Int. J. Plant Bas. Pharm. (2023) 3(2), 215-227

INTERNATIONAL JOURNAL OF PLANT BASED PHARMACEUTICALS
https://ijpbp.com

REVIEW

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Bioactive chemlali olive derivatives and compounds useful for pharmaceutical purposes: A review

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ARTICLE INFO

Article History:

Received: 04 May 2023
Revised: 14 October 2023
Accepted: 21 October 2023
Available online: 27 October 2023

Edited by: B. Tepe

Keywords:

Chemlali variety
Olive derivatives
Extraction
Chemical composition

Reviewed by:
Jawaher H. Al-Qahtani: King Saud University, Riyadh, Saudi Arabia
Teodora Todorova: Bulgarian Academy of Sciences, Sofia, Bulgaria

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e-ISSN: 2791-7509
doi: https://doi.org/10.29228/ijpbp.35

ABSTRACT

Several articles in the literature deal with and analyze components extracted from the olive tree, but these researches are mainly focused on the quality of olive oil and more precisely its volatile components, often related to their antioxidant properties, which denies other derivatives of the olive tree that are traditional sources of several healing broths. A thorough comparison of the richness of the different derivatives as well as the methods of extraction and identification will help to provide a useful decision-making tool. Olive tree derivatives were extracted in different ways to better understand the chemical composition of the Chemlali olive tree variety as well as to evaluate the efficiency of advanced extraction procedures. A comparison through the IC_{50} of the Chemlali variety with other olive cultivars was performed. It shows the richness of this variety in polyphenols. In addition, we present here a qualitative and quantitative table of basic chemicals for pharmacological use derived from olive oil, leaf, and bark that have been previously described in the literature. Diverse types and concentrations of phenolic compounds—an important class of natural antioxidants—can be found in different components of olives, including leaves, fruit, pits, seeds, bark, and paste. However, comprehensive studies are deficient in comparing the quantities extracted from these different sources. Indeed, this review attempt shows the diversity of Chemlali through the use of high-value-added molecules (VAT), which can be categorized as diterpenes such as tyrosol and phytol, triterpenes such as squalene, as well as cinnamates.

1. Introduction

The cultivation of olive trees has a long history in the Mediterranean region and has been a significant contributor to the economy of many countries. The olive tree is the most important oil-producing crop in this area, with approximately 90% of the world's olive trees grown in countries such as Spain, Italy, Tunisia, Morocco, and Greece (Kulak & Cetinkaya, 2018). Furthermore, the tree has a long lifespan, with some trees living for hundreds of years, making it a sustainable crop that provides income for generations.

Nowadays the cultivation of the olive tree has expanded into Australia, China, Latin America, Iran, South Africa, and the USA (Ghasemi et al., 2018; Obied et al., 2005; Pérez-Rodrigo & Aranceta, 2016; Tacer & Aşan Özüsağlam, 2022). The Mediterranean Basin is a climate change and biodiversity hot spot where substantial warming and water availability are predicted for the next few decades. The magnitude of the predicted climate change in natural and agroecosystems poses considerable challenges to their management (Gonçalves et al., 2020). In olive trees, an ancient crop with considerable ecological and socioeconomic importance in the Mediterranean area, the impacts derived from climate warming might lead to areas of decreased profitability **(Figure 1)**. Based on the study carried out by Brito et al. (2019), Mediterranean crops such as olive trees have natural protection mechanisms that help them

Please cite this article as: Ksibi, H. (2023). Bioactive chemlali olive derivatives and compounds useful for pharmaceutical purposes: A review. *International Journal of Plant Based Pharmaceuticals*, *3*(2), 215-227, https://doi.org/10.29228/ijpbp.35.

cope with typical summer stress factors such as drought, high irradiation levels, and heat.

Mediterranean olive-growing areas have diverse microclimates, soils, and rainfall patterns which result in a wide range of water needs for annual crops. Although olive trees are known to be drought-resistant, research has shown that irrigation can enhance the physiological performance of the trees. This includes better water status, higher stomatal conductance and photosynthetic rates, and crop yield (Gharsallaoui et al., 2011). However, it has also been discovered that irrigation has negative effects on both the yield and quality of olive oil. Increased amounts of water applied have been found to decrease the percentage of oil in the fruit, affecting both the total amount of phenolic compounds in virgin olive oils and their stability to autoxidation, sensory quality, and ultimately consumer acceptance (Calabriso et al., 2015; de Melo et al., 2012; Tamasi et al., 2016). Therefore, the decision to irrigate olive trees should be made judiciously, taking into account the potential trade-offs between the benefits and drawbacks of irrigation.

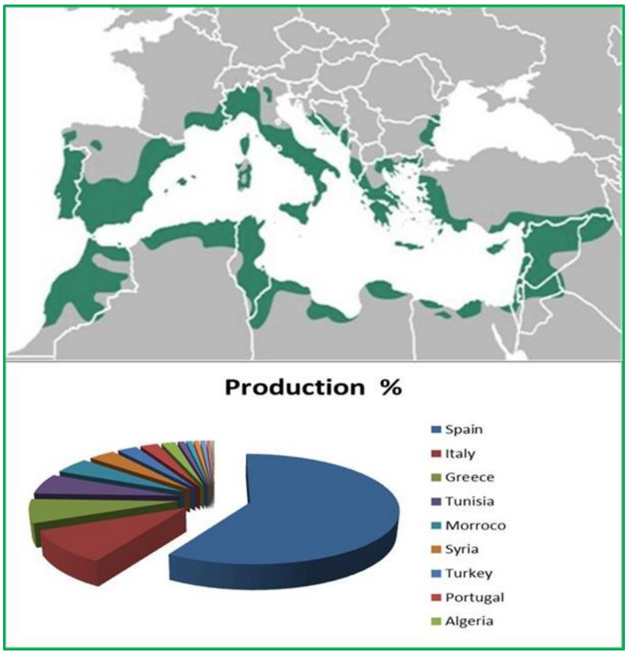

Figure 1. Olive production in the Mediterranean basin (IOC, 2022)

Modern agriculture techniques based on linear production have been widely adopted as a result of the globalization of the world and the necessity to generate ever-greater quantities of food and other products (Gharsallaoui et al., 2011). The accumulation of large amounts of waste from agro-industrial activities often leads to environmental issues when disposed of in landfills. As a result, researchers have begun studying methods to recover these by-products in a sustainable and circular bio-economy framework. This review summarizes natural derivatives extracted from the Chemlali olive tree variety by using published experimental papers analyzing the knowledge regarding the use of olive trees by-products for producing active ingredients, antioxidants, cosmetics, and several pharmaceutical products (Brahmi et al., 2012; Issaoui et al., 2017b; Jemai et al., 2008). Furthermore, the data regarding the potential biological activity of extracts from olive oil, leaves, bark, and stone were analyzed. Olive trees by-products are, indeed, rich in molecules with antioxidant (Wang et al., 2010), antimicrobial (El & Karakaya, 2009), cardioprotective (Manna et al., 2004), and anticancer activity (Rashed et al., 2022; Toric et al., 2019), representing a promising candidate for treating several human diseases.

In this paper, we describe high-performance liquid chromatography (HPLC) and liquid chromatography combined with LC/MS mass spectroscopy for the detection of flavonoids, secoiridoids, phenolic alcohols, terpenes, and phenolic acids. We also emphasize the significance of olive derivatives in traditional Mediterranean medicinal practices. We analyze the main by-products and natural antioxidants (Chemlali variety) that may be found in various olive matrices (leaves, fruits, pits, seeds, bark, and paste), including their types and quantities.

2. Historical aspect

The olive tree, a venerable plant with medicinal benefits, notably its oil, which is regarded as a healthy food, all contain components that are crucial for human well-being. The olive tree and its oil have played an important role in many cultures throughout history, from ancient times to the present day. The history of olive oil is closely linked to Mediterranean culture and the development of its agricultural industry. The history of olive oil is intertwined with that of bread and wine, and it is one of the staple foods of Mediterranean culture in coastal settlements. More than 2,000 years ago, people who lived along the Mediterranean Sea employed the olive tree's leaves, roots, and even bark as natural treatments. Even olive leaves were a component of the Egyptian method for keeping their mummies intact. For making teas to relieve colds and other discomforts, ancient societies used olive leaves. Olive oil was imported from Crete by the Egyptians during the time of the Pharaohs, who were known to be avid consumers of the oil for their funeral and purification rituals. One of the earliest trees to be domesticated is the olive tree, from whose fruit olive oil is made. It is a distinct oil manufactured entirely from vegetable juice. Some writers believe that the first olive oil production took place somewhere in the Mediterranean Levant between 6000 and 4000 years ago (Langgut et al., 2019). Early in the 1800s, European physicians used olive-leaf tea to treat malaria patients. Ancient and modern scientists both benefited from the naturally existing phytochemicals that nature provides and which naturally aid in protecting the olive tree from bacteria and insects (Senouci et al., 2020).

The cultivation and production of olive oil have become fundamental to the traditions and lifestyles of the nations and empires of the Mediterranean basin due to this extensive historical development. People naturally think of historical Mediterranean civilizations like the Carthaginians and Romans when they consider a Mediterranean diet and the health benefits of eating olives in the middle of meals. Olive oil was known for its various medicinal properties, as Hippocrates described over 60 different uses for treating wounds, infections, and burns. In ancient Greece, olive oil was used not only for medicinal purposes but also for coating the bodies of athletes before their competitions at the Olympic Games. It was also given as a reward to the victorious athletes who received a large quantity of it. However, its use was also influenced by the social status of individuals. Moreover, during funeral ceremonies, the deceased were offered olive oil along with other products like honey (Shaw, 2000).

3. Olive tree categories in Tunisia

The cultivation of olives has a long history in Tunisia, dating back to ancient times. The country's climate and geography provide an ideal environment for olive cultivation, with over 80% of its agricultural land dedicated to olive groves. Furthermore, the word zaytuna, which means "olive tree" in Arabic, is rooted at the heart of this 11 million-person country, just as this famous mosque known as "the olive tree" is at the center of Tunisia's history and daily life. Tunisia ranks among the world's leading producers of olive oil today. A century ago, semi-arid landscape predominated and there are now miles and miles of olive orchards. Tunisians are proud of their olive oil. The cultivation of olive trees is an integral part of Tunisia's agricultural industry, with approximately 82 million olive trees spread across almost 30% of the country's total agricultural land area (Mariem et al., 2019).

