Chemical analysis, biological and therapeutic activities of *Olea europaea* L. extracts

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Abstract

The *Olea europaea* L. is a very well known and widely used plant, especially for its alimentary qualities. Its extracts from leaves and fruits are widely used in contrasting and preventing various pathologies. In this review, we have collected several data to highlight the most important chemical analysis and biological effects of these plant extracts. It exhibits cholesterol-lowering, hypoglycemic, cytotoxic, antibacterial, neuroprotective, antioxidant, anti-inflammatory and hypotensive activities. The results show that extracts from *O. europaea* could be used as a food additive in the supplementary treatment of many diseases.

Keywords*: Olea europaea;* olive; extract; cytotoxic activity; anticholesterolemic; antihypertensive; antihyperglycemic; antimicrobial

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1. Introduction

Olea europaea L. is the best-known component of the *Olea* genus. It is the only species of this genus used for food and is a typical cultivar of the southern regions of Europe and North Africa. The olive tree has a large trunk, typically folded and almost twisted, which has branches and twigs with narrow, oblong-lanceolate leaves, with a sharp apex, pale green in the upper face, and whitish-silvery in the lower one. The fruit is small and fleshy, ovoid, violet or blackish when ripe, about 1-2.5 cm long (Bianco and Ramunno 2006c; Hashmi et al. 2015). *O. europaea* contains a significant amount of bioactive compounds such as flavonoids, secoiridoids, phenolic compounds and carotenoids. Polyphenols have a wide spectrum of beneficial properties, especially related to their antioxidant activity (Bianco et al., 2001; Bianco and Ramunno 2006c; Hashmi et al. 2015). Carotenoids, including vitamin A, are antioxidant compounds that are classified according to their structure in carotenes and xanthophylls and show a strong anti-inflammatory activity (Alesci et al. 2015; Aragona et al. 2017; Alesci et al. 2020a). Furthermore, the related extra virgin olive oil (EVOO) due to its peculiar constituents is one of the most important elements of many food regimens including the famous health benefit Mediterranean diet (Metro et al. 2018; Metro et al., 2020). Various beneficial activities have been ascribed to this plant, and its fruits, such as the reduction of glycaemia, cholesterol, uric acid, and blood pressure. It also shows anti-inflammatory, anti-diarrheal, anti-rheumatic, anti-cancer, vasodilator activities antibacterial and neuroprotective properties (Bianco and Ramunno 2006c; Hashmi et al. 2015; Ko et al., 2009; Visioli et al., 2002). The purpose of this review is to collect data demonstrating the importance of *O. europaea* chemical analyses and bioactivities against chronic and degenerative diseases.

