REVIEW

THE HEALTH EFFECTS OF OLEUROPEIN, ONE OF THE MAJOR PHENOLIC COMPOUNDS OF OLIVES, OLEA EUROPAEA *L.*

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ABSTRACT

Olives (*Olea europaea* L.) and olive oils have a significant place in the daily diet in the Mediterranean Region and contain significant amounts of polyphenolic compounds, of which oleuropein, hydroxytyrosol and tyrosol are dominant. Harvest time, geographical region and climate all affect the phenolic content. Experimental, clinical and epidemiological studies show the beneficial effects of oleuropein, such as antioxidant, antimicrobial, anticancer anti-inflammatory, antineuropathic and other properties. In particular, olive leaves, roots, virgin olive oil and olive mill waste (vegetation and wastewater) are potential sources of oleuropein. This review focuses on the pharmacological effects of oleuropein and extraction procedures for oleuropein.

Keywords: olive, phenolic compounds, oleuropein, pharmacological effects, *Olea europaea*, extract

1. INTRODUCTION

Olives are fruits and are a significant part of the daily diet, playing a vital role in the agricultural sector especially in the Mediterranean countries and having high economic value in this region. In addition to their popular usage as table olives, they have become extremely valuable because they have important nutrient properties and have shown positive benefits for human health. The majority of olives produced globally are used in oil production (approximately 85-90%), and the rest is used in the production of table olives. Although Turkey comes behind Spain, Italy and Greece in oil flesh and table olive production (FAO, 2011), It is reported that olive producing regions in this country are in order of Aegean (55.11%), Marmara (27.72%), Mediterranean (14.94%) and Black Sea region (2.22%) and that 75-80% of total oil production of olive oil is made in the Aegean region (ARSLAN and OZCAN, 2011). It is reported that as of 2010, Turkey has 6 million of olive trees and 2 million of table olive trees, and 73% of total olive areas and olive production is made of oil types and 27% comprises table olives. Olive oil production was approximately 1,040,000 tons and table olive production was 375,000 tons according to statistical data in 2011 (DOGAKA, 2011).

Olive trees (*Olea europaea L.*) are included in the Oleaceae family. The most important characteristic of olive fruits is their high oil content, chemical composition and a unique bitterness in comparison to other fruits with hard seeds (OTHMAN *et al*., 2008; MAFRA and BARROS, 2006). The typical bitterness of olive fruits results from their rich content of phenolic compounds, especially of oleuropein, in olive cultivars (Fig. 1). These phenolic compounds and oleuropein content of olive cultivars increase the interest in their antioxidant properties and their positive effects on health (ARSLAN and OZCAN, 2011; BARBARO *et al*., 2014;CARDENO *et al*., 2013). It has been reported that, in addition to their mono-unsaturated fatty acid content, lower-concentration compounds are the reason for their positive effects on nourishment. The amount of phenolic compounds ranges between 1-2% in fresh fruits, and that these are mainly secondary metabolites in olives (OTHMAN *et al*., 2008). Phenolic acids, phenolic alcohols, flavonoids and secoiridoids are also important phenolic compounds in olive cultivars. Iridoids and secoiridoids are compounds that are bound to glycosides and are formed from the secondary metabolism of terpenes as precursors of various indole alkaloids. It is reported that the secoiridoides in Oleaceae are usually derived from the oleoside type of glycosides, which are characterized by an exocyclic 8,9-olefinic functionality. Oleuropein is an ester that forms from 3-4-dihydroxyphenyl ethanol and hydroxytyrosol and has the oleosidic skeleton. It is reported that demethyloleuropein, oleuropein aglycone, elenolic acid and verbascoside are essential compounds of hydroxycinnamic acid derivative in olive cultivars (ROMANI *et al*., 1999; SERVILLI *et al*., 2004; ROMERO *et al*., 2002; ESTI *et al*., 1998; AMIOT *et al*., 1986).