Even babies are anointed with olive oil by some Tunisians. There are many different olive cultivars available now in Tunisia, each with a unique set of morphological and physiological traits that produce an array of extremely distinct attributes and applications. However, the Chemlali variety tends to be the most common (Figure 2). Classes of olives from various cultivars are naturally observed based on this variety's morphological characteristics and multivariate chemometric identification methods. The production of olive oil is supposedly almost totally restricted to the Mediterranean shore, where it is liberally sprinkled on a variety of meals. Numerous prospecting and characterization projects showed that the Tunisian olive grove is distinguished by a remarkable varietal richness in addition to the two main oil varieties (Chemleli and Chetoui).

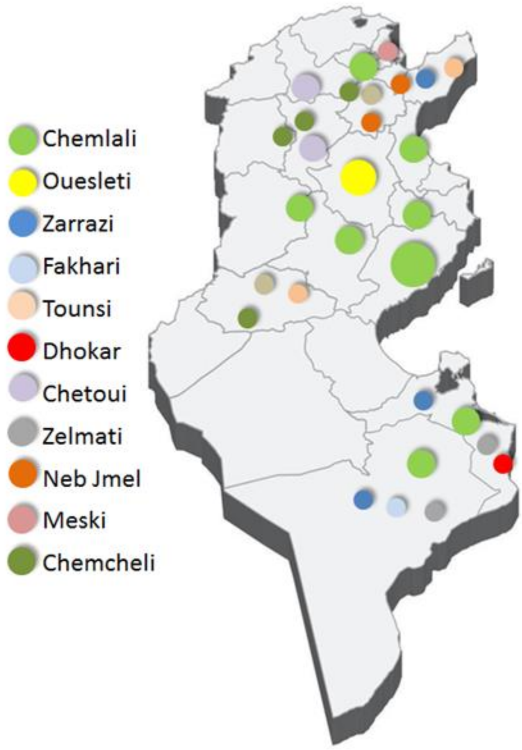

Figure 2. Olive tree varieties in Tunisia

Indeed, the cultivars Oueslati, Zalmati, Zarrazi, Chetoui, Fakhari, Tounsi, Chemcheli, Neb jmel, Meski, and other varieties represent some examples of the varieties offered by the local olive plantations (Mansour-Gueddes et al., 2020).

Tunisia has achieved a noteworthy diversification in olive oil production and a unique flavor that is recognized and admired in other countries by cultivating imported olive trees such as Arbequina and Arbosana from Spain and Koroneki from Greece (Abdelhamid et al., 2013).

In actuality, Tunisian history is extremely old in terms of olive crop methods and applications. There is surely a painting depicting the olive harvest at the entryway to the buildings that house the ONH (Mariem et al., 2019). The majority of the time, it is a vivid depiction of farmers in an olive grove at the start of a fresh harvest or during the extraction of this priceless oil. The olive tree and its derivatives have many benefits in Tunisia that have been traditionally acknowledged. Significant medical benefits are provided by the leaves, fruits, and oil. When infused or decocted, the leaves help regulate type II diabetes, decrease moderate hypertension, promote urine elimination, and protect against viral, bacterial, or fungal infections (Lo Giudice et al., 2021).

Tunisia, a country rich in phytogenetic resources, has been able to preserve its agricultural history via its agricultural tradition coupled with a program of prospection, acclimatization, and development of plants. These resources consist of various indigenous elements that are a source of future wealth and whose acknowledgment is a crucial factor in the growth of the olive industry (Debbabi et al., 2022). The olive variety is the most important factor in determining the olive oil taste.

Olives—and in particular olive oil—are Tunisia's unique and, oddly, unknown treasure. The Chemlali variety is planted in the South and the Middle mainly Sfax. 2/3 of the Tunisian olive grove is made up of Chemlali trees that were planted in the Sahel, the center, and the south. It is the most common kind, a particularly robust cultivar that adapts well to arid environments, and it is produced in large quantities in different ways. Overall, the olive tree and its products have become deeply intertwined with Tunisian life, reflecting the country's rich history, culture, and traditions.

4. Literature survey

The olive tree (*Olea europaea*) has gained popularity as a reliable source of traditional medicine for the treatment of a variety of diseases. The results of the phytochemical and pharmacological investigations summarized in this study will help to further develop the drug's therapeutic potential as well as convincingly support its possible use in clinical trials in the field of contemporary medicine.

Knowing that plants have therapeutic benefits, many scientists are eager to examine as many different plants as they can to learn vital information about their therapeutic capabilities. It applies to the pharmaceutical sector. Food by-products have drawn a lot of attention in the hunt for novel bioactive compounds made from natural raw materials due to their high potential as a source of phytochemicals, low cost, and significant environmental impact. For instance, in the olive oil sector, olive leaves obtained as biomass after pruning olive trees are one of the most promising sources of bioactives (Erbay & Icier, 2010). Because of the rising interest in the use of olive tree derivatives in a variety of industrial sectors, a systematic assessment of extracts for their qualities is becoming increasingly necessary.

Olive tree derivatives are genuinely gaining popularity across a wide range of industrial fields, so it has become increasingly important to conduct systematic evaluations of extracts for these qualities. Today, oleuropein, which has been widely utilized in folk medicine in Mediterranean regions, is primarily extracted from olive leaves (Brahmi et al., 2012). Nowadays, olive leaves as well as other derivatives have essentially been used for the extraction of oleuropein, it has been widely used in folk medicine in Mediterranean regions (Le Tutour & Guedon, 1992).

These organic substances are widely distributed in barks, fruits, roots, stems, leaves, and other plant parts. Numerous scientific terms for them include polyphenols, flavonoids, flavonols, pycnogenols, glucosinolates, isoprenoids, carotenoids, tocotrienols, and proanthocyanidins. The number of papers in the literature is meager that discuss the analysis of the phenolic elements of the olive tree, primarily oleuropein, olive oil (Bouaziz et al., 2005), and olive leaves (Brahmi et al., 2012; Pacheco et al., 2002), but the study of those taken from the olive tree bark is still minuscule. However, during the last decades, they have also been the focus of efforts to extract natural antioxidants and antibacterial agents via hydrodistillation (Issaoui et al., 2017a), solvent extraction (Issaoui et al., 2012), and, more recently, supercritical fluid extraction (Issaoui et al., 2017b; Le Floch et al., 1998), ultrasound (Japón-Luján et al., 2006), and maceration (Deng et al., 2017).

According to several studies, oleuropein and its derivatives play a variety of functions in the treatment of diseases, including anti-inflammatory and anti-thrombotic activities (de la Puerta et al., 2001). More specifically, oleuropein and one of its derivatives, hydroxytyrosol, inhibit the generation of leukotriene B4 and lipoxygenases and eicosanoid production (Manna et al., 2004). The oleuropein has antimicrobial activity against a variety of viruses, bacteria, yeasts, and molds, which is wider for hydroxytyrosol (Bisignano et al., 1999).

Luteolin is another key component having anti-inflammatory activity in animals (Ueda et al., 2002), and it also has antimutagenic properties and antitumorigenic (Issazadeh et al., 2012). Apigenin, extracted from the leaves, inhibits the inflammation mediators such as nitric oxide and prostaglandin E2. Among the most active flavonoids, mention rutin, catechin, and luteolin which exert antioxidant effects up to 2.5 times as vitamins C and E and are comparable to lycopene (Benavente-Garcia et al., 2000).

Recently, much attention has been paid to triterpene acids from the pharmaceutical viewpoint because of their anti-HIV, anti-inflammatory, and antitumor-promoting activities (Parra et al., 2014). A wide range of olive extract products offer antioxidant properties, guarding against oxidative damage by free radicals, which accelerate aging and disease. Nutrition plays a pivotal role in managing the severity of infectious diseases and is associated with various health benefits, such as improved maternal and child health due to components like oleanolic acid, fortified immune systems through pentacyclic triterpenes, reduced risk of non-communicable diseases, and increased longevity, as noted by Rufino-Palomares et al. (2022). Studies recently conducted have examined the anti-diabetic, anti-cancer, anti-microbial, anti-fungal, antiviral, antioxidant, antihypertensive, gastroprotective, anti-inflammatory, antinociceptive, neuroprotective, and cardioprotective actions. EVOO (extra virgin olive oil) as a valuable component of a Mediterranean diet (MED), can help keep the immune system strong and reduce the inflammation and oxidative stress that lead to a variety of diseases and conditions that increase the risk for serious cases of COVID-19 (Strub et al., 2022).

Nevertheless, there is an increase in demand for essential oils made from plant derivatives due to many variables, including an increase in the consumption of edible oil, the growth of the biofuel business, and the desire for green chemistry. In actuality, since ancient times, dried bark of various plants as well as leaves and their processed products have been widely employed as flavoring agents (de Melo et al., 2012; Issaoui et al., 2017b).

By directly regulating the activities of a variety of receptor tyrosine kinases, including the human epidermal growth factor receptor 2 (HER2), olive chemicals have recently demonstrated considerable anti-carcinogenic properties (García-Villalba et al., 2010; Menendez et al., 2008).

This residue is a very abundant vegetable material and it is supposed a potential source of polyphenols (De Leonardis et al., 2008). Constituents of olive leaves have shown antiviral (Lee-Huang et al., 2003), antimicrobial (Markin et al., 2003), antioxidant, anti-inflammatory (Bouaziz et al., 2005), neuroprotection (Angeloni et al., 2017), and anti-carcinogenic (Abaza et al., 2007; Bouallagui et al., 2011) activities. Various methods have been employed for the extraction of bioactive compounds from olive leaves, including conventional solid-liquid extraction using ethanol (Rada et al., 2007) or methanol-water (Bouaziz et al., 2005), and ultrasound-assisted extraction (García Pérez et al., 2011). Testing different methods of extraction is essential due to the significance of the extraction process as a means of isolating and purifying intriguing molecules from natural source materials.

In general, the consumption of olive byproducts is associated with a low incidence of cardiovascular diseases, neurological disorders, and breast cancer (da Silva, 2010; Hashmi et al., 2015; Oliveras-Ferraros et al., 2011). Several minor components have recently been related to the healthy properties, mainly polyphenols, of olive byproducts. These compounds also are associated with the oxidative stability and flavor characteristics of virgin olive byproducts. The phenolic composition of olive oil is a complex outcome of various factors, including genotype, environmental conditions, cultivation practices, and processing techniques. The qualitative and quantitative profile of phenolic compounds in olive oil can be significantly affected by multiple variables during the production process, such as the ripening stage of olives, harvesting methods, and storage conditions (Gharsallaoui et al., 2011).

