Minireview Article

Virgin Olive Oil, a rich source of functional bioactive compounds in the promotion of human health : An overview

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Abstract

The prevention of pathologies by diet management is an important public health challenge. The Mediterranean diet (Med diet) is considered one of the healthiest dietary patterns. According to current scientific research, this dietary pattern is linked to a decreased prevalence and incidence of various chronic diseases, including cardiovascular disease, diabetes, cancer, and age-related cognitive decline, as well as a lower overall mortality rate. In the Mediterranean diet, a high consumption of Virgin Olive Oil (VOO), fruit, nuts, vegetables, and grains is linked to a decreased incidence of many diseases and a longer life expectancy. According to numerous studies, VOO is the major food responsible for the health and nutritional benefits of the Mediterranean diet. VOO chemical compounds have been shown *in vitro* and *in vivo* investigations to have potentially favorable health benefits as a result of their biological activities. VOO composition has been linked to a variety of biological activities, including antioxidant, anti-inflammatory, anti-diabetic, anti-cancer, and antiviral characteristics. As a result, increasing attention is being paid to the composition of VOO and the identification of its biologically active components. Because of their antioxidant, anti-inflammatory, and anti-thrombotic properties, phytochemicals, particularly phenolic compounds, have health-promoting effects, especially on cardiovascular and metabolic illnesses. These characteristics are even more important in light of the COVID-19 severe illness's heightened inflammatory and pro-thrombotic consequences. This overview collects and discusses the scattered data available in the literature concerning VOO compounds of biological interest and highlights their possible mechanisms of action and effects on human health. *In vitro* studies and *in vivo* intervention trials were selected and included in the study after conducting literature searches through "PubMed" and "Web of Science." In the majority of investigations, the ability of VOO phytochemicals to prevent the oxidation process at both the initiation and promotion/progression phases of several pathologies has been verified. The health benefits discussed in this article support the prospective health benefits acquired from VOO as a possible candidate in developing pharmaceutical preparations and nutraceutical or functional foods for a variety of pathological disorders. This idea could pave the way for future *in vivo* research and, eventually, clinical trials. In addition, greater research into the mechanisms of action and efficacy is needed to clarify the real biological potential of VOO phytochemicals on humans by performing intervention studies on populations at high disorder risk.

Keywords: Virgin Olive Oil (VOO), bioactive compounds, biological activities, beneficial health effects

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Abbreviations

AChE: Acetylcholinesterase, **ACE2:** Angiotensin-Converting Enzyme 2, **BHT:** 2,6-di-tert-butyl-hydroxytoluene, **BuChE:** Butyrylcholinesterase, **CAT:** Catalase, **CNS:** Central nervous system, **COX:** Cyclooxygenase, **DNA:** Deoxyribonucleic acid, **EFSA:** European Food Safety Authority, **GSH:** Glutathione, **HDL:** high density lipoprotein; **HCV:** Hepatitis C virus, **HIV:** human immunodeficiency virus, **4-HNE:** 4- Hydroxynonenal, **HT:** Hydroxytyrosol, **•OH sacavenging:** Hydroxyl radical scavenging, **IOC:** International olive council, **5-LOX**: 5-Lipooxygenase, **LDL:** low density lipoprotein, **Med diet:** Mediterranean diet, **OA:** Oleocanthal, **OE:** Oleuropein, **ROS:** Reactive oxygen species, **SOD:** Superoxide dismutase, **TBARS:** Thiobarbituric acid-reacting substances, **T:** Tyrosol, **VOO:** Virgin olive oil.

1. Introduction

Worldwide olive oil production was about 3266.5-mile tons in 2019/2020, the majority of them in Mediterranean countries [1]. Spain, Italy, Tunisia, and Greece are the biggest olive oil-producing countries [1] (Fig. 1).

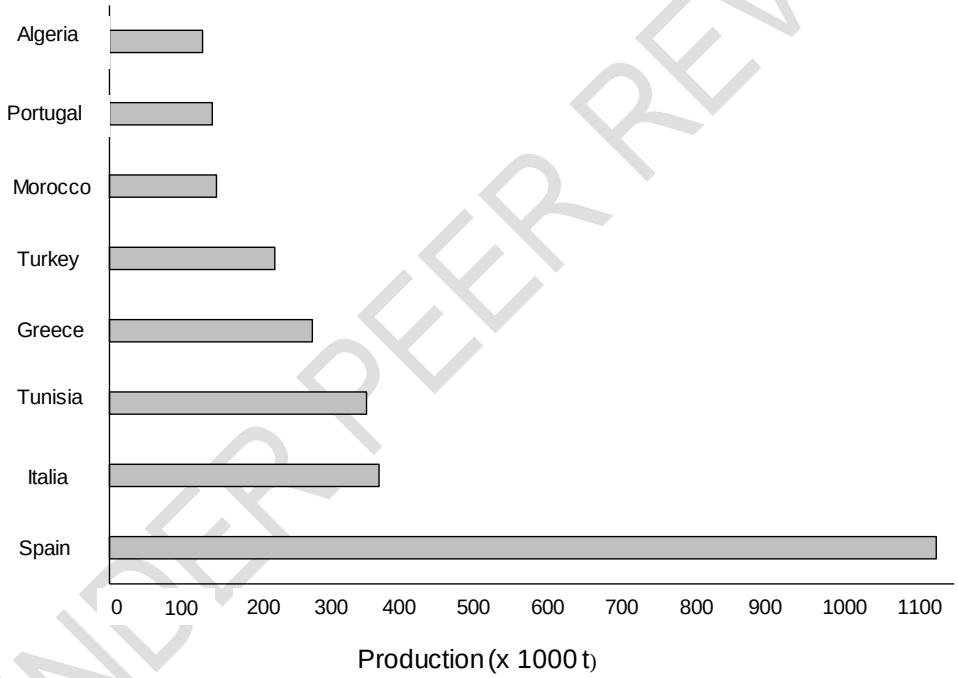

Fig. 1. Olive oil producing countries [1]

Because of the health benefits of olive oil [2], consumption is not restricted to the Mediterranean basin and is consumed in non-traditional producing countries, such as the United States (402.5-mile tons) [3]. Spain consumes the largest per capita amount of olive oil with over 521.6-mile tons and Italy with around 404.4-mile tons in 2019/2020 [3].

Virgin olive oil is the main fat of the Med diet. Virgin olive oil is oil which is obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and

which have not undergone any treatment other than washing, decantation, centrifugation and filtration [4].

Several epidemiological studies have shown that Med diet rich in VOO can diminish the incidence of chronic diseases [5, 6, 7]. The Med Diet is distinguished by a high consumption of phenolic compounds and unsaturated fatty acids (UFAs), which are abundant in the main key foods of this dietary pattern: VOO, legumes, vegetables, fruits, and whole-grain cereals. These phytochemicals are likely to be responsible for the Med Diet's positive effects. Their long-term consumption has been associated with decreased blood pressure and inflammation, an improved endogenous antioxidant system, and a lower risk of CVD and type 2 diabetes (Fig. 2) [5, 6, 7]. Furthermore, many experiences in human beings have revealed that the intake of VOO may be protective against these pathologies [8, 9, 10, 11].

