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Review Article

Effects of Hydroxytyrosol on Human Health

Alev Yüksel Aydar[1]*, Tülay Öncü Öner[2,3] and Elif Fatma Üçok[1]

[1]*Department of Food Engineering, Faculty of Engineering, Manisa Celal Bayar University, Muradiye, Manisa, Turkey*
[2]*Department of Bioengineering, Faculty of Engineering, Manisa Celal Bayar University, Muradiye, Manisa, Turkey*
[3]*Department of Medical Biology and Genetics, School of Medicine, Dokuz Eylul University, Inciralti, Izmir, Turkey*

***Corresponding Author**: Alev Yüksel Aydar, Research Assistant, Manisa Celal Bayar University, Manisa, Turkey.

Received: September 07, 2017; **Published:** October 10, 2017

Abstract

This review describes the biological activities of hydroxytyrosol which is the main phenolic compound of olive and olive oil and its effects on human health. In olive oil production process most of the polyphenols remain in waste water. Different industrial processes are developed to recover phenolic compounds from olive and olive oil byproducts including hydroxytyrosol, tyrosol and oleuropein with bioactive and antioxidant properties. Recent studies have shown that hydroxytyrosol has a role in osteoporosis prevention, anticancer, antioxidant and anti-inflammatory effects. According to *in vitro*- and animal-based studies, hydroxytyrosol has induced the cell growth arrest and apoptosis, inhibited tumor growth, stimulated the deposition of calcium and decreased H_2O_2 levels, inhibited the formation of multinucleated osteoclasts, suppressed the bone loss of trabecular bone, showed scavenging activity of superoxide formation, prevented the loss in polyunsaturated fatty acids and could decrease the risk of coronary heart disease. In this review, it was compiled these properties of hydroxytyrosol in detail. Thus, a better understanding of the effects of hydroxytyrosol on human health was provided. In conclusion, further studies are required to understand the mechanisms of hydroxytyrosol provided these effects.

Keywords: *Hydroxytyrosol; Anticancerogenic; Osteoporosis Prevention; Antioxidant; Anti-Inflammatory; Antimicrobial*

Introduction

The phenolic compounds which play an important role in the chemical, organoleptic and nutritional properties of foods are widely found in olive and olive oil [1]. The major phenolic compounds in olive oil are oleuropein, hydroxytyrosol (2-(3,4-dihydroxyphenyl)ethanol) and tyrosol (2-(4-hydroxyphenyl)ethanol) [2,3]. The chemical structures of these phenolic compounds which are strong antioxidants and radical scavengers and can protect the organism against the oxidative damage caused by oxidant agents (active oxygen, free radicals, etc.) are shown in figure 1 [4]. They also prevent the food deterioration by prevention of lipid oxidation. Thus, storage time of food products can be improved by using these natural compounds.

Oleuropein which is the major secoiridoid component of immature olive fruit reduces with maturation, while 3,4-DHPEA-EDA (dialdehydic form of elenolic acid linked to 3,4-dihydroxyphenylethanol), demethyloleuropein and the oleuropein aglycon risen with maturation. The predominant phenolic component in mature olives is hydroxytyrosol glucoside. The hydroxytyrosol, the major phenol found in olive tree, is a superior antioxidant and radical scavenger to oleuropein and tyrosol [6]. Table 1 shows the percentage of phenolics found in ripe olive pulp. The concentration of phenolic compounds in olive depends on the variety, maturity, harvesting method, season and climatology [7]

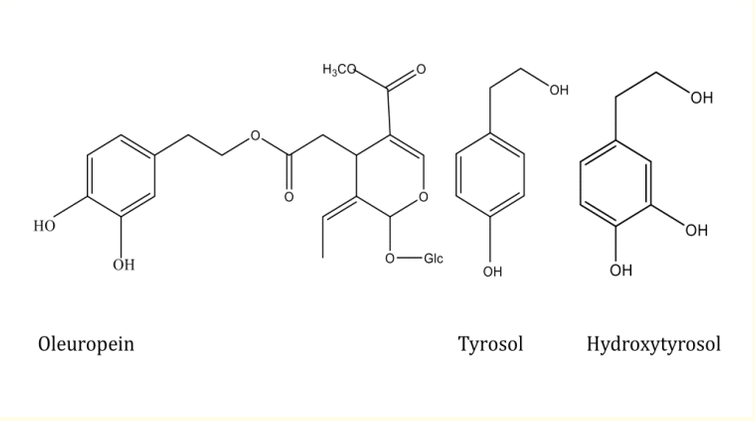

Figure 1: *Chemical structures of oleuropein, tyrosol and hydroxytyrosol [5].*

Phenolic Compound	Amount (%)
Hydroxytyrosol	50 - 70
Tyrosol	0.3
Oleuropein	5 - 10
Other polyphenols	Σ20
Total Phenols	100