5. State of art

In the past 30 years, olive oil production has spread to nontraditionally producing countries, leading to an increased olive and olive oil trade supported by high- and super-high-density production systems (Lavee & Avidan, 1993). However, this is not always accompanied by the production and marketing of high-quality olive oil, so great care must be taken to obtain olive oil with preserved content of minor components that are primarily responsible for the health benefits of olive oil.

Numerous publications about the study of olive extracts have been published recently, albeit these studies focus mostly on the volatile components because of their antioxidant activity (Naija et al., 2021). Beyond the leaves and the oil, a thorough evaluation of the various extraction techniques for the many olive tree materials was lacking. Therefore, it is necessary to present the main compounds frequently synthesized for pharmaceutical or food use, as well as an appropriate decision-making tool for the selection of the mode of extraction of olive tree substances.

However, comparative studies of different extracted amounts of such material are scarce in literature (Issaoui et al., 2017a; McDonald et al., 2001). Here, the authors deal with the partition of extracted phenolic compounds in olive tree derivatives through several experiment extractions. The analysis of the extracted compounds was performed using retention times and structural fragmentation. It's worth noting that the Chemlali variety is notably rich in oleuropein, squalene, and cinnamates, as previously documented in papers like those by Issaoui et al. (2017b), Lone et al. (2014), and Sellami et al. (2016). These compounds can be employed in the food sector with favorable effects on health as well as in the pharmaceutical and cosmetic industries because they limit the action of reactive species implicated in oxidative damage. The extracts also demonstrated antibacterial properties. Based on DPPH measurements, there is a noticeable and substantial increase in the total phenolic content in the Chemlali variety genotype.

6. Used extraction techniques

The treatment techniques used on olive derivatives can be divided into three groups and utilized alone or in combination. Here we discuss biological, chemical, and physical processes. Olive leaf physical processes mostly involve drying, with the goal of dehydration for preservation. Olive bark and leaves have also been subjected to pulverization and bleaching. The adsorption procedure, which tries to purify substances with medicinal or aesthetic interest, also piques our interest. The chemical techniques primarily involve the extraction of valuable chemicals from olive byproducts, including, flavonoids, and phenolic compounds. Moreover, treatments with acids or alkalis using the leaves or their extracts have been documented in the literature (Issaoui et al., 2017a; Romero & Brenes, 2012). The application of both endogenous and exogenous enzymes, as well as microorganisms, in the treatment of olive tree products, is known as a biological process (Khwaldia et al., 2022; Yuan et al., 2015).

In the literature, there are several techniques for vegetable materials extraction: solvent extraction (SE); hydrodistillation extraction (HDE), ultrasound extraction (UAE), and maceration (MCR). These different extraction methods can affect the quality, aroma, and appearance of oils, making it crucial to know the difference between them (Japón-Luján et al., 2006). In recent years, supercritical fluid extraction (SFE) has become an alternative to more conventional extraction techniques, chiefly because the dissolving power of supercritical fluids can be simply adjusted by regulation of pressure and/or temperature operational conditions (Ksibi, 2004; Reverchon & De Marco, 2006).

Clodoveo et al. (2021) provided an overview of the latest advancements in the use of innovative extraction methods, which offer promising efficiency and reduced solvent volume, such as $scCO_2$. This approach can address the issue of hazardous residual solvents in products and allows for lower temperatures during the extraction process, leading to less degradation of the extract's thermally labile components.

6.1. Sample preparation

The typical protocol for describing the extraction procedure is explained as follows: samples are consistently taken from the same tree but in different branches. They weighed hundreds of grams and were calibrated to compose a uniform batch of sheets that are formed for the extraction of phenolic compounds. Materials were boiled in distilled water for many hours and then filtered. The water was removed with a freeze-dryer to obtain the aqueous olive-dried extract. To prepare the solution, containing methanol or ethanol, along with acetonitrile and water as outlined in Ghomari et al. (2019), for the extraction of compounds from olive matrices (e.g., leaves, bark, stones, pulp, etc.) olive derivatives were separately macerated in either methanol or ethanol for several days in the dark at room temperature. The extract was generally separated by filtration and the solvent was evaporated under a vacuum as mentioned by Issaoui et al. (2012). In the case of supercritical extraction, the material was powdered and mixed with glass balls with a specific diameter to form a fixed bed (Issaoui et al., 2017b; Reverchon & De Marco, 2006).

6.2. Solvent extraction (methanol)

The extraction of bioactive compounds from natural materials has gained much interest from researchers as the demand for functional ingredients obtained via natural processes keeps increasing due to consumers getting more interested in functional foods. The commonly used conventional extraction method is soxhlet extraction, which applies in the extraction of several plant materials, knowing that solvents are not universal, different types of substances should be used in this technique to dissolve different solutes that compose the tree bark matrices. There are volatile essences and heavy organic molecules. The amount of solute that can dissolve in a given substance depends heavily on the temperature, volume or mass ratio, and various chemical properties of the substances involved (Sultana et al., 2009). Based on previous findings concerning the effects of parameter extraction on phenolic compounds, and taking into account that high temperatures, though causing an increase in total phenolics obtained, the extraction was performed at room temperature (25 °C), while the pH was adjusted to 2. These operational conditions were based on the fact that acidification of olive tree byproducts increases phenolic compounds' solubility. That implies more diffusion from the plant material through cell wall disintegration and simultaneously, more stability of the extracted phenolic compounds, (Issaoui et al., 2012; Malik & Bradford, 2008).

A commonly used protocol for extracting olive tree derivatives with methanol is based on the principle of "like dissolves like" for phenolic compounds (Ryan et al., 2002). This means that a polar solute is likely to dissolve in a polar solvent while a non-polar solute is likely to dissolve in a non-polar solvent. Other properties, such as the volume and temperature of the substances involved, are also major solubility determinants, but polarity is usually the most important factor. For these reasons, the powder thus obtained is taken up with methanol-water (80:20, v/v). The solution is placed generally, in a mechanical shaker for a long period to stir at room temperature and a speed of hundreds of rpm. The extract was filtered through a paper filter and then evaporated to dryness and the residue was taken up in methanol.

Typically, the purification of the phenolic compounds involves a two-step process. First, after the removal of solvent in vacuo the crude extract at a moderate temperature, the aqueous layer was partially defatted by extraction with hexane. This aqueous phase is extracted three times with ethyl acetate or ethyl ether (v/v 2:1).

The organic extracts are then combined and concentrated by a rotary evaporator to dryness to evaporate ethyl acetate. The residue is taken up in 5 ml of methanol for the identification of compounds present in the extract, for the determination of total phenolics, and for measurements of antioxidant activity (McDonald et al., 2001). The general pattern of extraction and separation of phenolic compounds from olive leaves is reported in Figure 3 (Ksibi & Ksibi, 2018).

6.3. Hydrodistillation

Hydrodistillation is one of the most used traditional methods of extraction, which is sometimes used instead of steam distillation. It is a technique that implies the plant material is boiled in water, using a hot source from below the vessel.

As mentioned by several authors such as Spadi et al. (2023) and Issaoui et al. (2017b), the extraction method involves soaking the powdered bark in water instead of passing steam through it in a hydro-distiller. The oil is then removed from the top of the resulting hydrosol after heating the container of water until steam escapes and cools down. Hydrodistillation is made with the clevenger-type apparatus at a scale lab. The combined effect of temperature and pressure explodes the secretory cells that release essential oils. Water vapor and volatile compounds are formed at the top of the column and passed into the condenser where they are liquefied. The volatile material is carried away in the steam through some tubes and becomes cool. The volatile oil is then removed from the top of the hydrosol. During hydrodistillation, the essential oils emerge from an azeotropic mixture with water. The aqueous phase was withdrawn and then the essential oil was recovered in a smoky glass bottle (protected against light). Finally, the bottle was sealed and kept in a refrigerator at 4 °C for chemical analysis (da Silva et al., 2012; Spadi et al., 2023).

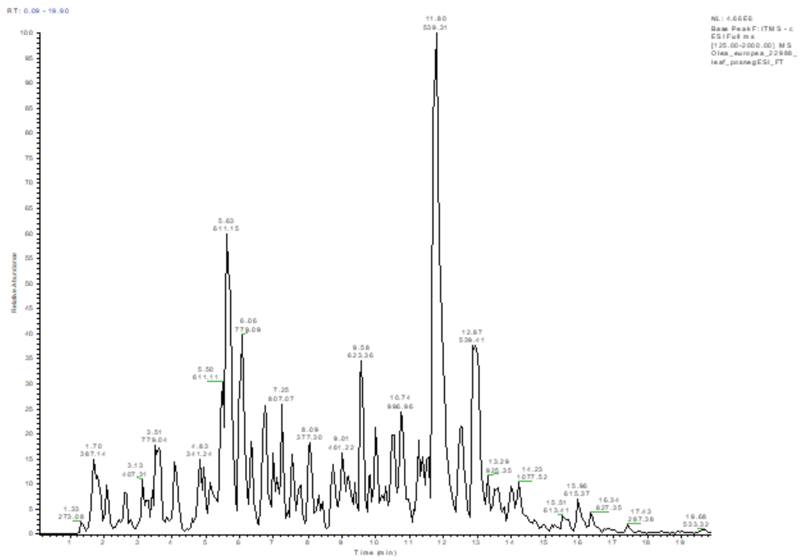

Figure 3. Profile of the chromatogram of the extract methanol/water of olive leaves by LC/DAD (Ksibi & Ksibi, 2018)

Most of these components do not mix well with water in the liquid phase so after condensation, they are separated by decantation. It is mentioned in the literature that the essential oil yield is often influenced by the extraction time (Zheljazkov et al., 2014). The volatile components obtained after trapping in diethyl-ether were dried under anhydrous sodium sulfate, evaporated, concentrated under a mild stream of N_2, and stored at 4 °C until tested and chemically analyzed.

The solution, which included phenolic compounds, was combined in a separatory funnel with ethyl acetate. The operation is repeated 2 or 3 times. The organic phase (ethyl acetate) was evaporated to dryness under reduced pressure at a moderate temperature (about 40 °C) and the residue was taken up by a few milliliters of methanol for the identification of compounds present in the extract for the determination of total phenolics and measures of antioxidant activity. The identification of the components was performed, by comparison of their retention times with those of pure authentic samples and through their linear retention indices (LRI) relative to the series of n-hydrocarbons.

