T.C.

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHYTOTHERAPY

COMPARATIVE ASSESSMENT OF ANTIOXIDANT CAPACITY OF DOMAT OLIVE LEAF AND SARI YAPRAK OLIVE LEAF

MASTER THESIS

Fatma Gül Yoldaş, Pharm.

İstanbul, 2019

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This study have approved as a Master Thesis in regard to content and quality by the Jury.

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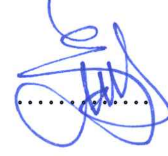
Chair of the Jury:	Prof. Dr. Erdem Yeşilada Yeditepe University, Faculty of Pharmacy, Dept. Pharmacognosy, Turkey	 ....
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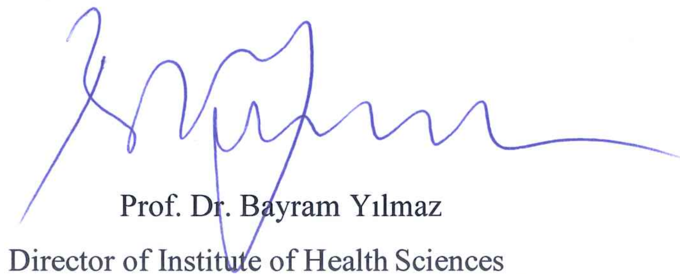
This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated .... and numbered ....



Prof. Dr. Bayram Yılmaz
Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

05.12.2019

Fatma Gül Yoldaş

To olive trees which are shelters for refugees,

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LIST OF SYMBOLS AND ABBREVIATIONS

3,4-DHPEA-EDA:	[2-(3,4-hydroxyphenyl)ethyl(3S,4E)-4-formyl-3-(2-oxoethyl)hex-4-enoate]
AAE:	Ascorbic Acid Equivalent
ABS:	Absorbance
ABST:	2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid)
ALP:	Linoleic Acid Peroxidation
ALT:	Alanine Aminotransferase
AST:	Aspartate Aminotransferase
BC:	Before Christ
BEA:	Benzene/ Ethanol/ Ammonium Solution
BHT:	Butylated Hydroxy Toluene
BUN:	Blood Urea Nitrogen
°C:	Celsius
CAE:	Caffeic Acid Equivalents
Cat:	Catalase
CEF:	Chloroform/ Ethyl acetate/ Formic Acid
COLE:	Chemlali Olive Leaf Extract
COX:	Cyclooxygenase
CPK:	Creatine Phosphokinase
CPK-MB:	Creatine Phosphokinase- Myoglobin Binding
CUPRAC:	Cupric Reducing Antioxidant Capacity
DOLE:	Dried Olive Leaf Extract
DPPH:	2,2-Diphenyl-1-picrylhydrazyl
DXR:	Doxorubicin
EDTA:	Ethylenediaminetetraacetic Acid
EMW:	Ethyl Acetate/ Methanol/ Water

ESI:	Electrospray Ionization
ESI-Q-TOF-MS:	Electrospray Ionization Quadrupole Time of Flight Mass Spectrometry
ERK1/2:	Extracellular Signal-Related Kinase
EtOH:	Ethanol
FCR:	Folin Ciocalteu Reagent
FRAP:	Ferric Reducing Antioxidant Power
GAE:	Gallic Acid Equivalents
GSH-Px:	Glutathione Peroxidase
HCD:	High Cholesterol-rich Diet
HIV:	Human Immunodeficiency Virus
HLD:	High Lipid Diet
HPLC:	High-Performance Liquid Chromatography
IL-1:	Interleukin-1
iNOS:	Inducible Nitric Oxide Synthase
I/R:	Ischemia/Reperfusion
i.p:	Intraperitoneal
IR:	Infrared
i.v:	Intravenous
LDH:	Lactate Dehydrogenase
LDL:	Low-Density Lipoprotein
L-NAME:	G-Nitro-L-Arginine-Methyl Ester
LPS:	Lipopolysaccharide
MAPK:	Mitogen-Activated Protein Kinase
MDA:	Malondialdehyde

MeOH:	Methanol
MFC:	Minimum Fungicidal Concentration
MIC:	Minimum Inhibitory Concentration
MTT:	Modified Microculture Tetrazolium
NMR:	Nuclear Magnetic Resonance
NO:	Nitric Oxide
OA:	Oleanolic Acid
OFE:	Olive Fruit Extract
OLE:	Olive Leaf Extract
OLME:	Olive Leaf Methanolic Extract
ORAC:	Oxygen Radical Absorbance Capacity
QE:	Quercetin Equivalents
QTOF-MS:	Quadrupole Time-of-Flight Mass Spectrometry
PI3K:	Phosphoinositide 3-Kinase
PUFA:	Polyunsaturated Fatty Acid
RNS:	Reactive Nitrogen Species
RP:	Reverse Phase
ROS:	Reactive Oxygen Species
S.D:	Standard Deviation
SFE:	Supercritical Fluid Extraction
SHR:	Spontaneously Hypertensive Rats
SOD:	Superoxide Dismutase
TAC:	Total Antioxidant Capacity
TC:	Total Cholesterol
TEAC:	Trolox Equivalent Antioxidant Capacity

TFC:	Total Flavonoid Content
TG:	Triglycerides
TLC:	Thin Layer Chromatography
TNF-α:	Tumor Necrosis Factor-α
TOAC:	Total Antioxidant Capacity
TPC:	Total Phenolic Content
TPTZ:	2,4,6-tripyridyl-s-triazine
UPLC-QTOF-MS:	Ultra-Performance Liquid Chromatography Ionization Quadrupole Time-of-Flight Mass Spectrometry
VHSV:	Viral Haemorrhagic Septicaemia Rhabdovirus
WKR:	Wistar Kyoto Rats
XO:	Xanthine Oxidase
YEF:	Yeditepe University Faculty of Pharmacy Herbarium

ABSTRACT

COMPARATIVE ASSESSMENT OF ANTIOXIDANT CAPACITY OF DOMAT OLIVE LEAF AND SARI YAPRAK OLIVE LEAF

Yoldaş, F G. (2019). Comparative Assessment of Antioxidant Capacity of Domat Olive Leaf and Sarı Yaprak Olive Leaf. Yeditepe University, Institute of Health Science, Department of Phytotherapy, MSc Thesis, İstanbul.

Oxidative stress plays a role in several degenerative conditions, including inflammation, cardiovascular diseases, and cancer. Organisms have a defensive capacity to defend themselves against oxidative stress, but it is not entirely effective. Besides, fruits and vegetables have excellent antioxidant properties. Olive, which has been planted because of its fruit and oil for ages, has antioxidant property in its leaves. Olive trees grow in a Mediterranean environment. There are many studies on the health benefits of olive leaf and how it is used in traditional treatment. The recognition of the health benefits of olive leaf has been on the rise. Different strains of olives are grown in different parts of Turkey. In this study, the antioxidant capacity of 80% methanolic extraction of different types of olive leaves was compared via DPPH radical scavenging activity, CUPRAC, and TOAC assays. At the same time, phytochemical properties of different olive leaves' strains were examined *in vitro* as well, with total phenolic content and total flavonoid content assays. The effect of growing region on how the antioxidant capacity of Sarı Yaprak olive leaf changed was investigated. As a result, different olive strains have different antioxidant capacity, and environmental conditions affect the total of phytochemical assays and antioxidant activities for Sarı Yaprak olive leaf.

Key words: *Olea europaea*, Domat, Sarı Yaprak, Phenolic Profile, Antioxidant Activity

ÖZET

DOMAT ZEYTİN YAPRAĞI İLE SARI YAPRAK ZEYTİN YAPRAĞININ ANTİOKSİDAN KAPASİTESİNİN KARŞILAŞTIRILMASI

Yoldaş, F G. (2019). Domat Zeytin Yaprağı ile Sarı Yaprak Zeytin Yaprağının Antioksidan Kapasitelerinin Karşılaştırmalı Değerlendirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Fitoterapi ABD., Master Tezi, İstanbul.

Oksidatif stres, inflamasyon, kalp-damar hastalıkları, kanser gibi dejeneretif durumlarda rol oynar. Canlıların oksidatif strese karşı koyma kapasitesine sahiptir ve bu tamamen etkili değildir. Meyve ve sebzeler iyi bir antioksidan özelliği olmasının yanı sıra yıllardır meyvesi ve yağı için dikilen zeytin yaprağı da iyi bir antioksidandır. Zeytin, Akdeniz havzasında yetişen bir ağaçtır. Zeytin yaprağının sıhhat üzerine faydaları ve geleneksel tedavide nasıl kullanıldığı hakkında birçok çalışma vardır. Zeytin yaprağının tıbbi faydasının bilinirliği artmaktadır. Türkiye'nin farklı bölgelerinde farklı zeytin türleri yetişmektedir. Bu çalışmada, farklı tür zeytin yapraklarının %80'lik metanolik ekstraksiyonunun antioksidan kapasiteleri DPPH radikal süpürücü aktivitesi, CUPRAC ve TOAC deneyleriyle karşılaştırmıştır. Aynı zamanda farklı zeytin türlerinin fitokimyasal özelliği total fenolik içerik ve total flavonoid içerik deneyleriyle *in vitro* olarak karşılaştırılmıştır. Yetiştirilme alanının Sarı Yaprak zeytin yaprağının antioksidan kapasitesini nasıl değiştiği araştırılmıştır. Sonuç olarak, farklı zeytin türleri farklı antioksidan kapasitesine sahiptir ve çevresel şartlar *in vitro* yapılan fitokimyasal deneyleri ve antioksidan aktivitenin sonuçlarını Sarı Yaprak zeytin yaprağı için etkilemiştir.

Anahtar kelimeler: *Olea europaea*, Domat, Sarı Yaprak, Fenolik Profil, Antioksidan Aktivite

1. INTRODUCTION and AIM

There has been considerable interest in the use of medicinal herbs to prevent the diseases and/or to assist the treatment. Traditional medicine is becoming popular throughout the world, compared to synthetic drugs that could pose and cause viable threats and side effects. Furthermore, a large number of researches are conducted on medicinal herbs used in traditional medicine in order to prevent diseases or to slow down the aging process. A high proportion of these effects are attributed to the phenolic compounds due to their antioxidant properties. Antioxidants are known for their abilities to scavenge free radicals, which are thought to be responsible for aging and systematic diseases. *Olea europaea* is considered to be among the richest sources of natural antioxidant sources. Olive is one of the oldest cultivated trees, and it has been cultivated since 2500 BC (1). Olive fruit and olive oil are widely consumed as food, and numerous studies have been conducted on their pharmacological properties (2). In addition to them, the leaves of *Olea europaea* have been the subject of many studies, most of which suggested a broad array of activity profiles. Recent studies have shown that olive leaves are a major source of antioxidant phenolic compounds.

Oxygen is compulsory for aerobic organisms to continue their lives. On the other hand, it might be responsible for causing hazardous effects for overall health since cells produce reactive oxygen species (ROS) when they consume oxygen. Organisms have their own defense system against ROS, but it is not sufficient due to the presence of exogenous oxidative stress factors such as UV rays, air pollution, etc. The danger of ROS mainly arises from damage to DNA, lipids, and proteins. Besides, ROS are known to trigger many acute diseases, chronic diseases, and cancers. Hence, it is crucial to maintain the equilibrium between oxidative stress and the antioxidant process to avert the deleterious effects of oxidative stress (3, 4).

As mentioned earlier in the text, olive (*Olea europaea* L.), which is a Mediterranean tree, is one of the significant antioxidant resources. Although its origin remains uncertain, the olive tree is thought to be first cultivated in Syria by Sami people due to its nutritious oil. The olive tree was then spread from Syria to Anatolia and Ancient Egypt via traders between 2000-1000 B.C (4, 5). Furthermore, olive leaves were found to be placed in the tombs of Ancient Egypt. The trees were later introduced to Spain and other countries located in the Mediterranean coasts via trade routes. The primary usage of olive trees was to gain oil and Romans succeeded in using the press method. The Spanish had supplied olive oil worldwide

since 1560. Also, olive trees are mentioned in Holy books like Quran, Bible, and Torah and called Holy Tree. Undoubtfully, the olive trees possess a vital place in Greek mythology. The city of Athens was believed to be founded when the olive tree was planted there by Athena, and olive leaves were used as a crown in that area. Moreover, olive leaves are a symbol of peace, as well (6).

Olive trees also have a major place in Anatolian culture. Some tools were found in Çukurova, related to obtain olive oil and dated back to BC 2000-1200 (4). Some olive trees are over a hundred years old in Turkey, and they were needed to be organized for proper harvesting. Bornova Olive Research Institution was founded by M. Kemal Atatürk's order in 1937(5, 6).

Olea europaea leaf has been used traditionally for reducing blood sugar, uric acid, cholesterol hypertension, inflammation, rheumatism, reducing fever, respiratory, urinary tract infections, asthma. It is useful for stomach and intestinal diseases, hemorrhoids. It is also known as laxative, diarrhea, cholagogue, mouth cleanser, anthelmintic, and vasodilator (3, 5). A formulation prepared from olive leaves was even used against malaria (4).

As stated earlier, *Olea europaea* has an immense economic value due to its fruit and olive oil, and there are many studies and articles about olive's fruit and oil. Also, studies on olive leaf have increased recently. Olives are not harvested, picking one by one for oil extraction. Special rake is used for olive harvesting. Moreover, nets are dispersed under trees. While olives are being harvested, their leaves are picked. The crop is obtained with olive leaves, and 10% weight of harvested olive consists of olive leaves (7). Furthermore, every year olive trees are trimmed, and the gathered leaves are not used and wasted.

