Turkish Journal of Agricultural and Natural Sciences 12 (2): xxx–xxx, 2025

https://doi.org/10.30910/turkjans.1655464

TÜRK TARIM ve DOĞA BİLİMLERİ DERGİSİ

TTDB

TURKISH JOURNAL of AGRICULTURAL and NATURAL SCIENCES

www.dergipark.gov.tr/turkjans

Original Article

Determination of Phenolic Content and Antioxidant Capacity of Olives in Fethiye Region

Ertan Şahinoğlu[1], Burcu Kabak[2], Erdal Kendüzler[2*]

[1]Republic of Türkiye Ministry of Trade, Fethiye Customs Directorate, 48300 Fethiye, Muğla, Türkiye

[2] Burdur Mehmet Akif Ersoy University, Faculty of Arts and Science, Chemistry Department,15100 Burdur, Türkiye

[1] https://orcid.org/0009-0003-4449-4006, [2] https://orcid.org/0000-0003-4217-1767, [2] https://orcid.org/0000-0002-9457-1503

✉: kenduzler@mehmetakif.edu.tr

ABSTRACT

In this study, the phenolic compound content and antioxidant activity of olive fruits collected from olive groves in the Fethiye region of Türkiye from different geographical directions (North, South, East and West) were investigated. Olive samples were extracted using different solvents, and the total phenolic content of the extracts was determined by spectrophotometric method. Individual phenolic compound analysis HPLC and antioxidant activities were determined by free radical cleaning analyses. The results showed that olive fruit has a wide range of phenolic compounds, such as katehin, ellagik, epicathery, gallic acid, protocolic, siriyngik, caffeic, sinnamic, vanilik, p-cumaric, ferulic, rintirinic, and routine. In addition, significant differences have been determined between breeding zones in terms of phenolic composition and antioxidant capacity due to genetic and environmental factors. These results reveal that olives in the Fethiye region can be considered as important functional food components in terms of health and contribute to the regional economy.

Key words: Olive, phenolic compound, antioxidant activity, DPPH, Fethiye

Fethiye Bölgesindeki Zeytinlerin Fenolik İçeriği ve Antioksidan Kapasitelerinin Belirlenmesi

ÖZ

Bu çalışmada, Türkiye'nin Fethiye bölgesindeki zeytin bahçelerinden farklı coğrafi yönlere (kuzey, güney, doğu ve batı) göre toplanan zeytin meyvelerinin fenolik bileşik içerikleri ve antioksidan aktiviteleri incelenmiştir. Zeytin örnekleri, farklı çözücüler kullanılarak ekstrakte edilmiş ve elde edilen özütlerin toplam fenolik içeriği spektrofotometrik yöntemle, fenolik madde profil analizi HPLC ile, antioksidan aktiviteleri ise serbest radikal süpürme analizleriyle belirlenmiştir. Sonuçlar, zeytin meyvesinin katehin, ellagik, epikateşin, gallik asit, protokateşüik, siriyngik, kafeik, sinnamik, vanilik, p-kumarik, ferulik, rozmariniik, rutin gibi geniş bir fenolik bileşikler yelpazesine sahip olduğunu göstermiştir. Ayrıca, yetiştirme bölgeleri arasında genetik ve çevresel faktörlere bağlı olarak fenolik kompozisyon ve antioksidan kapasite açısından önemli farklılıklar tespit edilmiştir. Bu sonuçlar, Fethiye bölgesindeki zeytinlerin sağlık açısından önemli fonksiyonel gıda bileşenleri olarak değerlendirilebileceğini ve bölgesel ekonomiye katkı sağlayabileceğini ortaya koymaktadır.

Anahtar kelimeler: Zeytin, Fenolik Bileşik, Antioksidan Aktivite, DPPH, Fethiye

INTRODUCTION

The olive tree (Olea europaea L.), indigenous to the Mediterranean region, has long been a significant agricultural and economic asset. Its cultivation can be traced back to 3000 BC, with ancient societies such as Crete and Egypt utilising it for farming and trade purposes (Rocha et al., 2020; Haddad et al., 2020). Today, the Mediterranean region remains a crucial hub for olive and olive oil production, accounting for 98% of global olive output (Haddad et al., 2020; Loureiro et al., 2006). Olive fruits are known for their distinctive bitter taste and high oil content. Olive oil is obtained by processing olive fruit and is one of the basic components of the

Mediterranean diet (Sánchez-Romero, 2021; Marcelino et al., 2019). Olive oil is rich in health-promoting phenolic compounds and unsaturated fatty acids, which are beneficial to heart health (Marcelino et al., 2019; Al-Asmari et al., 2021). In addition, olives and olive oil have historically played an important role in the diets of indigenous peoples in the Mediterranean region (Rocha et al., 2020; Maraulo et al., 2021). Olive trees are long-lived, resistant plants that make them attractive for agricultural production (Sánchez-Romero, 2021; Maesano et al., 2021). Olive cultivation is considered not only an economic activity but also a cultural heritage. Suitable conditions provided by the Mediterranean climate for olive cultivation allow a large part of the worldwide production of this plant to occur in this region (Haddad et al., 2020; Loureiro et al., 2006).

Phenolic compounds are recognised as important antioxidant molecules, owing to their ability to counteract oxidative stress. The hydroxyl (OH) groups in their structure provide the capacity to neutralise free radicals by donating electrons and hydrogen. The redox properties of phenolic compounds make them effective electron donors and free-radical scavengers, which forms the basis of their antioxidant activity (Chandra et al., 2014; Rudrapal et al., 2022). These properties enable the synthesis of phenolic compounds in response to ecological and physiological pressures in plants, thereby becoming an integral part of their defence systems (Khoddami et al., 2013). According to Duh and Yen (1995), the ability of phenolic compounds to inhibit lipid peroxidation is related to their antioxidant properties. It has been observed that the consumption of foods rich in phenolic compounds reduces the risk of cardiovascular diseases and slows down the development of atherosclerosis (Vázquez-Ruiz et al., 2022; Dueñas et al., 2004). In this context, the beneficial effects of phenolic compounds on cellular mechanisms stand out, particularly in protecting mitochondrial function and delaying cellular ageing. Studies have shown that, owing to their strong antioxidant activity, phenolic compounds provide protective effects by reducing cellular stress (Vázquez-Ruiz et al., 2022; Sharifi-Rad et al., 2020).

The phenolic composition of olives varies depending on various factors such as growing conditions, fruit maturity, and environmental stress factors. The techniques used during olive processing can also affect phenolic compound content. For example, it has been determined that different olive varieties and cultivation methods affect the total phenolic content in olive oil. In addition, the quality of olive oil is directly related to the presence of phenolic compounds; olive oils with higher phenolic contents have greater health benefits (Cerretani et al., 2010; Guasch-Ferré et al., 2014; Rohman et al., 2019). In recent years, with the increasing interest in olives and olive oil, scientific studies on the effects of these products on health have also increased. In particular, the positive effects of olive oil on cardiovascular health have been supported by large-scale studies such as the PREDIMED (PREvención con DIeta MEDiterránea) study. These studies show that olive oil, especially that with high phenolic content, supports heart health and reduces the risk of metabolic diseases (Guasch-Ferré et al., 2014; Yubero-Serrano et al., 2018).

