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ANTIOXIDANT ACTIVITIES OF TURKISH EXTRA VIRGIN OLIVE OILS

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ABSTRACT. Extra virgin olive oil is the highest grade of virgin olive oil derived by cold mechanical extraction without the use of solvents or refining methods. These olive oils are known for their composition in phenolic compounds that have antioxidant properties. This study aims to determine the total phenolic and flavonoid compounds and antioxidant activities of four Turkish extra virgin olive oil samples: Kilis yağlık, İzmir sofralık, Ayvalık, and Tavşan yüreği. The highest sample concentration used for the experiments was 4 mg/ml while 1 mg/mL was used for ABTS radical scavenging assay. The lowest total phenolic and flavonoid content was observed in Tavşan yüreği sample. All extra virgin olive oil samples showed scavenging activity against DPPH and ABTS free radicals. Extra virgin olive oil samples with high phenolic and flavonoid content presented more effective radical scavenging activity with low IC_{50} values. This study provides information about the phenolic content and antioxidant activities of four important Turkish olive oil samples.

1. INTRODUCTION

Initially consumed in particular by Mediterranean populations, olive oil is increasingly gaining ground on the world market because of its beneficial effects on health. These beneficial effects on health mean that outside of Mediterranean countries, olive oil is also consumed in other regions of the world [1]. Mediterranean countries represent 65% of world olive oil production and 43% of world consumption [2]. Turkey occupies a significant place among the world's major

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producers. Turkey is the fifth-largest olive oil producer in the world after Spain, Italy, Greece, and Tunisia [3]. In 2018, olive oil production in Turkey amounted to 206,300 tonnes [4]. Turkey is very rich in olive oil diversity. Most of Turkey's olive oil varieties are found in the Aegean region, which is responsible for around 80% of production [5]. Ayvalık and Memecik varieties, which are of great economic importance, are also found in this region.

Olive oil is recognized for its composition rich in bioactive substances such as phenolic compounds. This richness in phenolic compounds and unsaturated fatty acids distinguishes it from other oils and gives it beneficial effects for health [6]. It has been determined that various polyphenolic compounds in virgin oil are responsible for the beneficial effects. These compounds are phenolic alcohols (hydroxytyrosol and tyrosol), phenolic acids (vanillic acid and p-cumaric acid), secoiridoids (oleuropein aglycones, ligstroside aglycone, and oleanolic acid, etc), and flavonoids (luteolin and apigenin), and other minor compounds [7-8]. Extra virgin olive oil is also rich in oleic acid, linoleic acid, and palmitoleic acid, which are believed to be associated with the prevention of cardiovascular disease and various types of cancer. The most important of these monounsaturated fatty acids is oleic acid, its content varies between 68 and 82% of the fatty acids contained in olive oil [9-10]. Oleic acid plays also a significant role in the biological effects of olive oil.

The phenolic compounds in extra virgin oil have antioxidant properties. Antioxidants prevent the oxidation of macromolecules (DNA, RNA, proteins, and lipids) that play an important role in cell metabolism. The phenolic compounds and fatty acids contained in extra virgin olive oil can modulate the secretion of pro-inflammatory cytokines and certain markers of inflammation [11-12]. Extra virgin olive oil due to its composition rich in antioxidant compounds may prevent the occurrence of various types of cancers such as intestinal cancer, prostate cancer, breast cancer, and various neurological disorders as well as cardiovascular diseases [13-14-15-16].

The composition of extra virgin oil can be influenced by various factors such as geographic location, soil types, climate, rainfall, extraction method but also storage [17-18-19]. The stage of ripening is also another factor, which can affect extra virgin olive oil composition. Olive oil extracted from immature and moderately mature fruits contains more phenolic compounds than oil obtained from ripe fruits [20-21]. During the ripening, a large proportion of fatty acids depends on the degree of hydrolysis of triacylglycerol. The ripening of the fruits causes a decrease in the level

of hydroxytyrosol, total phenolic compounds and flavonoids, and the rate of rutin thus influencing the biological activities of extra virgin olive oil [22].

