Research Article | Open Access

Akbaba, 2023. 25 (3&4): 11-21. J. Environ. Agric. Sci. (ISSN: 2313-8629)

Health-Protecting Properties of Olive Leaves (*Olea europaea* L.) via Antioxidant Profile and Phenolic Composition

Emel Akbaba[1]

Edited by:
Muhammad Ammar Khan,
The Islamia University, Bahawalpur, Pakistan

Reviewed by:
Malik Muhammad Hashim,
Gomal University, D.I. Khan, Pakistan

Hafiz Talha Hafeez,
The Islamia University, Bahawalpur, Pakistan

Received
April 1, 2023
Accepted
June 5, 2023
Published Online
October 17, 2023

Abstract: Olive (*Olea europaea* L.) leaves are the major by-product of the olive oil industry, containing a variety of bioactive chemicals with significant potential health benefits. The major components of olives that are responsible for their therapeutic properties are phenolic compounds. An experiment was designed to determine phenolic and flavonoid contents, and antioxidant activity of methanolic extracts of olive leaves obtained using two techniques: maceration and microwave-assisted extraction. The antioxidant activities of the extracts were determined via ferric-reducing antioxidant power and phenanthroline-metal chelating capacity assays. The extract obtained via maceration yielded 37 mg g^{-1} of total phenolics, 16 mg g^{-1} total of flavonoids, 50 mg g^{-1} ferric-reducing antioxidant biomolecules, and 140 mg g^{-1} of phenanthroline metal chelating phytochemicals. However, microwave-assisted extraction yielded 38 mg g^{-1} of total phenolics, 21 mg g^{-1} of total flavonoids, 52 mg g^{-1} of ferric-reducing antioxidant biomolecules, and 142 mg g^{-1} of phenanthroline-metal chelating phytochemicals. In comparison to maceration, the microwave-assisted extraction method produced slightly higher quantities of bioactive compounds in a relatively short time, while using less solvent. This study showed that olive leaves have great potential for bioactive molecules and antioxidant profiles. This study also reports the importance of taking advantage of olive leaves, which are byproducts of factories and are generally considered waste. Therefore, olive leaves are highly recommended in the food industry as natural antioxidants rather than synthetic antioxidants, which can cause a variety of health problems.

Keywords: Microwave-assisted extraction, phenolic compounds, *Olea europaea*, antioxidant potential, olive.

***Corresponding author:** Emel Akbaba eakbaba@kku.edu.tr

Cite this article as: Akbaba, E. 2023. **Health-protecting properties of olive leaves (*Olea europaea* L.) via antioxidant profile and phenolic composition**. Journal of Environmental & Agricultural Sciences. 25 (3&4):11-21.

1. Introduction

Olive tree (*Olea europaea* L. Oleaceae) is native to the Mediterranean basin, and its leaves have been widely used as a folk remedy in traditional medicine (Barazani et al., 2023; Medina et al. 2019). Hence, olive is a key crop in the Mediterranean region and olive products are the key crops (Besnard et al., 2017; Šimat et al. 2022; Sofo et al. 2008). According to the Food and Agriculture Organization (FAO) of the United Nations, globally 23.6 million tons of olives were produced in 2020. The countries with the highest olive production are Spain, Italy, Greece, and Turkey (FAO, 2021). Olive leaves are by-products of the olive oil industry, including a variety of bioactive chemicals with many potential health benefits. Olive leaves account for 10 % of the weight of harvested olive fruits, as well as 25 % of the weight of a trimmed tree (Herrero et al. 2011). Therefore, olive leaves are cheap and rich source of natural bioactive phytochemicals, especially phenolics and flavonoids (Khemakhem et al., 2019). Although the utilization of natural products in the industry is not well practiced, however, some industries use natural products like olive leaves for food additives, dietary supplements, cosmetic and nutraceutical purposes, mainly due to their rich bioactive compounds (Medina et al. 2019).

[1]Department of Medical Biology, Faculty of Medicine, Kirikkale University, 71450, Kirikkale, Turkiye

The incorporation of olive leaves into food systems has the potential to reduce microbial load, increase the oxidative stability of edible oils, and modify the organoleptic and nutritional properties of olive oils. These uses include the addition of phenolic compounds from olive leaf extract to a variety of oils, including olive, sunflower, and soybean (Martín-García et al. 2020). Commercial olive leaf products include dietary supplements, tea, nutritional supplements and cosmetics (Oliveira et al., 2021; Tsimidou and Papoti, 2010). Thus, olive leaves may be a viable alternative to synthetic preservatives in the food sector (González-Burgos et al., 2011) Other potential uses for olive leaves include cosmetics, food preservatives and pharmaceutical products (Marchev et al. 2021). Olive leaf extracts may have synergistic effects with a variety of other health-promoting qualities. As a result, the development of an efficient and practical technique for extracting olive leaves is critical.

Traditional use of olive leaves and olive extracts has a long history, mostly associated with preservation and health. Egyptians, for example, are known to employ the extract of olive leaves in the process of mummification as a preservative agent (Marković et al. 2022; Soler-Rivas, et al., 2000). The health-promoting effects of olive leaves include antioxidant, anti-hypertensive, hypoglycemic, antibacterial, antiviral, anti-inflammatory, and anti-cancer activities (Ismail et al., 2021; Losada-Echeberría et al. 2017; Salem et al., 2015; Vezza et al., 2017). The primary elements of olives that provide them with their therapeutic benefits are phenolic compounds. Olive leaf oleuropein and hydroxytyrosol are abundant in fresh olive leaves. Additionally, they include flavonoids and polyphenols such as luteolin, rutin, catechin, apigenin, caffeic acid, coumaric acid, ferulic acid, gallic acid, vanillic acid are olive phenolic acids (Ozturk et al. 2021; Palmeri et al., 2022; Zhang et al., 2022).

In this study, microwave-assisted extraction (MAE) and maceration were used to compare the antioxidant profile and phenolic composition of olive leaf extract. The extracts were evaluated for their ferric-reducing antioxidant power (FRAP), phenanthroline-metal chelation capacity (MCC), total flavonoid (TFC) and phenolic content (TPC). These characteristics were suggested to be associated with both the food preservative and its contribution to health benefits. The extracts were compared regarding their bioactive contents, antioxidant profiles, extraction time, and the required solvent amount.

2. Materials and Methods

2.1. Plant material

In 2020, wild olive samples (as entire aerial parts) were collected in the Gaziantep region, Türkiye. Olive leaves were plucked and air-dried at room temperature in a shaded area for one week and then ground using a high-speed blender.

2.2. Maceration

Sampled plant material (10 g) was put in a flask with 75 cc methanol, and constantly stirred with a magnetic stirrer at room temperature (for 24 h). Subsequently, the collected samples were filtered using Whatman filter paper (125 mm, S-H Labware) and filtration was conducted in triplicate. Finally, obtained extracts were thoroughly mixed and allowed to evaporate at 40 ^{o}C under a pressure of 150 mbar using a rotary evaporator. Hexane was used to defat the extract after evaporation. After air drying, the extract was dried in a vacuum oven (Nuve 180, Ankara, Turkey). Obtained extract (0.1 g) was employed in 8 mL methanol as the starting material for the biochemical assays (Akbaba, 2021).

