

Neuroprotective Effects of Natural Extracts from Olive (*Olea europaea*) Plant Growing in Libya

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التأثيرات الوقائية العصبية للمستخلصات الطبيعية من نبات الزيتون (*Olea europaea*) الذي ينمو في ليبيا

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Abstract:

There is an increasing link between oxidative stress, inflammation, and neuronal damage in neurodegenerative dis-eases. Therefore, there is the need for safer natural neuroprotective drugs to protect neurons from damage. This study aimed to investigate the neuroprotective effect of natural extracts derived from *Olea Europaea* plants from Libya. Leaves and fruits of the olives were extracted using ethanol and methanol solvents. The extracts were then analyzed for the presence of phytochemicals and antioxidants, and neuroprotective activity was determined using neuronal cell models. Findings from the study show that olive extract was rich in phenols, flavonoids, oleuropein, and hydroxytyrosol in methanol extracts of leaves. There was a high free radical scavenging capacity as shown by antioxidant activity tests. Moreover, neuroprotective studies show that the extracts could in-crease neuronal cell viability and reduce intracellular reactive oxygen species when cells are exposed to oxidative stress. Inflammation markers such as TNF- α and IL-6 were also inhibited by the extracts.

Keywords: *Olea Europaea*, Neuroprotection, Antioxidant Activity, Olive Extracts, Neurodegenerative Diseases.

المخلص:

يتزايد الارتباط بين الإجهاد التأكسدي والالتهاب وتلف الخلايا العصبية في الأمراض التنكسية العصبية. لذا، تبرز الحاجة إلى أدوية طبيعية أكثر أماناً لحماية الخلايا العصبية من التلف. هدفت هذه الدراسة إلى بحث التأثير الوقائي العصبي لمستخلصات طبيعية مشتقة من نباتات الزيتون الأوروبي (*Olea Europaea*) من ليبيا. تم استخلاص أوراق وثمار الزيتون باستخدام مذيبات الإيثانول والميثانول. ثم تم تحليل المستخلصات للكشف عن وجود المركبات الكيميائية النباتية ومضادات الأكسدة، وتم تحديد النشاط الوقائي العصبي باستخدام نماذج الخلايا العصبية. أظهرت نتائج الدراسة أن مستخلص الزيتون غني بالفينولات والفلافونويدات والأولوروبين والهيدروكسي تيروسول في مستخلصات الميثانول من الأوراق. كما أظهرت اختبارات النشاط المضاد للأكسدة قدرة عالية على التخلص من الجذور الحرة. علاوة على ذلك، تُشير الدراسات الوقائية العصبية إلى أن المستخلصات قادرة على زيادة حيوية الخلايا العصبية وتقليل أنواع الأكسجين التفاعلية داخل الخلايا عند تعرضها للإجهاد التأكسدي. كما تم تثبيط علامات الالتهاب مثل TNF- α و IL-6 بواسطة المستخلصات

الكلمات المفتاحية: *Olea Europaea*، الحماية العصبية، نشاط مضاد للأكسدة، مستخلصات الزيتون، الأمراض التنكسية العصبية.

1. Introduction

Neurological disorders including Alzheimer's, Parkinson's, and other cognitive disorders have become serious public health issues because of their increased incidence and profound impact on quality of life. Such conditions are marked by neuronal degradation, oxidative damage, neuroinflammation, mitochondrial dysfunction, and accumulation of proteins in the brain

tissue. The aging process and current living standards in the modern world have caused a fast development of neurological disorders in both developed and developing countries. The problem is exacerbated in the latter since they lack medical resources necessary to cope with the challenge. Existing therapies of neurodegenerative diseases emphasize treatment of symptoms only, but not the total prevention or treatment of the disease. Besides, some artificial medicines prescribed to neurological patients can lead to adverse effects in case of prolonged treatment. As a result, there is an urgent need for discovering new neuroprotective drugs from natural sources. (Wang et al., 2024)

Amongst plants used in medicine, one of those which has received a lot of attention from scientists is the olive tree (*Olea europaea*), due to its phytochemical constitution and extensive medicinal applications throughout history. This tree is extensively found in Mediterranean countries, where it flourishes well in such a region with favorable conditions for growing olive trees. All different parts of this tree including leaves, fruits, seeds, and olive oil contain several bioactive phytochemicals such as polyphenols, flavonoids, secoiridoids, triterpenes, and phenolic acids. Bioactive components, like oleuropein, hydroxytyrosol, tyrosol, and oleocanthal, are proven to be effective as antioxidants, anti-inflammatories, antimicrobials, cardioprotectives, and antitumor agents. Furthermore, recent research has indicated that they can exert neuroprotective effects as well. (Elhrech et al., 2024)

Neuronal damage and neurodegeneration have been reported to be caused due to various pathological processes, but oxidative stress seems to be one of the major factors implicated in this regard. Increased levels of reactive oxygen species (ROS) can cause damage to neuronal lipids, proteins, and even the genetic material, thus causing neuronal dysfunction that ultimately results in cell death. Natural plant products that act as antioxidants have been proven to be efficacious treatment options as they are capable of quenching free radicals, and hence offer protection to neurons against oxidative damage. Phenolics from olive sources have been found to demonstrate potent antioxidative properties owing to their ability to scavenge ROS, induce endogenous antioxidative enzymes, and prevent lipid peroxidation. Anti-inflammatory property is another notable biological activity of olive extracts. (Marrero et al., 2024)

