and 21.47 and 15.83 mg/g DM using extraction with methanol-water and ethanol-water, respectively, for the "Chetoui" cultivar [55]. The variation in phenolic compounds among extracts is mainly due to their different chemical properties, which widely affect their solubilities in solvents with different polarities [56].

Similarly, the highest quantity of tannins was obtained in leaf ethyl acetate extracts (60 mg/g and 77.2 mg/g). These levels are higher than those reported by Afify et al. (2017), whose tannin content in the water extract of olive leaves was 21.57 mg TAE/g DW, and those mentioned by Guebebia et al. [57] in the leaves of the "Chemlali" variety (19 mg CE/100 g DM). These observations indicate that the total polyphenol, flavonoid, and tannin contents in olive leaves are higher under rainfed conditions compared to irrigated ones.

DPPH Radical Scavenging Activity

The existence of phenolic compounds (flavonoids, tannins, phenolic acids) in olive leaves means that this plant may be able to act as an antioxidant agent. Herein, the free radical scavenging activity of the DPPH radical in the methanolic extract of olive leaves (at a concentration of 50 μg/mL) was evaluated. Results are presented in Fig. 2. Similar to total phenolic compounds, the rainfed leaf extract showed the highest inhibition activity of the DPPH radical, with 90%, followed by irrigated extracts with 88%. Leaf extracts showed lower activity than those described in the "Chetoui" olive leaves using the methanol/water extract, in which the DPPH scavenging activity reached 93.7%, and higher than those found using ethanol/water as an extract (59.74%) [55]. The study by Saidana et al. [58] found that the DPPH of "Chemlali" olive leaves ranged from 59.83% to 89.33% under semi-arid conditions. In comparison, the "Chetoui" variety showed DPPH values ranging from 54% to 91.40% under similar conditions. This variation underscores the differing antioxidant capacities of these olive varieties when exposed to the same semi-arid environment. The antioxidant activity is enhanced when using methanol as an extraction solvent [59]. Therefore, methanolic extracts of olive leaves possess interesting free radical scavenging properties to varying degrees, and this tree can be used as an essential source of antioxidant molecules.

HPLC Analysis of Phenolic Compounds

Fifteen phenolic compounds from olive leaves were characterized and quantified by HPLC. The major compounds identified in the ethyl acetate extracts are given in Table 5. The phenolic profile included 10 flavonoids and 5 phenolic acids.

The analysis also showed that the identified flavonoids and their quantities depend on the irrigation system. Quercetrin (quercetin-O-rhamnose) and luteolin-7-O-glucoside were the most abundant flavonoids in olive leaves. A significant difference ($p<0.05$) in quercetrin content was observed between olive leaves from rainfed trees, which had 119.676 mg/100 g, and those from irrigated trees, which had 134.531 mg/100 g. Quercetin derivatives, commonly found in plants, have diverse biological activities against many diseases, such as cancer, cardiovascular diseases, and neurodegenerative diseases [60]. Luteolin-7-O-glucoside is well represented in leaves, with 127 and 141 mg/100 g of dried extract (Table 5). In the same context, Pereira et al. [61] detected and quantified luteolin-7-O-glucoside as highly represented (420.8 mg/g). Kaempferol is often acylated with caffeic acid, producing potent antioxidant activity [62]. The kaempferol amount varies between 18.88 and 22.63 mg/g in the rainfed and irrigated sites, respectively. Therefore, the results are consistent with those of Cittan et al. [62]. In this Turkish sample, the content is about 9.48 mg/100 g. The phenylpropanoid biosynthetic pathway and the levels of functional components, such as quercetin and kaempferol, were actively involved in olive plant defense against salinity stress [63]. Other flavonoids, such as flavonols (epicatechin), flavanones (naringenin), and flavone glycosides (apigenin-7-O-glucoside), were also identified. Previously, we identified similar compounds in whole olive leaves [64, 65].

In addition, flavonoid compounds have been detected in the ethyl acetate extract. Their substantial interest has been demonstrated because of their industrial potential and pharmacological value, such as antioxidant, antidiabetic, antiulcer, anticancer, antibacterial, and antifungal activities. Several studies have reported the

extensive use of these flavonoids in the pharmaceutical industry for their beneficial health effects.

