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Olive tree (*Olea europaea* L.) leaf as a waste by-product of table olive and olive oil industry: a review

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Abstract

Research into finding new uses for by-products of table olive and olive oil industry are of great value not only to the economy but also to the environment where olives are grown and to the human health. Since leaves represent around 10% of the total weight of olives arriving at the mill, it is worth obtaining high added-value compounds from those materials for the preparation of dietary supplements, nutraceuticals, functional food ingredients or cosmeceuticals. In this review article, olive tree (*Olea europaea* L.) leaf is reviewed as being a potential inexpensive, renewable and abundant source of biophenols. The importance of this agricultural and industrial waste is emphasised by means of describing its availability, nutritional and therapeutic effects and studies conducted on this field.
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Keywords: by-product; *Olea europaea* L.; biophenols; oleuropein; antioxidant activity; review

INTRODUCTION

Plant extracts are complex mixtures obtained from fruits, leaves, flowers, woods, roots, resins or seeds of plants with known medicinal properties or the history of traditional use.[1] Due to their natural antioxidative and antimicrobial activities, determination of biophenols in plants is of great significance. These compounds are synthesised against microorganisms and UV radiation as a defence mechanism, showing high antioxidant capacity in plants. Biophenols also have properties such as antimicrobial, antiviral and elimination of infections.[2–4] These valuable compounds in plant extracts are responsible for many health benefits (e.g. reduction of hypertension, prevention of cardiovascular problems, and suppression of different types of cancer and viral diseases). Therefore, there is a growing interest to utilise them in various industrial applications such as food supplements, cosmetic and pharmaceutical industries.[5–9] Those materials find application in food industry to eliminate the oxidation of lipids during processing and storage of foods. UV light, free radicals, heat, enzymes, micro-organisms and metals/metalloproteins might be the initiators of lipid oxidation. Finally, this problem gives rise to rancidity, potentially toxic oxidative products, degradation of colours, aroma, and nutritional quality, and decrease of shell life.[10–12] This general concern in consumer preferences towards preventing auto-oxidation is not restricted to the food safety. These high added value components are attracted by other industries such as the pharmaceutical and cosmetics as well. Thus, these three trade sectors focus on the products known as 'functional food', 'dietary supplements', 'phytochemicals', and 'nutraceuticals'.[13,14]

Olive leaf extracts are of special interest for their therapeutic effects. These extracts have different classes of biophenols including phenolic acids, phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside, luteolin 4-*O*-glucoside), and secoiridoids (oleuropein).[15,16] Oleuropein is the most abundant of all the constituents of olive leaf extract, having antioxidative, antimicrobial, antiviral[17] (even against the HIV virus),[18] anti-atherogenic, and cardioprotective (by protecting membrane lipid oxidation acting on coronary dilation and by antiarythmic action),[19] antihypertensive and anti-inflammatory properties.[20] Furthermore, it has hypocholesterolaemic and hypoglycaemic activities.[21] It is also known to improve lipid metabolism to alleviate obesity problems.[22] After all, using the extract without isolating the constituents might be recommended to achieve health because of the synergistic effects of all biophenols present in the extract.

Waste is a product that is thrown away or needed to be thrown away, while by-products are secondary products acquired from a process that can be useful and marketable or can be used in a secondary process to produce high-added value and marketable products.[23] On the other hand, the olive oil industry produces great quantities of solid waste as well as liquid waste. This review article mainly focuses on the solid residues from olive cleaning, olive leaves.

OLIVE LEAF

Olive tree comes from a genus of evergreen trees in the family Oleaceae containing 24 genus and 900 species. *Olea europaea* L. is in the same species such as lilacs, jasmine, *Forsythia* and the true ash trees (*Fraxinus*) (Table 1).[24]

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Table 1. Taxonomic classification of olive tree

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Oleaceae
Genus	Olea
Species	E. oleaster *E. sativa*
Binomial name	*Olea europaea*

