Pharmacology & Therapeutics 254 (2024) 108595

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Olea europaea L-derived secoiridoids: Beneficial health effects and potential therapeutic approaches

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ARTICLE INFO

Available online 30 January 2024

Associate editor: F Limana

Keywords:
Olea europaea L.
Secoiridoids
Oleuropein
Oleocanthal
Oleacein
Health beneficial effects

ABSTRACT

Over the years, health challenges have become increasingly complex and global and, at the beginning of the 21st century, chronic diseases, including cardiovascular, neurological, and chronic respiratory diseases, as well as cancer and diabetes, have been identified by World Health Organization as one of the biggest threats to human health. Recently, antimicrobial resistance has also emerged as a growing problem of public health for the management of infectious diseases. In this scenario, the exploration of natural products as supplementation or alternative therapeutic options is acquiring great importance, and, among them, the olive tree, *Olea europaea* L, specifically leaves, fruits, and oil, has been increasingly investigated for its health promoting properties. Traditionally, these properties have been largely attributed to the high concentration of monounsaturated fatty acids, although, in recent years, beneficial effects have also been associated to other components, particularly polyphenols. Among them, the most interesting group is represented by *Olea europaea* L secoiridoids, comprising oleuropein, oleocanthal, oleacein, and ligstroside, which display anti-inflammatory, antioxidant, cardioprotective, neuroprotective and anticancer activities. This review provides an overview of the multiple health beneficial effects, the molecular mechanisms, and the potential applications of secoiridoids from *Olea europaea* L.

Contents

1. Introduction

Over the years, health challenges have become increasingly complex and global and, at the beginning of the 21st century, chronic diseases have been identified by the World Health Organization as the biggest threat to human health. Indeed, cardiovascular diseases, cancer, diabetes, and chronic respiratory diseases are recognized as the leading cause of death and morbidity worldwide, with nearly 41 million deaths/year (World Health Organization, 2022). In the last years, neurologic disorders and inflammatory bowel diseases (IBD) as well are becoming a growing public health problem (Livingston et al., 2020; Wang, Li, Liu, & Zhang, 2023). A critical issue of these diseases is the multifactorial etiology, known to have a significant impact on their prevention and management since the concomitant use of different drugs may

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https://doi.org/10.1016/j.pharmthera.2024.108595

result in an increased risk of interactions and adverse effects (Siegel, 2011).

A further threat to human health is also represented by increasing antibiotic resistance, especially in ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) pathogens, with 4.95 million estimated deaths globally in 2019 and 10 million deaths/ year expected by 2050, a number greatly exceeding deaths from cancer (Bassetti et al., 2017; Huemer, Mairpady Shambat, Brugger, & Zinkernagel, 2020; Murray et al., 2022; World Health Organization, 2017).

Therefore, in this scenario, the exploration of natural products as supplementation or alternative therapeutic options, is acquiring great importance. Natural products are a preferable source of bioactive drugs because they have good efficacy and better safety profiles than synthetic drugs (Oliveira et al., 2021; Sessa et al., 2015; Sharifi-Rad et al., 2020). Natural products, mainly obtained from plants, possess multiple therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities (Di Pietro, De Santis, Schiavoni, Filardo, & Sessa, 2013; Filardo, Di Pietro, Mastromarino, & Sessa, 2020; Mattioli, Francioso, Mosca, & Silva, 2020; Sessa et al., 2017a; Sessa et al., 2017b; Theodoridis et al., 2022). The usefulness of natural products in disease treatment or prevention, is demonstrated by the fact that approximately one-half of all licensed drugs that were registered worldwide between 1980s and 2000s were natural products or their synthetic derivatives. The search for natural compounds active as potential therapeutic agents is more and more actively pursued, due to the rapid increase in the economic burden for the development of new drugs. Notably, many natural bioactive compounds are ingested *via* the intake of plant foods, which contain different substances in addition to nutrients, like large amounts of polyphenols, a wide class of phytochemical compounds endowed with well-known beneficial effects. Epidemiological studies have, indeed, suggested a causal relationship between consumption of phenolic-rich foods or beverages (fruits, vegetables, beans, cereals, tea, and wine) and a lower incidence of various diseases, such as stroke, cardiovascular diseases, cancer, and neurodegeneration (Del Bo' et al., 2019).

Phenolic compounds are present in *Olea europaea* L (olive tree) and derived products (*e.g.* olive oil). This plant is widespread in all Mediterranean countries and has been commonly used in traditional medicine since ancient times, specifically its leaves, fruits, oil, seeds, and bark (Acar-Tek & Ağagündüz, 2020; Hashmi, Khan, Hanif, Farooq, & Perveen, 2015). A particularly interesting class of bioactive polyphenols present in olive trees is represented by secoiridoids, *e.g.* oleuropein and ligstroside, which are iridoids (cyclopentane [c] pyran monoterpenoids) derivatives that are formed by cleavage of the cyclopentane ring and present a phenolic or catecholic moiety arising from the phenylpropanoid pathway.

This review provides an overview of beneficial health effects, molecular mechanisms, and potential applications of polyphenolic secoiridoids from *Olea europaea* L.

2. Bioactive compounds derived from *Olea Europaea* L

The cultivation of olive trees has its origins in prehistoric times and is one of the oldest known cultivated trees in the world. The plant is a species of small evergreen tree with firm branches and a grayish bark, belonging to the family Oleaceae, mainly found in the Mediterranean basin, though it is also present in North and South America, Australia, and New Zealand. Its fruit is the olive, a small, smooth, green, or purple drupe, that is used to produce olive oil.

Olive oil has been used for thousands of years in the countries surrounding the Mediterranean Sea as a food but also as a remedy for different pathological conditions, particularly skin affections. In the ancient world, olive oil was called "liquid gold" by Homer and "the great healer" by Hippocrates. There are many references to its use in history, literature and also in the mythology of the people of this area (Kapellakis, Tsagarakis, & Crowther, 2008). Nowadays, olive oil beneficial properties are well documented by sound science, and its consumption is strongly recommended in the context of a healthy diet (García-González, Quintero-Flórez, Ruiz-Méndez, & Perona, 2023). Its health promoting effects were attributed to its characteristic lipidic profile, which is rich in monounsaturated fatty acids; however, other oils rich in oleic acid do not exert the same beneficial effects (Isaakidis, El Maghariki, Carvalho-Barros, Gomes, & Correia, 2023). Indeed, an interesting characteristic of olive oil is the presence of minor constituents which are endowed with bioactive properties, besides conferring to olive oil the characteristic aroma and taste. Among these, we can include the following compounds: pigments (*e.g.* chlorophyll, carotenes), hydrocarbons (squalene), phytosterols (beta-sitosterol, campesterol, stigmasterol), lipophilic vitamins (tocopherols, carotenes), volatile compounds (aliphatic alcohols, aldehydes), triterpene alcohols (eritrodiol, uvaol), non-glyceride esters and waxes, and last but most importantly polyphenols (Isaakidis et al., 2023). This last class of compounds comprises at least 40 different chemical species whose bioactive properties have been extensively studied in the past decades (Zeb, 2021) and include: simple phenols (*e.g.* tyrosol, hydroxytyrosol), phenolic acids (hydroxycinnamic acid, hydroxybenzoic acid, vanillic acid), flavonoids (luteolin, apigenin), lignans (pinoresinol), isochromanes (1-phenyl-6,7-dihydroxyisochromane) and secoiridoids (oleocanthal, oleacein).

Among all the different phenolic compounds in olive oil the most interesting group is that of secoiridoids, comprising oleuropein and ligstroside, their respective aglyconic forms (3,4-DHPEA-EA and HPEA-EA) and dialdehydic derivatives (oleacein *i.e.*, 3,4-DHPEA-EDA, and oleocanthal, *i.e.* HPEA-EDA) (Fig. 1). Oleocanthal and oleacein, which are present exclusively in olive oil, are particularly interesting as they show remarkable anti-inflammatory and antioxidant activities. Oleuropein and ligstroside can be found in high concentrations in the leaves and in woody parts of olive tree, and recent reports indicate that oleuropein, ligstroside, oleocanthal and oleacein are also present in high amounts in the roots (Ben Brahim, Priego-Capote, & Bouaziz, 2022; Ortega-García & Peragón, 2010).

Secoiridoids present in olive tree and drupes are produced by the condensation of tyrosol or hydroxytyrosol precursors, produced by the phenylpropanoid metabolism, with intermediates, produced by the secoiridoid biosynthesis pathways, to form the glycosylated form of ligstroside and oleuropein (Fig. 2). During olive oil production, olives crushing liberates glycosidases which remove the sugar part of ligstroside and oleuropein, producing their respective aglycones. These latter compounds undergo demethylation and spontaneous decarboxylation, giving rise to the dialdehyde forms oleocanthal and oleacein, respectively. Hence, olive oil is the only source of these compounds.

Oleocanthal and oleacein are of particular interest and have attracted the attention of scientists who aim at studying their biological activity in detail. However, only limited information on their biological properties is available due to the difficulty of their purification in sufficient amount for extensive investigations. It could be speculated that the cost of industrial scale up of oleocanthal and oleacein production is very high, making it extremely expensive to set up experiments in animal models or in humans.

Oleuropein is the only phenolic secoiridoid which is present in large amount in olive trees, particularly in the leaves, and is easily obtained by extraction. Traditionally, oleuropein extracted from olive leaves or black olive drupes, has been utilized for the semisynthesis of oleacein (Costanzo et al., 2018). On the contrary, ligstroside and its derivative oleocanthal are less abundant and their isolation or chemical synthesis may be a cumbersome and expensive process (Scotece et al., 2015).