The Fethiye region has suitable climate and soil conditions for olive cultivation. Determining the phenolic content and antioxidant capacity of olives in the region will help to understand the potential health benefits of this valuable agricultural product and reveal its contribution to the regional economy. The aim of this study was to evaluate changes in the phenolic compound content and antioxidant activities of olive samples collected from different geographical locations and altitudes in the Fethiye region. In addition, the relationship between these changes and environmental factors such as altitude above sea level and geographical direction was investigated.

MATERIALS AND METHODS

Materials and Instruments

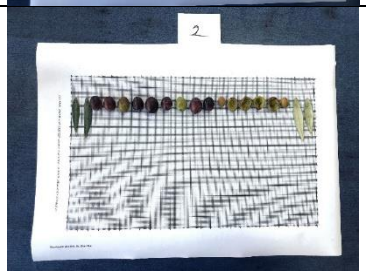
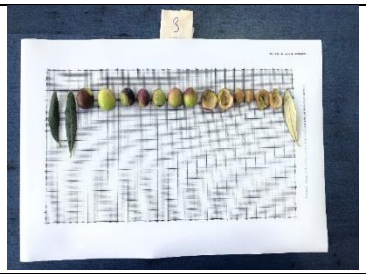
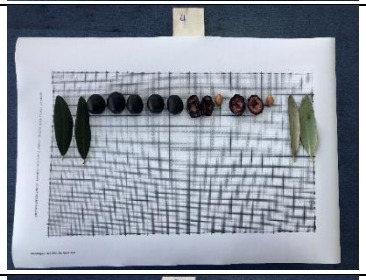
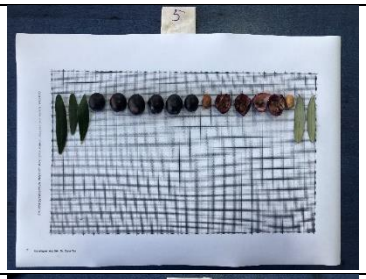
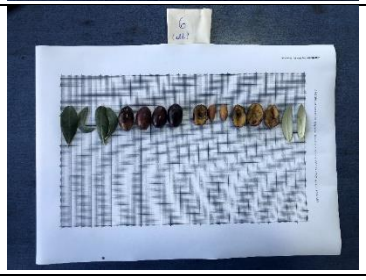
The chemicals used in this study were obtained from different suppliers. Folin reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH•) and sodium carbonate (Na_2CO_3) were supplied by Sigma-Aldrich; methanol, ethanol, acetone and gallic acid were supplied by Isolab. Catechin, ellagic acid, epicatechin, gallic acid, protocatechuic acid, syringic acid, caffeic acid, cinnamic acid, vanillic acid, p-coumaric acid, ferulic acid and rosmarinic acid used in HPLC analyses were supplied by Merck.

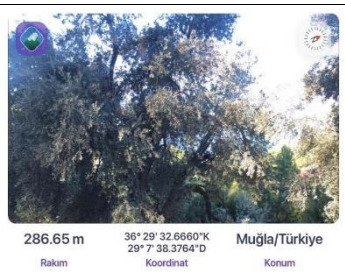
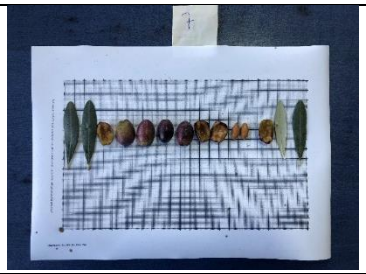
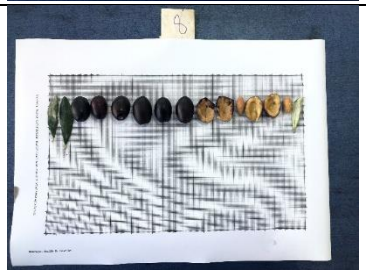
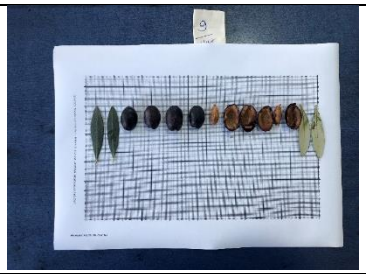
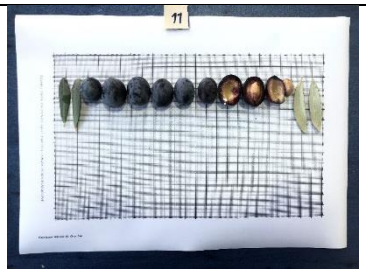
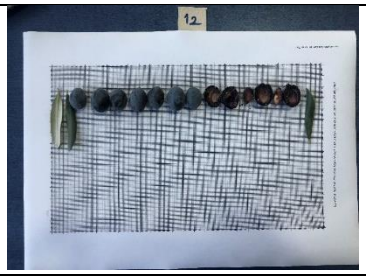
The devices used in the analyses included a PG Instruments T60 model spectrophotometer for absorption measurements, a Kern ACS 220-4 model precision balance for weighing, a Memmert UN 110 model oven for the heating and drying processes, and a Heidolph brand evaporator to remove the solvent after extraction. The HPLC system used for the determination of phenolic substances in olive extracts belongs to the Shimadzu Prominence brand and has a DAD detector, SPD-M20A system controller, SIL-20ACHT automatic sampler, and an LC-20 AT pump system. A Zorbax C18 (220 × 4.60 mm) 5 µm HPLC column was used.