2. Chemical analyses

Due to the biological, nutritional, and commercial worth of *O. europaea* contents, it is important to deeply understand the qualitative and quantitative constituents and their variability. The plant ability to produce the secondary metabolites is influenced by several factors, such as cultivar type, temperature, irradiance, seasonality, water availability, cultivation site, date of collection, drying condition, extraction condition, refining technology and storage procedures. All these factors may impact negatively or positively on the biosynthesis and recovery of the bioactive peculiar constituents, and thus on the overall quality, as demonstrated for *O. europaea* and many other plant species (Bianco et al., 2006a; Cicero et al. 2018; Fanali et al. 2018; Tardugno et al. 2020). Many phytochemical constituents of *O. europaea* have been identified so far, triacylglycerols (TAGs) and the derived fatty acid (FA) are the most abundant compounds followed by a complex mixture of minor compounds, represented by monoacylglycerols and diacylglycerols, alkyl esters, phytosterols, polyphenols, tocopherols, tocotrienols, pigments, minerals and flavour compounds (Bianco et al., 2001; Bianco et al., 2006a; Castellani et al., 2008; Saitta et al. 2009; Clodoveo et al. 2015; Cicero et al. 2018). Focusing on polyphenols in *O. europaea,* the total phenolic content (TPC) consists of various phenolic classes, such as phenolic acids (vanillic, coumaric, caffeic, protocatechuic, *p*-hydroxybenzoic, ferulic acid), lignans (acetoxypinoresinol, pinoresinol), flavones (apigenin, luteolin), flavone glycosides (luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside), phenolic alcohols (tyrosol, hydroxytyrosol), and secoiridoids (oleacein, oleocanthal, oleuropein) which are usually the most abundant phenolic compounds (Bianco et al., 2001; Bianco & Ramunno 2006c; Bellumori et al., 2019; Cicero et al. 2018; Fanali et al. 2018; Pedan et al. 2019). National and international organizations such as the United States Department of Agriculture, European Commission standards, Codex Alimentarius (Codex), IOC and many others worldwide defined a wide regulatory framework. The European Food Safety Authority has attributed the claim 'Healthy Extra Virgin Olive Oil' attributable to extra virgin olive oil (EVOO) containing at least 5 mg hydroxytyrosol and its derivatives (e.g., oleuropein and tyrosol) (Bajoub et al. 2018; Bellumori et al., 2019; Tsimidou et al. 2019). In this context, the extraction and chemical analysis play a key role in the determination of bioactive polyphenols in *O. europaea* olive fruit, leaves, pulp and pomace, olive tree wood, as well as olive oil wastewaters and by-products (Bianco et al., 2006b; Mulinacci et al., 2005). Several analytical methods have been described in the literature for identification and quantification. To avoid quality losses and undesirable phenolic degradation during storage before the chemical analyses, for instance, appropriate post-harvest processing and storage conditions of the samples should be considered as important operations. Olive leaves are washed are usually dried before extraction to avoid the interference of water in the process. While the olive oil matrix should generally be extracted, cleaned up and concentrated. Hexane is utilised in these steps to reduce the emulsifying effect and to improve the separation of the two-phases during the centrifugation (Pedan et al. 2019). Simple phenols, acids, lignans, secoiridoids and flavonoids are usually extracted by solvent extraction. Water, methanol, ethanol, acetone, as well as aqueous alcohol mixtures are commonly used. Applying liquid–liquid extraction (LLE) to the treatment of these compounds, the extraction solvents commonly employed are methanol/water mixtures. Indeed, LLE and solid phase extraction (SPE) showed to be the most effective procedures with no remarkable differences in extraction efficiency of the analysed phenolic compounds between the two methods (Cicero et al. 2018; Fanali et al. 2018). Ultrasound-assisted (UAE), supercritical fluid (SFE), pressurized liquid (PLE) and microwave-assisted (MAE) extractions have been also used in order to optimize the extraction conditions such as time, temperature and solvent use. Each extraction procedure (UAE, SFE, PLE, MAE) appeared to be more specific for a peculiar class of polyphenols (Abaza et al. 2015; Fanali et al. 2018). The colourimetric Folin-Ciocalteu assay is the most used and rapid quantitative technique for the determination of TPC in *O. europaea* products (Abaza et al. 2015; Fanali et al. 2018). Separation procedures follow the extraction in the analysis of individual compounds in complex mixtures, high-performance liquid chromatography and ultra-performance liquid chromatography (UPLC), coupled with different detectors, such as diode array (UV/DAD), fluorescence, electrospray ionization-mass spectrometry detection (ESI-MS, MS^2, QTOF) and nuclear magnetic resonance spectroscopy (NMR) are the techniques of election in this field in order to achieve the best quali- and quantitative chemical analysis of complex matrixes such as *O. europaea* products. Generally, the separation of the previously extracted polyphenols is performed in the reversed-phase mode with C_{18} as stationary phase, and water/methanol or water/acetonitrile acidified as mobile phases under linear gradients followed by column purging. Acids are added to the aqueous phase to maintain a low pH and avoid phenolic dissociation (Abaza et al. 2015; Bellumori et al., 2019; Cicero et al. 2018; Fanali et al. 2018; Tardugno et al. 2018). Detection with UV-DAD systems still remains one of the most used for *O. europaea* phenolic compounds with general use wavelengths (280, 320, 365 nm) and allows the identification of phenols which structurally fail the ionization process or suffering the matrix suppression effect, isomeric and glycosylated forms. Likewise, MS detection is performed on *O. europaea* extracts to fully characterize the chemical profile of the complex matrix, providing high accuracy (below 10 ppm) and new constituents identification. Electrospray ionization in the negative mode is mostly employed (Abaza et al. 2015; Bellumori et al., 2019; Cicero et al., 2018). Recently, also NMR spectroscopy techniques are used to acquire high-resolution (HR) spectra of heterogeneous samples including *O. europaea* products especially for the analysis of its liposoluble polyphenols (Corsaro et al. 2015; Rotondo et al. 2020). Nowadays, new derivatives from the complex matrix of *O. europaea* are still discovered by researchers with potential interesting bioactivities (Bianco et al., 2004; Serrilli et al., 2013; Venditti et al., 2013). Indeed, the development and validation of advanced extraction and analytical techniques with new the latest instruments available are even challenging and highly required for *O. europaea* fruits, leaves, roots, stems, and related byproducts.