Given the complicated steps in *de novo* chemical synthesis, an easier way to obtain purified secoiridoids is to isolate them from plant extracts. Many different technologies were applied to obtaining concentrated extracts from olive products or olive oil production wastes,

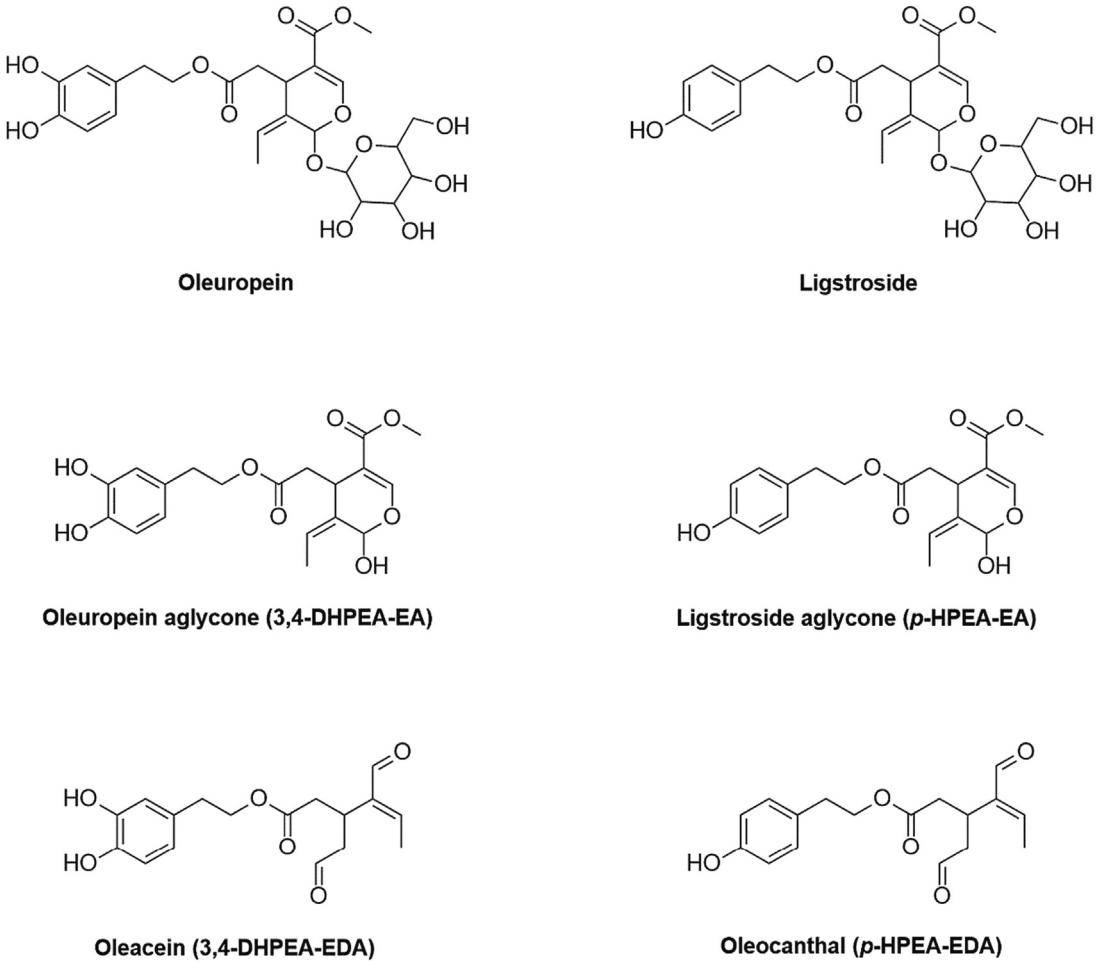

Fig. 1. Structures of secoiridoids present in olive tree and derived products (e.g. olive oil).

including organic solvents extraction, microwave-assisted extraction, supercritical fluid extraction, ultrasound-assisted extraction, and pressurized liquid extraction (Cör Andrejč, Butinar, Knez, Tomažič, & Knez Marevci, 2022; Tapia-Quirós et al., 2020). Recently a new strategy based on the use of Natural Deep Eutectic Solvents (NaDES) has been developed in order to obtain polyphenolic extracts from which the single polyphenols can be further isolated (Francioso et al., 2020; Rodríguez-Juan, Rodríguez-Romero, Fernández-Bolaños, Florido, & Garcia-Borrego, 2021) in a more economic and environmentally friendly way.

3. Beneficial health effects and potential therapeutic approaches of secoiridoids

It is well known that secoiridoids are active as antioxidant, anti-inflammatory, and immunomodulatory compounds, particularly oleocanthal and oleacein, which have been suggested as potential therapeutic agents in inflammatory and reactive oxygen species (ROS)-induced diseases (Castejón, Montoya, Alarcón-de-la-Lastra, & Sánchez-Hidalgo, 2020). Apart from being radical scavengers *in vitro*, oleocanthal and oleacein can alter the expression of antioxidant enzymes *via* Nuclear factor erythroid 2-related factor 2 (Nrf2) activation, which modulates the expression of glutathione- and thioredoxin-related enzymes, phase 2 detoxifying enzymes, NAD(P)H quinone oxidoreductase-1, heme oxygenase-1, *etc.* (Angeloni, Giusti, & Hrelia, 2019; Martínez-Huélamo, Rodríguez-Morató, Boronat, & de la Torre, 2017). The activation of Nrf2 pathway may be at the basis of all the health promoting activities exerted by olive oil secoiridoids.

3.1. Secoiridoids in neurologic disorders

Neurodegeneration refers to the chronic and progressive loss of neurons in the brain and spinal cord (Satyam & Bairy, 2022; Subhramanyam, Wang, Hu, & Dheen, 2019), typically associated with aging, and is a process that encompasses various diseases, the most represented being Alzheimer's and Parkinson's (Tiwari, Moin, Rizvi, Shahid, & Bajpai, 2021). Neurodegeneration and neuroinflammation are closely intertwined, and it is often challenging to determine which one is the cause, and which is the consequence of the other (Tiwari et al., 2021; Uddin, Yu, & Lim, 2021). For example, Alzheimer's is caused by the release of amyloid aggregates (amyloid-beta, Aβ, and tau protein) by neuronal cells, leading to a pro-inflammatory activation of microglial cells, ultimately resulting in neuronal death (Leng & Edison, 2021).

Currently, the cost of treatment for neurodegeneration-associated dementia disorders is around $52 billion for Parkinson's and $305 billion for Alzheimer's in the USA, with an expected surge to $1 trillion by 2050 (Al-kharboosh, Perera, Bechtle, Bu, & Quinones-Hinojosa, 2022). In this context, olive oil polyphenols could represent a new tool in both the prevention and treatment of these pathologies. In fact, according to a study by Berr and colleagues, based on a ten-year follow-up, a high consumption of olive oil is associated with a reduced risk of cognitive decline, stroke, and death (Berr et al., 2009).

Among olive oil polyphenols, secoiridoids have been investigated for their protective activity against neurological disorders, and, in particular, most studies focused on oleocanthal and oleuropein. As for oleocanthal, plenty of reports in the literature demonstrate its ability

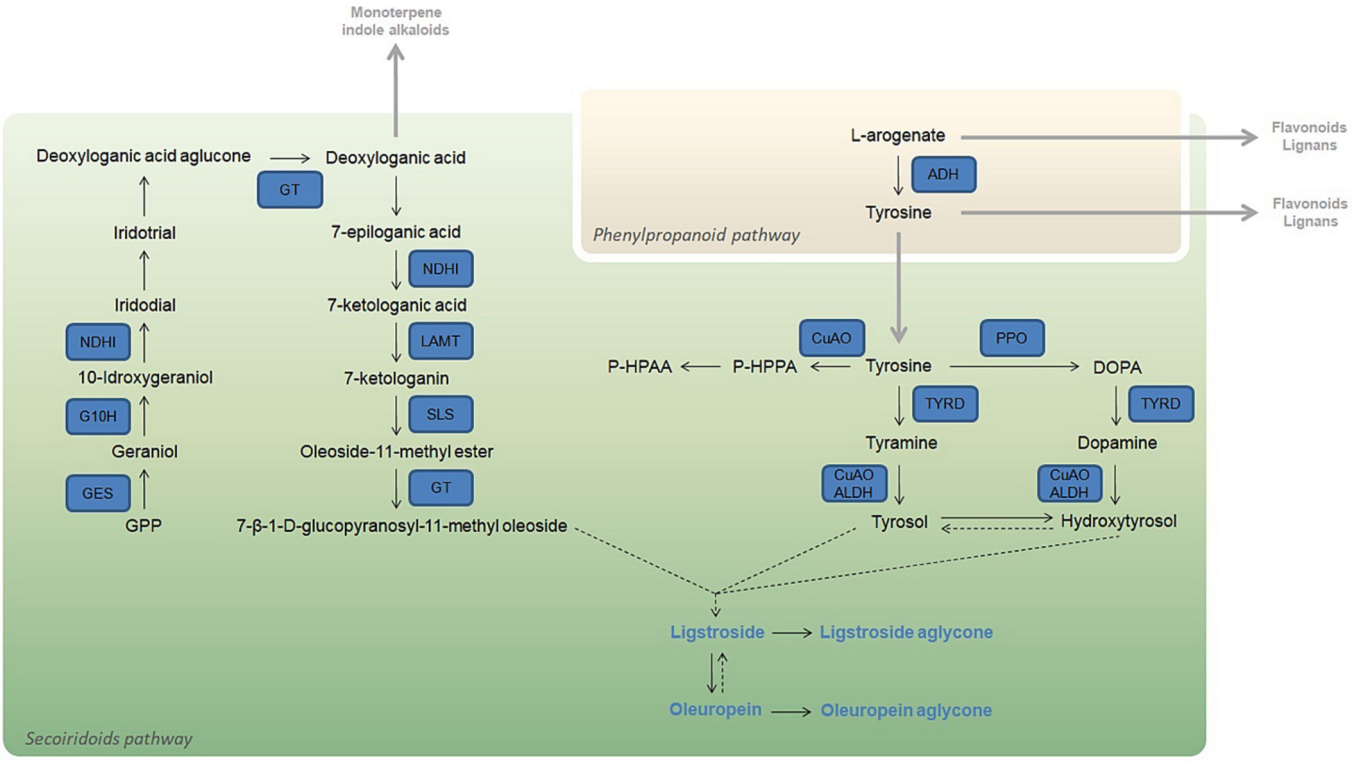

Fig. 2. Biosynthetic pathway of secoiridoids in olive tree. The enzymes which catalyze the biosynthetic steps are indicated in blue. ADH (Arogenate dehydrogenase); CuAO (Copper amine oxidase); PPO (Polyphenol oxidase); TYRD (Tyrosine/dopa decarboxylase); ALDH (Alcohol dehydrogenase); GES (Geraniol synthase); G10H (Geraniol 10-hydroxylase); NDHI (NADH dehydrogenase I); GT (Glucosyltransferase); LAMT (Loganic acid methyltransferase); SLS (Secologanin synthase). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to counteract the pathogenesis of Alzheimer's disease *via* different mechanisms:

- it interacts with the Tau protein, forming a Schiff base with lysine 18 (Li et al., 2009);
- it reduces oxidative and nitrosative stress, as demonstrated *ex vivo* (De La Cruz Cortés et al., 2021), *in vitro* on chondrocytes and neuronal SH-SY5Y cells (Iacono et al., 2010; Scotece et al., 2013) and in a rat model of brain injury (Mete et al., 2017);
- it interacts directly with Amyloid beta$_{1\text{-}42}$ (Aβ), already at concentrations of 10 nM, and reduces its aggregation, thereby preserving the dendritic spines of primary murine hippocampal cells (Pitt et al., 2009);
- when administered in powder and solid dispersion formulations by the oral route, it inhibits the C3AR1 factor upstream of STAT3 leading to improved cognitive performance in 5xFAD mice and to an attenuation of Aβ plaque formation (Tajmim et al., 2021);
- it induces the expression of proteins capable of ensuring adequate clearance of Aβ *via* apoE-dependent and independent pathways, also reducing astrocyte activation in TgSwDI mice (Qosa et al., 2015). Indeed, oleocanthal has also been shown to be an interesting modulator of these pathways in LS-180 adenocarcinoma lines (Abuznait et al., 2011), HeLa cells (Cassiano, Casapullo, Tosco, Monti, & Riccio, 2015) and SH-SY5Y neural cells, with increased ROS scavenging, phosphorylation of AKT and upregulation of Heat Shock Protein 90 (HSP90) (Giusti et al., 2018).

Beside oleocanthal, several authors highlighted the neuroprotective effects of oleuropein, showing that also this molecule was able to inhibit aggregation and Aβ-associated damage in different *in vivo* models (Henríquez, Gomez, Guerrero, & Narayan, 2020; Klimova, Novotný, Kuca, & Valis, 2019; Rigacci, 2015; Romero-Márquez et al., 2022).

Oleuropein aglycone was found to interact directly with Tau protein (Daccache et al., 2011), Aβ$_{1\text{-}40}$ (Caba, Ştefănescu, & Tamba, 2021) and alpha-synuclein (Borah, Sanjeev, & Mattaparthi, 2021), counteracting their aggregation and ameliorating the pathological condition. In particular, Brogi, Sirous, Calderone, & Chemi, 2020, conducted a very interesting *in silico* experiment where he showed that the likely point of interaction with Aβ is the 17-23 LVFFAED motif, from which the polyphenol can then move to the same motif in another chain (Brogi et al., 2020). This finding is in agreement with the previous work of Bazoti, Bergquist, Markides, & Tsarbopoulos, 2008, who demonstrated an interaction between Aβ and oleuropein (Bazoti et al., 2008). Galanakis et al., 2011, also showed how the region V12-N27, comprising LVFFAED motif, is the one that carries the greatest chemical shift in oleuropein binding by Nuclear Magnetic Resonance Spectroscopy (Galanakis et al., 2011).

The interaction between oleuropein and Aβ is also suggested by Elmazoglu et al., 2021, that outlined how the pretreatment with oleuropein of primary rat hippocampal cells subjected to hyperglycemia and Aβ$_{1\text{-}42}$ as inflammatory stimuli, led to antioxidant and anti-inflammatory effects by modulating the production of cytokines, the level of pro-oxidant markers, antioxidant enzymes and the mitochondrial membrane potential (Elmazoglu et al., 2021).

A recent work demonstrated oleuropein's ability to inhibit Aβ aggregation, reducing cellular mortality and associated oxidative stress in SH-SY5Y neural cells (Leri et al., 2021), and the amyloidogenic protein S100A9, which appears to be an important player in the neuroinflammatory cascade of Alzheimer's and Parkinson's disease, and traumatic brain injury (Leri, Chaudhary, et al., 2021). In addition, the combined treatment of oleuropein and hydroxytyrosol in a 1:1 ratio has been shown to activate autophagic flux when administered 24 h prior to treatment with amyloid oligomers or fibrils to SH-SY5Y neural cells (Leri, Bertolini, Stefani, & Bucciantini, 2021). More in-depth studies on oleuropein-modulated proteins identified inhibition of Beta Secretase 1 (BACE-1) (Omar, Scott, Hamlin, & Obied, 2018) and Histone

Deacetylase 2 (HDAC-2) (Luccarini et al., 2016; Omar et al., 2018) as key regulators. In line with this, Kostomoiri and coworkers had previously demonstrated how oleuropein increases the presence of sAPPalpha in HEK293 cells stably transfected with isoform 695 of human APP, *via* up-regulation of MMP-9 metalloprotease activity (Kostomoiri et al., 2013).

In *in vivo* models, Grossi et al., 2013, demonstrated that treatment with 50 mg/kg oleuropein for 2 months was able to reduce Aβ deposition in cortex and hippocampus of TgCRND8 mice through activation of the autophagic process, as demonstrated in both murine brain and N2A murine neuroblastoma cell cultures (Grossi et al., 2013). These data have been confirmed by Pantano et al., 2017, who demonstrated that supplementation with oleuropein (50 mg/Kg) improved behavioral performance and neuropathology in a dose dependent manner in the same mouse model (Pantano et al., 2017).

Yu et al., 2016, showed that intraperitoneal or intracerebroventricular injection of 100 mg/kg oleuropein was able to reduce ischemic damage in the brain following reperfusion injury (Yu et al., 2016). Similarly, Pourkhodadad et al., 2016, showed how a 10-day treatment of oleuropein (10-20 mg/Kg) in a rat model in which colchicine is administered at the hippocampal CA1 level to induce cognitive dysfunction, was able to reduce oxidative stress and apoptosis (Pourkhodadad et al., 2016). Finally, Wang, Ramasamy, Kang, & Jo, 2020, observed how oleuropein treatment (100 μM) led to an increase in long-term potentiation through the facilitation of calcium-permeable amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors trafficking and synaptic transmission in primary hippocampal cultures (Wang et al., 2020).

Diomede, Rigacci, Romeo, Stefani, & Salmona, 2013, used the *Caenorhabditis elegans* CL2006 strain (Alzheimer's disease model) to show that oleuropein (50 μM) is able to reduce Aβ aggregation, while Brunetti et al., 2020, demonstrates how treatment with 250 and 500 μg/ml oleuropein leads to a remarkable increase in survival of *C. elegans* following heat stress treatment (Brunetti et al., 2020; Diomede et al., 2013). This improvement is also seen in three *C. elegans* Parkinson's disease models (rotenone-induced, OW13 and UA44 strain) (Brunetti et al., 2020).

As for the secoiridoids oleacein and ligstroside, the investigation on their potential neuroprotective effects is still at a preliminary stage. On this regard, for example, Grewal et al., 2020, showed that ligstroside is able to increase ATP (through increased mitochondrial activity) in both neuroblastoma cells (SH-SY5Y-APP$_{695}$) and aged female NMRI mice resulting in improved spatial working memory and life extension, although it was unable to reduce A$\beta_{1\text{-}40}$ levels in the same cell line (Grewal et al., 2020).

Further evidence on the potential involvement of secoiridoids in the improvement of Alzheimer's disease-associated pathology and inflammation, came from *in vivo* and clinical trial studies investigating *O. europaea* L. extracts, derived from either EVOO or leaves, as well as more complex nutraceutical formulations, enriched with oleocanthal or oleuropein (Table 1).

3.2. Secoiridoids in metabolic disorders

The prevalence of metabolic syndrome is continuously growing globally and was estimated to be around 20–25% in 2021, with higher values in United States (Gallardo-Alfaro et al., 2020; Hirode & Wong, 2020; Zečević et al., 2023). Metabolic syndrome, defined by a cluster of several cardiometabolic conditions such as dyslipidemia, hyperglycemia, hypertension, and obesity, is well known to increase the risk of developing cardiovascular diseases, type 2 diabetes mellitus, non-alcoholic fatty liver disease, dementia, and cancer (Gallardo-Alfaro et al., 2020).

3.2.1. Dyslipidemia

The hypolipidemic effects of purified secoiridoids has been reported in different *in vitro* and *in vivo* models; *in vitro* studies evidenced a decreased free-fatty-acid-induced lipid accumulation, lipid droplet size and intracellular triglyceride content in hepatocytes after exposure to oleuropein (50 μmol/l) (Hur et al., 2012). In particular oleuropein (25 μM) inhibited the activity of several key enzymes involved in fatty acid biosynthesis (acetyl-CoA carboxylase), triglyceride synthesis (diacylglycerol acyltransferase) and in cholesterogenesis (3-hydroxy-3-methyl-glutaryl-CoA reductase) (Priore, Siculella, & Gnoni, 2014).

Importantly, different *in vivo* dyslipidemia models showed the ability of oleuropein, administrated at different doses (38 mg/kg/day-758 mg/kg/day) and times (8-12 weeks), to reduce body weight, adipose tissue, serum triglyceride and/or cholesterol levels, and/or liver steatosis (Hadrich et al., 2016; Park, Choi, Um, Yoon, & Park, 2011; van der Stelt et al., 2015). Similar findings have also been described for oleacein when administrated (20 mg/kg, 5 weeks) in mice fed with high fat diet (HFD) (Lombardo et al., 2018). Interestingly, different mechanisms as well as molecular targets have been described; for example, oleuropein (100 mg/kg per day, over the course of 6 weeks) has been demonstrated to act as a PPAR-α ligand, which up-regulates a broad spectrum of factors in the liver and white adipose tissue with essential roles in lipid synthesis, transport, metabolism, and clearance (Malliou et al., 2018).

