

ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *Olea europea* L. ‘DOMAT’

Berker Kocatürk^{1*}, Serap Doğan², Hamza Bayhan², Mehmet Emin Diken³

¹İzmir Kavram Vocational School, Medical Services and Technical, Izmir, Turkey

²Balıkesir University, Faculty of Science and Literature, Department of Molecular Biology and Genetic, Balıkesir, Turkey

³Balıkesir University, Science and Technology Application and Research Center, Balıkesir, Turkey

Abstract

Although there are many studies on the biological activities and chemical profiles of Olea europaea, changes in its activities and chemical contents can be observed according to the characteristics of the region where it grows (environmental conditions such as soil composition, climate type, altitude, rainfall etc.). Therefore, in this study, it was aimed that the antioxidant capacity and cytotoxic activities of leaves of the Domat cultivar of Olea europea L. collected in Akhisar province were determined by DPPH and ABTS radical scavenging activity and MTS methods, respectively. In addition, the phenolic constituents of the olive leaves were determined by HPLC. Cytotoxicity of the olive leaves prepared ethanol and methanol extracts (0-100 mg / L) on healthy human lymphocyte cells was investigated. The antioxidant activity results showed to be effective both of the DPPH and ABTS radicals. IC₅₀ values were determined for DPPH between 0.16– 0.20 mg/mL. IC₅₀ values were determined for ABTS between 0.023 – 0.079 mg/mL. The most effective extract was determined as ethanol extract. Moreover, all extracts of olive leaves were determined to scavenge both DPPH and ABTS radicals with quite strong activity. According to the MTS test results, it was observed that ethanol and methanol extracts of Olea europea preserved lymphocyte cell viability at all dose ranges, and cell viability varied only depending on the incubation period. It was observed that 0.5 mg/L dose of ethanol extracts increased lymphocyte cell viability in the 24-hour incubation period, and 1 mg/L dose in the 48-hour incubation period. Oleuropein, 5-hydroxytyrosol, tyrosol and rutin hydrate were found highest proportions as the phenolic constituents of the leaves, respectively.

Keywords: *Olea europea* L., Antioxidant activity, Cytotoxicity

1. INTRODUCTION

Olive is one of the richest plants in phenolic compounds and has been widely used from ancient times in different countries such as Spain, Italy, Greece, France, Israel, Algeria, Tunis, Turkey, and Mediterranean islands due to their nutritional and medicinal properties (Şahin et al. 2018) (Shalaby, Anwar, and Sallam 2018). An important agricultural waste obtained during the harvesting or processing of olive fruits is olive leaves. They are accumulated in high amounts especially during pruning of olive trees and are considered as a cheap raw material. Olive leaves are taken into account to be high value-added phytochemicals in the preparation of nutraceuticals and functional foods (Bouaziz et al. 2008) . In this scope, olive leaves are used as natural sources of bioactive phytochemicals such as oleuropein, hydroxytyrosol, tyrosol, phenolic acids, rutin, apigenin 7-o-glucoside, caffeic acid and luteolin 4-o-glucoside (Shalaby et al. 2018).

Since prehistoric times, olive leaves are widely used in traditional treatments in Mediterranean countries and Europe. Olive leaves, which are the richest part of the olive tree after its fruit, contain many phenolic compounds that show antioxidant properties. Olive leaves extract contains sequeroids, flavonoids, simple phenols, phenolic acids and terpenoids. Phenolic compounds showed protective effects against the harmful effects of genotoxic carcinogens by scavenging reactive oxygen species (ROS) and increasing host antioxidant defense systems. Therefore, it has been shown that antioxidant activities found in plant natural products can provide protective effects by preventing DNA oxidative damage. Phenols are one of the most important groups of natural antioxidants. They are found only in plant-derived samples and are known to protect easily oxidizable components from oxidation (Bouaziz

et al. 2008). The polyphenol content of olive leaves varies depending on their regional distribution. In addition, the antioxidant activities of polyphenols differ greatly according to their compositions (Şahin et al. 2018). Olive leaf extracts include many ingredients such as glycosides, flavonoids, simple phenols, phenolic acids and terpenoids. They are of commercial importance due to their rich content (Benavente-García et al. 2000). Olive leaf extracts, which are a natural source of antioxidants, have been widely used in human diet as concentrated liquid, powder, capsule or dry leaf tea.

Olive leaves can be used not only for therapeutic but also for cosmetic purpose. Besides, olive leaf extracts have been investigated as an additive supplemented to food products, such as oils and meats to extend shelf-life and impart the image of wholesomeness to consumers. Thus, they are commercialized according to several forms (such as intact leaves, powdered leaves, extract) for their advantages to health (Aouidi et al. 2011). For decades, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ter-butyl hydroquinone (TBHQ) are used as food additives to increase the oxidation resistance of vegetable and animal fats. However, synthetic antioxidants have been reported to have many health risks such as toxic, cancer and carcinogenic effects. Therefore, the most powerful synthetic antioxidant (TBHQ) is not allowed for food application in Japan, Canada, Europe and Turkey. Similarly, BHA has been removed from the list of safe compounds (Bouaziz et al. 2008).