In this research, the total phenolic and flavonoid contents as well as *in vitro* antioxidant capacity of extracts prepared from different cultivars named Domat olive leaf and Sarı Yaprak olive leaf were compared. Domat olive and Sarı Yaprak olive are cultivated in Akhisar, Turgutlu, and Manisa region, and according to previous research, Domat olive leaf has more antioxidant capacity according to other olives (8). For a better evaluation of antioxidant potentials, *in vitro* tests with various mechanisms such as DPPH radical scavenging activity and metal reducing activity (CUPRAC) were employed on samples.

2. GENERAL DESCRIPTION

2.1 *Olea* L.

2.1.1 Botanical Information

2.1.1.1 Oleaceae

The family of Oleaceae has approximately 25 genus and 600 species across the globe. It grows in temperate and sub-tropical areas (9). They are usually in the form of trees or shrubs. Leaves are exstipulate, deciduous or evergreen, simple, trifoliate or pinnate, opposite or whorled, and rarely alternate. The calyx is campanulate, mostly small or rarely absent. Flowers are hermaphrodite, rarely unisexual, 4(-9)-merous, actinomorphic. Coralla is sympetalous, polypetalous or absent. Stamens are 2, the anther cells back to back, filaments are adnate to corolla, or (when corolla absent) stamens free. The ovary is superior, 2- locular; styles are usually short or absent; stigma is thickened, capitate, or mostly shortly bifid; ovules are generally 2 per loculus, rarely 1 or 4-10. The fruit is a bilocular 2-valved capsule, samara, berry or dupe; seeds 1-4.

1. Leaves compound (in wild species)
  2. Leaves alternate (in wild species); corolla long-tubed; fruit a berry — ***1. Jasminum***
  2. Leaves opposite or whorled; corolla deeply divided or absent; fruit a samara — ***3. Fraxinus***
1. Leaves simple
  3. Fruit with lateral wings (samara) — **2. *Fontanesia***
  3. Fruit a drupe or berry
    4. Leaves with dense lepidote scales beneath — **5. *Olea***
    4. Leaves without dense lepidote scales beneath
      5. Flowers mostly in terminal panicles; corolla tube as long as — ***4. Ligustrum***
      5. Flowers in lateral clusters; corolla tube shorter than lobes
        6. Leaf lamina 4·5-17 cm; drupe 10-14 mm — **6. *Osmanthus***
        6. Leaf lamina less than 4 cm; drupe 3-8 mm — **7. *Phillyrea***

2.1.1.2. *Olea* species

They are evergreen trees and shrubs with terete or quadrangular twigs. Leaves are opposite, simple, lanceolate to obovate, usually entire, coriaceous. Flowers are hermaphrodite or polygamous, in axillary branching panicles or fascicles. Calyx short, 4-toothed. Corolla is with a short tube and 4 valvate lobes. Stamens are epipetalous, shorter than lobes; filaments are short, anthers large. The fruit is an oblong or ovoid drupe.

2.1.1.3. *Olea europaea* L.

The tree is 10-15 m, with a broad crown, often gnarled trunk, and thornless nearly terete branches; twigs lepidote, grey; buds very small, lepidote-sericeous, and greyish. Leaves are lanceolate or obovate, (8-) 20-86 x (4-)5-17(-24) mm, subsessile, mucronate, dark green and glabrous above, densely silvery-lepidote beneath. Panicles are shorter than leaves; flowers are white, fragrant, 3-4 mm, drupe is subglobose or oblong, it is about 6-35-40 x 5-20-25 mm, shining black, brownish-green or rarely ivory-white when ripe.

1. Leaves lanceolate, longer than 4 cm; twigs thornless, nearly terete; fruit large (to 35 mm).

var. *europaea*

2. Leaves obovate, shorter than 4 cm; lower twigs ± thorny and quadrangular; fruit small (to 15 mm)

var. *slyvestris*

The variety *europaea* is a cultivated form. Many cultivars are grown in Turkey, such as 'Ayvalık', 'Çakır', 'Çelebi', 'Çilli', 'Domat', 'Edinciksu', 'Erkence', 'Gemlik', 'Halhalı', 'Memecik', 'Memeli', 'Uslu', etc. They were selected for their edible fruit and oil (9).

Table 1: Classification of *Olea europaea* (10)

Rank	Scientific Name
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Scrophulariales or Lamiales
Family	Oleaceae
Genus	*Olea*
Species	*Olea europaea* L.

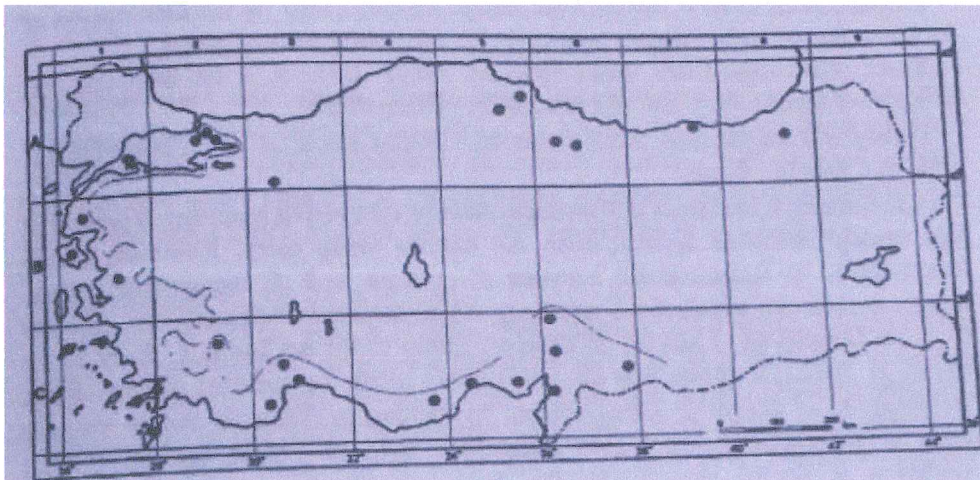


Figure 1: *Olea europaea* • var. sylvestris (9)

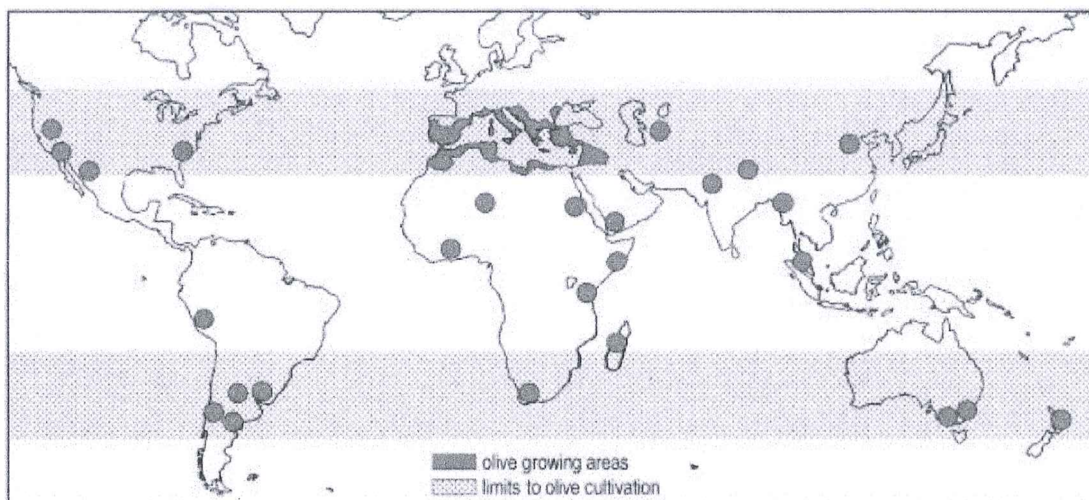


Figure 2: World distribution of *Olea europaea* L. collection sites (11, 12)

2.1.1.4 Traditional Usage of *Olea europaea* L. Leaf

Olea europaea L. is used in traditional medicine. For example, decoction of olive leaves is used internally for nodules in Gönen (13). In Turkey, 5% infusion prepared from the barks and leaves are used as an appetizer, diuretic, for reducing fever and constipation (14). Besides, a recent study demonstrated that decoction of olive leaves is used againt diabetes in Acıpayam district (15).

Generally, the leaves of olive are used as hypoglycemic, hypotensive, anti-inflammatory, mouth cleanser, and diuretic in traditional medicine. In Greece, for diminishing high blood pressure, a hot water extract of olive leaf is applied. Besides, fresh or dried boiled olive leaves are also used for the treatment of asthma in traditional Greek medicine (16).

The infusion of fresh leaves of olive is prepared for the anti-inflammatory effect (3). Moreover, in Italy, the tincture of olive leaves is febrifuge in internal usage and externally used for ingrown nails (17, 18). In Japan, leaves of olive are utilized for stomach and intestinal diseases per os (19). Olive leaf is applied in Mediterranean folk medicine to reduce symptoms gout (20). Moreover, olive leaf is used for its antibacterial effect against gingivitis, otitis, icterus, and cough in Tunisian folk medicine (21).

Table 2: The usage of *Olea europaea* leaf in traditional and contemporary medicine (3)

Preparation of *Olea europaea* leaf	**Usage**
Infusions and macerations of leaves	Hypoglycaemic, hypertensive, antidiabetic, antibacterial
Decoctions of dried leaf (per os)	Urinary tract infections, diarrhea, and respiratory
Boiled extract of green leaf (per os)	Asthma, to treat hypertension, eye infections treatment
Leaf extract in hot water	Diuretic
Infusion of leaf (per os)	Antipyretic, tonic, anti-inflammatory
Leaf preparations	Symptomatic treatment of gout
Decoction of leaf	Antihypertensive, antidiabetic
Fruits and leaf	Rheumatism, vasodilator, Haemorrhoids

2.1.2. Bioactivity Studies on *Olea europaea* L. Leaf

2.1.2.1. Studies on Anti-inflammatory and Analgesic Activities

Biopsies were taken via colonoscopy from 14 patients with ulcerative colitis to research the anti-inflammatory effect of OLE. Then, biopsies were placed into organ culture immediately. After that, 1 µg/mL of lipopolysaccharide from *Escherichia coli* was added to the organ cultures. 3 mM OLE was given to one of the organ cultures; none was added to the other. The interpretation of (COX)-2 and (IL)-17 were measured via Western blotting. OLE-treated colonic samples demonstrated a reduction of ranks of COX-2 and IL- 17 (22).

This research showed the topical effect of olive leaf extract ointment on oral mucositis on 5-fluorouracil injected golden hamsters. After oral mucositis occurred, olive leaf extract ointment was applied to the cheek pouch for five days. Blood examinations, histopathology evaluations, and tissue malondialdehyde rank were examined 1st, 3rd and 5th days after application. The histopathology amount and tissue malondialdehyde rank of the OLE- treated group were lower than the control (no ointment) group and base group (23).

J.Flemming et al. studied inhibition of xanthine oxidase, gout related enzyme, with *Olea europaea* leaf extract. Olive leaf extraction was prepared with 80% ethanol and 134 µM/g oleuropein. The activity of olive leaf extract was compared with allopurinol and K_1 values were checked by using Dixon and Lineweaver-Burk plot analysis. The 80% ethanolic OLE had potent activity against xanthine oxidase (21).

The chloroform and methanolic extraction olive leaf extract have shown the activity of anti-inflammatory analgesic. Inflammation occurred via the carrageenan-induced paw edema model in rats. The control group was given 2.5 mg/kg saline solution. 300 mg/kg acetylsalicylic acid or 1 mg/kg dexamethasone was given to the standard group. Chloroform and MeOH extracts at doses of 50, 100 and 200 mg/kg were injected to the test groups per i.p. After injection of 0.05 mL 1% carrageenan suspension, Wistar rats paws were measured via paleo thermometer at 0, 1, 2, 3, 4, and 5 hours after. For analgesic activity, abdominal writhes were used as a pain indicator. The anti-inflammatory effect of OLE was monitored from the first hour. The chloroformed extract had a better anti- inflammatory effect than methanolic extract. Also, OLE showed a notable analgesic effect (24).

2.1.2.2. Studies on Antioxidant Activity

Olea europaea L. cv. Leccino decoction of leaves was prepared by boiling 250 g of fresh olive in 2 litres of water for 30 min. Once cooled, it was filtered and extracted with butanol, chloroform, and n-hexane. These extractions' antioxidant capacities were measured by DPPH, TAC, XOI, and, ALP. After the experiments, the olive leaves extracted with water had a higher DPPH capacity, green olive leaves which were extracted with n-butanol had a higher total antioxidant capacity, and the decoction of olive leaves which was extracted with n-butanol showed higher total phenolic content (25).

In this research, *Olea europaea* leaves' extract was tested on pre-menopausal and postmenopausal women. The extract was prepared from 40 mg of olive leaves, extracted with 50% queous MeOH a solution. Women were chosen between 18 and 75 years old. Eight of them were premenopausal women, and 8 of them were postmenopausal women. They were given 250 mg OLE containing 40% oleuropein. Their blood samples were gathered t=0, 1, 2, 3, 4, 6, 8, 12, and 24 hours after and their urine samples were collected after 24 hours. Ferric reducing antioxidant capacity and MDA were computed from plasma. The result of this study showed that OLE decreases the MDA level in plasma (26).

Researchers wanted to demonstrate the ratio of phenolic compounds in olive leaves, which were extracted in a different portion of solvents and temperatures. HPLC-DAD detected phenolic compounds. First, dried olive leaves were extracted at 40°C for 2 hours. 20% aqueous ethanol solution was used, but the ratio of olive leaves and solvent was changed in 1:5, 1:6, 1:7, 1:8 and 1:10. Second, solid and the solvent ratio were 1:10. Also, the percentage of ethanol was changed to 20%, 40%, 55%, 70%, 80%, and 90%. Last, the temperature was changed to 40, 60, 65, 70, and 85°C. Antioxidant activity was detected with DPPH, and the Folin-Ciocalteau process was occupied to detect TPC. The higher yield was observed from 1:7 sample at which the solvent ratio was 70% aqueous ethanol. At multistage extraction, the highest extraction was obtained at 85°C. The TPC amount was increased by higher than 75% in steam distillation (27).

