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RESEARCH ARTICLE

Research on The Antioxidant Efficiacy of Olive (*Olea Europaea* L.) Leaf Using by *in vitro* Methods

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ABSTRACT

In this study, antioxidant properties of olive (*Olea europaea* L.) leaves containing oleuropein were investigated in *n*-hexane, ethyl acetate, and methanol based extracts obtained from dried olive leaves at different concentration by various *in vitro* methods. Percentage yields of the *O. europaea* were found for the *n*-hexane extract 13.21%, for ethyl acetate extract 26.15% and for methanol extract 34.59%, respectively. Total phenolic substance content (85.27±15.03%), linoleic acid reduction (89.52±9.77%) and reduction capacity (1.49±0.03) were the highest in the methanol extract. Finally, the DPPH radical scavenging efficiency (72.93±0.42%), reduction of iron (II) ions (50.53±5.53%) and superoxide radical scavenging activity (72.93±0.42%). were the highest in the ethyl acetate extract. In conclusion, it was seen that total phenolic substances, linoleic acid reduction and reduction capacity of the methanol extract of the antioxidant activity of the *O. europaea* was more active when equated with *n*-hexane and ethyl acetate extracts. Also, it was found out that the ethyl acetate extract was more effective in DPPH radical scavenging, iron reduction, linoleic acid reduction and superoxide radical scavenging activity. According to the data obtained, it is thought that olive leaf might be evaluated as a natural and cheap alternative antioxidant in different fields such as food, cosmetic, pharmaceutical industry and animal nutrition.

Keywords: Antioxidant activity, *Olea europaea* L., Oleaceae, Olive, Total phenolic substance

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Zeytin (*Olea Europea L.*) Yaprağının Antioksidan Etkilerinin *in vitro* Yöntemlerle Araştırılması

ÖZ

Bu çalışmada pek çok sekonder metabolitin yanında major madde olarak oleuropein içeren zeytin (*Olea europaea* L.) yaprağından elde edilen edilen *n*-hekzan, etil asetat ve metanol ekstrelerin farklı konsantrasyonlardaki antioksidan aktiviteleri çeşitli *in vitro* yöntemlerle incelenmiştir. Zeytin yaprağının *n*-hekzan, etil asetat ve metanollü ekstrelerinin yüzde verimleri sırasıyla %13.21, 26.15 ve %34.59 olarak bulunmuştur. Toplam fenolik madde içeriği (85.27±15.03%), linoleik asit indirgeme (89.52±9.77%) ve indirgenme kapasitesi (1.49±0.03) methanol ekstresinde en yüksek idi. Son olarak, DPPH radikali süpürücü aktivitesi (72.93±0.42%), demir (II) iyonlarını indirgeme (50.53±5.53%) ve süperoksit radikali giderme aktivitesi (72.00±1.35%) etil asetat ekstresinde en yüksekti. Sonuç olarak zeytin yaprağının metanollü ekstresinde toplam fenolik madde, linoleik asit indirgeme ve indirgenme kapasitesi diğer ekstrelere göre daha aktif bulunmuştur. DPPH radikali tutma, demir (II) şelatlama ve süperoksit radikali giderme aktivitesinde ise etil asetat ekstresinin daha etkili olduğu tespit edilmiştir. Elde edilen veriler doğrultusunda, zeytin yaprağının gıda, kozmetik, ilaç endüstrisi ve hayvan besleme gibi alanlarda doğal alternatif bir antioksidan olarak değerlendirilebileceği düşünülmektedir.

Anahtar Kelimeler: Antioksidan aktivite, *Olea europaea* L., Oleaceae, Toplam fenolik madde, Zeytin