6.4. SFE method

To avoid heat-sensitive degradation of materials, supercritical fluid extraction (SFE) represents a new extraction alternative at moderate temperatures and high pressure used in the medical and food industries. The second advantage of this method is that solvent recycling is more feasible in supercritical fluid extraction. This leads to an operating cost decrease. Additionally, supercritical fluid extraction offers a greater selectivity compared to other extraction methods, and the extraction yield and composition can be modified by adjusting the operating parameters. Carbon dioxide is one of the most common solvents in this process because of some advantages such as non-toxicity and non-flammability. It is an environmentally friendly solvent, too (Le Floch et al., 1998; Reverchon & De Marco, 2006).

Supercritical CO_2 extractions were performed using an extraction vessel of hundreds of cm^3, which operated in a single-pass mode by passing CO_2 through the fixed bed of vegetable materials. The extraction method utilized a semi-batch approach, where the vegetable matter was charged in batches while the solvent continuously flowed through the system. Temperatures and pressures were monitored during the extraction process using a thermocouple (Fe/Const 1/8) and Bourdon-tube test gauges, respectively. The pressure was manually controlled with high-pressure valves located at various points along the apparatus. According to previous kinetic studies, the overall extraction time was set to be 5 hours (Fornari et al., 2012).

There is a keen interest in isolating and identifying the natural essences found in olive tree bark. To achieve this, multiple extractions can be conducted using supercritical CO_2. The supercritical CO_2 extraction is not a spontaneous process because if the considered compound is very soluble in the extraction medium, it also has a considerable affinity for the matrix causing a slower migration solvent (CO_2). To track the yield variability of the olive tree bark powder and its chemical composition using a supercritical CO_2 extraction technique, a few grams of natural essences were retrieved following four hours of extraction, conducted at two distinct high-pressure levels, such as 90 and 250 bars, as detailed by Issaoui et al. (2017b).

The identification of the different components was made from their mass spectra and their retention indices according to Kovats compared with those of standard compounds of the bank of computerized data (Table 1). The qualitative chemical analysis of the obtained samples is necessary to adopt a technique that makes possible the separation and the identification of such components of the mixture via two independent methods.

6.5. Ultrasound-assisted extraction

For isolating bioactive compounds from plants, ultrasound extraction is the favored method. Complete extraction is made possible by sonication, leading to higher extraction yields in a remarkably short extraction period. Ultrasonic extraction is a time and money-saving extraction technique that yields high-quality extracts that are used in food, supplements, and pharmaceuticals.

This method is frequently applied when making olive oils. This analysis emphasizes the need for industrial scale-up of this novel technique since the majority of studies affirm the significant potential of ultrasound-assisted extraction (UAE) to shorten processing times, improve process sustainability, and produce oils with the desired nutritional and sensory quality (Ünver & Çelik, 2022). Ultrasound extraction enhances both the sensory attributes and the source purity of oil, provided it does not adversely affect customer perception. It is worth highlighting the acceptability of oils produced through UE treatments, as demonstrated by Martín-García et al. (2022). It can be applied by using an ultrasonic bath and probe systems. Nevertheless, with the assistance of probe systems, the effects of the majority of parameters on the extraction process have been investigated. In some circumstances, each extract compound can present significant challenges.

Table 1. IC_{50} values for selected olive genotypes in different derivatives

Cultivar	Leaf	Bark	Stone	Pulp	Reference
Chemlali	29.46	18.84	-	-	(Issaoui et al., 2017a)
	-	-	18	20	(Mansour-Gueddes et al., 2020)
	-	-	13.84	19.95	(Ben Mansour et al., 2015)
Arbequina	43.7	-	-	-	(Martín-García et al., 2022)
	-	-	31.97	21.48	(Mansour-Gueddes et al., 2020)
Koroneiki	-	-	27	40	(Mansour-Gueddes et al., 2020)
	36.7	-	-	-	(Martín-García et al., 2022)

6.6. Maceration

Maceration is especially recommended for roots and seeds. This process consists of soaking a plant in a chilly liquid (water, oil, alcohol, brine, etc.) at room temperature for anywhere between 30 minutes and 4 hours, to extract the cold soluble principles. Maceration can also be used so that the solid body absorbs the liquid and is perfumed. However, it's crucial to use the right quantity of alcohol; too little can lead to fermentation, and too much can have negative effects on the final product's strength and effectiveness. The total contents of bioactive compounds and pigments were increased by macerating fresh wild olive leaves in the literature, and the proportions of added leaves were statistically substantially linked with the total contents. 15 phenolic compounds from various phenolic types were identified, according to the findings (Dauber et al., 2022).

The plant is allowed to soak in a covered container, in a cold, dark environment. To avoid fermentation and/or degradation, water and alcohol are typically used as the solvent. The liquid is drained, and the wet marc is pressed, filtered, and placed in a bottle after the maceration time, which is unique to each plant. The result is typically referred to as a medicinal product. Gueboudji et al. (2023) gave a comparison between maceration and liquid-liquid extraction where they show phenolic compounds and antioxidant activities dependent on the extraction method.

7. Analysis techniques

Various analytical techniques are available to the experimenter for the separation of natural products such as high-performance liquid chromatography (HPLC), gas chromatography (GC), high-performance chromatography on thin layer (HPTLC), cons-current chromatography (CCC), supercritical fluid chromatography (SFC) or capillary electrophoresis (CE). Among these techniques, the first two, coupled with mass spectrometry, are the most used ones for our qualitative studies and/or quantitative different components of the leaves of the olive tree (Capasso et al., 1992).

Separation of phenolic compounds involves many qualitative techniques that enable the identification of one or more compounds of the unknown mixture injected. To do this, you have to have standard samples of suspected compounds in the mixture. For a given set of parameters (gas flow rate, temperature, speed, etc.), each parameter compound is characterized by its retention time, which is the time required to pass through the chromatographic column. The distance traveled by the compound is calculated from the time of injection until it appears in the detector. Other quantitative methods, assay for determining the concentration of an unknown mixture of the compound, once these compounds have been identified.

7.1. HPLC analysis

High-performance liquid chromatography (HPLC) is a method for separating the constituents of a mixture even if it is very complex, the sample is placed in a column filled with a stationary phase of small particle size and a high-pressure liquid (mobile phase). This decreases the time necessary for separating the components present in the stationary phase. These components, therefore, have less time to spread through the column, it occurs as a result of the narrow peaks with better sensitivity and selectivity.

The first step is the preparation of samples; products usually have to be subjected to a preliminary purification to remove unwanted mixing products. The extracts were filtered through a 0.5-micron membrane before being injected (Ryan et al., 1999).

The second waypoint is adjusting parameters (pressure and flow rate of eluent, eluent type, etc.) for the optimal separation of compounds of an unknown mixture. After injection, the standards and the unknown sample, proceed to step calculation for qualitative and quantitative analysis, and finally the interpretation of results. Phenolic compounds present in the extracts of olive leaves were analyzed by liquid chromatography high performance coupled with UV array detector diodes (HPLC-UV).

7.2. Analysis of extracts by LC/MS

Liquid chromatography coupled to mass spectrometry (LC/MS) is a technique that combines analytical chemistry with the physical capacity of liquid separation chromatography (HPLC) with mass spectrometry. LC/MS technique is used for many applications which have high sensitivity. In general, the application of the technique LC/MS relates to the detection and identification of chemical potential in the presence of other chemicals (Mohamed et al., 2018).

Issaoui et al. (2017b) performed LC-MS analysis using a Warters 600E pump system equipped with a Merck-Hitachi UV detector and a Lichrosphere 100 RP-18 column (250 x 4 mm). The system was connected to a Finnegan-MAT LCQ mass spectrometry instrument via an APCI interface (Atmospheric Pressure Chemical Ionization). In this model APCI eluent passes through a heater in the ionization chamber maintained at atmospheric pressure. Then the eluent is nebulized by the high temperature (500-600 °C) and a rebulsant gas (nitrogen). The ionization is produced by an electric discharge through a high-voltage needle (3000-4000 V) which forms the protonated molecular ions $(M + H)^+$. These ions pass through the sampling cone (cone sampling SPM) in a region at an intermediate pressure and through a skimming cone. An electric field between the two cones accelerates ions and causes collisions. The fragments' molecular and ionized ions arrive at the quadruple, maintained at a pressure of 10^{-4} torr.

7.3. DPPH test

Using a model system, the DPPH test can be used to measure the antiradical power of pure substances or plant extracts (organic solvent, room temperature). It gauges an antioxidant's ability to lower the DPPH chemical radical (generally, AH, phenolic substances) (2,2-diphenyl-1-picrylhydrazyl) by hydrogen transfer. The DPPH, initially purple, is transformed into DPPH-H, pale yellow. Using the stable free radical 2,2-diphenyl-1-picrylhydrazyl, the extracts' antioxidant activity was assessed based on hydrogen-donating or radical-scavenging capacity (DPPH). According to the investigation of Belaqziz et al. (2017), 3 ml of 0.04% DPPH methanolic solution and 0.1 ml of phenolic preparations were added. After carefully blending whatever together, the mixture was allowed to sit at room temperature for 60 minutes in the dark.

The IC_{50} represents the amount of antioxidant substance required to reduce 50% of the amount initially present in a DPPH solution. It is expressed in mcg/ml plant extract. The IC_{50} (%) is obtained by the equation of the straight average of the percentage of residual DPPH versus the logarithm of the antioxidant concentration. The highest anti-radical activity is a fraction that has the lowest IC_{50}. Their values were calculated by measuring the amount of DPPH inhibition brought on by olive leaf or bark preparations after 30 minutes.

From the literature, we collected IC_{50} values, in µg/ml for various olive tree derivatives, including leaf, bark, stone, and pulp (Table 1), these values represent the concentration of the extract giving 50% radical scavenging. Given values interest 3 kinds of an olive tree well planted in Tunisia, ie Chemlali, arbequina, and koroneiki. Table 1 shows the relationship between duration and concentration and the % inhibition of volatiles from the leaves, stems, and flowers of the considered cultivars. After only five minutes of contact with the radical cation $ABTS^+$, the volatile extracts from every section of the olive tree displayed an intriguing scavenging ability. With more contact time, this ability gradually grows.

According to those results, the IC_{50} value for olive bark is less than that estimated for leaf material. These findings demonstrate that bark has a greater potential for antioxidants than leaves. Oleuropein, which is present in greater concentration in the cortex than in the leaves, maybe one of the compounds present that have the potential to be active in these two olive bodies, explaining the variations in the polyphenol extracted from these two olive bodies' antioxidant capacity.

