

Investigation of The *In Vitro* Antioxidant Properties of Methanol Extract of *Olea europaea* L. (Olive) Leaf

Cenk GÜVEN¹ , Nurcan KILIÇ BAYGUTALP² , Lale DUYSAK² 

¹5th Grade Student of Faculty of Pharmacy, Ataturk University, Erzurum, Turkey

²Department of Biochemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey

ABSTRACT:

Olea europaea L. (Olive) plant has been used by humans for many years for the treatment of diseases. The aim of this study was to investigate the total phenolic compound and antioxidant activities of methanol extract of the leaves of *Olea europaea* L. (Olive) plant. Total phenolic content of *Olea europaea* L. (Olive) plant leaves was determined by a method using Folin Ciocalteu reagent. Antioxidant activities were determined by DPPH (1,1-diphenyl-2-picrylhydrazil) and FRAP (Iron ion reducing antioxidant power) methods. Different concentrations of reference samples between 1-100 µg/mL were prepared to determine the equivalent antioxidant capacity of the extract. The phenolic compound of methanol extract of the leaves of the *Olea europaea* L. (Olive) plant was the highest at a concentration of 1000 µl/ml. DPPH radical scavenging capacity (inhibition %) values were found to be significantly higher in the extract at 1000 µl/ml than the extracts at other concentrations. This study supports the potential use of *Olea europaea* L. (Olive) in folk medicine.

Keywords : Antioxidant, DPPH, FRAP, methanol extract, *Olea europaea* L.

Received

24.12.2022

Accepted

31.12.2022

Published

04.01.2023

To cite this article:

Güven C, Baygutalp KN, Duysak L. Investigation of The *In Vitro* Antioxidant Properties of Methanol Extract of *Olea europaea* L. (Olive) Leaf. International Journal of PharmATA. 2023; 3(1); 6-13.

1. INTRODUCTION

Plants have been used by humans to treat diseases since ancient times in the world. Considering the plant existence in our country, approximately 8500 plant species are grown and it is known that only 650 of them are used for therapeutic purposes. Some natural chemicals found in these plants, which have been the subject of research, draw attention as antioxidants that are very beneficial for human health, and these plants have been consumed by humans and animals since the first civilizations, and the demand for consumption is increasing day by day [1]. Turkey has a rich flora for medicinal plants and there are many plants used in traditional medicine [2]. Antioxidants are molecules that prevent the formation of radical groups in living metabolism and neutralize the resulting radicals. Preventing the spoilage of food and preserving the value of the nutrients in it is

* Corresponding Author: Tel : +90 5349878168
E-mail : cenkk.guven@gmail.com

another area of use. Fruits and vegetables are used in the treatment of some diseases due to these effects. Toxic substances that occur due to the applications in the production process of the foods we consume in our body increase the intake of free radicals to the organism and cause them to accumulate in our body. Therefore, it is extremely important to consume foods containing antioxidants in order to remove toxic substances from our body and protect them from their damaging effects. One of these plants is Olive (*Olea europaea* L.) and it is a plant belonging to the Oleaceae (Zeytingiller) family. Olive leaves (*Olea europaea* L. folium) are a natural source of bioactive components. It has been reported that many of the phenolic compounds contained in olive leaves have antioxidant effects [4]. In this study, it was aimed to determine the phenolic compound and antioxidant activity of olive (*Olea europaea* L. folium) leaf methanol extract. Although scientists have made various studies on many plant species, there are still many plant species that have not been researched yet. Our study differs from other studies in terms of the fact that the plant material used was collected from Antalya Finike district and the width of the tested concentration range.

2. METHODS

2.1. Plant Material

Olive leaves collected from Finike district of Antalya province in the Mediterranean Region of our country in September 2021 were cleaned and dried in the shade at room temperature.

2.2. Preparation of Plant Extracts

After drying the leaves of *Olea europaea* L. (Olive) plant, it was ground into powder in a porcelain mortar with liquid nitrogen. Olive leaves treated with methanol were extracted by filtering every 24 hours at 50°C in a shaker water bath for three days. The resulting filtrate was evaporated by an evaporator. After the methanol was removed, the extract remaining in the glass flask was taken into a glass petri dish, left to dry and stored in a refrigerator at +4°C for determinations.