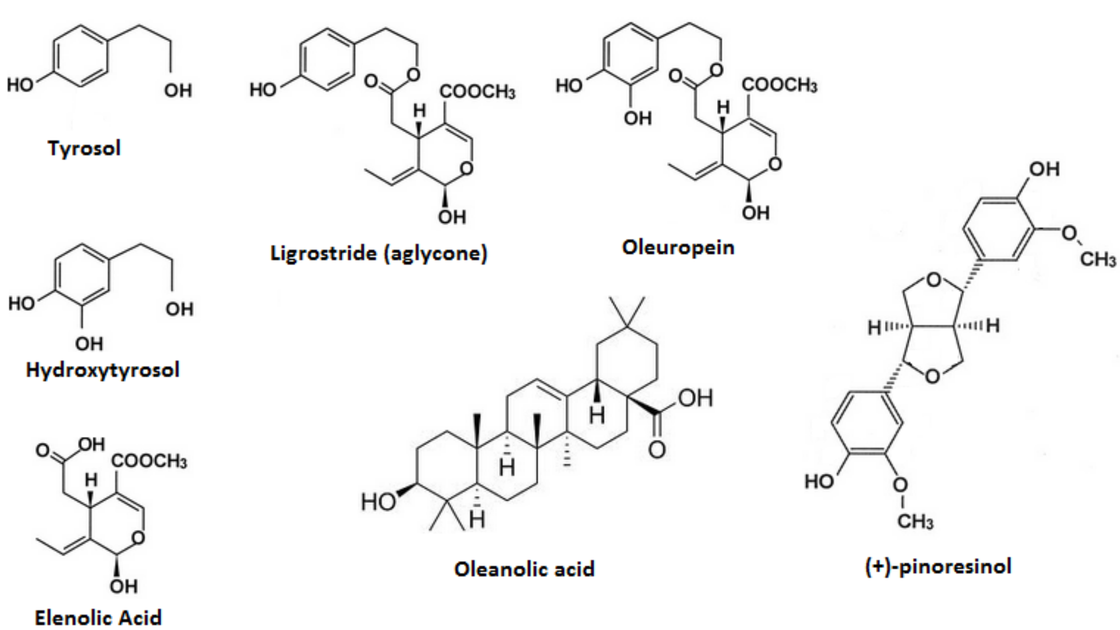

Figure 1: Chemical structures of some of the phenolic compunds that are found in the *Oleacea* family (YILDIZ and UYLASER, 2011).

Factors such as variety, geographical location, maturing progress, harvest time and seasonal conditions all affect phenolic substance amounts in olives. It was stated that the total precipitation content during the period until harvest time affected phenolic substances (ROMERO *et al.*, 2003; ESTI *et al.*, 1998), and there were decreases in phenolic concentrations of olive oils from fruits of trees cultivated in irrigated areas or under high rainfall conditions when compared to the oils from non-irrigated or low rainfall regions (TOVAR *et al.*, 2002; YOUSFI *et al.*, 2006; BOTIA *et al.*, 2001). Contrary to this situation, it was indicated that phenolic contents were higher in olives in the Alanya region, which receives a relatively high rainfall (ARSLAN and OZCAN, 2011). The phenolic contents of olives that were cultivated in higher altitude locations were greater than those from lower altitude regions (MOUSA *et al.*, 1996), and that high altitude did not affect the content of phenolic substances of Sariulak olives that were grown in different regions. It was stated that harvest time affected the phenolic substances in olives; and phenolic contents changed based on olive harvests made at different times during September-December months. It was indicated that at the second harvest period around the October-November months, rutin, verbascoides, taxifolin and tyrosol contents increased, and in addition, phenolic contents decreased toward the last periods of the harvest time. It was reported that among the phenolic substances present in Sariulak olives, apigenin, cinnamic acid and p-coumaric and 4-hydroxybiphenyl carboxylic acid contents decreased, whereas ferulic and vanillic acid contents increased as the harvest time progressed (ARSLAN and OZCAN, 2011).

