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Research Paper / Araştırma Makalesi

Effect of Collection Period and Irrigation Process on Antioxidant Activity and Phenolic Compounds of Olive Leaves

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ABSTRACT

Olive leaves have drawn attention because of their contents of bioactive compounds that exhibit antioxidant activity. The aim of this study was to investigate the impact of irrigation on the phenolic compounds and antioxidant activity of olive leaves belonged to different varieties collected between September and December in irrigated and rainfed orchards. Principal components analysis (PCA) was used to explain the effect of variables. The highest total phenolic content was determined in irrigated Ayvalık leaves (1945 mg/100g). Results showed that olive leaves contained significant amounts of flavonoids, ranging from 6379 to 159046 mg/100g. However, differences in antioxidant activity were generally between 1 and 2% regarding irrigation, collection time, and variety. Luteolin-7-glucoside (273-1461 mg/100g) was the main phenolic compound of olive leaves, followed by verbascoside (399-1002 mg/100g). The influence of irrigation showed differences in the amounts of phenolic compounds among the cultivars.

Keywords: Irrigation, Collection time, Olive leaf, Variety, Phenolic compounds

Zeytin Yapraklarının Antioksidan Aktivite ve Fenolik Bileşenleri Üzerine Sulama İşleminin ve Toplama Periyodunun Etkisi

ÖZ

Zeytin yaprakları, antioksidan aktiviteye sahip biyoaktif bileşenleri içermesinden dolayı dikkat çekmektedir. Bu çalışmada, sulanan ve sulanmayan bahçelerden Eylül-Aralık ayları arası toplanan farklı çeşitlere ait zeytin yapraklarının antioksidan aktiviteleri ve fenolik bileşenleri üzerine sulama işleminin etkisi araştırılmıştır. Değişkenlerin etkisini açıklamak için temel bileşenler analizi (PCA) kullanılmıştır. En yüksek toplam fenolik madde içeriği sulanan Ayvalık yapraklarında (1945 mg/100g) belirlenmiştir. Sonuçlar, zeytin yapraklarının 6379-159046 mg/100g arasında önemli miktarda flavonoid içerdiğini göstermiştir. Ancak çeşit, toplama zamanı ve sulama gibi faktörlere göre örneklerin antioksidan aktivitelerindeki farklılık genellikle %1-2 arasında bulunmuştur. Zeytin yapraklarının major fenolik bileşeni luteolin-7-glukozid (273-1461 mg/100g) bulunmuş ve bunu verbaskozid (399-1002 mg/100g) izlemiştir. Sulama işleminin fenolik bileşenlerin miktarı üzerine etkisi çeşitler arasında farklılık göstermiştir.

Anahtar Kelimeler: Sulama, Toplama zamanı, Zeytin yaprağı, Çeşit, Fenolik bileşikler

INTRODUCTION

Olive (*Olea europaea* L.) is one of the oldest cultivated and drought-tolerant trees [1]. After pruning process, the amounts of by-products such as twigs and leaves are annually about 25 kg/tree, which differ according to age of tree, culture and pruning applications [2]. Olive leaves are utilised for feeding animal and healing certain

diseases in traditional medicine from past to present [3]. In a study, published by Bouaziz et al. [4], it was informed that the extract of olive leaf can be added to improve the shelf life and stability of foods. Also, it was determined that olive leaf extract exhibited better antioxidant activity in comparison to both vitamins (C and E) and pure hydroxytyrosol [5]. Herbal teas and food supplements, which contain leaves or extract, are consumed throughout the world [6].

Phenolic compounds of olive by-products, especially olive leaf, has drawn increased attention in recent years [7]. Several factors as variety, origin, ripening degree cause significant differences in the phenolic composition of olive leaves [8-9]. Besides these factors, water stress is taken into consideration due to limit of the sustainable agriculture, and also affect the physicochemical characteristics of plant [10, 11]. The adaptation of olive leaves to water stress depens on changes in the leaf regarding morphological, anatomical and physiological properties [12, 13]. In olive trees, intense water loss is prevented by regulating tree transpiration through stomal closure in leaves [14]. However, the availability of water in the soil affects tree performance, including fruit development, fruit characteristics and oil quality. In addition, irrigation can directly influence yield factors as well as the vegetative growth [15]. Accordingly, the irrigation process of olive trees has been increasingly applied all around the world since the 1990s [16]. According to our knowledge, no studies about the effect of irrigation on the phenolic compounds of olive leaves are available. In this study, the influences of primarily irrigation and also variety and harvest time on antioxidant activity and phenolic compounds of olive leaves were investigated.