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INTRODUCTION

Olive and olive products have important place from in terms of Turkey economy, in which ranks 4th in the field of olive cultivation among the world countries (Keser and Bilal 2010). In Turkey, olive leaves remaining after harvesting the fruits are generally to be destroyed by burned due to they are waste. Investigations for evaluation of the biological activity have revealed that leaves of this plant to possess antibacterial, antifungal and antioxidant properties (Benavente-García et al. 2000, Ferreira et al. 2007, Lee et al. 2010). Olive, a valuable plant in terms of nutritional value, contains water (50%), lipid (22%), protein (1.6%), sugar (19.1%), cellulose (5.8%), minerals, hydrocarbons, tocopherols and polyphenols (Kiritsakis 1998). Olive leaf also contains high functional value phenolic compounds (tyrosol, coumaric acid, hydroxytyrosol, gallic acid, caffeic acid, ferulic acid), flavonoids (luteolin, quercetin, catechin, apigenin) and secoiridoids (oleuropein, ligstrocyte) (Dekanski et al. 2009, Talhaoui et al. 2015). Oleuropein, which causes bitter and acrid aroma of olive, is the main component of olive tree (Silva et al. 2006) and has antioxidant, antimicrobial, antiviral, anti-inflammatory, antiatrogenic, hypolipidemic, antiaging, and parkinson, alzheimer, skin, heart and cancer-protective effects (Ferreira et al. 2007, Omar 2010, Barbora et al. 2014).

Olive leaf extracts are used primarily in animal nutrition and biomass energy. In addition, it is assumed that presence of excessive level of cardiotonic substance oleanolic acid in olive leaves may prove it beneficial for hypertesnive associated cardiac arrests (Somove et al. 2003).

Leaves of olive usage in the food, cosmetics and pharmaceutical industry is becoming increasingly common (Spinelli et al. 2010, Rodrigues et al. 2015, Souilem et al. 2017). Although there are studies to reveal different biological effects of olive, which have been reported to have anti-inflammatory, antiallergic, antibacterial, antimycotic, immunoregulator, antidiabetic and hypolipemic effects (Omar 2010), researches on *in vitro* or *in vivo* effects of olive leaves are quite insufficient.

The objective of this study is to investigate the antioxidant properties of n-hexane, ethyl acetate and methanol based extracts obtained from olive leaves using *in vitro* methods.

MATERIALS and METHODS

Plant material

Olea europaea L. (Oleaceae) leaves were acquired from Selçuk district of İzmir province, Turkey in December 2017. The leaf samples after collection were first verified a by Prof. Dr. Esra Akkol, Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey and were deposited in the Herbarium of same Faculty.

Preparation of extracts

Leaves (400 g) of *O. europea* were grinded after comlete shade-driying. The grounded powder was later extracted with 2000 mL n-hexane, ethyl acetate and methanol each, for 5 days at room temperature. The collected extracts were first strained and later vaporized under low pressure at 40°C till there were completely dried. The percentage yields of each extract were calculated and were found to be 13.21% for *n*-hexane, 26.15% for ethyl acetate, and 34.59% for methanol, respectively.

HPLC analysis

The high-performance liquid chromatography (HPLC) analysis was conducted by using 1200 series HPLC system by Agilent Technologies (Palo Alto, CA, USA) according to method as described by Ansari et al. (2006). The system consisted of a quaternary pump, in-line degasser, column thermostat, and ultraviolet detector. Agilent Chemstation software was used for the data processing and acquisition. The chromatographic separation of the fractions was performed by a LiChrospher® 100 RP C-18 (film thickness, 5 μm; 250 × 4.0 mm) (Supelco Inc., Bellefonte, PA, USA) column using an isocratic elution of HPLC-grade water adjusted to pH 2.5 with *o*-phosphoric acid:acetonitrile (80:20 vol/vol). Detection wavelength was 280 nm, and the flow rate was optimized at 1 mL/minute. The stock solution (1 mg/mL) of oleuropein (Chromadex, Irvine, CA, USA) was prepared by dissolving it in HPLC-graded water and was used as reference. The calibration curve was achieved by diluting the stock seven times, and the equation was designed. The methanol extract was solubilized in methanol, while ethyl acetate extract was liquefied in a small quantity of ethyl acetate and then diluted with isopropanol to the desired volume. Oleuropein and the all other extracts were filtered by using a membrane filter (pore size, 0.45 μm). The reference compound and diluted extracts were inoculated for evaluation of their oleuropein concentration.

In vitro antioxidant property tests

Estimation of total phenolic substances

The overall phenolic substances of olive leaf extracts were measured using Folin-Ciocalteu's phenol reagent (FCR) as described by Singleton and Rossi (1965). The principle of this method rely on the formation of a blue colored compound as a result of the phenolic substances reducing FCR. The absorbance of blue compound is recordedat 760 nm. The total phenolic substances of olive leaf extracts are presented in terms of mg gallic acid equivalents (GAE) per g of extract.