Olive leaves, an agricultural waste by-product obtained during the harvesting or fabrication process of olive fruits, contain considerable biophenols as the other parts of olive tree. Those of leaves contribute to the colour and taste of the fruit depending on the nature and concentration. Their quantity also differs between several textures.[25,26] Biophenols in leaves are different from those of the flowers, fruits and the branch.[27]

Folklore reports about the therapeutic effects of tea made of olive leaves against sicknesses like coughing, sore throat, fever and cystitis in the Middle East culture for hundreds of years. Additionally, the leaf was used for dermatological diseases.[28] Olive leaf was first used medicinally in ancient Egypt, and was the symbol of heavenly power. The oil extracted from leaf was used in mummification ceremonies. Olive leaf has been beneficial in the treatment for conditions of influenza, common cold, malaria, dengue, severe diarrhoea, and dental, ear, urinary tract and surgical infections, especially in Mediterranean cultures as well. In the middle of the 19th century, olive leaf tea was utilised to counteract malaria. It became a very popular tea in England, since the English made a tea from olive leaves that was given to help sick sailors and colonists returning from tropical climates in colonial times.[29,30] Several brands of medical supplements containing olive leaf have been offered as liquid extracts or tablet forms against diabetes, high blood pressure, cardiovascular diseases, common cold, urinary tract infections, chronic fatigue syndrome, and to support time of recovery, immune system.[31] Products containing olive leaf extract are used for anti-ageing activities in the cosmetics industry. Drugs made of olive leaf extract are used not only for human health, but also for animal health due to their antibiotic and antiparasitic properties. Anti-HIV activity of olive leaf extract was also proved by modulation of host cell gene expression by HIV-1 infection.[18]

Antioxidant activity

Numerous phenolic components in olive leaf have a strong radical scavenging activity.[6,21,32–35] Furthermore, extracts may be more beneficial than isolated constituents, since a bioactive individual component can change its properties in the presence of other compounds present in the extracts. Flavonoids, rutin, catechin and luteolin have almost two and a half times more antioxidant activity than vitamins C and E.[36] Antioxidant activities of oleuropein and hydroxytrosol were found to have the same with those of vitamins C and E.[37] In another report, there was an order of radical scavenging ability as rutin > catechin > ≈ luteolin > olive leaf extract ≈ hydroxytrosol > diosmetin > caffeic acid > verbascoside > oleuropein > luteolin -7-glucoside ≈ vanillic acid ≈ diosmetin-7-glucoside > apigenin-7-glucoside > tyrosol > vanillin. The most striking point is that olive leaf extract shows more antioxidant activity than vitamins C and E, due to the synergy among flavonoids, oleuropeosids and phenols.[38] A study of antioxidant capacity change by leaf maturation shows that mature leaves have more antioxidant activity than young leaves.[39]

Anti-inflammatory and anti-thrombotic activity

In particular, beside the antioxidant activity, vasodilatatory, anti-platelet aggregation and anti-inflammatory effects have been assigned to the biophenols such as oleuropein and hydroxytyrosol in preclinical experimental models. They have been demonstrated to inhibit copper sulfate-induced oxidation of low-density lipoproteins.[40]

Antimicrobial activity

It is claimed that olive leaf might aid in the treatment of a broad range of infectious diseases caused by bacteria, viruses, yeasts and fungi.[39] Olive leaf extract is used for treatment of bacterial infections such as bronchitis and tonsillitis, fungal infections which occur in the vagina and viral infections like fever blister.[30,36,41] In an *in vitro* study, tyrosol was found to be the best antifungal compound, followed by catechin and oleuropein. Extracts may be more beneficial than isolated compounds, since a bioactive individual component can change its properties in the presence of other constituents present in the extracts. Therefore, the most effective olive leaf products on the market today are made directly from fresh-picked leaves providing the whole spectrum of natural biophenols, causing them to work together in natural synergy to maximise the health benefits.[7,21,42]

Antihypertensive effect

In vivo assays verify that olive leaf lowers the blood pressure. A prophylactic blood pressure lowering action of the olive leaf extract has been shown in a preclinical study with rats treated with L-NAME.[43] Antihypertensive and cholesterol-lowering actions of the olive leaf extract have been confirmed in humans.[44]

Hypoglycaemic activity

Gonzalez *et al.* reported that maximum hypoglycaemic activity was obtained from samples collected in the winter months.[45] One of the compounds responsible for this activity was oleuropeoside, which showed activity at a dose of 0.16 g kg^{-1}. This compound also demonstrated antidiabetic activity in animals with alloxan-induced diabetes. The hypoglycaemic activity of this compound may result from two mechanisms: (1) potentiation of glucose-induced insulin release, and (2) increased peripheral uptake of glucose.