Further evidence on the potential beneficial effects of secoiridoids towards dyslipidaemia came from studies investigating *O. europaea* L. leaf extracts (Table 1). More interestingly, a systematic review and meta-analysis, summarizing all the available human clinical trials on prehypertensive and hypertensive populations, evidenced a reduction in low density lipoprotein (LDL) levels and inflammatory markers for cardiovascular diseases [interleukin(IL)-6, IL-8, and tumor necrosis factor (TNF)-α] when olive leaf extract was administrated at 1000 mg/day and 500 mg/day, respectively (Ismail, Norhayati, & Mohamad, 2021).

3.2.2. Diabetes

Diabetes, characterized by chronic hyperglycemia, insulin resistance, and insufficient insulin secretion, is one of the leading causes of morbidity worldwide; in 2019, 463 million people were affected by diabetes, and approximately 700 million are expected by 2045 (Saeedi et al., 2019).

There are many studies showing the protective effects as well as molecular targets and mechanisms by which purified secoiridoids may control hyperglycemia and prevent diabetes. For example, an *in vitro* study on muscle cells showed that oleuropein (10 μM) induced the translocation of glucose transporter-4 (GLUT-4) into the cell membrane, through activation of adenosine monophosphate -activated protein kinase (MAPK), facilitating, hence, the glucose uptake (Fujiwara et al., 2017).

In addition to the regulation of the glucose uptake, secoiridoids, mainly oleuropein (1 μM) and ligstroside (10 μM), were able to promote glucose-stimulated insulin secretion in pancreatic β cell, through the activation of extracellular signal-regulated kinase (ERK)/MAPK signaling pathway (Wu, Velander, Liu, & Xu, 2017). Further mechanism by which oleuropein and its aglycone derivate may ameliorate hyperglycemia is to inhibit the formation and cytotoxicity of amylin deposits (IC50 100 μM), pathological hallmark of the pancreatic islet in type 2 diabetes and responsible for insulin resistance (Chaari, 2020). Lastly, oleuropein (IC50 13.1 μg/ml) has been also demonstrated to restrict the activity of carbohydrate digestive enzymes such as α-glucosidase within gastrointestinal tract, an enzyme that impedes the breakdown of complex carbohydrates slowing their absorption and, hence, reducing postprandial hyperglycemia (Haguet et al., 2023).

Of particular importance are the *in vivo* studies providing additional insight underlying the ability of purified secoiridoids to improve glucose tolerance as well as insulin sensitivity by using different diabetic animal models (Aggul et al., 2020; Al-Azzawie & Alhamdani, 2006; Lombardo et al., 2018); for example, oleacein (20 mg/kg, 5 weeks) was able to prevent the HFD-related hepatic insulin resistance by decreasing levels of important factors regulating the contribution of liver and adipose tissue

Table 1
Biological activities of *O. europaea* L. extracts.

Neurological disorders					
(Author, Year)	Study Design	*O. europaea* L. extract	Composition	Treatment Dose and time	Activity
(Barbalace et al., 2021)	SH-SY5Y neuroblastoma cells	EVOO dry extracts	Hill oil extract: hydroxytyrosol (0.68 mg/g), tyrosol (0.77 mg/g), oleacein (0.80 mg/g), oleocanthal (1.10 mg/g), pinoresinol (1.82 mg/g) acetoxypinoresinol (1.85 mg/g). Plain oil extract: hydroxytyrosol (2.17 mg/g), tyrosol (1.70 mg/g), oleacein (2.61 mg/g), oleocanthal (1.32 mg/g), pinoresinol (0.66 mg/g) acetoxypinoresinol (1.02 mg/g)	1-500 μg/ml	Antioxidant activity, *i.e.* positive modulation of antioxidant enzymes; neuronal plasticity enhancement, *i.e.* positive modulation of BDNF
(Batarseh & Kaddoumi, 2018)	5xFAD mouse model of AD	Oleocanthal-rich EVOO	oleocanthal (680 mg/kg)	0.7 g/kg/day (476 μg/kg/day oleocanthal), 4 months	Reduction of Aβ load
(Al Rihani, Darakjian, & Kaddoumi, 2019)	TgSwDI mouse model of AD	Oleocanthal-rich EVOO	oleocanthal (680 mg/kg), oleacein (315 mg/kg), hydroxytyrosol (389 mg/kg), tyrosol (994 mg/kg), oleuropein aglycon (72 mg/kg)	0.7 g/kg/day (476 μg/kg/day oleocanthal), 4 months	Restored BBB function and reduced AD-associated pathology
(Abdallah et al., 2023)	5xFAD mouse model of AD	Oleocanthal low EVOO or oleocanthal	oleocanthal (35 mg/kg), total phenolic compounds (540 mg/kg)	0.5 mg/kg/day, 3 months	Reduced AD-associated pathology and inflammation
(Romero-Márquez et al., 2022)	*Caenorhabditis elegans* model	Oleuropein-rich olive leaf extract	total phenols (212.1 mg/g), total flavonoids (388.1 mg/g), oleuropein (43% *w*/w)	oleuropein (100 μg/ml)	Reduction of oxidative stress and proteotoxicity related to Aβ and tau aggregation
(Marianetti, Pinna, Venuti, & Liguri, 2022)	Clinical trial - patients with mild AD	Nutraceutical formulation containing oleuropein	oleuropein (80 mg/cps), SAG (50 mg/cps), vitamin B6 (1 mg/cps), vitamin B12 (3 μg/cps), vitamin E (15 IU/cps), vitamin D3 (4 μg/cps), piperine (3 mg/cps), bacopa dry extract (100 mg/cps)	2 cps/day, 6 months	Improvement of measured neurocognitive parameters in all AD patients
Dyslipidaemia					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Olmez et al., 2015)	HCD fed rats	Olive leaf extract	total phenols (0.394 mg/100 mg), total flavonoids (0.558 mg/100 mg)	50 or 100 mg/kg/day, 8 week	Reduction of total and LDL cholesterol levels
(Fki et al., 2020)	HFD fed rats	Oleuropein-rich olive leaf extract	oleuropein (96.95% total phenols, 905.96 mg/g)	16 mg/kg/day, 2 months	Hypolipidemic and hepatoprotective effects
(Yoon, Liu, Park, & Kim, 2015)	Estrogen deficient rats	Olive leaf extract	Not reported	400 mg/kg/day, 10 weeks	Decreased liver and serum triglyceride and serum VLDL
Diabetes					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Kerimi et al., 2019)	Caco-2/TC7 cells	Bonolive powder	oleuropein (41.8 % w/w)	0.2-2.0 mg/ml oleuropein	Inhibition of glucose transporter-2 (EC50 0.5 mg/ml) and sucrase (IC50 1.28 mg/ml)
	Clinical trial - healthy volunteers			35-200 mg/day oleuropein	Attenuation of post-prandial blood glucose after consumption of 25 g sucrose
(Jemai, El Feki, & Sayadi, 2009)	Alloxan diabetic rats	Oleuropein-rich olive leaf extract	oleuropein (2.44% w/w)	16 and 8 mg/kg/day, 4 weeks	Decreased serum glucose and cholesterols levels and restored antioxidant perturbations
(Liu, Jung, Park, & Kim, 2014)	Streptozotocin and HFD–induced diabetes rats	Olive leaf extract	Not reported	400 mg/kg/day, 8 weeks	Attenuated insulin resistance by suppressing mRNA expression of proinflammatory cytokines and elevating of insulin receptor substrate 1 expression
(Murotomi et al., 2015)	Type 2 diabetic mice	Oleuropein-containing supplement OPIACE	oleuropein (35% w/w), other phenols (7% *w*/w)	0.2% w/w OPIACE in control diet (CE-2) twice daily, 20 weeks	Decreased hyperglycaemia and impaired glucose tolerance
(de Bock et al., 2013)	Clinical trial - overweight patients	Olive leaf extract	oleuropein (51.124 mg/4 cps), hydroxytyrosol (9.7 mg/4 cps), minor components (<1 mg/4 cps)	4 cps/day, 12 weeks	Improved insulin sensitivity and pancreatic β-cell secretory capacity
Hyperthension					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Romero et al., 2016)	*In vivo* - spontaneously hypertensive rats	Olive leaf extract	oleuropein (15% w/w), triterpenic acid (10% *w*/w), hydroxytyrosol (1% w/w)	30 mg/kg/day, 5 weeks	Reduced NOX-1 and NOX-2 mRNA levels, and increased eNOS activity

Table 1 (*continued*)