The gallic acid was used as standard solution. 0.5 mL of test extracts solution was stirred along with 0.5 mL FCR and incubated for 3 minutes. Then, 2% Na_2CO_3

was added and stored at room temperature for 2 h. After incubation period, the absorbance of reaction mixture was recorded at 760 nm against distilled water by using as blank. The results were computed by using the standard calibration curve of gallic acid (62.5-1000 µg/mL**)** and were demonstrated in terms of gallic acid equivalents (GAE µg/g).

Evaluation of DPPH radical scavenging activity

The DPPH scavenging activity of olive leaf extracts was established by using the technique of Blois (1958). The method works on the principle of removing DPPH, which is a stable free radical and has a dark purple colour. When the DPPH radical is scavenged, the colour of the reaction mixture shifts from purple to yellow and reduces absorbance at 517 nm.

Briefly, 0.5 mL olive leaf extracts (100-250-500-1000 µg/mL) were supplemented with DPPH (2 mL, 0.1 mM). Each mixture was placed in the dark for 30 min and the absorbance was recorded at 517 nm against ethanol using as blank. *A* blank is reffered as measure of the absorbance of the control reaction which contains all reagents except test compounds. *A* sample is measure of the absorbance of the olive leaf extracts only. The percentage of inhibition was calculated against blank.

Inhibition % = (*A* blank - *A* sample/ *A* blank) × 100

Antioxidant activity with ferric thiocyanate method in linoleic acid system

Antioxidant activity in a linoleic acid emulsion system was evaluated using the ferric thiocyanate method (Pan et al. 2007). According this method, peroxides, an oxidation product of linoleic acid, oxidize Fe^{2+} to Fe^{3+}. As a result, a decrease in absorbance reflects increased level of antioxidant activity.

The linoleic acid emulsion was developedby adding 77.5 µL of linoleic acid, 87.5 mg of Tween 20 and 25 ml of phosphate buffer (0.04 M, pH 7.0). Then the mixture was homogenized. Variable concentrations (100-250-500-1000 µg/mL) of olive leaf extracts (0.5 mL) were added into linoleic acid emulsion (1.25 mL, 0.2 M, pH 7.0) and phosphate buffer (0.75 mL, 0.04 M, pH 7.0). The blend was incubated at 37°C which accelerated the peroxidation process. The control composed of linoleic acid and phosphate buffer without adding extracts. After an interval of 24 h each, the readings were recorded. The mixture 0.05 mL of misture was removed and mixed thoroughly with 2.35 mL of 75% ethanol, 0.05 mL of 30% ammonium thiocyanate and after 3 minutes with 0.05 mL of 20 mM ferrous chloride in 3.5% HCl. After blendig with all component, the mixture was set out for 5 minutes at room temperature. The levels of peroxidation were calculated by measuring the absorbance at 500 nm.

The percentage of inhibition was determined against control by using following formula:

Inhibition % = 1-(*A* sample/ maximum control) × 100

Determination of iron (II) ions chelating activity

Iron (II) ions chelating activity of olive leaf extracts was performed conferring to protocol described by Dinis et al. (1994). This method works on the principle of inhibition in the formation of ferrous iron-ferrozine complex of chelating agents in the test tube. The decrease in red colour was determined by decrease in absorbance of the ferrous iron-ferrozine complex at 562 nm.

Concisely, altered concentrations of olive leaf extracts (100-1000 µg/mL) in 0.5 mL were mixed with a solution of 0.6 mM ferrous chloride (0.05 mL) and kept for 30 min. Later, the mixture was supplemented with ferrozine (5 mM, 0.1 mL). After 10 min, absorbance of the test tubes and EDTA as standards (50-250 µg/mL) were recorded spectrophotometrically at 562 nm. The control consisted of distilled water only without $FeCl_2$ and ferrozine. The percentageinhibition of iron chelating activity was recorded with following equation:

Inhibition % = 1-(*A* control - *A* sample/ *A* control) x 100

Measure of superoxide radical scavenging activity

The superoxide radical scavenging activity works on the principle that the superoxide radical produced by the NADH (nicotinamide adenine dinucleotide)/PMS (phenazine methosulfate)/O_2 complex reduces nitro blue tetrazolium (NBT) from yellow to purple-coloured formazone (Nishimikiet al. 1972). The decrease of the absorbance values indicates consumption of superoxide radical anions.