Recently, there has been a significant interest in finding natural antioxidants that can be used in foods or medicinal materials to replace synthetic antioxidants, which are restricted due to their carcinogenic effects (Celikler et al. 2009). Most medicinal plants consumed by humans are considered safe due to their long tradition of use (Verschaeve and Van Staden 2008). Also, the plants can produce naturally toxic, mutagenic or carcinogenic compounds (Pukalskienė et al. 2018). Therefore, the first priority in herbal research is to determine the safety of herbal products (Shafaei et al. 2015). All plants, including medicinal plants synthesize toxic substances that in nature serve as a defense against infections, insects and herbivores, but which usually affect organisms that feed on them. Therefore, evaluation of their cytotoxic potential is required to ensure the relatively safe use of medicinal plants. Among the methods frequently used to determine the cytotoxic effect of a substance such as a plant extract are tested with tetrazolium salts. One of them is the MTS tetrazolium salt. MTS assay is well-known in vitro cytotoxicity assay that has the indispensable characteristics of a good measurement system like being easy to use, a rapid and precise indicator of toxicity (Michelis et al. 2002), (Afonso et al. 2019). It is a colorimetric method for determining the number of viable cells in proliferation. MTS reagent is reduced by cells to an aqueous (soluble) formazan product in a culture medium and dehydrogenase enzymes found in metabolically active cells are responsible for this conversion (Shahneh et al. 2013). Therefore, cell viability is in direct proportion to the quantity of formazan product which is determined by the absorbance at 490 nm (Shahneh et al. 2013).

The aim of this work was to determine the ethanolic and methanolic extracts from the *Olea europaea* L. leaves of the Domat cultivar in Akhisar province by scavenging activities against DPPH and ABTS radicals and cytotoxic effect on lymphocyte cell lines isolated from healthy humans. Then, it was correlated these results to their phenolic profile based on the chromatographic determination by HPLC.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were purchased from Sigma-Aldrich and Merck, were analytical purity and used without further purification. The leaves of Domat olive variety were collected from Akhisar in Turkey during the pruning period. The leaves were the same colour and the same size. Collected olive leaves were washed five times with purified water to remove dust, separated into four parts and dried separately as following.

2.1.1. Preparation of olive leaves extracts

The extracts were prepared with methanol and ethanol solutions. 0,5 g of the powdered samples were added into tube 5 mL of methanol and ethanol was added into 0.5 g of each sample and kept in a fridge (+4°C) overnight. The samples were centrifugated for 15 min at 4500 rpm and supernatant was removed. Then, 5 and 2 mL methanol and ethanol were added into pellets and centrifugated under the same conditions, respectively. The supernatants were combined. The extracts were stored at -20 °C until analysis (Lee et al. 2009)

2.1.2. Preparation of Lymphocyte Culture from Blood

FBS and Penicillin/Streptomycin, which should be stored at -20 °C, were melted in an ultrasonic bath at 37 °C. In preparation of the medium, 100 ml of FBS containing growth factor for lymphocytes was added into 77.5 ml of RPMI 1640 medium. Against any contamination that may occur during the experiment, 0.5 ml of Penicillin/Streptomycin and 1 ml of Phytohemagglutinin, which will promote cleavage, were added to the medium. Removed for use at -20°C. 0.5 ml of blood was inoculated into the prepared cultures and cultured in a 37°C oven for 72 hours. (Yilmaz et al., 2018)

2.1.3. Determination of antioxidant capacity

2.1.3.1 DPPH

The antioxidant capacity of olive cultivars was determined using the radical scavenging activity of 1,1-diphenyl-2-picrylhydrazil (DPPH). DPPH (0.6 mM) was dissolved in MeOH in a flask. 0.05 mL of plant extract (0.1-100 mg/L), 2.5 mL of DPPH and 2.5 mL of MeOH were added to a test tube and kept in the dark for 30 min. Spectrophotometric measurements were performed at 517 nm, and IC₅₀ values of radical scavenging activity of the samples was calculated.(Genc et al. 2020).

2.1.3.2 ABTS

ABTS⁺ radical solution (2mM) was prepared using equal volumes of ABTS salt and ammonium persulfate and kept in the dark overnight. For sample analysis, 2.95 mL ABTS⁺ solution and 0.05 mL plant extract (0.1-100 mg/L) It was added to a 3 mL cuvette, and measurements were performed by UV-Visible spectrophotometer at 734 nm.(Genc et al. 2020).

2.1.4 MTS Assay

Tetrazolium salts are cyclic organic compounds. Tetrazolium salts are reduced by gaining electrons and cause a color change. Since the tetrazolium ring is only broken by the active mitochondria, the color change is observed only in living cells. In dead cells, tetrazolium compounds cannot be reduced and do not cause any color change. The test samples prepared certain concentrations were interacted with the newly isolated lymphocytes and no test sample was added for the lymphocyte solution used as a negative control. All samples and control groups were incubated at 37 °C in a 5% CO₂ environment for 2 days. At the end of the 24th and 48th hours, lymphocyte cells were added to the 96-Well Plate with 100 µL in each well and 20 µL of MTS reagent was added to them and the incubation process was started for 4 hours. Then, measurements were taken at 490 nm with a spectrophotometer with a microplate reader (Doğan et al. 2019).