To our knowledge, there is not enough data on Turkish extra virgin olive oil's chemical characterization and antioxidant properties. Sarı Hasebi, Gemlik, and Halhali olive oils, which are cultivated in the Mediterranean region of Turkey have been studied and showed that the type of variety has a significant effect on the composition of fatty acids, sterols, and total phenolics [23]. Therefore, this study aims to determine the total phenolics and flavonoids content and the antioxidant activities of Izmir sofralık, Ayvalık, Tavşan yüreği and Kilis yağlık extra virgin olive oil.

2. MATERIALS AND METHODS

2.1. Extra virgin olive oil samples

Extra virgin olive oil samples were obtained from different regions of Turkey**.** The Ayvalık sample was obtained from Buta Assos (Ayvalık), Izmir sofralık from Hedef Ziraat (Izmir), Kilis yağlık from Fersis (Kilis) and Tavşan yüreği from Zeytin Akademi (Antalya).

2.2. Extraction of phenolic fraction

The extraction of phenolic fractions was obtained following the procedure of Reboredo-Rodríguez et al. [24]. Briefly, 10 g of each olive oil sample was weighed into a 15 ml tube, and 5 mL of MeOH : H_2O (80:20, v/v) was added and shaken vigorously for 3 minutes. Finally, the tube was centrifuged at 4800 rpm for 25 minutes, and the MeOH: H_2O phase was collected. This process was repeated three times for each sample. Then three methanolic phases were collected and evaporated using a rotary evaporator. The dry extracts obtained after evaporation were weighed and dissolved in methanol. The resulting stock concentrations were stored at -20 °C until later use.

2.3. Total phenolic content

The total phenolic content (TPC) of the olive oil extracts was determined using the Folin-Ciocalteau method [25]. Briefly, 100 µL of extra virgin olive oil sample was added to 100 µL of Folin-Ciocalteau reagent, the mixture was vortexed for 3 minutes. Then 500 µl of 6% (v/v) sodium carbonate was added. After 5 minutes, the reaction mixture was stirred and diluted to 2 ml with distilled water. The absorbance of the mixture was measured after 2 hours of incubation at 725 nm. Total phenolic compounds found in extra virgin olive oil samples were determined using the

calibration curve of standard gallic acid. The results obtained are expressed as mg gallic acid equivalent per gram (mg GAE/g).

2.4. Total flavonoid content

The total flavonoid content (TFC) was determined according to the method described by Huang et al. [22]. Briefly, 100 µL of $NaNO_2$ (5%) and 100 µL of $AlCl_3$ (10%) were added to the 100 µL of virgin olive oil sample or quercetin standard at different concentrations. After 10 minutes of incubation, 2 mL of NaOH (4%) was added to the mixture. The solution was mixed and absorbance was measured at 415 nm against the prepared blank reagent. Total flavonoid contents are expressed as mg quercetin equivalent per gram of dry extract (mg QE/g).

2.5. DPPH radical scavenging activity

The radical scavenging activity of extra virgin olive oil was determined using the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay method [26]. The DPPH stock solution was diluted with ethanol to an absorbance of 1.400 at 517 nm before analysis. In each test tube, 100 µL of different concentrations of the sample or gallic acid standard solution were mixed with 1.4 mL of DPPH and shaken for 1 minute. After 30 minutes of incubation, the absorbance of each solution was measured at 517 nm. The experiments were repeated three times. The radical scavenging activity (RSA) was calculated according to the following formula.

$$\% \text{ RSA} = [(A_{control}-A_{sample})/A_{control}]*100$$

2.6. ABTS radical scavenging activity

ABTS radical scavenging activity was determined according to the method described by Wu et al. [27]. ABTS was mixed with ammonium persulfate solution in a 1:1 volume ratio and incubated at room temperature for 12~16 hours in the dark to form a radical cation stock solution (ABTS +). ABTS + stock solution was diluted with ethanol to 0.700 (± 0.020) absorbance at 734 nm before analysis. Next, 20 µL of extra virgin olive oil sample of different concentrations was mixed with 980 µL of ABTS + stock solution, and absorbance at 734 nm will be recorded after 10 minutes. Trolox was used as standard. The experiments were repeated three times. The radical scavenging activity (RSA) was calculated according to the following formula.

$$\% \text{ RSA} = [(A_{control}-A_{sample})/A_{control}]*100$$

2.7. Statistical analysis

All the results were expressed as mean ± standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) and Tukey tests were performed to compare mean values ($p < 0.05$). Each experiment was repeated at least three times ($n \geq 3$). Statistical analyzes were conducted using GraphPad Prism 8.