According to method, 0.5 mL NBT (156*µ*M) and NADH (468 *µ*M) in the sodium phosphate buffer (20 mM, pH 7.4) were added to different concentrations of extract solutions (0.5 mL, 100-250-500-1000 µg/mL) in phosphate buffer. The reaction began by supplementation of PMS (50µL, 60 µM) into the reaction mixture and incubated at room temperature for 5 minutes. Then, the absorbance was monitored at 560nm against the corresponding distilled water as control.

The percentage of inhibition was calculated by using following formula:

Inhibition % = (*A* control - *A* sample/ *A* control) × 100

Ferric ions reducing antioxidant power

Reducing capacity of olive leaf extracts was determined according to the developed method Oyaizu (1986). The method is based on Fe^{+3} ions are reduced to Fe^{+2} ions. When ferric chloride ($FeCl_3$) is added, the absorbance of the complex compound formed in Prussian blue colour is measured at 700 nm. The highest absorption values mean the highest reduction capacity for extracts.

A 0.5 mL solution of extracts (100-250-500-1000 µg/mL) was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. After incubating at 50°C for 20 minutes, the reaction mixture was further supplemented with 1.25 mL of 10% trichloroacetic acid and vortexed. The reaction mixture was centrifuged at 2500 rpm for 10 minutes. Later, 1.25 mL supernatant was collected and mixed with equal volume of distilled water and 0.25 mL $FeCl_3$ (0.1 %). It was kept for 10 minutes at room temperature and the absorbances were evaluated at 700 nm against distilled water as blank.

Statistical analysis

Antioxidant activity assays was conducted in triplicate. The results obtained were evaluated by SPSS 13.0 and given as mean ± standard error values. The statistical differences among groups were analysed through One way ANOVA analysis. Duncan test was used as a posthoc test. Statistical significance of all data was determined as $P < 0.05$ level.

RESULTS

The results obtained with the n-hexane, ethyl acetate and methanol extracts of the leaves of O. europea in the antioxidant activity tests are given in Table 1-6.

The olive leaf extracts were examined for their total phenolic substance amounts (Table 1) according to acquire from standard gallic acid curve graph (Figure 1). The highest phenolic content of methanolic extract was 85.27±15.03 mg GAE in 1000 µg/mL while the lowest value was 0.18±1.32 µg GAE/g extract in n-hexane extract (500 µg/mL).

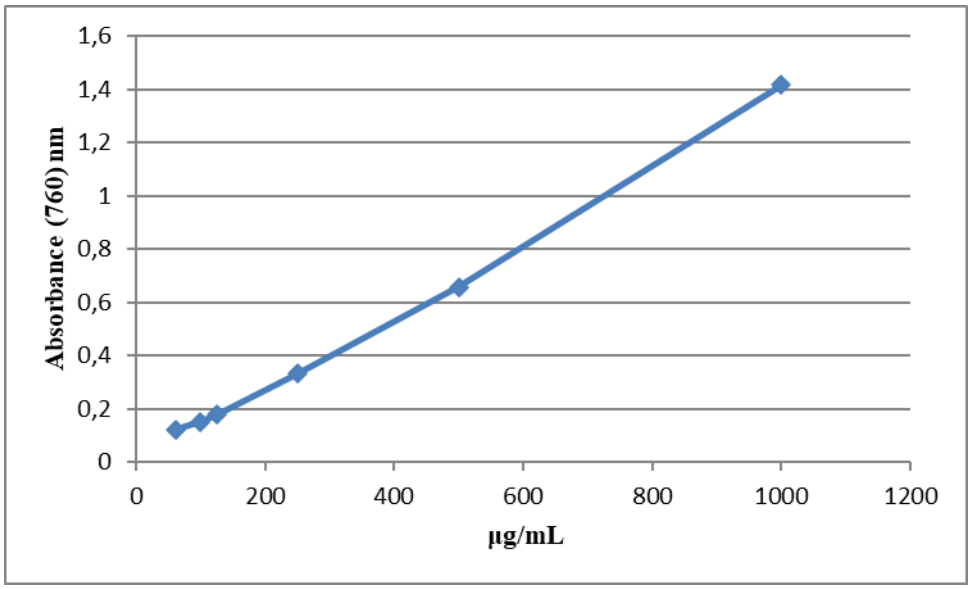

Figure 1. Standard gallic acid curve graphic

Table 1. Total phenolic substances concentration of the extracts obtained from O. europea leaves