Table 1: *Phenols in ripe olive [1].*

Recovering of hydroxytyrosol from olive by-products and olive oil waste water

During the mechanical processing of olive oil most of the phenolic compounds are found in the aqueous phase, but only a small percentage (1%) present in olive oil [8]. Recovering and isolation of water-soluble phenolic compounds from olive fruit has been determined in several studies [9-12]. The phenolic and volatile content and profiles of extra virgin olive oils depend on the initial composition of the olive fruits used for processing and on the technological variables used during the extraction process [2,13]. For this reason, the use of mechanical crushers results in a higher phenol extraction from fruit stones than traditional stone mills, resulting in oils with higher antioxidant capacity [14-17].

Olive oil and olive production wastes including olive leaf and olive mill waste water are good sources of hydroxytyrosol [18,19]. The isolation of hydroxytyrosol with a high degree of purity facilitates the examination of it stability, bioactivity, bioavailability and interaction with other food components, also helps to show its biological activity [1].

Several studies have been conducted to obtain high amount of highly purified hydroxytyrosol from olive by products [8,18-20]. Until now, several procedures for polyphenols recovery from olive products have been conducted, including a recent solvent free microwave extraction [21], ultrasound assisted extraction [18,22-24], solvent extraction [19,20], supercritical fluid extraction [25], microfiltration membrane extraction [26], and resin adsorption/desorption [27].

Effects of hydroxytyrosol on human health

The anticancerogenic effect

Cancer is a disease in which tissue growth regulation failure observed when the genes that regulate cell growth and differentiation are altered [28]. In cancers, abnormalities were found in the levels of cyclins or cyclin-dependent kinase inhibitors (CDIs), which are regulatory proteins of the cell cycle. Cyclin-dependent protein kinases (CDKs) control specific steps of the cell cycle progression such as M-G1, G1-S and G2-M transitions. CDKs activation require the binding to different cyclins called cyclins A, B, D and E which are timely expressed

during the cell cycle process. The CDK inhibitors (CKDi) also regulate CDK activity by binding and inactivating CDK-cyclin complexes. As it is known that inducing of the process of programmed cell death called apoptosis is a desirable condition in the treatment of cancer [29].

A small percentage of cancers (5 - 10%) are due to inherited genetic mutations, on the other hand a large percentage of cancers (90 - 95%) are due to non-hereditary epigenetic mutations which are caused by hormones, environmental or physical factors [28]. One of the main environmental cancer-causing factors is dietary habits. Lower cancer incidence has been observed in the populations living in the Mediterranean area compared to other regions. It has been suggested that this condition is based on the Mediterranean diet. Olive oil is a common component of this diet additionally wheat and grape are eaten up [30]. It was also reported that changing food intake may avoid over 30% of all cancers. For this reason, identification and characterization of food and food components are highly important to prevent several types of cancers [30].

Recently, many *in vivo* and *in vitro* studies have demonstrated the preservative and positive effect of hydroxytyrosol against cancer. Fabiani., *et al.* used human promyelocytic leukemia HL60 cell line in experiments and observed that hydroxytyrosol inhibited cell proliferation, arrested the cells in the G0/G1 phase, a concomitant diminish in the cell percentage in the S and G2/M phases. According to these results, authors suggested that hydroxytyrosol performed a protective effect against cancer [29,31].

Guichard., *et al*. investigated that hydroxytyrosol induced cell growth arrest and apoptosis in human colon carcinoma HT-29 cells. The hydroxytyrosol altered mitochondrial membrane permeabilization, stimulated caspase 3, induced ER (endoplasmic reticulum) stress, disturbed ER Ca2+ homeostasis, activated c-Jun NH2-terminal kinase and AP-1 (activator protein-1) transcription factor, inhibited TNF-α (tumor necrosis factor-α)-induced NF-κB activity and activated serine/threonine phosphatase 2A (PP2A) activity. They suggested that hydroxytyrosol has potent chemopreventive role because it targeted specific transcription factors, tumor suppressors and tumor-promoter-induced protein kinases as well as triggering apoptosis [32]. Terzuoli., *et al*. found hydroxytyrosol inhibited tumor growth in the same cell line HT-29. According to the *in vivo* experiments, it was showed that hydroxytyrosol inhibited the expression of HIF-1α (hypoxia inducible factor-1α), VEGF (vascular endothelial growth factor) and mPGEs-1 (microsomal prostaglandin-E synthase-1). Results of the *in vitro* experiments indicated that hydroxytyrosol inhibited ERK1/2 (extracellular signal-regulated kinase 1/2) phosphorylation and thus suppressed PGE-2/ERK1/2/HIF-1α signaling pathway [33]. Again Terzuoli., *et al*. showed that hydroxytyrosol downregulated EGFR (Epidermal growth factor receptor) expression in human colorectal adenocarcinoma cells HT-29, WiDr and CaCo2, and in HT-29 xenografts. It has been suggested that hydroxytyrosol-induced activation of the ubiquitin-proteasome/lysosomal axis decreased the oncogenic EGF (epidermal growth factor)/EGFR drive and attenuated colon tumour development [34].