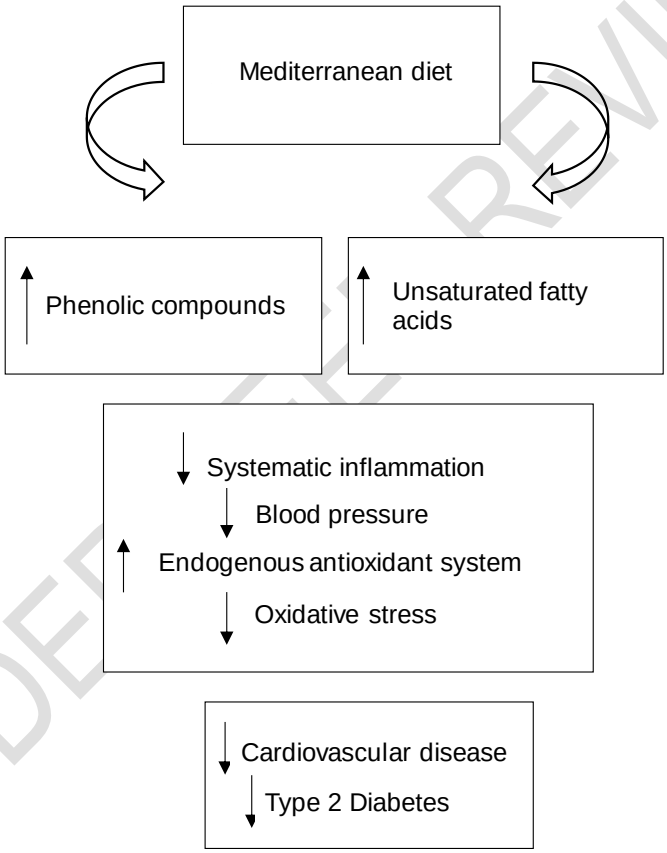

Fig. 2. The protective potential of the Mediterranean diet

It is well reported that in living organisms, the balance between reactive oxygen species (ROS) and endogenous antioxidants may be altered in some pathological and physio-pathological conditions, leading to a lower capacity of cellular defense against free radicals and/or increased production of ROS, thus creating cellular oxidative stress [12]. Oxidative stress occurs when cells are unable to eliminate free radicals known as reactive oxygen species (ROS) using the natural antioxidant defense system [12]. When the body's free radicals and antioxidants are out of equilibrium, oxidative stress can ensue. During regular

aerobic metabolism, free radicals are produced by the body's cells. Antioxidants, on the other hand, are produced by cells and neutralize free radicals. The body is capable of maintaining a balance between antioxidants and free radicals in general. However, oxidative stress and excessive free radical generation are caused by a number of factors, such as diet, lifestyle, and environmental pollution (Fig. 3). Target molecules for ROS are lipids, DNA, and proteins. Oxidative stress associated with aging appears to be involved in several chronic pathologies like atherosclerosis, Alzheimer's disease, type 2 diabetes mellitus, and cancer [13].

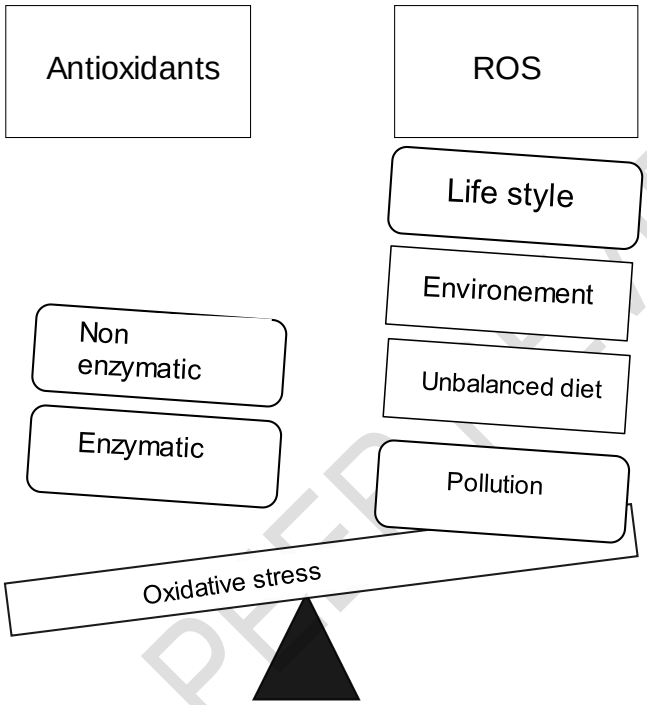

Fig. 3. Oxidative stress

Exploring the chemical composition of our diet, which has enhanced our overall standard of health, is one of the latest themes in food science. Food chemical compounds, in fact, intervene in biological processes to deliver a variety of health benefits. Bioactive chemicals of interest could be examined to see if they have a wide range of biological applications. As a result, there is a massive list of bioactive compounds that have already been published in the literature, and new compounds are being introduced on a regular basis. VOO, on the other hand, is one of the greatest sources of phytochemicals with biological activities known to date, as well as the only food with EFSA-approved health benefits [14]. VOO has been extensively researched for a variety of health advantages over the years. The health-promoting characteristics of VOO have sparked a lot of curiosity. Following research (both *in vivo* and *in vitro*) has shown that VOO bioactive chemical compounds have beneficial effects on physiological parameters, potentially lowering the risk of chronic diseases linked to oxidative stress [15]. As a source of bioactive compounds, it plays an important role as the main food of the Med diet [16, 17], which is associated with a lower incidence of several diseases [18, 19, 20]. With the advancement in scientific research, VOO bioactive compounds were identified, as well as their applications in the biological system [8, 21, 22, 23]. Monounsaturated fatty acids (especially oleic acid), phenolic compounds, and squalene are some of the main active components found in this matrix [24].

VOO is primarily composed of mono- and polyunsaturated fatty acids, accounting for more than 98% of the total weight. VOO mainly contains palmitic acid (C16:0), hypogeic acid (C16:1ω9), palmitoleic acid (C16:1ω7), margaric acid (C17:0), margaroleic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1ω9), cis-vaccenic acid (C18:1ω7), linoleic acid (C18:2), arachidic acid (C20:0), alpha-linolenic acid (C18:3), eicosenoic acid (C20:1), behenic acid (C22:0), and lignoceric acid (C24:0). Out of the given fatty acids, oleic acid is found in the highest concentration [23]. This fatty acid represents the most abundant fatty acid in VOO. Its concentration ranges from 56% to 84% of total fatty acids [23].

Many experimental studies on human beings have disclosed that the intake of monounsaturated fatty acids may be protective against chronic diseases. Extensive evidence points to a series of potential health effects of oleic acid in VOO. Indeed, Bermudez et al. [29] demonstrated the relevance of oleic acid in VOO on different nutrition-related issues and discussed the impact of oleic acid in olive oil and its clinical relevance to major risk factors for chronic diseases. In this respect, Soriguer et al. [9] showed that the regular consumption of VOO exerts a healthy effect on several cardiovascular danger factors, particularly in the presence of obesity, a reduced glucose tolerance, or a sedentary way of life. Besides, Kien et al. [30] reported that replacing dietary palmitic acid with oleic acid reduces the blood LDL concentration and whole-body fat oxidation. Montserrat-De La Paz et al. [31] reported that monounsaturated fatty acids present in VOO prevent atherosclerosis by interfering with various inflammatory responses. Lamy et al. [32] demonstrated that oleic acid may be useful in cancer chemoprevention.