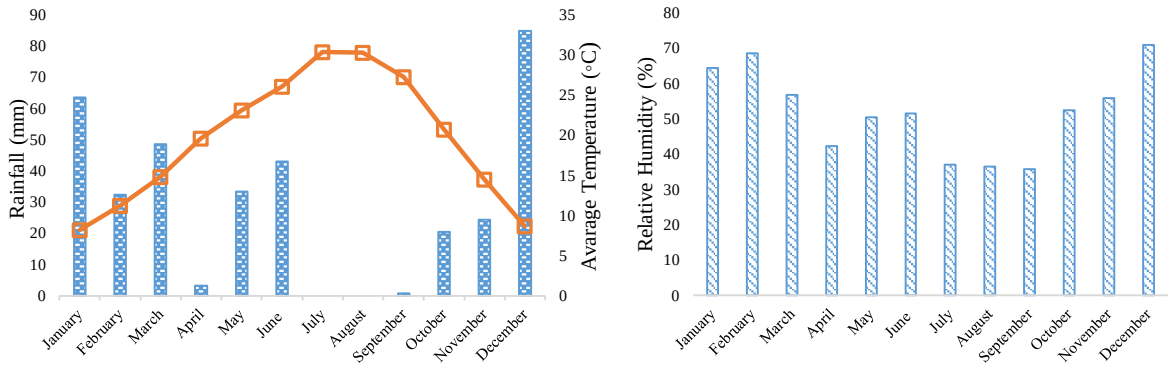
MATERIALS and METHODS

Collection of Olives Leaves

Olive leaves belonged to Ayvalık, Çöpaşı, Gemlik and Yağlık varieties grown in irrigated (altitude: 280 m) and arid (altitude: 307 m) orchards in Mersin location were collected from each side of the three trees, and at 20 days intervals between Sebtember and December in 2018. Olive trees of different varieties were located in the same orchard. Samples were transferred to the laboratory in paper bags and they were dried at room temperature prior to analyses. The collection times of olive leaves were September 15th, October 6th, October 27th, November 17th, December 8th, and December 29th, respectively.

Climatic Conditions

The average monthly humidity, temperature, and total monthly rainfall graphs of the location where the olives leaves were collected in 2018 are shown in Figure 1.



a b

Figure 1. Average temperature, total rainfall (a) relative humidity (b) for the collection region of olive leaves in 2018

Irrigation Process

Irrigation was carried out with a drip irrigation system which was applied once a month for a total of 4 hours. The total amount of irrigation water was 640 L/month. Irrigation process was applied till October.

Methods

Moisture Content

Moisture amounts of olive leaves were determined by drying in an oven (Nüve FN055 Ankara, Turkey) at 105°C until a constant weight was obtained.

Extraction Process

Olive leaves (0.5 g), which were ground into powder by a grinder, were mixed with 5 mL of methanol/water (80:20, v/v). The mixture was stirred for 1 min using vortex, followed by sonication in water bath for 10 min and centrifugated at 6000 rpm for 10 min. Supernatant was removed, and these steps were repeated twice. Finally, extract was filtered using a 0.45 µm syringe filter before analysis [17].

Total Phenolic Content

Total phenol contents of olive leaf extracts were determined using Folin Ciocalteu (FC) reagent [18].

Extract (0.5 mL) was mixed with 2.5 mL of Folin Ciocalteu reagent and 1.5 mL of sodium carbonate solution (7.5%). The absorbance values of the samples, which were stored for 2 hours at room temperature and in the dark, were measured at 725 nm. Gallic acid was used as standard, and results were given in mg GAE/100g (fresh weight).

Total Flavonoid Content

Total flavonoid contents of olive leaf extracts were determined spectrophotometrically according to the method of Hogan, et al. [19]. Extract (1 mL) was mixed with 0.3 mL of $NaNO_2$ (5%), 0.3 mL of $AlCl_3$ (10%) and 2 mL of NaOH (4%), respectively. The absorbance of mixture was recorded at 510 nm by a spectrophotometer. Results were given as mg catechin (CE)/ 100g of fresh weight.

Antioxidant Activity (DPPH Free-radical Scavenging Activity)

The antioxidant activities of olive leaf extracts were determined using 2,2-diphenyl-1-picrazil (DPPH) according to method described by Lee, et al. [20]. The extracts (0.1 mL) were mixed with 2 mL of 0.1 mM DPPH solution and the absorbance values of the samples, which kept in the dark for 30 min at room temperature, were measured at 517 nm. Inhibition values (%) corresponding to each sample volume were calculated according to the equation given below.

$$\text{Antioxidant activity (\%)} = (A_{Control} - A_{Sample})\ x\ 100/A_{Control}$$

(A: Absorbance)

Determination of Phenolic Compounds

The phenolic compounds of olive leaf extracts were performed at 280 and 330 nm using HPLC.
Working conditions are presented below:

Instrument	Shimadzu LC 10A vp, Kyoto, Japan
Software	PC running Class VP chromatography manager software (Shimadzu, Japan)
Injection volume	40 µL
Column	Inertsil ODS3 analytical column (GL Sciences, Japan), (5 µm, 25 cm x 4.6 mm)
Mobile phase	A (2% formic acid aqueous solution), B (methanol)
Flow rate	0.85 mL / min
Detector	Shimadzu SPD-M20 A Diode Array Detector
Temperature	40°C

Statistical Analysis

Statistical analyses of the results were performed by using SPSS-Statistics-22 statistical program. The means of significant variation sources were compared to Duncan Multiple Comparison Test with the help of MSTAT program. The significance level was given as $p<0.01$ unless otherwise stated. The analyses were repeated three times (n =3). PCA was applied using XLSTAT software.

RESULTS and DISCUSSION

Moisture contents of olive leaves ranged from 7.94% to 9.68% for Ayvalık variety; from 8.01% to 9.16% for Gemlik variety; from 7.78% to 8.92% for Yağlık variety; from 7.77% to 9.48% for Çöpaşı variety, as are shown in Table 1.