The antioxidant activity of olive leaf extract was adjusted by the nitrite-scavenging method and DPPH. OLE was prepared according to Lee et al. procedure in 2009. All phenolic except vanillin had higher nitrite-scavenging capacity. In the DPPH method, the phenolics mixture showed a higher DPPH scavenging activity after caffeic acid (28).

In this study, 200 g of fresh olive leaves were washed and dried. 80% aqueous methanol solvent was used as 1:10 and 1:20 as a sample: solvent ratio. 15, 30, 60, 90, and 120 min were the extraction time. Phenolic compounds were higher in the first 15 min.

The DPPH method showed a better antioxidant capacity in comparison to BHT in olive leaf extract (29).

One gram of the sample was extracted 10 mL of solvents, which were chloroform, ethyl acetate, chloromethane, acetone, hexane, EtOH, MeOH, butanol, and water. The antioxidant activities of these extracts were measured by TLC. Three different solutions were prepared for TLC; these were BEA, CEF, and EMW. 2% DPPH in methanol was sprayed onto TLC plates, and yellow points were observed (30).

Olive leaves, stems, and seeds were extracted by maceration with different solvents like; hexane, ethyl acetate, and MeOH. Their antioxidant capacities were measured by FRAP, DPPH, ORAC, and β-carotene-linoleic acid bleaching assay. The different extracts prepared from the same organ were demonstrated to be affected in terms of antioxidant, and found as such: methanol/water > methanol > ethyl acetate > hexane (31).

This study showed that the amount of oleuropein was not the same during a year in olive leaves. Oleuropein and total phenolic content were measured by HPLC. Folin-Ciocalteu approach was operated to regulate TPC. The levels of oleuropein were higher in January; there was no significant change between March and April, but the levels decreased after the becoming of the olive fruit. DPPH, FRAP, ABTS, nitrite-scavenging assays, total reducing power were exploited to determine the antioxidant capacity of the olive leaves. They were extracted with 80% methanol-water, and its ratio was 1:25. The total antioxidant capacity of oleuropein was higher than BHT (32).

Olive leaves and fruit were extracted with 75% ethanol. Ethanol was evaporated and the extracted material was lyophilized. The antioxidant properties of olive leaves and fruit were measured using reducing power assay, DPPH, ABTS radical+ scavenging capacity assay, hydroxyl radical-scavenging activity capacity, and superoxide anion radical scavenging ability. OLE (olive leave extract) showed a significant antioxidant effect than OFE (olive fruit extract) in every experiment applied in this research (33).

In another study, four different cultivars were studied. These were Biancolilla, Coratina, Nocellara, and San Benedettese. The olive leaves were dried at 40°C and extracted, and samples were prepared 5 g in 100 ml of hot water and stored in darkness. TPC, DPPH, and TEAC were applied to determine the antioxidant capacity. The cultivar of Bioncollila had a higher level in TPC and DPPH. Bioncolilla and Nocellara were the same antioxidant value in the ABTS assay (34).

Three cultivars which were Chondrolia, Halkidiki, Kalamon, and Koroneiki were studied. Each sample was extracted with 70% MeOH and evaporated. The dry extract was diluted with 70% methanol. DPPH, ABTS, FRAP, CUPRAC, HPLC assays were applied

for antioxidant capacity. Also, TPC was measured. Higher antioxidant capacity was determined in Chonrolia, and Halkidiki *in vitro*. In HPLC assay, different phenolics were seen in all cultivations (35).

Antioxidant effects of olive leaf extract and olive cake, which was residue after olive oil extraction, in different solvents were shown in this research. Furthermore, olive leaf containing packages can be used as a radical scavenger. As solvent, ethanol (50% and 70%), acetone (50% and 70%) and distilled water were used, and UPLC-QTOF-MS assay was adjusted to figure out the results of the assays. Antioxidant capacity of 50% and 70% of ethanol was higher than acetone (50% and 70%) in both leaf and cake extract. In ORAC assay, olive leaves had higher value than olive cake and it was chosen to be used as antioxidant in active film (36).

2.1.2.3. Studies on Antihyperlipidemic Activity

Oleuropein was insulated from *Olea europaea* and purified via RP-HPLC with a thermal finning SPECTRA system. This was used to check hypolipidemic effects in anesthetized rabbits. 52 rabbits were split into 8 groups. During 6 weeks, 10 and 20 mg/kg of oleuropein were given to them. Cholesterol, triglycerides, SOD activity were measured by HNMR spectra. The activity of oleuropein was discernibly higher than the control groups (37).

OLE was prepared with 75% aqueous ethanol (1:20) to study the atherogenic lipid profile in rats which were fed a high cholesterol diet. Forty of male Wistar rats were split into 8 groups, and they are nourished by 2% cholesterol- enriched or standard eats for eight weeks. 20 mg/kg Atorvastatin and 50 or 100 mg/kg OLE were given the rats. Serum samples of the rats were analyzed to determine lipid levels and it was observed that both atorvastatin and OLE significantly decreased the lipid levels in the rats' serums, which were nourished with high cholesterol diet (38).

In this research, the hypolipidemic effect of Chemlali OLE was studied *in vivo*. 50 Wistars rats were split into 5 groups. The premier group was bolstered with a standard laboratory diet, the Group 2 was sustained with HCD, Groups 3 was nourished with HCD and olive leaf extract. Group 4 was given. HCD with acid hydrolysate extract, and group 5 was given enzymatic hydrolysate extract (quantity succeeding to 3 mg/kg of body weight oleuropein, hydroxytyrosol and, oleuropein aglycone) for 16 weeks. Phenolic content of olive leaf extract decreased the lipid levels in serum and showed lipid-lowering effect and vigilant outcomes against hypercholesterolemia (39).

OLE has an anti-atherosclerotic effect and in this research was studied *in vivo*. 24 healthy male New Zealand rabbits were split into 3 groups. These were control group, HLD group and HLD with OLE group which was given 100 mg/kg. The atherosclerotic markers were examined at 2nd, 4th and 6th week. Atherosclerotic lesions were observed both HLD and HLD with OLE but the thickness of intima in HLD with OLE was thinner than HLD groups (0.10 ± 0.03 mm for HLD with OLE and 0.31 ± 0.26 in the HLD group) (40).

2.1.2.4. Studies on Antidiabetic Activity

Polyphenols of olive leaf (51.1 mg oleuropein and 9.7 mg hydroxytyrosol) were used to assess insulin action in 46 middle-aged overweight men. OLE and placebo were given once a day for 12 weeks. After 6 weeks and the washout period, groups were crossed over. Then, 12 weeks treatment process began. At the end of the trial, the insulin resistance effect of OLE showed a 15% improvement compared to the placebo (41).

Oleuropein was purified from *O.europapea* leaves of labeeb form and detected via TLC, UV, IR, and NMR. During 16 weeks, alloxan-induced rabbits were given 20 mg/kg oleuropein. The level of blood glucose of oleuropein-treated rabbits significantly fall at 16 weeks (42).

The antihyperglycemic activity of *Olea europaea* was studied as a TGR5 against. For *Olea europaea* extraction was gained 80% aqueous ethanol by maceration. Male (57BL/6) mice were split into 2 groups. 5 of them were nourished a chow diet and 16 of them were fed with a high-fat diet for ten weeks. The group which was nourished the high-fat diet was separated into 2 groups. First groups (n= 8) continued to be fed high-fat diet and the other group was fed on some food reinforced with oleanolic acid at a 100 mg/kg day dose. For the glucose tolerance test, 2 g of glucose/kg was injected and tail vein blood was collected at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. Glucose level at the blood in mice who were nourished high-fat diet with OA lower than mice fed on high fat diet (43).

Songi et al. studied the antihyperglycemic effect at oleuropein. *Olea europaea* L. leaves were extracted with 70% ethanol. For animal experiment 36 male Wistar rats were used 30 of them were diabetes via injection of streptozotocin. 3 and 5 mg/kg were injected by intraperitoneal when the level of fasting blood glucose level was ruled. Treatment was given along 56 days. Oleuropein showed a notable decline in blood glucose levels. It occurred in the 4th week and at a dose of 3 mg/kg and 5 mg/kg (44).

2.1.2.5. Studies on Antimicrobial Activity

The antimicrobial effect of Tunisian cultivations of *Olea europaea* leaf was studied. The volatile fraction obtained from both fresh and dried leaves were used. The volatiles of the fresh leaf showed stronger activity against *Escherichia coli* and *Pseudomonas aeruginosa* than volatiles of the dried leaf. Otherwise, the volatile of the dried leaf was more effective against *Staphylococcus aureus* than the volatiles of dried olive leaf (45).

The antimicrobial activity of olive leaf extract was proven against foodborne bacterial pathogens such as *Listeria monocytogenes, Escherichia coli* and *Salmonella enteritidis.* 62.5 mg/mL OLE which was bought as a commercial OLE product or extracted with 80% ethanol inhibits those pathogens. Moreover, OLE decreased the mobility of *Listeria monocytogenes* and inhibited the biofilm formation of *Listeria monocytogenes and Salmonella enteritidis* (46).

The activities of OLEs from 5 different Tunisian types of olive trees were studied on *Acanthamoeba castellanii* Neff. Alamar Blue was used for determination of the anti-*A*canthamoeba activities. Water, EtOH, MeOH and a mixture of alcohol were used as a solvent and mixture of alcohol extract of Dhokkar showed the highest anti-*Acanthamoeba* effect. The same research continued to find which compounds are effective in OLE as the anti- *Acanthamoeba*. Oleanolic acid, maslinic acid, and oleuropein showed the highest activity against *Acanthamoeba castellanii* (47).

In this study, the synergic interaction of olive phenols and ampicillin was observed *in vitro* against *Escherichia coli* and *Staphylococcus aureus.* Phenolic compounds were disclosed via HPLC in the commercial OLE. Hydroxytyrosol demonstrated an apparent synergistic effect with ampicillin (48).

The antifungal activity of OLE was studied against *Candida albicans* and *Candida dubliniensis*. The trypan blue exclusion approach and fluorescent die exclusion approach were used to test the antifungal effect. Hydroxytyrosol showed more antifungal effect than OLE which was aqueous extraction of olive leaf (49).

2.1.2.6. Studies on Anticancer Activity

Goldsmith et al. performed the anti-proliferative activity of OLEs on pancreatic cancer cells in this research. Olive leaves were collected two varieties Olea *europaea* L. which were *Corregiola* and *Frantoio.* 50% methanol, 50% EtOH, and aqueous extracts of *Corregiola* and *Frantoio* of *Olea europaea* were searched of their TPC, TFC, and oleuropein content, and anti-proliferative activity against MiaPaCa-2 pancreatic cancer cells. Concentrations of OLE were 50, 100 and 200 μg/mL. The water extraction of

Corregiola form showed a remarkable negative effect on MiaPaCa-2 pancreatic cancer cells at 50 µg/mL. Overall, ethanol extraction of both varieties of *Olea europaea* L. had higher cytotoxicity on pancreatic cells contrasted to gemcitabine at its IC_{50} (50).

Supercritical fluid extraction of Tunisian olive form 'El Hor' was used to analyze potential antiproliferative mechanisms on the JIMT-1 breast cancer cell line. In this research, major elements were detected via HPLC and ESI-Q-TOF-MS after SFE extract incubation of JIMT-1 breast cancer cells. These analyses were applied on cell cycle, MAPK, and PI3K proliferation pathways. Especially, diosmetin was found as the principal metabolite in the intercellular target. 'El Hor-SFE' olive extract showed cytotoxicity effect on MAPK route at the ERK1/2 which mediated partially G_1 phase (51).

The effect of OLE in Human Chronic Myelogenous Leukemia K562 cells was studied in this research. Chemlali form of olive leaf was collected and olive leaves were extracted by ethanol 70% (1/10, w/v) in darkness. The duration of extraction was 2 weeks at 23°C. Oleuropein was detected at 254 nm via HPLC. COLE (Chemlali Olive Leaf Extract) concentrations were 50 to 150 µg/mL and they were given every 24 hours. At 72nd hours 150 µg/ml of COLE inhibited 17% of leukemia cells (52).

Nashwa et al. studied the anticancer influence of olive leaf in 2014. The olive leaves were extracted with water (1:10) and 80% methanol (1:20) at the room temperature. Four different cancer cell lines, breast, colon, hepatocellular and cervical carcinoma, were used, and a reference drug, vinblastine was applied. The potent effect of OLE was shown against HEPG-2 cancer cell line (29).

Commercial olive leaf extract powder was mixed in dimethylsulfoxide in a stock solution of 300 mg/ml. HPLC-ESI-QTOF-MS gauged the uptake and metabolism of phenolic compounds from olive leaves in SKBR3 cells. First, HPLC-ESI-QTOF-MS analyzed olive leaf extract than, SKBR3 cells were incubated with 200 µg/ml of OLE at 15 min, 1, 2, 24 and 48 hours. After the incubation, intercellular phenolic compounds were checked by HPLC-ESI-QTOF-MS. Oleuropein, luteolin-7-O-glucoside, and metabolites of luteolin-7-O-glucoside were found in the cells. These compounds were responsible for antitumor activity (53).

The anticancer effect of dried olive leave extract (DOLE) was searched on B16 mouse melanoma cell line *in vitro*. For DOLE, EFLA® 943 which was gathered from the dried leaves of *Olea europaea* L. into ethanol (80% w/w) was used. The extract was standardized to 19.8% oleuropein. Proliferation of B16 mouse melanoma was inhibited DOLE-created B-16 cells stopped in G_0/G_1 phase of the cell cycle (54).