Table 1. Fatty acid composition of virgin olive oil

Source of Virgin Olive Oil	Localization	GPS coordinate	Fatty acid composition (%)							Reference
			C16:0	C16:1 ω7	C17:0	C18:0	C18: 1 ω9	C18:2 ω6	C18:3 ω3	
Tunisian varieties										
Chemlali	Sfax	34°44'28.0N 10°45'35.1"E	15.81 ±0.60	3.61 ±0.15	0.03 ±0.002	1.77 ±0.08	61.92 ±2.7	15.77 ±1.02	0.56 ±0.03	[25]
Chetoui	Oueslatia	35°51'02.7N 9°35'10.4"E	11.53 ±0.80	0.39 ±0.03	n.d	2.99 ±0.12	64.24 ±1,22	19.63 ±0.62	0.68 ±0.15	[26]
Zalmati	Medenine	33°20'46.3N 10°29'24.6"E	17.00 ±1.10	1.93 ±0.38	0.03 ±0.05	2.06 ±0.01	63.43 ±2,31	14.47 ±0.6	0.51 ±0.08	[25]
Italian varieties										
Coratina	Calabria	38°06′37″ N 15°39′40″ E	12.62 ± 1.32	0.93 ± 0.03	0.02 ± 0.00	1.74 ± 0.24	71.70 ± 4.50	10.42 ± 0.93	0.80 ± 0.02	[27]
Leccino	Calabria	38°06′37″ N 15°39′40″ E	14.25 ± 1.21	1.31 ± 0.04	0.02 ± 0.00	2.50 ± 0.84	70.52 ± 3.90	10.43 ± 0.62	0.67 ± 0.04	[27]
Ottobratica	Calabria	38°06′37″ N 15°39′40″ E	15.12 ± 2.22	1.44 ± 0.13	0.02 ± 0.00	2.23 ± 0.40	69.60 ± 6.21	9.92 ± 0.70	0.90 ± 0.02	[27]
Nocellara del Belice	Calabria	38°06′37″ N 15°39′40″ E	11.14 ± 1.01	0.60 ± 0.01	0.08 ± 0.00	2.24 ± 0.41	76.30 ± 4.52	8.12 ± 0.70	0.58 ± 0.03	[27]
Spanish varieties										
Arbequina	Extremedura	39°31'55.5N 6°23'38.4"W	15.32 ±1.41	1.64 ±0.24	0.11 ±0.01	1.33 ±0.48	67.25 ±4.31	12.66 ±2.60	0.59 ±0.13	[28]
Cornicabra	Extremedura	39°31'55.5N 6°23'38.4"W	12.12 ±2.06	1.00 ±0.32	0.05 ±0.01	2.44 ±1.10	77.36 ±6.07	5.32 ±4.25	0.67 ±0.25	[28]
Picual	Extremedura	39°31'55.5N 6°23'38.4"W	11.62 ±1.18	1.02 ±0.17	0.04 ±0.01	1.96 ±0.88	80.67 ±1.95	3.08 ±0.46	0.65 ±0.12	[28]

Data are presented as the mean of the number ± Standard deviation of different samples analyzed.

Fatty acids: C16:0, palmitic acid (hexadecanoic acid); C16:1ω7, palmitoleic acid (9-hexadecenoic acid); C17:0, margaric acid (heptadecanoic acid); C18:1ω9, oleic acid (9-octadecenoic acid); C18:2ω6, linoleic acid (9,12-octadecadienoic acid); C18:3ω3, linolenic acid (9,12, 15-octadecatrienoic acid).

Secondary metabolites with at least one aromatic ring and one or more hydroxy substituents are known as phenolic compounds [33]. In VOO, more than 36 phenolic compounds have been discovered [34, 35, 36, 37]. The main phenolic compounds identified in VOO are phenolic acids (benzoic acid derivatives, cinnamic acid derivatives, and other phenolic acids and derivatives), phenolic alcohols (tyrosol and hydroxytyrosol), secoiridoids (oleuropein and ligstroside), hydroxy-isocromans (3,4-dihydro-1H-benzo[c]pyran derivatives), flavonoids (flavones and flavanols) [34, 38]. Fig. 4 shows the chemical structures of various phenolic compounds of VOO.

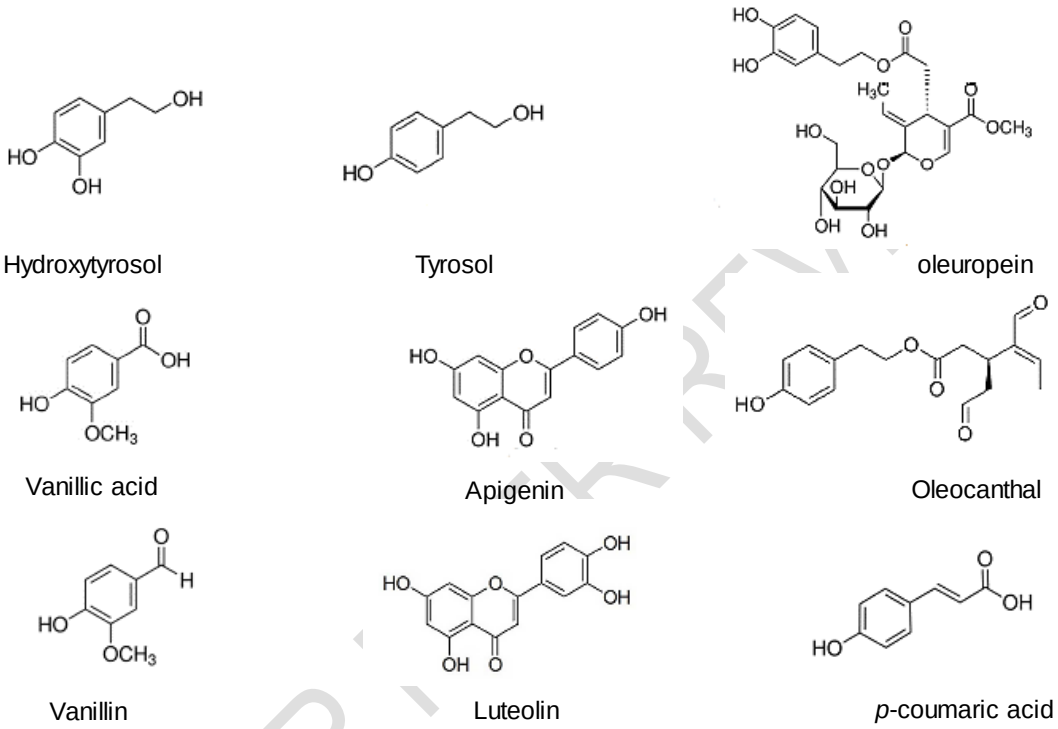

Fig. 4. Chemical structures of phenolic compounds of virgin olive oil

Numerous factors influence the concentration of phenolics in VOO, including agronomic factors such as the area of origin, cultivar, and stage of fruit ripening, as well as agronomic practices and the technological and operational circumstances of the oil extraction process [39, 40, 41]. Table 2 shows the phenolic content of virgin olive oils from Tunisian, Italian, and Spanish varieties.