Total phenolic contents of Ayvalık, Gemlik, Yağlık and Çöpaşı olive leaves collected at different periods depending on irrigation and rainfed conditions are given in Table 2. Total phenolic contents of irrigated and rainfed Ayvalık, Gemlik, Yağlık and Çöpaşı leaves were determined between 1658.75 and 1945.00 mg/100g, 1418.75 and 1902.50 mg/100g, 1485.00 and 1903.75 mg/100g, 1685.00 and 1835.00 mg/100g, respectively. The highest total phenolic amount was found in both irrigated (1945.00 mg/100g) and rainfed (1910.00 mg/100g) Ayvalık olive leaves collected on December 29 (6th harvest). Collection time of leaves affected the total phenolic content, and the highest values were generally observed in 4th (November 17) or 6th (December 29) harvests. The reason of increase in total phenolic content was explained as the increase of polyphenol oxidase (PPO) content and activity in olive leaves. It was informed that the PPO in leaves contributed to the synthesis and modification of several compounds such as phenols during fruit development [21]. In addition, the effect of irrigation process showed differences regarding variety and collection time. For Gemlik leaves, irrigation process caused reduction, except 4th and 6th harvests, and the major decrease from 1741.25 mg/100g to 1418.75 mg/100g was detected in 3rd harvest (October 25). In leaves of Yağlık variety, there was no significant difference in total phenolic content for 1st, 2nd, and 4th harvests, while the highest reduction from 1768.75 mg/100g to 1485.00 mg/100g was observed in 3rd harvest when the leaves were irrigated. The total phenolic amounts of leaves belonged to Çöpaşı variety showed decrease in 2nd, 4th and 5th harvests or no change in 1st, 3rd, and 6th harvests. In Ayvalık leaves, the irrigation process caused a fluctuation in total phenolic contents during collection period. The main difference was determined in 2nd harvest, and total phenolic content of Ayvalık leaves increased from 1658.75 mg/100g to 1838.75 mg/100g with irrigation process. In previous study, Salah, et al. [22] informed that total phenolic contents of olive leaves belonged to different varieties were found between 73.05 mg GAE/g (Sevillane variety) and 144.19 mg GAE/g (Limouni variety). In another study, which were in accordance with current results, total phenolic contents of olive leaf extracts ranged from 13.23 mg GAE/g to 24.09 mg GAE/g [23]. Total phenolic contents of Chemlali (464.27 mg/100g) and Neb jmel (270.53 mg/100g) olive leaves collected in January were higher than leaves collected in October (219.85 mg/100g for Chemlali and 197.60 mg/100g for Neb jmel) [24].

Table 1. Moisture contents of olive leaves belonged to different varieties collected from irrigated and rainfed orchards during olive harvest period (%)[1,2]

Process	Variety	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Irrigated	Ayvalık	8.25±0.06	8.51±0.13	8.98±0.67	8.84±0.66	8.67±0.29	9.59±0.25
	Gemlik	8.82±0.16	8.70±0.06	8.50±0.00	8.46±0.06	8.21±0.30	8.95±1.34
	Yağlık	8.00±0.47	7.99±0.22	8.52±0.68	8.19±0.33	8.92±0.70	8.48±0.67
	Çöpaşı	8.57±0.11	7.77±0.41	8.36±0.15	8.42±0.06	9.48±0.04	8.21±0.30
Rainfed	Ayvalık	8.79±1.08	8.78±0.08	8.44±0.03	7.94±0.08	8.13±0.23	9.68±0.25
	Gemlik	8.30±0.39	8.01±0.30	9.16±0.29	8.73±0.32	8.21±0.30	8.98±0.67
	Yağlık	8.83±0.31	7.78±0.57	8.46±0.64	7.98±0.03	8.42±0.76	8.23±0.33
	Çöpaşı	8.41±0.49	8.13±0.81	8.68±0.32	9.09±0.31	8.44±0.03	8.00±0.71

[1]mean ± standard deviation (n=3). [2]Average values were found statistically insignificant ($p>0.05$).

Table 2. Total phenolic contents of olive leaves belonged to different varieties collected from irrigated and rainfed orchards during olive harvest period (mg/100g)[1,2]

Process	Variety	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Irrigated	Ayvalık	1718.75±7.01^{ABCD}	1838.75±5.53^{AB}	1745.00±6.97^{ABCD}	1792.50±4.25^{ABC}	1786.25±8.88^{ABC}	1945.00±5.28^{A}
	Gemlik	1662.50±7.39^{BCDE}	1538.75±7.99^{CDEF}	1418.75±9.24^{F}	1902.50±7.87^{AB}	1503.75±6.73^{DEF}	1786.25±4.95^{ABC}
	Yağlık	1845.00±6.93^{AB}	1807.50±6.64^{AB}	1485.00±6.91^{EF}	1873.75±5.33^{AB}	1761.25±6.20^{ABC}	1903.75±5.56^{AB}
	Çöpaşı	1835.00±6.97^{AB}	1745.00±4.42^{ABCD}	1705.00±5.68^{ABCDE}	1752.50±4.44^{ABCD}	1685.00±5.54^{ABCD}	1771.25±4.14^{ABC}
Rainfed	Ayvalık	1871.25±8.21^{AB}	1658.75±8.87^{BCDE}	1706.25±7.18^{ABCDE}	1818.75±5.22^{AB}	1886.25±4.47^{AB}	1910.00±7.30^{AB}
	Gemlik	1755.00±11.48^{ABC}	1751.25±9.92^{ABCD}	1741.25±8.27^{ABCD}	1846.25±5.21^{AB}	1781.25±6.15^{ABC}	1747.50±6.88^{ABCD}
	Yağlık	1843.75±5.34^{AB}	1796.25±7.87^{AB}	1768.75±8.21^{ABC}	1876.25±7.39^{AB}	1671.25±4.10^{BCDE}	1777.50±9.51^{ABC}
	Çöpaşı	1822.50±8.76^{AB}	1822.50±7.49^{AB}	1698.75±6.96^{ABCDE}	1835.00±4.14^{AB}	1768.75±3.76^{ABC}	1791.25±4.42^{ABC}

[1]mean ± standard deviation (n=3). [2]The properties in the line of harvest applications (1st-6th harvests) were compared with Duncan test, and average values indicated with different letters between A and F were found statistically significant ($p<0.01$).