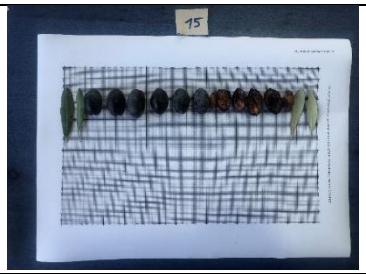
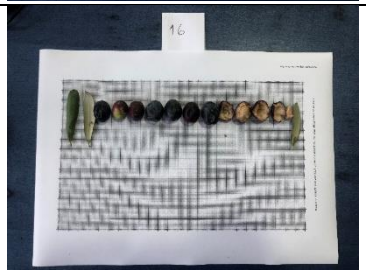
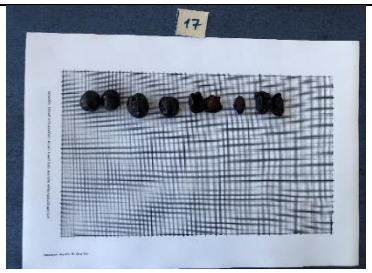
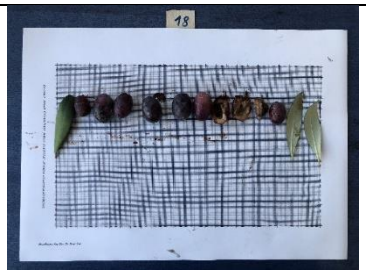
Collection of Olive Fruits and Preparation of Extracts

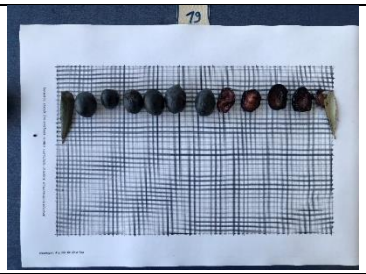
Olive fruits were collected from olive groves in the Fethiye region, taking into account different geographical directions (north, south, east, and west) and elevations above sea level, and stored at -16°C until extraction. The location of the olive samples is presented in Table 1.

Table 1. Locational Information of Collected Olive Samples

Address	Direction	Coordinate	Olive Samples
Karagözler neigh.	North		
Kayaköy neigh.	South east		
Kayaköy neigh.	West		
Ölüdeniz neigh.	South		
Ölüdeniz neigh.	North east		
Kelebek Valley	South		

Faralya neigh.	West		
Kabak Cove	South		
Ovacık neigh.	South west		
Taşyaka neigh.	North		
Günlükbaşı neigh.	North		
Yeşilüzümlü neigh.	North		

Yeşilüzümlü neigh.	North		
Yeşilüzümlü neigh.	North		
İncir Village	South		
Karaçulha neigh.	South west		
Karagedik neigh.	South		
Çiftlik neigh.	North		

Yanıklar	East		
Göcek	South		

In this study, four different solvents with different polarities (ethanol, methanol, acetone, and water) were used in the extraction process, and the bioactive performance of the obtained liquid extracts was compared. The extraction processes performed with mixtures of solvents with the highest bioactive activity prepared with different water ratios were also evaluated, and the most suitable solvent-water mixture was determined. The solvent-water mixture obtained as a result of this optimization was also applied to other olive samples. The extraction process for the preparation of olive extracts was performed in a double-necked flask. In this process, 3 g of a fresh olive sample was mixed with 30 mL of the selected solvent and extracted at 30°C for 45 min. After the extraction was complete, the filtrate was passed through a blue band filter paper to separate the supernatant and evaporated under vacuum at 40°C to remove the solvent.

Determination of Total Phenolic Substance and Antioxidant Activity (DPPH Radical Scavenging Test)

Total phenolic substance amounts in olive samples was determined according to the method reported by Singleton and Rossi (1965). For this purpose, 5 mL of 0.2 N Folin Ciocelteau reagent was added to 100 µL of olive extract and the mixture was kept in the dark for 5 min. Then, 4 mL of 7.5% Na_2CO_3 and 900 µL of distilled water were added to the mixture, which was tightly closed, vortexed, and kept in the dark for 2 h at room temperature (20-25 ºC). At the end of two hours, the absorbance values of the samples were determined using a UV-vis spectrometer at a wavelength of 765 nm. For the gallic acid calibration curve, methanol solutions of gallic acid were prepared at 5 different concentrations (0.5-5 mg/L). The aforementioned procedures were also applied to gallic acid solutions of different concentrations prepared for the calibration curve. Total phenolic substance amounts were calculated as mg GAE/kg using a calibration chart prepared with gallic acid standard. Each measurement was repeated thrice.

The antioxidant capacity of olive extracts was determined based on the DPPH radical scavenging activity (Mishra et al., 2012). Different volumes of the extraction product (25, 50, 100, 200, 400, and 600 µL) were placed in glass tubes and 600 µL of 1 mM DPPH radical solution was added. The final volume was adjusted to 6 mL with ethyl alcohol. The mixtures were then incubated in the dark for 15 min at room temperature. The absorbance was measured at a wavelength of 517 nm against the prepared control solution (pure ethyl alcohol and DPPH radicals). The results are expressed as % inhibition and IC_{50} values. The individual inhibition values for each olive sample were calculated using the following equation:

$$\%\ \boldsymbol{inhibition} = \frac{\boldsymbol{A_0 - A_1}}{\boldsymbol{A_0}} \boldsymbol{X100}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the reaction mixture.

HPLC Analysis

For the HPLC analysis of phenolic compounds in plant extracts, calibration graphs of 15 different phenolic compound standards were drawn separately. Two different solvents were used as the mobile phases in the analyses (A: 3% formic acid and B: Methanol). The gradient program was as follows: 93% A + 7% B for three minutes, 72% A + 28% B for 28 min; 67% A + 33% B for 60 min; 58% A + 42% B for 62 min; 50% A + 50% B for 70 min; 30% A + 70% B for 75 min; and 93% A + 7% B for 90 min (Gomes vd.,1999). The results obtained from the HPLC analysis are given in micrograms/gram with a 95% confidence interval.

Statistical Analysis

The total phenolic content of olive fruits collected from olive groves in the Fetiye region considering geographical directions (north, south, east, and west) and altitudes above sea level were applied by both One-Way ANOVA and Kruskal-Wallis tests according to which group the samples belonged to (e.g., altitude groups, direction groups, etc.). These analyses allowed us to determine which factors (altitude, direction, etc.) created statistically significant differences in the composition of olives.

RESULTS AND DISCUSSION

Determination of Total Phenolic Substance Quantity

The total phenolic content of olive extracts was determined using the Folin-Ciocaltaeu method. The gallic acid solutions used as standards were prepared in ethanol at five different concentrations. Absorbance readings were recorded at a wavelength of 765 nm. A gallic acid calibration graph is shown in Figure 1.