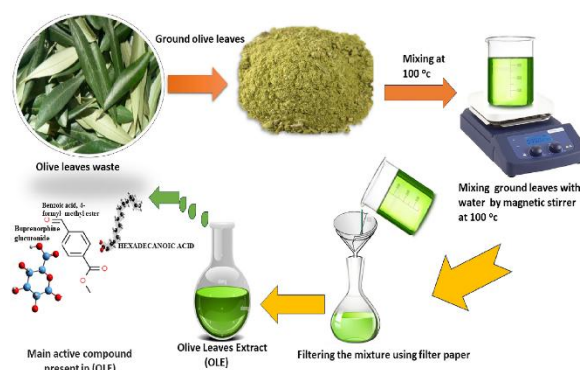


Figure 1. The water extract process of olive leaves (Elhady et al., 2023)

Libyan olive cultivation and the extraction of oil from this tree constitute part of the traditions observed in other North African nations. The growth environment of Libyan olive plants varies significantly, as these trees grow in arid regions and soils rich in minerals that may impact their chemical content. Various types of Libyan olive plants can be harvested and utilized for nutrition and medicinal benefits by local residents. Despite the significance of olives in the economy and agriculture of Libya, research into the pharmacological and neuroprotective qualities of this plant species has not been conducted extensively. The majority of existing research on neuroprotection by olives refers specifically to European or other Mediterranean varieties of the olive tree, while there is a lack of research related to the biological activity of

Libyan olive cultivars. This constitutes an important gap in knowledge, especially with regard to the discovery of new neuroprotective compounds. (Lagili et al., 2023)

In addition, another critical issue lies in the rise of the number of neurodegenerative diseases across the globe but with the lack of adequate and affordable neuroprotective medicines. Most of the synthetically developed neuroprotective drugs tend to be less effective or produce toxicity and adverse reactions. Hence, there exists a dire necessity for exploring the other therapeutic options from natural products, which are non-toxic and exhibit multiple biological activities. The olive extracts can be considered as one such option as they not only have antioxidant activity but can also reduce inflammation and apoptosis. Unfortunately, there are not enough research studies that have investigated the neuroprotective potential of olives, grown in the Libyan environment. Moreover, little information is available about the link between the phytochemistry of Libyan olive extracts and their probable neuroprotective mechanisms. (Angeloni et al., 2023)

Previous scientific evidence has shown that olive leaf extract and olive oil extract might help enhance cognitive ability and provide protection against neurons degeneration within animal models of neurodegenerative conditions. Oleuropein and hydroxytyrosol have been found to have inhibitory effects on the accumulation of amyloid-beta peptides, inhibit oxidative stress and inflammatory mechanisms linked to Alzheimer's disease. Some previous researches have pointed out that polyphenols from olive can be able to enhance mitochondrial function and suppress neuronal cell death. However, most previous studies concentrated on isolated compounds or commercially available products made of olives instead of studying crude extracts derived from locally cultivated olive plants. Besides, previous studies have been done elsewhere than in North Africa and hence there is an unexplored territory regarding the biological effects of olive varieties found in Libya. Stressful environmental conditions prevailing in Libya might influence phytochemical synthesis in these plants. (Gonçalves et al., 2024)

This current investigation attempts to bridge some of the above gaps through examining the neuroprotective properties of natural extracts obtained from Libyan olives. The uniqueness of this work is based on studying the neuroprotective properties of various types of olives produced in Libya. In addition, this investigation attempts to differentiate itself from previous works which have focused more on studying the effects of either olive oil or specific chemical compounds in terms of neuroprotection. This investigation focuses on evaluating the neuroprotective properties of natural extracts from various parts of olive plants such as the leaves and fruits, thereby identifying their biological active compounds and assessing their effects on neurons. This research will also attempt to investigate the mechanism behind the neuroprotective properties of Libyan olives. One such mechanism might include antioxidant and anti-inflammatory properties. This research may be able to add useful scientific knowledge about the potential therapeutic properties of medicinal plants in Libya. (Bolgaseem et al., 2024)

Olive leaves and fruits aren't the only valuable things from Libya's olive trees; extra virgin olive oil is huge too. It's made mostly in the western and northern parts of the country and plays a big role in both meals and money matters there. Studies show this oil's got tons of monounsaturated fatty acids, vitamin E, and phenolic compounds, making it a powerful antioxidant and anti-inflammatory agent. Because of these healthy bits, it guards neuronal cells from oxidative stress, which helps protect against neurodegenerative diseases. (Elhafi et al., 2024)

Research in western Libya shows that how long olive oil is stored and the conditions it's kept in really affect its chemistry and antioxidants. This means its health benefits can change over time. Also, reviews show that in Libya, eating olive oil is tied to lower chances of heart and nerve problems. It does this by fighting free radicals and reducing swelling. So, Libyan olive

oil isn't just good for nutrition; it could also team up with other olive parts to protect against nerve cell damage. (Arunachalam et al., 2023; Lamma, AVVS, & Alhadad, 2019)

The importance of this study is not only confined to its academic value but could serve as a foundation for the development of safer, cost-effective, and local neuroprotective agents. Identification of effective neuroprotectants in the olives of Libya would facilitate advances in pharmacology as well as functional food manufacturing and improve public health. Furthermore, this study would be important in increasing the economic value of olive plantations in Libya due to the introduction of medical uses of olives (Gadhavé et al., 2024).

Thus, the major objective of this research work is to study the neuroprotective activity of natural extracts derived from olive plants (*Olea europaea*) grown in Libya. In particular, the current research is intended to focus on the phytochemistry of olive extracts, assessment of the antioxidant and neuroprotective activity of the extracts, and possible mechanisms for neuroprotection. The above-stated objectives are expected to contribute to validating the use of Libyan olive plants as potent herbal medicines against neurodegeneration.