2.3. Determination of Total phenolic content

Total phenolic compound content of olive (*Olea europaea* L.) leaf methanol extract was determined by Folin-Ciocalteu method (FC) method [4]. The amount of total phenolic compounds in the plant methanol extract was determined by using the modified version of the method developed by Slinkard and Singleton [5]. First, 50 ml of 7.5% Na₂CO₃ was prepared. Then, after weighing 25 mg of Gallic acid for the standard, it was completed with methanol to 25 ml in a test tube. Finally, Folin Ciocalteu reagent was taken into beaker for phenolic compound determination. Stock solutions were prepared and necessary dilutions were made. First, 40 µL of the sample was pipetted onto the plates. Then, 200 µL of Folin & Ciocalteu reagent was pipetted and incubated for 5 minutes. Finally, 160 µL of Na₂CO₃ was pipetted and incubated for 30 minutes. After the incubation period was over, absorbance was measured at 765 nm with a spectrophotometer device.

Using the standard graph prepared using gallic acid, the results were given as mg Gallic Acid equivalent (GAE)/g.

2.4. Antioxidant Capacity Tests

Total antioxidant activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazil) and FRAP Iron(III) Ion Reduction methods.

2.5. Determination of DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging activity

The DPPH radical scavenging capacities of the methanol extract obtained from the leaves of *Olea europaea* L. plant were determined according to the Brand Williams method [6]. After the DPPH solution was prepared according to this method, stock solutions of plant extracts in the range of 2-20 $\mu\text{L}/\text{mL}$ were prepared. First, 210 μL of extract sample was pipetted into the plate wells, and then 70 μL of DPPH solution was pipetted into each well. The plate was mixed for 1 minute with the help of a stirrer and incubated for 30 minutes. Trolox was used as the standard antioxidant for the control sample. All samples were measured absorbance at 515 nm against a blank consisting of methanol, and the results were calculated as percent inhibition.

2.6. Fe^{+3} TPTZ reduction capacity according to FRAP method

The antioxidant capacity determination method of the extracts obtained from the leaves of *Olea europaea* L. plant, based on electron transfer, was applied by Huang et al. [7]. First, 300 mmol/L acetate buffer (pH=3.6) was prepared. 10 mM TPTZ was taken into a 100 mL flask, 40 mM HCl was added and the final volume was made up to 100 mL. Finally, 20 mmol/L FeCl_3 solution was prepared. A total of 30 mL of FRAP solution was obtained by taking 2.5 mL of TPTZ, 2.5 mL of FeCl_3 and 25 mL of acetate buffer from these prepared solutions. 10 μL of the extract sample and 200 μL of FRAP solution were pipetted into the plate wells and allowed to incubate for 30 minutes, and then the absorbance was measured at 593 nm.

3. RESULTS

3.1. Total Phenolic Compound Quantification

The amount of total phenolic compounds of the methanol extract prepared from the leaves of *Olea europaea* L. (Olive) plant was determined by Folin-Ciocalteu Reagent (FCR). Gallic acid was plotted with calculations using a standard phenolic compound (Figure 1). The total phenolic compound amounts of the methanol extract prepared from the leaves of *Olea europaea* L. (Olive) plant were calculated as gallic acid equivalents (GAE) (Table 1).

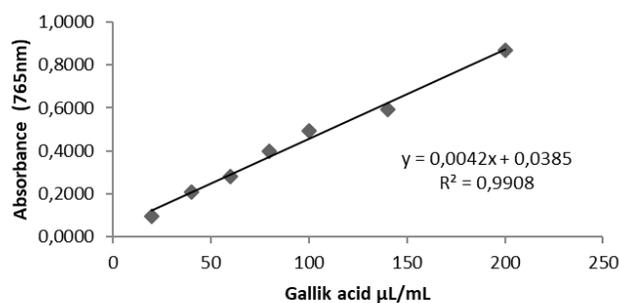


Figure 1. Standard graph of gallic acid

Table 1. Total phenolic compound amounts of methanol extract of *Olea europaea* L. (Olive) leaves

Concentration µg/mL	Total Phenolic Compound (µg GAE/mg extract)
250	14.976
500	26.555
1000	63.103

GAE: Gallic acid equivalent

Total phenolic contents of methanol extract of *Olea europaea* L. (Olive) leaves were determined at different concentrations. Accordingly, the highest total phenolic content was determined at the concentration of 1000µg/mL.

3.2. Antioxidant Capacity Findings

3.2.1. DPPH radical scavenging studies

The analyzed concentration range (1-100 µg/mL) was determined as a result of studies in the literature and preliminary trials on standard antioxidant compounds. As a standard antioxidant, the radical scavenging effect of trolox DPPH reached its highest level at a concentration of 20 µg/mL (Figure 2).