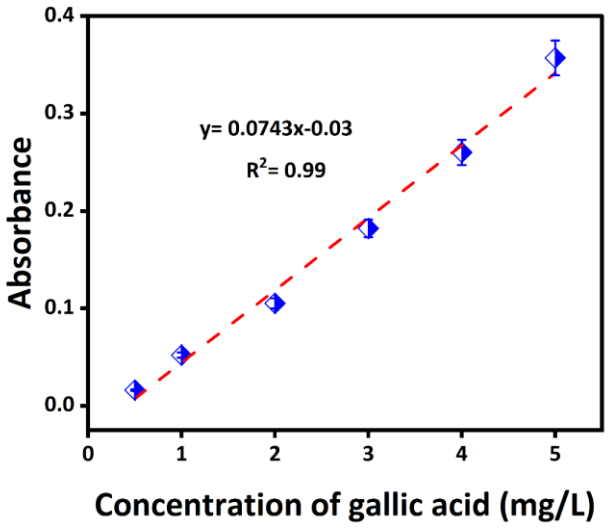


Figure 1. Calibration graph of gallic acid

Various phenolic compounds with varying polarity properties exist in the olive fruit. Depending on their structure, these compounds show better solubility in solvents with different polarities. For example, polar solvents such as water are effective in the extraction of more polar phenolic compounds (such as hydroxytyrosol), whereas less polar solvents such as ethanol, methanol, or acetone provide better extraction of less polar phenolic substances (such as oleuropein). The purpose of the extraction process using solvents of different polarities is to obtain the widest possible range of phenolic compounds in the olive fruit. Thus, the substance content and antioxidant capacity of olive oil or other olive products can be determined more comprehensively. In addition, extracts obtained with different solvent polarities can be compared in terms of their phenolic profiles and bioactivity. This allowed for the determination of the most effective antioxidant compounds in olive fruit suits. For this purpose, firstly, the solvent type with the highest phenolic composition was determined by using extracts obtained using four different solvents (water, methanol, ethanol, and acetone) for an olive fruit sample. The results obtained are listed in Table 2.

Table 2. Phenolic substance amounts of extracts obtained with different solvents

Solvents	Total Phenolic Substance Amount (mg GA/g)
Water	0.74± 0.04
Ethanol	0.68± 0.03
Methanol	1.68± 0.06
Acetone	0.75± 0.04

As shown in Table 2, the highest gallic acid content was obtained using methanol as the solvent, yielding 1.193 ± 0.059 GA mg/g, whereas the lowest content was recorded with ethanol, at 0.684 ± 0.034 GA mg/g. According to the obtained results, the methanol extract was richer in terms of phenolic substances than the other solvent extracts. Some compounds may have higher solubilities in mixed solvent systems than in pure solvents. The polarity of the solvent system was optimized by changing the methanol/water ratio. This can lead to a solvent system with a polarity that matches well with the target compounds and more efficient extraction (Zuorro et al., 2014). For this purpose, the most efficient solvent system was determined for the extraction of olive fruits using different methanol-water fractions (75%, 50%, and 25% v/v). The obtained data are presented in Table 3.

Table 3. Phenolic substance amounts in extracts obtained in different methanol-water fractions

Solvent ratio	Total Phenolic Substance Amount (mg GA/g)
Methanol (100%)	1.75± 0.09
Methanol-water (75% v/v)	4.19± 0.21
Methanol-water (50% v/v)	3.88± 0.19
Methanol-water (25% v/v)	2.39± 0.12

When Table 3 is examined, it is seen that the highest value in terms of phenolic substance was obtained with 75% methanol-water ratio. In line with the obtained results, extracts of other olive fruits were obtained using a solvent system with a 75% methanol-water ratio, and the results are given in Table 4.

Table 4 presents the TPSA of olive samples collected from various locations within the Fethiye region, expressed as mg GA/g. The results demonstrated significant variation in phenolic content based on geographical location and altitude. Notably, samples collected from higher altitudes such as Yeşilüzümlü (505 m, 8.70 mg GA/g), İncir (653 m, 5.21 mg GA/g), and Karagedik (42 m, 6.82 mg GA/g) exhibited higher phenolic contents compared to those from lower altitudes like Kelebek Valley (362 m, 1.99 mg GA/g) and Faralya (287 m, 1.91 mg GA/g). This suggests that environmental stressors commonly associated with higher altitudes, such as increased exposure to ultraviolet (UV) radiation, temperature fluctuations, and reduced oxygen levels, may enhance phenolic biosynthesis as part of the plant's natural defense mechanisms. Additionally, the orientation of olive cultivation sites plays a critical role in the phenolic content, as regions exposed to more sunlight generally show higher TPSA values. For instance, samples from southern-oriented locations, such as Yeşilüzümlü and Karaçulha, demonstrated higher TPSA values than those from shaded or less exposed areas. Furthermore, regional differences may be attributed to variations in soil composition, microclimate conditions, and other environmental factors specific to each area. This variability highlights the complexity of phenolic compound production in olives and underscores the importance of environmental factors in determining phenolic profiles. In addition, one-way ANOVA and Kruskal-Wallis tests were applied to the results obtained for a more detailed examination. The results are presented in Table 5.

When the data in Table 5 were examined, there was a statistically significant difference between the altitude groups for both analysis models ($p<0.05$), while there was no statistically significant difference between the direction groups. As a result, the total phenolic content of plant samples in the Fethiye region was closely related to the altitude of the samples but was not affected by geographical orientation. Such regional differences are important in terms of showing the extent to which the secondary metabolite content of plants is affected by environmental factors.

Table 4. Total Phenolic Substance Amounts (TPSA) of Olive Fruits According to Their Locations

Number	Address	Height Above Sea Level (m)	Direction	Coordinate	TPSA (mg GA/g)
1	Karagözler neigh.	~90	North	36°36'59.1396"N 29°6'37.3842"E	3.62±0.18
2	Kayaköy neigh.	~169	South east	36°35'18.8264"N 29°5'44.9359"E	4.04±0.21
3	Kayaköy neigh.	~151	West	36°35'10.1508"N 29°5'46.3700"E	3.66±0.18
4	Ölüdeniz neigh.	~29	South	36°33'3.2981"N 29°7'20.7996"E	2.89±0.14
5	Ölüdeniz neigh.	~15	North east	36°31'58.1260"N 29°7'34.3226"E	2.23±0.11
6	Kelebek Valley	~362	South	36°30'4.8178"N 29°7'38.4199"E	1.99±0.09
7	Faralya neigh.	~287	West	36°29'32.6660"N 29°7'38.3764"E	1.91±0.08
8	Kabak Cove	~82	South	36°27'51.9167"N 29°7'44.3890"E	2.81±0.14
9	Ovacık neigh.	~334	South west	36°24'14.3364"N 29°8'38.3174"E	3.87±0.19
10	Taşyaka neigh.	~18	North	36°37'22.3386"N 29°8'48.7133"E	2.91±0.14
11	Günlükbaşı neigh.	~10	North	36°38'44.0104"N 29°8'6.1419"E	4.61±0.23
12	Yeşilüzümlü neigh.	~505	North	36°44'50.7658"N 29°14'15.1012"E	8.70±0.43
13	Yeşilüzümlü neigh.	~500	North	36°44'50.7708"N 29°14'14.0436"E	3.19±0.16
14	Yeşilüzümlü neigh.	~633	North	36°46'21.7411"N 29°13'4.3288"E	4.71±0.23
15	İncir Village	~653	South	36°46'33.9318"N 29°13'3.5350"E	5.21±0.26
16	Karaçulha neigh.	~97	South west	36°38'53.9388"N 29°12'41.6915"E	4.31±0.22
17	Karagedik neigh.	~42	South	36°41'2.5263"N 29°7'24.2712"E	6.82±0.34
18	Çiftlik neigh.	~40	North	36°42'4.9921"N 29°5'29.0978"E	4.63±0.23
19	Yanıklar	~16	East	36°42'5.4239"N 29°4'11.5638"E	6.44±0.33
20	Göcek	~5	South	36°45'26.5765"N 28°56'18.4578"E	3.44±0.17