Table 1 depicts how many distinct olive tree species have had their IC_{50} values determined. The three categories provide an IC_{50} in the range (13-43). The IC_{50} index for Chemlali changes only slightly between different derivatives of the olive tree.

7.4. Composition and fragmentation

Several applications within the pharmaceutical sector provide an opportunity to investigate technologies for processing plant biomass. Fatty acids, polyphenols, flavonoids, triterpenes, and various other compounds undergo chemical fragmentation through the solvolysis of chemlali olive derivative biomass, as detailed by Ksibi and Ksibi (2018). Based on the knowledge gained from traditional medicine, the phenolic fragments from this plant matrix that were thusly solubilized can be employed as precursors for the development of medications. They may also result in fresh formulations of antioxidants and active components, the existence of which depends on the variety.

The recognition of retention times stated in the analytical techniques used to compare the given extract to standards forms the basis for the identification of compounds. Several compounds, including oleuropein, phenolic alcohols, phenolic acids, and other substances in very small percentages, are mentioned in many papers regarding the extraction of the Chemlali variety (Bouaziz et al., 2005; Essafi et al., 2019; Issaoui et al., 2017a; Mechi et al., 2023).

Figure 3 shows the chromatogram of the extract of olive leaves (Ksibi & Ksibi, 2018). This chromatogram clearly shows that oleuropein is the predominant compound. Other phenolic acids were identified and quantified in Chemlali olives. Among these compounds, it has there vanillic acid and *p*-coumaric acid, which have an average concentration. Additional phenolic monomers such as tyrosol, *p*-hydroxyphenylacetic acid, caffeic acid, ferulic acid, and vanillin exhibit low concentrations, they are present as minor compounds in the leaves of the variety of Chemlali. These results agree well with those found in Spanish olive varieties (Brenes-Balbuena et al., 1992).

Mass spectrometry is a technique for chemical characterization that measures the ratio of the mass/charge (*m/z*) of atoms or molecules in the sample to identify and determine its chemical structure. The extract of olive leaves was analyzed by LC-MS/MS instrument using the ionization system with electrospray (ESI) in negative mode. Identification of phenolic compounds of olive leaf variety Chemlali was released by the explanation of the different fragments found in mass spectra MS1 and MS2 in comparison with the literature. The obtained chromatogram obtained allowed us to determine the structures of the compound present in the sample based on the high specificity of the analysis by mass spectrometry.

7.5. Principle extracted compounds

Various extracted substances from olive tree bark are recognized for their antioxidant capacity (using the DPPH radical scavenging) and total phenols amount. The most active extracts were obtained with hydrodistillation (HD) and solvent extraction (SE). Among the components identified by GC-MS, it can be noticed that benzyl cinnamate is a medicine used as a treatment against scabies (Gunning et al., 2012). Furthermore, we recognize the significant presence of VAT compounds such as hydroxytyrosol, tyrosol, and oleuropein. The study of these volatile compounds is primarily linked to their antioxidant properties. Several reports in the literature also describe the antimicrobial properties of phenolic compounds in olive tree products referring to compounds obtained from olive tree derivatives extraction. Following a few details concerning more known compounds (Table 2).

Table 2. The retention time and concentration for selected compounds in different derivatives

Compound	Cultivar	Derivative	Retention time	Concentration	Reference
Oleuropein	Chemlali	Bark	36.46	3.9	(Issaoui et al., 2017a)
	Chemlali	Leaf	-	4.32	(Jemai et al., 2008)
	-	Leaf	18.42	-	(Ghomari et al., 2019)
	-	VOO	6.98	-	(García-Martínez et al., 2016)
	-	Bark	13.1	8.52	(Tóth et al., 2015)
	Chemlali	-	-	8.48	(Mechi et al., 2023)
	Chemlali	-	-	15.6	(Essafi et al., 2019)
Cinnamic acid	-	Leaf	-	0.05-1.09	(Özcan & Matthäus, 2017)
	Arbequina	-	16.62	-	(Quirantes-Piné et al., 2013)
Benzyl cinnamate	Chemlali	Bark	46.92	1.46	(Issaoui et al., 2017a)
Cinnamyl cinnamate	Chemlali	Bark	55.87	1.6	(Issaoui et al., 2017a)
Hydroxytyrosol	Chemlali	Bark	12.66	0.028	(Issaoui et al., 2017a)
	-	Leaf	07.66	0.025	(Ghomari et al., 2019)
	Arbequina	VOO	2.74	0.019	(García-Martínez et al., 2016)
	Arbequina	VOO	9.70	0.016	(Romero & Brenes, 2012)
	-	Pomace	3.19	-	(Dierkes et al., 2012)
	-	Bark	4.03	-	(Tóth et al., 2015)
	Chemlali	-	-	4.19	(Mechi et al., 2023)
	Chemlali	VOO	-	0.092	(Essafi et al., 2019)
Tyrosol	Chemlali	Bark	15.33	0.164	(Issaoui et al., 2017a)
	-	Leaf	10.68	0.2-1.95	(Ghomari et al., 2019)
	Arbequina	EVOO	3.49	2.46	(García-Martínez et al., 2016)
	-	Pomace	11.54	0.298	(Owen et al., 2004)
	-	VOO	10.45	-	(Mateos et al., 2005)
	Arbequina	VOO	14.9	0.1779	(Romero & Brenes, 2012)
	Chemlali	-	-	0.177	(Mechi et al., 2023)
	Chemlali	Leaf	-	0.041	(Essafi et al., 2019)
Phytol	Chemlali	Bark	47.7	0.198	(Issaoui et al., 2017a)
	-	-	10.8	-	(Vetter et al., 2012)
Squalene	Chemlali	Bark	67.35	1.796	(Issaoui et al., 2017a)
	Arbequina	VOO	33.5	0.8-1.2	(Lanzón et al., 1994)
	-	VOO	53.41	0.2-16.2	(Aresta et al., 2020)
	Chemlali	Leaf	-	3.26	(Issaoui et al., 2017b)

VOO: Virgin Olive Oil, EVOO : Extra Virgin Olive Oil

7.5.1. Phytol ($C_{20}H_{40}O$)

Phytol is a significant acyclic diterpene alcohol that is present in all plants with esterified chlorophyll and can be employed as a precursor to vitamins E and K, as well as a component of chlorophyll. In actuality, the molecules of chlorophyll are made up of methanol and phytol derivatives known as esters of phytol. Phytol is naturally present in oil sediment and is the most promising precursor of the isoprenoid hydrocarbons found in oil shale.

Concerning olive tree derivatives extraction, the phytol component was detected using a chromatography column at tr 10.8 min by Vetter et al. (2012). In contrast, according to Issaoui et al. (2017a), its peak may be seen in the chromatogram at about 47 min. The extracted pythol content in this instance may be greater than 0.9 mg/g DW.

7.5.2. Cinnamyl cinnamate ($C_{18}H_{12}O_2$)

Cinnamyl cinnamate is an element of the group of organic substances called cinnamic acid esters. It is an odoriferous ingredient found in many fragrance compounds. In addition to domestic cleansers and detergents, it can be found in decorative cosmetics, shampoos, and bathroom soaps. It naturally exists in *Goniothalamus borneensis*, *Populus candicans*, *Cinnamomum cassia*, Peruvian white balsam, and other organisms' bark. According to Issaoui et al. (2017a), the component cinnamyl cinnamate was found using a chromatography column at tr 55.87 min during the extraction of olive bark, with a concentration of roughly 1.46 mg/g DW.

7.5.3. Oleuropein ($C_{25}H_{32}O_{13}$)

Oleuropein (or oleopicrin) is a phytochemical compound found in olive leaves, raw olive, and olive oil, as well as in argan oil and privet leaves. It is an ester of elenolic acid and hydroxylated and glycosylated tyrosol, constituting a tannin that gives the raw olive a burning taste and makes it inedible. Together with hydroxytyrosol, its degradation metabolite, it has powerful antioxidant properties and gives extra virgin olive oil its bitter taste.

Oleuropein and its metabolite hydroxytyrosol exhibit strong antioxidant activity, which may contribute to the antioxidant, anti-inflammatory, and disease-fighting properties of olive oil. In particular, oleuropein is known for its ability to reduce blood pressure. Chemlali leaves shows also secoiroids mainly oleuropein, verbascoside, and oleoside. Oleuropein is the dominant phenolic compound in the different varieties of olives and reaches a concentration from leaves, in dry weight of about 4 mg/g as shown in several papers (Issaoui et al., 2017b; Jemai et al., 2008).

7.5.4. Benzyl cinnamate ($C_{16}H_{14}O_2$)

Benzyl cinnamate is an ester that is obtained by the condensation of benzyl alcohol and cinnamic acid. It is one of the fragrance compounds, that is widely used in food and pharmaceutics (Bhatia et al., 2007). Benzyl cinnamate is a fragrance ingredient commonly used in heavy oriental perfumes as well as in cosmetics for its discrete scent. Its natural occurrence in the trunk of olive trees and roots of certain plants remains hidden until its scent is revealed. Benzyl cinnamate finds also applications as a fixative and in the production of strong oriental fragrances. It is used as a flavoring agent. From olive bark, benzyl cinnamate was extracted via hydrodistillation technique at 1.46 mg/g in dry weight (Issaoui et al., 2017a). However, according to (Özcan & Matthäus, 2017), it is primarily produced synthetically and has a concentration that ranges from 0.005 to 1.09 mg per gram when extracted from leaves.

7.5.5. Cinnamic acid ($C_9H_8O_2$)

The natural compound cinnamic acid is a white crystalline substance that has limited solubility in water but is easily soluble in many organic solvents. It is considered an unsaturated carboxylic acid and can be found in various plants. It serves as a crucial intermediate in the biosynthesis of various natural products, such as lignols (which are the precursors to lignin and lignocellulose), flavonoids, isoflavonoids, coumarins, aurones, stilbenes, catechin, and phenylpropanoids. This versatile compound is derived from the shikimic acid pathway and is a precursor to the biosynthesis of other important compounds in plants. Cinnamic acid is also used in the food, pharmaceutical, and cosmetic industries due to its antioxidant, antimicrobial, and anti-inflammatory properties.

Cinnamic acid was identified by Quirantes-Piné et al. (2013) in an olive leaf extraction of the arbequina variety using a chromatography column at tr 16.62 min. A variable concentration of cinnamic acid from leaves can approach 1 mg/g DW (Özcan & Matthäus, 2017), according to previous results provided in the literature, although the extracted cinnamic acid content was around 0.83 mg/g DW (Yorulmaz et al., 2012).