The order of phenolic acids based on the most abundant component in this study is as follows: quinic acid > p-coumaric acid > trans-ferulic acid > caffeic acid > 4-O-caffeoyl acid (Table 5). Thus, quinic acid was characterized as the main phenolic compound in olive leaves at 560 mg/100 g dried extract. These concentrations are nearly 4-fold higher than those reported by Sabella et al. [66], who found a 136 mg/100 g quinic acid concentration in olive leaves from a resistant Italian cultivar. As for p-coumaric acid detected in this study, the concentration was more noticeable in the irrigated trees (22.45 mg/100 g) compared with the rainfed trees (14.21 mg/100 g). Abdel-Aziz et al. [67] also confirmed this component's presence in olive leaf extract. However, for caffeic acid, they showed that its amount in the olive leaves was lower than that detected in the present study. Accumulation of phenolic compounds has been suggested to be a known adaptation mechanism in olive trees to water scarcity [36, 68]. Olive leaves, rich in phenolic compounds, are recognized for their health benefits, including antioxidant, antibacterial, and antifungal properties, making them valuable for both human health and the food industry [69]. However, the olive oil production process generates significant biowastes, such as olive mill wastewater (OMW) and solid olive husk (SOH) [70]. These effluents can pose environmental risks due to their high phytotoxicity [71], raising concerns about the sustainability of intensive olive cultivation, especially as climate change increases irrigation demands. Many factors can qualitatively and quantitatively modify the composition of the olive leaves in terms of phenolic compounds [72-74]. Climate change is expected to increase the demand for irrigation, leading to greater degradation of land and water resources in affected areas. This degradation includes over-extraction of groundwater, soil salinization, and erosion, primarily driven by the development of intensive orchards that use poor-quality water [9]. Additionally, field trials have shown a reduction in phenol content with higher amounts of applied water [9]. The amount and composition of polyphenols in olive leaves can vary due to several factors, including sampling time, water deficiency, salinity, geographical zone, and light exposure [75]. These are well-documented abiotic factors that affect phenolic composition. However, there are limited data on the impact of mineral fertilization on the phenolic composition of olive leaves [76]. Phytochemicals in olive leaf extracts offer various health benefits, making them valuable for nutritional and pharmaceutical applications [31]. Phenolic acids, in particular, are utilized as adjuvants in food and cosmetic technologies and therapeutic treatments. According to Oteros et al. [77], these acids are gaining prominence in research due to their anti-inflammatory, antimicrobial, antioxidant, and hypoglycemic properties. Notwithstanding these significant findings, the present study has some limitations that may affect the generalizability of its conclusions. It focused on a single olive cultivar ("Chemlali"), grown in a specific arid region of southern Tunisia, which may limit the applicability of the results to other cultivars. Previous research has demonstrated considerable variability in antioxidant capacities and phenolic compound accumulation among olive cultivars and geographical origin [78, 79]. Moreover, the sampling was conducted at a single time point (February), although it is well established that the phytochemical composition of olive leaves fluctuates throughout the growing season in response to developmental stages and environmental conditions [80, 81]. In addition, the analysis of irrigation effects was limited to two contrasting water regimes and did not account for intermediate irrigation levels, variations in water quality, or the seasonal dynamics of water use efficiency, factors that are known to influence plant physiology and secondary metabolism [82, 83].

Conclusions

The medicinal properties of the olive tree are attributed to its leaves, which are now the subject of many scientific studies. This study assessed olive leaves grown in rainfed and irrigated sites as a potential source of natural antioxidants. Total phenolic, flavonoid, condensed tannin, DPPH antioxidant capacity, sugar, mineral, and α-tocopherol contents were deeply investigated. The olive leaves exhibited high polyphenol and flavonoid contents correlated with antioxidant capacity. The phenolic profile differed between the two sites and revealed an abundance of flavonoids, mainly luteolin-7-O-glucoside. These findings provide scientific evidence of the benefits of traditional medicine and indicate promising potential for isolating natural antioxidant agents from olive leaves. Future studies should include multiple cultivars, varied irrigation strategies, and temporal monitoring to better capture the complexity of olive tree responses to water availability under changing climatic conditions.

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Conflict of interest

The authors declare no conflict of interest.