2. OLEUROPEIN AND ITS FORMATION

Olive fruits contain a high level of mono-unsaturated oils and there are significant amounts of phenolic substances. Phenolic compounds are good antioxidants and contribute to the prevention of illnesses due to their important biological activity characteristics (OTHMAN *et al.*, 2008). The antioxidant capacities of phenolic substances

originate from their reducing characteristics, which enable them to react with mono oxygen (RICE-EVANS *et al*., 1997).
It is stated that there are approximately 40 phenolic compound when olive plants are considered as a whole as a tree, branch, root, and olive fruit. Among the phenolic compounds the concentrations of, especially, oleuropein, hydroxytyrosol, lutolein, rutin, trans cinnamic acid and tyrosol are higher compared to other phenolic substances (YORULMAZ *et al*., 2013; ARSLAN, 2012; ARSLAN and OZCAN. 2011). Oleuropein, dimethyl-oleuropein, ligstride and oleoside compounds indicate dominance of phenolic oleosides in olive fruits and, in addition to this, verbascoside is the main derivative of hydroxycinnamic acid in olives. Oleuropein is the most important phenolic compound in olive cultivars and its concentrations can reach up to 140 mg g^{-1} (db) levels in some olive species; and the content ranged between 60-90 mg g^{-1} (db) of dry matter in young olive cultivars (AMIOT *et al*., 1986; LE TUTOUR and GUEDON, 1992).
Oleuropeins, classified within the secoiridoids, exist in varying concentrations in olive plants, mostly in tree stems, branches, leaves and olive fruits. Iridoid and secoiridoids that are bound to glycosides are compounds formed in the secondary metabolism of terpenes which are the precursors of various indole alkaloids. It is reported that oleuropein (Fig. 2) is an ester of 2-ethanol (3,4-dihydroxyphenyl) and oleuropein aglycone have an oleoside structure known as secoiridoide glucoside (SOLER-RIVAS *et al*., 2000).

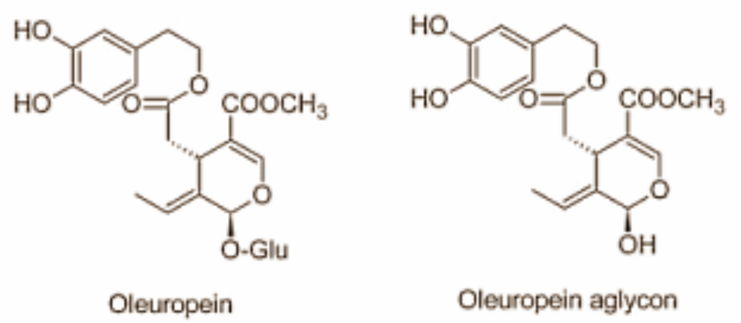

Figure 2: Chemical structures of oleuropein and oleuropein aglycone (Yıldız and Uylaser, 2011).

Oleuropein production in olives (Fig. 3) occurs by branching in the mevalonic acid cycle in the secondary metabolism of terpenes during the formation of oleosides (DAMTOFT *et al*., 1992). Secoiridoids are derived from these compounds and the carbon skeleton is derived from mevalonic acid. Geraniol, 10-hydroxygeraniol and iridoidal are regarded as the precursors of loganin. Deoxyloganic acid, 7-epiloganic acid and loganic acids are the precursors of oleuropein, which are incorporated in to the ligstrosides (DAMTOFT *et al*., 1993).