Table 2. Phenolic content of virgin olive oils from some Tunisian, Italian and Spanish varieties

Source of virgin olive oil	Localization	GPS coordinate	Phenolic content (mg GAE/kg)*	Reference
Tunisian varieties				
Chemlali	Sfax	34°44'28.0"N 10°45'35.1"E	266.00 ± 5.76	[25]
Chétoui	Oueslatia	35°51'02.7"N 9°35'10.4"E	800.00 ± 3.32	[26]
Zalmati	Medenine	33°20'46.3"N 10°29'24.6"E	189.63 ± 4.67	[25]
Italian varieties				
Colozzese	Lecce	40◦21’17.32” N, 18◦10’20.78” E	251.00 ±12.00	[42]
Barone di Monteprofico	Lecce	40◦21’17.32” N, 18◦10’20.78” E	202.00 ± 14.00	[42]
Cellina di Nardò	Lecce	40◦21’17.32” N, 18◦10’20.78” E	253.00 ± 7.00	[42]
Spanish varieties				
Arbequina	Extremedura	39°31'55.5"N 6°23'38.4"W	234.00 ± 2.4.00	[43]
Manzanilla	Extremedura	39°31'55.5"N 6°23'38.4"W	210.70 ± 13.20	[43]
Picual	Extremedura	39°31'55.5"N 6°23'38.4"W	590.30 ± 2.20	[43]

*Data are presented as the mean of the number of different samples analyzed; *: mg Gallic Acid Equivalent*

This article will focus on the most existing features of virgin olive oil's health benefits, which are mostly due to the presence of phenolic compounds.

2. Methodology

The current study was based on literature that is already available on various scientific databases and highlighted the health benefits of VOO. It compiles, critically interprets, and presents the data gathered from these various sources. A systematic electronic literature search was conducted using PubMed and Web of Science. The search included articles written in English. Studies related to VOO as a rich source of functional bioactive compounds in the promotion of human health were selected, including data available from organizations and books. The following search key terms were used to find original studies addressing the biological activities of VOO and its phytochemicals: "hydroxytyrosol" OR "*p*-hydroxyphenyl-ethanol" OR "*p*-HPEA" OR "tyrosol" OR "secoiridoid" OR "oleuropein" OR "oleocanthal" OR "olive oil phenolics" OR "tocopherols" OR "phytosterols" OR "triterpenic acids" AND biological potential OR "bioavailability" Additional relevant publications were found by looking through the reference lists of included articles and recent noteworthy reviews. Published data on the *in vitro* effects of VOO and its phytochemicals were considered. *In vivo* trials were also taken into account if they revealed outcomes directly related to oxidative stress, essentially referring to neurodegenerative disorders, cardiovascular disease, or type 2 diabetes. From the *in vitro* studies, the first author's last name, the year of publication, the damage agent, the tested VOO or its phytochemicals, dose, and effects are retrieved. Further information was gathered from the *in vivo* investigations as follows: VOO or its phytochemical exposure duration and mechanisms in tested model systems. All authors participated in the literature search, data extraction, and synthesis. An initial screening was conducted on the basis of the abstract and title. Exclusions were made, taking into consideration the exclusion criteria and in order to avoid redundancy of cited material. Exclusion criteria are the application of bioactive compounds extracted from VOO as food antioxidants, olive byproducts and their bioactive compounds, and the effect of processing technology and storage conditions on VOO phytochemicals with biological significance. The Critical Appraisal Skills Programme (2018) CASP Checklist was used to critically appraise and assess the quality of each included study.

3. Results and discussion

After deleting duplicates (n = 35) from the original literature search using PubMed and Web of Science databases (n = 405) and other sources (n = 26), 120 records were omitted based on the title, abstract, or exclusion criteria. To reduce duplicates of the mentioned content, 87 items were eliminated. The reference list includes the eligible reports for analysis (n = 93) based on the PRISMA flowchart for the report selection process (Fig. 5).

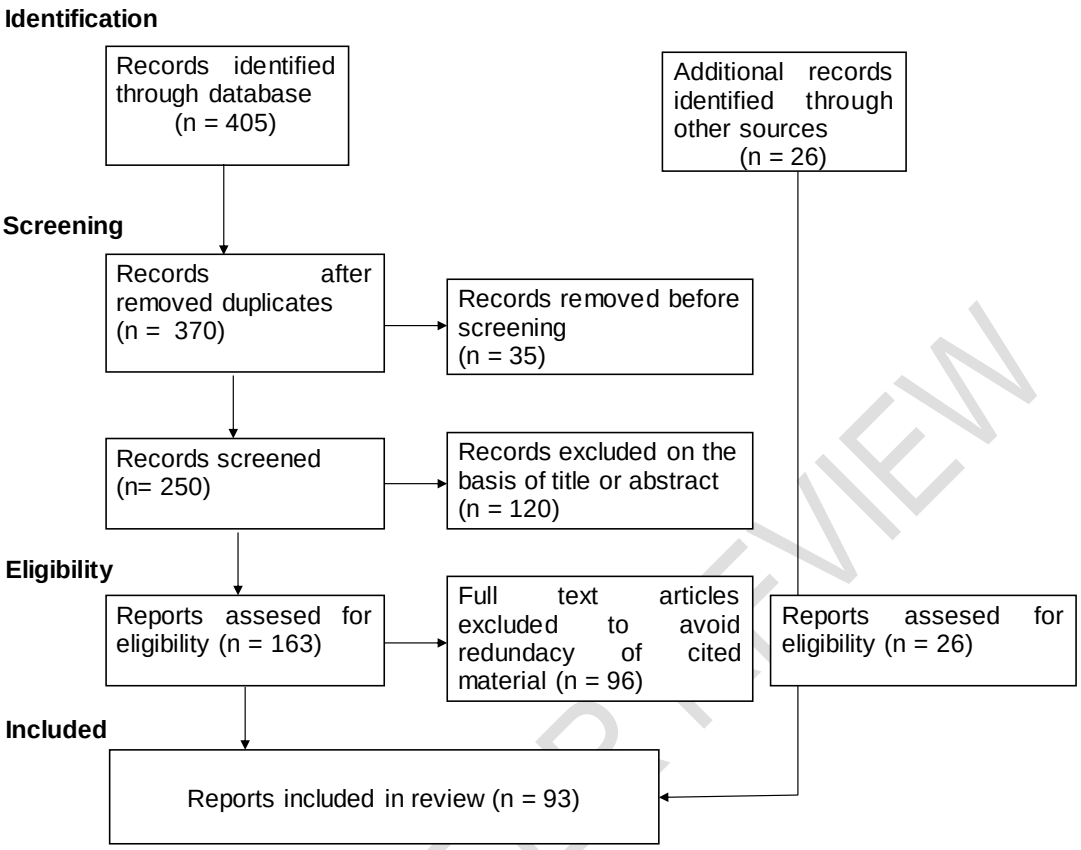

Fig. 5. A flowchart of the report selection process

The main features of eligible reports are summarized in Table 3. Single phytochemicals, such as oleuropein, hydroxytyrosol, and oleocanthal, were evaluated in several scientific studies. Phenolic extracts derived from VOO or VOO have also been investigated in other research. The effects of VOO enriched with naringenin, diallyl sulfide, and camel milk have been documented in several studies. This review was registered in the PROSPERO International Prospective Register of Systematic Reviews, identified under ID313430.

3.1 Biological activities of phenolic compounds

VOO phenolics have been shown to have positive effects on specific physiological parameters and decrease the risk of chronic diseases in both *in vitro* and *in vivo* studies [34, 44, 45, 46, 47, 48, 49]. Furthermore, VOO phenolic compounds are highly bioavailable, thus reinforcing their potential health-promoting properties [50, 51, 52].