Extracts	Total phenolic substances (µg GAE/g ± SEM)			
	100µg mL^{-1}	250µg mL^{-1}	500µg mL^{-1}	1000µg mL^{-1}
***n*-Hexane**	0,4177±2,34^{d}	2,79±0,71^{d}	0,18±1,32^{d}	12,53±3,82^{c,d}
Ethyl acetate	13,49±8,22^{c,d}	14,91±3,59^{c,d}	19,89±2,29^{c,d}	33,67±4,12^{b,c}
Methanol	11,58±0,63^{c,d}	26,78±3,14^{c}	48,39±1,42^{b}	85,27±15,03^{a}

^{a,b,c,d} Different letters in the same column represent the statistically significant difference between the mean values P<0,001
SEM: Standard error meaning

The percentages of DPPH inhibition of olive leaf extracts at different concentrations are shown in Table 2. According to the results, DPPH radical scavenging activity was found to be highest in ethyl acetate extract (72.93 ± 0.42%) at a concentration of 500 µg/mL, the lowest value in n-hexane extract (100 µg/mL) was 13.77 ± 1.23%.

Table 2. DPPH radical scavenging activity of the extracts obtained from *O. europea* leaves

Extracts	DPPH radical scavenging activity (Inhibition % ± SEM)			
	100µg mL^{-1}	250µg mL^{-1}	500µg mL^{-1}	1000µg mL^{-1}
***n*-Hexane**	13,77±1,23^{f}	43,67±1,04^{e}	64,10±4,95^{a,b,c}	71,17±2,93^{a}
Ethyl acetate	42,37±3,34^{e}	61,50±0,79^{b,c,d}	68,40±0,67^{a,b}	72,93±0,42^{a}
Methanol	55,77±1,58^{c,d}	55,67±2,83^{c,d}	54,43±5,17^{d}	60,53±4,33^{b,c,d}

a,b,c,d,e,f Different letters in the same column represent the statistically significant difference between the mean values P<0,001
SEM: Standard error meaning

As shown in Table 3, the highest value linoleic acid reduction was obtained as 89.52±9.77% in methanolic extract at 250 µg/mL concentration, the lowest value as 61.19±4.05% in ethyl acetate extract at 100 µg/mL concentration.

Table 3. Antioxidant activity in linoleic acid system of the extracts obtained from *O. europea* leaves

Extracts	Antioxidant activity in linoleic acid system (Inhibition % ± SEM)			
	100µg mL^{-1}	250µg mL^{-1}	500µg mL^{-1}	1000µg mL^{-1}
***n*-Hexane**	76,19±2,03^{a,b,c}	76,19±5,61^{a,b,c}	75,24±1,26^{a,b,c}	71,67±1,26^{b,c}
Ethyl acetate	61,19±4,05^{c}	63,09±0,48^{c}	65,24±6,67^{c}	86,67±7,63^{a,b}
Methanol	83,57±2,70^{a,b}	89,52±9,77^{a}	74,79±3,93^{a,b,c}	67,67±2,34^{c}

^{a,b,c} Different letters in the same line represent the statistically significant difference between the mean values P<0,001
SEM: Standard error meaning

The effects of various amounts of olive leaf extracts on reduction of iron (II) ions are given in Table 4. While it was found 50.53±5.53% in ethyl acetate extract at 1000 µg/mL concentration, the lowest value was 8.16±0.43% in n-hexane extract 100 µg/mL concentration.

Table 4. Iron (II) ions chelating activity of of the extracts obtained from *O. europea* leaves

Extracts	Iron (II) ions chelating activity (Inhibition % ± SEM)			
	100µg mL^{-1}	250µg mL^{-1}	500µg mL^{-1}	1000µg mL^{-1}
***n*-Hexane**	8,16±0,43^{e}	8,95±0,14^{d,e}	9,05±0,20^{d,e}	8,85±0,18^{d,e}
Ethyl acetate	14,87±1,22^{c,d,e}	16,91±5,22^{b,c,d,e}	25,62±5,90^{b}	50,53±5,53^{a}
Methanol	11,61±0,24^{d,e}	15,33±0,20^{c,d,e}	17,70±0,33^{b,c,d}	23,26±0,31^{b,c}

^{a,b,c,d,e,} Different letters in the same column represent the statistically significant difference between the mean values P<0,001
SEM: Standard error meaning

As shown in Table 5, the superoxide radical scavenging activity was detected 72.00±1.35% in ethyl acetate extract (250 µg/mL), the lowest value as 13.76±2.24% in n-hexane extract at 500 µg/mL concentration.