References

1. FERNÁNDEZ J.E. Understanding olive adaptation to abiotic stresses as a tool to increase crop performance. Environmental and Experimental Botany. **103**, 158, **2014**.
2. ZEEV W. Desert olive oil cultivation: advanced bio technologies. Academic Press. **3**, 1, **2009**.
3. ARTEMIOS M BOSABALIDIS G.K. Comparative effects of drought stress on leaf anatomy of two olive cultivars Plant Science. **163** (2), 375, **2002**.
4. DICHIO B., XILOYANNIS C., NUZZO V., MONTANARO G., PALESE A.M. Postharvest regulated deficit irrigation of peach tree in a Mediterranean environment: effects on vegetative growth and yield. International Society for Horticultural Science (ISHS), Leuven, Belgium, **2004**.
5. ONAGRI Observatoire National de l'Agriculture Tunisie. Situation des barrages. International Journal Water Sciences and Environment Technologies. **viii** (2), 28, **2023**.
6. MARIA K. Olive heritage and tourism in Messenia: a path to sustainable development. Sustainable Development, Culture, Traditions Journal. **4**, 16, **2024**.
7. HANNACHI H., MARZOUK S. Flowering in the wild olive (*Olea europaea L.*) tree (oleaster): Phenology, flower abnormalities and fruit set traits for breeding the olive. African Journal of Biotechnology. **11** (32), 8142, **2012**.
8. RAJHI H., BARDI A., AJMI L., HAZOUG M., DES TUREAUX T.H., IZHARUL HAQ I., BOUSNINA H., ABICHOU M., PODWOJEWSKI P. Effect of multi sourced compost on a rainfed olive tree grove in an arid land: Management practices to adapt to global climate change. Journal of Arid Environments. **218**, 105058, **2023**.
9. TRABELSI L., NCUBE B., BEN HASSENA A., ZOUAIRI M., BEN AMAR F., GARGOURI K. Comparative study of productive performance of two olive oil cultivars Chemlali Sfax and Koroneiki under arid conditions. South African Journal of Botany. **154**, 356, **2023**.
10. GAALOUL N., ESLAMIAN S., KATLANCE R. Impacts of climate change and water resources management in the southern mediterranean countries. Water Productivity Journal. **1** (1), 51, **2021**.
11. VANELLA D., CONSOLI S., CONTINELLA A., CHINNICI G., MILANI M., CIRELLI G.L., D'AMICO M., MAESANO G., GENTILE A., LA SPADA P., SCOLLO F., MODICA G., SIRACUSA L., LONGO-MINNOLO G., BARBAGALLO S. Environmental and Agro-Economic Sustainability of Olive Orchards Irrigated with Reclaimed Water under Deficit Irrigation. Sustainability. **15** (20), 15101, **2023**.
12. FENDRI M.J.F., OUESLATI H., MAHMOUD L.B, KHLIFI A., LAABIDI O., BOUDHRIOUA N. Évaluation de l'infrastructure de transformation dans les differents terroirs du nord de la tunisie pour la production d'huiles d'olive labelisées. Revue Ezzaïtouna. **16**, **2020** [in French].
13. DEBBABI O.S., RAHMANI M.S., BEN AMAR F., BEN NACEUR M., MONTEMURRO C., MIAZZI M.M. Applications of Microsatellite Markers for the Characterization of Olive Genetic Resources of Tunisia. Genes. **12**, 286, **2021**.
14. BEN ALI S., BOUSSORA F., BEN MOHAMED M., TRIKI T., BENNANI L., HASHEM A., AVILA-QUEZADA G.D., ABD ALLAH E.F., GRATI KAMMOUN N., GUASMI F. Fatty acid profiles and differential genes expression in Tunisian olive cultivars: a study of fruit ripening and quality attributes. Euro-Mediterranean Journal for Environmental Integration. 1, **2024**.