3.1.1 Antioxidant activity

Interest in phenolic compounds is related primarily to their antioxidant activity, which may contribute to countering many chronic diseases that may be related to oxygen radical

formation, oxidative damage, and aging [53]. Phenolic compounds work inside cellular compartments as the first line of defense against free radicals due to their capacity to stabilize free radicals through the formation of intramolecular hydrogen bonds. In this context, many studies disclose the important antioxidant potential of VOO phenolics [42, 54, 55, 56].

The antioxidant properties of phenolic compounds can be achieved in a variety of ways. They can chelate metallic ions in oxidative systems using transition metals, preventing their involvement in Fenton reactions, which can generate significant amounts of hydroxyl radicals [57, 58]. Furthermore, the most essential antioxidant activity is connected to the ability to scavenge free radicals by interrupting the chain of reactions set off by free radicals. Indeed, the potential of phenolics to promote radical stability by creating an intramolecular hydrogen link between the free hydrogens of their hydroxyl groups and the phenoxyl radicals is well recognized [59]. *In vivo* studies confirmed that VOO phenolic compounds appear to regulate the oxidative/antioxidative balance and explained that the mechanisms by which phenolic compounds of VOO can protect lipids and DNA are primarily related to their abilities to counteract metal- and radical-dependent oxidation and to act as chelating agents, thus breaking the chain-like propagation of the lipid peroxides [60, 61]. Moreover, an important claim by the EFSA [14], based on scientific reports, supported the effectiveness of the ingestion of VOO phenolics at a concentration evaluated to be 5 mg/day in protecting against oxidative stress. The most extensively investigated VOO phenolics are hydroxytyrosol (HT) and oleuropein (OE), and their antioxidant potentialities have been extensively reported in the scientific literature. Indeed, many authors have indicated that HT and OE are potent scavengers of free radicals [21]. Also, HT and OE inhibit copper sulfate-induced oxidation of LDL, according to Visioli et al. [63] and Visioli et al. [64]. The protective effects of HT and OE have been demonstrated through the assessment of various markers of LDL oxidation, including the thiobarbituric acid-reacting substances (TBARS), lipid peroxides, and 4-hydroxynonenal (4-HNE) adducts [63, 64].

On the other hand, VOO phenolics enhance the endogen antioxidant system, thereby improving the antioxidant enzymes including catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) [65]. According to the previous report by Chashmi et al. [66], these effects are mainly attributed to HT, which increases SOD and glutathione (GSH) activities. In the same context, Pérez-Bonilla et al. [67] found that oleocanthal (OA) could display a higher anti-oxidative effect against the free radical DPPH than the reference 2,6-di-tert-butyl-hydroxytoluene (BHT).

3.1.2 Anti-atherosclerosis activity

Considering the role of oxidative stress in the pathogenesis of atherosclerosis, previous studies evaluated the protective effect of VOO phenolics. Based on various studies performed *in vitro* and *in vivo*, phenolic compounds of VOO play a primordial role in the prevention of atherosclerosis through their inhibition of LDL oxidation [68, 69, 70]. Many studies have shown that VOO phenolic compounds can bind to LDL and thus protect them against oxidation [71]. In this respect, some studies have focused attention on the antioxidant power of VOO phenolics on plasma LDL. Plasma was incubated with various VOO phenolics to simulate an in vivo situation. LDL was then isolated and submitted to the action of free radicals. The obtained results showed that HT and OE are more effective than tyrosol and ligstroside aglycone [72]. Another important risk factor for the onset of atherosclerosis is a high blood concentration of cholesterol. Benkhalti et al. [73] reported that VOO phenolics can interfere with 3-hydroxy3-methylglutaryl (HMG)-CoA reductase, a key enzyme involved in the synthesis of cholesterol.

3.1.3 Cardioprotective activity

Some clinical trials that have examined the effects of VOO on cardiovascular disease have indicated that the consumption of VOO can protect various aspects of cardiovascular disease [18, 74, 75]. According to Bullota et al. [76], VOO phenolics play a significant role in improving cardiovascular health in adherents to the MED diet. Besides, phenolics have been shown to improve vascular function and reduce inflammation in heart tissues [77, 78, 79].

3.1.4 Anti-cancer activity

Studies have corroborated the protective effect of olive oil against various forms of cancer. VOO consumption and the incidence of many forms of cancer, including colon, breast, and skin cancer, have been inversely correlated [47]. Recent studies attribute this effect to the inhibitory potential of phenolic compounds on tumor growth [80, 81, 82, 83]. These bioactive substances appear to be able to influence gene expression, reduce DNA damage caused by ROS, and alter tumor eicosanoid biosynthesis and cell signaling pathways. Among the phenolic compounds possessing anticancer activity, HT is of special interest. Deiana et al. [84] highlighted that the presence of HT reduced the biochemical effects of peroxynitrites, such as oxidizing methionine and protecting DNA against damage. Della Ragione et al. [85] studied the anticancer effect of HT on the proliferation of human promyelocytic leukemia cells and highlighted its anti-cancer potential. HT delivered orally was shown to decrease breast tumor volume [86]. According to García–Villalba et al. [87], HT and luteolin have been shown to exhibit anti-cancerous effects against breast cancer cell lines. In the same context, some scientific studies have found that lignans inhibit the growth of various types of tumors, including cutaneous, mammary, colonic, and pulmonary tumors [88]. According to Akl et al. [89], OA was found to suppress tumor cell growth in a model of breast cancer. More recently, López de las Hazas et al. [90] reported the potential apoptotic and antiproliferative effects of VOO phenolics.

3.1.5 Anti-inflammatory activity

Natural inhibitors of cyclooxygenase (COX), important enzymes involved in the management of inflammation, can be an effective therapeutic approach. Some studies have found that VOO phenolics have an inhibitory effect on COX [91, 92, 93]. The anti-inflammatory activity of HT is similar to that of aspirin, a drug that is well known for its inhibitory potential on COX activity [91]. As reported by Facchini et al. [94], HT has also been shown to decrease the activity of COX-2. OA was found to have a similar anti-inflammatory role to that of the drug Ibuprofen [95]. Procopio et al. [96] explained that the anti-inflammatory activity of OA could be achieved by inhibiting COX enzymes. Therefore, OA can play an effective role in anti-inflammatory activities. According to the studies of De La Puerta [92] and Barbaro et al. [97], OE has been found to inhibit 5-lipoxygenase (5-LOX) activity, an enzyme with an important role in inflammation.

3.1.6 Antimicrobial activity

VOO is a promising source of bioactives with antimicrobial properties; the majority of them act as antimicrobial agents against a wide range of pathogenic microorganisms (bacteria, fungi, viruses, and protozoa) [98]. Phenolic compounds found in VOO have been shown to possess antimicrobial potential against several strains of bacteria [37]. OE can inhibit the

development and production of enterotoxin B by *Staphylococcus aureus*, the development of Salmonella enteritidis, and the germination of spores of *Bacillus cereus* [99, 100, 101]. OE and other olive oil phenolic compounds (*p*-hydroxybenzoic, vanillic, and *p*-coumaric acids) inhibit the proliferation of *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus cereus* [102]. HT shows a highly antibacterial effect against *Pseudomonas syringae* pv *savastanoi* and *Corynebacterium michiganense* [103]. Hydroxytyrosol and oleuropein have been shown to inhibit the growth of *Staphylococcus aureus* and *Candida albicans* [98]. The dialdehydic form of the decarboxymethyl oleuropein aglycon, OA, HT, and T have been shown to possess potent activity against several strains of bacteria responsible for intestinal and respiratory infections *in vitro* [104]. As reported by Romero et al. [105], Oleocanthal has also been found to inhibit the growth of *Helicobacter pylori*, a bacterium associated with peptic ulcer and gastric cancer development. According to Karaosmanoglu et al. [106], the antimicrobial activities of VOO phenolics were reported against three foodborne pathogenic bacteria: *Escherichia coli* O157: H7, *Listeria monocytogenes*, and *Salmonella enteritidis in vitro*.