7.5.6. Tyrosol ($C_8H_{10}O_2$)

Tyrosol is a natural phenolic antioxidant found in olive leaves and olive oil and to a lesser extent in argan oil and white wine. It is also found in extracts in the form of glucosides. Tyrosol was shown to be an effective cellular antioxidant, probably due to intracellular accumulation, despite its weak antioxidant activity. Tyrosol, the major olive oil biophenol, protects against oxidative stress that causes cellular damage, tyrosol, a phenolic compound, ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. According to Ghomari et al. (2019), olive leaves contain roughly 0.2-1.95 mg/g of tyrosol, while olive bark contains about 0.164 mg/g via hydrodistillation as mentioned by Issaoui et al. (2017a).

7.5.7. Hydroxytyrosol ($C_8H_{10}O_3$)

Hydroxytyrosol, which is among the major phenolic compounds found in olives, virgin oil, and wastewater produced during olive oil manufacturing, is mainly present in an esterified form in fresh virgin oil. However, over time, non-esterified hydroxytyrosol becomes more dominant due to hydrolytic reactions (Angerosa et al., 1995; Cinquanta et al., 1997).

Hydroxytyrosol is phenolic alcohol and has been hypothesized to exert a wide range of biological effects, including cardioprotective, anticancer, neuroprotective antimicrobial, beneficial endocrine, and other effects. Despite this, it is believed that the antioxidant properties of hydroxytyrosol are one of the main reasons for its health-promoting effects. It has been shown to protect cells from oxidative damage caused by free radicals, which are unstable molecules that can harm cells and contribute to the development of diseases such as cancer, Alzheimer's, and heart disease.

Despite numerous studies, the precise molecular mechanisms responsible for these effects are still not fully understood.

7.5.8. Squalene ($C_{30}H_{50}$)

Squalene is one of the substances derived from derivatives of olives. It is a naturally occurring 30-carbon lipid that was first obtained commercially from shark liver oil (hence the name), but it is now also obtained from plant sources, primarily vegetable oils, such as amaranth seeds, rice bran, and wheat germ, as well as in smaller amounts (0.1–0.7%) from olive oil and other cereal oils (Akgün, 2011). As long as it has a significant capacity to absorb oxygen, it is a crucial step in the production of cholesterol. Squalene serves as the building block for stigmasterol in plants (Popa et al., 2015). It is the precursor to ergosterol in some fungi. Squalene, however, is not produced by blue-green algae or all bacteria; therefore, if they require it, they must obtain it from the environment (Martínez-Beamonte et al., 2020).

As mentioned by Aresta et al. (2020), squalene occurs in olive oil at concentrations in the range of 0.2 to 16.2 mg/g. According to Issaoui et al. (2017b), the supercritical fluid extraction method was used to extract the squalene from the Chemlali leaf, and the peak was attained after 67.35 minutes as seen in the chromatogram (**Figure 3**). Additionally, the concentration increased from 1.8 mg/g (at P=25 MPa) to 3.27 mg/g (at P=30 MPa) by using supercritical fluid extraction.

4. Conclusions

Recent studies reveal an interest in applying innovative extraction strategies to acquire bioactive byproducts, specifically phenolic chemicals, from olive tree derivatives. The use of non-standardized extraction processes, on the other hand, might result in variances in the chemical makeup of various plant sections. We summarized alternative extraction procedures for phenolic compounds from olive leaf, stone, pulp, and bark and assessed their antioxidant activity using the DPPH test. Upon comparing the excessively planted olive cultivars in Tunisia, namely koroneiki, arbequina, and chemlali, it is apparent that the chemlali variety exhibits a higher content of polyphenols and triterpenes. With an IC_{50} of 14.84 mg/ml, the Chemlali bark extract has a stronger antioxidant capacity than the leaf extract and virgin olive oil. Hydrodistillation appears to be a suitable method for extracting small quantities of benzyl and cinnamyl cinnamates from the Chemlali variety. With SF extraction, it was shown that derivatives treated with supercritical carbon dioxide can produce from Chemlali olive genotype different extracts with high VAT such as squalene and cinnamates where yields increase significantly at a moderate pressure of 90 bar.

Furthermore, it is observed that the main active principles and substances extracted from the Chemlali variety are comparable in quantity to those extracted from other varieties, although the richness and diversity of the Chemlali variety remain distinct.

Acknowledgments

None.

Conflict of interest

The author confirms that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Funding

None.

CRediT authorship contribution statement

Hatem Ksibi: Investigation, Methodology, Data collection, Writing, Formal analysis

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Supplementary File

None.

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References

Abaza, L., Talorete, T. P., Yamada, P., Kurita, Y., Zarrouk, M., & Isoda, H. (2007). Induction of growth inhibition and differentiation of human leukemia HL-60 cells by a Tunisian gerboui olive leaf extract. *Bioscience, Biotechnology, and Biochemistry, 71*(5), 1306-1312.

Abdelhamid, S., Gouta, H., Gharsallaoui, M., Ghrab, M., Kwon, Y., Yoon, I. S., & Byun, M. O. (2013). A review on current status of olive and olive oil production in Tunisia. *Journal of the Korean Society of International Agriculture, 25*, 351-357.

Akgün, N. A. (2011). Separation of squalene from olive oil deodorizer distillate using supercritical fluids. *European Journal of Lipid Science and Technology, 113*(12), 1558-1565.

Angeloni, C., Malaguti, M., Barbalace, M. C., & Hrelia, S. (2017). Bioactivity of olive oil phenols in neuroprotection. *International Journal of Molecular Sciences, 18*(11), 2230.

Angerosa, F., d'Alessandro, N., Konstantinou, P., & Di Giacinto, L. (1995). GC-MS evaluation of phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry, 43*(7), 1802-1807.

Aresta, A., Damascelli, A., de Vietro, N., & Zambonin, C. (2020). Measurement of squalene in olive oil by fractional crystallization or headspace solid phase microextraction coupled with gas chromatography. *International Journal of Food Properties, 23*(1), 1845-1853.

Belaqziz, M., Tan, S. P., El-Abbassi, A., Kiai, H., Hafidi, A., O'Donovan, O., & McLoughlin, P. (2017). Assessment of the antioxidant and antibacterial activities of different olive processing wastewaters. *PLoS One, 12*(9), e0182622.

Ben Mansour, A., Porter, E. A., Kite, G. C., Simmonds, M. S., Abdelhedi, R., & Bouaziz, M. (2015). Phenolic profile characterization of Chemlali olive stones by liquid chromatography-ion trap mass spectrometry. *Journal of Agricultural and Food Chemistry, 63*(7), 1990-1995.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuño, A., & del Rio, J. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chemistry, 68*(4), 457-462.

Bhatia, S., Wellington, G., Cocchiara, J., Lalko, J., Letizia, C., & Api, A. (2007). Fragrance material review on cinnamyl cinnamate. *Food and Chemical Toxicology, 45*(1), S66-S69.

Bisignano, G., Tomaino, A., Cascio, R. L., Crisafi, G., Uccella, N., & Saija, A. (1999). On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. *Journal of Pharmacy and Pharmacology, 51*(8), 971-974.

Bouallagui, Z., Han, J., Isoda, H., & Sayadi, S. (2011). Hydroxytyrosol rich extract from olive leaves modulates cell cycle progression in MCF-7 human breast cancer cells. *Food and Chemical Toxicology, 49*(1), 179-184.

Bouaziz, M., Grayer, R. J., Simmonds, M. S., Damak, M., & Sayadi, S. (2005). Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar Chemlali growing in Tunisia. *Journal of Agricultural and Food Chemistry, 53*(2), 236-241.

Brahmi, F., Flamini, G., Issaoui, M., Dhibi, M., Dabbou, S., Mastouri, M., & Hammami, M. (2012). Chemical composition and biological activities of volatile fractions from three Tunisian cultivars of olive leaves. *Medicinal Chemistry Research, 21*, 2863-2872.

Brenes-Balbuena, M., Garcia-Garcia, P., & Garrido-Fernandez, A. (1992). Phenolic compounds related to the black color formed during the processing of ripe olives. *Journal of Agricultural and Food Chemistry, 40*(7), 1192-1196.

Brito, C., Dinis, L. T., Moutinho-Pereira, J., & Correia, C. M. (2019). Drought stress effects and olive tree acclimation under a changing climate. *Plants, 8*(7), 232.

Calabriso, N., Scoditti, E., Pellegrino, M., & Carluccio, M. A. (2015). Olive oil. In V. Preedy & R. Watson (Eds.), *The Mediterranean Diet* (pp. 135-142): Elsevier.

Capasso, R., Evidente, A., & Scognamiglio, F. (1992). A simple thin layer chromatographic method to detect the main polyphenols occurring in olive oil vegetation waters. *Phytochemical Analysis, 3*(6), 270-275.

Cinquanta, L., Esti, M., & Notte, E. L. (1997). Evolution of phenolic compounds in virgin olive oil during storage. *Journal of the American Oil Chemists' Society, 74*, 1259-1264.

Clodoveo, M. L., Crupi, P., Annunziato, A., & Corbo, F. (2021). Innovative extraction technologies for development of functional ingredients based on polyphenols from olive leaves. *Foods, 11*(1), 103.

da Silva, J. C. E. (2010). Chemometric classification of cultivars of olives: Perspectives on Portuguese olives. In V. Preedy & R. Watson (Eds.), *Olives and olive oil in health and disease prevention* (pp. 33-42): Elsevier.

da Silva, M., Freitas, A. M. C., Cabrita, M., & Garcia, R. (2012). Olive oil composition: Volatile compounds. In D. Boskou (Ed.), *Olive Oil-Constituents, Quality, Health Properties and Bioconversions* (pp. 17-46): IntechOpen.

Dauber, C., Carreras, T., González, L., Gámbaro, A., Valdés, A., Ibanez, E., & Vieitez, I. (2022). Characterization and incorporation of extracts from olive leaves obtained through maceration and supercritical extraction in Canola oil: Oxidative stability evaluation. *LWT, 160*, 113274.

de la Puerta, R. O., Domínguez, M. E. M. N., Ruíz-Gutíerrez, V., Flavill, J. A., & Hoult, J. R. S. (2001). Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrergic neurotransmission. *Life Sciences, 69*(10), 1213-1222.