15. SELIM S., ALBQMI M., AL-SANEA M.M., ALNUSAIRE T.S., ALMUHAYAWI M.S., ABDELGAWAD H., AL JAOUNI S.K., ELKELISH A., HUSSEIN S., WARRAD M., EL-SAADONY M.T. Valorizing the usage of olive leaves, bioactive compounds, biological activities, and food applications: A comprehensive review. Frontiers in Nutrition. **9**, 2022, **2022**.
16. TABREZ S., PRIYADARSHINI M., UROOJ M., SHAKIL S., ASHRAF G.M., KHAN M.S., KAMAL M.A., ALAM Q., JABIR N.R., ABUZENADAH A.M., CHAUDHARY A.G., DAMANHOURI G.A. Cancer chemoprevention by polyphenols and their potential application as nanomedicine. Environmental Science and Health, Part C: Toxicology and Carcinogenesis. **31** (1), 67, **2013**.
17. CASTEJÓN M.L., MONTOYA T., ALARCÓN-DE-LA-LASTRA C., SÁNCHEZ-HIDALGO M. Potential Protective Role Exerted by Secoiridoids from *Olea europaea* L. in Cancer, Cardiovascular, Neurodegenerative, Aging-Related, and Immunoinflammatory Diseases. Antioxidants (Basel). **9** (2), **2020**.
18. SÁNCHEZ-GUTIÉRREZ M., BASCÓN-VILLEGAS I., ESPINOSA E., CARRASCO E., PÉREZ-RODRÍGUEZ F., RODRÍGUEZ A. Cellulose Nanofibers from Olive Tree Pruning as Food Packaging Additive of a Biodegradable Film. Foods. **10** (7), 1584, **2021**.
19. TOPUZ S.B.M. Oleuropein extraction from leaves of three olive varieties *(Olea europaea* L.): Antioxidant and antimicrobial properties of purified oleuropein and oleuropein extracts. Journal of Food Processing and Preservation. **46** (6), 15697, **2022**.
20. BAHLOUL N., BOUDHRIOUA N., KOUHILA M., KECHAOU N. Effect of convective solar drying on colour, total phenols and radical scavenging activity of olive leaves (*Olea europaea* L.). International Journal of Food Science and Technology. **44** (12), 2561, **2009**.
21. BACCOURI O., GUERFEL M., BACCOURI B., CERRETANI L., BENDINI A., LERCKER G., ZARROUK M., MILED D.D.B. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. Food Chemistry. **109** (4), 743, **2008**.
22. EL RIACHY M., BOU-MITRI C., YOUSSEF A., ANDARY R., SKAFF W. Chemical and Sensorial Characteristics of Olive Oil Produced from the Lebanese Olive Variety 'Baladi'. Sustainability. **10** (12), 4630, **2018**.
23. CHARTZOULAKIS K.S. Salinity and olive: Growth, salt tolerance, photosynthesis and yield. Agricultural Water Management. **78** (1-2), 108, **2005**.
24. AL-SHOWIMAN S. Chemical composition of date palm seeds (*Phoenix dactylifera* L.) in Saudi Arabia. Journal of the Chemical Society. **12**, 15, **1990**.
25. BOOIJ I., PIOMBO G., RISTERUCCI A.-M., COUPÉ M., THOMAS D., FERRY M. Etude de la composition chimique de dattes à différents stades de maturité pour la caractérisation variétale de divers cultivars de palmier dattier (*Phoenix dactylifera* L.). Fruits. **47** (6), 667, **1992**.
26. BACCOURI B., RAJHI I., THERESA S., NAJJAR Y., MOHAMED S.N., WILLENBERG I. The potential of wild olive leaves (*Olea europaea* L. subsp. oleaster) addition as a functional additive in olive oil production: the effects on bioactive and nutraceutical compounds using LC-ESI-QTOF/MS. European Food Research and Technology. **248**