Neurological disorders					
(Author, Year)	Study Design	*O. europaea* L. extract	Composition	Treatment Dose and time	Activity
(Lockyer, Rowland, Spencer, Yaqoob, & Stonehouse, 2017)	Clinical trial - pre-hyperthensive subjects	Phenolic-rich olive leaf extract	oleuropein (6.81 mg/ml), oleoside (0.73 mg/ml), hydroxytyrosol (0.32 mg/ml), minor components (<0.5 mg/ml)	oleuropein 136 mg/day, hydroxytyrosol 6.4 mg/day, 6 weeks	Reduction of systolic and diastolic blood pressure
(Susalit et al., 2011)	Clinical trial - stage-1 hyperthensive patients	Olive leaf extract	oleuropein (19.9% *w*/w)	1000 mg/day, 8 weeks	Reduction of systolic and diastolic blood pressure
(Ivanov et al., 2018)	*In vivo* - spontaneously hypertensive rats	Olive leaf extract	oleuropein (17% w/w), total polyphenols (40.5% *w*/w)	25 or 50 mg/kg	Improved systemic as well as carotid and renal haemodynamics, and reduced peripheral and regional vascular resistance at 25 mg/kg. Improved blood pressure at 50 mg/kg.
Obesity					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Vezza et al., 2019)	HFD fed mice	Olive leaf extract	oleuropein (10% w/w), other phenols (2% w/w)	25 mg/kg/day, 5 weeks	Reduced body weight gain, basal glycaemia and insulin resistance, and improved plasma lipid profile and inflammation; reduced dysbiosis in gut microbiota
Cardiovascular diseases					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Agrawal et al., 2017)	Clinical trial - healthy volunteers	Oleocanthal- and oleacein- rich EVOOs	D2i0.5 composition: oleocanthal (310 mg/kg), oleacein (150 mg/kg); D2i2 composition: oleocanthal (172 mg/kg), oleacein (312 mg/kg)	40 ml EVOO, 4 non consecutive days	Inhibition of platelet aggregation
(Dell'Agli et al., 2006)	Human umbilical vascular endothelial cells	EVOO extract	oleuropein aglycone (0.380 mg/ml), hydroxytyrosol (0.170 mg/ml)	0.25-25 μM expressed as hydroxytyrosol equivalent	Reduced cell surface expression of ICAM-1 and VCAM-1 (IC50 < 1 μM)
Cancer					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Peri et al., 2022)	Gastric adenocarcinoma cell line (AGS) wild type, or chemoresistant to 5-fluorouracil (5FUr) and Paclitaxel (TAXr)	Oleocanthal-enriched EVOO extract fraction	oleocanthal (189.16 mg/g), 10-hydroxyoleocanthal (287.62 mg/g), oleuropein aglycone (95.44 mg/g), ligstroside (23.29 mg/g), secoiricoidic derivatives (171.99 mg/g), other compounds (98.07 mg/g)	3.75 - 240 μM OCF	Cell cycle and ROS production inhibition at 60 μM
Inflammatory Bowel Diseases					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Elmaksoud, Motawea, Desoky, Elharrif, & Ibrahimi, 2021)	Induced ulcerative colitis rats	Olive leaf extract supplemented with 25% hydroxytyrosol	Not reported	150 mg/kg/day, 7 days	Reduced oxidative stress and inflammation in colon tissue (decreased MDA, MPO and NO levels and increased SOD, CAT and GPX levels)
(Mattioli et al., 2022)	Caco-2 cells	Olive leaf extract	total secoiridoids (309.83 mg/g), oleuropein and its isomers (85.91%)	217 and 433 μg/ml, 24 h	Reduced cell viability (IC50 433 μg/ml)
	Ileum and colon portion from guinea pigs			0.1-10 mg/ml, 5 min	Spasmolytic activity on Ileum (IC50 0.77 mg/ml) and Colon (IC50 0.13 mg/ml) tissues
(Fakhraei et al., 2014)	Acetic acid-induced ulcerative colitis rats	Olive leaf extract	oleuropein (356 mg/g), tyrosol (3.73 mg/g), hydroxytyrosol (4.89 mg/g), caffeic acid (49.41 mg/g)	250, 500 and 750 mg/kg, 3 days	Reduced severity of the ulcerative lesions and inflammation (dose-dependent effect)
(Vezza et al., 2017)	Mice models of colitis (DSS and DNBS)	Olive leaf extract	total phenols (10.64% w/w), oleuropein (82.5%)	0.5, 1, 10, and 25 mg/kg/day, 6 days	Reduced expression of proinflammatory mediators, and improved intestinal epithelial barrier integrity (lowest active concentration 1 mg/kg)
	Mucosal explants of healthy donors and Chron's disease patients			100 ng/ml, 24 h	Decreased proinflammatory mediators
Skin Disorders					
(Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Segura Palacios et al., 2019)	Clinical trial - patients diagnosed with actinic keratosis/field cancerization	Oily emollient fluid enriched with olive oil extract	oleocanthal (50% w/w), oleacein (45% *w*/w), olive oil lipids (5% w/w)	Topical application three times/day, 1 week	Reduced cutaneous inflammation and better response to photodynamic therapy

(*continued on next page*)

Table 1 (*continued*)

Neurological disorders					
(Author, Year)	Study Design	*O. europaea* L. extract	Composition	Treatment Dose and time	Activity
Infectious Diseases (Author, Year)	**Study Design**	***O. europaea* L. extract**	**Composition**	**Treatment Dose and time**	**Activity**
(Di Pietro et al., 2022)	*In vitro*	EVOO extract	total polyphenols (52.9-68.8 mM), oleocanthal (5.2-9.3 mM), oleacein (6.3-6.6 mM)	225-7200 μg/ml	Anti-microbial activity against antibiotic-resistant human clinical isolates (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*), MIC 900-2340 μg/ml.
(Silvan, Guerrero-Hurtado, Gutierrez-Docio, Prodanov, & Martinez-Rodriguez, 2022)	*In vitro*	Oleuropein-rich olive leaf extract	oleuropein (20,471 mg/100 g), other phenolic and secoiridoid compounds (8684 mg/100 g)	0.1 - 2 mg/ml	Anti-microbial activity againsts zoonotic antibiotic resistant strains of *Campylobacter jejuni* and *Campylobacter coli* (MIC 2 mg/ml)
(Silvan et al., 2021)	*In vitro*	Oleuropein-rich olive leaf extract	oleuropein (20,471 mg/100 g), other phenolic and secoiridoid compounds (8684 mg/100 g)	2 mg/ml	Anti-microbial activity againsts different human clinical isolates of *Helicobacter pylori*
(Pereira et al., 2007)	*In vitro*	Olive leaf extract	total phenols (36,051.8 mg/kg), oleuropein (26,471 mg/kg)	0.05 - 5 mg/ml	Anti-microbial activity against standard bacterial strains (*Bacillus subtilis, Bacillus cereus, S. aureus, E. coli, K. pneumoniae*) (IC25 0.63-3.22 mg/ml) and fungal strains (*Candida albicans* and *Candida neoformans*) (IC25 0.85-3.0 mg/ml)
(Lee-Huang, Zhang, Lin Huang, Chang, & Huang, 2003)	*In vitro*	Olive leaf extract	oleuropein (12% w/w)	0.1 - 1000 μg/ml	Anti-HIV activity (EC50 0.23 μg/ml)
(Ahmadpour et al., 2023)	Clinical trial - COVID19 patients	Olive leaf extract capsules	oleuropein (30% w/w)	250 and 500 mg/12 h, 5 days	Improved clinical status and decreased length of hospitalization (250 mg)

FAD, familial Alzheimer's disease mutation; AD, Alzheimer's Disease; EVOO, extra virgin olive oil; Aβ, amyloid β protein; BBB, blood brain barrier; IU, international unit; cps, capsules; HCD, high cholesterol diet; LDL, low density lipoprotein; HFD, high fat diet; VLDL, very low density lipoprotein; EC50, half-maximal effective concentration; IC50, half-maximal inhibitory concentration; NOX, nitrogen oxides; eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; ROS, reactive oxygen species; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; MIC, minimum inhibitory concentration; OCF, Oleocanthal-enriched EVOO extract fraction.

to lipogenesis (sterol regulatory element-binding transcription factor-1 and fatty acid synthase) as well as the levels of phospho-ERK (Lombardo et al., 2018). Differently, oleuropein (5-10 mg/Kg/day) reduced blood glucose, insulin, and hepatic glycogen levels in mice with gestational diabetes, by attenuating oxidative stress and inflammation *via* the activation of the AMPK signaling pathway (Zhang, Zhao, & Wang, 2021). Very recently, oleuropein (200 mg/kg body weight/day, 15 weeks) has been described to counteract gut microbiota dysbiosis in type 2 diabetic db/db mice, known to play a key role in the regulation of metabolic pathways including those related to glucose (Howard, Lam, & Duca, 2022; Zheng et al., 2021).

More importantly, clinical trials have shown the potential application of oleuropein alone in the control of blood glucose; for example, Carnevale et al., 2018, reported the ability of oleuropein (20 mg, single dose) to lower postprandial glycaemia by counteracting oxidative stress mediated mechanisms underlying insulin secretion in healthy subjects, as well as in patients with impaired fasting glucose (Carnevale et al., 2018). Specifically, oleuropein inhibited the production of NADPH oxidases (NOX)-2 derived ROS and, consequently, the activity of dipeptidyl-peptidase 4 protein, resulting in increased glucagon-like peptide-1 mediated insulin secretion (Carnevale et al., 2018). Also extracts from *O. europaea* L. leaves, as well as dietary supplements, mostly rich in oleuropein, showed significant improvement in insulin sensitivity, post prandial blood glucose response and decreased blood glucose levels, *via in vitro* or *in vivo* studies and clinical trials (Table 1).

3.2.3. Hypertension

Hypertension is the most important risk factor for cardiovascular diseases and, according to the WHO, approximately 1.28 billion adults aged 30-79 worldwide have arterial hypertension (World Health Organization, 2022). *In vitro* studies have demonstrated several mechanisms by which purified secoiridoids may have antihypertensive or vasodilator activities; in particular, oleacein inhibited angiotensin-converting enzyme (IC50 26 μM) (Hansen et al., 1996) and had a direct vasodilatory effect on vascular smooth muscle cells (Zarzuelo, Duarte, Jiménez, González, & Utrilla, 1991). However, the antihypertensive activity of secoiridoids has also been highlighted in animal models, although most studies focused on *O. europaea* L. leaf extract containing oleuropein alongside other phenols (Table 1).

Of high clinical significance are the findings from the systematic review and meta-analysis including pre-hypertensive and hypertensive populations evidencing that 500 mg per day of olive leaf extract significantly reduced the systolic blood pressure over a period of at least 8 weeks of follow up (Ismail et al., 2021).

3.2.4. Obesity

Obesity is responsible for over 2.8 million deaths each year globally (World Health Organization, 2021) and it is characterized by an excessive growth and expansion of the adipose tissue responsible for metabolic dysfunction, linked to several comorbidities, including insulin resistance and type 2 diabetes, cardiovascular diseases, and cancer (Jin et al., 2023).

By using an *in vitro* human adipose tissue model mimicking inflammation related to obesity, purified oleacein (25 μmol/l) and oleocanthal (25 μmol/l) have been demonstrated to blunt adipocyte dysfunction and the associated pro-inflammatory cascade through different mechanisms: i. downregulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and consequent decreased expression of TNF-α-induced genes and miRNAs involved in inflammation (chemokines like monocyte chemoattractant protein-1, the pro-

inflammatory enzyme cyclooxygenase COX-2, IL-1β, *etc.*), angiogenesis (pro-angiogenic vascular endothelial growth factor, metalloproteinase-2, *etc.*), and oxidative stress (NOX, superoxide dismutase-2, *etc.*); ii. upregulation of the anti-inflammatory/metabolic effector PPAR-γ expression (Carpi et al., 2019). Moreover, oleacein (20 mg/kg/day, 13 weeks) in already obese mice, has been described to neutralize some of the adverse effects induced by HFD diet, including the increase in body weight, mainly due to lesser liver steatosis enlargement, and the increase in total serum cholesterol levels, although it was not able to ameliorate the plasma glucose and triglyceride serum levels (Lombardo et al., 2018).