De Leonardis, A., Aretini, A., Alfano, G., Macciola, V., & Ranalli, G. (2008). Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea europaea* L.) and evaluation of its antioxidant properties and bioactivity. *European Food Research and Technology, 226*, 653-659.

de Melo, M. M., Oliveira, E. L., Silvestre, A. J., & Silva, C. M. (2012). Supercritical fluid extraction of triterpenic acids from *Eucalyptus globulus* bark. *The Journal of Supercritical Fluids, 70*, 137-145.

Debbabi, O. S., Amar, F. B., Rahmani, S. M., Taranto, F., Montemurro, C., & Miazzi, M. M. (2022). The Status of Genetic Resources and Olive Breeding in Tunisia. *Plants, 11*(13), 1759.

Deng, J., Xu, Z., Xiang, C., Liu, J., Zhou, L., Li, T., Yang, Z., & Ding, C. (2017). Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives. *Ultrasonics Sonochemistry, 37*, 328-334.

Dierkes, G., Krieger, S., Dück, R., Bongartz, A., Schmitz, O. J., & Hayen, H. (2012). High-performance liquid chromatography–mass spectrometry profiling of phenolic compounds for evaluation of olive oil bitterness and pungency. *Journal of Agricultural and Food Chemistry, 60*(31), 7597-7606.

El, S. N., & Karakaya, S. (2009). Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. *Nutrition Reviews, 67*(11), 632-638.

Erbay, Z., & Icier, F. (2010). The importance and potential uses of olive leaves. *Food Reviews International, 26*(4), 319-334.

Essafi, H., Trabelsi, N., Benincasa, C., Tamaalli, A., Perri, E., & Zarrouk, M. (2019). Phytochemical profile, antioxidant and antiproliferative activities of olive leaf extracts from autochthonous Tunisian cultivars. *Acta Alimentaria, 48*(3), 384-390.

Fornari, T., Vicente, G., Vázquez, E., García-Risco, M. R., & Reglero, G. (2012). Isolation of essential oil from different plants and herbs by supercritical fluid extraction. *Journal of Chromatography A, 1250*, 34-48.

García-Martínez, O., De Luna-Bertos, E., Ramos-Torrecillas, J., Ruiz, C., Milia, E., Lorenzo, M. L., Jimenez, B., Sánchez-Ortiz, A., & Rivas, A. (2016). Phenolic compounds in extra virgin olive oil stimulate human osteoblastic cell proliferation. *PLoS One, 11*(3), e0150045.

García-Villalba, R., Carrasco-Pancorbo, A., Nevedomskaya, E., Mayboroda, O. A., Deelder, A. M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Exploratory analysis of human urine by LC–ESI-TOF MS after high intake of olive oil: Understanding the metabolism of polyphenols. *Analytical and Bioanalytical Chemistry, 398*, 463-475.

García Pérez, J., Mulet Pons, A., & Carcel Carrión, J. (2011). Ultrasound-assisted extraction of natural products. *Food Engineering Reviews, 3*, 108-120.

Gharsallaoui, M., Benincasa, C., Ayadi, M., Perri, E., Khlif, M., & Gabsi, S. (2011). Study on the impact of wastewater irrigation on the quality of oils obtained from olives harvested by hand and from the ground and extracted at different times after the harvesting. *Scientia Horticulturae, 128*(1), 23-29.

Ghasemi, S., Koohi, D. E., Emmamzadehhashemi, M. S. B., Khamas, S. S., Moazen, M., Hashemi, A. K., Amin, G., Golfakhrabadi, F., Yousefi, Z.*, et al.* (2018). Investigation of phenolic compounds and antioxidant activity of leaves extracts from seventeen cultivars of Iranian olive (*Olea europaea* L.). *Journal of Food Science and Technology, 55*, 4600-4607.

Ghomari, O., Sounni, F., Massaoudi, Y., Ghanam, J., Kaitouni, L. B. D., Merzouki, M., & Benlemlih, M. (2019). Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. *Biotechnology Reports, 23*, e00347.

Gonçalves, A., Silva, E., Brito, C., Martins, S., Pinto, L., Dinis, L. T., Luzio, A., Martins-Gomes, C., Fernandes-Silva, A.*, et al.* (2020). Olive tree physiology and chemical composition of fruits are modulated by different deficit irrigation strategies. *Journal of the Science of Food and Agriculture, 100*(2), 682-694.

Gueboudji, Z., Kadi, K., Mahmoudi, M., Hannachi, H., Nagaz, K., Addad, D., Yahya, L. B., Lachehib, B., & Hessini, K. (2023). Maceration and liquid–liquid extractions of phenolic compounds and antioxidants from Algerian olive oil mill wastewater. *Environmental Science and Pollution Research, 30*(2), 3432-3439.

Gunning, K., Pippitt, K., Kiraly, B., & Sayler, M. (2012). Pediculosis and scabies: a treatment update. *American Family Physician, 86*(6), 535-541.

Hashmi, M. A., Khan, A., Hanif, M., Farooq, U., & Perveen, S. (2015). Traditional uses, phytochemistry, and pharmacology of *Olea europaea* (olive). *Evidence-Based Complementary and Alternative Medicine, 2015*, 541591.

IOC. (2022). International Olive Council. Retrieved from https://www.internationaloliveoil.org/the-world-of-olive-oil/.

Issaoui, A., Ksibi, H., & Ksibi, M. (2017a). Comparison between several techniques of olive tree bark extraction (Tunisian Chemlali variety). *Natural Product Research, 31*(1), 113-116.

Issaoui, A., Ksibi, H., & Ksibi, M. (2017b). Supercritical fluid extraction of triterpenes and aliphatic hydrocarbons from olive tree derivatives. *Arabian Journal of Chemistry, 10*, S3967-S3973.

Issaoui, A., Mahfoudh, A., Ksibi, H., & Ksibi, M. (2012). Composition of the olive tree bark: Richness in Oleuropein. *Trends in Chemical Engineering, 14*, 65-69.

Issazadeh, K., Aliabadi, M. A., Darsanaki, R. K., & Pahlaviani, M. R. M. K. (2012). Antimutagenic activity of olive leaf aqueous extract by Ames test. *Advanced Studies in Biology, 4*(9), 397-405.

Japón-Luján, R., Luque-Rodríguez, J., & De Castro, M. L. (2006). Dynamic ultrasound-assisted extraction of oleuropein and related biophenols from olive leaves. *Journal of Chromatography A, 1108*(1), 76-82.

Jemai, H., Bouaziz, M., Fki, I., El Feki, A., & Sayadi, S. (2008). Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. *Chemico-Biological Interactions, 176*(2-3), 88-98.

Khwaldia, K., Attour, N., Matthes, J., Beck, L., & Schmid, M. (2022). Olive byproducts and their bioactive compounds as a valuable source for food packaging applications. *Comprehensive Reviews in Food Science and Food Safety, 21*(2), 1218-1253.

Ksibi, H. (2004). The solvent-solute interaction in supercritical solution at equilibrium: modeling and related industrial applications. *International Journal of Thermodynamics, 7*(3), 131-140.

Ksibi, H., & Ksibi, M. (2018). Structural analysis of the main phenolic compounds in olive leaves (Tunisian Chemlali variety). *Trends in Chemical Engineering*, 1-16.

Kulak, M., & Cetinkaya, H. (2018). A systematic review: polyphenol contents in stressed-olive trees and its fruit oil. In J. Wong (Ed.), *Polyphenols* (pp. 3-20): IntechOpen.

Langgut, D., Cheddadi, R., Carrión, J. S., Cavanagh, M., Colombaroli, D., Eastwood, W. J., Greenberg, R., Litt, T., Mercuri, A. M.*, et al.* (2019). The origin and spread of olive cultivation in the Mediterranean Basin: The fossil pollen evidence. *The Holocene, 29*(5), 902-922.

Lanzón, A., Albi, T., Cert, A., & Gracián, J. (1994). The hydrocarbon fraction of virgin olive oil and changes resulting from refining. *Journal of the American Oil Chemists' Society, 71*, 285-291.

Lavee, S., & Avidan, N. (1993). *Protein content and composition of leaves and shoot bark in relation to alternate bearing of olive trees (Olea europaea L.).* Paper presented at the II International Symposium on Olive Growing 356.

Le Floch, F., Tena, M., Rios, A., & Valcarcel, M. (1998). Supercritical fluid extraction of phenol compounds from olive leaves. *Talanta, 46*(5), 1123-1130.

Le Tutour, B., & Guedon, D. (1992). Antioxidative activities of *Olea europaea* leaves and related phenolic compounds. *Phytochemistry, 31*(4), 1173-1178.

Lee-Huang, S., Zhang, L., Huang, P. L., Chang, Y. T., & Huang, P. L. (2003). Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. *Biochemical and Biophysical Research Communications, 307*(4), 1029-1037.

Lo Giudice, V., Faraone, I., Bruno, M. R., Ponticelli, M., Labanca, F., Bisaccia, D., Massarelli, C., Milella, L., & Todaro, L. (2021). Olive trees by-products as sources of bioactive and other industrially useful compounds: A systematic review. *Molecules, 26*(16), 5081.

Lone, R., Shuab, R., & Koul, K. (2014). Role of cinnamate and cinnamate derivatives in pharmacology. *Journal of Pharmacology, 8*, 328-335.

Malik, N., & Bradford, J. (2008). Stability of oleuropein and other phenolic compounds during various extraction and processing methods of olive leaves. *Journal of Food, Agriculture, and Environment, 6*, 8-13.

Manna, C., Migliardi, V., Golino, P., Scognamiglio, A., Galletti, P., Chiariello, M., & Zappia, V. (2004). Oleuropein prevents oxidative myocardial injury induced by ischemia and reperfusion. *The Journal of Nutritional Biochemistry, 15*(8), 461-466.

Mansour-Guedes, S. B., Saidana, D., Jabnoun-Khiareddine, H., Bchir, A., Daami-Remadi, M., & Braham, M. (2020). Chemical composition and biological activities assess-ment of olive fruit volatiles from different varieties grown in Tunisia. *Acta Scientiarum Polonorum Hortorum Cultus, 19*(4), 3-20.

Mariem, Z., Zied, D. M., Ooussema, J., & Salah, B. (2019). Assessment of the Tunisian olive oil value chain in the international markets: Constraints and Opportunities. *FARA Research Result, 4*(2), PP36.

Markin, D., Duek, L., & Berdicevsky, I. (2003). In vitro antimicrobial activity of olive leaves. Antimikrobielle Wirksamkeit von Olivenblättern in vitro. *Mycoses, 46*(3-4), 132-136.