Table 5. Statistical analysis of olive samples according to TPSA altitude and directions

Variables	ANOVA		Kruskal-Wallis	
	F- statistic	p-value	H- statistic	p-value
Height Above Sea Level	4.805	0.039	7.211	0.027
Direction	2.375	0.122	4.745	0.191

DPPH• (1,1-Diphenly-2-picrylhydrazyl) Radical Scavenging Effect

The DPPH• radical-scavenging assay is a frequently used method to evaluate antioxidant activity. DPPH• is a chemical compound with free-radical properties that causes its color to change by reacting with an

antioxidant substance. The basic principle of this method is based on the scavenging of DPPH• radicals using antioxidant compounds. An antioxidant substance donates a hydrogen atom or electron to the DPPH• radical and converts it into a stable compound. As a result, the purple color of DPPH• becomes lighter and its absorbance decreases (Mishra et al., 2012). Olive samples of different concentrations (25, 50, 100, 200, 400, and 600 µL) were mixed with DPPH• solution and incubated for a certain period. After incubation, the decrease in absorbance of DPPH• was measured spectrophotometrically. The results obtained were evaluated by creating dose-response curves, and the parameters representing antioxidant activity were calculated as EC_{50} (Effective Concentration 50). The results are presented in Table 6.

Table 6. EC_{50} values of olive fruits according to their location

Number	Address	Height Above Sea Level (m)	Direction	Coordinate	EC_{50} (g)
1	Karagözler neigh.	~90	North	36°36'59.1396"N 29°6'37.3842"E	0.36±0.02
2	Kayaköy neigh.	~169	South east	36°35'18.8264"N 29°5'44.9359"E	0.59±0.03
3	Kayaköy neigh.	~151	West	36°35'10.1508"N 29°5'46.3700"E	0.91±0.04
4	Ölüdeniz neigh.	~29	South	36°33'3.2981"N 29°7'20.7996"E	0.13±0.01
5	Ölüdeniz neigh.	~15	North east	36°31'58.1260"N 29°7'34.3226"E	0.33±0.02
6	Kelebek Valley	~362	South	36°30'4.8178"N 29°7'38.4199"E	0.70±0.04
7	Faralya neigh.	~287	West	36°29'32.6660"N 29°7'38.3764"E	0.63±0.03
8	Kabak Cove	~82	South	36°27'51.9167"N 29°7'44.3890"E	0.22±0.01
9	Ovacık neigh.	~334	South west	36°24'14.3364"N 29°8'38.3174"E	0.18±0.01
10	Taşyaka neigh.	~18	North	36°37'22.3386"N 29°8'48.7133"E	0.41±0.02
11	Günlükbaşı neigh.	~10	North	36°38'44.0104"N 29°8'6.1419"E	0.28±0.01
12	Yeşilüzümlü neigh.	~505	North	36°44'50.7658"N 29°14'15.1012"E	0.06±0.01
13	Yeşilüzümlü neigh.	~500	North	36°44'50.7708"N 29°14'14.0436"E	0.17±0.01
14	Yeşilüzümlü neigh.	~633	North	36°46'21.7411"N 29°13'4.3288"E	0.16±0.01
15	İncir Village	~653	South	36°46'33.9318"N 29°13'3.5350"E	0.14±0.01
16	Karaçulha neigh.	~97	South west	36°38'53.9388"N 29°12'41.6915"E	0.11±0.01
17	Karagedik neigh.	~42	South	36°41'2.5263"N 29°7'24.2712"E	0.12±0.01
18	Çiftlik neigh.	~40	North	36°42'4.9921"N 29°5'29.0978"E	0.27±0.01
19	Yanıklar	~16	East	36°42'5.4239"N 29°4'11.5638"E	0.09±0.01
20	Göcek	~5	South	36°45'26.5765"N 28°56'18.4578"E	0.24±0.01

The analysis of Table 6 reveals that EC_{50} values range from 0.06 g to 0.91 g, with lower EC_{50} values indicating greater antioxidant activity. The most potent antioxidant activity was observed in samples from the Yeşilüzümlü neighbourhood (EC_{50}=0.06 g) and Karaçulha neighbourhood (EC_{50}=0.11 g). Overall, specimens

collected at higher elevations demonstrated enhanced antioxidant properties, which can be attributed to the tendency of high-altitude environments to stimulate the production of secondary metabolites in plants.

The comparison of data presented in Table 4 (Total Phenolic Substance Amounts, TPSA) and Table 6 (DPPH Radical Scavenging Activity - EC_{50}) reveals a noticeable relationship between the total phenolic content of olive samples and their antioxidant activity. Generally, lower EC_{50} values indicate better antioxidant activity, whereas higher TPSA values suggest a richer phenolic profile. In most cases, the samples exhibiting high TPSA values also demonstrated strong antioxidant activity, as reflected by the low EC50 values. For instance, the samples collected from Yeşilüzümlü (505 m) with a TPSA of 8.70 mg GA/g displayed the most potent antioxidant activity, with an EC_{50} value of 0.06 g. Similarly, samples from Karaçulha (97 m) with a TPSA of 4.31 mg GA/g also exhibited strong antioxidant activity, with an EC_{50} value of 0.11 g. However, some exceptions are observed. For example, samples from Günlükbaşı (10 m), with a relatively high TPSA value of 4.61 mg GA/g, displayed moderate antioxidant activity with an EC_{50} value of 0.28 g. Another noticeable mismatch occurred with samples from Kayaköy (169 m) which had a TPSA of 4.04 mg GA/g, but demonstrated poor antioxidant activity with an EC_{50} value of 0.59 g. This indicates that a high total phenolic content does not always correspond to a high antioxidant activity (Shim, 2011; Kosanić et al., 2011; Kamdem et al., 2012; Susanti, 2019). The discrepancy between TPSA and EC_{50} values can be explained by several factors. First, not all phenolic compounds contributed equally to antioxidant activity. The DPPH assay primarily measures the hydrogen-donating capacity of phenolics, which may not reflect the overall antioxidant potential if the dominant phenolic compounds in the sample have weak radical scavenging abilities. Additionally, the structural characteristics of phenolic compounds, including their hydroxyl group positioning, conjugation, and glycosylation, significantly influence their effectiveness as antioxidants. Compounds with more hydroxyl groups generally exhibit higher antioxidant activities, whereas glycosylated or esterified forms may display reduced activities. Moreover, the interactions between different phenolic compounds may play a critical role in determining the antioxidant potential of an extract. Some compounds may work synergistically to enhance antioxidant effects, while others may have antagonistic effects that reduce the overall efficacy. Furthermore, the solvent system used for extraction was optimised for total phenolic determination, which may not have selectively extracted phenolic compounds with high antioxidant potential (Solar & Stampar, 2011; Sheik & Chandrashekar, 2014; Makhafola et al., 2016).