3.1.7 Anti-diabetic activity

VOO bioactive compounds have been investigated for their contribution to the prevention and/or treatment of type 2 diabetes mellitus. Rigacci et al. [74] concluded that OA could prevent or retard the development of type 2 diabetes. Loizzo et al. [107] reported that phenol-rich VOO extracts from "Frantoio," "Ortice," and "Ortolana" produced in Campania (Italy) showed antidiabetic activity. HT has been found to prevent high-fat diet adverse effects such as obesity, diabetes, and insulin resistance. It also increases glucose consumption and inhibits adipogenesis [72, 108]. In fact, HT and OE could act as α-glucosidase and α-amylase inhibitors, according to Hadrich et al. [109]. Besides, OE has been shown to protect β cells from cytotoxicity induced by amylin amyloids that are characteristic of type 2 diabetes [110, 111]. In the same context, Collado-González et al. [24] reported that luteolin is the main phenolic compound that contributes to the antidiabetic activity of VOO.

More recently, Figueiredo-González et al. [112] revealed the antidiabetic potential of phenol-rich extracts from monovarietal VOOs (obtained from 'Cornicabra' and 'Picual' varieties, two of the most representative Spanish cultivars), through inhibition of α-amylase and α-glucosidase, key enzymes involved in the treatment of diabetes mellitus. In this respect, it is interesting to mention that many phenolic compounds have been described as inhibitors of key enzymes in the management of diabetic complications [24, 109, 113, 114, 115].

3.1.8 Neuroprotective activity

VOO phenolics are increasingly being investigated for their potential to manage the devastating central nervous system (CNS) [116]. HT has been shown to protect brain cells from free radical damage that contributes to neurodegenerative processes [117]. HT has also been shown to protect against mitochondrial dysfunction in rat brains [118]. It induces neuroprotection by increasing the activity of detoxifying enzymes, such as the GSH enzyme [119]. OE has demonstrated protective effects on dopaminergic neurons, implying possible roles in preventing or treating Parkinson's disease [97]. OE interacts with some of the biochemical processes behind the formation and deposition of β-amyloid plaques associated with Alzheimer's disease [97]. OA also promotes neuroprotection in cultured neuronal cells, according to Barbaro et al. [97], by interfering with Amyloid aggregation, diminishing aggregate cytotoxicity, and counteracting related neuroinflammation. Another noteworthy study shows that VOO restores cholinergic deficits by restoring acetylcholine levels by inhibiting the two main forms of cholinesterases (BuChE and AChE) [112].

3.1.9 Protective activity against Covid-19

SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) is a hazardous infection that is causing global health problems. It causes coronavirus disease 2019 (Covid-19), which has a high rate of morbidity and fatality. The coronavirus disease (Covid-19) is a pathogenic virus caused by SARS-CoV-2 that has spread fast around the world. Antiviral bioactivities of phenolics have been identified against HCV, HIV, and other viruses. Protease inhibitors, such as phenolic compounds, have already been demonstrated to be particularly effective in suppressing virus-induced infections in multiple investigations [120, 121, 122, 123, 124]. There is currently insufficient data from *in vivo* studies indicating correlations between phenolics and ACE2 expression downregulation. Nonetheless, phenolics have recently been discovered to interact with SARS-CoV-2 viral proteins and their cellular targets [125]. As a result, the potential modification of immune response by these natural bioactive chemicals might be viewed as a positive element of the human body's defence against Covid-19. According to Paraiso et al. [125], phenolic compounds have been shown to have an effect on various stages of the SARS-CoV-2 life cycle. According to El-Missiry et al. [126], phenolics could be used as dietary adjuvants to target Covid-19. The health-promoting benefits of these natural substances on Covid-19 may be attributable to the body's anti-inflammatory and antioxidant defenses against viral infection, according to the researchers. Other approaches, such as targeting virus proteins and/or inhibiting cellular receptors, can still be effective in preventing virus entry and multiplication in host cells [126]. Although robust *in vivo* evidence for the efficacy of phenolics against the Covid-19 pandemic is still lacking, the preliminary findings can pave the way for more systematic and advanced experimental research into the efficacy of phenolic compounds derived from natural sources in the prevention and/or treatment of Covid-19. Covid-19 patients may benefit from natural bioactive compounds in their dietary control [127].

Since ancient times, virgin olive oil (VOO) has been used for therapeutic purposes as a valuable component of MED human diets. During Covid-19, VOO is regarded as a nutritional therapeutic immuno-enhancer since it can help keep the immune system healthy and minimize inflammation and oxidative stress, which can lead to a number of diseases and conditions, raising the likelihood of major Covid-19 instances [128].

Table 3. The characteristics of some selected studies dealing with several biological activities and health benefits of virgin olive oil and its phytochemicals

Source	Biological activity	Mechanisms	References
Phenolic extract of VOO	Antioxidant activity	Reduction of reactive oxygen species, lipid peroxidation, and $NO^{\cdot}$ overproduction through modulating inducible nitric oxide synthase protein levels after H_2O_2 induced oxidative stress.	[129]
OE		Protection of L-0_2 cells from H_2O_2-induced oxidative stress by boosting SOD, GPx and CAT expression	[130]
VOO		Improvement of antioxidant status in plasma and cell blood	[65]
VOO	Anti-obesity activity	Dietary 5% VOO increased serum HDL concentrations while decreasing triglyceride levels. Hydroxytyrosol modulated adipocyte gene expression.	[131] [132]
VOO	Immunological resistance activity	Increased immune system activity aids in immune resistance.	[133]
Phenolic compounds		White blood cell proliferation and the production of cytokines, which enhance immune resistance	[134]
VOO	Renoprotective activity	Ethopone-induced nephrotoxicity was reduced by 2mL/Kg/day (150 mg per kg daily).	[135]

VOO VOO + naringenin		Inhibition of amikacin-induced nephrotoxicity. During a 45-day treatment period, the combined use of olive oil (1.25 ml/kg/day virgin olive oil) and naringenin (100 mg/kg/day naringenin) reduced the cyclosporine-induced (25 mg/kg/day cyclosporine) nephrotoxicity by improving renal functionality and lowering serum urea and creatinine concentrations in rats.	[136] [137]
VOO VOO + phenolic compounds Secoiridoids VOO MUFAs	Anti-cancer activity	Protection against mammary carcinogenesis in both *in vitro* and *in vivo* studies. VOO and its phenolic components, such as HT, OE, and OA, have been shown to have anti-cancer properties. Secoiridoids found in olive oil have been demonstrated to have anti-cancer and anti-preventive properties against a variety of human malignancies. VOO helps to prevent cancers of the breast, prostate, and digestive system. MUFAs can potentially inhibit tumor growth and proliferation.	[138] [139] [140] [133] [138]