2. Literature Review

2.1. Neurodegenerative Diseases and Neuroprotection

Neurodegenerative diseases are those diseases where there is a progressive decline in both structure and function of neurons of the brain. Some examples of neurodegenerative diseases are Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Neurodegenerative diseases are usually age-related diseases, and they are becoming more and more common throughout the world. Mechanisms that contribute to neurodegenerative diseases include oxidative stress, neuroinflammation, mitochondrial dysfunction, protein aggregation, excitotoxicity, and neuronal apoptosis. Reactive oxygen species can cause cell damage by oxidizing lipids, proteins, and nucleic acids resulting in neuron death. (Gadhavé et al., 2024)

Neuroprotection entails the use of techniques or substances that can prevent, retard, or minimize nerve cell injury and degeneration. Neuroprotectants can achieve their functions by using a number of approaches, such as acting as antioxidants, reducing inflammation, blocking apoptosis, affecting neurotransmitter levels, and guarding mitochondria against dysfunction. Over the past decade, there has been much research into the role played by plant products in providing neuroprotection owing to their biological functions as well as lower toxicity when compared to synthetic drugs. The importance of antioxidants obtained from medicinal plants is due to their ability to counteract free radicals. (Shoaib et al., 2023)

2.2. Olive Plant (*Olea europaea*)

The olive tree, whose scientific name is *Olea europaea*, is one of the oldest crops cultivated in the Mediterranean area. It belongs to the Oleaceae family and is mainly cultivated for its fruit and oil. The olive plant is of great economic value in Mediterranean and North African regions, including Libya. Various parts of the olive plant, including leaves, fruit, seeds, bark, and olive oil, have been used in traditional medicine to treat diseases, including hypertension, diabetes, inflammation, microorganisms, and heart disease. (Elhrech et al., 2024; Emhmd et al., 2022)

Phenols, flavonoids, triterpenes, and secoiridoids are examples of the many biologically active compounds present in the olive plant. Phytochemicals that have been isolated from olive extracts include oleuropein, hydroxytyrosol, tyrosol, oleocanthal, luteolin, and apigenin. The biological activities associated with these compounds include antioxidative and anti-inflammatory properties. Factors such as the climate, soil composition, irrigation techniques, and other factors might affect the phytochemical composition of the olive plant. Olive plants grown in Libya face environmental stresses due to hot and dry climatic conditions. (Ali et al., 2024)

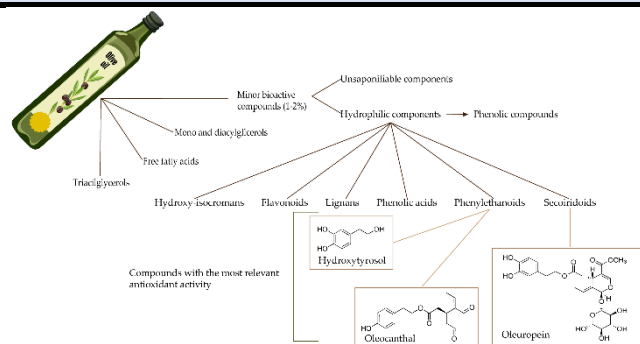


Figure 2. Graphical representation of olive oil composition (Gonçalves et al., 2024)

2.3. Phytochemical Constituents of Olive Extracts

Phytochemicals are bioactive compounds present in plants that play an essential role in giving medicinal properties to plants. The extracts of olives contain many polyphenols that are regarded as some of the most potent antioxidants found in nature. Oleuropein is a major phenolic compound present in olives and their leaves. The compound has received numerous scientific studies due to its antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic, cardioprotective, and neuroprotective properties. Hydroxytyrosol, which is another major phenol found in olives, acts as a powerful scavenger of free radicals. (Kumar et al., 2023)

Phytochemicals such as flavones like luteolin and apigenin contained in olive extracts are also active antioxidants and anti-inflammatories. Besides, triterpenes such as oleanolic acid and maslinic acid also protect from inflammations and cellular stress. These phytochemicals can act collectively to increase the bioactivity of the olive extracts. These phytochemicals are not present in constant concentrations in olive plants; rather, they vary according to the variety, maturity stage, extraction techniques, and environmental conditions of the olives. Thus, the study of the phytochemical content of the Libyan olives is significant to determine their medicinal value. (Romero-Márquez et al., 2023)

2.4. Oxidative Stress and Antioxidant Mechanisms

The occurrence of excessive oxidative stress arises when there is a dis-balance between reactive oxygen species production and the body's antioxidant system. The neurons are highly susceptible to oxidative damage due to their high oxygen metabolism, lipid-rich content, and relatively low antioxidant capabilities. Oxidative stress is a key factor in the development of several neurological conditions through mechanisms such as lipid peroxidation, mitochondrial dysfunction, DNA damage, and apoptosis induction. (Jomova et al., 2023)

Antioxidants are substances that have the capacity to inhibit or counteract the formation of free radicals or reactive oxygen species. Natural antioxidants found in medicinal plants can help improve the antioxidant protective system in the body and prevent oxidation damage. The antioxidants in olive phenolic compounds act through different processes, including free radical scavenging, metal ion chelation, inhibition of lipid peroxidation, and stimulation of endogenous antioxidants like superoxide dismutase, catalase, and glutathione peroxidase. The neuroprotection provided by olive extracts is attributed to their antioxidant properties. (Chaudhary et al., 2023)