In general, the findings suggest that although there is a strong positive correlation between high TPSA and low EC_{50} values, this relationship is not absolute. The antioxidant capacity of olive samples is influenced by the type and structural characteristics of the phenolic compounds, their interactions, and the extraction method employed. Further studies involving the identification and quantification of individual phenolic compounds are necessary to better understand the relationship between the phenolic content and antioxidant activity.

HPLC Analysis

HPLC analysis of the olive fruit extracts revealed the presence of 13 distinct phenolic compounds. The identified compounds are listed in Table 7. The presence of ellagic acid (EA) in olives indicates its potential as a natural source of this compound. Ellagic acid is predominantly recognized for its antioxidant, anti-inflammatory, and potential anticancer properties, making its identification in olives important for nutritional and therapeutic applications. Previous studies have corroborated the notion that olives and their related products are rich in bioactive phenolic compounds. For instance, research has previously linked ellagic acid with preventive effects against various cancers and oxidative stress-related disorders, emphasizing the functional relevance of olives in disease prevention and health promotion (Hsieh et al., 2016; Mishra & Vinayak, 2015). The identification of high levels of EA aligns with findings in other fruits, where similar beneficial effects of ellagic acid have been documented, thereby illustrating the potential of olives as functional foods (Neveu 2010; Djurić et al., 2014). In addition, the notable concentrations of epicatechin (EC) found in olive samples significantly contribute to their antioxidant potential. Epicatechin, a well-established polyphenolic compound, exhibits strong free-radical scavenging activity and has been associated with various health benefits, including cardiovascular protection and anticancer effects. The predominance of EC in olives compared to other phenolic compounds—such as rosmarinic acid (RA) and rutin (RU)—underscores olives' potential as a functional food ingredient, which can further promote overall health and facilitate disease prevention (Singh et al., 2011; Khanal et al., 2010). The health benefits of epicatechins have been extensively documented in the literature, suggesting their role in mitigating chronic diseases (Khan and Mukhtar, 2018; Gorai et al., 2020). Moreover, the significant amounts of caffeic acid (CA) and cinnamic acid (CI) observed in olive fruits reinforce their role as sources of hydroxycinnamic acids.

Tablo 7. Phenolic compound content of olive fruits (mg/kg)

No	CA	EA	EC	GA	PA	SA	CA	CI	VA	Pc	FE	RA	RU
1	4.12	459	6.3	0.07	0.04	1.62	40	62	1.33	0.02	0.01	27	0.09
2	1.03	8.8	0.11	0.12	0.19	0.18	69.81	142.45	13.2	3.24	0.01	0.93	0.09
3	1.24	18.3	6.3	0.11	0.83	0.13	76.80	8.85	0.18	0.79	0.51	2.82	0.11
4	0.23	78.6	16.4	0.13	0.26	0.36	23.77	2.47	0.85	0.13	0.65	0.12	0.08
5	0.56	58.7	2.79	0.04	0.17	0.09	17.22	0.80	3.58	1.04	0.23	0.09	0.05
6	0.20	2.3	0.83	0.03	010	0.39	2.47	0.31	0.80	0.05	3.22	0.44	0.04
7	0.21	9.6	1.5	0.10	0.11	0.05	2.15	0.07	0.49	0.13	0.95	1.92	0.05
8	1.18	91.8	29.7	0.16	0.10	0.64	9.43	0.26	1.10	0.02	0.01	1.26	3.67
9	2.46	85.2	18	0.02	0.31	0.06	20.87	1.55	0.35	0.03	3.79	1.84	2.84
10	2.45	215	3.65	0.14	0.62	0.96	59.90	0.46	0.08	0.03	0.73	0.34	0.85
11	1.61	345.6	13.4	0.35	0.90	2.27	49.67	0.003	0.25	0.04	3.97	1.99	1.67
12	1.95	0.67	51.3	0.58	0.11	2.16	79.87	1.73	21.2	0.04	1.12	3.03	1.55
13	0.34	79.4	19.6	0.25	0.15	1.88	18.55	0.27	3.49	0.08	1.69	0.10	4.24
14	0.39	25.4	7.49	0.01	0.05	0.96	1.62	3.61	11.7	0.05	1.64	0.37	0.08
15	0.98	16.3	29.8	0.14	0.16	0.28	4.95	23	5.89	1.21	18.8	0.74	0.11
16	1.92	58.4	5.65	0.34	0.40	2.07	35.11	3.52	2.77	0.84	52.9	0.26	0.30
17	1.64	156	32.8	0.60	0.39	0.22	22.34	1.69	27.4	4.00	1.13	0.15	0.15
18	1.38	250	4.94	0.04	0.27	406	85.38	18.84	3.51	0.01	411	24.72	0.10
19	2.48	27.8	35	0.22	0.16	0.34	61.4	2.31	0.55	0.02	43.5	0.28	0.20
20	0.55	43	4.85	0.01	0.06	0.41	23.21	0.25	0.50	0.30	0.20	2.0	0.12

CA: Catechin, EA: Ellagic, EC: Epicatechin, GA: Gallic, PA: Protocatechuic, SA: Syringic, CA: Caffeic, CI: Cinnamic, VA: Vanillic, Pc: p-Coumaric, FE: Ferulic, RA: Rosmarinic:, RU: Rutin

These compounds are known for their potent antioxidant and antimicrobial activities. Numerous studies have highlighted the efficacy of phenolic acids, such as caffeic acid, in preventing oxidative damage and inhibiting microbial growth, thus expanding the potential applications of olives in food preservation and nutraceutical formulations (Giampieri et al., 2015; Redford et al., 2021). The antioxidant capabilities of these phenolic acids have been further validated in various studies, which emphasize their importance in enhancing the preservation of food products and contributing to health benefits (Ingólfsson et al., 2011; Hsieh et al., 2016). In conclusion, the findings related to ellagic acid, epicatechin, caffeic acid, and cinnamic acid concentrations in olives underscore their considerable value as sources of bioactive phenolic compounds. This position is not only nutritionally beneficial but also therapeutically relevant in the realm of functional foods, offering a range of health benefits owing to their rich phytochemical profile.