In Table 3, total flavonoid contents of leaves collected from Ayvalık, Gemlik, Yağlık and Çöpaşı varieties ranged between 58156.66 mg/100g and 159045.56 mg/100g, 6378.89 mg/100g and 118378.89 mg/100g, 37267.78 mg/100g and 134267.78 mg/100g, 34378.89 mg/100g and 132712.22 mg/100g, respectively. Olive leaves of irrigated Ayvalık variety collected on December 29 (6th harvest) had the highest total flavonoid content (159045.56 mg/100g), followed by rainfed Yağlık olive leaves (134267.78 mg/100g) picked at 4th harvest (November 17). Similar to the results of total phenolic content, there are notably differences in total flavonoid amounts regarding to variety and collection time. It could be seen that the maximum total flavonoid contents of Ayvalık, Gemlik and Çöpaşı olive leaves were found in both irrigated and rainfed samples picked on December 29. In Yağlık olive leaves, the highest total flavonoid content was determined in 5th harvest (113356.08 mg/100g) for irrigated samples; in 4th harvest (134267.78 mg/100g) for rainfed leaves. The increase in phenolic compounds of olive leaves could originate from L-phenylalanine ammonia lyase (PAL) enzyme activity, which increases in cold [26]. Concerning the effect of irrigation, the major decrease from 103490.00 mg/100g to 6378.89 mg/100g was detected in Gemlik olive leaves collected on December 8 (5th harvest). For Ayvalık and Çöpaşı olive leaves when the influence of irrigation was evaluated, total flavonoid content fluctuated in regard to collection time. However, irrigation caused a reduction in both Gemlik (except 1st harvest) and Yağlık (except 5th and 6th harvests) olive leaves. A study of the olive leaves belonged to different olive varieties indicated that total flavonoid amounts were found as 125.65 mg CE/g (Gerboua variety), 120.88 mg CE/g (Limouni variety), 94.03 mg CE/g (Chetoui), 82.74 mg CE/g (Chemlali), 56.75 mg CE/g (Sevillane), 97.74 mg CE/g (Lucques), 76.01 mg CE/g (Rosicola), and 91.32 mg CE/g (Meski) [22]. Abaza, et al. [23] reported that total flavonoid contents of olive leaf extracts varied from 11.78 mg CE/g to 21.47 mg CE/g. Total flavonoid content of Chemlali olive leaves (98.4 mg CE/100g) was lower than Neb jmel olive leaves (119.28 mg CE/100g) when collected in October, while Chemlali olive leaves (377.06 mg CE/100g) had higher total flavonoid content than Neb jmel (147.96 mg CE/100g) when collected in January [24]. In another study, total flavonoid contents of irrigated and rainfed olive leaves were reported as 53.94-92.14 mg QE/g for Kilis Yağlık variety and 58.13-89.79 mg QE/g for Gemlik variety; 54.79-89.74 mg QE/g for Kilis Yağlık variety and 34.53-88.01 mg QE/g for Gemlik variety, respectively [25]. Moreover, similar to current results, total flavonoid contents were higher than total phenolic contents in olive leaves (422.9 mmol RE/kg and 353.6 mmol GAE/kg) studied by Lama-Muñoz, et al. [27]; in leaves of *Timonius celebicus* (721.39 mg QE/g and 258.76 mg GAE/g), *Psychotria celebica* (288.91 mg QE/g and 157.40 mg GAE/g), and *Gardenia mutabilis* 426.82 mg QE/g and 89.486 mg GAE/g) reported by Pratiwi, et al [28].

Table 3. Total flavonoid contents of olive leaves belonged to different varieties collected from irrigated and rainfed orchards during olive harvest period (mg/100g) [1,2]

Process	Variety	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Irrigated	Ayvalık	91712.22±59.46^{B-L}	100934.44±44.88^{B-L}	66045.56±50.75^{I-Q}	65490.00±52.44^{J-Q}	63378.89±79.57^{K-Q}	159045.56±76.15^{A}
	Gemlik	42156.66±26.33^{M-R}	23490.00±15.00^{QR}	14045.55±16.44^{R}	61712.22±58.15^{K-Q}	6378.89±18.59^{R}	87823.33±44.58^{D-L}
	Yağlık	102712.22±61.92^{B-K}	63601.11±46.80^{K-Q}	88601.11±31.20^{C-L}	110823.33±76.28^{B-I}	113356.08±67.63^{B-H}	110045.56±51.64^{B-J}
	Çöpaşı	105267.78±42.01^{B-K}	39045.56±53.61^{M-R}	105490.00±57.66^{B-K}	117156.67±75.20^{B-G}	34378.89±29.71^{O-R}	132712.22±66.88^{ABC}
Rainfed	Ayvalık	58156.66±53.22^{L-Q}	80601.11±45.53^{F-N}	70045.56±28.06^{H-P}	92601.11±64.55^{B-L}	122601.11±86.26^{A-F}	126601.11±81.78^{A-E}
	Gemlik	30378.89±16.16^{PQR}	57045.56±37.11^{L-Q}	70934.44±24.64^{H-P}	106156.67±88.76^{B-K}	103490.00±61.89^{B-K}	118378.89±91.70^{A-G}
	Yağlık	108490.00±72.83^{B-J}	81378.89±66.92^{F-M}	109490.00±70.78^{B-K}	134267.78±90.44^{AB}	37267.78±38.31^{N-R}	82156.67±65.13^{E-M}
	Çöpaşı	95156.66±43.63^{B-L}	89712.22±54.39^{B-L}	77378.89±63.26^{G-O}	105378.89±84.19^{B-K}	87378.89±47.84^{D-L}	127156.67±66.67^{A-D}

[1]mean ± standard deviation (n=3). [2]The properties in the line of harvest applications (1st-6th harvests) were compared with Duncan test, and average values indicated with different letters between A and R were found statistically significant (p<0.01).