2.3. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) was performed using a microwave oven with a closed vessel. Subsequently, dried samples (0.1 g) were put in a polytetrafluoroethylene tank (70 mL) and extracted with 8 mL of solvent (70% methanol in water) under different time durations and temperatures. Temperature of system was adjusted at 60 ^{o}C, 70 ^{o}C, and 85 ^{o}C. Moreover, three different microwave powers were utilized (200, 300, and 400 W). The ramp duration has been increased to ten minutes. The duration of the hold varied between eight, fourteen, and twenty minutes. The vessels were allowed to cool after extraction and then centrifuged at 3000 rpm (10 min). Biochemical analyses were performed on the supernatant.

2.4. Determination of Total Phenolic Content

Total phenolic contents (TPC) in olive leaf extracts were determined through the Folin-Ciocalteu test, which is based on the Singleton's Folin-Ciocalteu reagent's reduction by phenolic compounds in alkaline conditions (Magalhaes et al. 2008; Singleton, et al., 1999). Sample or standard solutions (gallic acid) (0.5 g) were placed in test tubes containing water (1.5 ml) and Folin-Ciocalteu reagent (0.25 m). Later, tubes were vortexed and allowed to settle for 5 min. Prior to the one-h incubation at room temperature in the dark, 1.5 ml of 10% Na_2CO_3 was applied. Samples were processed to record absorbance at 760 nm using a spectrometer. The findings are presented as gallic acid equivalents on a dry plant basis (mg GAE g^{-1} dw),

using a calibration curve produced with a genuine reference chemical (5, 10, 20, 50, 100, and 200 ppm).

2.5. Determination of Total Flavonoid Content

The colorimetric aluminum chloride technique was used to measure total flavonoid content. In test tubes, sample or standard solutions (quercetin) (0.5 ml) was added and then 10% $AlCl_2$ (0.6 ml), 1M KCH_3COO (0.1 ml), and methanol (2.7 ml) were added, the mixture was incubated for one h. At 420 nm, the absorbances were determined. The findings are presented as quercetin equivalents (mg QE g^{-1} dw), using a calibration curve produced with a genuine reference chemical (5, 10, 20, 50, 100, and 200 ppm).

2.5. Ferric-Reducing Antioxidant Power Assay

Reducing power of methanolic olive extracts, based on their antioxidant properties, was evaluated by their ability to make colorful complexes with potassium ferricyanide. Final product, Prussian blue was spectrophotometrically measured as a proxy of antioxidants' reducing potency. Antioxidants may either reduce the reduction of ferricyanide in the solution to ferrocyanide, by antioxidants, which then bind the free Fe^{3+} in the solution to formation of Prussian blue. Moreover, reduction of iron(III) to iron(II), which then precipitates the ferricyanide in the solution to formation of Prussian blue (Berker et al., 2010). The findings are given in terms of quercetin equivalents per dry weight of the plant (mg QE g^{-1} dw). The calibration curve was constructed using quercetin concentrations ranging from 5-200 ppm. The absorbances were determined at a wavelength of 700 nm.

2.6. Phenanthroline Metal Chelating Capacity

Reducing capacity of plant extracts was determined by using the phenanthroline assay (Szydłowska-Czerniak et al., 2008). Herein, the samples were first mixed with ferric chloride. The antioxidant molecules in the plant extracts reduce the Fe^{3+} to Fe^{2+}. Then a 1,10-phenanthroline solution was added. The reduced Fe^{2+} forms a complex that has an orange-red color. Fe3+ does not form this complex with 1,10-phenanthroline solution (Brandt et al., 1954). The absorbance of the orange-red solution was measured at 510 nm against a blank control (Trifa et al. 2020). The calibration curve was constructed using values ranging from 50 to 800 ppm. $FeSO_4$, as the standard, forms a complex with 1,10- phenanthroline and is measured at the same wavelength. The calibration curve, therefore, was generated. The findings are presented as $FeSO_4$ equivalents (mg $FeSO_4$ g^{-1} dw) on a dry plant basis.

2.7. Statistical Analysis

Each chemical test was performed in triplicate. The one-way ANOVA and Tukey's *post hoc* test for multiple comparisons were used for statistical comparison of obtained results. The statistical significance level was set at $p<0.05$.

3. Results and Discussion

In this study, the total contents of flavonoids and phenolics were investigated in olive leaf extracts. The olive leaves were extracted via two methods and under various conditions because the extraction procedures could affect the phytochemical profile of the extracts. Furthermore, sustainable recovery of natural biomolecules from olive leaves comes with several challenges, including a time-consuming extraction process and the stability of biomass and leave extracts (Markhali et al., 2020).

3.1. Total phenolic content (TPC)

One-way ANOVA revealed overall significant differences [$F(9,20)=44.43$, $p<0.0001$] in the total phenolic content of olive leaf extract. Tukey's *post hoc* analysis also revealed significant differences between conventional extraction vs MAE 20 min 85 °C ($p<0.0001$), MAE 20 min 70 °C vs MAE 20 min 85 °C ($p<0.0001$), and MAE 20 min´ 70 °C vs MAE 20 min 85 °C ($p<0.0001$). In this study, the total content of phenolic and flavonoid substances as well as the antioxidant capacity were determined to be similar in both extracts. However, MAE offers fast and cost-effective procedures, a short extraction time, the flexibility to perform several extractions concurrently, and a reduced solvent need. The primary disadvantage of MAE is that it produces a small amount of extract due to the small volume of the tank.

The quantities of phenolic components in olive extract produced through MAE (38 mg g^{-1}) were found to be somewhat higher than those obtained via the traditional technique (37 mg g^{-1}) (Fig 1). On the other hand, the phenolic compounds produced by MAE were substantially reduced (25 mg g^{-1}) when heated to 85 °C for 20 minutes, indicating that the total phenolic components are destroyed at high temperatures.

On the other hand, this phenomenon was not seen at the same temperature for 8 or 14 minutes, indicating that phenolic degradation is also dependent on the extraction duration. Therefore, a longer procedure at high temperatures is not recommended for extraction via MAE. Also, no significant differences were obtained between 14 and 8 minutes of extraction at all studied temperatures. Thus, the optimum conditions to extract phenolic compounds in olive leaves, seem to be 14-minutes at 60 °C and 70 °C.