2.1.5 HPLC analysis

The phenolic constituents of the olive leaves were performed by HPLC (An Agilent 1260 Infinity LC system equipped with a UV-Visible detector and Thermo Scientific C18 column (150 x 4.6 mm, 3 µm particle size)). Mobile phases were prepared with water (2% acetic acid) and acetonitrile (0.5% acetic acid). The separation was achieved using the following elution gradient conditions: 10- 35% solution B for 15 min, 35- 55% solution B for 10 min, 55- 65% solution B for 5 min. The injection volume and flow were adjusted 20 µL and 1 mL min⁻¹, respectively. The detector was set at 280 nm for the phenolic compounds. After the calibration of the standards mixture was defined, the retention time was used to identify the phenolics in the plant extracts (De villiers et al., 2004, Dogan et al., 2022).

3. RESULTS

In this study, the antioxidant capacity of the Domat cultivar of *Olea europea* L. was determined by using DPPH and ABTS, and its cytotoxic effects of olive leaves ethanol extracts (0-100 mg / L) on healthy human lymphocyte cells were investigated by MTS test.

3.1. Antioxidant capacity of olive leaves

The antioxidant capacities of Domat olive leaves were performed in DPPH and ABTS radical scavenging methods.

Table 1. Antioxidant activity of Domat olive leaves (IC₅₀: mg /mL)

	DPPH	ABTS
	Radical scavenging activity	Radical scavenging activity
Ethanol extract	0.16 ±0.02	0.023±0.002
Methanol extract	0.20 ±0.02	0.035± 0.003

3.2. Cytotoxic effects of olive leaves

The data of the cytotoxic analyzes performed by the MTS method are presented in Figures 1 and 2. Extracts prepared with ethanol and methanol (8 different doses such as 0.1, 0.25, 0.50, 1, 10 and 25 mg/L) were added to the cultures prepared with human lymphocyte cells. Incubations for 24 and 48 hours were then performed and statistical analysis was performed compared to the negative control.

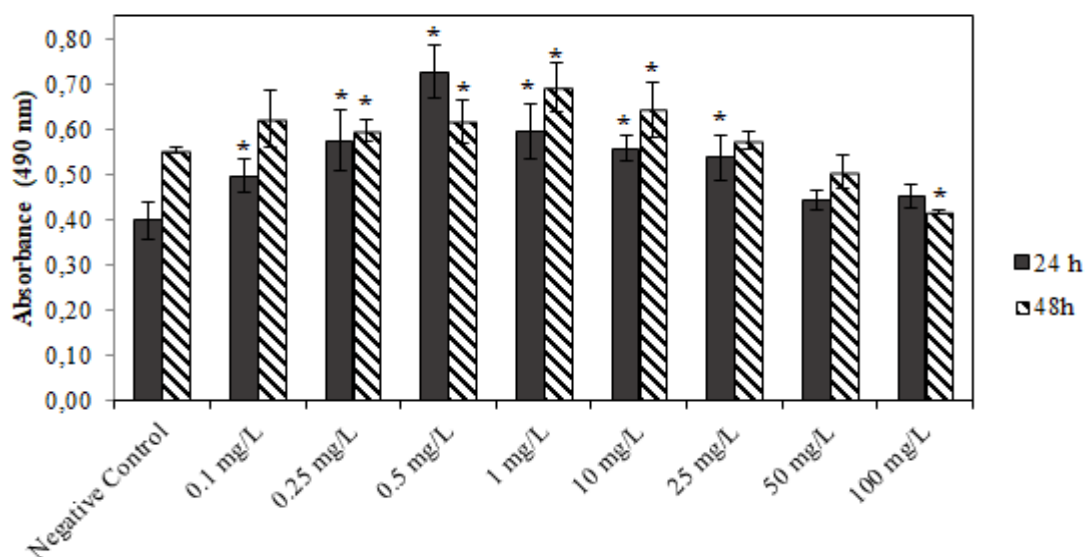


Fig. 1. Cytotoxicity results of ethanolic extract. *indicates statistical significance ($p < 0.05$).