Other properties

Olive leaf is also told to be an astringent and antiseptic in traditional texts. Olive leaf extract is also known to be beneficial for treating common cold, cough and flu.[46] Furthermore, García-Villalba *et al.* investigated the bioavailability and metabolism of this material in addition to other properties.[47] They suggested that postmenopausal women could be a target population for the intake of olive phenolics in order to prevent age-related and oxidative stress-related processes such as osteoporosis.

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BIOACTIVE CONSTITUENTS IN OLIVE LEAF

Olive tree is continuously exposed to environmental stresses such as high temperature and UV radiation like many other plants. In order to defend itself against this situation, the plant produces various biophenols like phenolic acids, phenolic alcohols, sekoiridoids and flavonoids. Quantitative and qualitative changes of these constituents depend on olive tree cultivar, maturation degree of the leaf, infestation caused by *Dacus olea,* a kind of olive fly, climate and geographical origin. Biophenols constitute the majority of olive leaf content.[48] Additionally, it is possible to find traces of other components such as terpenes and lipophilic compounds.[49–52]

While flavonoids, phenolic acids and alcohols are found in various fruit and vegetables, secoiridoids like oleuropein only exist in plants belonging to the family of Oleaceae. These compounds are both terpenic and hydroxy aromatic secondary metabolites. Their molecular structures are characterised by elenolic acid and its derivatives. Oleuropein, dimethyl oleuropein and ligtroside are examples of this kind. Terpenic groups such as α-amyrin, maslinic acid and oleanolic acid are also found in olive leaves. Oleanolic acid constitutes approximately 30 g kg^{-1} dried matter.[29,46] Olive leaf contains lipohilic compounds like saturated hydrocarbons, squalene, wax, α-tocopherol, triglycerides, β-carotene, linear alcohols and fatty acids (palmitic, oleic and linoleic acids) at ppm level. The number of hydrocarbon changes between 29 and 33 (C_{29}–C_{33}). Squalene, α-tocopherol, β-carotene, α-and β-amyrine analysed in hexane extract are bioactive compounds, which can modulate human physiological system. Therefore, these components have applications in drug, cosmetic and food industries.[36] Olive leaf also involves mannitol, which is a sugar alcohol, synthesised only by the Oleaceae family. This compound has the potential of sweetening corresponding 70% of sucrose. It does not lead to tooth decay, and is convenient for diabetics, since its metabolism in human beings does not depend on insulin. This carbohydrate is found between 10 and 20 g kg^{-1} olive leaf.[30]

Biophenols mentioned are significant factors to evaluate the quality of virgin olive oil, since these constituents are partially responsible for the auto oxidation stability and organoleptic qualities of olive oil.[34]

Apart from all the constituents, oleuropein is the most prominent biophenol in olive leaf extract.[50,53–55]

Oleuropein

Oleuropein was first isolated in olive fruit by Bourquelot and Vintilesco in 1908. They defined this biophenol as soluble in water and alcohol and non-soluble in ether, a non-crystalline and an intensely bitter glucoside. Subsequent studies also proved its ester structure consisting of elenolic acid and hydroxytyrosol.[38,39,56] Most of the researchers are in agreement that the health benefits of olive leaf are mostly attributed to oleuropein, its major bioactive compound, since hydrolysis products of this compound also has significant pharmacological effects.[29,30,32,47,57,58] Furthermore, leaves have the highest level of oleuropein of olive tree. Being a polar compound, it is expected to gain much more oleuropein from olive mill waste water than that of non-polar olive oil. Its level can reach from 10 to 140 g kg^{-1} olive leaf.[59,60] After its biological activities against to diseases such as cardiac, cancer, obesity, diabetes and DNA damage were proved, many other sources of oleuropein such as ligustrum, jasminum and syringa have been investigated other than olive leaf.[55,61]