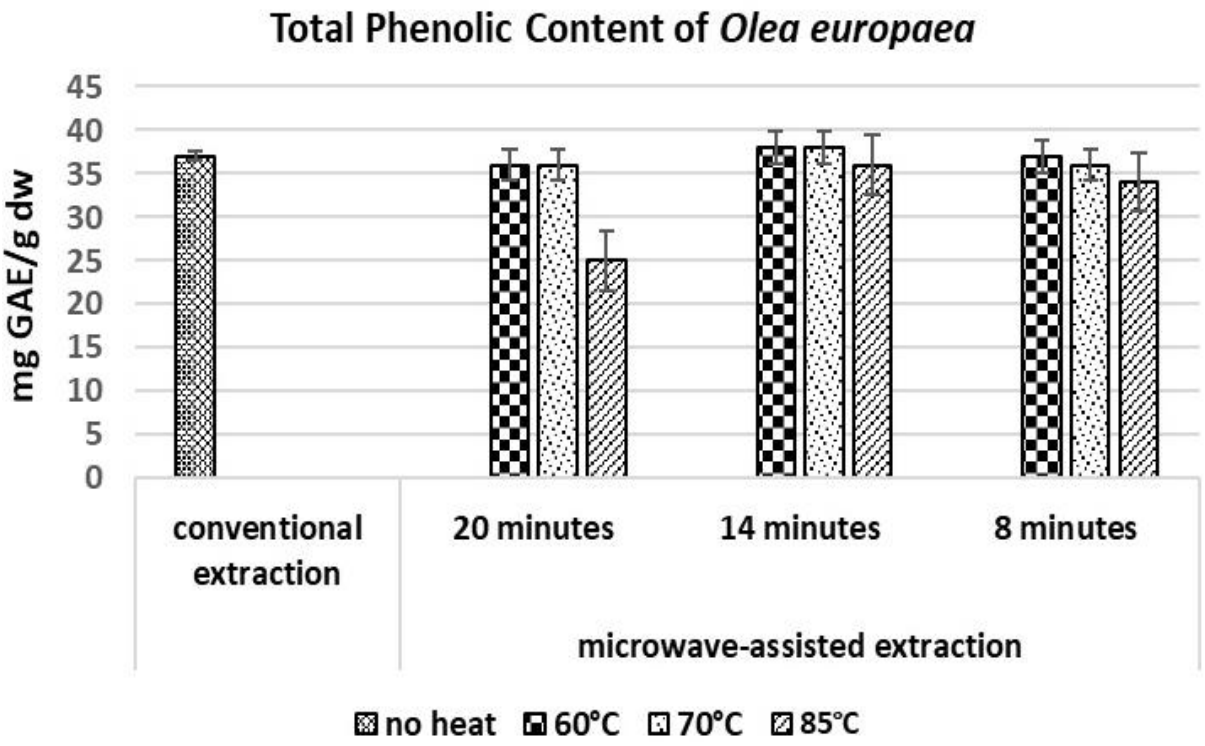

Fig. 1. Total phenolic content of *Olea europaea* leaf extract. (mg GAE g^{-1} dry plant samples).

Under various conditions via different extraction techniques, the total contents of phenolic compounds within olive leaves have been reported to be between 1.6 and 113 mg g^{-1}. The TPC value of olive leaves extracted using ethanol as a solvent for one hour at 50 °C was reported to be 61 mg g^{-1} (Kashaninejad et al., 2020). On the other hand, 24 h of the same extraction method at 25 °C, using water as the solvent, revealed a 57 mg g^{-1} TPC value (Altiok et al., 2008). In this study, 37 mg g^{-1} TPC value was obtained from the methanolic extract of olive leaves, under the conditions of no heat, for 72 h. Therefore, these data suggest that moderate heat during extraction via maceration may increase the content of phenolic compounds. On the other hand, 3 minutes of MAE at 86 °C, using water as the solvent was shown to reveal 104 mg g^{-1} TPC (Altiok et al., 2008). In this study, a 38 mg g^{-1} TPC value was determined in the olive leaf extract obtained via MAE at 60 °C or 70 °C for 14 minutes of extraction. The relatively low amount of total phenolics in this study could be attributed to the 14-minute extraction procedure, which most likely resulted in phenolic constituent degradation. This fact could also be the result of degradation after the extraction process. Furthermore, other methods such as Soxhlet-assisted extraction (4 h) were reported to reveal a 42.5 mg g^{-1} TPC value with ethanol as the solvent (Lama-Muñoz et al. 2020).

Ultrasonic-assisted extraction for 29 minutes at 27 °C using water as the solvent was shown to reveal an 81 mg g^{-1} TPC value (Rosa et al. 2021). However, using methanol as solvent via the same method was reported to reveal 39 mg g^{-1} (Bilgin and Sahin, 2013). Freeze-drying (50 °C, one h) and pressurized liquid extraction methods (190 °C, 5 minutes) using ethanol as the solvent yielded 113 mg g^{-1} (Kashaninejad et al., 2020) and 41.9 mg g^{-1} (Lama-Muñoz et al. 2020) TPC values, respectively, suggesting that moderate temperature is necessary for the extraction, however, extremely high temperature degraded the phenolics. The homogenizer-assisted extraction procedure at 25 °C for 30 minutes was shown to reveal 47 mg $g^{-1}$and 48 mg g^{-1} TPC values using methanol and ethanol as solvents, respectively (Monteleone et al. 2021).

The same method using methanol as a solvent for one minute and 1.3 minutes of extraction was reported to reveal 1.6 mg g^{-1} (Hayes et al. 2011) and 62 mg g^{-1} (Bilgin and Sahin, 2013) TPC values, respectively. The same method was shown to reveal 10 mg g^{-1} TPC value ethanol as the solvent for 24 h of the extraction procedure (Altiok et al., 2008). Recently, using natural deep eutectic solvents (NADES) for plant extraction has gained great interest. Aqueous olive leaf extract produced with the help of NADES was reported to yield 51.8 mg g^{-1} total phenolics.