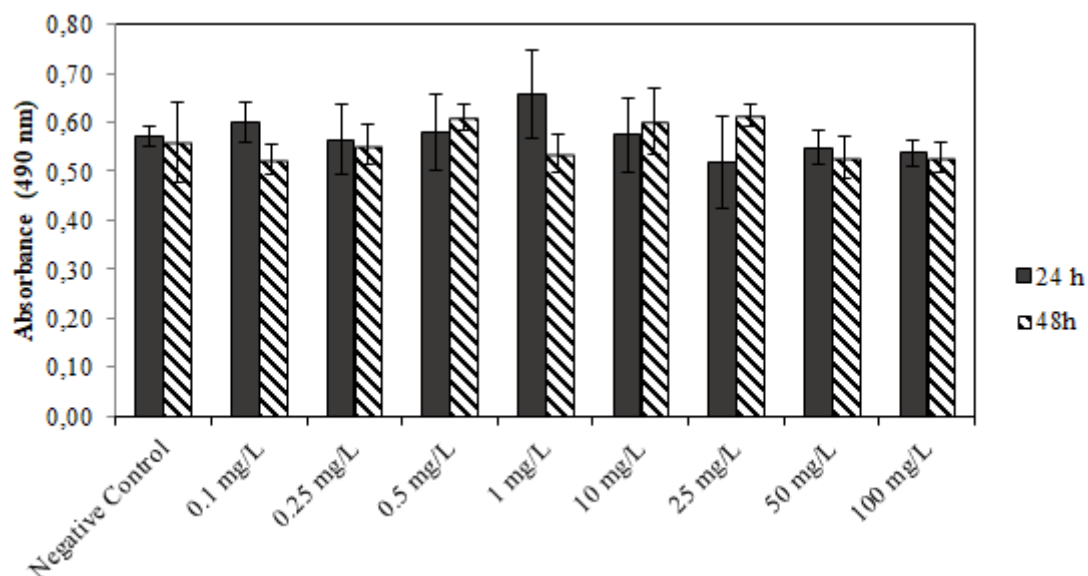


Fig. 2. Cytotoxicity results of methanolic extract. *indicates statistical significance ($p < 0.05$).

3.3. Phenolic constituents

The phenolic constituents of the leaves of the Domat olive cultivar were analyzed by HPLC, and they were listed in Table 2. Nine phenolic compounds of the tested olive cultivar were identified and quantified by this analysis.

Table 2. The phenolic constituents of the leaves of *Olea europaea* 'Domat' cultivar.

Phenolic Constituents	Amounts ($\mu\text{g/g}$)
Oleuropein	2726.91 $\pm$ 33.61
Hydroxytyrosol	426.38 $\pm$ 7.96
Tyrosol	288.75 $\pm$ 20.74
Rutin hydrate	274.8 $\pm$ 6.87
Ferulic acid	6.41 $\pm$ 0.21
<i>p</i> -coumaric acid	2.99 $\pm$ 0.27
Quercetin	2.61 $\pm$ 0.22
Caffeic acid	1.64 $\pm$ 0.12
Vanilic acid	1.06 $\pm$ 0.14

4. DISCUSSION

DPPH and ABTS are indicator radicals that are commonly used to evaluate of antioxidant activity of samples. According to Table 1, it can be said that the radical scavenging activities of ethanol extract are higher than methanol extract. In addition, the ABTS radical scavenging activity of both extract types was found to be higher than DPPH radical scavenging activity. In the literature, Abaza et al. determined the antioxidant capacity of Chétoui olive leaves as 17 $\mu\text{g}/\text{mL}$ in methanolic extracts and 97 $\mu\text{g}/\text{mL}$ in aqueous extracts (Abaza et al. 2011). In the study carried out by Lfitat et al. in 2021, they determined the antioxidant capacity of olive leaves of the Moroccan Picholine cultivar as 0.490

mg/mL in methanolic extracts and 0.705 mg/mL in aqueous extracts (Lfitat et al. 2021). Khaliq determined the antioxidant capacity of 8 different olive cultivars (Dolece-Agogia, Mission, Moriolo, Maurino, Carotina, Leccino, Gemlik, Uslu) prepared with water as 22.46 $\mu\text{g}/\text{mL}$ and 115.5 $\mu\text{g}/\text{mL}$ (Khaliq et al. 2015). Efssafi et al. determined antioxidant capacity between 41.56 $\mu\text{g}/\text{mL}$ and 63.52 $\mu\text{g}/\text{mL}$ in methanol extracts of 4 different olive cultivars in Tunisia (Essafi et al. 2019). Zairi et al. reported that ABTS and DPPH radical scavenging activities (IC_{50} values) of olive leaves aqueous extracts of Tunisian chemlali and meski olive cultivars were determined as 0.13 and 0.19 mg/mL for DPPH, and 0.30 and 0.78 mg/mL for ABTS, respectively (Zaïri et al. 2020). In another study, Kalaycioğlu et al., presented the results of antioxidant activity (DPPH radical scavenging activity) of olive leaves of 7 different provinces in Turkey, including Bursa, Izmir, Manisa, Istanbul, Balikesir, Yalova and Mugla, calculated as IC_{50} ($\mu\text{g}/\text{mL}$). The DPPH scavenging activities of the methanol-water (v/v, 80/20) extracts were between 63.2 and 163 $\mu\text{g}/\text{mL}$ (Kalaycioğlu, Kopar, and Erim 2020) In the present study, it was observed that methanolic and ethanolic extracts showed better antioxidant activity than aqueous extracts compared with the literature. In addition to the compatibility of our results with the literature, it was stated that the Domat cultivar showed better activity than olive cultivars in other regions.