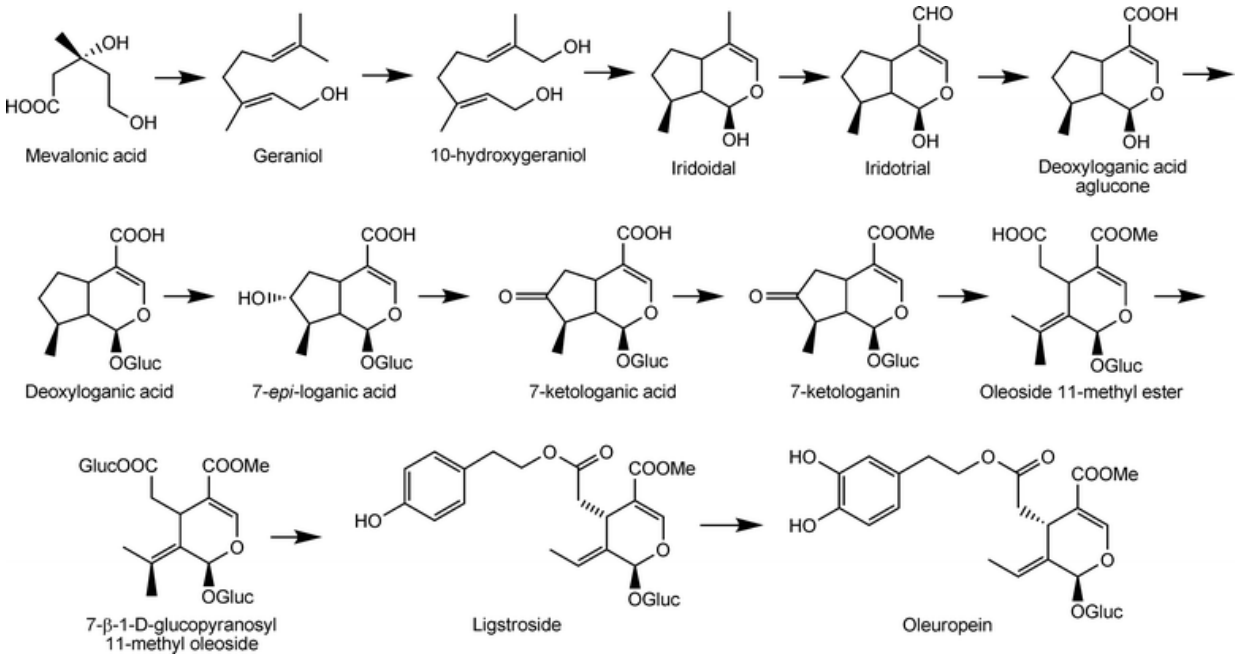

Figure 3: Formation of oleuropein in the Olea family (YILDIZ and UYLASER, 2011).

In studies conducted on the contents of oleuropein which is responsible for the bitter taste in olive cultivars, its content ranged between 2.1-134 mg g^{-1}(db) in olive leaves, 11-18.9 mg g^{-1}(db) in olive branches, 1.9-6 mg g^{-1} (db) in olive roots, 0.3-21.7 mg g^{-1} (db) in olive fruits and 0-11.2 mg g^{-1} (db) in extra virgin olive oil (ANSARI et al., 2011; SAVOURNIN *et al.*, 2001; TAYOUB *et al.*, 2012; MALIK and BRADFORD, 2006; ALTINYAY and ALTUN, 2006; YORULMAZ *et al.*, 2013). In non-virgin oils, oleuropein contents range between 0-0.4 mg g^{-1} after oil extraction and oleuropein is also found in pomace and in the liquid portion (vegetation and waste water) except for oil obtained as a result of olive oil pressing that is found in ratios between 6.5-8.7 mg g^{-1} (GOLDSMITH *et al.*, 2014; ALLOUCHE *et al.*, 2004; YILDIZ and UYLASER, 2011). Oleuropein formation in olive fruits takes place at the order of growth stage, green maturation stage and black maturation stage. During the growth stage, oleuropein accumulates in olive fruits and amounts of chlorophyll and oleuropein decrease at the green maturation stage. At the black maturation stage in olives, a decrease in oleuropein contents takes place and anthocyanin pigments are formed (AMIOT *et al.*, 1989). It is proposed that oleuropein content is high at the beginning of the fruit growth in young olive fruits (YORULMAZ *et al.*, 2013). Although there is a significant amount of oleuropein in olives that are harvested when the olive fruits are green, the oleuropein content decreases rapidly with the black maturation phase (BIANCO *et al.*, 1993). Although phenolic compounds in table olives are initially higher, in the production of table olives the oleuropein concentration decreases during maturation and disappears at full ripeness. The oleuropein concentration also decreases sharply after salt treatment and disappears after lye treatment. It is also reported that the phenolic contents of table olives showed different phenolic profiles such as hydroxytyrosol concentration of olive drupes, which decline rapidly during the black maturation phase, which are explained by the fact of climate, soil type and growing region (OTHMAN *et al.*, 2008).