2.5. Neuroinflammation and Anti-inflammatory Activity

The role of neuroinflammation cannot be overlooked when discussing the pathogenesis of neurodegenerative disorders. Microglia and astrocyte cells activated by continuous stimulation generate excess levels of inflammatory products like cytokines, nitric oxide, and prostaglandins. Neuroinflammation can contribute to neuron dysfunction and promote neurodegeneration. This implies that targeting inflammation could be an effective neuroprotective strategy. (Adamu et al., 2024)

Various studies have shown the presence of anti-inflammatory activity in oleaginous compounds. For instance, oleocanthal is believed to display similar anti-inflammatory activities to nonsteroidal anti-inflammatory drugs since it inhibits the cyclooxygenase enzymes. In addition, olive polyphenols can help reduce the levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6. As a result of their antioxidant and anti-inflammatory capabilities, olive extracts may be considered neuroprotective agents. (Karagiannis et al., 2024)

3. Methodology

Research Design

Experimental laboratory-based research design was used in this research in order to understand the neuroprotective properties of extracts extracted from the olive tree plant. The research incorporated phytochemical studies and antioxidant studies along with neuroprotective activity tests in order to assess the biological potentials of olive extracts. In this research, the bioactive components contained in the leaves and fruits of olive trees were assessed, along with the impacts that they could have on neurodegenerative diseases. Quantitative techniques were used for assessment of antioxidants, phytochemicals, and neuroprotective activities. (Papadopoulou et al., 2024)

Study Area and Sample Collection

Samples of olive plants were harvested from selected areas of olive cultivation in Libya during the harvesting period. Healthy leaves and fruits from olive plants were collected from local sources. The samples were identified botanically by a plant taxonomist to ascertain the exact identity of the plants. The plant samples were carried to the laboratory in sterile bags and washed thoroughly with distilled water to eliminate dust particles and other contaminants. Air drying was done under shade to maintain heat-sensitive phytochemicals. The dried plant samples were grinded to powder form with the aid of a laboratory grinder and kept in tightly closed containers for extraction.

Preparation of Olive Extracts

Natural extracts were obtained through solvent extractions from powdered leaves and fruits of olives. Approximately 100 g of powdered plant material was extracted using ethanol or methanol solvents at room temperature for 72 hours with periodic stirring for efficient extraction of bioactive molecules. Subsequently, the mixture was subjected to filtration with Whatman filter paper to get liquid extract free of plant particles. Finally, the filtrate was concentrated under vacuum with the help of a rotary evaporator to obtain crude extracts. The dried extracts obtained from plants were stored in the refrigerator at 4 °C until use in biological and phytochemical studies. Different solvents were selected during extraction process because the polarity of solvent affects the recovery of phenolic compounds and flavonoids from plant materials. Extraction yield was estimated by the ratio of dried extract mass to the initial mass of dried plant material.

Phytochemical Screening

Phytochemical analysis was performed as a preliminary study in order to know about the major bioactive components in the olive extracts. Qualitative standard procedures were carried out to test for the presence of phenols, flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides, and steroids. The total phenol content was estimated by Folin-Ciocalteu method, whereas total flavonoids by aluminum chloride colorimetric assay. Important components like oleuropein, hydroxytyrosol and other phytochemicals present in the olive extracts were quantified using high-performance liquid chromatography (HPLC) technique.

Antioxidant Activity Assay

The antioxidant effect of olive extracts was evaluated based on standardized in vitro antioxidant tests. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test was performed for

determining the free radical scavenging ability of the extracts. During the DPPH test, various concentrations of olive extracts were combined with DPPH solution, and decrease in absorbance was measured by spectrophotometry at wavelength 517 nm. Other antioxidant tests like FRAP (Ferric Reducing Antioxidant Power) and Hydrogen Peroxide Scavenging Activity were also conducted for assessing the antioxidant effect of the extracts in different ways.

Evaluation of Neuroprotective Activity

Assessment of the neuroprotective effect of olive extracts involved the use of neuronal cells placed in an environment that causes oxidative stress. This was done by culturing human neuroblastoma cells or other suitable neuronal cell lines under standard cell culture conditions. Neuronal oxidative stress was induced through exposure of cells to hydrogen peroxide or any other neurotoxin that would cause damage to neurons like in neurodegenerative diseases. Olive extracts were then administered at different concentrations on either side of neuronal oxidative stress induction, before and after, respectively. Viability of cells was assessed using the MTT assay based on mitochondrial metabolic activity. The effect of the extract was assessed by comparing cells under treatment and without treatment. Changes in neuronal morphology were studied using microscopy while intra-cellular reactive oxygen species generation was studied as well.

Anti-inflammatory Activity Assessment

In order to elucidate the principles of neuroprotection, the anti-inflammatory effect of the extracts of olive fruit was also examined. Levels of inflammatory markers like TNF- α , IL-6, and nitric oxide were analyzed by means of ELISA methods. Anti-inflammatory potential of the olive fruit extracts in the neurons was viewed as a vital parameter for their efficacy.

Data Analysis

All experiments were carried out in triplicate to ensure reliability of results. Data obtained was expressed as mean \pm standard deviation. Statistical analysis was done with the help of Statistical Package for Social Sciences (SPSS) software or GraphPad Prism software. Differences in experimental groups were tested by ANOVA test and followed by an appropriate post-hoc test. Statistical significance was determined by a probability value of $p < 0.05$. Data was displayed in form of tables, graphs and charts for better interpretation of neuroprotective effect of Libyan olive extracts.