3. Cytotoxic activity

Several studies have shown the effectiveness of the use of extracts of *O. europaea*, both from fruits and from leaves, on different types of cancer cancer cell line. Randon et al. (2007) demonstrated oleuropein stimulate lymphocyte proliferation. The beneficial effects of oleuropein were treated in research by Acquaviva et al. (2012), on prostate cancer. It induces an antioxidant effect on non-malignant cells and a prooxidant effect on cancer cells, confirming its use as adjuvant therapy for prostatitis. It effectively induced cell death in melanoma, as seen from the analysis of apoptotic markers (Ruzzolini et al. 2018). Furthermore, oleuropein is capable of inhibiting angiogenesis (Samara et al. 2017; Song et al. 2017). In 2017, Zeriouh et al. evaluated the effects of *O. europaea* subsp. *Sylvestris* leaf extract on colon cancer HCT116, noting its ability to limit neoplastic growth and induce apoptosis (Zeriouh et al. 2017). Oleuropein and hydroxytyrosol have also been used to treat the same type of cancer, HT29and neoplastic cells of MCF7, both arrest uncontrolled cell growth, inducing apoptosis by the p53 pathway and blocking the cell cycle in G1 (Han et al. 2009; Cárdeno et al. 2013). Furthermore, Liman and co-workers conducted a study to evaluate the effects of oleuropein in MCF7, showing the antiangiogenic, apoptotic and genotoxic ability of this molecule towards cancer cells. Since it has high antioxidant power, it plays a protective role of the cells against the genetic damage that induces neoplastic transformation (Liman et al. 2017). A study by Kimura, used oleuropein in the treatment of UV exposure, which is the leading cause of skin cancer. The administration of oleuropein significantly decreased the thickness of the skin and inhibited the reduction of skin elasticity, preventing the expression of the matrix metalloproteins (MMP2, 9 and 13), of the vascular endothelial growth factor (VEGF) and of cyclooxygenase -2 (COX-2). After histological analysis, a reduction in the expression of Ki-67 and CD31, normally induced after irradiation, was seen. Since Ki-67 is a marker of cell proliferation, and CD31 is a marker of angiogenesis, oleuropein has shown an important anti-carcinogenic and anti-angiogenic effect (Kimura and Sumiyoshi 2009). An interesting study conducted by Muscoli et al. (2014) showed that oleuropein extract of *O. europaea* can affect not only cancer but also the pain it causes. Pretreatment with an extract from *O. europaea* maintained the normal pain threshold and inhibited the onset of oxidative stress, avoiding lipid peroxidation. These data suggested that oleuropein extract from *O. europaea* may restore the analgesic effect of morphine, limiting its side effects (Muscoli et al. 2014). The ethanolic extract of the Lebanese *O. europaea* leaves, showed to induce apoptosis on human leukemia cells (Fares et al. 2011). A study in 2013 evaluated the antitumor effect of oleanolic acid on hepatocellular carcinoma, highlighting the inhibitory effect on cell multiplication, thanks to apoptotic induction and the arrest of the cell cycle (Wang et al. 2013). Maslinic acid component of *O. europaea* leaves and fruits has been studied and has proven its antineoplastic effect in the treatment of colorectal carcinoma HT29, due to its antiproliferative properties (Reyes-Zurita et al. 2009), stopping the cell cycle in G1 (Rufino-Palomares et al. 2013). Erythrodiol, uvaol, oleanolic acid and maslinic acid have shown a cytotoxic effect on cancer cells, inhibiting their proliferation (Allouche et al. 2011). The phenolic compounds blocked the cells in G1, reducing the expression of cyclooxygenase-2 (COX-2) and cyclin D1, increasing the antioxidant activity, inducing apoptosis of MCF7 cells (Milanizadeh et al. 2014)(Table S1).