Notably, also an oleuropein-rich olive leaf extract, in an HFD-induced obesity mouse model, showed a significant reduction in body weight and adipose tissue by acting on multi-targets related to insulin resistance, including glucose uptake, lipid metabolism and inflammation (Table 1) (Vezza et al., 2019). Particularly intriguing was also the beneficial effect of oleuropein rich-olive leaf extract towards the intestinal microbiota in the same murine model, counteracting gut dysbiosis (Vezza et al., 2019).

3.3. Secoiridoids in cardiovascular disorders

Cardiovascular diseases (CVDs) are the leading cause of death globally, accounting for an estimated 17.9 million deaths in 2020 (World Health Organization, 2023), and the main pathological process underlying this disease is the atherosclerosis. Atherosclerosis typically begins with endothelial dysfunction, followed by foam cell formation, vascular smooth muscle cell proliferation, and platelet adhesion and aggregation resulting in an unstable atherosclerotic plaque, responsible for myocardial infarction and stroke (Wojtasińska et al., 2023).

Over the last decade, a growing number of *in vitro* studies have highlighted beneficial effects of purified secoiridoids on the early stages of atherosclerosis. First, oleuropein and the respective aglycone, oleocanthal and oleacein inhibited ROS production and, hence, LDL oxidation and foam cell formation (Masella et al., 2004; Rosignoli, Fuccelli, Fabiani, Servili, & Morozzi, 2013; Visioli, Bellomo, Montedoro, & Galli, 1995). Oleacein (50 μM) also attenuated the foam cell formation by decreasing the expression of receptors (Scavenger receptor A1, CD36, lectin-like oxLDL receptor) involved in binding and uptake of oxidized LDL in macrophage (Filipek, Mikołajczyk, Guzik, & Naruszewicz, 2020). Second, oleuropein and oleocanthal were able to scavenge free radicals or HOCl, responsible for oxidation of apoproteic component of LDL (Cuffaro et al., 2023; Saija, 1998; Visioli, Bellomo, & Galli, 1998). Lastly, oleuropein and oleacein (50- 100 μM) inhibited the expression of endothelial leukocyte adhesion molecules (vascular cell adhesion molecule, VCAM-1, intercellular adhesion molecule, ICAM-1, L-selectin, *etc.*), thus decreasing monocyte cell adhesion to human vascular endothelial cells (Carluccio et al., 2003; Czerwińska, Kiss, & Naruszewicz, 2012; Czerwińska, Kiss, & Naruszewicz, 2014; Sindona et al., 2012).

Interestingly, purified secoiridoids have also been demonstrated to prevent the late stages of atherosclerotic process. Oleuropein (100 μM), indeed, inhibited vascular smooth muscle cell proliferation (Abe et al., 2011) as well as platelet aggregation (Zbidi et al., 2009). Oleacein (10-20 μM) attenuated the destabilization of atherosclerotic plaque and, hence, prevented its rupture by inhibiting multiply molecular targets (high-mobility group protein-1, matrix metalloproteinase 9, matrix metalloproteinase 9/ neutrophil gelatinase-associated lipocalin complex and tissue factor) (Czerwińska et al., 2014; Filipek, Czerwińska, Kiss, Polański, & Naruszewicz, 2017). Further mechanisms, preventing atherosclerotic plaque rupture, have been described for oleacein; specifically, it (50 μM) was able to inhibit apoptosis of macrophages induced by oxLDL in advanced atherosclerotic plaques (Filipek et al., 2020), as well as to change macrophage phenotype from pro-inflammatory M1, the predominant population in unstable plaques, to anti-inflammatory M2 with anti-hemorrhagic properties, *via* the IL-10 receptor/JAK/STAT3 pathway (Filipek et al., 2020).

The cardioprotective effects of purified secoiridoids, as well as *O. europaea* L. extracts (Table 1), have been furtherly supported by *in vivo* studies and clinical trials, highlighting their potential utility in the primary prevention of cardiovascular disease for their ability to improve the cardiovascular risk factors, including hypertension, dyslipidemia, and obesity, *etc.*, as above described.

3.4. Secoiridoids in cancer

Cancer is currently one of the leading causes of death worldwide (CDC, 2023) and, in 2020, there were approximately 19.3 million new cancer cases globally, including breast cancer (11.7%), lung cancer (11.4%), colorectal cancer (10.0%), prostate cancer (7.3%), and stomach cancer (5.6%), with variations in incidence between industrialized and developing countries (Sung et al., 2021).

There is a growing body of scientific evidence that suggests that consuming extra virgin olive oil (EVOO) can have a number of beneficial effects on human health, including reduced risk of cancer (Markellos et al., 2022; Toledo et al., 2015). In an attempt to exclude the potentially confounding effects of the different components of EVOO, such as fatty acids, carotenoids, and chlorophylls, an increasing number of studies are focusing on the role of olive oil polyphenols in counteracting or preventing various forms of cancer. In particular, EVOO secoiridoids seem to exert anticancer activity by limiting cell replicative capacities, by inhibiting cellular divisions, and by increasing apoptotic phenomena, through three main mechanisms of action: *i.* by exerting pro-oxidant activity and interfering with inflammatory responses; *ii.* by altering Ca^{++} levels and its homeostasis; *iii.* by modulating specific signaling pathways involved in the processes of proliferation, differentiation, and apoptosis.

The administration of purified oleocanthal, not combined with other polyphenols or confounding elements of EVOO, has shown to have an anticancer effect on hepatocellular carcinoma (HCC) and colorectal carcinoma (CRC) cell lines, as well as on colon cancer cell lines. Oleocanthal is capable of inducing apoptosis through the production of reactive oxygen species (ROS) and consequent DNA damage. Interestingly, it appears that oleocanthal is not able to exert its cytotoxic effect on normal primary human hepatocyte cells, indicating a specificity of action limited to tumor cells (Cusimano et al., 2017). Recent experiments conducted on gastric cancer cell lines (GC) confirmed the role of oleocanthal-enriched EVO extract in the production of ROS and consequently in the inhibition of cell growth and proliferation (Peri et al., 2022) (Table 1). On colon cancer cells, the administration of oleocanthal had the ability to inhibit cell proliferation and viability through an increase in AMPK (adenosine monophosphate-activated protein kinase) and, subsequently, by suppressing the expression of COX-2. Additionally, the administration of Compound C (an AMPK inhibitor) to colon cancer cells, blocked the antiproliferative action of oleocanthal (Khanal et al., 2011). Similarly, several studies demonstrated that also oleuropein exerted its anti-cancer activity *in vitro* by blocking cell division and inducing apoptosis, involving the production of ROS (Yan, Chai, Cai, Miao, & Ma, 2015), as well as by abrogating NF-κB activation cascade and affecting inflammatory process (Liu et al., 2019).

As for the modulation of Ca^{++} levels, oleocanthal treatment reduced the expression of the TRPC6 channel (a non-selective cation channel that allows the influx of Ca^{++}) in breast carcinoma cells (MDA-MB-231 and MCF7), leading to compromised proliferation and viability of these cells, as well as reduced migration capacity. Differently, non-tumor (MCF10A) cells did not appear to be sensitive to the treatment, since these cells do not overexpress the TRPC6 channel, highlighting a specificity of action (Diez-Bello et al., 2019).

Concerning the last mechanism of action, literature data indicate that secoiridoids such as oleocanthal, oleacein, and oleuropein altered the signaling pathways and development of ERK1/2 and phosphatidylinositol 3-Kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR), involved in the processes of proliferation, differentiation, and

apoptosis (Bulotta et al., 2013; Fogli et al., 2016; Polini et al., 2018; Scotece et al., 2013). In addition, olive oil secoiridoids can interact with other factors or pathways directly or indirectly connected to the ERK1/2 and PI3K/AKT/mTOR pathways. This is the case with the c-Met kinase, which controls proteins such as MAPK, RAS, ERK on the one hand, and PI3K, AKT, mTOR on the other hand, and its phosphorylation was altered by oleocanthal, on breast and prostate cancer cells, and ligstroside on breast cancer cells (Busnena, Foudah, Melancon, & El Sayed, 2013; Elnagar, Sylvester, & El Sayed, 2011). In addition to interfering with the phosphorylation of c-Met kinase, ligstroside aglycone was also capable of inducing apoptosis in breast carcinoma cells through the alteration of Human Epidermal Growth Factor Receptor 2 (HER2), a tyrosine kinase receptor highly expressed in some cancer forms (Menendez et al., 2008). Also, oleuropein was demonstrated to deregulate several factors, including MAPK p38 (W. Wang et al., 2019), p53 and hypoxia-inducible factor (HIF)-1α (Cárdeno, Sánchez-Hidalgo, Rosillo, & de la Lastra, 2013), c-Jun e c-Fos (Goldsmith et al., 2018), in various cellular tumor models. Intriguingly, the modulation of some of these pathways can also occur through the involvement of miRNA following the treatment with oleuropein and oleacein (Abtin et al., 2018; Carpi et al., 2020). For example, oleacein appears to be able to alter the expression of numerous miRNAs, such as miR-193a-3p targeting MCL1, c-KIT, and K-RAS, miR-193a-5p targeting PIK3R3 and mTOR, miR-34a-5p targeting Bcl2 and c-KIT, miR-16-5p targeting Bcl2, K-RAS, and mTOR, and miR-214-3p targeting BAX. Consistently, melanoma cancer cells treated with micromolar concentrations of oleacein showed a G1/S phase arrest, downregulation of anti-apoptotic genes Bcl2 and MCL1, and proliferative genes c-KIT, K-RAS, PIK3R3, and mTOR, while exhibiting an increase in the transcriptional levels of pro-apoptotic genes such as BAX (Carpi et al., 2020).

To date, limited evidence is available *in vivo* and in clinical trials in humans, although the evidence tends to align with what has been observed in cellular models. According to Fabiani's systematic review on the anti-cancer properties of EVOO secoiridoids, there are 16 animal studies and 5 human studies reported in the literature. The majority of animal studies confirm the ability of these molecules to counteract the tumorigenic process. Regarding human studies, most reports showed a significant effect of secoiridoids in preventing DNA damage in terms of reducing the levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine in urine, mitochondrial DNA of mononuclear cells, and lymphocyte DNA (Fabiani, 2016).