Ethical Considerations

In this study, all laboratory practices regarding safety and ethics have been adopted as per the established standards of biology studies. Plants have been collected after securing the proper permissions from the relevant agricultural authorities whenever required. Culturing of the cells has been done in accordance with the biosafety protocols.

4. Result and Discussion

4.1. Result

The current part includes the research results of the phytochemical, antioxidant, anti-inflammatory, and neuroprotective activities carried out on natural extracts extracted from the plants of Libyan olive (*Olea europaea*). In order to show how effective is the extraction process, what are the phytochemical properties of the olive extracts, as well as their antioxidant, anti-inflammatory, and neuroprotective activities, all experimental data has been collected and summarized into tables. The provided information will prove that there is scientific significance in using Libyan olive extracts due to the beneficial effect these extracts have on neuronal cells protection against neurodegenerative diseases.

Table 1. Extraction Yield of Olive Plant Extracts

Plant Part	Solvent Used	Dry Weight of Plant Material (g)	Extract Yield (g)	Extraction Yield (%)
Olive Leaves	Ethanol	100	18.5	18.5
Olive Leaves	Methanol	100	21.3	21.3
Olive Fruits	Ethanol	100	14.7	14.7
Olive Fruits	Methanol	100	17.1	17.1

In Table 1 is illustrated the extraction yield from olive leaves and olive fruits using ethanol and methanol solvents. It can be seen that extraction using methanol solvent generated greater extraction yield in comparison to extraction using ethanol solvent. Of all the samples examined, it was found that methanol extract of olive leaf had the greatest extraction yield, which was equal to 21.3%, whereas ethanol extract of olive fruits had the lowest extraction yield, which was equal to 14.7%. It was possible to conclude that methanol solvent was superior for extraction of bioactive molecules due to greater polarity. Moreover, it can be noted that extracts from olive leaves had better extraction yields than extracts from olive fruits.

Table 2. Preliminary Phytochemical Screening of Olive Extracts

Phytochemical Constituents	Leaf Extract	Fruit Extract
Phenols	+++	++
Flavonoids	+++	++
Tannins	++	++
Saponins	+	+
Alkaloids	+	-
Terpenoids	++	++
Glycosides	++	+
Steroids	+	+

(+) Present in low amount (+++) Highly present

(++) Moderately present (-) Absen

The results of preliminary phytochemical testing of olive leaf and fruit extracts are shown in Table 2. It was found that there were a number of bioactive substances in those samples such as phenols, flavonoids, tannins, terpenoids, glycosides, saponins, and steroids. Leaf extracts showed higher amounts of phenols and flavonoids than fruit ones, which can confirm that they have a more rich phytochemical content. Alkaloids were found only in leaf extracts while the tannin and terpenoid contents of both extracts can be described as moderate. It should be noted that a high amount of phenol and flavonoid is an indicator of great biological activity due to their antioxidant and neuroprotective properties.

Table 3. Total Phenolic and Flavonoid Content of Olive Extracts

Extract Sample	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)
Olive Leaf Ethanol Extract	185.4 ± 2.6	96.8 ± 1.9
Olive Leaf Methanol Extract	201.7 ± 3.1	104.5 ± 2.3
Olive Fruit Ethanol Extract	142.3 ± 2.1	73.6 ± 1.5
Olive Fruit Methanol Extract	156.9 ± 2.8	81.4 ± 1.7

Results. Table 3 shows the total phenol and flavonoid content of different olive extracts. The methanolic leaf extract contained the highest total phenolic (201.7 mg GAE/g extract) and flavonoid (104.5 mg QE/g extract) content, while fruit extracts showed a relatively low concentration. From the data obtained, it can be inferred that leaves of the olive have higher phytochemical antioxidants compared to olive fruits. In light of the significant contribution of phenolic compounds and flavonoids in antioxidant potential, the high amount of these phytochemicals observed in leaf extracts might facilitate their function in scavenging free radicals.

Table 4. HPLC Analysis of Major Olive Phytochemicals

Phytochemical Compound	Leaf Extract (mg/g)	Fruit Extract (mg/g)
Oleuropein	85.6 ± 1.4	42.8 ± 1.1
Hydroxytyrosol	24.5 ± 0.8	18.7 ± 0.6
Tyrosol	11.2 ± 0.5	9.4 ± 0.4
Oleocanthal	7.6 ± 0.3	5.8 ± 0.2
Luteolin	13.1 ± 0.4	8.9 ± 0.3
Apigenin	9.8 ± 0.3	6.5 ± 0.2

Table 4 below shows the results of HPLC analysis of the major phytochemicals found in olive extracts. Oleuropein is found to be the most prevalent compound in these extracts, especially the olive leaf extract, followed by hydroxytyrosol, luteolin, apigenin, tyrosol, and oleocanthal. It can be seen that the presence of more amounts of oleuropein in leaf extracts makes olive leaves a potential source of neuroprotective polyphenols. Neuroprotection is a term used for biological compounds which have antioxidant and anti-inflammatory properties. Thus, neuroprotective polyphenols work together synergistically to give neuroprotective activity.