The results of the ethanol extracts prepared from the leaf parts of the *Olea europea* 'Domat' plant are given in Figure 1. It was observed that the various doses in 24 hours incubation caused statistically significant increases in absorbance when compared to the negative control ($p < 0.05$). It was observed that the 0.50 mg/L dose supported lymphocyte cell viability by giving the highest absorbance value when compared to the negative control. Although 50 and 100 mg/L doses gave absorbance values similar to the negative control, these changes were not statistically significant ($p > 0.05$). In 48 hours of incubation, 0.25, 0.50, 1 and 10 mg/L doses caused statistically significant increases in absorbance when compared to the negative control, while 100 mg/L doses caused a statistically significant decrease in absorbance. Most doses (0.1, 0.25, 1, 10 mg/L) at 48 hours of incubation were observed to increase cell viability compared to 24 hours of incubation. It was observed that methanol extracts of the plant gave absorbance values similar to the negative control at the other doses except for 25, 50 and 100 mg/L doses after 24 hours of incubation (Figure 2). Although the 1 mg/L dose caused an increase in absorbance compared to the negative control, these observed changes were not statistically significant ($p > 0.05$). It was observed that all doses gave values similar to the negative control during the 48-hour incubation period. These similarities were not determined statistically significant ($p > 0.05$). In the literature, although there is no study on the cytotoxic activity of ethanol and methanol extracts prepared from the leaf parts of the *Olea europea* 'Domat' plant on lymphocyte cells, there are few studies on the cytotoxic activity of the plant. For example; in a study by Cumaoglu et al., they isolated oleuropein from *Olea europea* L. leaf and fruit and investigated the viability of cytokine-induced insulin-producing β -cells (INS-1) with MTT test. Researchers reported that oleuropein did not reduce the amount of both apoptotic and necrotic cells, but significantly protected the cells against cytokine-induced death (Cumaoglu et al. 2011). Chatzikonstantinou et al. investigated the cytotoxic activity of water extracts prepared from the leaf parts of *Olea europea* plant by MTT test on human embryonic kidney 293 cells (HEK293, ATCC®CRL-1573) and leiomyosarcoma cells (LMS) isolated from leiomyosarcoma tumors in Wistar rats. Researchers reported that water extracts did not cause cytotoxic effects on HEK293 and LMS cells in 24 hours of incubation, but increased cell proliferation at the highest doses (500 $\mu\text{g}/\text{mL}$) (Chatzikonstantinou et al. 2022). Belscak-Cvitanovic et al. investigated the cytotoxic activity of water extracts of *Achillea millefolium* L., *Crataegus oxyacantha* L., *Glechoma hederacea* L. and *Olea europea* L. on human laryngeal cancer (HEp2) cells by NR test. Researchers have shown that these plants reduce cell viability and the formation of reactive oxygen species in a dose- and time-dependent manner (Belščak-Cvitanovic et al. 2014) Bouallagui et al. isolated the hydroxytyrosol polyphenol compound from *Olea europea* plant and investigated the cytotoxic activity of 2, 2.2, 2.4, 2.6, 2.8 and 3 mg/mL doses in MCF-7 cell line with MTT and neutral red tests. Researchers reported that the hydroxytyrosol polyphenol compound inhibited the growth of MCF-7 cells depending on the dose amount (Bouallagui et al. 2011) .Chiaino et al. investigated the cytotoxic activity of PRES (extracts from *Olea europaea* L. leaves and *Hibiscus sabdariffa* L. flowers) on human neuroblastoma cells (SH-SY5Y) by MTT assay. Researchers have observed that PRES

reduces cell viability due to its concentration. When the cytotoxic activity of *Olea europea* L. and *Hibiscus sabdariffa* plants were examined separately, it was found that they caused significantly lower toxicity than the cytotoxic activity caused by PRES (Chiaino et al. 2020).

It was mentioned in the introduction section that olive leaf extracts are rich in phenolic contents. In this study, the concentrations of nine different compounds in olive leaves were determined as a result of chromatography analysis. When Table 2 is examined, the highest concentration of oleuropein was found, followed by hydroxytyrosol, tyrosol, rutin hydrate, ferulic acid, p-coumaric acid, quercetin, caffeic acid and vanillic acid. In previous studies related to olive leaf extracts, it was found similar and different results to this work. For instance, Zairi et al. found in their study that oleuropein was the predominant phenolic compound in olive leaf extracts. But, hydroxytyrosol and tyrosol were noted a small concentration in phenolic composition (Zaïri et al. 2020). In our results, although the oleuropein concentrations were similar in the tested cultivars, hydroxytyrosol and tyrosol proportions were found higher concentrations than in the above-cited work. It is well known that oleuropein, hydroxytyrosol and tyrosol were revealed to possess antibacterial, antioxidative, and anti-inflammatory properties, and also trigger cell proliferation (Antonioni and Hull 2021). Boruga et al. profiled the phenolic content of the olive leaves' ethanolic extract obtained with the maceration method by LC/MS. As a result of the experiment, they found that luteolin and rutin were the main components of the extract. Also, they determined many components, including caffeic acid, ferulic acid, p-coumaric acid, etc (Borugă et al. 2021). Sarikurkcu et al. determined verbascoside, luteolin 7-hexosides and apigenin 7-hexosides as the main components of the extract of olive leaves obtained with boiled water. They determined the presence of many components including vanillic acid, caffeic acid, p-coumaric acid, ferulic acid and quercetin by LC-ESI-MS/MS (Sarikurkcu et al. 2020). There are many factors for the differences in concentration of the constituents both in the literature and in this work. These differences may vary depending on the experimental alternative such as extraction method and solvent type, as well as geographical features where the plant grows such as soil composition, moisture and climate (Kalaycıoğlu et al. 2020).