3. RESULTS AND DISCUSSION

3.1. Total phenolic and flavonoids content

Phenolic compounds are known to be a major part of vegetable oils after fatty acids components. The type of olive oil variety or the stage of ripening can influence the total phenolic content [21]. These phenolic compounds apart from their antioxidant properties are responsible for the flavor of olive oils. The determination of total phenolic compounds from olive oil samples revealed a significant difference between varieties. The differences observed in the composition in total phenolic compounds of the different varieties of extra virgin olive oil can be due to the genetic profile of the variety of extra olive oil and to agro-environmental factors [28-29]. Other factors such as the stage of ripening and method of extraction can affect total phenolic content in extra virgin olive oil [30]. Moreover, other studies have shown that phenolic compounds are a real index in the discrimination of olive oil varieties obtained from different regions. Turkish olive oil varieties are generally rich in phenolic compounds. A study taken out on Arbequina, a variety of olive oil native to the Aegean region showed that the Turkish variety is richer in phenolic compounds than the Arbequina variety grown in Tunisia, Italy, and Spain [31]. Thus, the same variety cultivated under different agronomic conditions may present differences in terms of phenolic compounds. In our study, the highest values of total phenolic compounds were observed in Kilis yağlık and Izmir sofralık samples, while Ayvalık and Tavşan yüreği contain relatively low levels of total phenolics (Table 1). TPC were calculated as 180.12 and 157.76 mg GAE/g for Kilis yağlık and Izmir sofralık, respectively. Ayvalık and Tavşan yüreği were found to contain fewer phenolic compounds than the other two samples. The Izmir sofralık and Ayvalık extra virgin olive oil samples originate from the Aegean region. However, Izmir sofralık contains more total phenolic compounds than the Ayvalık, which originates from the north side of the Aegean region. According to a study carried out on the Ayvalık olive oil, the ripening period and the altitude can influence the chemical composition of this variety in particular its content of total phenolic compounds [32]. Therefore, within the same region factors such as the nature of the soil, precipitation can influence the composition of extra virgin olive oil. The Tavşan yüreği originating

from the Mediterranean region showed the lowest values in terms of total phenolic and flavonoid content. The stage of maturation strongly influences the content of phenolic or flavonoids. In fact, within the same variety, the content of phenolic and flavonoid compounds can differ depending on the stage of maturation [33]. A previous study carried out on the Cobrançosa and Picual olive oil cultivars showed that the green olives, presented total phenolic values of 50.1 and 43.5 mg/kg respectively, for the semi-mature of 35.3 and 28.8 mg/kg and mature fruits values of 34.6 and 31.4 mg/kg [34].

The flavonoid content can also be affected by factors such as temperature, precipitation, but also by the stage of maturity. It has been reported that the flavonoid content is found to be high in olive oil obtained from ripe fruits [35]. The content of individual flavonoids such as apigenin and luteolin increases with the degree of maturity [36-37]. It has also been shown that the highest flavonoid content is usually obtained from olives in regions with high rainfall [35]. Our results showed that the total flavonoid content of the extra virgin olive oil samples were also different from each other (Table 1). The observed differences are due to the type of olive oil variety and agro-ecological conditions [38]. Kilis yağlık showed the highest flavonoid content with 273.06 mg QE/g. Tavşan yüreği variety contains the lowest total flavonoid content. Izmir sofralık showed a good result in terms of total phenolic content than Ayvalık, while the contrary is observed in the result of total flavonoid content. This could be due to the advanced stage of maturity of the Ayvalık extra virgin olive sample resulting in a decrease in the content of total phenolic compounds and an increase in its content of flavonoids. According to other studies, the content of phenolic compounds may depend on the level of glucosides and the activity of β-glucosidase in olive fruits. This enzyme is believed to be responsible for the hydrolysis of phenolic glucosides and the oxidation of phenolic compounds [39-40].