Table 5. Antioxidant Activity of Olive Extracts

Extract Sample	DPPH Radical Scavenging Activity (%)	FRAP Value (µmol Fe ²⁺ /g)	H ₂ O ₂ Scavenging Activity (%)
Olive Leaf Ethanol Extract	78.4 ± 2.2	685.3 ± 12.4	72.6 ± 1.9
Olive Leaf Methanol Extract	84.7 ± 2.5	742.8 ± 14.1	79.3 ± 2.1
Olive Fruit Ethanol Extract	65.2 ± 1.8	574.6 ± 10.8	61.7 ± 1.6
Olive Fruit Methanol Extract	70.9 ± 2.0	618.2 ± 11.5	67.5 ± 1.8

Results. Table 5 highlights the antioxidant activities of the various olive extracts analyzed through DPPH, FRAP, and hydrogen peroxide scavenging methods. The methanolic leaf extract was found to have the best antioxidant activity based on all three methods by having the highest scavenging activity towards the DPPH (84.7%), the highest FRAP value (742.8 µmol Fe²⁺/g), and highest hydrogen peroxide scavenging activity (79.3%). These findings suggest that the olive leaves contain great abilities of neutralizing free radicals. These antioxidants are likely to be due to the presence of high amounts of phenolic compounds and flavonoids. Oxidative stress is among the major factors leading to neuronal degeneration.

Table 6. Effect of Olive Extracts on Neuronal Cell Viability (MTT Assay)

Experimental Group	Cell Viability (%)
Control Group	100.0 ± 0.0
H ₂ O ₂ -Treated Group	46.3 ± 2.1
H ₂ O ₂ + Leaf Ethanol Extract	71.8 ± 2.4
H ₂ O ₂ + Leaf Methanol Extract	81.5 ± 2.7
H ₂ O ₂ + Fruit Ethanol Extract	64.7 ± 2.0
H ₂ O ₂ + Fruit Methanol Extract	69.2 ± 2.3

Results. The results shown in Table 6 depict the influence of olives extracts on viability of neuronal cells in oxidative stress state created with the help of hydrogen peroxide. Hydrogen peroxide led to significant decrease in cell viability, decreasing it up to 46.3% due to oxidative neuronal cell damage. In spite of the above-mentioned decrease, olives extract showed high efficacy in increasing cell viability, particularly methanolic leaf extract improved cell viability up to 81.5%. The results presented above clearly evidence the effectiveness of neuroprotective properties of olives extracts in terms of their ability to protect from oxidative stress.

Table 7. Intracellular Reactive Oxygen Species (ROS) Levels in Neuronal Cells

Experimental Group	ROS Level (% of Control)
Control Group	100.0 ± 0.0

H ₂ O ₂ -Treated Group	228.6 ± 5.4
H ₂ O ₂ + Leaf Ethanol Extract	151.4 ± 4.2
H ₂ O ₂ + Leaf Methanol Extract	132.8 ± 3.9
H ₂ O ₂ + Fruit Ethanol Extract	169.5 ± 4.7
H ₂ O ₂ + Fruit Methanol Extract	158.3 ± 4.4

As shown in Table 7, oxidative stress-induced intracellular production of reactive oxygen species (ROS) was significantly elevated by hydrogen peroxide treatment, which reached 228.6% above the control level, indicating that severe oxidative stress occurred in neuronal cells. The application of olive extracts resulted in a significant reduction of ROS levels, with the methanolic extract of leaves producing the highest suppression effect. This indicates that the extracts of olives successfully counteract intracellular free radicals, thereby lowering oxidative stress. This is especially noteworthy since excess production of ROS leads to neuronal damage and degeneration.

Table 8. Effect of Olive Extracts on Antioxidant Enzyme Activity

Experimental Group	Superoxide Dismutase (U/mg protein)	Catalase (U/mg protein)	Glutathione Peroxidase (U/mg protein)
Control Group	18.5 ± 0.7	26.3 ± 1.1	14.8 ± 0.5
H ₂ O ₂ -Treated Group	9.2 ± 0.4	12.7 ± 0.6	6.9 ± 0.3
H ₂ O ₂ + Leaf Methanol Extract	16.8 ± 0.6	23.5 ± 0.9	13.1 ± 0.4
H ₂ O ₂ + Fruit Methanol Extract	14.9 ± 0.5	20.6 ± 0.8	11.7 ± 0.4

The effects of olive extracts on the activities of endogenous antioxidant enzymes are shown in Table 8. These enzymes include superoxide dismutase, catalase, and glutathione peroxidase. Hydrogen peroxide-induced oxidative stress led to a reduction in the activity of these antioxidant enzymes. Nevertheless, the use of olive extracts helped restore the activities of these enzymes. In particular, cells exposed to methanolic leaf extract exhibited a restoration of endogenous antioxidant enzymes. Thus, it can be concluded that olive phytochemicals can exert dual mechanisms of action: they can function as antioxidants and also stimulate endogenous antioxidants.

Table 9. Anti-inflammatory Activity of Olive Extracts

Experimental Group	TNF- α (pg/mL)	IL-6 (pg/mL)	Nitric Oxide (μ M)
Control Group	18.4 ± 0.8	12.6 ± 0.5	8.3 ± 0.3
H ₂ O ₂ -Treated Group	52.7 ± 2.1	44.8 ± 1.8	26.5 ± 1.1
H ₂ O ₂ + Leaf Methanol Extract	24.6 ± 1.0	18.7 ± 0.7	11.9 ± 0.5
H ₂ O ₂ + Fruit Methanol Extract	29.3 ± 1.2	22.5 ± 0.9	14.7 ± 0.6

Anti-inflammatory effect of olive extracts is shown through the analysis of inflammatory mediators including TNF- α , IL-6, and nitric oxide as shown in Table 9. Treatment with hydrogen peroxide resulted in increased concentration of inflammatory mediators suggesting that inflammation was stimulated in neurons. Olive extracts caused significant reduction in the synthesis of inflammatory mediators with methanolic extract of leaves having the greatest anti-inflammatory activity. It can be seen that olive extracts have powerful anti-inflammatory activity. In view of the fact that chronic neuroinflammation has been found to be highly correlated with the development of Alzheimer's and Parkinson's disease, inhibition of inflammatory response can delay neurodegeneration.