2.1.2.7. Studies on Hepatoprotector Activity

Dried olive leaf was extracted with 80% methanol and its efficiency on a liver was studied with rabbits which were induced hepatic damage by CCl_4. Rabbits were divided into 5 groups. OLME was compared with Silymarin. The experiment existed for 4 weeks 50 mg/kg OLME and 100 mg/kg silymarin were used after the process, blood samples were taken from rabbits and AST and ALT levels were measured. AST levels were the same at groups that received OLME and silymarin but OLME reduced ALT level more than silymarin (55).

In this research, oleuropein which was isolated from *Olea europaea* leaf was studied *in vivo*. Thirty-three Sprague-Dawley rats were split aimlessly into four groups. The control group received 1 ml of normal saline, the second group was given 4 g/kg ethanol, third group accepted 15 mg/kg oleuropein, and the last group was allowed 4 g/kg ethanol after 120 min 15 mg/kg oleuropein. The experiment lasted 4 weeks. After 4 weeks, blood samples and liver tissues were collected from rats. Concentrations of TC, TG, ALT, and AST was controlled. Oleuropein reduced the level of ALT, AST, TG, and TC. Moreover, the result of oleuropein and ethanol group was close to the result of control group (56).

2.1.2.8. Studies on Cardiovascular Activity

The OLE was studied renal ischemia-reperfusion on injured rats. Sevillano form of *Olea europaea* was extracted with 80% ethanol. 40 male Sprague Dawley rats were segregated into 5 groups which were control, I/R+0.5 ml water by gavages, I/R+OLE in 3 dissimilar doses (25, 50 and 100 mg/kg) one time in a day. It lasted for 2 weeks. Serum BUN and creatinine MDA were measured. Three different doses of OLE improved the kidney functions (57).

In this study, eighteen healthy volunteers and nine females joined the research to show OLE effect on vascular function. This study continued for nine weeks. They consumed OLE which had 151 mg oleuropein and 10 mg hydroxytyrosol and they were separated by four weeks washout between June and September 2011. Their vascular function was observed via the digital volume pulse. OLE improved vascular function. Arteriole stiffness of OLE consumed group was distinguishably lower than the control group (58).

Synergic protective effect of *Olea europaea* L. leaf extract which was extracted with 80% ethanol and *Hibiscus sabdariffa* L. flower extract (13:2 w/w) was studied in this research. The cardiac and vascular effects were examined via isolated guinea-piglet

endothelial cells right atria and aorta. The mixture of both extracts showed the highest cytoprotective effect that was detected via MTT assay on vascular smooth muscle (59).

In this research, the effect of oleuropein on cardiotoxicity was studied. Oleuropein was isolated from *Olea europaea* L. via CH_2Cl_2/methanol (98:2) and it was detected via RP-HPLC. Fifty rats were split into 6 groups and 2 ml normal saline was injected intraperitoneal. The first group was injected only DXR (20 mg/kg) the others were 100 mg/kg oleuropein and 20 mg/kg DXR, 200 mg/kg oleuropein and 20 mg/kg DXR, 100 mg/kg oleuropein, 20 mg/kg DXR, and 200 mg/kg oleuropein, separately. After 72 hours, blood samples were obtained from DXR treated rats and CPK, CPK-MB, LDH, AST and ALT values were measured. Oleuropein reduced the myocardial necrosis far all doses. The formation of vacuolation decreased for rats that were treated with oleuropein. The rate at PCC and nitrotyrosine fell in rats that oleuropein was used (60).

Four triterpenoid derivations that were isolated from Cape cultivar of *Olea europaea* L. were studied on heart rate, β-adrenolytic, inotropic and dromotropic effect. In addition, antidysrhythmic effects of oleanolic acid and ursolic acid were compared by using antidysrhythmic drugs. The experiment lasted 60 min and every 10 min data were recorded. Oleanolic acid (40 mg/kg) per intraperitoneal showed a significant effect on heart rate decrease at 20 min. Furthermore, the effects of propranolol i.v. (2 mg/kg) an oleanolic acid i.p. (40 mg/kg) were similar to ischemia-reperfusion dysrhythmia (61).

Antithrombotic effect of 99% ethanol extracted local *Olea europaea* L. leaves were tested on rabbits. The method of thrombosis bred via ligature of the vena cava anal intravenous administration of tissue thromboplastin was used. Warfarin was the positive control group 100 and 200 mg/kg OLE were used for the other groups respectively. Moreover, water was given to negative control groups for 8 weeks. There was no significant effect OLE on prolongation on prothrombin time but there was a slight effect of OLE at 200 mg/kg which prolonged prothrombin time (62).

2.1.2.9. Studies on Hypotensive Activity

In this research anti-hypertension effect of *Olea europaea* L. extract was studied. The olive leaf was extracted in 80% ethanol (w/w). Twenty four-week-old male SHR were used. They were dived into three groups, and they were fed on 5, 25 and 50 mg/kg, respectively. Moreover, one group (n= 7) was fed on 10 mg/kg oleuropein and 0.2 ml saline was given to the control group (n= 7). Carotid blood flow was measured. 25 mg/kg OLE decreased blood pressure. Furthermore, despite OLE 50, OLE 25 did not change cardiac output and heart rate (63).

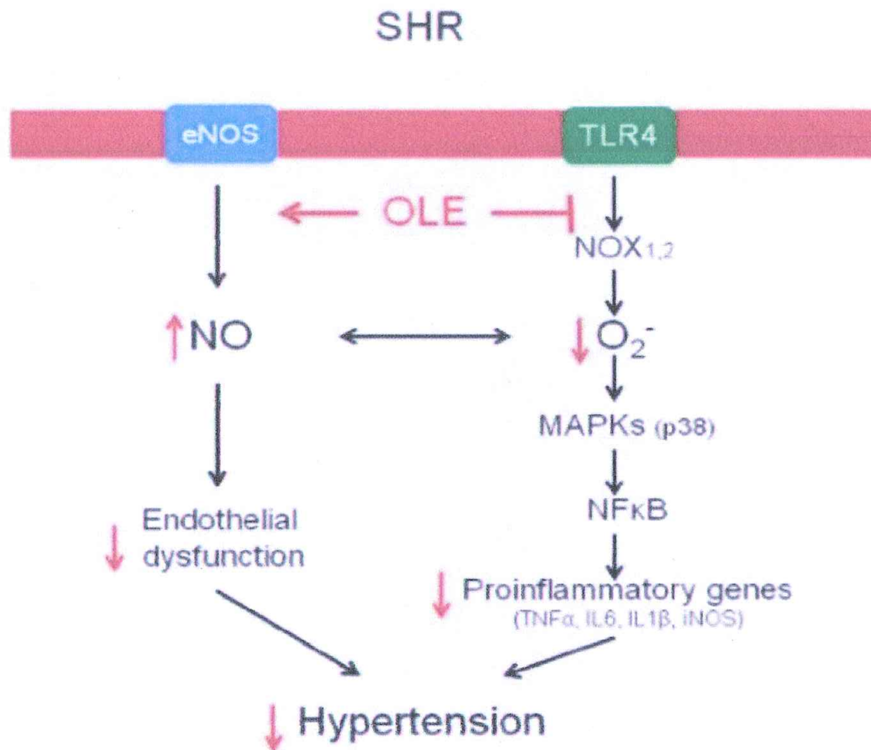


Figure 3: Mechanism of action of OLE on hypertension (Adapted from 63)

In this study, 10 WKR and 20 SHR were used to show the antihypertensive effect of oleuropein-enriched olive leaf extract. Water was accepted as a vehicle to WKR group and SHR control group and SHR group receive OLE which was standardized 15% oleuropein, 10% medial triterpenic acid content and 1% hydroxytyrosol for 5 weeks. Tail-cuff plethysmography measured blood pressure and heart rate quantified at room temperature. OLE defeated the increase in aortic superoxide ranks and decreased the heightened NADPH oxidase activity. This affected the reduction of NOX-1 and NOX-2 mRNA ranks in SHR (64).

E. Susalit et al. compared the antihypertensive effect of OLE and captopril. Patients were stage-1 hypertension (232 patients) were equally divided into 2 groups. After four weeks washout period, the first group received 500 mg OLE (EFLA® 943) twice a day for 8 weeks. After 2 weeks, a dose of captopril titrated 25 mg twice a day. At the end of the eight weeks, there was no momentous variation in the reduction of SBP in both groups (65).

OLE (EFLA® 943) was accepted as a food supplement to 40 borderline hypertensive monozygotic twins and they were between 16 and 60 years old. The antihypertensive effect of OLE was studied for 8 weeks. Every sibling of twins was elected to different groups. Those groups were given 500 and 1000 mg/day. Their heart rate, blood pressure, weight, and glucose were checked every 2 medications. 1000 mg/day dose of OLE showed a better antihypertensive effect than 500 mg/day dose of OLE (66).

In this study, orally 100 mg/kg olive leaf extract (EFLA® 943) was given to L- NAME induced hypertensive Wistar rats for 4 weeks. During 12 weeks, OLE was given with L-NAME. OLE treatment decreased the blood pressure significantly in the 12th week (67).

2.1.2.10. Studies on Antifungal Activity

The antifungal effect of olive extract and seven phenolic compounds were compared against 30 fungal strains by using disk diffusion assay. Olive leaf extract was prepared via using water, acetone, methanol and ethyl acetate. Aqueous OLE was the highest effect on fungal strains. On the other hand, methanol extract had the lowest antifungal effect. Oleuropein had the best antifungal activity at the lowest test concentration (0.1%) (68).

Olive leaves were collected from Trilye Region from Turkey, and it was extracted with water, EtOH, acetone, ethyl acetate in the Soxhlet apparatus. MIC, MFC, and dose diffusion assay were used to detect antifungal activity. Except for the water extract, none of the extracts did not show antifungal sensitivity to the yeasts. Aqueous extract of olive leaf (15%) was effective against *Candida albicans* within 24 hours. *Saccharomyces uvarum* and *Candida aleophila* effected from acetone extracts according to MIC value. *S. cerevisiae* was influenced by none of the extracts expect ethyl acetate (69).

2.1.2.11. Studies on Other Activities

The antiviral effect of OLE was studied against VHSV and solenoid rhabdovirus. Viral infection activity decreased 10 and 30% before OLE was applied (70).

OLE was used as a supplement to assess the effect on peripheral blood mononuclear cell gene expression. 20 ml OLE was given 15 individuals and 14 people took the placebo for about 8 weeks. Then gene expression figures of peripheral blood mononuclear cells were analyzed genes that responded to OLE. Real-time PCR detected the genes which were related to inflammation and cancer (71).

In this research, the effect of DOLE on adrenaline generated DNA damage of human peripheral leukocyte was studied. DOLE was given adrenaline-induce human leukocytes, which were 9 collected from 6 volunteers as pretreatment and a post-treatment at 0.125, 0.5 and 1 mg/ml concentration. After that single cell gel electrophoresis, all concentrations of DOLE showed DNA protective effect (72).

The thyroid-stimulating activity of DOLE was studied. Lyophilized aqueous extract of dried olive leaf was supplied Wistar rats about 100 μg, 200 μg and 500 μg orally for 14 days. Then their blood was collected to check hormones. OLE has broadened the rank of T_3 and T_4 in rats' blood (73).

The treatment effect of OLE on cyclosporine-induced nephrotoxicity was studied. Olive leaf was extracted with Soxhlet extraction processing with 70% ethanol. Thirty Wistar rats were split into five groups. The first group was provided with normal saline. The second group was treated with 25 mg cyclosporine. The other three groups were given OLE at 40 mg/kg, 80 mg/kg and 120 mg/kg, respectively. The body weight and kidney weight significantly increased at concentrations of 80 mg/kg and 120 mg/kg (74).

Antidiarrheal effect of *Olea europaea* L. subsp. Africana (Mill.) leaf was studied on castor oil-induced diarrhea in mice. Olive leaf was extracted methanol (70 g of dried powder and 750 ml of methanol). Then, the concentration of dried olive leaf extract was 2.5-7.5 mg/ml, which was given to mice per oral. The results of olive leaf methanol extract were compared with loperamide, and its effect was not good as loperamide (75).

The effect of OLE on Parkinson's disease was tested on rats that were injected intrastriatal of 6-hydroxydopamine in this research. OLE was given 50, 100, 150 mg/kg for seven weeks. The motor coordination and balance behavior of rats well via pretreatment of OLE. The narrow beam test showed significant improvement. Moreover, MDA and glutathione decreased and the activity of CAT was lower than the control group (76).

The immunomodulation effect of OLE was studied in this research. Peritoneal macrophages BALB/C were used for this experiment. Concentrations of 6.25, 12.5 and 25 μg/ml OLE were continued the experiment at 12, 24 and 72 hours. Furthermore, 25 μg/ml of OLE concentration showed a decrease of IL-10 level and an increase of IL-12 level (77).

2.1.3. Side Effects and Intoxications in *Olea europaea* L. Leaf

In vitro studies, olive leaf extract at doses 1 mg/ml did not show a toxic effect on human cells. There is no enough study about the security of OLE in pregnant and lactating women (78).