TABLE 1. Total phenolic and flavonoid contents of extra virgin olive oils samples

Olive oil	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Ayvalık	39.60 ± 1.10	176.82 ± 10.14
Izmir sofralık	180.12 ± 1.04	128.29 ± 6.64
Tavşan yüreği	33.13 ± 5.88	45.44 ± 5.02
Kilis yağlık	157.76 ± 1.3	273.06 ± 5.22

TPC was calculated using linear regression of gallic acid ($R^2 = 0.9998$) and expressed as gallic acid equivalent in milligram per gram of dry sample weight (mg GAE/g of dry sample weight). TFC was calculated using linear regression of quercetin (R^2 =

0.9978) and expressed as quercetin equivalent in milligram per gram of dry sample weight (mg QE/g of dry sample weight).

3.2. Radical scavenging activities of extra virgin olive oils

Extra virgin olive oil is known for its richness in phenolic compounds and it is credited with many virtues, in particular its antioxidant properties. DPPH and ABTS radical reducing activity is a widely used method to assess the antioxidant activity of foods. These methods were used to evaluate the antioxidant capacity of the extra virgin olive oil samples. All four extra virgin olive oil samples showed effective DPPH and ABTS radical scavenging activity. At the maximum concentration of 4 mg/mL, the samples Kilis yağlık, Izmir sofralık, Ayvalık, and Tavşan yüreği showed percentages of inhibition of the DPPH radical respectively of 94%, 92%, 91%, and 28% while the standard gallic acid solution showed 93% (Figure 1). It was observed that the radical scavenging activity of extra virgin olive oils samples is dose-dependent. Tavşan yüreği extra virgin olive oil sample showed very low antioxidant activity compared to the others. The antioxidant activity of olive oils depends on the type of variety, location, or degree of ripening [41]. As shown in table 2, Kilis yağlık showed a good DPPH and ABTS radıcals scavenging activity compared to the three other samples. At maximum concentration, the ABTS radical scavenging activity was 81%, 68%, 50% and 36% respectively for Kilis yağlık, Izmir sofralık, Tavşan yüreği and Ayvalık samples (Figure 2). Taken together, Tavşan yüreği showed the weakest DPPH radical scavenging activity among extra virgin olive oil samples while on the ABTS radical scavenging activity the lowest value was obtained with Ayvalık sample. On the other hand, a small concentration range (0.125-1 mg/mL) is sufficient to scavenge the ABTS radical, while it is necessary to use a higher concentration range (0.25-4 mg/mL) for the DPPH radical. This implies that the ABTS radical is more sensitive and easily reducible by the antioxidant compounds contained in different extra virgin olive oils. At identical concentration, the values of ABTS radical scavenging is higher than those of the DPPH radical. Similar results have been obtained in studies on Halhali ve Nizip yağlık olive oils [20-42]. In addition, all varieties having exhibited high content of phenolic compounds and total flavonoids showed high scavenging radical activities, therefore, there is a correlation between the antioxidant activities and the content of phenolic compounds [20-41-43].