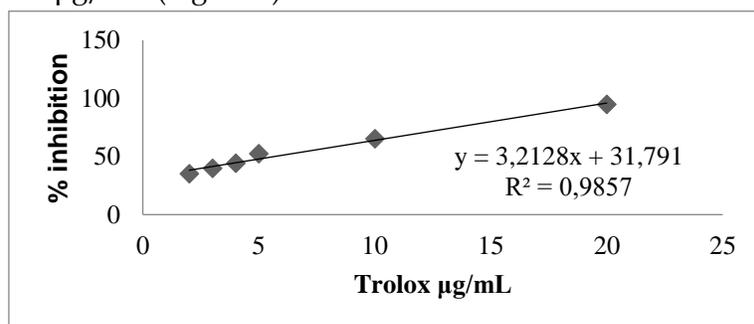


Figure 2. Concentration-% inhibition plot of Trolox

DPPH radical scavenging capacities in the range of methanol extract (250-1000 µg/mL) of *Olea europaea* L. (Olive) leaves are shown in Table 2 as % inhibition.

Table 2. DPPH % Inhibition Values

Concentration (µg/mL)	Trolox Eq (µL/mL)
250	4.774
500	9.682
1000	17.620

Olea europaea L. (Olive) leaves showed the highest DPPH free radical scavenging effect of methanol extract at 1000 µg/mL concentration of the extract.

3.2.2. Iron ion reducing antioxidant power (FRAP)

Spectrophotometric measurement of iron (III) reduction/antioxidant equivalent absorbance at 595 nm of methanol extract of *Olea europaea* L. (Olive) leaves and standard antioxidant compounds was performed. As a result of the studies, the analyzed concentration value range (1-100 µg/mL) was made over standard antioxidant compounds. Since the antioxidant power capacity of trolox, which is one of the standard antioxidant compounds, reached the maximum level at a concentration value of 40 µg/mL, the study was performed in the range of 1-100 µg/mL (Figure 3).

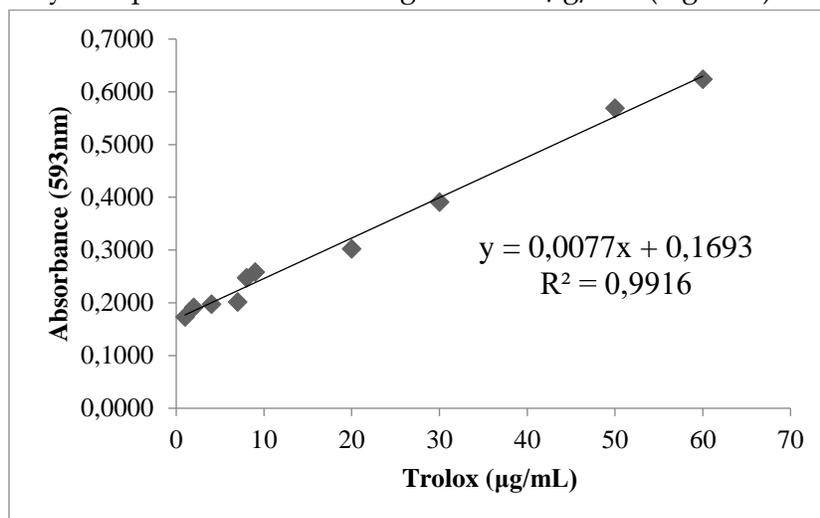


Figure 3. Standard graph of Trolox

The comparison of the iron (III) reducing/antioxidant powers of *Olea europaea* L. (Olive) leaves at 593 nm by spectrophotometric method in terms of µg/mL Trolox equivalent Antioxidant Capacity (TEAC) is shown in Table 3.

Table 3. FRAP method Equivalent Trolox Eq Values

Concentration ($\mu\text{g/mL}$)	Trolox Eq $\mu\text{g/mL}$
250	3.316
500	6.259
1000	11.324

4. DISCUSSION

In this study, the total phenolic compound amount and antioxidant capacity (DPPH, FRAP) of the methanol extract of the leaves of *Olea europaea* L. (Olive) plant were determined. Accordingly, the phenolic content was found to be 63.103 $\mu\text{g GAE/mg}$ at 1000 $\mu\text{g/mL}$ concentration. DPPH, FRAP tests carried out to determine the antioxidant capacities of the methanol extract obtained from the leaves of *Olea europaea* L. (Olive) plant showed that, antioxidant activity was found to be higher at 1000 $\mu\text{g/mL}$ concentration. The antioxidant effects were found to be 1000 $\mu\text{g/mL}$ 17.620 Trolox Eq ($\mu\text{L/mL}$) in the DPPD method and 1000 $\mu\text{g/mL}$ 11.324 Trolox Eq ($\mu\text{L/mL}$) in the FRAP method.