4. Antihypercholesterolemic activity

Hypercholesterolemia is one of the leading causes of cardiovascular disease death. The use of *O. europaea* extracts from leaf, fruit and plant has been shown to combat the damage induced by excess cholesterol. Wani et al., have shown that olive oil can reduce triglycerides in the blood, lowering the accumulation of fat in the liver, blocking inflammatory processes. They also showed how consuming olive oil reduces body weight, which is the main monitoring method for NAFLD (Wani et al. 2015). Hydroxytyrosol and oleuropein can inhibit the oxidation of LDL and HDL (Visioli et al. 2002). EVOO increases the serum concentration of HDL by affecting the LDL/HDL ratio (Covas et al. 2006). Furthermore, it improves the Reverse Cholesterol Transport (RCT) making HDL more efficient in mediating the outflow of cholesterol (Helal et al., 2013). The polyphenols contained in olive oil also improve the stability of HDL

(Hernáez et al. 2014). Coni et al., Have also shown that adding 10% olive oil to the standard diet can decrease total blood cholesterol and assist LDL against lipid oxidation (Coni et al. 2000), as well as improving the activity of antioxidant enzymes (Fki et al. 2005). In 2006, Andreadou et al. conducted a study to evaluate the biological effect of oleuropein on oxidative, ischemic and hypercholesterolemic damage. A reduction in total cholesterol and circulating triglycerides has been noted, also providing cardiovascular protection, counteracting ischemic damage (Andreakos et al. 2004). It has also been shown that tyrosol and hydroxytyrosol improve the resistance of LDL and HDL to lipid peroxidation, eliminating oxygen radicals, reactive and harmful species for cells. EVOO can therefore improve the antiatherogenic properties of HDL (Berrougui et al. 2015). El-Gengaihi et al. carried out a study on olive, *Eruca sativa* and borage oils. These three oils contain a high percentage of quantities of fatty acids (UFA). Furthermore, oleic acid has led to a decrease in LDL, triglycerides and total cholesterol (El-Gengaihi et al. 2004). An interesting data was provided by a study by Alesci et al. (2014), on the effect of an extract of exhausted olive oil from *O. europaea*. It has been tested on the steatotic liver, experimentally induced by hypercholesterolemic diet, with the formation of foam cells in the intestine, typical features of atherosclerosis (Lauriano et al. 2016), identified by Toll-Like Receptor (TLR) (Lauriano et al. 2014; Alesci et al. 2020b). The histological and immunohistochemical analysis showed the regression of damage both in the parenchyma and in the endothelium of the hepatic vessels, suggesting the biological hypocholesterolemic effect of the polyphenols of the exhausted extract in the treatment of pathologies of lipid accumulation (Alesci et al. 2014). The polyphenols in olive oil act not only on the levels of cholesterol in the blood and tissues but also its oxidized products, the oxysterols. These molecules can alter the intestinal mucosa, favouring the onset of chronic inflammatory pathologies. The phenolic extract of olive oil counteracted the formation of oxysterols and inhibited the induction of iNOS, maintaining normal NO levels (Serra et al. 2018) (Table S2).