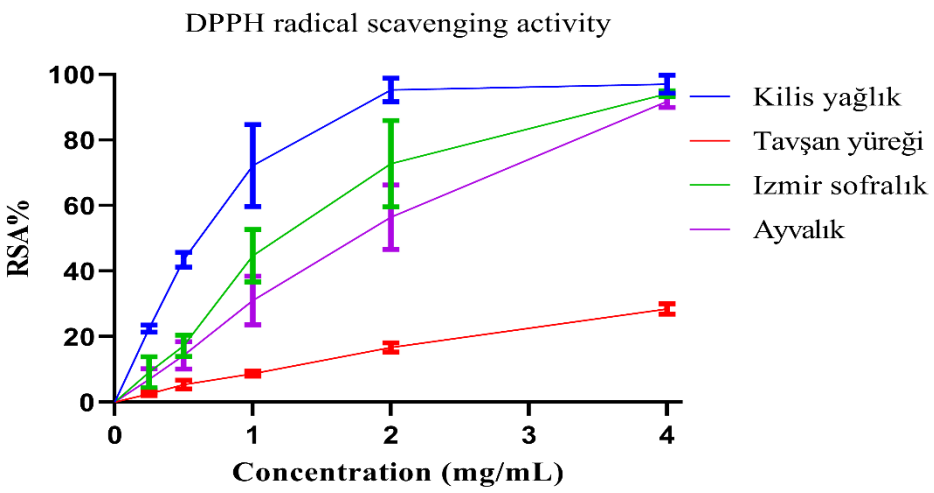

FIGURE 1. DPPH free radical scavenging activity of extra virgin olive oil samples.

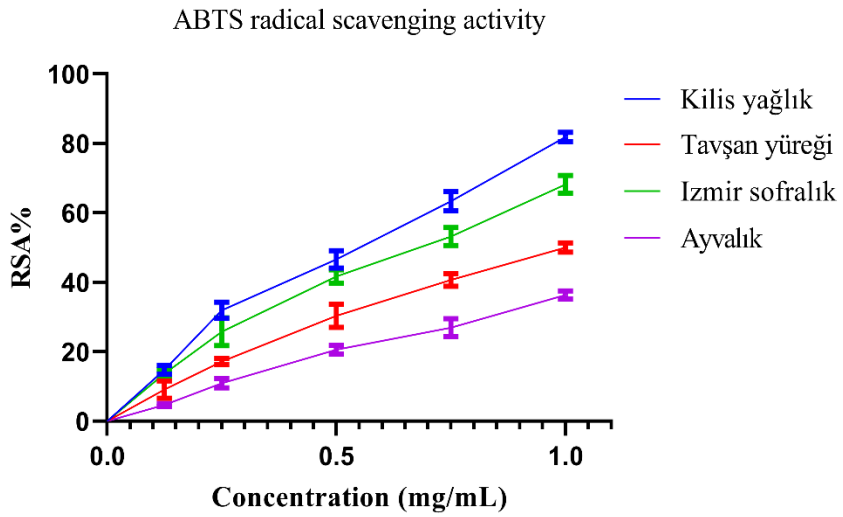

FIGURE 2. ABTS free radical scavenging activity of extra virgin olive oil samples.

TABLE 2. IC_{50} (mg/ml) values of DPPH and ABTS radical scavenging activity of extra virgin olive oil samples

	Kilis yağlık	**Tavşan yüreği**	**Izmir sofralık**	**Ayvalık**	**Gallic acid**	**Trolox**
DPPH	0.82 ±0.03	7.07 ± 0.41	1.20 ± 0.08	1.83 ± 0.04	0.009 ± 0.06	-
ABTS	0.56 ± 0.01	0.94 ±0.01	0.69 ± 0.03	1.40 ± 0.05	-	0.002 ± 0.01

4. CONCLUSION

This study focused on the determination of the total phenolic and flavonoid content as well as the antioxidant activities of extra virgin olive oils from Turkey. There is a significant difference between the total phenolic and flavonoid content of the extra virgin olive oil samples. Kilis yağlık sample, which was rich in total phenolic and flavonoid content (157.76 mg GAE/g and 273.06 mg QE/g respectively), also showed the highest DPPH and ABTS radical scavenging activity (94% and 81% respectively). Therefore, the type or the location of extra virgin olive oil affects their phenolic content and antioxidant activity. Our study provided preliminary information on the phenolic content and antioxidant properties of these different extra virgin olive oils. Further studies including the determination of phenolic composition using chromatographic methods and the stage of maturity could help to better understand the variation of phenolic compounds and the antioxidant properties of these four extra virgin olive oils.

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Declaration of Competing Interests The authors declare no conflict of interest.

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