3.5. Secoiridoids in inflammatory bowel diseases

Inflammatory bowel diseases, encompassing Chron's disease and ulcerative colitis, are life-long and life-limiting disorders, characterized by chronic inflammation of gastrointestinal tract. Over the past decades, there has been an increasing prevalence of these diseases with >6.8 million affected people, most likely associated with industrialization (Kaplan & Windsor, 2021).

Few data are reported, to date, in the literature concerning the beneficial effects towards inflammatory bowel diseases, and these are mainly focused on olive oil industry by-products or olive leaf extracts containing secoiridoids alongside a mix of polyphenols and other minor compounds (Table 1).

Only one *ex vivo* study have provided evidence on the beneficial effects of purified oleuropein against the inflammation correlated to IBD; specifically, oleuropein treatment of LPS-challenged colonic biopsies from patients with active ulcerative colitis showed an amelioration of inflammatory damage with reduced infiltration of CD3, CD4 and CD20 cells (Larussa et al., 2017).

3.6. Secoiridoids in skin disorders

If we exclude tumors such as melanoma, which have already been discussed in this review, many skin disorders and diseases such as redness, psoriasis, and atopic dermatitis have a genetic but also oxidative or inflammatory basis. Although there is not extensive scientific literature describing the effects of individual secoiridoids from EVOO (Extra Virgin Olive Oil) or their blends on skin disease models, some studies seem to highlight potential beneficial effects of oleuropein and oleocanthal.

The skin, in addition to limiting excessive water evaporation, serves as the body's first barrier against various external agents, whether biotic, such as pathogens, or abiotic, such as chemicals or UV rays. The skin is composed of the epidermis and dermis; the epidermis, in turn, consists of different cellular layers, each with a specific function. The outermost layer is the stratum corneum, rich in ceramides and populated by a complex bacterial network called skin microbiota. Similar to the intestinal microbiota, the skin microbiota is of fundamental importance for skin health (Byrd, Belkaid, & Segre, 2018). Below the stratum corneum, there are other layers, including the granular layer composed of cells containing the proteins filaggrin and loricrin, which play a barrier function (Furue & Kadono, 2017). The layer closest to the dermis is called the basal or germinative layer, and it is composed of stem cells that give rise to the cells of the more superficial layers. Below the epidermis, separated by the basement membrane, lies the dermis, which is populated by a complex network of fibroblasts and immune cells such as macrophages and lymphocytes. Therefore, what may appear to be a simple organ turns out to be an extremely complex one, and its integrity is crucial for the overall health of our body (Furue & Kadono, 2017; Guttman-Yassky & Krueger, 2017).

In 2008, Perugini and colleagues tested the efficacy of oleuropein in reducing the effects caused by UV-B irradiation on the skin of 10 female subjects 20-30 years old with Fitzpatrick skin types II and III (Perugini et al., 2008). An artificial UV-B lamp (305/350 nm) was used to irradiate 7 areas of the skin measuring 1 cm^2 each, with the aim of inducing cutaneous erythema. The protective or soothing effect of oleuropein was evaluated through the measurement of parameters such as barrier function, skin color, and microcirculation. The administration of oleuropein was able to reduce erythema and water loss by approximately 22% and 35%, respectively, and improved blood flow by about 30%. Although the mechanism of action was not studied in this work, the authors speculate on the possible ability of the oleuropein catechol moiety to act as a scavenger against oxygen free radicals or reactive nitrogen species, including the radical nitric oxide (NO•) (Perugini et al., 2008).

A similar effect to that obtained through UV irradiation is achieved through Photodynamic Therapy (PDT), an effective treatment against cancer and currently under investigation for the treatment of psoriasis and acne (Nimbalkar, Yawalkar, Mahajan, & Dhoble, 2021). Segura Palacios and colleagues observed that the administration of an oily emollient fluid enriched with oleocanthal and oleacein was able to reduce inflammation following PDT, the main side effect of this therapy (Segura Palacios et al., 2019).

Oleuropein has also shown activity against skin disorders such as atopic dermatitis (Huang et al., 2022) and psoriasis (El-Gogary, Ragai, Moftah, & Nasr, 2021). In particular, in a 2,4-dinitrochlorobenzene-induced atopic dermatitis-like mouse model, topical application of oleuropein at two different concentrations (5 and 10 mg/Kg) induced a significant reduction in the expression of Th2-related pro-inflammatory cytokines IL-4, IL-5, IL-6, as well as COX-2, and ICAM-1. Furthermore, the production of IgE and IgG1, as well as the infiltration of mast cells and eosinophils, was markedly decreased in the group treated with oleuropein compared to the control group. Finally, the disease manifestations, including swelling and thickening of the epidermis and dermis in the ear and back areas, were also reduced (Huang et al., 2022). Taken together, these findings suggest that oleuropein may act by penetrating the different layers of the epidermis and inhibiting the Th2-driven inflammatory axis (Huang et al., 2022).

Nanoformulations of oleuropein loaded into nanoparticles with a diameter of 30.25 nm, created from ethyl oleate, Capryol 90, and

Transcutol, have shown greater efficacy in counteracting psoriasis than Dermovate cream containing clobetasol propionate, a corticosteroid used to alleviate symptoms of dermatological conditions (El-Gogary et al., 2021). In particular, the treatment of psoriatic patients for 8 weeks with oleuropein-based nanoformulations led to significant clinical improvements, as assessed by the Psoriasis Area Severity Index score, as well as by a reduction in disease features such as scaling and thickening of the epidermis.

3.7. Secoiridoids in infectious diseases

The widespread use of antimicrobials in clinical and community settings, livestock and crop production, is considered one of the main drivers of phenomenon of antimicrobial resistance, as well as the emergence of novel pathogens (SARS-CoV-2, *Candida auris*) (Zhu, Yang, Wang, & Liu, 2023).

Recently, the antimicrobial activity of extracts from EVOO or olive leaves, rich in secoiridoids (Table 1), as well as of purified oleuropein, oleocanthal and oleacein has been increasingly reported (Di Pietro et al., 2022; Karygianni et al., 2019; Pereira et al., 2007). Of high clinical relevance is the antibacterial activity of purified secoiridoids observed against several human clinical isolates, including drug resistant strains. For example, oleacein in choline/propylene glycol based NaDES showed a high antibacterial activity towards several ESKAPE pathogens such as *P. aeruginosa* and *K. pneumoniae* (MIC 404 μg/ml) (Di Pietro et al., 2022), whereas oleuropein has been described to inhibit several isolates of Salmonella *spp.*, Vibrio *spp.*, as well as penicillin resistant *S. aureus* (MIC 31.25-250 μg/ml) (Bisignano et al., 2010).

Lastly, purified secoiridoids have been also demonstrated to inhibit several viruses; in particular, oleuropein showed antiviral activity against respiratory syncytial virus and parainfluenza type 3 virus (IC50 23.4 and 11.7 μg/ml, respectively) (Ma et al., 2001), and, more recently, molecular docking and molecular dynamics aided virtual search of OliveNet identified olive secoiridoids as inhibitors of SARS-CoV-2 entry and replication and associated hyperinflammatory response (Thangavel, Al Bratty, Al Hazmi, Najmi, & Ali Alaqi, 2021).

The potential application of secoiridoids in clinical setting came from *in vivo* and *ex vivo* studies; in an experimental model of sepsis induced by multidrug-resistant isolates of *P. aeruginosa*, oleuropein decreased mortality and showed synergism with amikacin, probably by promoting phagocytosis or inhibiting biosynthesis of proinflammatory cytokines (Giamarellos-Bourboulis et al., 2006). An *ex vivo* study suggested oleuropein (0.25 or 0.5 μM) as promising immune reconstitution strategy for the treatment of chronic infection by Hepatitis B, as evidenced by its ability to significantly improve mitochondrial, proteostasis and antiviral functions of HBV specific CD8 T cells (Acerbi et al., 2021).

Recently, olive leaf extract capsules containing 30% oleuropein have been suggested as a promising approach in COVID-19 disease management by ameliorating respiratory rate, pulse rate, body temperature, blood oxygen saturation and decreasing erythrocyte sedimentation rate, C-reactive protein levels, as well as duration of hospitalization (Ahmadpour et al., 2023) (Table 1).

4. Bioavailability of secoiridoids

The oral bioavailability of dietary phenolic compounds is rather poor. This is due to various reasons: metabolism of these compounds in the gut by microbiota, poor absorption, and more importantly, extensive first pass metabolism in the intestinal epithelium and in the liver. In the past it was supposed that the resulting metabolites, mainly sulphate, methylated, and glucuronide conjugates, were biologically inactive. However, there is some evidence that some kind of conjugates may still retain biological activity, while others in some cases could be metabolized releasing back the active form (Islam et al., 2015; Terao, 2017). Unfortunately, regarding oleacein and oleocanthal, whose availability on the market is limited, detailed studies about their bioavailability are poor.

The bioavailability of secoiridoids has been recently well described in different comprehensive reviews (Huang, Guan, Zhang, Wang, & Li, 2024; Nikou, Sakavitsi, Kalampokis, & Halabalaki, 2022; Rivero-Pino, 2023). Briefly, once ingested, the secoiridoids present in olive oil are subjected to the same fate of polyphenols: they are absorbed in limited amount probably *via* passive diffusion, and extensively metabolized by intestine and liver, as well as by the colonic microflora (Lozano-Castellón et al., 2022; Nikou et al., 2022). As a consequence, the part of secoiridoids that is absorbed as such and reaches the plasma level is quite low. Due to extensive metabolization, the therapeutic potential of these molecules could be limited. However, the potential synergy among various polyphenols, including the secoiridoids, which are ingested daily cannot be excluded, and may increase their efficacy. Another reason why oleocanthal and oleacein as such have not been detected in biological fluids is a consequence of their extreme reactivity. These molecules are highly instable due to the presence of two aldehyde moieties (Soler-Rivas, Espín, & Wichers, 2000) which can readily form Shiff base adducts with primary amines. Indeed, the detection of oleocanthal in cell culture medium or biological fluids is virtually impossible due to its rapid conjugation with proteins or amino acids, as demonstrated by Darakjian and coworkers (Darakjian et al., 2021). In their study, these researchers demonstrated that oleocanthal spontaneously reacts with amino acids, particularly with glycine, and assessed the pharmacokinetics of this adduct in mice and in cell cultures, revealing the ability of the formed derivative to cross physiological barriers such as the blood brain barrier (BBB). The formation of adducts could explain the difficulty in detecting this substance in biological fluids and could open up the possibility of designing other molecules that may be more stable and long-lasting in the body.