5. Antihypertensive activity

Hypertension is a pathological condition that can lead to the onset of heart disease, the stroke of the arteries and chronic diseases. Oral administration of olive oil has been noted to be helpful in the prophylaxis of hypertension (Khayyal et al. 2002). Oleanolic acid and uvaol have demonstrated a vasodepressor effect, confirming that olive oil is a useful adjuvant in controlling blood pressure (Somova et al. 2004). Susalit et al. (2011)

tested the effect of extracts from olive leaves, comparing it with the effect of captopril, one of the drugs of choice in the treatment of hypertension, the patients treated with the extract showed better results. Perrinjaquet-Mocetti et al. (2008) conducted research on the effect of an olive leaf extraction the blood pressure of 40 hypertensive monozygotic twins. They were given two different dosages. Patients who received the highest daily dose showed better blood pressure and a reduction of lipid concentration. In addition, olive oil extract can suppress the channels for calcium causing a decrease in systolic pressure and heart rate (Scheffler et al. 2008). Ivanov et al. in 2018, carried out research to evaluate the antihypertensive action, profile and lipid peroxidation, with the treatment at different doses of an oil extract from *O. europaea*, enriched with phenolic compounds. The minimum dose tended to normalize the pressure, while the maximum dose determined an improvement in the pressure range while maintaining high endothelial resistance. The intermediate dose was the one that was the best in regulating blood pressure and lipid profile, counteracting the onset of cardiovascular diseases (Ivanov et al. 2018) (Table S3).

6. Antihyperglycemic activity

Diabetes Mellitus (DM) is a metabolic pathology that occurs with chronic hyperglycaemia accompanied by imbalances in lipid, protein, secretion, and insulin action. It is estimated that over 300 million people will have diabetes in 2025 (Park et al. 2011). There are many medicinal plants in the plant kingdom, whose extracts can be used in the treatment of diabetic pathology. In particular, the *O. europaea* has been widely used for this purpose (El-Rahman Abd 2016). Indeed, several studies have highlighted the hypoglycaemic properties of the components of the leaf extract from *O. europaea* (Laaboudi et al. 2016). Many experimental studies have been conducted on animals, with the administration of streptozotocin (STZ), to induce hypoglycemia (Rahmanian et al. 2015). Al-Attar and Alsalmi (2019) conducted a study in rats with diabetes experimentally induced. Physiological, molecular, and histological improvements have been observed after administration of *O. europaea* leaf extract. The use of low and high doses of extract improved glycemic and insulinemic control, lipid, and protein profiles, restoring the normal morphology of the pancreas, histologically observed (Al-Attar and Alsalmi 2019). In addition, administration of *O. europaea* leaf extract inhibited alpha-amylase and alpha-glucosidase (Temiz and Temur 2019). *O. europaea* also works by increasing the enzymatic activity against diabetes-induced lipid peroxidation, by improving the efficacy of superoxide dismutase (SOD) (Cui et al. 2009; Mansouri et al. 2015). The treatment with *O. europaea* leaf extract is also able to restore the normal morphological conditions of the liver and pancreas, suppressing the secretion of insulin by cells exposed to peroxide radicals (Cumaoğlu et al. 2011), thanks to the presence of phenolic compounds which combat the toxicity of diabetes (Sakr et al. 2016). The olive leaf extract also leads to an important reduction in the digestion and absorption of starch, resulting in better glucose homeostasis in experimental animal models (Wainstein et al. 2012). Oleuropein can also inhibit intestinal maltase and human sucrase (Kerimi et al. 2019); it is able to restore blood glucose values close to the norm, acting as a hypoglycemic agent and reducing the oxidative stress that causes lipid peroxidation, due to its antioxidant property (Al-Azzawie and Alhamdani 2006). This, together with hydroxytyrosol, also normalized serum cholesterol levels, bringing enzymatic activities back to normal (Jemai et al. 2009). The effect of *O. europaea* leaf extract has been compared to the effect of Glibenclamide, showing the extract more effective than the drug. In fact, in addition to lowering the levels of sugar, cholesterol, urea and uric acid in the blood, the extract also implemented serum insulin levels (Eidi et al. 2009), as also demonstrated by de Bock et al. (2013) (Table S4).