5. CONCLUSIONS

Since most of the developed and developing countries depend on plants for health services, it has become common to investigate the effects of plant extracts on healthy cells (Grujičić et al. 2020). Therefore, in this study, the antioxidant capacity of ethanol and methanol extracts of *Olea europea* 'Domat' leaves and their cytotoxic effects on lymphocyte cells were investigated. When the antioxidant capacity results were examined, it was found that both extracts showed strong radical scavenging activity. When the antioxidant capacity results were examined, the samples prepared with ethanol showed the best results in both DPPH and ABTS tests. According to cytotoxicity test results, ethanol and methanol extracts of *Olea europea* 'Domat' plant preserved the viability of healthy human lymphocyte cells at all doses and did not cause a cytotoxic effect. Also, increased variability of cell viability was observed with respect to the amount of extract dose and incubation times. In order to define total extracts such as oleuropein, tyrosol, hydroxytyrosol, caffeic acid, etc., the main components of olive leaf were measured in the same experimental system, and the sources of strong antioxidant and non-cytotoxic activities were proven. This study highlight for further studies and adds new information to the literature about the *Olea europaea* 'Domat' plant, especially with its cytotoxic test.

REFERENCES

- Abaza, Leila, Nabil Ben Youssef, Hédia Manai, Faouzia Mahjoub Haddada, Kaouther Methenni, and Mokhtar Zarrouk. 2011. "Chétoui Olive Leaf Extracts: Influence of the Solvent Type on Phenolics and Antioxidant Activities." *Grasas y Aceites* 62(1):96–104. doi: 10.3989/gya.044710.
- Afonso, Andrea F., Olívia R. Pereira, Ângela S. F. Fernandes, Ricardo C. Calhelha, Artur M. S. Silva, Isabel C. F. R. Ferreira, and Susana M. Cardoso. 2019. "The Health-Benefits and Phytochemical

Profile of Salvia Apiana and Salvia Farinacea Var. Victoria Blue Decoctions.” *Antioxidants* 8(8). doi: 10.3390/antiox8080241.

Antoniou, Chrystalla, and Jonathon Hull. 2021. “The Anti-Cancer Effect of Olea Europaea L. Products: A Review.” *Current Nutrition Reports* 99–124. doi: 10.1007/s13668-021-00350-8.

Aouidi, Fathia, Samia Ayari, Hana Ferhi, Sevastianos Roussos, and Moktar Hamdi. 2011. “Gamma Irradiation of Air-Dried Olive Leaves: Effective Decontamination and Impact on the Antioxidative Properties and on Phenolic Compounds.” *Food Chemistry* 127(3):1105–13. doi: 10.1016/j.foodchem.2011.01.109.

Belščak-Cvitanovic, Ana, Ksenija Durgo, Arijana Bušić, Jasna Franekić, and Draženka Komes. 2014. “Phytochemical Attributes of Four Conventionally Extracted Medicinal Plants and Cytotoxic Evaluation of Their Extracts on Human Laryngeal Carcinoma (HEp2) Cells.” *Journal of Medicinal Food* 17(2):206–17. doi: 10.1089/jmf.2013.0071.

Benavente-García, O., J. Castillo, J. Lorente, A. Ortuño, and J. A. Del Rio. 2000. “Antioxidant Activity of Phenolics Extracted from Olea Europaea L. Leaves.” *Food Chemistry* 68(4):457–62. doi: 10.1016/S0308-8146(99)00221-6.

Borugă, Mădălina, Virgil Enătescu, Iulia Pînzaru, Camelia Szuhaneck, Daliana Minda, Iasmina Marcovici, Daniela Radu, Daniela Marți, Brigitha Vlaicu, and Oana Suciu. 2021. “Assessment of Olive Leaves Extract – Cytotoxicity in Vitro and Angiogenesis in Ovo.” *Farmacia* 69(1):38–43. doi: 10.31925/farmacia.2021.1.5.

Bouallagui, Zouhaier, Junkuy Han, Hiroko Isoda, and Sami Sayadi. 2011. “Hydroxytyrosol Rich Extract from Olive Leaves Modulates Cell Cycle Progression in MCF-7 Human Breast Cancer Cells.” *Food and Chemical Toxicology* 49(1):179–84. doi: 10.1016/j.fct.2010.10.014.