Martín-García, B., De Montijo-Prieto, S., Jiménez-Valera, M., Carrasco-Pancorbo, A., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M. (2022). Comparative extraction of phenolic compounds from olive leaves using a sonotrode and an ultrasonic bath and the evaluation of both antioxidant and antimicrobial activity. *Antioxidants, 11*(3), 558.

Martínez-Beamonte, R., Sanclemente, T., Surra, J. C., & Osada, J. (2020). Could squalene be an added value to use olive by-products? *Journal of the Science of Food and Agriculture, 100*(3), 915-925.

Mateos, R., Goya, L., & Bravo, L. (2005). Metabolism of the olive oil phenols hydroxytyrosol, tyrosol, and hydroxytyrosyl acetate by human hepatoma HepG2 cells. *Journal of Agricultural and Food Chemistry, 53*(26), 9897-9905.

McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry, 73*(1), 73-84.

Mechi, D., Baccouri, B., Martín-Vertedor, D., & Abaza, L. (2023). Bioavailability of phenolic compounds in Californian-Style table olives with Tunisian aqueous olive leaf extracts. *Molecules, 28*(2), 707.

Menendez, J. A., Vazquez-Martin, A., Garcia-Villalba, R., Carrasco-Pancorbo, A., Oliveras-Ferraros, C., Fernandez-Gutierrez, A., & Segura-Carretero, A. (2008). TabAnti-HER2 (erbB-2) oncogene effects of phenolic compounds directly isolated from commercial Extra-Virgin Olive Oil (EVOO). *Bmc Cancer, 8*, 377.

Mohamed, M. B., Guasmi, F., Ali, S. B., Radhouani, F., Faghim, J., Triki, T., Kammoun, N. G., Baffi, C., Lucini, L.*, et al.* (2018). The LC-MS/MS characterization of phenolic compounds in leaves allows classifying olive cultivars grown in South Tunisia. *Biochemical Systematics and Ecology, 78*, 84-90.

Naija, D. S., Gueddes, S. B. M., Flamini, G., Khiareddine, H. J., Remadi, M. D., Mariem, F. B., Ghariani, W., & Braham, M. (2021). Assessment of Antioxidant and Antimicrobial Compounds of Volatiles from Leaves, Stems and Flowers of Olives. *Polish Journal of Environmental Studies, 30*(2), 1325–1338.

Obied, H. K., Allen, M. S., Bedgood, D. R., Prenzler, P. D., & Robards, K. (2005). Investigation of Australian olive mill waste for recovery of biophenols. *Journal of Agricultural and Food Chemistry, 53*(26), 9911-9920.

Oliveras-Ferraros, C., Fernandez-Arroyo, S., Vazquez-Martin, A., Lozano-Sanchez, J., Cufi, S., Joven, J., Micol, V., Fernandez-Gutierrez, A., Segura-Carretero, A.*, et al.* (2011). Crude phenolic extracts from extra virgin olive oil circumvent de novo breast cancer resistance to HER1/HER2-targeting drugs by inducing GADD45-sensed cellular stress, G2/M arrest and hyperacetylation of Histone H3. *International Journal of Oncology, 38*(6), 1533-1547.

Owen, R., Haubner, R., Würtele, G., Hull, W., Spiegelhaider, B., & Bartsch, H. (2004). Olives and olive oil in cancer prevention. *European Journal of Cancer Prevention, 13*(4), 319-326.

Özcan, M. M., & Matthäus, B. (2017). A review: Benefit and bioactive properties of olive (*Olea europaea* L.) leaves. *European Food Research and Technology, 243*, 89-99.

Pacheco, A., Barros, L., Freitas, M., Reis, M., Hipólito, C., & Oliveira, O. (2002). An evaluation of olive-tree bark for the biological monitoring of airborne trace-elements at ground level. *Environmental Pollution, 120*(1), 79-86.

Parra, A., Martin-Fonseca, S., Rivas, F., Reyes-Zurita, F. J., Medina-O'Donnell, M., Martinez, A., Garcia-Granados, A., Lupianez, J. A., & Albericio, F. (2014). Semi-synthesis of acylated triterpenes from olive-oil industry wastes for the development of anticancer and anti-HIV agents. *European Journal of Medicinal Chemistry, 74*, 278-301.

Pérez-Rodrigo, C., & Aranceta, J. (2016). Olive oil: Its role in the diet. 158-166.

Popa, O., Băbeanu, N. E., Popa, I., Niță, S., & Dinu-Pârvu, C. E. (2015). Methods for obtaining and determination of squalene from natural sources. *BioMed Research International, 2015*, 367202.

Quirantes-Piné, R., Lozano-Sánchez, J., Herrero, M., Ibáñez, E., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2013). HPLC–ESI–QTOF–MS as a powerful analytical tool for characterising phenolic compounds in olive-leaf extracts. *Phytochemical Analysis, 24*(3), 213-223.

Rada, M., Guinda, Á., & Cayuela, J. (2007). Solid/liquid extraction and isolation by molecular distillation of hydroxytyrosol from *Olea europaea* L. leaves. *European Journal of Lipid Science and Technology, 109*(11), 1071-1076.

Rashed, S. A., Saad, T. I., & El-Darier, S. M. (2022). Potential aptitude of four olive cultivars as anticancer and antioxidant agents: Oleuropein content. *Rendiconti Lincei. Scienze Fisiche e Naturali, 33*(1), 195-203.

Reverchon, E., & De Marco, I. (2006). Supercritical fluid extraction and fractionation of natural matter. *The Journal of Supercritical Fluids, 38*(2), 146-166.

Romero, C., & Brenes, M. (2012). Analysis of total contents of hydroxytyrosol and tyrosol in olive oils. *Journal of Agricultural and Food Chemistry, 60*(36), 9017-9022.

Rufino-Palomares, E. E., Pérez-Jiménez, A., García-Salguero, L., Mokhtari, K., Reyes-Zurita, F. J., Peragón-Sánchez, J., & Lupiáñez, J. A. (2022). Nutraceutical role of polyphenols and triterpenes present in the extracts of fruits and leaves of *Olea europaea* as antioxidants, anti-infectives and anticancer agents on healthy growth. *Molecules, 27*(7), 2341.

Ryan, D., Antolovich, M., Prenzler, P., Robards, K., & Lavee, S. (2002). Biotransformations of phenolic compounds in *Olea europaea* L. *Scientia Horticulturae, 92*(2), 147-176.

Ryan, D., Robards, K., & Lavee, S. (1999). Changes in phenolic content of olive during maturation. *International Journal of Food Science & Technology, 34*(3), 265-274.

Sellami, M. K., Khemakhem, I., Mkadmini, K., & Bouzouita, N. (2016). Chemical composition, antioxidant potential and phenolic profile of oil mill waste water from Tunisian olive varieties (Chetoui and Chemlali). *Mediterranean Journal of Chemistry, 5*(6), 605-614.

Senouci, H., Benyelles, N. G., Dib, M. E., Costa, J., & Muselli, A. (2020). *Ammoides verticillata* essential oil as biocontrol agent of selected fungi and pest of olive tree. *Recent Patents on Food, Nutrition & Agriculture, 11*(2), 182-188.

Shaw, T. M. (2000). Ascetic Eucharists: Food and Drink in Early Christian Ritual Meals. *Journal of Early Christian Studies, 8*(4), 608-609.

Spadi, A., Angeloni, G., Cecchi, L., Corti, F., Balli, D., Guerrini, L., Calamai, L., Parenti, A., & Masella, P. (2023). Exploiting Virgin Olive Oil By-products Using Hydrodistillation. *Waste and Biomass Valorization, 14*(6), 1931-1944.

Strub, D. J., Talma, M., Strub, M., Rut, W., Zmudzinski, M., Brud, W., Neyts, J., Vangeel, L., Zhang, L., *et al.* (2022). Evaluation of the anti-SARS-CoV-2 properties of essential oils and aromatic extracts. *Scientific Reports, 12*(1), 14230.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules, 14*(6), 2167-2180.

Tacer, S., & Aşan Özüsağlam, M. (2022). The Potential Uses of Olive Leaf Extracts in Various Areas. *Eurasian Journal of Food Science and Technology, 6*(1), 8-22.

Tamasi, G., Bonechi, C., Belyakova, A., Pardini, A., & Rossi, C. (2016). The olive tree, a source of antioxidant compounds. *Journal of the Siena Academy of Sciences, 8*(1), 10-29.

Toric, J., Karkovic Markovic, A., Jakobusic Brala, C., & Barbaric, M. (2019). Anticancer effects of olive oil polyphenols and their combinations with anticancer drugs. *Acta Pharmaceutica, 69*(4), 461-482.

Tóth, G., Alberti, Á., Sólyomváry, A., Barabás, C., Boldizsár, I., & Noszál, B. (2015). Phenolic profiling of various olive bark-types and leaves: HPLC–ESI/MS study. *Industrial Crops and Products, 67*, 432-438.

Ueda, H., Yamazaki, C., & Yamazaki, M. (2002). Luteolin as an anti-inflammatory and anti-allergic constituent of *Perilla frutescens*. *Biological and Pharmaceutical Bulletin, 25*(9), 1197-1202.

Ünver, N., & Çelik, Ş. (2022). Optimization of ultrasound-assisted extraction of olive leaf (var. *halhali*) extracts. *Hacettepe Journal of Biology and Chemistry, 50*(2), 173-184.

Vetter, W., Schröder, M., & Lehnert, K. (2012). Differentiation of refined and virgin edible oils by means of the trans-and cis-phytol isomer distribution. *Journal of Agricultural and Food Chemistry, 60*(24), 6103-6107.

Wang, W., Tai, F., & Hu, X. (2010). Current initiatives in proteomics of the olive tree. In V. Preedy & R. Watson (Eds.), *Olives and olive oil in health and disease prevention* (pp. 25-32): Elsevier.

Yorulmaz, A., Poyrazoglu, E. S., Ozcan, M. M., & Tekin, A. (2012). Phenolic profiles of Turkish olives and olive oils. *European Journal of Lipid Science and Technology, 114*(9), 1083-1093.

Yuan, J. J., Wang, C. Z., Ye, J. Z., Tao, R., & Zhang, Y. S. (2015). Enzymatic hydrolysis of oleuropein from *Olea europea* (olive) leaf extract and antioxidant activities. *Molecules, 20*(2), 2903-2921.

Zheljazkov, V. D., Astatkie, T., & Schlegel, V. (2014). Hydrodistillation extraction time effect on essential oil yield, composition, and bioactivity of coriander oil. *Journal of Oleo Science, 63*(9), 857-865.