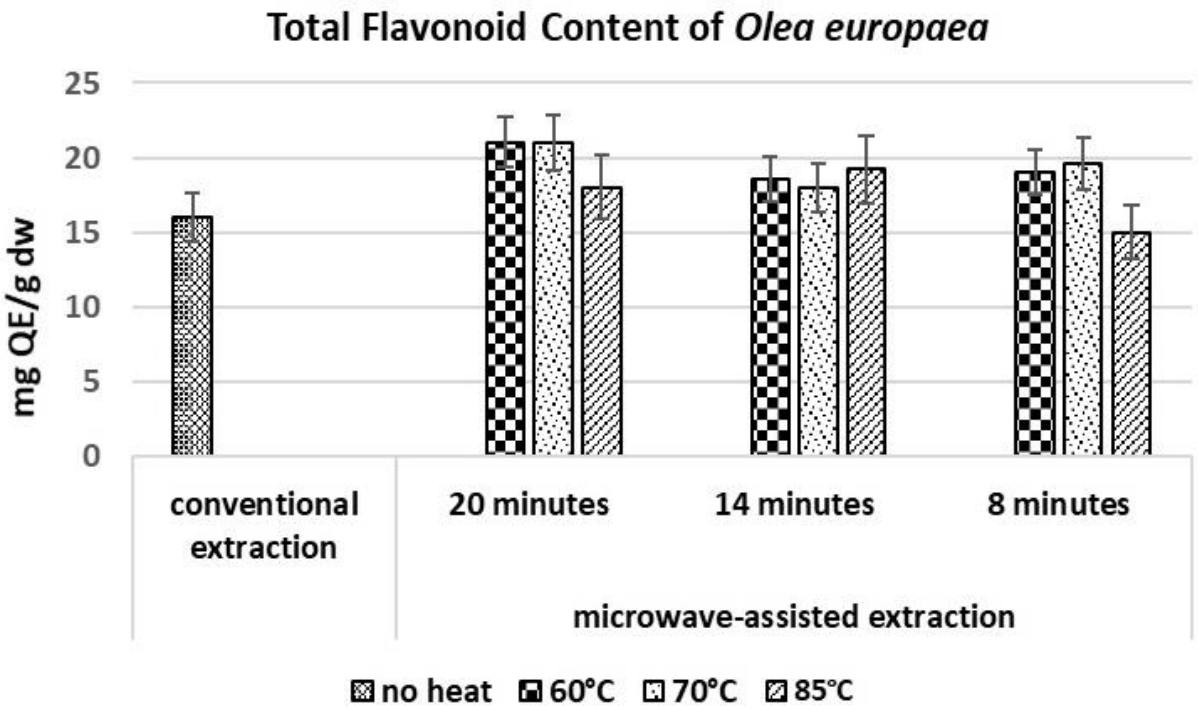

Fig. 2. Total flavonoid content of *Olea europaea* leaf extract (mg QE g^{-1} dry plant samples).

However, in the same study, methanolic and ethanolic extracts were shown to yield 26.4 mg g^{-1} and 26.9 mg g^{-1} of total phenolic composition, respectively (Yao et al. 2019). Although other factors such as the solvent and duration of the process were reported to be different in the above-mentioned study, these data suggest utilizing NADES in extraction increases the yield by almost 2-times.

3.2. Total Flavonoid Content (TFC)

One-way ANOVA revealed overall significant differences [F (9,20)=29.56, p<0.0001] in the total flavonoid content of olive leaf extract. Multiple comparisons of Tukey *post hoc* test revealed significant differences between conventional extraction vs MAE 20´ 60 °C (p<0.0001), conventional extraction vs MAE 20´ 70 °C (p<0.0001), conventional extraction vs MAE 14´ 60 °C (p<0.01), conventional extraction vs MAE 14´ 85 °C (p<0.001), conventional extraction vs MAE 8´ 60 °C (p<0.001), conventional extraction vs MAE 8´ 70 °C (p<0.001), MAE 20´ 60 °C vs MAE 20´ 85 °C (p<0.0001), MAE 20´ 70 °C vs MAE 20´ 85 °C (p<0.0001), MAE 8´ 60 °C vs MAE 8´ 85 °C (p<0.0001), MAE 8´ 70 °C vs MAE 8´ 85 °C (p<0.0001).

Flavonoid concentration (21 mg g^{-1}) obtained in this research through MAE (60 °C, 20 min) was found to be substantially greater than the values (16.0 mg g^{-1}) obtained via maceration (Fig 2). It was discovered that many of the values obtained by MAE were either higher or very similar to those obtained by the conventional method. In 8-minutes of procedure at 85 ^{o}C only, a slightly lower concentration of TFC was obtained as compared to the value obtained via the classical method.

Phenolic compounds are powerful antioxidants that have garnered significant attention lately due to their potential to benefit human health by combating degenerative illnesses. There is a strong interaction between medicinal plant intake and the prevention and treatment of a variety of health issues (cancer, cardiovascular, and neurological disorders). Phenolic substances found in foods and medicinal plants significantly reduce the harmful effects of chemically reactive species on the normal physiological processes of humans (Perron and Brumaghim, 2009).

An aromatic ring containing one or multiple hydroxyl groups is structurally called a phenolic compound. These chemicals are found in plants in free form and also as bounded structure. The phenolic compounds are ester-attached to cell wall components such as xylans, pectin, and lignin, or are covalently connected to sugars in the form of depsides or simple glycosides (Gupta and De, 2017). Therefore, an effective method to extract free and bound-form phenolics is significantly important.

The effects of olive leaf extracts are also attributed to their flavonoid content. A wide range of total

flavonoid compounds within olive leaf extracts have been reported in the literature. The olive leaf extract produced via maceration (1 h at 50 °C) to yield 6.9 mg g^{-1} TFC has the lowest data in the literature. The relatively low concentration of total flavonoids is probably due to the high temperature of extraction (Kashaninejad et al., 2020). On the other hand, homogenizer-assisted extraction was reported to reveal 15 mg g^{-1} and 17 mg g^{-1} TFC, using methanol and ethanol as the solvent, respectively, under the conditions of 25 °C and 30 minutes of duration (Monteleone et al. 2021). However, the freeze-drying method (1 h at 50 °C) was shown to yield 13.3 mg g^{-1} total flavonoids using ethanol as the solvent (Kashaninejad et al., 2020). The flavonoids were clearly degraded during the lengthy extraction at high temperatures. In the meantime, the highest amount of flavonoids was reported to be 57.3 mg g^{-1} in the aqueous olive leaf extract, obtained with the help of NADES via ultrasound-assisted extraction (Yao et al. 2019). However, the above-mentioned extraction method was shown to yield 45.0 mg g^{-1} when using methanol as the solvent at room temperature after 15 minutes of time. Changing the solvent to ethanol and the conditions to 34 °C for one h, on the other hand, revealed 41.6 mg g^{-1} TFC in the same study (Yao et al. 2019). Therefore, to yield a high concentration of total flavonoids from olive leaf extract, using NADES as solvent via an ultrasound-assisted extraction procedure at a low or medium temperature could be suggested.

3.3. Ferric Reducing Antioxidant Power Assay (FRAP)

One-way ANOVA revealed overall significant differences [F (9,20)=245.8 p<0.0001] in FRAP analysis. Tukey's multiple comparisons assay revealed significant differences between conventional extraction vs MAE 20´ 60 °C (p<0.0001), conventional extraction vs MAE 20´ 85 °C (p<0.0001), conventional extraction vs MAE 14´ 60 °C (p<0.0001), conventional extraction vs MAE 14´ 70 °C (p<0.0001), conventional extraction vs MAE 14´ 85 °C (p<0.0001), conventional extraction vs MAE 8´ 60 °C (p<0.01), MAE 20´ 60 °C vs MAE 20´ 70 °C (p<0.0001), MAE 20´ 70 °C vs MAE 20´ 85 °C (p<0.0001), MAE 14´ 60 °C vs MAE 14´ 70 °C (p<0.0001).