2.1.4. Chemical Compound of *Olea europaea* L. Leaf

2.1.4.1. Flavonoid Compounds

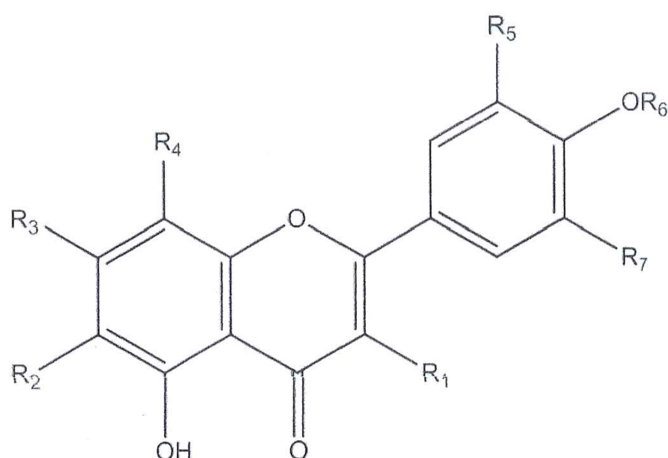


Table 3: Flavonoids from *Olea europaea* leaf

Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Ref.
Quercetin	OH	H	H	H	OH	H	H	(3)
Quercetin-7-*O*-glucoside	Glucose	H	OH	H	OH	H	H	(3)
Rutin	Rutinose	H	OH	H	OH	H	H	(3)
Apigenin-7-*O*-glucoside	H	H	Glucose	H	OH	H	H	(3)
Luteolin-7,4'-*O*-diglucoside	H	H	Glucose	H	H	Glucose	H	(3)
Apigenin	H	H	OH	H	H	H	H	(79)
Luteolin	H	H	OH	H	OH	H	H	(79)
Myricetin	OH	H	OH	H	OH	H	OH	(79)
Luteolin-7-*O*-glucoside	Glucose	H	H	H	OH	H	H	(80)
Hesperetin-7-rutinoside	H	H	Rutinose	H	OH	CH_3	H	(80)
Apigenin-7-*O*-rutinoside	H	H	Rutinose	H	H	H	H	(81)
Quercetin-3-rhamnoside	OH	H	Rhamnose	H	OH	H	H	(81)
Chrysoeriol	H	H	OH	H	H	H	CH_3	(81)
Chrysoeriol-7-*O*-glucoside	H	H	Glucose	H	H	H	H	(81)
Diosmetin	H	H	H	H	H	CH_3	H	(82)
Catechol	OH	H	OH	H	OH	H	H	(82)

2.1.4.2. Secoiridoid Compounds

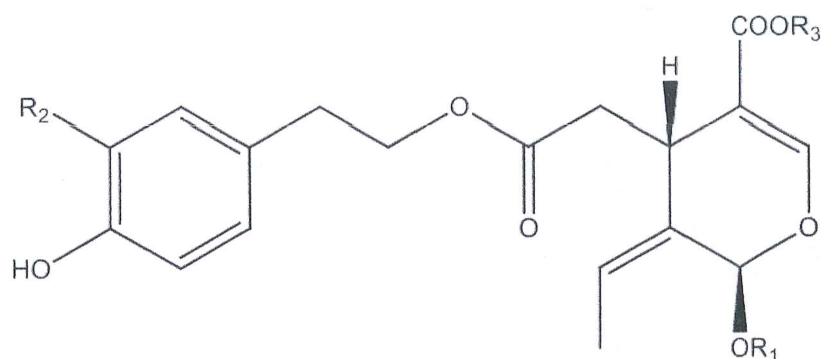


Table 4: Secoiridoids from *Olea europaea* leaf

Compound	R_1	R_2	R_3	Ref
Oleuropein	Glucose	OH	CH_3	(83)
Ligstroside	Glucose	H	CH_3	(84)
Oleuricine	6-O-β-D-glucopyranose	H	CH_3	(3)

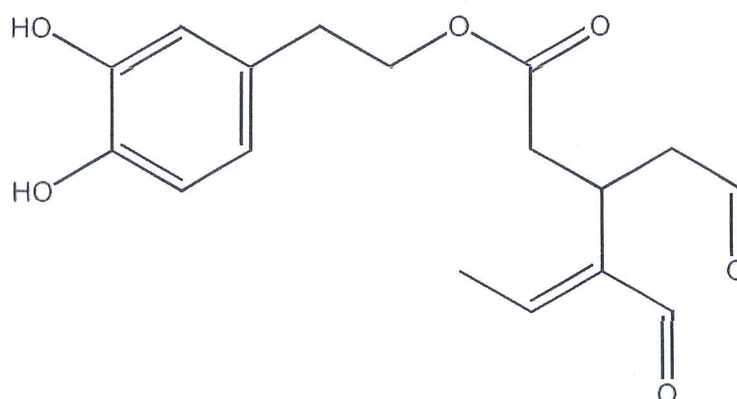


Table 5: Secoiridoids from *Olea europaea* leaf

Compound	Ref
3,4-DHPEA-EDA	(85)

2.1.4.3. Triterpenic Compounds

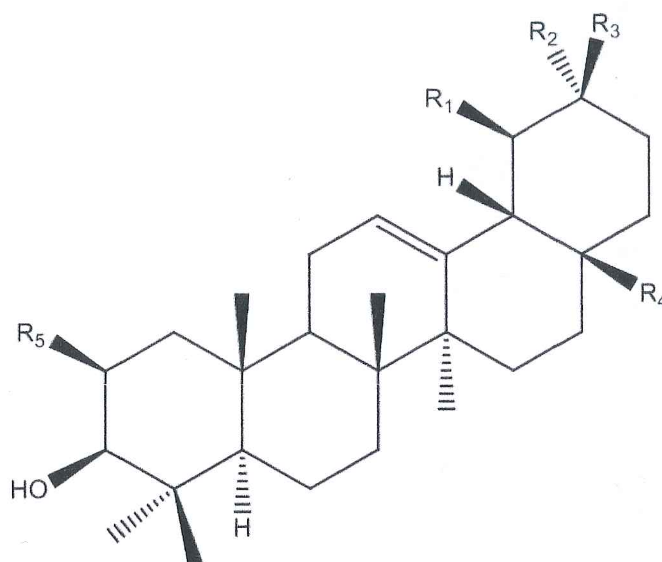


Table 6: Triterpens from *Olea europaea* leaf

Compound	R_1	R_2	R_3	R_4	R_5	Ref
Uvaol	CH_3	CH_3	H	CH_2OH	H	(3)
Oleanolic acid	H	CH_3	CH_3	COOH	H	(3)
Erythrodiol	H	CH_3	CH_3	CH_2OH	H	(86)
Maslinic acid	H	CH_3	CH_3	COOH	OH	(86)
β-amyrin	H	CH_3	CH_3	CH_3	H	(86)
Oleic acid	H	H	H	COOH	H	(86)
Ursolic acid	CH_3	CH_3	H	COOH	H	(61)
Methyl Maslinate	H	H	H	CO_2CH_3	OH	(61)

2.1.4.4. Phenolic Compounds

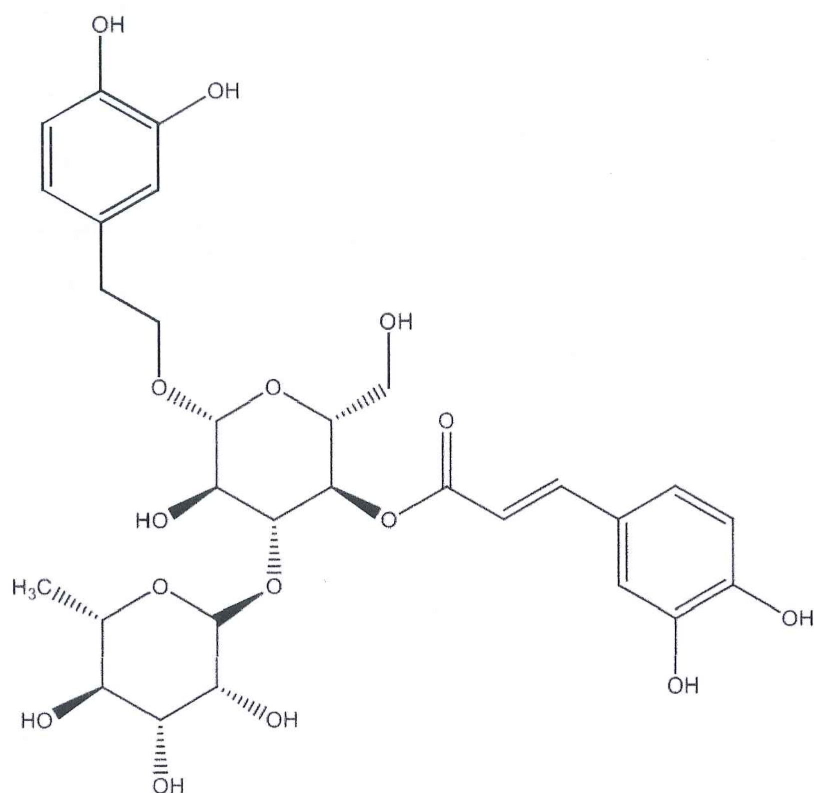


Table 7: Phenolic Compounds from *Olea europaea* leaf

Compound	Ref.
Verbascoside	(87)

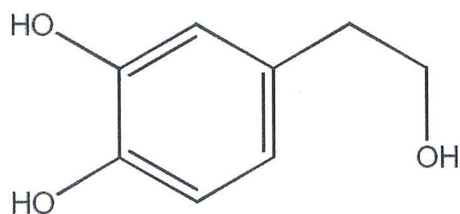


Table 8: Phenolic Compounds from *Olea europaea* leaf

Compound	Ref
Hydroxytyrosol	(88)

2.1.3.5. Nonphenolic Compounds

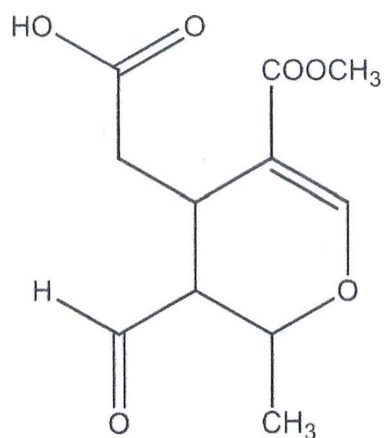


Table 9: Nonphenolic Compounds from *Olea europaea* leaf

Compound	**Ref**
Elenolic Acid	(89)

2.2. Oxidative stress

Human metabolism produces ROS and RNS. ROS causes aging and degenerative diseases in the human body. In a number of diseases, including cancer and atherosclerosis, there is evidence that ROS-mediated mechanisms, in form of DNA damage, oxidation of low-density lipoprotein (LDL), or cell membrane changes, play a significant role in pathogenesis (90, 91).

One focus of current research is to inhibit or prevent these oxidative processes. Defensive biological systems exist and in addition may be supplemented by dietary components. Vitamins, flavonoids, carotenoids and polyphenols are the example of antioxidants which are taken via foods. Moreover, some studies are proven the remarkable antioxidant effect of phenolic compounds (92, 93).

2.2.1. Molecular Oxygen and Reactive Oxygen Species

Free radicals are an atom which has unsubstituted (unpaired) electrons. ROS are occurred by the attraction of molecular oxygen (O_2) to free radicals. The result of this union, radical oxygen appears and it tends to react with other free radicals while, the radical oxygen crawls to react when it encounters non-radical substances (94).

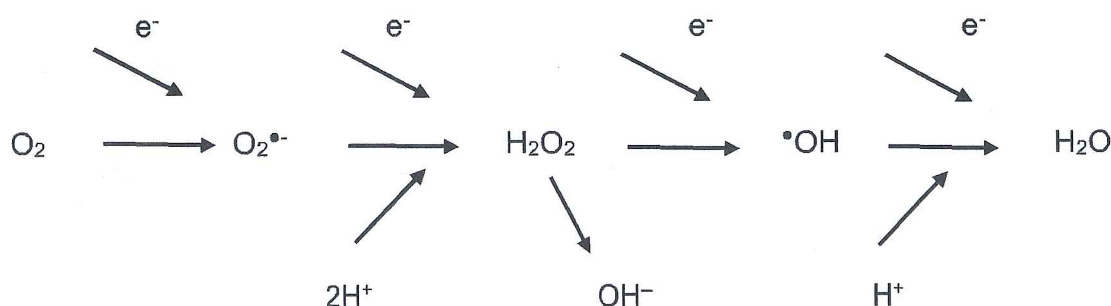


Figure 4: Molecular Oxygen and Reactive Oxygen Species diagram

The example of reactive oxygens is superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), lipid peroxides (LOOH), peroxyl radicals ($ROO^{\bullet}$) and peroxynitrite ($^{-}OONO$). These are seldom seen in normal oxygen metabolism. They are hazardous and they tend to attack protein and DNA, they lead to cardiovascular diseases and cancer (93).

Table 10: Reactive species (95, 96)

	Radicals		Non-radicals	
Reactive oxygen species	Superoxide	O_2	Hydrogen peroxide	H_2O_2
	Hydroxyl	$OH^{\bullet-}$	Hypochlorous acid	HOCl
	Peroxyl	$ROO^{\bullet}$	Hypobromous acid	HOBr
	Alkoxyl	$RO^{\bullet}$	Singlet oxygen	$^1O_2$
	Hydroperoxyl	$HO_2^{\bullet-}$	Ozone	O_3
Reactive nitrogen species	Nitrogenoxide	$NO^{\bullet-}$	Nitrous acid	HNO_2
	Nitrogendioxide	$NO_2^{\bullet-}$	Diazotrioxide	N_2O_3
			Peroxynitrite	$ONOO^-$
			Alkylperoxynitrite	LOONO

2.2.1.1. Oxygen Derived Free

Radicals Superoxide Anion Radical

Molecular oxygen accepts an electron, it reduces and superoxide anion radical ($O_2^{\bullet}$). It occurs via the reduction of molecular oxygen (97). The superoxide anion radical or the superoxide radical acts weakly as an oxidant. On the contrary, it is a strong reducer, superoxide dismutase (SOD) enzyme inhibits the oxygen toxicity effect of superoxide radicals and it protects the living system. $O_2^{\bullet-}$ may cause oxidative stress by damaging the cell. For example; OH can occurs via O_2 and H_2O_2 if there is iron the medium. This reaction is called the Haber-Weiss reaction.

$$O_2^{\bullet-} + H_2O \longrightarrow O_2 + OH^{\bullet} + OH^-$$

Hydroxy Radical (OH^-)

The hydroxyl radical derives from hydroxide ion. It has two significant properties. These are the most reactive radicals and the most robust oxidant (98, 99). There are many ways to form hydroxyl (98, 99). It has a high affinity to other molecules such amino acids, phospholipid, sugars, organic acid, and DNA. For example; they may emerge when water is exposed to high energy ionizing radiation. Hydrogen peroxide reacts with transistane metals (Fenton Reaction), when peroxynitrite degrades or UV rays form homolytic fission of the O-O bonding H_2O_2 (100, 101).