Bouaziz, Mohamed, Ines Fki, Hedy Jemai, Mohamed Ayadi, and Sami Sayadi. 2008. “Effect of Storage on Refined and Husk Olive Oils Composition: Stabilization by Addition of Natural Antioxidants from Chemlali Olive Leaves.” *Food Chemistry* 108(1):253–62. doi: 10.1016/j.foodchem.2007.10.074.

Celikler, Serap, Ozgur Vatan, Gamze Yildiz, and Rahmi Bilaloglu. 2009. “Evaluation of Anti-Oxidative, Genotoxic and Antigenotoxic Potency of Codium Tomentosum Stackhouse Ethanolic Extract in Human Lymphocytes in Vitro.” *Food and Chemical Toxicology* 47(4):796–801. doi: 10.1016/j.fct.2009.01.010.

Chatzikonstantinou, Alexandra V., Archontoula Giannakopoulou, Stamatia Spyrou, Yannis V. Simos, Vassiliki G. Kontogianni, Dimitrios Peschos, Petros Katapodis, Angeliki C. Polydera, and Haralambos Stamatis. 2022. “Production of Hydroxytyrosol Rich Extract from Olea Europaea Leaf with Enhanced Biological Activity Using Immobilized Enzyme Reactors.” *Environmental Science and Pollution Research* 29(20):29624–37. doi: 10.1007/s11356-021-17081-6.

Chiaino, Elda, Matteo Micucci, Sandro Cosconati, Ettore Novellino, Roberta Budriesi, Alberto Chiarini, and Maria Frosini. 2020. “Olive Leaves and Hibiscus Flowers Extracts-Based Preparation Protect Brain from Oxidative Stress-Induced Injury.” *Antioxidants* 9(9):1–15. doi: 10.3390/antiox9090806.

Cumaoglu, Ahmet, Nuray Ari, Murat Kartal, and Çimen Karasu. 2011. “Polyphenolic Extracts from Olea Europea L. Protect against Cytokine-Induced β -Cell Damage through Maintenance of Redox Homeostasis.” *Rejuvenation Research* 14(3):325–34. doi: 10.1089/rej.2010.1111.

Doğan, E. E., P. Tokcan, M. E. Diken, B. Yilmaz, B. K. Kizilduman, and P. Sabaz. 2019. “Synthesis, Characterization and Some Biological Properties of PVA/PVP/PN Hydrogel Nanocomposites: Antibacterial and Biocompatibility.” *Advances in Materials Science* 19(3):32–45. doi: 10.2478/adms-2019-0015.

Essafi, H., N. Trabelsi, C. Benincasa, A. Tamaalli, E. Perri, and M. Zarrouk. 2019. “Phytochemical Profile, Antioxidant and Antiproliferative Activities of Olive Leaf Extracts from Autochthonous

Tunisian Cultivars.” *Acta Alimentaria* 48(3):384–90. doi: 10.1556/066.2019.48.3.14.

Genc, Nusret, Ilyas Yildiz, Radia Chaoui, Ramazan Erenler, Cengiz Temiz, and Mahfuz Elmasas. 2020. “Biosynthesis, Characterization and Antioxidant Activity of Oleuropein-Mediated Silver Nanoparticles.” *Inorganic and Nano-Metal Chemistry* 51(3):1–9. doi: 10.1080/24701556.2020.1792495.

Grujičić, Darko, Aleksandra Marković, Jovana Tubić Vukajlović, Milan Stanković, Marina Radović Jakovljević, Andrija Ćirić, Katarina Djordjević, Nevena Planojević, Milena Milutinović, and Olivera Milošević-Djordjević. 2020. “Genotoxic and Cytotoxic Properties of Two Medical Plants (Teucrium Arduini L. and Teucrium Flavum L.) in Relation to Their Polyphenolic Contents.” *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 852(September 2019):503168. doi: 10.1016/j.mrgentox.2020.503168.

Kalaycıoğlu, Zeynep, Merve Kopar, and F. Bedia Erim. 2020. “Oleuropein Levels of Anatolian Olive Leaves and Correlated Antioxidant, Antidiabetic, and Anti-Inflammatory Activities.” *Journal of Chemical Metrology* 14(2):133–41. doi: 10.25135/jcm.51.20.04.1632.

Khaliq, Abdul, Syed Mubashar Sabir, Syed Dilnawaz Ahmad, Aline Augusti Boligon, Margareth Linde Athayde, Abdul Jabbar, Imtiaz Qamar, and Asmatullah Khan. 2015. “Antioxidant Activities and Phenolic Composition of Olive (*Olea Europaea*) Leaves.” *Journal of Applied Botany and Food Quality* 88(1):16–21. doi: 10.5073/JABFQ.2015.088.004.

Lee, Ok Hwan, Boo Yong Lee, Junsoo Lee, Hee Bong Lee, Jong Youn Son, Cheon Seok Park, Kalidas Shetty, and Young Cheul Kim. 2009. “Assessment of Phenolics-Enriched Extract and Fractions of Olive Leaves and Their Antioxidant Activities.” *Bioresource Technology* 100(23):6107–13. doi: 10.1016/j.biortech.2009.06.059.