The antioxidant capacity of olive extract produced by MW-assisted extraction (52 mg g^{-1}) was found to be somewhat greater than that of the traditional approach (50 mg g^{-1}) in this research (Fig 3). On the other hand, the lowest FRAP value was achieved using the MW-assisted extraction method after 14 minutes at 60 °C. Additionally, throughout the 20-minute process at 60 °C, lower FRAP values were found which are likely due to inadequate temperature. This indicates that the chemicals responsible for FRAP in olive leaves are very temperature sensitive. The highest concentration was achieved after eight minutes of extraction at 70 °C. These findings indicate that the antioxidant molecules used in the FRAP test are very sensitive to changes in extraction time and temperature.

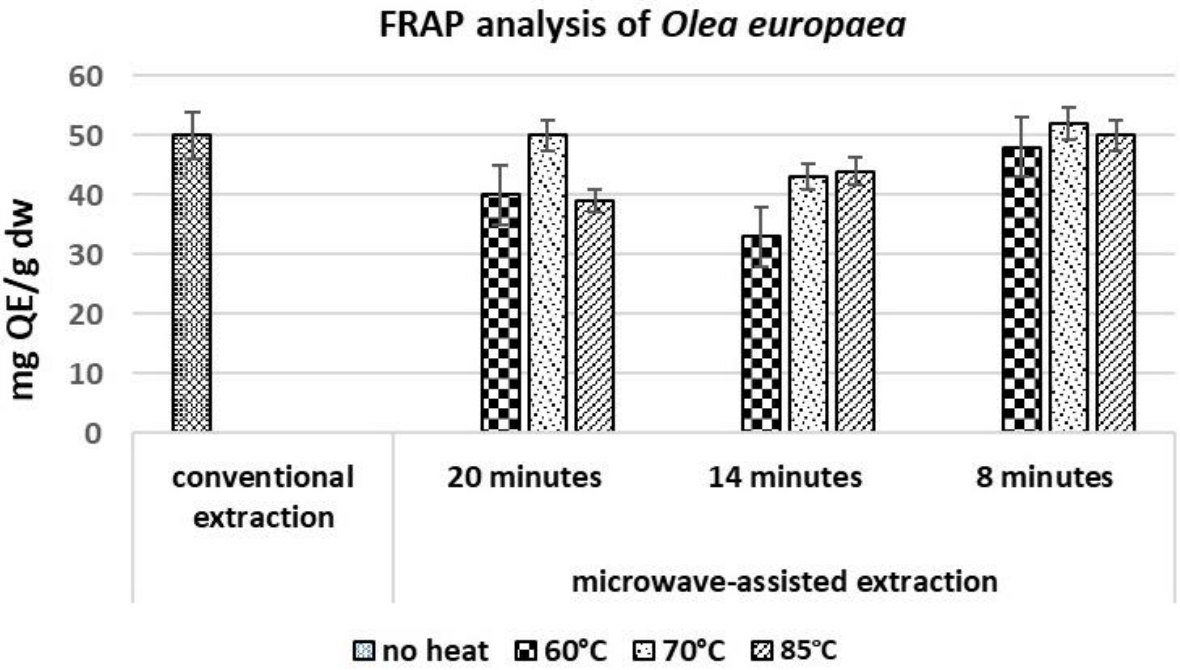

Fig. 3. FRAP analysis of *Olea europaea* extract. (mg QE g^{-1} dry plant samples).

The FRAP technique quantifies an antioxidant's capacity to act as a reducing agent. In this study, the concentrations of FRAP values within the olive leaf extract obtained via maceration and MAE were found to be close to each other. However, in the literature, a wide range of FRAP values have been reported. For example, ultrasound-assisted extraction of olive leaves was reported to yield 58 mg g^{-1} FRAP value with 50% acetone at 60 °C for 10 min of extraction (Irakli et al., 2018). On the other hand, high FRAP values were reported in the ethanolic olive leaf extracts obtained via maceration and freeze-drying methods (134 mg g^{-1} and 435 mg g^{-1}, respectively) at 50 °C for one h (Kashaninejad et al., 2020). Furthermore, homogenizer-assisted extraction was shown to yield a 300 mg/g FRAP value using ethanol at 50 °C for one h (Hayes et al., 2011). In addition, the FRAP values of olive leaf extract obtained via conventional and ultrasound-assisted extraction at 60 °C for one h with ethanol were reported to be 552 µM and 619 µM TE g^{-1}, respectively (Giacometti et al., 2021). Interestingly, in the above-mentioned studies, high temperature during extraction did not cause degradation of the molecules contributing to FRAP.

3.4. Phenanthroline metal chelating capacity (MCC)

Significant overall differences were observed via one-way ANOVA [$F(9,20)=1464$ $p<0.0001$]. Further multiple comparison test revealed significant differences between conventional extraction vs MAE 20´ 60 °C ($p<0.0001$), conventional extraction vs MAE 20´ 70 °C ($p=0.0013$), conventional extraction vs MAE 20´ 85 °C ($p<0.0001$), conventional extraction vs MAE 14´ 60 °C ($p<0.0001$), conventional extraction vs MAE 14´ 70 °C ($p<0.0001$), conventional extraction vs MAE 8´ 60 °C ($p<0.0001$), conventional extraction vs MAE 8´ 70 °C ($p<0.0001$), conventional extraction vs MAE 8´ 85 °C ($p<0.0001$), MAE 20´ 60 °C vs MAE 20´ 70 °C ($p<0.0001$), MAE 20´ 60 °C vs MAE 20´ 85 °C ($p<0.0001$), MAE 20´ 70 °C vs MAE 20´ 85 °C ($p<0.0001$), MAE 14´ 60 °C vs MAE 14´ 70 °C ($p<0.0001$), MAE 14´ 60 °C vs MAE 14´ 85 °C ($p<0.0001$), MAE 14´ 70 °C vs MAE 14´ 85 °C ($p<0.0001$), MAE 8´ 60 °C vs MAE 8´ 70 °C ($p<0.0001$), MAE 8´ 60 °C vs MAE 8´ 85 °C ($p<0.0001$).

The highest MCC value obtained via MAE was 142 mg g^{-1}, which was slightly higher than the value obtained via the classical method (140 mg g^{-1}). On the other hand, the lowest MCC values determined via MAE were obtained at 8-minutes of procedures, suggesting that reduced time was not sufficient to extract the molecules contributing MCC assay (Fig 4).