Table 4. Antioxidant activities of olive leaves belonged to different varieties collected from irrigated and rainfed orchards during olive harvest period (%)[1,2]

Process	Variety	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Irrigated	Ayvalık	78.85±0.08^{A-D}	76.17±0.40^{G}	78.43±0.15^{A-E}	77.59±0.23^{EF}	78.17±0.45^{A-F}	79.17±0.23^{A-E}
	Gemlik	78.70±0.08^{A-E}	78.59±0.15^{A-E}	78.96±0.09^{A-D}	78.91±0.30^{A-D}	79.01±0.10^{ABC}	78.64±0.50^{A-E}
	Yağlık	78.01±0.24^{A-F}	73.86±0.17^{H}	78.28±0.15^{A-F}	77.96±0.32^{A-F}	78.70±0.08^{A-E}	78.17±0.24^{A-F}
	Çöpaşı	77.75±0.18^{DEF}	77.85±0.08^{C-F}	78.43±0.15^{A-E}	78.49±0.23^{A-E}	77.90±0.23^{B-F}	79.11±1.73^{AB}
Rainfed	Ayvalık	77.96±0.31^{A-F}	77.59±0.23^{EF}	76.12±0.50^{G}	78.59±0.42^{A-E}	79.06±0.27^{ABC}	78.80±0.39^{A-E}
	Gemlik	78.80±0.17^{A-E}	78.70±0.32^{A-E}	78.96±0.09^{A-D}	78.80±0.24^{A-E}	78.43±0.41^{A-E}	78.69±0.65^{A-E}
	Yağlık	78.96±0.17^{A-D}	78.01±0.39^{A-F}	78.80±0.32^{A-E}	78.70±0.08^{A-E}	78.48±0.41^{A-E}	78.75±0.28^{A-E}
	Çöpaşı	77.86±0.10^{C-F}	77.17±0.31^{F}	78.43±0.15^{A-E}	77.75±0.18^{DEF}	78.54±0.23^{A-E}	78.17±0.70^{A-F}

[1]mean ± standard deviation (n=3). [2]The properties in the line of harvest applications (1st-6th harvests) were compared with Duncan test, and average values indicated with different letters between A and G were found statistically significant (p<0.01).

Antioxidant activities of olive leaves belonged to different olive varieties are shown in Table 4. The antioxidant activity values ranged from 76.12% to 79.17% for Ayvalık leaves; from 78.43% to 79.01% for Gemlik leaves; from 73.86% to 78.96% for Yağlık leaves; from 77.17% to 79.11% for Çöpaşı leaves. Similar to total phenolic and flavonoid results, Ayvalık leaves exhibited the best antioxidant activity (79.17%) when collected on December 29 (6th harvest). The factors such as variety, collection time and irrigation process caused minor differences on antioxidant activity of olive leaves. The highest reduction in antioxidant activity when the irrigation was applied was observed in Yağlık olive leaves (from 78.01% to 73.86%) collected on October 6. The radical scavenging activities determined using DPPH of Arbequina, Sikitita and Picual olive leaves were reported as 7.2 µg/mL, 11.3 µg/mL and 12.3 µg/mL, respectively [17]. In another study, published by Brahmi, et al. [24], the olive leaves harvested in January showed higher antioxidant activity (98.2% for Chemlali and 97.5% for Neb jmel) than leaves collected in October (79.8% for Chemlali and 58.75% for Neb jmel).

The phenolic compounds of irrigated and rainfed olive leaves are illustrated in Figure 2. The hydroxytyrosol contents of Ayvalık, Gemlik, Yağlık and Çöpaşı olive leaves varied between 149.05 mg/100g and 361.05 mg/100g; 217.02 mg/100g and 427.59 mg/100g; 240.61 mg/100g and 522.77 mg/100g; 195.53 mg/100g and 525.29 mg/100g, respectively. Çöpaşı olive leaves contained the highest amount of hydroxytyrosol (525.96 mg/100g) collected in rainfed tree on September 15 (1st harvest), followed by Yağlık olive leaves (522.77 mg/100g). The rainfed Yağlık and Çöpaşı olive leaves collected in early-season had higher hydroxytyrosol content than those in late-season leaves. Additionally, a decrease was observed in Yağlık olive leaves, while an increase was mostly determined in Ayvalık, Gemlik and Çöpaşı olive leaves with irrigation. In a previous study, the hydroxytyrosol content was found as 0.67 mg/100g in Neb jmel olive leaves collected in October; 0.74 mg/100g in the same variety collected in January [24]. Ghomari, et al. [29] revealed that olive leaves had 0.02 mg/g hydroxytyrosol contents obtained by maceration; 15.17 mg/g hydroxytyrosol contents obtained by sonication. The tyrosol amounts of olive leaves ranged from 134.49 mg/100g to 328.72 mg/100g for Ayvalık variety; from 166.34 mg/100g to 346.44 mg/100g for Gemlik variety; from 190.66 mg/100g to 305.30 mg/100g for Yağlık variety; from 158.71 mg/100g to 324.71 mg/100g for Çöpaşı variety. Irrigation process caused an increase in tyrosol concentration of olive leaves. Moreover, no regular increase or decrease was determined in tyrosol content during collection time. According to the study of Lorini, et al. [30], tyrosol contents of Arbequina, Manzanilla, and Picual olive leaves were recorded as 6.43 µg/g, 13.11 µg/g, and 10.82 µg/g in autumn; 7.40 µg/g, 9.94 µg/g, and 7.26 µg/g in winter; 9.20 µg/g, 12.20 µg/g, and 9.70 µg/g in spring; 4.55 µg/g, 7.99 µg/g, and 1.29 µg/g in summer, respectively, and these results were lower than current study. The highest caffeic acid contents of Ayvalık (185.93 mg/100g), Gemlik (183.20 mg/100g), Yağlık (191.91 mg/100g) and Çöpaşı (217.38 mg/100g) varieties were detected in irrigated leaves collected