Oleuropein, the main component of olive biophenols is a heterosidic ester of β-glucosylatedelenolic acid and hydroxytyrosol.[53,55,62]

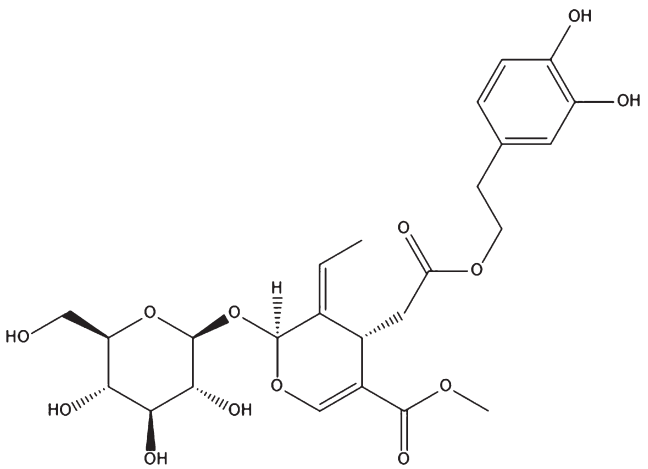


Figure 1. Molecular structure of oleuropein.

Therefore, oleuropein is responsible for the existence of hydroxytyrosol, another valuable natural antioxidant. It is the bitter principle of immature olives, since the oleuropein level of olive decreases by ripening of the fruit.[34] The molecular formula and molecular weight of oleuropein are $C_{25}H_{32}O_{13}$ and 540.514 g mol^{-1}, respectively. Its molecular structure is drawn in Fig. 1.

Oleuropein has antimicrobial activity against viruses, bacteria, yeasts, fungi, moulds and other parasites.[63–66] It was found, *in vitro*, that elenolic acid inside oleuropein had a synergic effect on this activity. Calcium elenolate obtained by hydrolysis has also been proved to have antiviral and antibacterial activities.[31,67] It is used in many medical treatments due to its natural antioxidant property as well. It protects enzymes in cancer patients, and prevents cell death in arterial hypertension.[68–71] It protects cells and organisms by preventing platelet segregation and eicosanoid production. It may be effective in cardiological diseases by preventing membrane lipid oxidation acting in coronary dilation.[40,72,73] It enhances lipid metabolism in order to overcome obesity problems.[74]

Its high antioxidant capacity is ascribed to *o*-dihyroxy substitution (catechol) in the ring structure.[75] Furthermore, oleuropein was found to have higher antioxidant activity than natural and synthetic antioxidants on DNA and lipid oxidation.[76] Although oleuropein has a very distinctive antioxidant capacity, the pure substance has a restricted effect. This might be explained by degradation of this compound to a stronger biophenol, hydroxytyrosol, leading to increase of total antioxidant capacity in the extract. Another explanation might be the synergistic action of each biophenol in the extract.

STUDIES ON OLIVE LEAF

In the literature there are many studies related to olive leaf, and its extraction, analysis, and antioxidant and antimicrobial properties and potential uses.

Stability of olive leaf extract

Pre-treatments before extraction processes affect the amount of target compounds substantially. In order to estimate the effect of drying temperature on oleuropein content, olive leaves were dried between 25 and 150 °C, and freeze-dried. A specific drying temperature from 65 to 80 °C revealed a declining concentration of