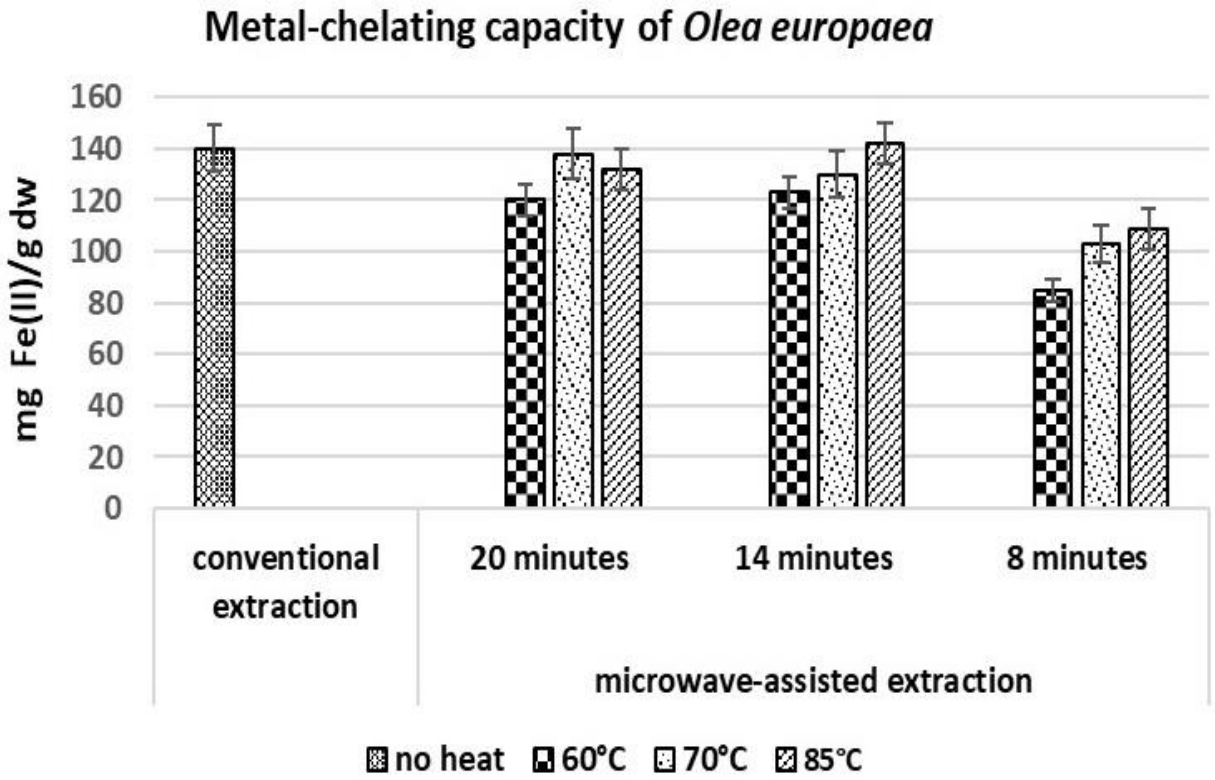

Fig. 4 Metal-chelating activity of *Olea europaea* extract. (mg Fe(II)/g dry plant samples).

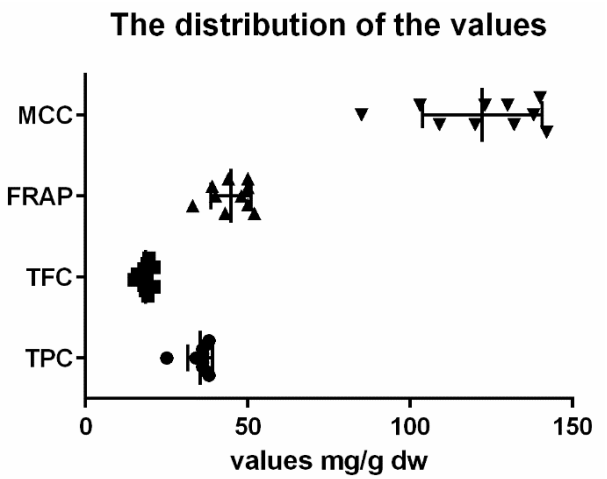

Fig. 5. Distribution of values obtained in each assay. TPC (total phenolic content), TFC (total flavonoid content), FRAP (ferric reducing antioxidant power assay), and phenanthroline MCC (metal chelating capacity**).**

Distribution of all values obtained in each assay is presented in Fig. 5. Interestingly, TFC was found to be the least distributed assay, followed by TPC and FRAP assays. One of the values in the TPC assay was observed to be significantly lower than the other values which were clustered together. However, in the MCC assay, the values were highly distributed as compared to the other assays. Therefore, these results suggest that the total flavonoid content of olive leaves is not significantly affected due to extraction conditions. However, the molecules contributing to the phenanthroline-metal chelating assay seem more sensitive to the extraction conditions. Supposedly, not all phenolic compounds contribute to the FRAP and MCC assays. Furthermore, although phenolics and flavonoids are known to be temperature-sensitive, this study shows the opposite.

The leaves of olives are generally considered waste products in the industry, although they are rich in phenolic compounds. Olive leaves with tremendous health-promoting activities as antioxidants are highly recommended to take advantage of in the food industry. Lately, many studies have suggested the advantages of using olive leaf extracts on foods and food packages. Indeed, there is a great need to increase the antioxidant properties of cooking oil, particularly when using deep frying or high temperatures (Sánchez de Medina et al. 2011). In this regard, several studies revealing the consequences of olive leaf extracts in edible oils have been reported. The addition of olive leaf extract to vegetable oils has been shown to boost the phenolic content and shelf life of the oil (Chiou et al., 2007). Furthermore, the addition of olive leaf extracts to palm and olive oil has been reported to increase the antioxidant potential of the oils owing to their increased polyphenolic composition (Paiva-Martins et al. 2007). Recently, the addition of olive leaves and oleuropein to yogurt was reported to improve firmness, but not affect the taste. Therefore, olive leaf extracts have been suggested to be incorporated into foods to produce novel functional foods (Zoidou et al. 2017). Olive leaf extracts have also been reported to be a good way to produce environmentally safe food packages with antimicrobial and antioxidant properties to increase shelf-life (Khwaldia et al., 2022).

Hydroxytyrosol, the other main component of olive leaves, has the potential to be used as an ingredient in functional foods (Liamin et al., 2023). The European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) both classify hydroxytyrosol as Generally Recognized as Safe (GRAS) (Monteleone et al., 2021). According to the 2011 EFSA decision and EC regulation 432/2012, hydroxytyrosol is the only "food" that is legally recognized as having an evident health benefit when ingested regularly (Silva et al., 2020). Numerous patented hydroxytyrosol-rich extracts have been marketed as dietary supplements or as additives in healthy foods. These extracts have been suggested as functional components in a variety of food matrices, including edible oils, drinks, and baked goods (Caponio et al. 2019; Cedola et al. 2020; Guglielmotti et al., 2020; Kranz et al. 2010).

4. Conclusion

In this research, traditional and microwave-assisted techniques were compared for the extraction of olive leaves. Microwave-assisted extraction was found to be an efficient, rapid and economical method as compared to maceration. High temperature during extraction supposedly degrades the phenolic compounds in olive leaves. Therefore, the extraction of olive leaves is suggested to be somewhat higher than room temperature, but not above 50 °C. It could be also possible to apply high temperatures during extraction, only if the duration is relatively short. Furthermore, olive is recommended for use in the food sector as a natural antioxidant rather than synthetic ones in order to avoid health-damaging consequences.