Lfitat, Aziza, Hind Zejli, Fatima Zohra Bousraf, Abdelkamel Bousselham, Yassine El Atki, Abdelkader Gouch, Badiaa Lyoussi, and Abdelfattah Abdellaoui. 2021. “Comparative Assessment of Total Phenolics Content and in Vitro Antioxidant Capacity Variations of Macerated Leaf Extracts of *Olea Europaea* L. And *Argania Spinosa* (L.) Skeels.” *Materials Today: Proceedings* 45:7271–77. doi: 10.1016/j.matpr.2020.12.990.

Michelis, Fotios, Ekaterini Tiligada, Helen Skaltsa, Diamanto Lazari, Alexios Leandros Skaltsounis, and Andreas Delitheos. 2002. “Effects of the Flavonoid Pilloin Isolated from *Marrubium Cylleum* on Mitogen-Induced Lymphocyte Transformation.” *Pharmaceutical Biology* 40(4):245–48. doi: 10.1076/phbi.40.4.245.8472.

Pukalskienė, Milda, Gražina Slapšytė, Veronika Dedonytė, Juozas Rimantas Lazutka, Jūratė Mierauskienė, and Petras Rimantas Venskutonis. 2018. “Genotoxicity and Antioxidant Activity of Five *Agrimonia* and *Filipendula* Species Plant Extracts Evaluated by Comet and Micronucleus Assays in Human Lymphocytes and Ames Salmonella/Microsome Test.” *Food and Chemical Toxicology* 113(2018):303–13. doi: 10.1016/j.fct.2017.12.031.

Şahin, Selin, Elaf Elhussein, Mehmet Bilgin, Jose M. Lorenzo, Francisco J. Barba, and Shahin Roohinejad. 2018. “Effect of Drying Method on Oleuropein, Total Phenolic Content, Flavonoid Content, and Antioxidant Activity of Olive (*Olea Europaea*) Leaf.” *Journal of Food Processing and Preservation* 42(5). doi: 10.1111/jfpp.13604.

Sarikurcu, Cengiz, Marcello Locatelli, Angela Tartaglia, Vincenzo Ferrone, Aleksandra M. Juszczak, Mehmet Sabih Ozer, Bektas Tepe, and Michał Tomczyk. 2020. “Enzyme and Biological Activities of the Water Extracts from the Plants *Aesculus Hippocastanum*, *Olea Europaea* and *Hypericum Perforatum* That Are Used as Folk Remedies in Turkey.” *Molecules* 25(5):1–15. doi: 10.3390/molecules25051202.

Shafaei, Armaghan, Kameh Esmaili, Elham Farsi, Abdalrahim F. A. Aisha, Amin Malik Shah Abul Majid, and Zhari Ismail. 2015. “Genotoxicity, Acute and Subchronic Toxicity Studies of Nano Liposomes of *Orthosiphon Stamineus* Ethanolic Extract in Sprague Dawley Rats.” *BMC Complementary and Alternative Medicine* 15(1):1–14. doi: 10.1186/s12906-015-0885-z.

- Shahneh, Fatemeh Zare, Behzad Baradaran, Mona Orangi, and Fatemeh Zamani. 2013. "In Vitro Cytotoxic Activity of Four Plants Used in Persian Traditional Medicine." *Advanced Pharmaceutical Bulletin* 3(2):453–55. doi: 10.5681/apb.2013.074.
- Shalaby, Ali Ragab, Mervat Mohamed Anwar, and Essam Mohamed Sallam. 2018. "Improving Quality and Shelf-Life of Minced Beef Using Irradiated Olive Leaf Extract." *Journal of Food Processing and Preservation* 42(11):1–10. doi: 10.1111/jfpp.13789.
- Verschaeve, L., and J. Van Staden. 2008. "Mutagenic and Antimutagenic Properties of Extracts from South African Traditional Medicinal Plants." *Journal of Ethnopharmacology* 119(3):575–87. doi: 10.1016/j.jep.2008.06.007.
- Yilmaz, Begümhan, Serap Doğan, and Serap Çelikler Kasimoğullari. 2018. "Hemocompatibility, Cytotoxicity, and Genotoxicity of Poly(Methylmethacrylate)/Nanohydroxyapatite Nanocomposites Synthesized by Melt Blending Method." *International Journal of Polymeric Materials and Polymeric Biomaterials* 67(6):351–60. doi: 10.1080/00914037.2017.1331349.
- Zaïri, Amira, Sahar Nouir, Amira Zarrouk, Houda Haddad, Améni khélifa, and Lotfi Achour. 2020. "Phytochemical Profile, Cytotoxic, Antioxidant, and Allelopathic Potentials of Aqueous Leaf Extracts of *Olea Europaea*." *Food Science and Nutrition* 8(9):4805–13. doi: 10.1002/fsn3.1755.