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oleuropein in the leaves. When the leaves were treated with boiling water, the oleuropein level was restored. Enzyme activities might be responsible for this decrease, which would be inactivated with boiling water.[77] These results were confirmed in another study.[78] Oleuropein was quite stable in aqueous extracts for 7 days when stored at room temperatures but degraded after 17 days. Other biophenols were less stable in aqueous extracts and started to show some degree of degradation after 7 days, and were completely degraded after 17 days. Compounds in methanol extract were quite stable for 30 days when stored at room temperature. Kamran *et al.* found that oven-dried leaves (105 °C) showed the highest total phenol recovery and increased oleuropein recovery up to 38-fold compared with fresh olive leaves.[79] Amazingly, they recommended olive leaves should be dehydrated at 105 ° C for 3 h before extraction to obtain the maximum recovery of oleuropein and other biophenols. The effect of drying and storage conditions was also evaluated. Olive leaves were hot air dried (120 °C) and freeze dried. The extracts, in liquid and powder forms, were stored at 4, 25 and 35 °C for 4 weeks. Drying the material affected both the initial extract composition and the bioactivity properties during storage. Finally, storage conditions (temperature and extract form) did not have a significant influence on the antioxidant activity.[80]

Determination of biophenols in olive leaf extract

A group of researchers analysed the biophenols present in olive leaves by HPLC/DAD, and evaluated the extracts antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. Seven biophenols (caffeic acid, verbascoside, oleuropein, luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside and luteolin 4′-*O*-glucoside) were identified and quantified. As expected, oleuropein was the compound present in the highest amount, representing almost 730 g kg^{-1} of the total identified compounds.[21] Determination of oleuropein content in olive leaf from five Tunisian cultivars (Chemlali, Chetoui, Meski, Sayali and Zarrazi) and one French cultivar (Bouteillan) was performed by mid-infrared spectroscopy as a rapid tool.[81] One hundred and five samples were analysed by HPLC and mid-infrared spectroscopy. Olive leaves of Spanish cultivars were found to contain important amounts of oleanolic acid and mannitol as well as oleuropein by means of solid phase extraction and HPLC methods.[82] The combined use of HPLC-MS/MS and NMR spectroscopy was also utilised for complete assignment and structural determination of analytes in complex mixture of olive leaf extract.[83]

Pharmacological effects of olive leaf

The antiviral activity of olive leaf extract standardised by liquid chromatography–coupled mass spectrometry against HIV-1 infection was examined.[18] The results exhibited the anti-HIV activity of olive leaf extract, and provided insight into its molecular mechanisms of action. On the other hand, Zorić *et al.* evaluated the antifungal activity of olive leaf extract against *Candida albicans* ATCC 10231 and *C. dubliniensis* CBS 7987 strains.[48] They verified the extract's cytotoxic effect on the viability of tested yeast strains depending on the concentration.

Both luteolin and oleanolic acid of olive leaf extract had an inhibitory effect on postprandial blood glucose increase in diabetic rats.[24] Olive leaves suppressed the elevation of blood glucose after oral administration of starch in borderline volunteers, and was recommended as a useful food supplement for the prevention of diabetes.

Antihypertensive, diuretic, anti-atherosclerotic, antioxidant and hypoglycaemic effects of authentic oleanolic and ursolic acid and the three isolates of olive tree leaves were investigated in a Dahl salt-sensitive, insulin-resistant rat genetic model of hypertension. All three isolates for 6 weeks treatment prevented the development of severe hypertension and atherosclerosis and improved the insulin resistance of the experimental animals.[84]

Since opioids used for treating moderate to severe pain gave rise to induction of side effects such as antinociceptive tolerance, physical dependence, emesis, constipation and drowsiness, olive leaf extract and its chief constituent were tested to show anti-tolerant effects against the development of tolerance to the antinociceptive effect of morphine in rats.[85] Ethanol extract of olive leaves and its oleuropein proved to be a significant inhibitory effect against morphine antinociceptive tolerance in rats.

Fakhraei *et al.* studied on the possible protective effect of dry olive leaf extract against acetic acid-induced ulcerative colitis in rats in order to indicate its anti-inflammatory and antioxidative properties.[86] The findings showed that the extract probably decreased intestinal damage through the antioxidant and antiinflammatory effects of biophenols, mainly, oleuropein and hydroxytyrosol. Another study in conjuction with the relationship between olive leaf extract intake and potential biological effects was carried out.[87] The authors supplied novel data with respect to the positive effects of acute consumption of olive biophenols on the human vasculature.