CONCLUSION

In this study, the phenolic compound contents and antioxidant capacities of olive fruits collected from different geographical locations and altitudes in the Fethiye region were evaluated. The results showed that the methanol-water (75%) mixture provided the highest phenolic compound extraction, and that the samples collected from high altitudes were richer in phenolic substance content. In particular, the total phenolic content of olives collected from the Yeşilüzümlü Neighborhood was significantly higher than that in other regions. In addition, the low EC_{50} values in antioxidant activity assessments revealed that phenolic compounds can effectively neutralize free radicals. This finding supports the hypothesis that high altitude and environmental stress factors increase secondary metabolite production in olives. HPLC analysis revealed the presence of various phenolic compounds, such as catechin, ellagic acid, epicatechin, gallic acid, protocatechuic acid, cinnamic acid, and ferulic acid in olives. In particular, the high concentrations of ellagic acid and epicatechin highlight the potential health benefits of these olives. Previous studies have shown that ellagic acid has antioxidant and anticancer properties and epicatechin has positive effects on cardiovascular health by effectively scavenging free radicals. These results show that olives grown in the Fethiye region not only have regional economic value, but may also be an important source of nutritional and pharmacological terms.

Acknowledgements
I would like to thank the Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordination Unit for financially supporting my thesis with Project 0908-YL-23.

Declaration of interests
The authors declare that they have no conflicts of interest.

Author Contributions
The authors declare that they contributed equally to this work.

ORCID

Ertan ŞAHİNOĞLU: https://orcid.org/0009-0003-4449-4006
Burcu KABAK: https://orcid.org/0000-0003-4217-1767
Erdal KENDÜZLER: https://orcid.org/0000-0002-9457-1503

Article History

Submission received:	11.03.2025
Revised:	02.04.2025
Accepted:	05.04.2025

REFERENCES

Al-Asmari, K.M., Abu Zeid, I.M., Altayb, H.N., Al-Attar, A.M., Alomar, M.Y. (2021).Alleviation of Malathion Toxicity Effect by Coffea arabica L. Oil and Olea europaea L. Oil on Lipid Profile: Physiological and In Silico Study. *Plants*, 10, 2314. https://doi.org/10.3390/plants10112314

Cerretani, L., Giuliani, A., Maggio, R.M., Bendini, A., Toschi, T.G. & Cichelli, A. (2010). Rapid FTIR determination of water, phenolics and antioxidant activity of olive oil. *European Journal of Lipid Science and Technology*, 112, 1150–1157. https://doi.org/10.1002/ejlt.201000356

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M.H., ElSohly, M.A., & Khan, I.A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-Based Complementary and Alternative Medicine,* Article ID 253875. http://dx.doi.org/10.1155/2014/253875

Djurić, M., Maškovic, P., Murtić, S., Veljković, B., Ćurčić, S., Paunović, G., & Rakočević, L.B. (2014). Quantitation of ellagic acid in blackberries. *Hemijska Industrija*, 68(2), 241-245. https://doi.org/10.2298/HEMIND130306048D

Dueñas, M., Fernańdez, D., Hernańdez, T., Estrella, I., & Munõz, R. (2005). Bioactive phenolic compounds of cowpeas (*Vigna sinensis* L). Modifications by fermentation with natural microflora and with Lactobacillus plantarum ATCC 14917. *Journal of the Science of Food and Agriculture*, 85, 297–304. https://doi.org/10.1002/jsfa.1924

Duh, P.-D., & Yen, G.-C. (1995). Changes in antioxidant activity and components of methanolic extracts of peanut hulls irradiated with ultraviolet light. *Food Chemistry*, 54, 127-131. https://doi.org/10.1016/0308-8146(94)00148-X

Giampieri, F., Forbes-Hernandez, T.Y., Gasparrini, M., Alvarez-Suarez, J.M., Afrin, S., Bompadre, S., Quiles, J.L., Mezzetti, B., & Battino, M. (2015). Strawberry as a health promoter: an evidence based review. *Food Function*, 6, 1386-1398. https://doi.org/10.1039/C5FO00147A

Gomes, T., Caponio, F.ve Alloggio, V. (1999). Phenolic compounds of virgin olive oil: influence of paste preperation techniques. *Food Chemistry*, 64, 203-209. https://doi.org/10.1016/S0308-8146(98)00146-0

Gorai, T., Sakthivel, S., & Maitra, U. (). An inexpensive paper-based photoluminescent sensor for gallate derived green tea polyphenols. *Chemistry an Asian Journal*, 15(23), 4023-4027. https://doi.org/10.1002/asia.202001054

Guasch-Ferré, M., Hu, F.B., Martínez-González, M.A., Fitó, M., Bulló, M., Estruch, R., Ros, E., Corella, D., Recondo, J., Gómez-Gracia, E., Fiol, M., Lapetra, J., Serra-Majem, L., Muñoz, M.A., Pintó, X., Lamuela-Raventós, R.M., Basora, J., Buil-Cosiales, P., Sorlí, J.V., Ruiz-Gutiérrez, V., Martínez, J.A. & Salas-Salvadó, J. (2014). Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC Medicine*, 12, 78. http://www.biomedcentral.com/1741-7015/12/78

Haddad, B., Gristina, A.S., Mercati, F., Saadi, A.E., Aiter, N., Martorana, A., Sharaf, A.,& Carimi, F. (2020). Molecular analysis of the ocial algerian olive collection highlighted a hotspot of biodiversity in the central mediterranean basin. *Genes*, 11, 303; https://doi.org/10.3390/genes11030303

Hsieh, S.-C., Hsieh, W.-J., Chiang, A.-N., Su, N.-W., Yeh, Y.-T., & Liao, Y.-C. (2016). The methanol-ethyl acetate partitioned fraction from Chinese olive fruits inhibits cancer cell proliferation and tumor growth by promoting apoptosis through the suppression of the NF-κB signaling pathway. *Food & Function*, 7, 4797–4803. https://doi.org/10.1039/c6fo01202g

Ingólfsson, H.I., Koeppe II, R.E., & Andersen, O.S. (2011). Effects of green tea catechins on gramicidin channel function and inferred changes in bilayer properties. *FEBS Letters*, 585(19), 3101-3105. https://doi.org/10.1016/j.febslet.2011.08.040

Kamdem, J.P., Stefanello, S.T., Boligon, A.A., Wagner, C., Kade, I.O., Pereira, R.P., Preste, A.S., Roos, D.H., Waczuk, E.P., Appel, A.S., Athayde, M.L., Souza, D.O., & Rocha, J.B.T. (2012). In vitro antioxidant activity of stem bark of Trichilia catigua Adr. Juss. *Acta Pharmaceutica*, 62, 371–382. https://doi.org/10.2478/v10007-012-0026-x

Khan, N., & Mukhtar, H. (2019). Tea polyphenols in promotion of human health. Nutrients, 11, 39. https://doi.org/10.3390/nu11010039

Khanal, R.C., Howard, L.R., Wilkes, S.E., Rogers, T.J., & Prior R.l. (2010). Urinary excretion of (Epi)catechins in rats fed different berries or berry products. *Journal of Agricultural and Food Chemistry*, 58, 11257–11264. https://doi.org/10.1021/jf102916m

Khoddami, A., Wilkes, M.A., & Roberts, T.H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, 18, 2328-2375. https://doi.org/10.3390/molecules18022328