Hydroxyl Radical ($OH^{\bullet}$)

The $OH^{\bullet}$ radical, which reacts immediately with some molecules such as, amino acids, sugars, phospholipids, organic acids and DNA, is a highly reactive oxidant radical. Hydroxyl radicals occur in different ways, such as exposing to high-energy ionizing radiation of water, reacting with the transition metals of hydrogen peroxide (Fenton Reaction), degradation of the peroxynitrite radical, homolytic fission of the O-O bond in H_2O_2 formed by UV rays (100, 101).

2.2.1.2. Oxygen Derived Non-Free Radicals

Hydrogen peroxide

Hydrogen peroxide occurs in the dismutation reaction when O_2^- is presence.

$$2O_2 + 2H_2 \longrightarrow H_2O_2 + O_2$$

There is no unpaired electron on hydrogen peroxide. So, it cannot be defined as free radical but it has potential to produce free oxygen radicals. Hydrogen peroxide can generate hydroxyl radicals (OH^-) when Fe^{+2} is in the medium or other transition metals by the Fenton reaction and the superoxide if there is superoxide radical while the Haber-Weiss reaction is occurring. Furthermore, in the body myeloperoxidase this is in leukocyte transforms hydrogen peroxide into hypochlorous acid (HOCl) (102).

2.2.1.3. Reactive Nitrogen Species (RNS)

Recent studies provided that there are free radicals which are non-reactive and non-short lived. Nitric oxide is a significant example of not very reactive free radicals (103).

Nitrogen Oxide

Nitric oxide (NO) is synthesized from L-arginine and it is used for pathogens by macrophages. Peroxynitrite radical emerges when nitrogen oxide reacts with free oxygen radicals. NO has an important role against bacteria, fungi, virus and, parasite in the host immune. NO regulates some physiological functions in the body. For example; it dilates blood vessels and it is a neurotransmitter (104, 105).

Peroxynitrite

Nitric oxide and superoxide form peroxynitrite. It is not stable and thiyl radicals are the formation of peroxynitrite and thiols. Peroxynitrite can attack big and important molecules such as protein, lipid and, DNA (103).

2.2.2. Damage of Free Radicals in Tissue and Cell

2.2.2.1. Lipid Peroxidation

PUFAs which are located in cell membranes, endoplasmic reticulum and mitochondria cause lipid peroxidation through the free radical oxidation (106, 107).

Exposure of light, ionization of light and metal ion or metalloproteinase catalyst can all lead to radical formation and oxidation of polyunsaturated lipids. Subsequent cell membrane damage can harm the cell and trigger cell death (108).

2.2.2.2. Protein Oxidation

Amino acids have a high tendency to be oxidized lipids due in part to the presence of carboxyl and amino groups. Amino acids contain reduced carbon atoms and can be subjected to oxidative transformation in the side chain. Some proteins have unsaturated bonds and sulphur. These proteins such as tryptophan, tyrosine, phenylalanine, histidine, methionine and cysteine, can all be readily oxidized and cross-linked free radicals. Attachment of free radicals to proteins, leads to protein damage, reduction of enzyme activity, cytolysis and cell death. For example; Parkinson's disease, Alzheimer's disease, diabetes type 2, renal tumour formulation, and rheumatoid arthritis all feature a role for protein oxidation in their pathogenesis (109, 110).

2.2.2.3. Oxidative Damage of DNA

ROS can injure every element of DNA. If ROS attaches the base the integrity of the strands is disrupted. $OH^{\bullet}$ radical can harm double bonds between DNA bases. Furthermore, high amounts of reactive oxygen species can activate the cell transcription process. It means cell proliferation can start (111).

2.2.2.4. Oxidative Damage in Carbohydrates

α-hydroxyalkyl radicals occur when hydrogen atoms remove from C-H bonds. α-hydroxyalkyl radicals react with C-OH bonds in the acid and base-catalyzed rearrangement. As a consequence of this reaction glycosidic bonds break down and they form peroxyl radicals when reacting O_2 (112).

2.2.3. Antioxidants and Defence Mechanisms

Antioxidants are the first step of inhibition of oxidation. They can be effective at low concentration. The importance of antioxidants is realized recently.

Antioxidants are known as a helper to prevent degenerative diseases which are associated with ROS, RNS and reactive chlorine species in the body (108).

The effects of antioxidants can be shown in the following ways:

1. Enzymatic antioxidant defence mechanisms or direct
2. Non- enzymatic antioxidant defence mechanisms
3. Natural antioxidant molecules (108).

2.2.3.1. Enzymatic Antioxidant Defence Mechanisms

One of the antioxidant enzyme is SOD and it modifies superoxide free radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (113).

Glutathione inhibits or decreases the destructive effect of free radicals. It works as a substrate for enzymes like transferases, peroxidases. Glutathione prevents lipid peroxidation on the biological membrane. For example; Glutathione peroxidase (GSH-Px) is responsible for the degradation of hydroperoxides (114).

Superoxide dismutase transforms the superoxide anion radical into H_2O_2. H_2O_2 is unstable and it can convert into OH radical. That's why the catalase enzyme (CAT) is in the medium to remove H_2O_2 in the medium (97).

2.2.3.2. Non-Enzymatic Endogenous Antioxidant Defence Mechanisms

Melatonin, glutathione, bilirubin and uric acid are the examples of non-enzymatic endogenous antioxidants. Melatonin is a lipophilic antioxidant and its effect is too strong to remove the hydroxyl free radical ($OH^{\bullet}$) (114).

Glutathione inhibits the oxidative damage in the cells via reacting radicals and peroxides. Bilirubin scavenges the peroxide and hydroxyl radicals to stop lipid peroxidation. Uric acid suppresses the prooxidation of vitamin C by binding the iron and copper ions but it does not prevent the lipid peroxidation (114).

2.2.3.3. Non-Enzymatic Exogenous Antioxidant Defence Mechanisms

Vitamin C or ascorbic acid dissolves in the water and it is the one of the vitamins which is found in the human plasma, cerebrospinal fluid, aqueous humor of the eye, gastric juice, seminal fluid and, lung lining fluid. Majority of organism can synthesis vitamin C but human cannot produce it. Due to the reason, the human should take it with diet. Ascorbic acid is one of the strongest reducing agent. It scavenges $O_2^{\bullet}$, singlet oxygen and $OH^{\bullet}$ immediately. Furthermore, Vitamin C can scavenge water soluble peroxyl radicals. It prevents the damages which OH^- or LOO^- reacts with urate radicals (115, 116, 117).

Vitamin E protects lipids from oxidative damage. The significant protection of vitamin E defences PUFA within phospholipids of the biological membrane and in plasma lipoproteins against lipid peroxidation (115, 116).

Simple phenols, benzoic acid derivatives, phenyl propanoides, flavonoids, tannins, stylbens, lignins and lignans are phenolic compounds. They have an antioxidant effect and they are founded in plants and foods. In 1947, Boland and Ten have performed antioxidant effect of phenolic compounds and they are great sources of hydrogen. Moreover, phenolic compounds donate electron easily to lipids radicals (108, 116).

3. MATERIALS and METHODS

3.1. Materials

3.1.1. Plant Material

The leaves of *Olea europaea* L. were collected from İzmir, Olive Research Centre and olive field of researcher's parents (NL 38° 28' 40'' and EL 27° 44' 21'') which was determined from İzmir, Olive Research Centre on September 2017. The plant material was corroborated by Prof. Dr. Erdem Yeşilada, Voucher specimens were installed in the herbarium of Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey. (Herbarium No:17101)

3.1.2. Chemicals & Solvents

2, 2-diphenyl picryl hydrazil	Sigma Aldrich; 056K1147
Butylated hydroxytoluene	Sigma Aldrich; MKBD8339
Methanol	Sigma Aldrich; SZE9365S
Neocuproine	Sigma Aldrich; 120M1890V
Ammonium acetate	Carlo Erba; 313507
Ascorbic acid	Sigma Aldrich; 065K0003
Copper sulphate	Carlo Erba; 364757
Ethanol	Sigma Aldrich; 46139
Sodium acetate trihydrate	Riedel de Haen; 33450
Sodium phosphate monobasic	Riedel de Haen; 62840
Ammonium molybdate tetrahydrate	Riedel de Haen; 30590
Sulfuric acid (98%)	Riedel de Haen; 62260
Quercetin dehydrate	Sigma Aldrich; 116K1836
Aluminum chloride	Merck; 8.01081.1000
Sodium carbonate	Riedel de Haen; 2217A
Folin-Ciocalteu Reagent (FCR)	Sigma Aldrich; BCBD5119
Gallic acid	Fluka; 1126284
Sodium molybdate dihydrate	Riedel de Haen; 10102406

3.1.3. Equipments

Balance	Ohaus Explorer
Beaker (50, 100, 250 mL)	
Graduated cylinder (25, 50, 100 mL)	
Lyophilizator	Christ Alpha 2-4 LD
Micropipette (100-1000 microlt)	Isolab
Micropipette (20-200 microlt)	Transferpette
Microplate reader	Thermo Multiskan Ascent
Milli Q water device	Millipore
Refrigerator	Arçelik
Rotatory evaporator	Buchi, Heidolph
Volumetric flasks (50, 200 mL)	
Vortex	Heidolph Reax
Water bath	GFL

3.2. Method

3.2.1. Chemical Studies

3.2.1.1. Extraction

The dried and powdered leaves of *Olea europaea* (50 g) were macerated with 1000 ml of 80% aqueous MeOH for 1 day at 23°C. The resulting total extracts were filtered, and methanol was completely evaporated in a rotatory evaporator under diminished pressure. The remaining parts were frozen and then lyophilized.

Table 11: The extraction yields with 80% MEOH (g/g)

Plant Name	Part Used	Yield (%)
Domat	Leaf	24,81
Sarı Yaprak	Leaf	25,02
Uslu	Leaf	22,73
Sarı Yaprak (Osmancık)	Leaf	25,7

3.2.2. *In Vitro* Antioxidant Activity Studies

3.2.2.1. Determination of Total Phenolic Content

The approach of Singleton and Rossi was applied to the determination of TPC of samples (118). This is a colorimetric assay. It is depended on the set of a blue molybdenum-tungsten complex in the existence of phenolic using Folin-Ciocalteu Reagent (FCR). It is possible to define this complex with spectrophotometry at 765 nm.

The Chemicals and Reagents

- Na_2CO_3 20% in H_2O
- Folin-Ciocalteu Reagent (FCR)
- Gallic acid

The Experimental procedure

The concentration of all samples was 1 mg/ml. First, 20 µl of each sample was added then, 75 µl of Na_2CO_3 and 100 µl of FRC were done addition respectively. Then the mixture was incubated at 23°C in the dark for half an hour. The absorbance was measured at 690 nm at the spectrophotometry through using 96-well micro plate reader. Gallic acid was standard item which was dissolved in the water the curve of calibration was operated in the concentrations of 50, 100, 125, 250, 500 µg/ml. All assays were repeated in triplicate. The total phenolic content of the samplings was recorded as mg gallic acid equivalents (GAE) per 1 g dry extract.

3.2.2.2. Determination of Total Flavonoid Content

TFC of the samplings was figured according to the aluminium chloride colorimetric approach flourished by Woisky and Salatino (119). This method is determined the construction of acid decided compounds by $AlCl_3$ with the C-4 keto group and either C-3 or C-5 hydroxyl groups of flavones or flavonols. Moreover, $AlCl_3$ also creates acid unstable compounds with ortho-dihydroxyl groups in the A- or B- ring of flavonoids.

The Chemicals and Reagents

- Ethanol 75%
- Aluminum chloride 5% in H_2O
- Potassium acetate 1 M
- Quercetin

The Experimental procedure

All samples were diluted to a concentration of 1 mg/ml. 50 µl of samples were stirred with 150 µl of 75% ethanol, 10 µl of 5% aluminium chloride solution, 10 µl of 1M potassium acetate. The blend was waited at 23°C in the dark for half an hour. The absorbance was checked spectrophotometrically at 405 nm. Quercetin was reference, dissolved in methanol, and applied as the standard substance. The concentrations were 12.5, 25, 50, 75, 100, and 125 µg/ml and the calibration curve was plotted in those concentrations. All detections were executed in three times. TFC of the sampling was asserted as quercetin equivalents (QE) per 1 g dry extract.