3. OLEUROPEIN AND ITS PHARMACOLOGICAL EFFECTS

The significant effects of phenolic compounds that are present in plants have increased interest for the use of these substances in the preparation and processing of foodstuffs. Olive fruits and oil that are produced in the Mediterranean region have a key position in the daily diet and they are good resources in terms of oleuropein, which is the most prominent phenolic substance (ARSLAN and OZCAN, 2011). Oleuropein, which is found in varying quantities in olive fruits, branches, leaves and roots of olive trees, has many positive effects on human health according to clinical, epidemiological and experimental investigations conducted on the health effects, such as antioxidant (VISIOLI *et al*., 2002; GONZALES-HIDALGO *et al*., 2012), anti-atherogenic, anti-inflammatory (VISIOLI *et al*., 1998), anti-cancer, antimicrobial and antiviral effects (TRIPOLI *et al*., 2005; FREDRICKSON and GROUP, 2000).

Effects of oleuropein on lipid oxidation are shown in the decrease in by-products (malondialdehyde and 4-hydroxynonenal) that react with 2-thiobarbituric acid (TBA) and produced by oxidation. It is stated that antioxidant potential of oleuropeins is related to the ability of radical stability development by forming a hydrogen bond within the molecule between the free hydrogen of hydroxyl groups and phenoxy radicals and the higher content of oleic acid that are present in virgin oils compared to other edible oils and the presence of high level of antioxidant compounds in olives and olive oils (hydroxytyrosol and oleuropein) have less sensitivity to oxidation in comparison to n-6 unsaturated fatty acids. (BARBARO *et al*., 2014). Oleuropein has protective effects in the decrease of plasmatic levels of cholesterol in the total, free and ester forms in rabbits for improving the resistance of low density lipoproteins to oxidation (CONI *et al*., 2000) and also oleuropeins have the ability to clear nitric oxide (NO). In addition, this causes an increase in inducible nitric oxide synthesis in cells (DE LA PUERTA *et al*., 2001). Hypochlorous acid, which is formed as a result of oxidation by neutrophil myeloperoxidases at the site of inflammation and can cause damage to proteins including enzymes (VISIOLI *et al*., 2002).

There are many studies that have considered the effects of oleuropein on health. The consumption of virgin olive oil, which is rich in nutrients, have an observed antitumor effect and decrease the proliferation of cancer cells, especially of the colon, breast and skin (CARDENO *et al*., 2013; PSALTOPOULOU *et al*., 2011). It is reported that an application of a 400 μM dose of oleuropein and hydroxytyrosol cause a significant decrease in the cell proliferation of colon cancer (HT-29) 24-hour after treatment (CARDENO *et al*., 2013). It is stated that the oleuropein aglycone compound is the most effective phenolic compound for decreasing the viability of cancer cells in breast, colon and kidney cancer types (MENENDEZ *et al*., 2007;HAMDI and CASTELLON, 2005). Oleuropein that has been extracted from olive leaves has marked antitumor effects on prostate, breast and hepatoma cancer cells, depending on the amount of dose, 24-72 hours after application (KOCKAR *et al*., 2010). The effects of oleuropeins on the various cancer types and cells are shown in Table 1.

Table 1: Oleuropein-induced anti-tumor effects in different cancer cells.