(11), 2809, **2022**.
27. ÖNDER D E.Ü., ÖNDER S. Comparison of biochemical and antioxidant activities of ultrasonic-assisted extraction with different solvents in olive leaf. Biotech Studies. **32** (1), 31, **2023**.
28. ANTUNES M.D., GAGO C., GUERREIRO A., SOUSA A.R., JULIÃO M., MIGUEL M.G., FALEIRO M.L., PANAGOPOULOS T. Nutritional Characterization and Storage Ability of *Salicornia ramosissima* and *Sarcocornia perennis* for Fresh Vegetable Salads. Horticulturae. **7** (1), 6, **2021**.
29. OUESLATI A., MONTEVECCHI G., ANTONELLI A., MANSOUR H.B. Short-time irrigation on young olive tree *(Olea europaea* L. cv. Chemlali) with untreated industrial poultry wastewater: investigation of growth parameters and leaves chemical composition. Environmental Science and Pollution Research. **28** (36), 50420, **2021**.
30. IOC Trade Standard Applying To Olive Oils and Olive-Pomace Oils. **2019**.
31. MANSOUR H.M.M., ZEITOUN A.A., ABD-RABOU H.S., EL ENSHASY H.A., DAILIN D.J., ZEITOUN M.A.A., EL-SOHAIMY S.A. Antioxidant and Anti-Diabetic Properties of Olive (*Olea europaea*) Leaf Extracts: In Vitro and In Vivo Evaluation. Antioxidants (Basel). **12** (6), **2023**.
32. DE OLIVEIRA A.P.S., AGRA-NETO A.C., PONTUAL E.V., DE ALBUQUERQUE LIMA T., CRUZ K.C.V., DE MELO K.R., DE OLIVEIRA A.S., COELHO L.C.B.B., FERREIRA M.R.A., SOARES L.A.L. Evaluation of the insecticidal activity of *Moringa oleifera* seed extract and lectin (WSMoL) against *Sitophilus zeamais*. Journal of Stored Products Research. **87**, 101615, **2020**.
33. HANNACHI H., WALID E., MAJDA L., IMEN E., FETHI M.R., AND NAGAZ K. Chemical Profiles and Antioxidant Activities of Leaf, Pulp, and Stone of Cultivated and Wild Olive Trees (*Olea Europaea* L.). International Journal of Fruit Science. **20** (3), 350, **2020**.
34. SALWA MAGDICH W.A., MAKKI B., BÉCHIR BEN R., EMNA A. Effects of long-term olive mill wastewater spreading on the physiological and biochemical responses of adult Chemlali olive trees (*Olea europaea* L.). Ecological Engineering. **97**, 122, **2016**.
35. MECHRI B., BOUSSADIAO., ATTIA F., BEN MARIEM F., BRAHAM M., HAMMAMI M. Effects of agronomic application of olive mill wastewater in a field of olive trees on carbohydrate profiles, chlorophyll a fluorescence and mineral nutrient content. Environmental and Experimental Botany. **71** (2), 184, **2011**.
36. CETINKAYA H., KULAK M. Monitoring of mineral and polyphenol content in olive leaves under drought conditions: Application chemometric techniques. Industrial Crops and Products. **88** (0926-6690), 78, **2016**.
37. MARKHALI F.S., TEIXEIRA J.A., ROCHA C.M.R. Olive Tree Leaves—A Source of Valuable Active Compounds. Processes. **8** (9), 1177, **2020**.
38. LABANI M.M., REZAEE R., SAEEDI A., AL HINAI A. Evaluation of pore size spectrum of gas shale reservoirs using low pressure nitrogen adsorption, gas expansion and mercury porosimetry: A case study from the Perth and Canning Basins, Western Australia. Journal of Petroleum Science and Engineering. **112**, 7, **2013**.
39. BOUSSADIA O., STEPPE K., ZGALLAI H., BEN EL HADJ S., BRAHAM M., LEMEUR R. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski' and 'Koroneiki'. Scientia Horticulturae. **123** (3), 336, **2010**.