Table 5. Superoxide radical scavenging activity of the extracts obtained from *O. europea* leaves

Extracts	Superoxide radical removal activity (Inhibition % ± SEM)			
	100µg mL^{-1}	250µg mL^{-1}	500µg mL^{-1}	1000µg mL^{-1}
***n*-Hexane**	13,76±2,24^{f}	31,53±1,97^{e}	39,96±3,52^{d}	51,02±0,46^{c}
Ethyl acetate	70,78±2,99^{a}	72,00±1,35^{a}	63,88±1,65^{a}	67,29±2,23^{a}
Methanol	59,02±2,51^{a,b}	56,90±0,85^{b}	67,02±1,47^{a}	68,82±0,65^{a}

^{a,b,c,d,e,f} Different letters in the same column represent the statistically significant difference between the mean values P<0,001
SEM: Standard error meaning

Finally, according to the results while the highest value in the reduction capacity of extracts was found 1.49±0.03 in methanol extract at 1000 µg/mL concentration and the lowest value was in ethyl acetate extract (100 µg/mL) (Table 6).

Table 6. Reduction capacity of the extracts obtained from *O. europea* leaves

Extracts	Reduction capacity (Absorbance ± SEM)			
	100µg mL^{-1}	250µg mL^{-1}	500µg mL^{-1}	1000µg mL^{-1}
***n*-Hexane**	0,25±0,01^{e,f}	0,23±0,02^{e,f}	0,24±0,01^{e,f}	0,28±0,00^{d,e}
Ethyl acetate	0,19±0,01^{f}	0,25±0,01^{e,f}	0,21±0,00^{d}	0,44±0,01^{c}
Methanol	0,33±0,00^{d}	0,54±0,01^{b}	0,90±0,04^{a}	1,49±0,03^{a}

^{a,b,c,d,e,f} Different letters in the same column represent the statistically significant difference between the mean values P<0,001
SEM: Standard error meaning

To determine the oleuropein concentration in the extracts, HPLC analysis was used. In order to determine the oleurpeins's concentration in all extracts, calibration curve and the equation were first proved by using oleuropein as reference compound. Retention time was recorded as 9.1 ± 3 min for the oleuropein. The reference oleuropein and the all extracts were inoculated respectively to confirm if the detected major peak is oleuropein or not. The repetitive increase in the area of the same peak confirmed the idea that the major peak was oleuropein in the methanol extract. Thus, it has been measured that 1 g of methanol extract contains 24.12 ± 2.87 mg of oleuropein.

DISCUSSION and CONCLUSSION

In the current study, it was recorded that the yield of extraction of olive leaves of the Memecik cultivar was 34.59% in methanol, 26.15% in ethyl acetate and 13.21% in *n*-hexane extracts. Orak et al. (2019) reported the yield of olive leaf extracts varied from 24.26-29.87% and also the yield of methanol extract of Memecik type was found at the level of 27.11%. In addition, the oleuropein in methanol extract was determined as 24.12 ± 2.87 mg / g in this study. The result obtained for oleuropein in the methanol extract is in accordance with previous report which state that oleuropein, the main phenolic component for all genotypes, range from 21.0 to 98.0 mg /g in the methanolic extract (Orak et al. 2019). Orak et al. (2019) reported that oleuropein found at 38.2±1.9 for the methanol extract of Memecik type olive leaf. Moreover, hydroxytyrosol, verbascoside, luteolin 7-*O*-glucoside and luteolin 4′-*O*-glucoside have been widely contained in olive leaves (Talhaoui et al. 2015).