Table 10. Statistical Analysis of Experimental Results

Parameter	F-value	p-value	Significance
Total Phenolic Content	18.72	<0.05	Significant

DPPH Activity	21.45	<0.05	Significant
Cell Viability	26.13	<0.05	Significant
ROS Production	19.88	<0.05	Significant
TNF- α Level	17.56	<0.05	Significant

Analysis of the experimental results based on one-way ANOVA is given in Table 10. Statistical differences ($p < 0.05$) have been found in all analyzed parameters – phenolics content, antioxidant capacity, cell viability, ROS levels, and TNF- α levels between the experimental groups. This proves that the obtained biological activities of the olive extracts were not accidental but statistically significant. The statistics obtained validate the experiment by confirming the fact that the extracts have a certain antioxidant, anti-inflammatory, and neuroprotective effect. Thus, the research results prove the hypothesis according to which natural substances found in the plants of Libyan olives have good prospects in the therapy of neurodegenerative diseases.

4.2. Discussion

4.2.1. Extraction Yield and Phytochemical Composition

In this study, it was found out that there are large quantities of phytochemicals that have medicinal value in the Libyan-grown olive fruits and leaves. It was observed that methanol gave higher yields when compared to ethanol in both olive fruits and leaves. The results indicated that methanol had better solvency properties for polar phytochemicals like phenols and flavonoids. From the phytochemical screening, some important secondary metabolites were identified to include phenols, flavonoids, tannins, terpenoids, and glycosides. In addition, it can be concluded from this study that there is a greater quantity of phenolic compounds in olive leaves since these showed a greater biological activity. Moreover, the environmental conditions of Libya, which include high temperatures, drought conditions, and nutrient-rich soils, induce the production of secondary metabolites by the olive plant. This phenomenon has been seen in the case of Mediterranean olive varieties under stressful environmental conditions, which led to enhanced phenolic biosynthesis. Thus, Libyan olive plants are excellent sources of potent phytochemicals. (Jarwan et al., 2023)

4.2.2. Antioxidant Activity of Olive Extracts

From the antioxidant activity measurements, it is clear that the extracts from olive have potent free radical scavenging and reducing power, especially the methanol extract of the leaves. The extremely high value of DPPH scavenging activity and FRAP shows that olive extracts donate electrons and neutralize reactive oxygen species very well. It is well known that oxidative stress is a major pathophysiological cause of neurodegenerative disease since ROS damages lipids, proteins, and DNA in the neurons. From the current findings, it can be inferred that the potent antioxidant activity in the olive extracts is due to the presence of oleuropein, hydroxytyrosol, and flavonoids in the extracts. It has been shown that these phytochemicals in olive plants help reduce lipid peroxidation and increase antioxidants. The findings are similar to those obtained in earlier studies showing that phenolic compounds present in the olive have significant antioxidant activity. (Dash et al., 2024)

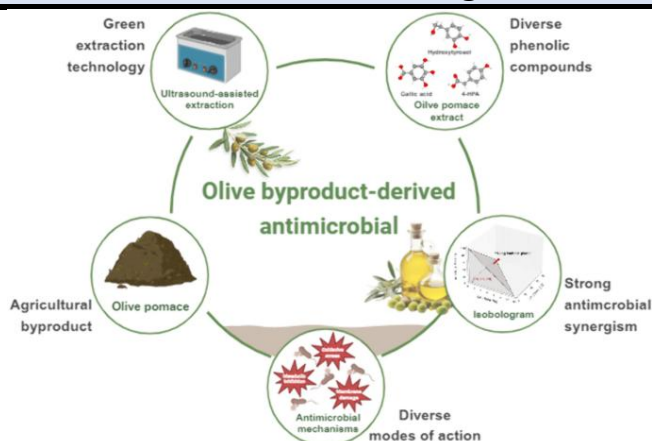


Figure 3. Olive byproduct-derived antimicrobials (Kim et al., 2024)

4.2.3. Neuroprotective Effects on Neuronal Cells

Neuroprotective analysis results showed that olive extracts could significantly increase neuronal cell viability after hydrogen peroxide treatment-induced oxidative stress. Hydrogen peroxide treatment induced neuronal cell damage and lower levels of viability; however, olive extract treatment increased the cell viability rate, particularly for the methanolic leaf extract treatment group. This indicates that phytochemicals found in olives have the capability to provide neuroprotection against neuronal cell damage due to oxidative stress. This might be due to the presence of antioxidants and antiapoptotic components found in olive extracts. For instance, it has been shown that oleuropein and hydroxytyrosol exert their protective effects by stabilizing mitochondria and reducing ROS accumulation, which leads to inhibiting the apoptotic signaling pathways. Further, the lower levels of ROS seen in cells treated with olive extracts indicate that olive extracts help directly reduce oxidative damage within the cells. As neuronal degeneration is often associated with oxidative stress and dysfunction of mitochondria, olive extracts could be used as a natural source of protection against such conditions. (Paié-Ribeiro et al., 2024)