40. BUSTAN A., AVNI A., LAVEE S., ZIPORI I., YESELSON Y., SCHAFFER A.A., RIOV J., DAG A. Role of carbohydrate reserves in yield production of intensively cultivated oil olive (*Olea europaea* L.) trees. Tree Physiol. **31** (5), 519, **2011**.
41. DEBBABI M., NURY T., ZARROUK A., MEKAHLI N., BEZINE M., SGHAIER R., GRÉGOIRE S., MARTINE L., DURAND P., CAMUS E., VEJUX A., JABRANE A., BRETILLON L., PROST M., MOREAU T., AMMOU S.B., HAMMAMI M., LIZARD G. Protective Effects of α-Tocopherol, γ-Tocopherol and Oleic Acid, Three Compounds of Olive Oils, and No Effect of Trolox, on 7-Ketocholesterol-Induced Mitochondrial and Peroxisomal Dysfunction in Microglial BV-2 Cells. International Journal of Molecular Sciences. **17** (12), 1973, **2016**.
42. NIELSEN N.S., PEDERSEN A., SANDSTRÖM B., MARCKMANN P., HØY C.E. Different effects of diets rich in olive oil, rapeseed oil and sunflower-seed oil on postprandial lipid and lipoprotein concentrations and on lipoprotein oxidation susceptibility. British Journal of Nutrition. **87** (5), 489, **2002**.
43. FAGHIM J., BAGUES M., GUASMI F., TRIKI T., NAGAZ K. Irrigation effects on phenolic profile and extra virgin olive oil quality of "Chemlali" variety grown in South Tunisia. South African Journal of Botany. **141** (0254-6299), 322, **2021**.
44. BOTSOGLOU E., GOVARIS A., AMBROSIADIS I., FLETOURIS D. Lipid and protein oxidation of α-linolenic acid-enriched pork during refrigerated storage as influenced by diet supplementation with olive leaves (*Olea europaea* L.) or α-tocopheryl acetate. Meat Science. **92** (4), 525, **2012**.
45. TARCHOUNE I., SGHERRI C., EDDOUZI J., ZINNAI A., QUARTACCI M.F., ZARROUK M. Olive Leaf Addition Increases Olive Oil Nutraceutical Properties. Molecules. **24** (3), 545, **2019**.
46. DE LUCAS A., DE LA OSSA E.M., RINCÓN J., BLANCO M.A., GRACIA I. Supercritical fluid extraction of tocopherol concentrates from olive tree leaves. The Journal of Supercritical Fluids. **22** (3), 221, **2002**.
47. PAIVA-MARTINS F., BARBOSA S., PINHEIRO V., MOURÃO J.L., OUTOR-MONTEIRO D. The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. Meat Science. **82** (4), 438, **2009**.
48. MEZGHANI M.A.T.M., MGUIDICH A., ZOUARI I., AYADI M., ELLOUMI O., MECHRI B. How different amounts of leaves added during the extraction process affect the biochemical composition of Chemlali olive oil cultivar? Journal of Food Measurement and Characterization. **17** (1), 751, **2023**.
49. JAYAPRAKASHA G.K., GIRENNAVAR B., PATIL B.S. Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different in vitro model systems. Bioresource Technology. **99** (10), 4484, **2008**.
50. EL MANSOURI F., LAVILLO P.L., EL FARISSI H., OUFDOU H., BRIGUI J. Extraction, analysis of polyphenols and antioxidant properties of morrocan barley seed extracts (*Hordeum vulgare* L.). Materials Today: Proceedings. **43** (2), 1896, **2021**.
51. TAPIA-QUIRÓS P., MIR-CERDÀ A., GRANADOS M., SENTELLAS S., SAURINA J. From waste to resource: exploring green approaches for phenolics recovery from olive leaves. Antioxidants. **14** (2), 136, **2025**.
52. SALAH M.B., ABDELMELEK H., ABDERRABA M. Study of Phenolic Composition and Biological Activities