7. Antimicrobial activity

Several studies have demonstrated the antimicrobial and antibacterial effect of *O. europaea* (Adnan et al. 2014). Olive brines, for example, have an antibacterial effect against the fermentation of lactic acid. The dyaldehydic form of decarboxymethyl-elenolic acid linked to hydroxytyrosol has shown the ability to inhibit bacterial growth during the fermentation of olives (Medina et al. 2007). Furthermore, the phenolic compounds of the olives have shown good antibacterial activity, such that they can be used in the treatment of infections of the gastrointestinal and respiratory tract (Pereira et al. 2007). Olive leaf extract has shown strong antibacterial activity against *S. tiphymurium*, *E. Coli*, *S. aureus*, *B. cereus*, *L. monocytogenes* and *P. aeruginosa* (Ko et al. 2009). Faiza et al., conducted a study to test the antibacterial effect of *O. europaea* olive extract. These molecules particularly inhibited *B. cereus* and *E. coli* (Faiza et al. 2011). However, the phenolic compounds of the extract also showed bactericidal action against *S. aureus*, *K. pneumoniae*, *S. tiphy* and *V. parahaemoliticus* (Owen et al. 2003; Gómez-Ruiz et al. 2007). Furthermore, oleuropein and hydroxytyrosol inhibited the growth rate of several pathogens of the gastrointestinal and respiratory tract, such as *H.*

influenzae, *M. catarrhalis*, *V. cholerae*, *V. alginolyticus*, *S. aureus* and *S. tiphy* (Bisignano et al. 1999). Leaves extracts from *O. europaea*have proven to be very effective against gram + but less effective against gram- (Malik 2015). Furthermore, the phenolic compounds of *O. europaea* were able to inhibit the growth of flagella and the motility of *L. monocytogenes* (Liu et al. 2017). Research was carried out to evaluate the antibacterial effects of polyphenolic extracts of EVOO, obtained from three varieties of *O. europaea*: Ruvea, Ravece and Ogliara. All these extracts have inhibited bacterial growth already at low concentrations (Nazzaro et al. 2019)(Table S5).

8. Conclusion

Outstanding signs of progress were achieved regarding the chemical investigation in *O. europaea* products in the last years, thanks to the modern powerful techniques. Nevertheless, *O. europaea* products are complex matrixes with still many "unknown" compounds, which require deep investigations. Scientific research has amply demonstrated that *O. europaea* is a plant with great therapeutic capacity, fruit and leaves extracts have beneficial activities useful to fight various pathologies. Nevertheless, many biological studies still use extracts without a clear chemical profile. The correlation between the chemical characterization and the biological activities of standardised phytocomplexes will play a key role in the development of new food supplements with high therapeutic values. Additional studies regarding chemically analysed and standardised extracts of *O. europaea* and its main components especially in the clinical field are required to achieve the rational and targeted use of this precious plant.

Appendices

Tables related to this article are available online (see supplementary data).

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Table S1. *O. europaea* cytotoxic activities.

Table S2. *O. europaea* anticholesterolemic activities.

Table S3. *O. europaea* antihypertensive activities.

Table S4. *O. europaea* antihyperglycemic activities.

Table S5. *O. europaea* antibacterial activities.