4.2.4. Effects on Antioxidant Enzyme Activity

The experiment showed that the extracts of olive increased the activity of endogenous enzymes of antioxidants in neuronal cells subjected to oxidative stress. Treatment with hydrogen peroxide resulted in the significant reduction of activities of superoxide dismutase, catalase, and glutathione peroxidase, thus implying a dysfunction of the antioxidant defense system in the cells. But treatment with olive extracts led to the normalization of the activity of the mentioned enzymes. In this way, the experiment revealed not only the ability of olive extracts to function as antioxidants but also their ability to enhance the antioxidant defense in cells. This is a crucial point since antioxidants play an essential role in neutralizing reactive oxygen species. Thus, the protection against oxidative stress by means of activation of endogenous antioxidant defense mechanisms might be the second way of neuroprotection provided by olives. Earlier studies also demonstrated that olive phenolic compounds were able to activate such a response. Thus, it is quite possible that neuroprotective activity of Libyan olive extracts is determined by the dual mechanism of action. (Mairuae et al., 2023)

4.2.5. Anti-inflammatory Activity and Neuroprotection

Neuroinflammation is recognized as an important contributor to the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. In the current investigation, it was seen that olive extracts greatly decreased the secretion of inflammatory mediators like TNF- α , IL-6, and nitric oxide in neurons under oxidative stress. These results suggest that the extracts obtained from Libyan olives have excellent anti-inflammatory actions that could play a role in protecting neurons. Polyphenolic compounds present in the olive like oleocanthal and oleuropein have earlier been found to block inflammatory pathways and also reduce the generation of pro-inflammatory cytokines. It is possible that the suppression of inflammatory mediators in the present experiment would prevent neuronal dysfunctions and thereby neurodegeneration. As oxidative stress and neuroinflammation are associated with each

other, antioxidant and anti-inflammatory actions of the olive extracts can serve to protect neurons. (Gonçalves et al., 2024)

OLE administration alleviated oxidative stress by restoring antioxidant biomarker levels, maintaining AChE close to normal, and preserving brain histology, while also improving rat behavior and learning. OLE can protect brain tissue against simultaneous exposure to noise and toluene. Importantly, these results have significant implications for the prevention and treatment of neurotoxicity associated with exposure to toluene and noise, highlighting the potential practical applications of our research. (Attia et al., 2024)

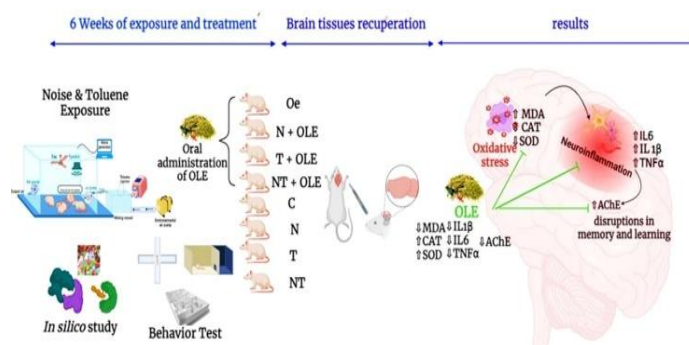


Figure 4. Neuroprotective effects of *Olea europaea L.* leaf extract (Tylutka et al., 2024)

4.2.6. Significance of Libyan Olive Plants as Natural Neuroprotective Agents

This study provides important insights into the medicinal value of olive species grown under the Libyan environment. The unique climate stresses, such as aridity and temperature highs that characterize Libya, could be responsible for increased levels of biosynthesis of plant chemicals. Hence, Libyan olive species have unique biological characteristics different from those of other olive species grown elsewhere.³¹ The antioxidant, anti-inflammatory, and neuroprotective properties exhibited by the study can aid in the usage of olive preparations as medicine and in the potential pharmaceutical application. Moreover, the utilization of naturally occurring neuroprotective compounds from plants can provide alternative approaches in managing neurodegenerative diseases that are cost-effective and less hazardous, especially in developing nations where access to quality healthcare services is not readily available. This research can contribute significantly to the body of science about North African herbal medicines. Nonetheless, further research is needed through additional experiments, both in vitro and in vivo, to explore fully the effects of olive extract on neurological disorders. (Martins et al., 2024)

5. Conclusion

In the current study, it has been proved that natural extracts of Libyan olives (*Olea europaea*) are effective in demonstrating antioxidant, anti-inflammatory, and neuroprotective properties. The phytochemical analysis indicated the presence of bioactive chemicals such as oleuropein, hydroxytyrosol, flavonoids, and phenols, among which the highest levels were observed in methanolic leaf extracts. It was found that the extracts have high antioxidant potential, since they were effective in scavenging freeradicals. Besides, it was confirmed that Libyan olive extracts can increase neuronal cell viability and decrease cellular damage due to oxidative stress. In addition, it was found that extracts can decrease inflammatory markers such as TNF- α , IL-6, and nitric oxide, demonstrating strong anti-inflammatory activity. Thus, the combination of biological activities proves that Libyan olive extracts could be used as natural remedies for the treatment of neurodegenerative disorders.

From the findings of the present study, there are several recommendations that can be made for future studies in order to increase knowledge regarding the neuroprotective properties of olive plants grown in Libya. Specifically, animal experiments, as well as clinical trials, will need to be performed to validate the effects, the safety, and the mechanism of action of the olive extracts

in relation to neurodegenerative dis-eases. Further molecular studies will also be necessary in order to clarify the exact signaling mechanisms involved in the antioxidative and anti-inflammatory properties of the plants. Also, comparative studies between olive plants from different parts of Libya and under various environmental conditions will allow the identification of the elements that influ-ence their composition and activity. Lastly, more effective methods of extraction should be employed in order to separate neuroprotective components such as oleuropein and hy-droxytyrosol. Pharmaceutical products and functional foods based on the extracts of Libyan olive plants can also be developed.

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