VOO		Olive oil's phenolic components, which scavenge free radicals and other reactive oxygen species, may help to slow or stop cancer progression.	[141]
HT		HT exerted an anti-proliferative impact on human colon cancer growth.	[142]
Secoiridoids		In several cancer cell lines, VOO secoiridoids suppressed cell proliferation and triggered apoptosis.	[140]
OE		The mammary tumor MCF-7 cell line was used to demonstrate that OE had powerful anti-breast cancer effects.	[143] [144]
VOO+ diallyl sulfide	Hepatoprotective activity	In mice, VOO combined with diallyl sulfide protects them from CCl_4^- induced acute liver damage.	[145]
VOO		In animal studies and cellular cultures, VOO and its phytochemicals exert a protective effect on the liver.	[146]
HT		HT helps to avoid liver steatosis and the mitochondrial dysfunction that comes with it, both of which are caused by a high-fat diet.	[147]
VOO + camel milk		Because of its strong antioxidant properties, a combination of VOO and camel milk showed hepatoprotective	[148]

VOO		effects in mice against acetaminophen-induced hepatotoxicity at a single dose (500 mg/kg). Deltamethrin-induced hepatotoxicity was minimized when VOO was consumed.	[149] [150]
VOO HT OE	Anti-inflammatory activity	VOO (0.3 g/animal/day) showed cell membrane protecting and anti-inflammatory properties. In a mouse model of systemic inflammation, HT has anti-inflammatory and anti-oxidant properties. By lowering the secretion of COX inflammatory enzymes, OE has the power to break the inflammatory cascade.	[151] [152] [153]
MUFAs VOO	Cardioprotective activity	A higher concentration of MUFAs (such as oleic acid) benefited the male albino rats' cardiovascular systems. Olive oil is linked to a lower risk of cardiovascular disease and stroke. With a daily intake of 20 to 30 g, the greatest effect might be reached.	[154] [155]
HT	Anti-Neurodegenerative activity	In a mouse model of amyloid deposition, HT supplementation improves brain disease and recovers cognitive skills. Polyphenols from VOO can affect a	[156]

Phenolic compounds		different cellular pathway associated with the disease's onset and progression.	[157]
OE		In an in *vitro* model of Parkinson's disease, oleuropein has been proven to diminish cell damage, apoptosis, and oxidative stress in PC12 cells generated by 6-OHDA.	[158]
OE		On PC12 cells exposed to 150 mM 6-OHDA, OE (20 and 25 g/ml) showed neuroprotective effects.	[159]
VOO	Antidiabetic activity	In type 1 diabetes, VOO has been linked to reduced postprandial glycemia.	[160]
VOO		In patients with impaired fasting glucose, VOO improves post-prandial glycemic and lipid profiles.	[161]
HT	Antimicrobial activity	Inactivation of *S. aureus* as well as the enterotoxin A	[162]
VOO		*In vitro* antimicrobial activities of the *Listeria monocytogenes* DSM 20600, *Staphylococcus aureus* DSM 20231, *Escherichia coli* DSM 30083, *Salmonella bongori* DSM13772, *Lacticaseibacillus* casei *Shirota*, *Limosilactobacillus*	[163]

		reuteri DSM 17938, *Lacticaseibacillus* casei RI4, and the yeast *Candida albicans* 3393	

VOO: virgin olive oil; OE: oleuropein; MUFAs: Monounsaturated fatty acids; HT: hydroxytyrosol; H_2O_2: Hydrogen peroxide, a destructive oxidant; $NO^{\cdot}$: nitric oxide, In states of oxidative stress, NO production increases considerably and, in conjunction with other ROS, contributes to oxidative stress. Nitric oxide (NO) is synthetized enzymatically from l-arginine (l-Arg) by NO synthase; L-0_2: Normal Liver Cell Line, cells Human; SOD: Superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase, Superoxide dismutase, catalase and glutathione peroxidase are antioxidant enzymes which constitute a very important antioxidant defense against oxidative stress in the body; OA: oleocanthal; MCF-7: human breast cancer cell line; COX: cyclooxygenase; PC12 cells: pheochromocytoma cells, Pheochromocytoma is a type of neuroendocrine tumor that grows from cells called chromaffin cells. ; 6-OHDA: 6-Hydroxydopamine, it forms free radicals and it is a potent inhibitor of the mitochondrial respiratory chain complexes.

3.1.10. Bioavailability

The bioavailability of VOO bioactive compounds refers to the degree to which they are extracted from this food matrix and absorbed by the body [164]. Bioavailability is essential in determining the beneficial health effects. Indeed, achieving an effect in specific tissues or organs depends on the bioavailability of VOO bioactive compounds. The scientific community has looked at the metabolic fate of phenolic substances after consumption. Phenolics are known to have strong metabolism in phases I and II, during which they are hydrolyzed (phase I) and then conjugated (phase II) into their glucuronidated, methylated, and sulfated forms, which are then absorbed [165].

The majority of studies regarding the bioavailability of VOO bioactive compounds have focused on phenolics. Some studies have shown that HT and T are absorbed after ingestion in a dose-dependent manner [62, 63, 166]. Vissers et al. [167] reported that the absorption of administered ligstroside-aglycone, HT, T, and OE aglycone was as high as 55–66% in human subjects. According to Vissers et al. [167], the polarities of the phenolics seemed to play a key role in the absorption of these compounds. In this context, Manach et al. [165] explained that the absorption of T and HT occurs via passive diffusion. However, OE-glycoside may be absorbed via a different mechanism to T and HT. Edgecombe et al. [168] explained that OE-glycoside may diffuse through the lipid bilayer of the epithelial cell membrane and be absorbed via a glucose transporter.

On the other hand, some work has been conducted to evaluate the quantity of VOO phenolics excreted. A smaller quantity of HT and T was recovered in urine (5%–16%) [167]. Casas et al. [18] revealed that approximately 24% of administered T was excreted. Visioli et al. [62] reported that the excretion of administered HT and T was evaluated at 30–60% and 20–22% of the total ingested by human subjects, respectively. These findings demonstrate that humans absorb a significant quantity of the dietary VOO phenolics.

3.2 Other bioactive compounds and their biological activities

3.2.1 Tocopherols

Tocopherols are hetero acid compounds with a high molecular weight that have been designated as α-, β-, γ- and δ-tocopherols. 90 % of the tocopherols in VOO are α-tocopherol, which has a concentration of up to 300 mg/kg, while others (β-, γ-, and δ-) are present in small amounts, up to 25 ppm [44, 169]. Tocopherols are particularly important bioactive constituents in VOO mainly due to their antioxidative effects [170]. They can act as antioxidants by two mechanisms. The first is a chain-breaking electron donor mechanism in which tocopherol donates its hydrogen atom to lipid radicals. The second is a chain-breaking acceptor mechanism that includes singlet oxygen scavenging or quenching and inhibits oxidation induced by excited singlet oxygen. High levels of α-tocopherol prevent the oxidation of polyunsaturated fatty acids in plasma lipoproteins, which causes atherosclerosis and modifies numerous pathways implicated in atherogenesis prevention [171]. Furthermore, α-tocopherol has been shown to inhibit prostate cancer cell growth in animal model experiments [172], decrease the progression of Alzheimer's disease [173], and protect against upper respiratory tract infections [174]. Fig. 6 shows the chemical structure of α-tocopherol.