Kosanić, M., Ranković, B., & Stanojković, T. (2011). Antioxidant, antimicrobial, and anticancer activity of 3 umbilicaria species. *Journal of Food Science,* 77(1), 20-25. https://doi.org/10.1111/j.1750-3841.2011.02459.x

Loureiro, J., Rodriguez, E., Costa, A., Santos, C. (2007). Nuclear DNA content estimations in wild olive (*Olea europaea* L. ssp. *europaea* var. *sylvestris* Brot.) and Portuguese cultivars of *O. europaea* using flow cytometry. *Genetic Resources and Crop Evolution*, 54, 21–25. https://doi.org/10.1007/s10722-006-9115-3

Maesano, G., Chinnici, G., Falcone, G., Bellia, C., Raimondo, M., & D'Amico, M. (2021). Economic and environmental sustainability of olive production: A case study. *Agronomy*, 11, 1753. https://doi.org/10.3390/agronomy11091753

Makhafola, T.J., Elgorashi, E.E., McGaw, L.J., Verschaeve, L., & Eloff, J.N. (2016). The correlation between antimutagenic activity and total phenolic content of extracts of 31 plant species with high antioxidant activity. *BMC Complementary and Alternative Medicine*, 16, 490. http://dx.doi.org/10.1186/s12906-016-1437-x

Maraulo, G.E., Ferreira, C.S., Mazzobre, M.F. (2021). β-cyclodextrin enhanced ultrasound-assisted extraction as a green method to recover olive pomace bioactive compounds. *Journal of Food Processing and Preservation*, 45, e15194. https://doi.org/10.1111/jfpp.15194

Marcelino, G., Hiane, P.A., Freitas, K.C., Santana, L.F., Pott, A., Donadon, J.R., & Guimarães, R.C.A. (2019). E ects of olive oil and ıts minor components on cardiovascular diseases, ınflammation, and gut microbiota. *Nutrients*, 11, 1826; https://doi.org/10.3390/nu11081826

Mishra, K., Ojha, H., & Chaudhury, N.K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chemistry, 130(4), 1036-1043. https://doi.org/10.1016/j.foodchem.2011.07.127

Mishra, S., & Vinayak, M. (2015). Role of ellagic acid in regulation of apoptosis by modulating novel and atypical PKC in lymphoma bearing mice. *BMC Complementary and Alternative Medicine*, 15, 281. https://doi.org/10.1186/s12906-015-0810-5

Neveu, V., Perez-Jimeńez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D., & Scalbert, A. (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database,* Article ID bap024. https://doi.org/10.1093/database/bap024

Redford, K.E., Rognant, S., Jepps, T.A., & Abbott, G.W. (2021). KCNQ5 potassium channel activation underlies vasodilation by tea. *Cellular Physiology and Biochemistry*, 55(S3), 46-64. https://doi.org/10.33594/000000337

Rocha, J., Borges, N., & Pinho, O. (2020). Table olives and health: a review. *Journal of Nutritional Science*, 9(57), 1-16. https://doi.org/10.1017/jns.2020.50

Rohman, A., Riyanta, A.B., Lukitaningsih, E. & Riyanto, S. (2020). Olive (*Olea europea*) oil: physico-chemical characterization and antioxidant activities in vitro and in vivo. *Food Research*, 4 (3), 563 – 570. https://doi.org/10.26656/fr.2017.4(3).220

Rudrapal, M., Khairnar, S.J., Khan, J., Dukhyil, A.B., Ansari, M.A., Alomary, M.N., Alshabrmi, F.M., Palai, S., Deb, P.K., & Devi, R. (). Dietary polyphenols and their role in oxidative stress-ınduced human diseases: ınsights ınto protective effects, antioxidant potentials and mechanism(s) of action. *Frontiers in Pharmacology*, 13, 806470. https://doi.org/10.3389/fphar.2022.806470

Sánchez-Romero, C. (2021). Somatic embryogenesis in olive. *Plants*, 10, 433. https://doi.org/10.3390/plants10030433

Sharifi-Rad, M., Kumar, N.V.A., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Fokou, P.V.T., Azzini, E., Peluso, I., Mishra, A.P., Nigam, M., El Rayess, Y., El Beyrouthy, M., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A.O., Setzer, W.N., Calina, D., Cho, W.C., & Sharifi-Rad, J. (2020). *Frontiers in Physiology*, 11, 694. https://doi.org/10.3389/fphys.2020.00694

Sheik, S. & Chandrashekar, K.R. (2014). Antimicrobial and antioxidant activities of Kingiodendron pinnatum (DC.) Harms and Humboldtia brunonis Wallich: endemic plants of the Western Ghats of India. *Journal of the National Science Foundation of Sri Lanka*, 42 (4), 307-313. http://dx.doi.org/10.4038/jnsfsr.v42i4.7729

Shim S.-M. (2011). Changes in profiling of phenolic compounds, antıoxidatıve effect and total phenolic content ın smilax China under in vitro physiological condition. *Journal of Food Biochemistry,* https://doi:10.1111/j.1745-4514.2011.00589.x

Singh, B.N., Shankar, S., & Srivastava, R.K. (). Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and clinical applications. *Biochemical Pharmacology*, 82(12), 1807-1821. https://doi.org/10.1016/j.bcp.2011.07.093

Singleton, V. L. ve Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158. https://doi.org/10.5344/ajev.1965.16.3.144

Solar, A., & Stampar, F. (2011). Characterisation of selected hazelnut cultivars: phenology, growing and yielding capacity, market quality and nutraceutical value. *Journal of the Science of Food and Agriculture*, 91, 1205–1212. https://doi.org/10.1002/jsfa.4300

Susanti, H. (2019). Total phenolic content and antioxidant activities of binahong (Anredera cordifolia.). *Indonesian Journal of Medicine and Health*, 10(2), 171-175. https://doi.org/10.20885/10.20885/JKKI.Vol10.Iss2.art9

Vázquez-Ruiz, Z., Toledo, E., Vitelli-Storelli, F., Goni, L., de la O, V., Bes-Rastrollo, M., & Martínez-González, M.Á. (2022). Effect of dietary phenolic compounds on ıncidence of cardiovascular disease in the SUN Project; 10 Years of Follow-Up. *Antioxidants*, 11, 783. https://doi.org/10.3390/antiox11040783

Yubero-Serrano, E.M., Lopez-Moreno, J., Gomez-Delgado, F., & Lopez-Miranda, J. (2019). Extra virgin olive oil: More than a healthy fat. *European Journal of Clinical Nutrition*, 72, 8–17. https://doi.org/10.1038/s41430-018-0304-x

Zuorro, A., Maffei, G., & Lavecchia, R. (2014). Effect of solvent type and extraction conditions on the recovery of phenolic compounds from artichoke waste. *Chemical Engineering Transactions*, 39. https://doi.org/10.3303/CET1439078