Effect of agronomical and technological factors on composition of olive leaf extract

The amount of oleuropein in the collected Italian olive leaves was markedly modified by the colour/age and genetic factors, whereas meaningless variations were ascribable to the quantification method and the collecting period factors.[19] The samples of leaves collected in October seemed to contain slightly lower amounts of oleuropein than those collected in March, likely due to a higher degradation rate of this glycoside in autumn and in addition to lower production of green young leaves during this season with respect to the spring period. Differences between cultivars of olive trees (Alameño, Arbequina, Azulillo, Chorna, Hojiblanca, Lechín, Manzanillo, Negrillo, Nevadillo, Ocal, Pierra, Sevillano and Tempranillo) from the same cultivation zone, and of Arbequina olive trees cultivated in different geographical zones (Córdoba, Mallorca, Ciudad Real, Lleida and Navarra) were achieved by HPLC-DAD analysis of extracts (ethanol/water) from olive leaves obtained by the assistance of microwaves.[88] The most influential variables were found to be the main biophenols (oleuropein, verbacoside, apigenin-7-glucoside, and luteolin-7-glucoside) in olive leaves. This shows that the relative concentrations of these compounds might also be useful to differentiate both varieties and cultivation zones. The impact of sampling parameters such as cultivar, leaf age, and sampling date, on the radical scavenging potential of olive leaf extracts was also examined via several antioxidant capacity assays such as DPPH, ABTS, ORAC and CBA. Total phenolic content and radical scavenging potential values were found to be safe criteria for the selection and appreciation of olive leaf batches for further usage. Establishment of olive leaf as a robust source for radical scavengers throughout the year was concluded to be promising for its commercialisation.[89] The oleuropein content varied with 0.127, 0.115 and 0.144 g kg^{-1} in Mary Zeitoon, Koroneiki and Sevillana varieties cultivated in three different regions of Iran after collection in two different seasons. In general, the oleuropein concentration was significantly higher in the cold season and in the tropical regions than in the hot season and the temperate region.[90] Leaves from 20 olive cultivars (San Felice, Galleaga, Frantoio,

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Maurino, Luccino, Arbasana, Arbequina, Empeltre, Koroneiki, Taggiasca, Grappolo, Manzanilla, Ouslati, Santa Caterina, Ligurian, Itrana, Pendolino, Sevillano, Chitoni, Picual, Chemlali, Coratina, Mission, Aglandau and Nichitskaia) collected from San Antonio, Texas, were compared with respect to oleuropein, verbascoside, luteolin 7-*O*-glucoside and luteolin 4-*O*-glucoside, respectively.[91] The genetic make-up of the cultivars and geographical location showed profound effects on levels of major biophenols in olive leaves. The levels of all the individual biophenols were significantly lower in summer samples compared to spring (for example, oleuropein levels in June sample of 'Arbasana' leaves were 98.4% lower relative to February samples). Bilgin and Şahin also investigated differences in six cultivars grown in Turkey but of different origin (Bursa, Mardin, Ayvalik, Kas, Tekirdag and Canakkale).[25]

A research group evaluated phenolic contents and compositions of olive leaf extracted by several solvent methods and to further establish their antioxidant activities using various radical scavenging systems. Total flavonoid and phenolic contents were observed significantly higher in the 80% ethanol extract, butanol, and ethyl acetate fractions than hexane, chloroform and water fractions. Oleuropein was identified as the major phenolic compound with considerable contents in those major three fractions that correlated with their higher antioxidant and radical scavenging. The results indicated that olive leaf contained significant amounts of oleuropein and phenolics, important factors for antioxidant capacity, which could be substantially modified by different extraction methods.[7] Furthermore, the same group showed that both the individual and combined phenolics exhibited good radical scavenging abilities. In terms of antimicrobial activity, both oleuropein and caffeic acid showed inhibition effects against microorganisms. Additionally, the antimicrobial effect of the combined biophenols was significantly higher than those of the individual ones.[92]

There are also reports on investigation of extraction media affecting the extract both qualitatively and quantitatively. Within this scope, effects of pH, extraction time and ethanol concentration on extracted biophenols were studied in an extracting media composed of water/ethanol/citric acid.[93] The results showed that the ethanol concentration was the primary factor that affected the yield, whereas the pH of the media was less significant. Increasing the percentage of ethanol from 40% to 50% produced a decrease in biophenols yield, whereas a further increase from 50% to 60% promoted the biophenol yield. As for the pH effect, increasing the pH from 2 to 6 provoked an almost linear decline in biophenol extraction.