The potential application of secoiridoids as neuroprotective agents is supported by evidence that these compounds are able to bypass the BBB. For instance, sulphate and glucuronate derivatives of hydroxytyrosol can cross the BBB in rats (Galmés, Reynés, Palou, Palou-March, & Palou, 2021; López de las Hazas et al., 2015). Serra and colleagues demonstrated that it only takes one hour after ingestion of a mixture of polyphenols for them to cross the BBB, and they were able to identify in plasma and in the brain the oleuropein derivatives, and tyrosol and hydroxytyrosol sulphate (originating from the degradation of ligstroside and oleuropein, respectively) (Serra et al., 2012). On the other hand, Lopez-Yerena and colleagues demonstrated that in the rat brain, although hydroxytyrosol cannot be quantified, metabolites are present that can be traced back to first and second phase metabolism (methylated, hydrated, hydrogenated and glycuronate forms of oleocanthal or oleacein), after ingestion of an olive oil enriched in oleocanthal or oleacein (López-Yerena et al., 2021).

An interesting approach is the topical application of polyphenols. Indeed, topical treatment may spare secoiridoids from extensive metabolism and allow them to exert full potential. Given their anti-microbial and anti-inflammatory activity (this latter well documented by their potent COX-inhibiting capacity) topical application of these compounds in specific formulations may be beneficial in the treatment of skin diseases or mucosal affections, as reported above.

The possibility of effectively delivering bioactive molecules and more easily overcoming the barriers offered by the organism (BBB, gastro-intestinal tract, epidermis *etc.*) to enhance bioavailability, is a challenge that in recent years has been pursued applying nanotechnological approaches. Up to now, some evidence is available about the efficacy of nanoformulations with oleuropein. For example, Huguet-Casquero and colleagues tested nanostructured lipid carriers (NLC) loaded with oleuropein, on lung epithelial cells, in order to verify the biocompatibility and antioxidant activity (Huguet-Casquero, Moreno-Sastre, López-Méndez, Gainza, & Pedraz, 2020). The same nanoparticles have been used also in *in vivo* model (C57BL/6 mice) and tested for their anti-inflammatory action (Huguet-Casquero, Xu, Gainza, Pedraz, &

Beloqui, 2020). In both cases, the experiments provided encouraging results. Finally, as reported above, nanoparticles loaded with oleuropein, are tested successfully also counteracting skin disorders (El-Gogary et al., 2021). On the contrary, nanoformulations with oleacein and oleocanthal, described in the literature, have yet to be tested on *in vitro* and/or *in vivo* systems (Li et al., 2022).

5. Discussion

The search for natural bioactive compounds as potential therapeutic agents is more and more actively pursued, due to the several side effects of available drugs, forcing an exponential increase in the economic burden for the development of new alternatives.

Olive tree products have a clear history of beneficial properties for human health, typically associated with the phenolic content in leaves, drupes and EVOO; in 2011, the European Food Safety Authority released a unique health claim, declaring the efficacy of oil phenols, at a specific dosage (5 mg/day of hydroxytyrosol and secoiridoid oleuropein aglycone per 20 g of EVOO), in protecting low-density lipoprotein from oxidation, and, hence, reducing the cardiovascular risk (EFSA, 2011).

Among the secoiridoids, evidence on the beneficial effects of oleuropein, as a purified compound or enriched extract from olive leaves or drupes, has increased over the years, as demonstrated by numerous studies evidencing its ability to modulate the molecular mechanisms underlying the development of metabolic, cardiovascular, and neurological diseases, as well as cancer; oleuropein has also been suggested as potential strategy towards inflammatory bowel diseases and skin disorders, although more studies are needed. Emerging evidence suggests that also other secoiridoids, like oleocanthal and oleacein, may help in improving human health; specifically, oleocanthal has been widely studied concerning its potential activity towards neurological disorders and cancer, whereas the study of oleacein is still at an early stage.

The powerful picture of multiple health benefits of oleuropein, oleacein and oleocanthal is mainly due to their strong antioxidant and anti-inflammatory activities, although they have also been demonstrated to act on other molecular targets. Besides these numerous activities, oleacein and oleocanthal, as well as oleuropein rich olive leaf extract, have been recently showed, for the first time, to counteract the alteration of two metabolic "organs", the adipocyte tissue, and the gut microbiota, involved in obesity-related complications. More recently, it is emerging the very promising potential of these compounds as alternative anti-microbial agents able to counteract the antimicrobial resistance, considered as one of the most serious public health threats of modern times.

The larger amount of evidence on oleuropein, as compared to oleocanthal and oleacein, is due to poor availability of the two latter compounds. Indeed, both oleocanthal and oleacein are components exclusively found in EVOO, usually at very low concentrations, cannot be easily obtained *via* chemical synthesis, and their isolation and purification from EVOO is cumbersome. Indeed, several methods have been used to isolate oleocanthal and oleacein from EVOO with higher yields by using large amounts of organic solvents, some of them toxic, carcinogenic, and non-biodegradable, and, hence, harmful for both human health and the environment. To overcome these limitations, a recent rapid and economic approach is represented by green and environmental-friendly solvents, namely the NaDES, capable to isolate oleocanthal and oleacein with a good overall yield (200 mg of oleocanthal and oleacein from 1 kg of EVOO) (Francioso et al., 2020). However, as research in this area continues, it is likely that new and even more efficient and environmentally friendly methods of polyphenol extraction will be developed.

An alternative approach to the use of raw extracts or purified molecules, which is much less expensive in terms of resources, involves the use of *in silico* investigations through docking analyses and dynamic simulations. Indeed, several authors have focused on studying potential interactions between the secoiridoids in EVOO and various proteins and/or enzymes involved in different developmental processes or underlying the onset of different pathologies. For instance, Cuyàs and colleagues investigated potential interactions between oleacein and LSD1 (lysine-specific histone demethylase 1 A), a central epigenetic regulator associated with several human degenerative disorders such as obesity, neurodegeneration and cancer (Cuyàs et al., 2019). Similarly, it has been found that oleacein can interact with catechol-*O*-methyltransferase (COMT), an enzyme that degrades catecholamines by adding a methyl moiety to the hydroxyl group of the catecholic ring. In particular, oleacein could be superimposed onto the catechol-binding site of the enzyme. All these computational predictions have been experimentally verified through *in vitro* enzymatic inhibition tests (Cuyàs et al., 2019).

Given the significant antitumor properties of oleocanthal and the role played by the mTOR pathway in the development and progression of various forms of cancer, some authors have investigated the possibility of interaction between oleocanthal and PI3K-γ, a factor that is part of the PI3K/AKT/mTOR pathway and important in the regulation of the cell cycle. *In silico* analyses have shown that oleocanthal shares 9 out of 10 interactions with a dual mTOR/PI3K-γ inhibitor. Further *in vitro* analyses have confirmed this prediction, in fact, oleocanthal was able to inhibit the enzyme's activity with an IC_{50} value of 708 nM. These pieces of evidence add another small part to the mechanism of action through which oleocanthal may exhibit its antitumor effects (Khanfar, Bardaweel, Akl, & El Sayed, 2015).

Despite the great potential of secoiridoids to prevent and/or counteract several chronic pathologies of high impact on public health as well as infectious diseases, more *in vitro* and *in vivo* studies alongside human trials are required to advance the knowledge on their beneficial effects as well as to support their application in a clinical setting. To reach this goal, several challenges remain to be addressed; first, standardized and appropriately designed studies should be performed since, up to now, they have been extremely variable both in formulation type, study population, animal models, administrated amounts, and treatment duration. For example, the clinical studies investigating oleuropein alone, as well as in *O. europea* L. enriched extracts, in the control of hyperglycemia and the prevention of diabetes included different populations, like patients with type 2 diabetes, patients with impaired fasting glucose, overweight patients, or healthy subjects, and treatment dose ranged from 20 mg (single dose) to 51 mg/day for 12 weeks. Similarly, in *in vivo* studies, different animal models including rats, mice, and rabbit were used. Extracts from olive leaves or purified compounds were administrated, and treatment dose ranged from 8 to 16 mg/Kg/day for 4 weeks in alloxan-diabetic rats to 225 mg/Kg/day for 4 weeks in streptozotocin-diabetic rats. Second, more studies concerning the bioavailability and metabolism of secoiridoids, alone or in enriched formulations, as well as their safety profiles in humans, are required in view of their interesting biological activities investigated to date. Lastly, a chemical analysis of extracts as well as an accurate quantification of secoiridoid metabolites in human biological samples after ingestion should be performed to correlate a clear chemical profile to the biological activity and, hence, to identify the formulations with potential therapeutic value.

CRediT authorship contribution statement

Simone Filardo: Conceptualization, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Mattioli Roberto:** Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Daniel Di Risola:** Formal analysis, Writing – review & editing. **Luciana Mosca:** Conceptualization, Formal analysis, Supervision, Validation, Writing – original draft, Writing – review & editing. **Marisa Di Pietro:** Conceptualization, Formal analysis, Supervision, Validation, Writing – original draft, Writing – review & editing. **Rosa Sessa:** Formal

analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

SF and RM are recipients of funding from FSE REACT-EU, within the program PON "Research and Innovation" 2014–2020 (PON R&I), Action IV.6 "Contratti di ricerca su tematiche Green"; LM received grants from Sapienza Ateneo, grants RM12117A439CD073 and RM120172A35E81B1, and from Ministero dell'Istruzione, dell'Università e della Ricerca, Progetti di Ricerca di Interesse Nazionale (PRIN) 2022, grant number 2022B9WP2K.

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