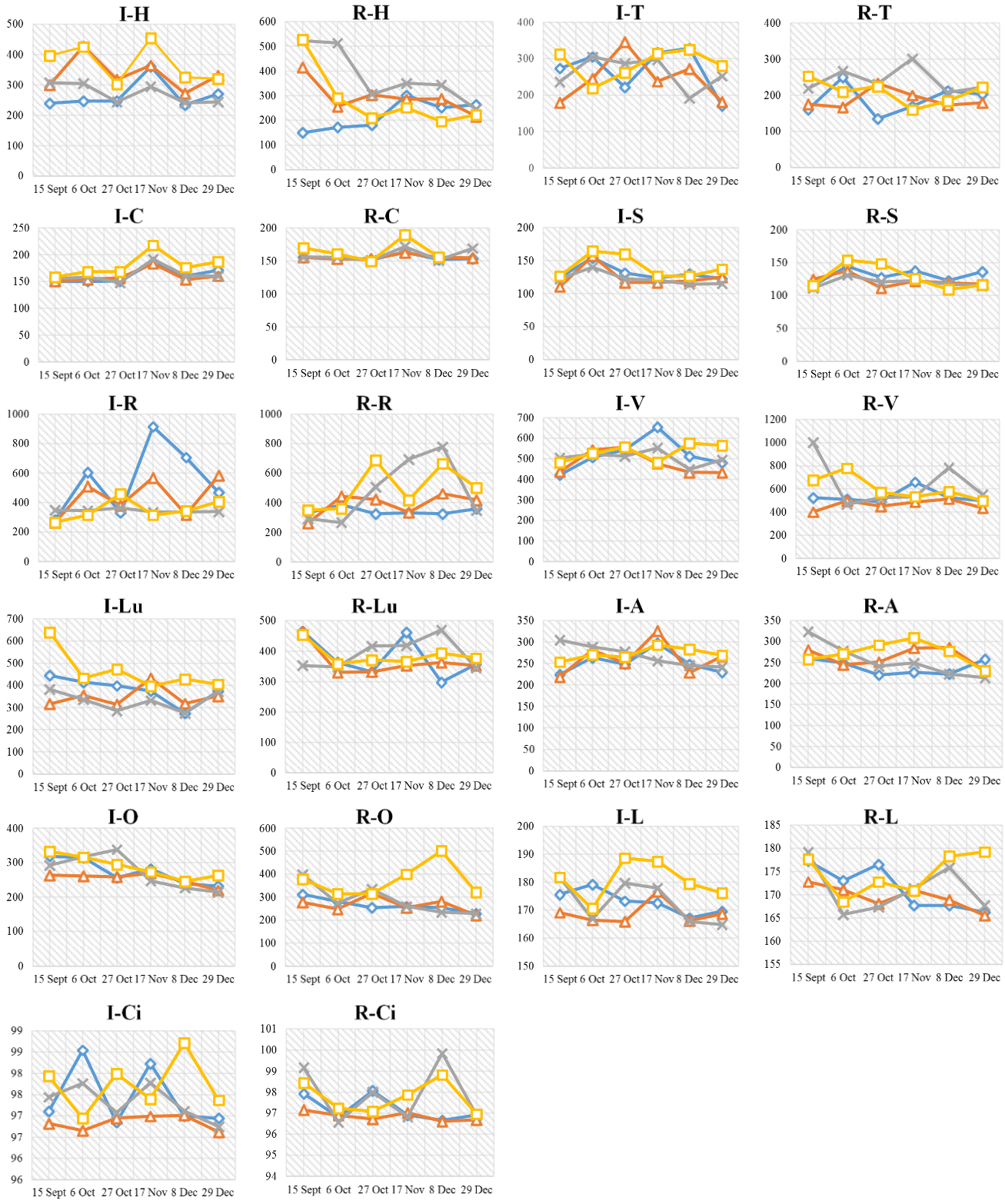


Figure 2. Phenolic compounds of olive leaves belonged to different varieties collected from irrigated and rainfed orchards during olive harvest period (mg/100g). ◊:Ayvalık, Δ: Gemlik, ⋆ : Yağlık, □: Çöpaşı, I: Irrigated, R: Rainfed, H: Hydroxytyrosol, T: Tyrosol, C: Caffeic acid, S: Syringic acid, R: Rutin, V: Verbascoside, Lu: Luteolin-7-glucoside, A: Apigenin-7-glucoside, O: Oleuropein, L: Luteolin and Ci: cinnamic acid. Values are the average of 3 measurements (n=3).