Studies on olive leaf extraction through supercritical fluid extraction are scarce in the literature. In order to extract tocopherol content from olive tree leaves, a residue obtained during the harvest of olives for oil production were extracted with supercritical carbon dioxide. The effect of pressure (25–45 MPa), particle size (0.25–1.5 mm), solvent flow (0.5–1.5 SL min^{-1}) and temperature (40–60 °C) on the extraction rates of oil and tocopherol were detected. Optimum conditions led to high valuable extracts of 745 and 971 g kg^{-1} tocopherol concentration, respectively.[94] Counter-current supercritical fluid extraction at a pilot scale plant was used for fractionation of waxes, hydrocarbons, squalene, β-carotene, triglycerides, α-tocopherol, β-sitosterol, and alcohols from a raw extract of olive leaves in hexane. The influences of extraction pressure (75–200 bar), extraction temperature (35–50 °C) and ethanol as modifier (0–10%) were examined. The experimental results were evaluated regarding extraction yield, enrichment, recovery, and selectivity. The optimum conditions were found as 75 bar, 35 °C, and 10% ethanol.[95] Olive tree leaves from the Aegean region of Turkey were extracted by means of Soxhlet and supercritical fluid extraction methods. Effect of pressure (100–300 bar), temperature (50–100 °C) and type of co-solvent (ethanol, methanol and water) on the amount of both extract and oleuropein were evaluated. While the highest oleuropein yield (37.84 g kg^{-1} dried leaf) was achieved with the Soxhlet method through methanol, CO_2 modified by methanol at 300 bar and 100 °C yielded the best oleuropein yield.[96] Another study on supercritical fluid extraction was carried out in the presence of water and ethanol as co-solvent by the same group.[97] The influences of operating parameters by means of co-solvent content (0–1 mL min^{-1}), temperature (50–100 °C) and pressure (100–300 bar) on both extract and oleuropein yields were investigated. CO_2 modified by water and ethanol showed nearly the same extract performance, while CO_2 modified by water yielded higher oleuropein content. Taamalli *et al.* compared the supercritical fluid extraction of phenolic compounds from leaves of six Tunisian olive varieties with those of microwave-assisted and pressurised liquid extractions.[98] As expected, supercritical fluid extraction was found to be more efficient to extract compounds with less polarity such as apigenin, luteolin, or diosmetin. Issaoui *et al.* extracted triterpenes and aliphatic hydrocarbons from olive leaves by means of supercritical fluid extraction using carbon dioxide at 40 and 60 °C, and 90, 250 and 300 bar.[99] In addition to this advenced technology, supercritical antisolvent processing of natural products is also a very effective novel technique. A supercritical antisolvent process was carried out to obtain precipitates with potent antioxidant activity.[100] The findings showed that antioxidant activities were much higher for the fine particles obtained by this technique than for the extracts obtained by supercritical fluid extraction. Furthermore, they also used the same technology for assessment the possibility of producing biophenols with controlled size and size distribution.[101]