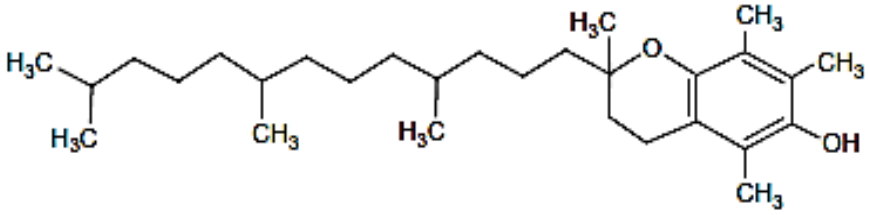

Fig. 6. Structure of α-tocopherol

3.2.2 Carotenoids

Carotenoid pigments are responsible for the color of olive oil. Their content does not usually exceed 10 mg/kg, with lutein being the major carotenoid, followed by β -carotene. They are substances with antioxidant activity [170]. In this context, β-Carotene is known to be a potent singlet oxygen quencher and free radical scavenger.

3.2.3 Hydrocarbons

Hydrocarbons are always present in VOO. Squalene is the most important hydrocarbon of VOO. Therefore, VOO can be considered as significant dietary source of squalene [170]. It is composed of approximately 0.7% squalene [171]. The biological activities of squalene are well-known. Indeed, the high squalene level of VOO, when compared with other foods, may account for the low incidence of certain tumors in the Mediterranean area [172, 173]. In vitro and in vivo animal model studies have shown inhibition of chemically induced colon and lung cancer formation by squalene [175, 176]. Squalene appears to be critical for reducing free radical oxidative damage. According to Kelly [177] and Huang et al. [178], it seems to function in the skin as a quencher of singlet oxygen, thus inhibiting lipid peroxidation due to exposure to UV light and other factors of oxidative stress. The chemical structure of squalene is provided in Fig. 7.

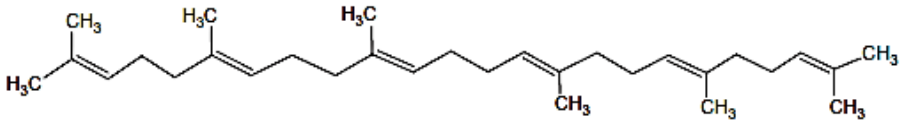

Fig. 7. Structure of squalene

3.2.4 Phytosterols

VOO has between 1000 and 2300 ppm of sterols, with β-sitosterol being the most abundant [169]. According to several epidemiological investigations [179, 180], phytosterols have been shown to have immunological and anticancer properties in animal models, both in vitro and in vivo. Beta-sitosterol substantially suppressed the tumor growth of a human colon cancer cell line [181]. Epidemiological investigations linking dietary phytosterol intake to a lower risk of a variety of diseases corroborated these findings. In the same vein, multiple epidemiological studies suggest that having them in one's diet is linked to a lower risk of common malignancies like colon, breast, and prostate cancer [170].

Furthermore, phytosterols act as cholesterol-lowering agents by reducing cholesterol absorption [170]. Consumption of phytosterol may also increase the activity of antioxidant enzymes, reducing oxidative stress [180]. Fig. 8 shows the chemical structure of β - sitosterol.

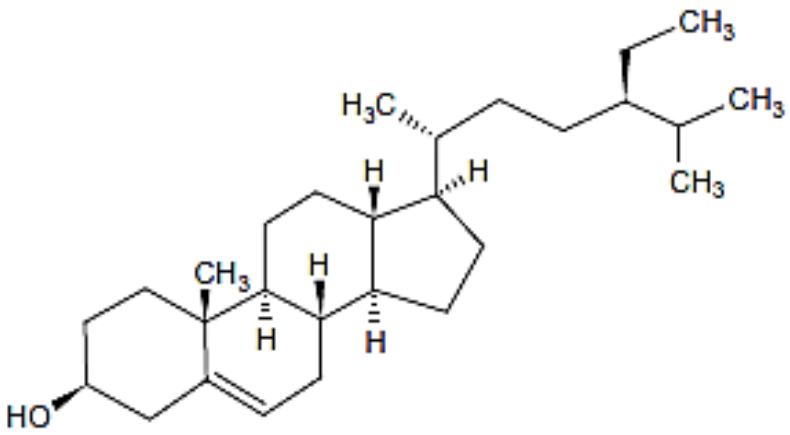

Fig. 8. Structure of β-sitosterol

3.2.5 Triterpenic Acids

Oleanolic acid, maslinic acid, ursolic acid, and betulinic acid are triterpenic acids that are present in small amounts. Prades et al. [182] indicated that these acids could act as anti-inflammatory, antioxidant, antihypertensive, antihyperlipidemic, antidiabetic, antiviral, and antitumoral agents. Additionally, some studies have reported their neuroprotective effect. Additionally, it has been described that oleanolic acid protects the brain during hypoxic injury in rats and that ursolic acid protects the brain against ischemic injury in mice via Nrf2 activation [183, 184, 185]. According to Andrikopoulos et al. [186], oleanolic acid was described as a protective agent against LDL oxidation. Oleanolic acid is also involved in atherosclerosis protection, with antihyperlipidemic effects in animal models. Previous studies of isolated oleanolic acid describe its antitumoral activity.

Maslinic acid strongly inhibits *in vitro* LDL oxidation. Maslinic acid has previously been shown to have cardioprotective properties. This chemical may also protect against atherosclerosis by lowering insulin resistance in a mouse model, with potential antioxidant and hypoglycemic effects. Different research has focused on different aspects of the role that maslinic acid appears to play in cancer [187, 188, 189].

4. Critical perspective

The data allows us to show that VOO has a preventive impact on a variety of diseases. An overview of the literature reveals that the vast majority of the stated research works on VOO biological activities were carried out *in vitro* and *in vivo* models. Similarly, while research on VOO phytochemical substances is promising, the majority of their protective properties have only been established *in vitro* and *in vivo* models. However, it is critical that the investigations of this matrix and its phytochemicals continue to follow the proper phases of efficacy and safety testing in more *in vivo* models and eventually clinical trials in order to

validate this knowledge for the development of functional foods and nutraceuticals. Furthermore, more research is required to determine the precise mechanism of action of these chemicals. According to the experimental investigations described in this study, hydrocarbons and triterpenic acids have not been researched as completely as phenolic chemicals and fatty acids.

5. Conclusion

In terms of culture and health, olive oil consumption is a significant advantage for many countries. Consuming VOO, which is high in phenolic compounds and other physiologically active substances, may help to lower the risk of developing a variety of diseases. The quest for natural, biologically active substances is becoming increasingly popular, and academics are becoming increasingly interested in the subject. Dietary management as a means of preventing disease is a major public health concern around the world. VOO has been proven to be a significant source of bioactive chemicals with a variety of biological effects in both *in vitro* and *in vivo* experimental models. The health benefits outlined in this review lead us to believe that virgin olive oil is one of the healthiest foods available.

The potential health benefits gained from VOO as a possible candidate in developing pharmaceutical preparations and nutraceutical or functional foods for numerous pathological conditions are supported by the health benefits outlined in this article. This hypothesis could lead to further research concentrating on more *in vivo* and, eventually, clinical trials. Furthermore, more thorough research on the mechanisms of action and efficacy is required.

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