November 17 (4th harvest), while the syringic acid amounts of Ayvalık (154.05 mg/100g), Gemlik (156.97 mg/100g), Yağlık (140.22 mg/100g) and Çöpaşı (164.64 mg/100g) olive leaves were found the maximum when collected from irrigated trees on October 6 (2nd harvest). For caffeic and syringic acid, there is no significant differences in regard to variety, collection time and irrigation process ($p>0.05$). Pereira, et al. [31] informed that olive leaves contained 220.5 mg/kg caffeic acid. According to Medina, et al. [32], the commercial olive leaf extracts contained 0.04-0.10 g/kg caffeic acid. The rutin content of Ayvalık olive leaves (914.64 mg/100g) collected from irrigated trees on November 17 (4th harvest) was found higher than other varieties, followed by Yağlık olive leaves (780.18 mg/100g) picked from rainfed trees on December 8 (5th harvest). In Çöpaşı olive leaves, irrigation reduces the rutin amount during collection period. Additionally, the rutin content significantly increased from 331.71 mg/100g to 914.64 mg/100g in Ayvalık olive leaves and also decreased from 780.19 mg/100g to 333.64 mg/100g in Yağlık olive leaves because of irrigation process. The rutin contents of olive leaves were detected as 0.651 mg/kg for Arbequina variety; 0.319 mg/kg for Sikitita variety; 0.289 mg/kg for Picual variety in a study recorded by Talhaoui, et al. [17], while Pereira, et al. [31] determined the content of rutin as 495.9 mg/kg. The verbascoside concentrations of Ayvalık, Gemlik, Yağlık and Çöpaşı olive leaves varied between 420.24 mg/100g and 659.61 mg/100g; 399.09 mg/100g and 556.51 mg/100g; 446.69 mg/100g and 1001.94 mg/100g; 482.02 mg/100g and 777.23 mg/100g, respectively. It was observed that the richest leaves in terms of verbascoside content belonged to Yağlık variety and the highest amount of this hydroxycinnamic acid was found in leaves collected from rainfed trees on September 15. Moreover, irrigation process caused the reduction from 1001.94 mg/100g to 503.91 mg/100g in the same variety and collection time. Looking to similar studies in literature, the verbascoside contents of olive leaves were recorded as 1.127-4.069 mg/kg [17] and 966.1 mg/kg [31]. In another study, verbascoside contents of commercial olive leaves were determined between 0.36 and 2.31 g/kg [32]. The Ayvalık olive leaves contained the highest amount of luteolin-7-glucoside (1460.95 mg/100g), followed by Yağlık olive leaves (1345.08 mg/100g) when both of them were collected from rainfed trees. A significant reduction from 1460.95 mg/100g to 374.48 mg/100g for Ayvalık, and from 1345.08 mg/100g to 372.65 mg/100g for Yağlık olive leaves was detected with irrigation. Generally, the irrigation application increased the luteolin-7-glucoside contents of Çöpaşı olive leaves, while it was observed the opposite trend in Yağlık olive leaves during harvest period. In previous studies, luteolin-7-glucoside amounts of olive leaves were found as 0.94-4.65 g/kg [27]; 4208.9 mg/kg [31]; 8.27-819.32 mg/kg [33]. Apigenin-7-glucoside concentrations of Ayvalık, Gemlik, Yağlık and Çöpaşı olive leaves were determined as 220.52-297.22 mg/100g, 219.52-326.42 mg/100g, 214.20-323.81 mg/100g and 230.56-309.34 mg/100g, respectively. The apigenin-7-glucoside levels showed a minor differences according to collection time and irrigation process.

In an experiment carried out by Pereira, et al. [31], apigenin-7-glucoside amount of olive leaves was found as 2333.1 mg/kg. Moreover, a similar apigenin-7-glucoside content (2475.53 mg/kg) in olive leaf belonged to Zalmati Zarzis cultivar was identified by Ben Mohamed, et al. [33]. The highest oleuropein contents of Ayvalık, Gemlik, Yağlık and Çöpaşı olive leaves were detected as 317.53 mg/100g (1st harvest), 317.08 mg/100g (3rd harvest), 397.85 mg/100g (1st harvest), and 499.72 mg/100g (5th harvest), respectively. The oleuropein amounts of early-season leaves collected from irrigated trees were higher than late-season leaves. However, there is no regular increase or decrease in oleuropein levels of olive leaves picked from rainfed trees. The Çöpaşı olive leaves affected from irrigation process, especially in the last three harvest, and the maximum reduction from 499.72 mg/100g to 245.87 mg/100g was determined when collected on December 8. Similar oleuropein contents were obtained by Ben Mohamed, et al. [33] who dedected as 3146.06 mg/kg in Dokhar el Gorthab cultivar, and 4741.62 mg/kg in Fougi cultivar. Salah, et al. [22] determined the oleuropein contents between 30.76 mg/g and 57.24 mg/g. The oleuropein concentrations were found as 25.08 mg/100g in Neb jmel olive leaves collected in October; 19.93 mg/100g in the same variety collected in January [24]. In another study, oleuropein contents of dry olive leaves were recorded as 2.337 mg/kg for Arbequina variety; 2.110 mg/kg for Sikitita variety; 2.100 mg/kg for Picual variety [17]. The oleuropein content of olive leaves was reported as 26471.4 mg/kg by Pereira, et al. [31]. Luteolin contents of olive leaves varied from 166.30 mg/100g to 179.14 mg/100g for Ayvalık variety; from 165.63 mg/100g to 176.64 mg/100g for Gemlik leaves; from 164.83 mg/100g to 181.53 mg/100g for Yağlık samples; from 168.51 mg/100g to 188.55 mg/100g for Çöpaşı leaves, while cinnamic acid contents of olive leaves were determined between 96.58 mg/100g and 99.84 mg/100g. The collection time and irrigation did not significantly affect both luteolin and cinnamic acid contents of olive leaves. In a previous study, luteolin contents of Arbequina, Sikitita, and Picual varieties were recorded as 0.394 mg/kg, 0.367 mg/kg, and 0.497 mg/kg, respectively [17]. Ben Mohamed, et al. [33] reported that luteolin amounts of olive leaves of 21 studied genotypes varied between 1.79 mg/kg (Chemlali djerba) and 273.96 mg/kg (Zalmati zarzis). Olive leaves have been found to be rich in phenolic compounds and a comparison of their values with those in literature reveal differences. These differences can be attributed to growing temperature of plant, variety, ecological factors, collection period, maturation, soil structure, and analytical factors. Additionally, the structure or properties of the plant material and bioactive compounds may likely cause differences in the effects of the irrigation and collection period on phenolic compounds. Also, biochemical reactions and enzymatic activities in growing period of olive leaves depending on irrigation and dry agriculture applications can cause differences in phenolic compound types, and amounts. The varitial difference among plants can also be an important contributing factor for determining the phenolic profile. The presence of these important phenolic compounds having important biological activities show

the importance of leaves from different olive varieties for use in the development of functional and nutraceutical products.