3.2.2.3. Determination of DPPH Radical-Scavenging Activity

It is an approach, the scavenging effects of DPPH radical. DPPH is durable organic nitrogen radical of antioxidants is used. The expression of the scavenging activity of the samples against DPPH radical depends on the approach interpreted by Akter et al. (120, 121). This radical is reduced hydrazine when it interacts with hydrogen donors. Purple- colored DPPH radical is observed maximum absorption at 517 nm. The absorbance decreases while the color is changing from purple color to yellow color via adding antioxidant to the DPPH solution. This method is easy for evaluating the scavenging ability

of antioxidants. On the other hand, this method is not convenient for some compounds like carotenoids because their spectrum is measuring with DPPH at 517 nm and it is difficult to interpret. Moreover, most of them enter the slow reaction with DPPH because of the steric

hindrance. Thus, the method does not give an accurate assessment of the antioxidant capacity of the antioxidants entering the reaction with DPPH. In addition, DPPH is sensitive to light, air oxygen, humidity and pH. So, it is hard to obtain reproducible results the color of DPPH.

The reaction mechanism is as follows.

$DPPH^{\bullet}$ + Antioxidant-H → DPPH-H + $A^{\bullet}$

The Chemicals and Reagents

- DPPH 0.1 mM in MeOH
- BHT

The Experimental procedure

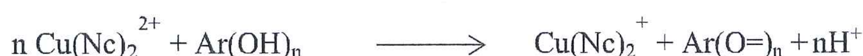
All samplings were diluted to a condensation of 1 mg/ml. 25 μl of cases were solely added to 200 μl 0.1 mM DPPH solution braced in MeOH just before use. The mixture was incubated at room temperature in the dark for 50 min. The absorbance was gauged at 540 nm. The control group was MeOH. BHT was the reference substance. The calculation of DPPH radical-scavenging activity of the control group, the samples and the reference substance were as below:

$$\text{DPPH radical-scavenging activity} = \frac{(ABS_{Control} - ABS_{Sample})}{ABS_{Control}} \times 100$$

"$ABS_{Control}$" is the absorbance value of the control group; "ABS_{Sample}" is the absorbance of the samples. The activity was asserted as EC_{50} values corresponding to the concentration which shows 50% activity.

3.2.2.4. Determination of Cupric Reduced Antioxidant Capacity (CUPRAC)

Cupric reduced antioxidant capacity activity was recorded regarding to the approach found by Apak et al. with some changings made by Celep et al. (121, 122). This experiment is established the formation of a colored complex by reducing Cu (II) -neocuproine complex to Cu (I) - neocuproine in the presence of antioxidant and absorbance is measured at 450 nm since maximum absorption gives in this absorbance.



$$n\,Cu(Nc)_2^{2+} + Ar(OH)_n \longrightarrow Cu(Nc)_2^{+} + Ar(O{=})_n + nH^+$$

The Chemicals and Reagents

- $CuSO_4$ 1 mM
- Neocuproine 7.5 mM in MeOH
- Ammonium acetate buffer 1000 mM, pH 7.0
- Ascorbic acid

The Experimental procedure

85 µl of every of 10 mM $CuSO_4$, 7.5 mM neocuproine, and 1 M ammonium acetate buffer (pH 7.0) solutions were stirred in a micro plate. Later, 51 µl of distilled water and 43 µl of samples were added properly. The mixture was rested at 23°C for 1 hour. After, the incubation period, absorbance was observed at 450 nm. Ascorbic acid was adopted as the standard substance, and a calibration curve was plotted in concentrations of 12.5, 25, 50, 100, 200 and 400 µg/ml. The outcomes were displayed as mg ascorbic acid equivalent (AAE) per g material.

3.2.2.5. Determination of Total Antioxidant Capacity (TOAC)

The TOACs of the samplings were gauged with regard to phosphomolybdenum approach found by Prieto et al. and small arrangements were made by Celep et al. (121, 123). The process is depended on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and consecutive composition of a green phosphate/ Mo (V) complex at acidic pH.

The Chemicals and Reagents

- The Reagent Solution:
    - Sodium phosphate monobasic 28 mM
    - Ammonium molybdate 4 mM
    - Sulfuric acid 600 mM
- Ascorbic acid

The Experimental procedure

All samplings were diluted to a concentration of 1 mg/ml. 300 µl of the reagent solution was stirred with 30 µl of warrantable diluted samples. The micro plate containing the mixture was incubated at 95°C for 90 min in a water bath. Then, the incubation period, the samplings were waited to cool until they reached to 23°C. Then, absorbance was recited at 690 nm. Ascorbic acid was the standard substance and a calibration curve was plotted in the concentrations of 12.5, 25, 50, 100, 200, 400 and 500 µg/ml. The outcomes were asserted as mg ascorbic acid equivalent (AAE) per g material.

3.2.2.6. Statistics

The procedures were applied in three times. The outcomes were displayed as mean ± standard deviation. Statistical contrasts were composed applying one-way analysis of variance (ANOVA) followed by Students–Newman–Keuls post hoc test for multiple contrasts. Also, Pearson correlation coefficients were computed. Statistically meaningful dissimilarity was illustrated.

4. RESULTS

4.1. Results of *In vitro* Activity Studies

4.1.1. Total Phenolic Content of *Olea europaea* L. Leaf Extracts

The total phenolic content of 80% methanolic extracts of *Olea europaea* leaves are given in Table 12, Figure 5. A standard calibration curve was plotted using Gallic acid in H_2O (50-1000 µg/ml) (y= 0.0013x - 0.0087; R^2= 0.9969).

Table 12: The total phenolic acid content *Olea europaea* leaves

	DOM[C]	SARA	US	SAROS
Total Phenolic Content[A]	77.85 ± 1.63[B,a]	85.41 ± 1.6[b]	80.15 ± 0.54[c]	90.15 ± 0.54[d]

[A] Total phenolic content was displayed as mg gallic acid equivalents (GAE) in 1 g dry extract.

[B] Outcomes were displayed as the mean of three times ± standard deviation (S.D.)

[a-d] Values with dissimilar letters within a row were significantly dissimilar ($p < 0.05$)

[C] The abbreviations for samples are DOM: Domat olive, SARA: Sarı Yaprak olive, US: Uslu olive, SAROS: Sarı Yaprak olive which was gathered Osmancık district.

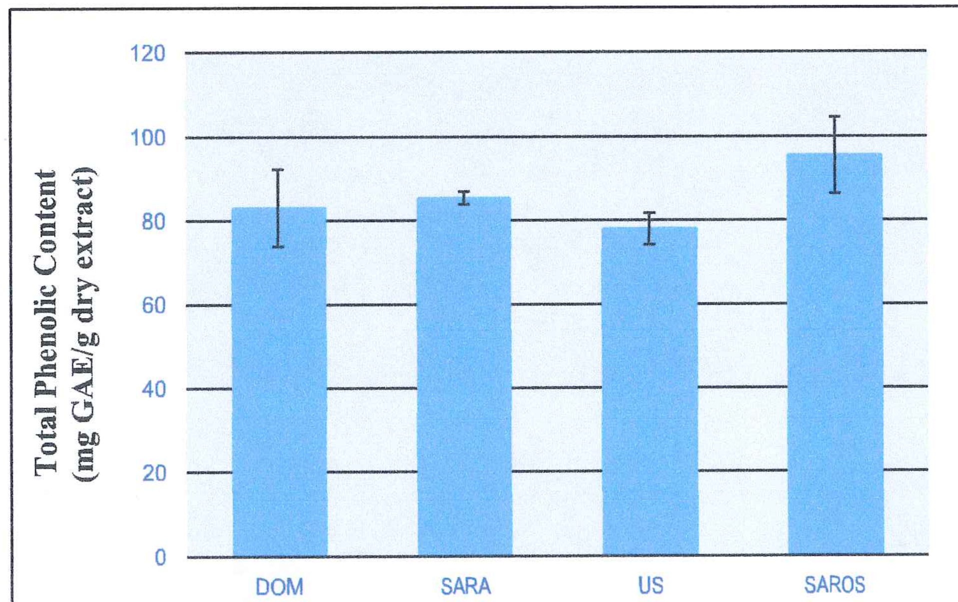


Figure 5: The total phenolic content of *Olea europaea* leaves. The results are explained as mg gallic acid equivalent per 1 g dry extract

4.1.2. Total Flavonoid Content of *Olea europaea* L. Leaf Extract

The TFC of 80% MeOH extracts of *Olea europaea* leaves is given in Table 13, Figure 6. A standard calibration curve was plotted using quercetin in MeOH (12.5-125 µg/ml) ($y= 0.0049x + 0.0539$; $R^2= 0.9993$).

Table 13: The total flavonoid content of *Olea europaea* leaves

	DOM[C]	SARA	US	SAROS
Total Flavonoid Content[A]	32.37 ± 0.72[B,a]	47.88 ± 1.29[b]	46.55 ± 0.15[b]	59.82 ± 2.88[c]

[A] Total flavonoid content was displayed as mg quercetin equivalents (QE) in 1 g dry extract.

[B] Outcomes were displayed as the mean of three times ± standard deviation (S.D.)

[a-c] Values with dissimilar letters within a row were significantly dissimilar ($p< 0.05$)

[C] The abbreviations for samples are DOM: Domat olive, SARA: Sarı Yaprak olive, US: Uslu olive, SAROS: Sarı Yaprak olive which was gathered Osmancık district

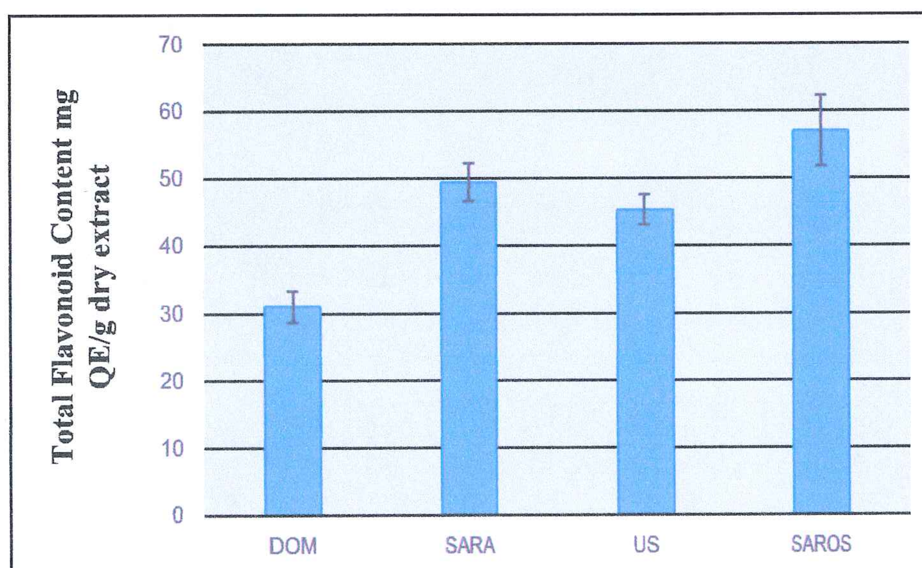


Figure 6: The total flavonoid content of *Olea europaea* leaves. The outcomes are shown as mg quercetin equivalent per 1 g dry extract.

4.1.3. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical-Scavenging Activity

DPPH radical-scavenging activity of 80% MeOH extracts of *Olea europaea* leaves are given in Table 14, Figure 7. The outcomes are expressed as "half maximal effective concentration" (EC_{50}). Although Sarı Yaprak olive leaves and Domat olive leaves displayed high DPPH radical-scavenging activity, the BHT applied as reference substance presented the best activity.

Table 14: DPPH radical-scavenging activity of *Olea europaea* leaves

	DOM[C]	SARA	US	SAROS	BHT[*]
DPPH radical-scavenging activity[A]	$566 \pm 0.004^{B,a}$	572 ± 0.015^{b}	594 ± 0.024^{c}	564 ± 0.002^{d}	500 ± 0.15^{e}

[A] DPPH radical scavenging activity was displayed as EC_{50} in µg/ml.

[B] Outcomes were displayed as the mean of three times ± standard deviation (S.D.)

[a-e] Values with dissimilar letters within a row were significantly dissimilar ($p < 0.05$)

[C] The abbreviations for samples are DOM: Domat olive, SARA: Sarı Yaprak olive, US: Uslu olive, SAROS: Sarı Yaprak olive which was gathered Osmancık district

[*] Butylated hydroxytoluene

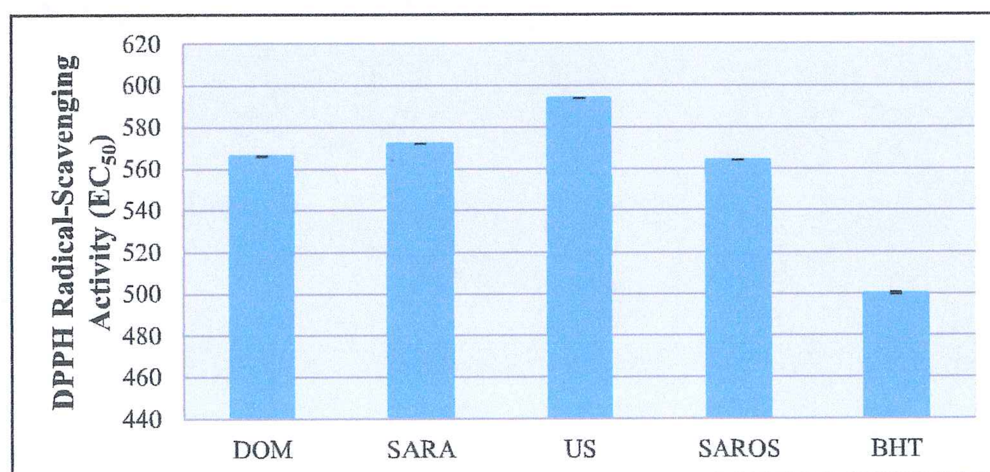


Figure 7: DPPH radical-scavenging activity of *Olea europaea* leaves. The outcomes are displayed as EC_{50} in µg/ml.