The maximum amount of total phenolic substances was determined as 85.27 ± 15.03 in methanol extract (1000 µg / mL), the lowermost value was found as 0.18 ± 1.32 mg gallic acid (GAE) / g in *n*-hexane extract (500 µg / mL). This value in the methanol extract was found to be lower than 209 ± 3.4 mg GAE/g reported by Orak et al. (2019) for Memecik cultivar olive leaf. Orak et al. (2019) described that total phenolic substances was found as a 110-268 mg GAE/g in the methanol extract of leaves of other *Olea* species growing in Turkey. These differences stem from cultivated conditions, natural habitats of the olive tree.

In this study, the highest value in the DPPH radical scavenging activity was measured 72.93±0.42% for ethyl acetate extract at 500 µg/mL concentration and the lowest value was 13.77±1.23% in *n*-hexane (100 µg/mL). Addition, it was found in this study that when the concentration of extracts in methanol, ethyl acetate and *n*-hexane groups decreased, DPPH radical scavenging activity was found to decreasing accordingly. Hayes et al. (2009), olive leaf methanol extract DPPH radical scavenging activity increased from 100 ppm to 400 ppm and this increase reached 94% at 1000 ppm. Our results were consistent with these reported results. Stupans et al. (2002) reported that this activity was due to hydroxytyrosin and oleuropein, a radical scavenging agent in olive leaves. Non-enzymatic lipid peroxidation caused by free radicals begins with lipid peroxidation of H atoms containing an electron from conjugated double bonds in fatty acids and the fatty acids in the membrane are converted to lipid free radicals. These radicals are unstable and are converted to oxygen by lipid peroxide radical (LOO·) and then by electron from other fatty acids to lipid peroxides (LOOH). Malondialdehydes are orginated as a result of peroxidation of fatty acids while chaining continues (Halliwel and Gutterigde 1990). In our study, the highest value linoleic acid reduction was found as 89.52±9.77% in methanol extract at 250 µg/mL concentration, the lowest value as 61.19±4.05% in ethyl acetate extract at 100 µg/mL concentration. Danahaliloğlu et al. (2018) found that the % inhibition values in the methanolic extract of the Halhalı type olive leaf ranged between 72.89-41.32%. The highest value in reduction of iron (II) ions was found as 50.53±5.53% in ethyl acetate at 1000

µg/mL concentration, the lowest value as 8.16±0.43% in *n*-hexane 100 µg/mL concentration. Hayes et al. (2009) reported that iron chelating activity was found in methanolic extracts as ellagic acid>sesamol> olive leaf extract> lutein, respectively.

It is known that superoxide dismutase (SOD) is a key antioxidant enzyme and catalyses the dismutation of the superoxide (O_2^-) radical into eithermolecular oxygen (O_2) or hydrogen peroxide (H_2O_2). SOD has three isozymes as Mn-SOD, Fe-SOD and CuZn-SOD, which localized in different cell compartments of olive leaf (Corpas et al. 2006; Halliwell et al. 2000). According to our findings, the highest superoxide radical scavenging activity was found 72.00±1.35% in ethyl acetate extract (250 µg/mL), and was measured the lowest value as 13.76±2.24% in n-hexane extract (500 µg/mL). Lee and Lee (2010) reported that SOD activity was determined at 18% in oleuropein of olive leaves, 67% in routine, 83% in caffeic acid and 19% in their mixtures. Finally, the highest reduction capacity was found 1.49 ± 0.03 in methanol extract (1000 µg / mL) and the lowest value was 0.19 ± 0.01 in ethyl acetate extract (100 µg /mL) in our study. Danahaliloğlu et al. (2018) reported that FRAP values for methanol extracts of olive leaves of Halhali type were between 0.96-0.11.

Various antioxidant activity differences observed in the olive leaf extracts are thought to be due to the level of polyphenolic compounds passing into the solvent used and the difference in their chemical structure. Considering the extracts according to polarity, it was found that phenolic substances dissolved in methanol extract alone or together synergistically reduce free radicals, chelate metal ions, prevent lipid peroxidation and have metal-reducing capacities.

As a result, it was determined that the methanolic extract of olive leaf was found to be more active than other extracts in terms of total phenolic substances, linoleic acid reduction and reduction capacity. Considering this study data, where the antioxidant activities of olive leaves in different extracts were determined, it was concluded that olive leaf might be regarded as a natural alternative antioxidant source in food, cosmetics, pharmaceutical and animal nutrition fields.

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Conflict of Interest: The authors declare that they have no conflict of interest

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