Principal Components Analysis of Bioactive Properties

Concerning eigenvalues, the first two components accounted for 36.24% of total variance (22.63% for PC1; 13.61% for PC2). Phenolic compounds, total phenolic and total flavonoid contents were placed in positive side of PC1, as is illustrated in Figure 3a. Antioxidant activity showed a negative correlation with PC1; positive correlation with PC2. Additionally, the samples of YR-1, ÇR-1, ÇI-1 and Ç1-4 were located in positive area of both PC1 and PC2. GI-1, GI-3, GI-2 and GI-5 samples were found in negative area of PC1 and PC2 (Figure 3b). It can be concluded that there was a positive correlation between all of the phenolic compounds and Çöpaşı leaves, while negative area of PC1 axis was correlated with Gemlik leaves (except GI-4).

Table 5. Eigenvalues and correlations between bioactive properties and principal components (PC1 and PC2)

	PC1	PC2
Eigenvalue	3.168	1.906
Variability (%)	22.630	13.612
Cumulative (%)	22.630	36.242
Correlations	**PC1**	**PC2**
Hydroxytyrosol	0.487	-0.113
Tyrosol	0.313	-0.373
Caffeic acid	0.437	0.350
Syringic acid	0.111	-0.358
Rutin	0.203	-0.089
Verbascoside	0.585	-0.112
Luteolin-7-glucoside	0.542	0.117
Apigenin-7-glucoside	0.601	-0.080
Oleuropein	0.601	-0.052
Luteolin	0.720	0.073
Cinnamic acid	0.755	-0.143
TPC	0.233	0.804
TFC	0.124	0.839
AA	-0.270	0.288

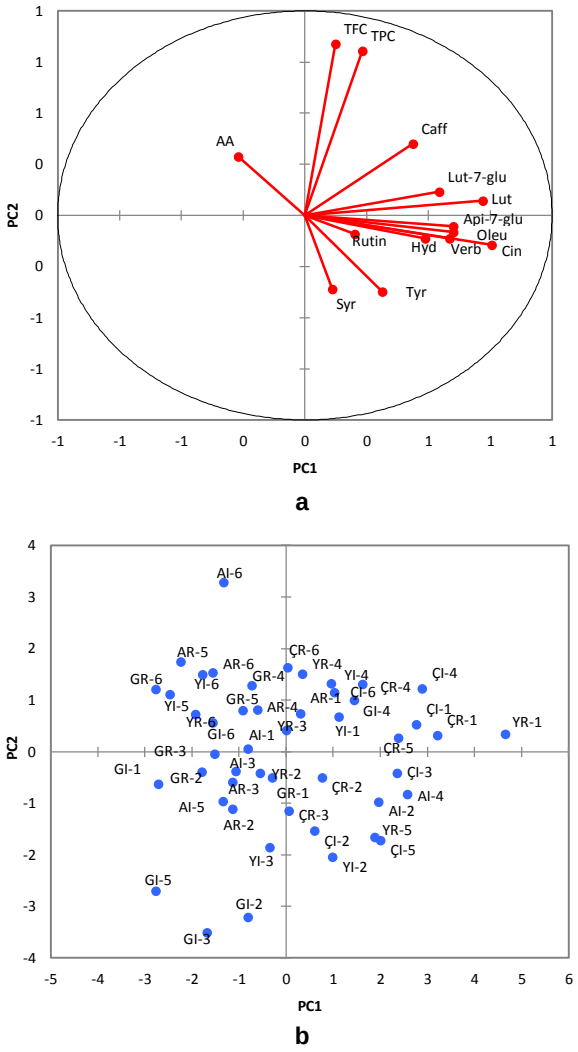


Figure 3. Principal components analysis (a: Loading plot, b: Score plot) of bioactive properties of olive leaves belonged to different varieties collected from irrigated and rainfed orchards during olive harvest period. AA: Antioxidant activity, TFC: Total flavonoid content, TPC: Total phenolic content, Caff: Caffeic acid, Lut-7-glu: Luteolin-7-glucoside, Lut: Luteolin, Api-7-glu: Apigenin-7-glucoside, Oleu: Oleuropein, Cin: Cinnamic acid, Verb: Verbascoside, Hyd: Hydroxytyrosol, Tyr: Tyrosol, Syr: Syringic acid, A: Ayvalık, G: Gemlik, Ç: Çöpaşı, Y: Yağlık, R: Rainfed, I: Irrigated.

CONCLUSION

Irrigation process is applied in several olive orchards on account of the fact that it causes positive differences in certain properties (such as fruit growing) of olives, although the olive tree is drought resistant. The effect of irrigation on the physicochemical properties and bioactive compounds of olives was reported in several studies. However, there is no study on the influence of irrigation on the phenolics of olive leaves, which are rich in bioactive compounds and are consumed as extracts or leaves. According to results obtained, not only variety and harvest time, but also irrigation significantly affected the total phenolic content, total flavonoid amounts, and antioxidant activities of olive leaves. In addition, the irrigation process increased the rutin and luteolin-7-glucoside contents of Çöpaşı leaves, and hydroxytyrosol amounts of Yağlık variety, while the reduction was observed in tyrosol and luteolin-7-glucoside contents of Yağlık leaves; hydroxytyrosol and oleuropein contents of Çöpaşı leaves, and tyrosol contents of Gemlik variety during the harvest period from September to December. In Ayvalık variety, the concentrations of phenolic compounds fluctuated regarding to collection time. In view of the results, responses of olive leaves to irrigation process showed differences according to variety during harvest period.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared toinfluence the work reported in this article.

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