Cell line	Cancer type	References
MCF-7;MDA;T-47D	Breast adenocarcinoma and ductal carcinoma	(HAMDI and CASTELLON, 2005)
786-O	Renal cell adenocarcinoma	(HAMDI and CASTELLON, 2005)
RPMI 7951	Melanoma	(HAMDI and CASTELLON, 2005)
HT-29,Caco-2,LoVo	Colorectal adenocinoma	(CARDENO *et al.*, 2013;CORONA *et al.*, 2007)
PC-3,MCF-7,HEP3b	Prostate,Breast,Hepatoma	(KOCKAR *et al.*, 2010)
LNCaP and DU145	Prostate cancer	(ACQUAVIVA *et al.*, 2012)
A549	Lung carcinoma	(MAO *et al.*, 2012)

The antitumor effect of oleuropein is related to its anti-angiogenic function. There are many different varieties of tumors that are connected with other cells and these are formed by complex interactions in a permeable microstructure. The development of a tumor mass is formed in the 'nutrition' environment, and in growing initially forms a deoxygenated environment. The formation of new blood vessels occurs by stimulating multiplication and activities of endothelial cells (BARBARRO *et al*., 2014). Skin that is exposed to solar UV radiation is damaged and also skin thickness and elasticity are reduced, which makes it more vulnerable to skin cancers. It is reported that oleuropein and olive leaf extracts from were administered orally twice for 30 weeks in hairless mice. Both the extract and oleuropein (0.3 and 1 g kg^{-1}) significantly inhibit the increases in skin thickness, reductions in skin elasticity, skin carcinogenesis and tumor growth (KIMURA and SUMIYOSHI, 2009).

Antimicrobial agents are used to control microbial growth and many synthetic antioxidant substances are used for this purpose. There is an increase in the use of natural substances instead of synthetic substances due to their side effects on human health. Oleuropein has been considered as a natural antioxidant substance to reduce the growth rate of microorganisms. It has been reported that the phenolic compound oleuropein in virgin olive oil has antimicrobial activity towards *L. plantarum*, *B. cereus* and *S. enteritidis* among both Gram positive and Gram negative bacteria (CICERALE *et al*., 2012; AZIZ *et al*., 1998). The toxic effect of oleuropein is higher for Gram (+) bacteria than for Gram (-) bacteria, especially *S. aureus* and *E. coli* (FURNERI et al., 2002). Oleuropein also has antimycoplasmal properties and consequently has positive effects against mycoplasma bacteria strains, which are resistant to antibiotic applications (FURNERI *et al*., 2002). Although the antimicrobial activity mechanism of the oleuropein component has not been explained completely, it has been suggested that the microbial activity mechanism of oleuropein originates from the presence of an ortho-diphenolic (catechol) system in the environment and that it changed the ability of a glycoside group, enabling it to enter through the cell membrane and reach the target region (SAIJA and UCCELLA, 2001). It was expressed that oleuropein that had been extracted from olive leaves also has antimicrobial characteristics towards *Campylobacter jejuni*, *Helicobacter pylori* and *Staphylococcus aureus*, which are resistant to methicillin, and extracts of olive leaves played a crucial role in the arrangement of the composition of stomach flora by reducing *H. pylori* and *C. jejuni* microorganisms (SUDJANA *et al*., 2009).

4. DIFFERENT ANALYTICAL APPROACHES FOR OLEUROPEIN EXTRACTION FROM OLIVE LEAVES

Oleuropein is the most significant component among phenolic compounds present in olive cultivars. It is present in a significant amount in the development phases of olive fruits, but its content starts to diminish along with maturation and decreases to near zero after the olive fruits turn from green to black. There is a demand for extracting and purifying of oleuropein existing in the olive cultivars, especially from olive leaves, because of their pharmacological characteristics and favorable effects on health. There are many methods for extracting such phenolic substances from olive leaves, such as extraction by using organic solvents at high temperature and pressure, liquid-liquid extraction (JAPON-LUJAN and LUQUE DE CASTRO, 2006), super-critical liquid extraction (SAHIN *et al*., 2011), polar substances extraction (MALIK and BRADFORD. 2008), solid phase extraction, acidified de-ionized water extraction (STAMATOPOULOS *et al*., 2012), steam blanching, hot water and ultrasound extraction (ANSARI *et al*., 2010).