List of Abbreviations: EFSA, European Food Safety Authority; FAO, Food and Agriculture Organization; FDA, Food and Drug Administration; FRAP, ferric reducing antioxidant power assay; MAE, microwave-assisted extraction; MCC, metal chelating capacity; TFC, total flavonoid content; TPC, total phenolic content.

Acknowledgment: Author would like to thank Prof. Dr. Mehmet Yaman, from the Department of Chemistry, Firat University Elazig, Türkiye for technical support and allowing the use of laboratory equipments for chemical analysis.

References

Akbaba, E. 2021. Characterization of bioactive and antioxidant composition of mountain tea (*Sideritis montana* ssp. montana): Microwave-assisted technology. Int. J. Second. Metabol. 8: 159-171.

Altıok, E., D. Bayçın, O. Bayraktar and S. Ülkü, 2008. Isolation of polyphenols from the extracts of olive leaves (*Olea europaea* L.) by adsorption on silk fibroin. Separation and Purification Technol. 62: 342-348.

Barazani, O., A. Dag and Z. Dunseth. 2023. The history of olive cultivation in the southern Levant. Front. Plant Sci. 14: 1131557.

Berker, K.I., K. Güçlü, İ. Tor, B. Demirata and R. Apak. 2010. Total Antioxidant Capacity Assay Using Optimized Ferricyanide/Prussian Blue Method. Food Analytical Methods. 3: 154-168.

Besnard, G., J.-F. Terral and A. Cornille. 2017. On the origins and domestication of the olive: a review and perspectives. Ann. Bot. 121: 385-403.

Bilgin, M. and S. Şahin. 2013. Effects of geographical origin and extraction methods on total phenolic yield of olive tree (*Olea europaea*) leaves. J. Taiwan Inst. Chemical Eng. 44: 8-12.

Brandt, W. W., F. P. Dwyer and E. D. Gyarfas. 1954. Chelate complexes of 1,10-phenanthroline and related compounds. Chem. Rev. 54: 959-1017.

Caponio, F., G. Difonzo, M. Calasso, L. Cosmai and M. De Angelis. 2019. Effects of olive leaf extract addition on fermentative and oxidative processes of table olives and their nutritional properties. Food Res. Int. 116: 1306-1317.

Cedola, A., C. Palermo, D. Centonze, M. A. Del Nobile and A. Conte. 2020. Characterization and bio-accessibility evaluation of olive leaf extract-enriched "Taralli". Foods. 9: 1268.

Chiou, A., F. N. Salta, N. Kalogeropoulos, A. Mylona, I. Ntalla and N. K. Andrikopoulos. 2007. Retention and distribution of polyphenols after pan-frying of french fries in oils enriched with olive leaf extract. J. Food Sci. 72: S574-S584.

FAO. 2021. "Http://Www.Fao.Org/Faostat/En/#data/QCL/Visualize." 2021.

Giacometti, J., S. Milovanović, D. Jurc\ʿić Momc\ʿilović and M. Bubonja-S\ʿonje. 2021. Evaluation of antioxidant activity of olive leaf extract obtained by ultrasound-assisted extraction and their antimicrobial activity against bacterial pathogens from food. Int. J. Food Sci. Technol. 56: 4843-4850.

González-Burgos, E., M. E. Carretero and M. P. Gómez-Serranillos. 2011. Sideritis spp.: Uses, chemical composition and pharmacological activities—A review. J. Ethnopharmacol. 135: 209-225.

Guglielmotti, M., P. Passaghe and S. Buiatti. 2020. Use of olive (*Olea europaea* L.) leaves as beer ingredient, and their influence on beer chemical composition and antioxidant activity. J. Food Sci. 85: 2278-2285.

Gupta, P., and B. De. 2017. Differential responses of cell wall bound phenolic compounds in sensitive and tolerant varieties of rice in response to salinity. Plant Signal. Behav. 12 (10): e1379643.

Hayes, J. E., P. Allen, N. runton, M.N. 'Grady and P. Kerry. 2011. Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: olive leaf extract (*Olea europaea* L.), lutein, sesamol and ellagic acid. Food Chem. 126 (3): 948–55.

Herrero, M., T. N. Temirzoda, A. Segura-Carretero, R. Quirantes, M. Plaza and E. Ibañez. 2011. New possibilities for the valorization of olive oil by-products. J. Chromatog. A. 1218: 7511-7520.

Irakli, M., P. Chatzopoulou and L. Ekateriniadou. 2018. Optimization of ultrasound-assisted extraction of phenolic compounds: Oleuropein, phenolic acids, phenolic alcohols and flavonoids from olive leaves and evaluation of its antioxidant activities. Ind. Crops Prod. 124: 382-388.

Ismail, M. A., M. N. Norhayati and N. Mohamad. 2021. Olive leaf extract effect on cardiometabolic profile among adults with prehypertension and hypertension: a systematic review and meta-analysis. PeerJ. 9: e11173.

Kashaninejad, M., M. T. Sanz, B. Blanco, S. Beltrán and S. M. Niknam. 2020. Freeze dried extract from olive leaves: Valorisation, extraction kinetics and extract characterization. Food Bioprod. Process. 124: 196-207.

Khemakhem, I., A. Fuentes, M. J. Lerma-García, M. A. Ayadi, M. Bouaziz and J. M. Barat. 2019. Olive leaf extracts for shelf life extension of salmon burgers. Food Sci. Technol. Int. 25: 91-100.

Khwaldia, K., N. Attour, J. Matthes, L. Beck and M. Schmid. 2022. Olive byproducts and their bioactive compounds as a valuable source for food packaging applications. Comprehensive Rev. Food Sci. Food Saf. 21: 1218-1253.

Kranz, P., N. Braun, N. Schulze and B. Kunz. 2010. Sensory quality of functional beverages: bitterness perception and bitter masking of olive leaf extract fortified fruit smoothies. J. Food Sci. 75: S308-S311.

Lama-Muñoz, A., M. d. M. Contreras, F. Espínola, M. Moya, I. Romero and E. Castro. 2020. Content of phenolic compounds and mannitol in olive leaves extracts from six Spanish cultivars: Extraction with the Soxhlet method and pressurized liquids. Food Chem. 320: 126626.

Liamin, M., Lara, M.P., Michelet, O., Rouault, M., Quintela, J.C., Le Bloch, J. 2023. Olive juice dry extract containing hydroxytyrosol, as a nontoxic and safe substance: Results from pre-clinical studies and review of toxicological studies. Toxicol Rep. 10: 245-260.

Losada-Echeberría, M., A. Taamalli, V. Micol, M. Herranz-López, D. Arráez-Román and E. Barrajón-Catalán. 2017. P 215 - Antioxidant enriched olive leaf extracts show antiproliferative activity in cellular models of breast cancer. Free Radic. Biol. Med. 108: S92.

Magalhães, L. M., M. A. Segundo, S. Reis and J. L. F. C. Lima. 2008. Methodological aspects about in vitro evaluation of antioxidant properties. Anal. Chim. Acta. 613: 1-19.