It is also possible to see studies on olive leaf extracts obtained by novel technologies other than supercritical fluid extraction. A dynamic ultrasound-assisted extraction with ethanol-water mixtures as the solvent was applied to olive leaves, which was faster (25 min vs. 24 h) and more efficient than traditional methods such as maceration/stirring. Oleuropein, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside in the extract were not degraded, even though there was an increase in the temperature caused by ultrasonic irradiation.[102] Another study on ultrasound-assisted extraction of olive leaves was performed in order to optimise the extraction parameters such as solvent concentration (0–100% EtOH, v/v), the ratio of solid to solvent (25–50 g L^{-1}) and extraction time (20–60 min) by means of response surface methodology. On the other hand, solvent concentration was found to be the most effective parameter, influencing the extraction performance.[103] [a]ahin *et al.* used ultrasound for the olive leaf extract rich in biophenol and flavonoid with high antioxidant capacity.[104] A temperature of 34.18 °C, EtOH concentration of 43.61% (v/v), and extraction time of 59.99 min were optimal operating conditions in order to extract the greatest TPC, while the maximum yield of TFC was predicted under 34.44 °C of temperature, 70% (v/v) of EtOH, and 60 min of extraction time through response surface methodology. A combined method of ultrasound-assisted and reduced-pressure boiling extractions was also tried to extract oleuropein from Frantoio olive leaves.[105] High oleuropein yield was achieved due to the broken cells under the optimal conditions of the method: ethanol concentration 75% (v/v), extraction temperature 50 °C, ultrasonic power 600 W, extraction time 3 min, liquid-to-solid ratio 30:1 (mL

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g^{-1}), and extraction pressure 25 kPa. A kinetic study of oleuropein extraction from olive leaves by ultrasound is also suggested.[106]

Another novel method was applied to olive leaves by microwave-assisted extraction.[107] The best extract yield was achieved by ethanol/water solvent system for 8 min, which was even better than ultrasound-assisted extraction. The same researchers also extracted oleuropein, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside from olive leaves by superheated liquid extraction without degradation of the analytes for only 13 min [lower than ultrasound-assisted extraction (25 min), in contrast to microwave-assisted extraction (8 min)].[108]

Recently, there has been an increasing interest in the membrane technologies for separation and purification. Olive leaf extract was fractionated by membrane separation techniques through a sequence of microfiltration, ultrafiltration and nanofiltration systems.[109] Nanofiltration retentate exhibited high inhibitory activities with high oleuropein content making this technology useful in the development of new products with functional properties suitable for the cosmetics, food and pharmaceutical industries.

Application of olive leaf extract

The development of fast methods to enrich edible oils with olive leaf biophenols is attracting great interest. A liquid–liquid extraction method was developed to enrich olive, sunflower, and soy oils with biophenols from olive leaf extracts.[34] A continuous approach assisted by ultrasound for direct enrichment of edible oils (olive, sunflower, and soya) with the main biophenols in olive leaves (oleuropein, verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside) was also developed.[52]

Olive leaf extract also used as an antioxidant supplement in meat stored under refrigerated conditions.[110] Olive leaf extract at 0.2 or 0.3 g GAE kg^{-1} inhibited lipid and protein oxidation in pork patties.

Marcos *et al.* developed antioxidant polyester films with α-tocopherol and olive leaf extract.[111] Incorporation of antioxidants to polyester films modified their physical properties in addition to the antioxidant activity *in vitro*. Recently, Mohammadi *et al.* applied nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil.[112] These authors recommended nano-encapsulation of olive leaf extract a suitable novel technique to improve the antioxidant capacity of natural ingredients.

CONCLUSION

Olive leaf is a potential inexpensive, renewable and abundant source of biophenols as an agricultural and industrial waste. These valuable phenolic compounds in olive leaf powders and leaf extracts are responsible for many health benefits. Therefore, there is a growing interest to utilise olive leaf powders or extracts in various industrial applications such as food supplements, cosmetic and pharmaceutical industries. However, the biophenolic profile of olive leaves is known to be affected by technological parameters, which affect industrial processes employed for extraction such as preliminary preparations, particle size of the extracted material, solvent type, solvent composition, solid-to-solvent ratio, extraction temperature and pressure, extraction time and pH. In addition to those parameters, there are agronomical factors such as leaf age, degree of ripeness, geographical origin, cultivar, and phonological stage during sampling, proportion of brunches on the tree, moisture content and degree of contamination with soil. In general, the studies reported here show that both technological and agronomical parameters could make significant differences in leaf biophenols. There appears to be a general trend that biophenols tend to decrease in hot summer days. Therefore, sampling of leaves in spring is likely to yield higher amounts of biophenols. Care must be taken in harvesting the leaves as well as selecting proper cultivars for obtaining specific types of biophenols.

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