Oleuropein is more readily extracted from olive leaves due to their higher content of oleuropein compared to the fruits. Prior to the extraction of oleuropein, olive leaves are dried at temperatures not exceeding 60°C and then the dried leaves are crushed to granules with their dimensions ranging between 0.9-2 mm. Following this, the phenolic substances and oleuropein are extracted. The stability and content of oleuropein differs, depending on the extraction method used. It is reported that a higher amount of oleuropein is extracted by using 80% methanol compared to extraction by hexane, ethanol, hot water or a mixture of methanol/hexane (SAHIN *et al*., 2011; MALIK and BRADFORD, 2008). The oleuropein is quite stable for 30 days at room conditions and also stable in aqueous extracts for 7 days when stored at room temperatures but is degraded after 17 days (MALIK and BRADFORD, 2008). Drying of olive leaves at 25°C has no effects on the oleuropein and verbascosides contents at room conditions but drying leaves at greater than 60°C causes a decrease in phenolic substances (MALIK and BRADFORD. 2008). The extraction of oleuropein from leaves by using acidified de-ionized water (pH: 3, 60°C) has a marked effect on the color of the leaf extract (green) and oleruopein content; and also this method has the advantage of the non-toxic characteristics of acidified de-ionized water (ANSARI *et al*., 2011). Among the treatments of steam blanching, hot water and UV-C irradiation, the steam extraction method provides an increase in oleuropein yield from 25 to 35 times compared to non-steam blanching samples whereas antioxidant activity increases from 4 to 13 times. The particle size of leaves (1-3 mm) used affects the extraction of phenolic contents and oleuropein and the ethanolic extraction of hot water blanched leaf fractions produces higher amounts of oleuropein compare to the untreated samples (STAMATOPOULOS *et al*., 2012). Ethanol, methanol and water are considered as co-solvents in 20% (v/v) for the extraction of oleuropein from olive leaves, whereas the maximum oleuropein yield is obtained by supercritical fluid (CO_2) extraction modified with 20% methanol (SAHIN *et al*., 2011).

5. RESULTS AND CONCLUSIONS

Olives have a crucial place in the daily diet in the Mediterranean region. When they are examined in terms of ingredients, in addition to mono-unsaturated fatty acids, there are 15-40 phenolic substances in olive fruits, leaves, branches and roots; and oleuropein is found to be the most prominent substance among the phenolic compounds. The amount of rainfall, geographical region, harvest time and altitudes affect the phenolic contents in olive cultivars and there is a close relationship between phenolic compounds and olive maturation. It is crucial that when prompted to maximum extract of oleuropein, color changes of olive fruits should be monitored in terms of phenolic contents and oleuropein. Oleuropein has many pharmacological effects such as antioxidant, antimicrobial, anti-inflamatory, antitumor and neuroprotective effects on neural disorders emerging with aging (Table 2).

Table 2: Biological activities of oleuropein that found in olive fruits,leaf and virgin olive oils.

Activity	**Effects**
Antioxidant	Inhibition of oxidation of LDL,improvement of radical stability
Anti-tumor	Antiproliferative,anti-migration,angiogenesis and apoptosis effect
Antimicrobial	Bacterial cell membrane damage
Neuroprotective	Tau fibrilization inhibition,oxidative stress reduction
Anti-inflammatory	Lipoxygenase and cytokine inhibition

It has been observed that oleuropein extracted from olive cultivars can be used in the prevention of proliferation of different cancer cells such as breast, skin, lung, colon cancers and also neuronal disorders occurring due to aging. It is possible to obtain oleuropein naturally and due to its pharmacological effects it is likely to be used as an antioxidant and antimicrobial agent in the food preparation or preservation and health sectors. Among the available extraction methods of oleuropein from olive cultivars, especially from leaves, polar substances are being used according to color, antioxidant activity and stability of oleuropein at room conditions. Some pre-treatments of the extraction procedure can be applied to increase extraction yield from olive leaves such as use of supercritical fluid, steam blanching and grinding dried olive leaves to the size of 0.9-3 mm.

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