Olea europaea L. (Olive) is a rich source of active compounds and has been used in folk medicine in Turkey as well as worldwide. Antiarrhythmic and hypotensive effects of olive leaf have been demonstrated, as demonstrated in animal studies. In the literature, it has been stated that the extract made by drying the olive leaf can regulate blood coagulation and circulation and therefore has preventive effects on heart diseases. In another aspect, it has been determined that it has an effect on regulating blood pressure and preventing cardiovascular diseases by preventing low-density lipoprotein (LDL) oxidation. It has been determined that it has an inhibitory effect on free radicals that occur as a result of inflammation in the lung epithelial cells, and it has been suggested to be used in the treatment of the disease. As a result of experiments in mice, genetic structures were also examined in mice in the reproductive and gestational period, and no acute or chronic toxic effect was detected in the study. Oleuropein, one of the main compounds of olive leaf, has been shown to have anti-inflammatory, anti-bacterial and antitumor properties, and in addition, it has strong antioxidant activity due to its binding to endogenous peptides [8]. In this study, it was determined that the methanol extract of the leaves of *Olea Europaea* L. (Olive) plant was rich in high antioxidant activity and total phenolic compounds. We think that this feature is due to the compounds contained in the leaves of the *Olea Europaea* L. (Olive) plant. The reason why methanol extract has been studied is because it has been studied in detail at different concentrations from a single substance (methanol). In addition, in the extract studies in the literature, it is given with the comparison of the methanol extract made with ethanol, water and other different substances. This study was conducted in order to find studies based on these comparisons, to show that the antioxidant activity capacity and the amount of phenolic compounds in

the methanol extract are intense, and to draw attention to the biological effects of olive leaf, which is produced in many countries around the world and has a place in the field of health. Lee and Lee studied the antioxidant and antibacterial effects of phenolic compounds in olive leaf extract and determined that olive leaf extract has radical quenching effects such as superoxide dismutase (SOD) [9]. Since methods for determining antioxidant activity depend on various parameters, there is no single standard method for determining the antioxidant activity of a compound. For this reason, many methods are used to measure antioxidant activity. Different results in the literature may be due to different methods, growing and drying conditions of plants.

5. CONCLUSIONS

The methanol extract obtained from the leaves of the *Olea europaea* L. (Olive) plant analyzed in this study was rich in antioxidant activity and amount of phenolic compounds. It has been determined that olive tree leaves can prevent the formation of damaged cells caused by free radicals due to their high antioxidant effect, and can also be used as an alternative to the standard antioxidants BHA and BHT for various purposes because they are cheap, safe and easily accessible. It is thought that this study will contribute to the studies on *Olea europaea* L. (Olive) plant.

Conflict of Interest

The authors of the article declare that there is no conflict of interest.

Author Contributions

The authors declare that they have contributed equally to the article. This study was presented as a graduation Project of first author (Cenk Güven) in Ataturk University Faculty of Pharmacy in December 2022.

REFERENCES

1. Li FS, Weng JK. Demystifying traditional herbal medicine with modern approach. *Nat Plants*. 2017; 3: 17109.
2. Sezik E, Yeşilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey X. Folk medicine in Central Anatolia. *J Ethnopharmacol*. 2001; 75: 95-115.
3. Alkadi H. A Review on Free Radicals and Antioxidants. *Infect Disord Drug Targets*. 2020; 20: 16-26.
4. Ferreira IC, Barros L, Soares ME, Bastos ML, Pereira JA. Antioxidant activity and phenolic contents of *Olea europaea* L. leaves sprayed with different copper formulations *Food Chem*. 2007; 103: 188-195.
5. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic*. 1977; 28: 49-55.
6. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol Int*. 1995; 28: 25-30. Doi: 10.1016/S0023-6438(95)80008-5.

7. Huang DJ, Ou BX, Prior RL. The chemistry behind antioxidant capacity assays. *J Agr Food Chem.* 2005; 53: 1841- 56
8. Gorzynik-Debicka M, Przychodzen P, Cappello F, Kuban-Jankowska A, Marino Gammazza A, Knap N et al. Potential Health Benefits of Olive Oil and Plant Polyphenols. *Int J Mol Sci.* 2018; 19: 686. Doi: 10.3390/ijms19030686.
9. Lee OH, Lee BY. Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract. *Bioresour Technol.* 2010; 101: 3751–3754.