Assessment of Olive Leaves from different Varieties Grown in Tunisia. Medicinal Chemistry. **2012**, **2012**.
53. EL-SAYED O.M., EL-GAMMAL O.H.M., SALAMA A.S.M. Effect of ascorbic acid, proline and jasmonic acid foliar spraying on fruit set and yield of Manzanillo olive trees under salt stress. Scientia Horticulturae. **176**, 32, **2014**.
54. BRAHMI F., DABBOU S., DHIBI M., HAMMAMI M. The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. Industrial Crops and Products. **38**, 146, **2012**.
55. ABAZA L., BEN YOUSSEF N., MANAI H., MAHJOUB HADDADA F., METHENNI K., ZARROUK M. Chétoui olive leaf extracts: influence of the solvent type on phenolics and antioxidant activities. Grasas y Aceites. **62** (1), 96, **2011**.
56. CHAN A.W., WHITNEY R.A., NEUFELD R.J. Semisynthesis of a Controlled Stimuli-Responsive Alginate Hydrogel. Biomacromolecules. **10** (3), 609, **2009**.
57. GUEBEBIA S., GHARSALLAOUI A., DUMAS E., BAGHI F., ZOURGUI L., ROMDHANE M., AGUSTI G., GHNIMI S. Microencapsulation of phenolic compounds extracted from okra (*Abelmoschus esculentus* L.) leaves, fruits and seeds. Applied Sciences. **13** (22), 12273, **2023**.
58. SAIDANA NAIJA D., BEN MANSOUR GUEDDES S., BRAHAM M. Effects of water scarcity and salinity on the anatomy of the Tunisian table olive cultivar 'Meski'. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. **49** (4), 12157, **2021**.
59. BARKU V., OPOKU-BOAHEN Y., OWUSU-ANSAH E., MENSAH E. Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of *Amaranthus spinosus*. Asian Journal of Plant Science and Research. **3** (1), 69, **2013**.
60. ANAND DAVID A.V., ARULMOLI R., PARASURAMAN S. Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. Pharmacognosy Reviews. **10** (20), 84, **2016**.
61. PEREIRA A.P., FERREIRA I.C., MARCELINO F., VALENTÃO P., ANDRADE P.B., SEABRA R., ESTEVINHO L., BENTO A., PEREIRA J.A. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules. **12** (5), 1153, **2007**.
62. CITTAN M., ÇELIK A. Development and Validation of an Analytical Methodology Based on Liquid Chromatography-Electrospray Tandem Mass Spectrometry for the Simultaneous Determination of Phenolic Compounds in Olive Leaf Extract. J Chromatogr Sci. **56**, (4), 336, **2018**.
63. ROSSI L., BORGHI M., FRANCINI A., LIN X., XIE D.Y., SEBASTIANI L. Salt stress induces differential regulation of the phenylpropanoid pathway in *Olea europaea* cultivars Frantoio (salt-tolerant) and Leccino (salt-sensitive). Journal of Plant Physiology. **204**, 8, **2016**.
64. NASIR S.A., MALIK J.M.B. Changes in oleuropein levels during differentiation and development of floral buds in 'Arbequina' olives. Scientia Horticulturae. **110** (3), 274, **2006**.
65. LAGUERRE M., LʹOPEZ GIRALDO L.J., PIOMBO G., FIGUEROA-ESPINOZA M. C., PINA M., BENAISSA M., COMBE A., ROSSIGNOL CASTERA A., LECOMTE J., VILLENEUVE P. Characterization of Olive-Leaf Phenolics by ESI-MS and Evaluation of their Antioxidant Capacities by the CAT Assay. Journal of the American Oil Chemists' Society. **86** (12), 1215, **2009**.
66. SABELLA E., LUVISI A., APRILE A., NEGRO C., VERGINE M., NICOLÌ F., MICELI A., DE BELLIS L. *Xylella fastidiosa* induces differential expression of lignification related-genes and lignin accumulation in tolerant olive trees cv. Leccino. Journal of Plant Physiology. **220**, 60, **2018**.
67. ABDEL-AZIZ M.E.D., DARWISH M.S., MOHAMED A.H., EL-KHATEEB A.Y., HAMED S.E. Potential Activity of Aqueous Fig Leaves Extract, Olive Leaves Extract and Their Mixture as Natural Preservatives to Extend the Shelf Life of Pasteurized Buffalo Milk. Foods. **9** (5), **2020**.
68. PETRIDIS A., THERIOS I., SAMOURIS G., KOUNDOURAS S., GIANNAKOULA A. Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four Greek olive (*Olea europaea* L.) cultivars. Plant Physiol Biochem. **60**, 1, **2012**.
69. EL ADNANY E.M., ELHADIRI N., MOURJANE A., OUHAMMOU M., HIDAR N., JAOUAD A., BIGAUD E., BITAR K., MAHROUZ M. Impact of heat treatment and granulometry of olive leaf powder (Moroccan picholine) on its phenolic compounds, antioxidant activity and physical–chemical properties. Euro-Mediterranean Journal for Environmental Integration. **9** (2), 721, **2024**.
70. LOUKIL E., BEN MAHMOUD I., GARGOURI K., BEN MBAREK H., ALLOUCHE N., AYEDI M., TRABELSI L., CHAKER R., RIGANE H. Assessment of polyphenols decomposition during olive mill wastewaters drying in storage basin. Euro-Mediterranean Journal for Environmental Integration. **9** (3), 1619, **2024**.
71. ELKADRI A., SAHNOUN H., ELFKIH S., ABICHOU M. Assessing the sustainability of olive mill wastewater storage tank locations in Tunisia. Euro-Mediterranean Journal for Environmental Integration. **8** (2), 255, **2023**.
72. LEONARDO DI DONNA F M., NACCARATO A., SALERNO R., TAGARELLI A., TAVERNA D., SINDONA G. Secondary metabolites of *Olea europaea* leaves as markers for the discrimination of cultivars and cultivation zones by multivariate analysis,. Food Chemistry. **121** (2), 492, **2010**.
73. MEHMET B., SELIN Ş. Effects of geographical origin and extraction methods on total phenolic yield of olive tree (*Olea europaea)* leaves. Journal of the Taiwan Institute of Chemical Engineers. **44** (1), 8, **2013**.
74. KÖSEOĞLU O., GÜNGÖR F.Ö., KAYA Ü., ÇAMOĞLU G., AKKUZU E., SEVIM D., AŞIK Ş. Effect of Different Irrigation Treatments on Quality and Antioxidant Compounds of Virgin Olive Oils from cv. "Memecik". Akademik Gıda. **23** (2), 91, **2025**.
75. VARDAKAS A., KAPRANOU M.A., STATHOPOULOS P., XENAKI M., PROESTOS C., GIANNAKAS A.E. Evaluation of Olive Leaves of Different Varieties and Climatic Cultivation Conditions as a Source of Bioactive Substances Using Sustainable Extraction Methods. **2025**. Preprints 2025,2025072574. https://doi.org/10.20944/preprints202507.2574.v1
76. ABDELJELIL Z.B., TEKAYA M., ELMSELLEM H., MECHRI B., HAMMAMI M. Impact of season and foliar fertilisers on phenolics of leaves of Chemlali olive cultivar. Moroccan Journal of Chemistry. **5**, **2017**.
77. OTEROS J., ORLANDI F., GARCÍA-MOZO H., AGUILERA F., DHIAB A.B., BONOFIGLIO T., ABICHOU M., RUIZ-VALENZUELA L., DEL TRIGO M.M., DIAZ DE LA GUARDIA C. Better prediction