4.1.4. Cupric Reducing Antioxidant Capacity (CUPRAC)

CUPRACs of 80% methanolic extracts of *Olea europaea* leaves are given in Table 15, Figure 8. The outcomes were displayed as mg ascorbic acid equivalents (AAE) per g dry extract. A standard calibration curve was plotted using ascorbic acid in H_2O (12.5-400 μg/ml) ($y= 0.0076x + 0.189$; $R^2= 0.9997$).

Table 15: CUPRAC of of *Olea europaea* leaves

	DOM[C]	SARA	US	SAROS
Cupric reducing antioxidant capacity[A]	351.91 ± 29.12[B,a]	302.43 ± 3.07 [b]	391.65 ± 25.4[a]	347.7 ± 5.5[a]

[A] CUPRAC activity was displayed as mg ascorbic acid equivalents (AAE) in 1 g dry extract.

[B] Outcomes were displayed as the mean of three times ± standard deviation (S.D.)

[a-b] Values with dissimilar letters within a row were significantly dissimilar ($p < 0.05$)

[C] The abbreviations for samples are DOM: Domat olive, SARA: Sarı Yaprak olive, US: Uslu olive, SAROS: Sarı Yaprak olive which was gathered Osmancık district

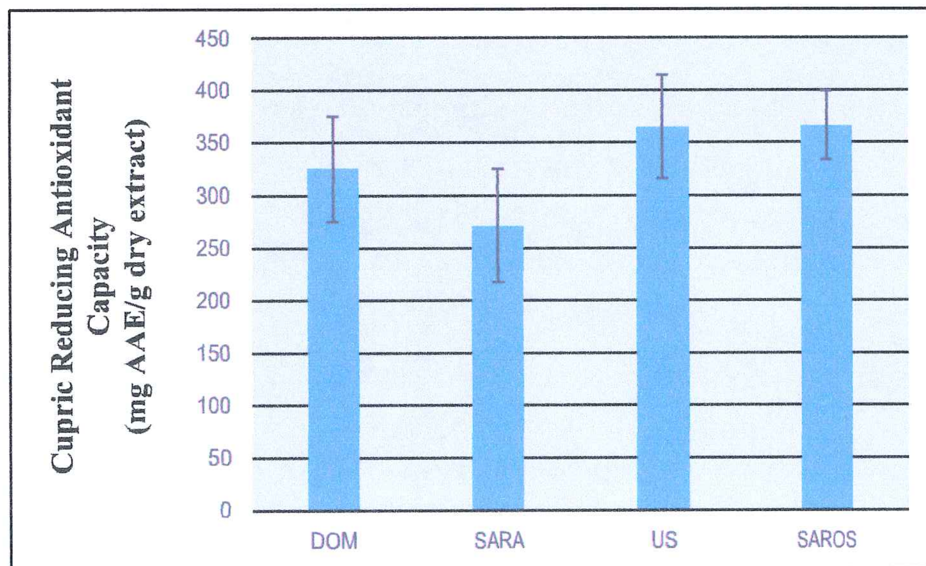


Figure 8: CUPRAC of *Olea europaea* leaves. The outcomes are displayed as mg ascorbic acid equivalent in 1 g dry extract.

4.1.5. Total Antioxidant Capacity (TOAC)

TOAC of 80% methanolic extracts of *Olea europaea* leaves is given in Table 16, Figure 9. The outcomes were displayed as mg ascorbic acid equivalents (AAE) per g dry extract. A standard calibration curve was plotted using ascorbic acid in H_2O (100-400 μg/ml), ($y = 0.0049x + 0.0539$; $R^2 = 0.9993$).

Table 16: TOAC of 80% methanolic extracts of *Olea europaea* leaves.

	DOM[C]	SARA	US	SAROS
Total antioxidant capacity[A]	149 ± 1.86[B,a]	158.47 ± 0.37[b]	139.8 ± 3.7[c]	186.1 ± 8.1[d]

[A] Total antioxidant activity was displayed as mg ascorbic acid equivalents (AAE) in 1 g dry extract.

[B] Outcomes were displayed as the mean of three times ± standard deviation (S.D.)

[a-d] Values with dissimilar letters within a row were significantly dissimilar ($p < 0.05$)

[C] The abbreviations for samples are DOM: Domat olive, SARA: Sarı Yaprak olive, US: Uslu olive, SAROS: Sarı Yaprak olive which was gathered Osmancık district

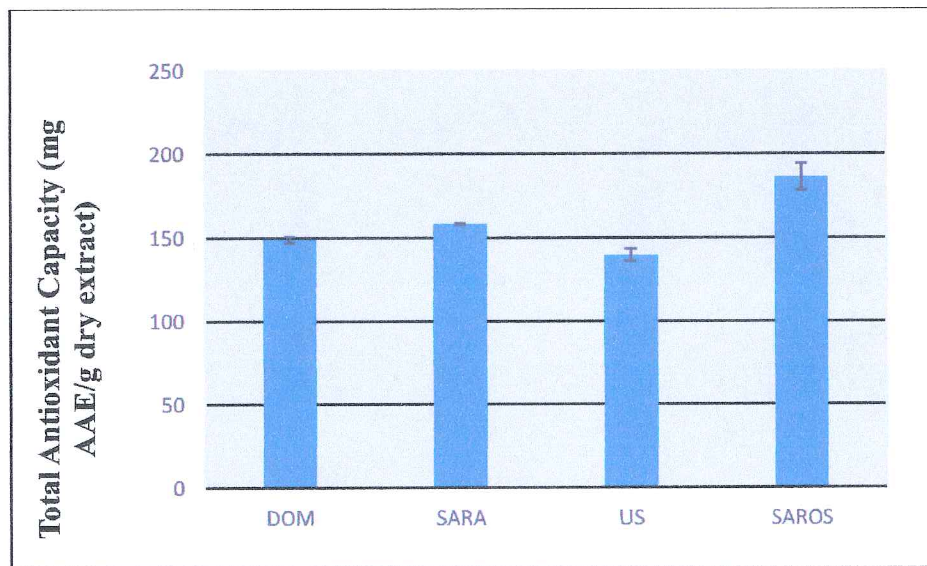


Figure 9: TOAC of 80% methanolic extract of *Olea europaea* leaves. The results are displayed as mg ascorbic acid equivalent per 1 g dry extract.

5. DISCUSSION

Every living organism, except anaerobic microorganism, uses oxygen in its molecular form for energy production. Oxygen is also a powerful oxidant, and it can cause secondary oxidation reaction in the regular physiological metabolism. Oxygen-derived radicals that are also named reactive oxygen species (ROS) are noxious to the living organism (115).

Oxidative stress has a crucial role in many diseases. Cardiovascular disease, cancer, diabetes, inflammatory diseases, ischemia/reperfusion injury, Alzheimer's disease, immune diseases, and eye diseases are examples of diseases that oxidative stress causes (124). Furthermore, the aging process and the generative diseases of aging have a direct connection with oxidative stress (125).

There are two defense systems in the human body against oxidative stress. These are the enzymatic and non-enzymatic endogenous antioxidant systems. Unfortunately, these defense systems might not be successful to scavenge the oxidative stress, because there are many sources of free radicals such as UV exposure, air pollutants, and tobacco smoke, etc. (126).

Due to these facts, the human body needs help to combat oxidative stress. Fruits and vegetables are one of the best resources for antioxidants. Moreover, Black et al. reported that fruits and vegetables constitute a good option to reduce the risk of several cancers like lung, colon, oral, stomach (127). On the other hand, they are not the only sources of plant-derived antioxidants.

Olea europaea is one of the characteristic plants of Turkey. It is mainly cultivated in the Aegean and Mediterranean coastlines, though it can be seen everywhere in Turkey. There are many cultivars of *Olea europaea* in Turkey. The chemical composition of *Olea europaea* mainly consists of oleuropein, hydroxytrosol, verbascoside, and luteolin- 7-glucoside. These compounds are generally responsible for the antioxidant, anticancer, hypoglycemic, antiatherosclerotic, cardioprotective effects of *Olea europaea* leaves (128). Orak et al. also studied the antioxidant capacities of aqueous extracts obtained from the leaves of 21 different cultivars (8).

The main objective of this study was to compare the antioxidant potential of Sarı Yaprak olive leaf with Domat olive leaf. Uslu olive leaf and Sarı Yaprak olive leaf, which were cultivated in Osmancık District, Turgutlu, and Manisa, were included in the study as well. Uslu olive leaves showed the lowest scavenging activity on DPPH radical scavenging assay. Uslu and Domat olive leaves were compared with Sarı Yaprak olive leaf extract. Sarı Yaprak olive leaves from Osmancık region extract were also studied to observe the

geographic effect on antioxidant properties.

In a similar study, five different *in vitro* methods were applied for a comprehensive evaluation of the antioxidant activity. 80% methanolic extract (OLE) demonstrated the highest antioxidant activity when compared to those of aqueous extract, 70% ethanol and 80% acetone (129). The authors suggested that such differences might occur due to differences in the polarities of the solvents.

DPPH scavenging activity method and CUPRAC test were applied to measure the overall free radical-scavenging antioxidant capacity of OLE in another study. The authors reported a high level of activity in both tests (130).

In the current study, TPC assay and TFC assay were applied to show the phytochemical profile of the samples. Among the extracts, Sarı Yaprak olive leaves from Osmancık District had higher total phenolic content than Sarı Yaprak olive leaves from Kemalpaşa, İzmir. These results suggest that variations in geography and altitude might lead to different total phytochemical contents. Besides, total flavonoid contents of Sarı Yaprak olive leaf (Osmancık) (59.82 ± 2.88) were shown to be higher than other extracts. Also, Uslu olive leaf (46.55 ± 0.15) showed a higher content than Domat olive leaf extract (32.37 ± 0.72), similar to the results reported by Orak et al. (8). They investigated DPPH scavenging activity of different varieties of olive leaf and Domat olive showed the highest DPPH scavenging activity (EC_{50}= 59 µg/mL) The cultivar variations of *Olea europaea* might cause differences in total phenolic assay. In addition, Sarı Yaprak olive (Osmancık) leaf might have different amount of oleuropein and hydroxytyrosol rather than Domat olive leaf and Sarı Yaprak olive leaf (Kemalpaşa) due to the climate and content of the soil.

Similarly, CUPRAC, a metal ion reducing test, is based on the reduction of Cu^{2+} to Cu^{+} occurs in the presence of antioxidant in the medium. Sarı Yaprak olive leaf extract (Osmancık) showed the highest cupric reducing power, but the cupric reducing power of Uslu olive leaf extract was closer to the data of Sarı Yaprak olive leaf extract (Osmancık).

Interestingly, Sarı Yaprak olive leaf extract (Kemalpaşa) had the lowest cupric reducing power. According to these results, the number of phenolic compounds may be different not only among cultivars but also among different locations of the same cultivar of *Olea europaea.*

The total antioxidant capacity of Sarı Yaprak (Osmancık) is the highest followed by Sarı Yaprak (Kemalpaşa). Domat showed the lowest total antioxidant capacity.

80% methanolic extract of Sarı Yaprak (Osmancık) displayed the highest TPC, TFC, and TOAC. Antioxidant capacity of *Olea europaea* may be derived from the level of total

phenolic content. Furthermore, the solvent of OLE, and collection time of olive leaf have an important role in antioxidant activity (31, 129, 131). For example, dried olive leaves were reported to show higher TPC than fresh leaves, but oleuropein content was found to be higher in fresh olive leaves (131). On the contrary, Domat olive leaf extract displayed higher TPC and DPPH scavenging activity (132). Moreover, the best total phenolic content was observed when olive leaves were collected in January (32).

Briefly, Sarı Yaprak olive leaf possessed a good antioxidant potential. It was compared with Domat olive leaf and Uslu olive leaf. Besides, the geographical conditions are important for antioxidant content. Both samples of Sarı Yaprak olive leaf extract belong to the Aegean region, raised in Kemalpaşa, İzmir and Turgutlu, Manisa. Both places are close to each other, but Sarı Yaprak (Osmancık) showed higher antioxidant content than Sarı Yaprak from Kemalpaşa. Geographical conditions seem to have a direct influence on the chemical composition of plants and change the antioxidant effect of samples.

This study is the first to analyze Sarı Yaprak olive leaf and this thesis will be a pioneer for future researchers to investigate the antioxidant mechanism of Sarı Yaprak olive leaf *in vivo*.

6. CONCLUSION

This study was designed to evaluate both the antioxidant potential and their phenolic content of 80% methanolic extract of Domat olive leaf and Sarı Yaprak olive leaf *in vitro*. The reason for this research was that there have been many reports regarding the traditional usage of olive leaves on health care. Moreover, Domat and Sarı Yaprak olive are endemic varieties of Akhisar, Turgutlu. Uslu and another Sarı Yaprak olive, which were cultivated in Osmancık, were studied to compare the amount of total flavonoid and geographical effect on Sarı Yaprak olive leaf, respectively.

The results displayed that these four samples of olive leaves had antioxidant activity potential. The *in vitro* tests showed samples had free radical scavenging activity with DPPH assay and the capacity to reduce metal ions (cupric reducing antioxidant capacity and TOAC).

At the same time, TPC and TFC of Domat olive leaf and Sarı Yaprak olive leaf were displayed. The fresh or dried olive leaf should affect the results.

The alteration in the antioxidant activity of *Olea europaea* L. depends on the variation, altitude, and climate. Sarı Yaprak Olive leaf from Osmancık District was shown to possess the highest antioxidant activity in the experiments. This activity not might only be related to only phenolic compounds. Also, non-phenolic compounds might increase the antioxidant effect. The chemical compounds of Sarı Yaprak olive leaf and Domat olive leaf should be analyzed. The amount of oleuropein, hydroxytrosol, verbascoside, and luteolin should be studied. Moreover, the antioxidant effect of Domat olive leaf and Sarı Yaprak olive leaf should be tested *in vivo* in further studies.

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