Marchev, A. S., L. V. Vasileva, K. M. Amirova, M. S. Savova, I. K. Koycheva, Z. P. Balcheva-Sivenova, S. M. Vasileva and M. I. Georgiev. 2021. Rosmarinic acid - From bench to valuable applications in food industry. Trend. Food Sci. Technol. 117: 182-193.

Markhali, F. S., J. A. Teixeira and C. M. R. Rocha. 2020. Olive tree leaves—a source of valuable active compounds. Processes. 8: 1177.

Marković, M., E. Mezzatesta, S. Porcier, C. Vieillescazes and C. Mathe. 2022. Rethinking the process of animal mummification in ancient Egypt: Molecular Characterization of embalming material and the use of brassicaceae seed oil in the mummification of Gazelle Mummies from Kom Mereh, Egypt. Molecules. 27: 1532.

Martín-García, B., S. Pimentel-Moral, A.M. Gómez-Caravaca, D. Arráez-Román and A. Segura-Carretero. 2020. Box-Behnken experimental design for a green extraction method of phenolic compounds from olive leaves. Ind. Crops Prod. 154: 112741.

Medina, E., C. Romero, P. Garcia and M. Brenes. 2019. Characterization of bioactive compounds in commercial olive leaf extracts, and olive leaves and their infusions. Food Function. 10 (8): 4716–24.

Monteleone, J.I., E. Sperlinga, L. Siracusa, G. Spagna, L. Parafati, A. Todaro and R. Palmeri. 2021. Water as a solvent of election for obtaining oleuropein-rich extracts from olive (*Olea europaea*) leaves. Agronomy. 11 (3): 465.

Oliveira, A. L. S., S. Gondim, R. Gómez-García, T. Ribeiro and M. Pintado. 2021. Olive leaf phenolic extract from two Portuguese cultivars –bioactivities for potential food and cosmetic application. J. Environ. Chem. Eng. 9: 106175.

Ozturk, M., V. Altay, T.M. Gönenç, B.T. Unal, R. Efe, E. Akçiçek and A. Bukhari 2021. an overview of olive cultivation in Turkey: botanical features, eco-physiology and phytochemical aspects. Agronomy. 11(2): 295.

Paiva-Martins, F., R. Correia, S. Félix, P. Ferreira and M.H. Gordon. 2007. Effects of enrichment of refined olive oil with phenolic compounds from olive leaves. J. Agric. Food Chem. 55 (10): 4139–43.

Palmeri, R., L. Siracusa, M. Carrubba, L. Parafati, I. Proetto, F. Pesce and B. Fallico. 2022. Olive leaves, a promising byproduct of olive oil industry: Assessment of metabolic profiles and antioxidant capacity as a function of cultivar and seasonal change. Agronomy. 12: 2007.

Perron, N.R., and J.L. Brumaghim. 2009. A Review of the antioxidant mechanisms of polyphenol compounds related to ıron binding. Cell Biochem. Biophys. 53 (2): 75–100.

Rosa, G.S., Martiny, T.R., Dotto, G.L., Vanga, S.K., Parrine, D., Gariepy, Y., Lefsrud, M., and Raghavan, V. 2021. Eco-Friendly extraction for the recovery of bioactive compounds from Brazilian olive leaves. Sustain. Mat. Technol. 28: e00276.

Salem, M.B., H. Affes, K. Ksouda, Z. Sahnoun, K.M. Zeghal and S. Hammami. 2015. Pharmacological activities of *Olea europaea* leaves. J. Food Process. Rreserv. 39 (6): 3128–36

Sanchez de Medina, V., Priego-Capote, F., Jiménez-Ot, C., and Luque de Castro, M.D. 2011. Quality and stability of edible oils enriched with hydrophilic antioxidants from the olive tree: The role of enrichment extracts and lipid composition. J. Agric. Food Chem. 59 (21): 11432–41.

Silva, A.F.R., D. Resende, M. Monteiro, M.A. Coimbra, A.M.S. Silva and S.M. Cardoso. 2020. Application of hydroxytyrosol in the functional foods field: From ingredient to dietary supplements. Antioxidants. 9 (12): 1–23.

Šimat, V., D. Skroza, G. Tabanelli, M. Čagalj, F. Pasini, A. M. Gómez-Caravaca, C. Fernández-Fernández, M. Sterniša, S. Smole Možina, Y. Ozogul and I. Generalić Mekinić. 2022. Antioxidant

and antimicrobial activity of hydroethanolic leaf extracts from six Mediterranean olive cultivars. Antioxidants 11: 1656.

Singleton, V.L., R. Orthofer and M.B.T. Rosa. 1999. Methods in enzymology lamuela-raventós. analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. Methods Enzymol. 299: 178–84.

Sofo, A., S. Manfreda, M. Fiorentino, B. Dichio and C. Xiloyannis. 2008. The olive tree: A paradigm for drought tolerance in Mediterranean climates. Hydrol. Earth Syst. Sci. 12 (1): 293–301.

Soler-Rivas, C., J.C. Espin and H.J. Wichers. 2000. Oleuropein and Related Compounds. J. Sci. Food Agric. 80 (7): 1013–23.

Szydłowska-Czerniak, A., C. Dianoczki, K. Recseg, G. Karlovits and E. Szłyk. 2008. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. Talanta. 76 (4): 899–905.

Trifa, W., S. Akkal, M. Lefahal, L. Benmekhebi and S. Khennouf. 2020. Preliminary screening of extracts for determination of antioxidant activity by different methods. Curr. Iss. Pharm. Med. Sci. 33 (1): 32–37.

Tsimidou, M.Z., and Papoti, V.T. 2010. Chapter 39 - Bioactive Ingredients in Olive Leaves. In Olives and Olive Oil in Health and Disease Prevention, edited by V.R. Preedy and R. Ross B T - Olives and Olive Oil in Health and Disease Prevention Watson, p: 349–56.

Vezza, T., F. Algieri, A. Rodriguez-Nogales, J. Garrido-Mesa, M.P. Utrilla, N. Talhaoui, A.M. Gomez-Caravaca, et al. 2017. Immunomodulatory properties of *Olea europaea* leaf extract in ıntestinal ınflammation. Mol. Nutrit. Food Res. 61: 10.

Yao, Q., Y.F. Shen, L. Bu, P. Yang, Z.P. Xu and X.Q. Guo. 2019. Ultrasound-assisted aqueous extraction of total flavonoids and hydroxytyrosol from olive leaves optimized by response surface methodology. Prep. Biochem. Biotechnol. 49 (9): 837–45.

Zhang, C., X. Xin, J. Zhang, S. Zhu, E. Niu, Z. Zhou and D. Liu. 2022. Comparative evaluation of the phytochemical profiles and antioxidant potentials of olive leaves from 32 cultivars grown in China. Molecules. 27: 1292.

Zoidou, E., E. Melliou, G. Moatsou and P. Magiatis. 2017. Preparation of Functional Yogurt Enriched With Olive-Derived Products. In: Yogurt in Health and Disease Prevention, p:203–220.