of Mediterranean olive production using pollen-based models. Agronomy for Sustainable Development. **34**, 685, **2014**.

78. KHELOUF I., KAROUI I.J., LAKOUD A., HAMMAMI M., ABDERRABBA M. Comparative chemical composition and antioxidant activity of olive leaves *Olea europaea* L. of Tunisian and Algerian varieties. Heliyon. **9** (12), **2023**.
79. BORGHINI F., TAMASI G., LOISELLE S.A., BAGLIONI M., FERRARI S., BISOZZI F., COSTANTINI S., TOZZI C., RICCABONI A., ROSSI C. Phenolic profiles in olive leaves from different cultivars in Tuscany and their use as a marker of varietal and geographical origin on a small scale. Molecules. **29** (15), 3617, **2024**.
80. BRAHMI F., MECHRI B., DABBOU S., DHIBI M., HAMMAMI M. The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. Industrial Crops and Products. **38**, 146, **2012**.
81. UGOLINI T., CECCHI L., SANI G., DIGIGLIO I., ADINOLFI B., CIACCHERI L., ZANONI B., MELANI F., MULINACCI N. Seasonal and Cultivar-Dependent Phenolic Dynamics in Tuscan Olive Leaves: A Two-Year Study by HPLC-DAD-MS for Food By-Product Valorization. Separations. **2025**.
82. VIDOVIĆ N., PASKOVIĆ I., MARCELIĆ Š., LUKIĆ I., BRKIĆ BUBOLA K., KLISOVIĆ D., NOVOSELIĆ A., PALČIĆ I., POLIĆ PASKOVIĆ M., HERAK ĆUSTIĆ M., PETEK M., JUKIĆ ŠPIKA M., PECINA M., PONGRAC P., GORETA BAN S. Effect of Combined Sulfur and Nitrogen Foliar Supply on Olive Oil Volatile Compounds and Sensory Attributes. Horticulturae. **8** (10), 912, **2022**.
83. PARRI S., CAI G., ROMI M., CANTINI C., PINTO D.C., SILVA A.M., DIAS M.C.P. Comparative metabolomics of leaves and stems of three Italian olive cultivars under drought stress. Frontiers in Plant Science. **15**, 1408731, **2024**.