Corona., *et al*. showed that hydroxytyrosol induced inhibition of the proliferation in human colon carcinoma cells $Caco_2$ because of the induction of a G2/M phase cell cycle block. Hydroxytyrosol inhibited activity of p38 and CREB (cyclic adenosine mono phosphate response element binding protein) and thus expression of COX-2 (cyclooxygenase-2) decreased. It has been reported COX-2 is overexpressed in colorectal cancer cells and this situation is associated with colorectal neoplasia, by promoting cell growth, survival, angiogenesis, invasion and migration [35]. Corona., *et al*. used again $Caco_2$ cells and indicated that hydroxytyrosol showed antiproliferative effects because of inhibition of ERK1/2 phosphorylation additionally reduction the level of cyclin D1 expression. Activation of ERK is required for promoting cell proliferation and also cyclin D1 expression which regulates cell cycle [36].

Fabiani., *et al*. researched the effects of hydroxytyrosol on proliferation, apoptosis, cell cycle progression and also differentiation of human promyelocytic leukemia cells HL60. They suggested that hydroxytyrosol affected the expression of genes which regulated tumor cell proliferation and differentiation. According to the obtained data, hydroxytyrosol diminished the level of cyclin-dependent kinase 6 (CDK6) and also increased the level of cyclin D3, CDK inhibitors $p21^{WAF1/Cip1}$ and p^{27Kip1}. As a result, hydroxytyrosol inhibited the proliferation of HL60 cells and induced an accumulation of cells in the G0/G1 phase [29].

Han., *et al.* reported that hydroxytyrosol reduced cell viability, induced apoptosis and inhbited proliferation in human breast cancer cells MCF-7. Hydroxytyrosol blocked cell cycle at G1 to S phase transition [37]. Bouallagui., *et al.* also researched potential anti-tumoral activities of hydroxytyrosol on human breast adenocarcinoma cells MCF-7. It has been shown that hydroxytyrosol exhibited cell cycle blocking in the G1 phase because of a down-expression of the peptidyl-prolyl cis–trans isomerase Pin1 which decreased the level of cyclin D1 [38].

Granados-Principal., *et al*. revealed that hydroxytyrosol inhibited cell growth and proliferation in mammary tumours in female Sprague-Dawley rats. Hydroxytyrosol altered the expression of genes associated with apoptosis, proliferation, survival, Wnt signaling pathway, which is an ancient and evolutionarily conserved pathway that regulates critical aspects of cell fate decision [39], and also promoting a high expression of Srfp4 that is an extracellular Wnt antagonist [40].

Notarnicola., *et al*. showed in human colon cancer cells SW620 that hydroxytyrosol inhibited proliferation via inhibition of FAS (fatty acid synthase) expression and its enzymatic activity. It has been signified that high expression of FAS is observed in colorectal cancer [41].

Li., *et al*. investigated the effect of hydroxytyrosol on cholangiocarcinoma (CCA) which is a malignant tumor with a high rate of mortality. It has been showed that hydroxytyrosol inhibited the proliferation of the KMBC, GBS-SD and TFK-1 cell lines and also inhibited CCA xenograft growth in mice. Additionally, they observed apoptosis increased via the mitochondrial cell death pathway and G2/M phase cell cycle arrest, and also a time-dependent and dose-dependent inhibition of phospho-ERK. The levels of cleaved caspase-3, caspase-9, PARP and Bax increased, while the levels of pro-PARP, cyclin B1, Bcl-2 and p-Cdc2 were decreased by hydroxytyrosol treatment [42].

Martínez-Martos., *et al*. demonstrated that hydroxytyrosol inhibited the tumor growth in Wistar rats subjected to C6 glioma cell implantation. Moreover hydroxytyrosol decreased protein oxidation and lipid peroxidation levels [43].

Oktay LM studied to investigate of the effects of hydroxytyrosol on cytotoxicity, apoptosis, PI3K/Akt and ERK1/2 pathways in ovarian cancer cell culture. It was observed that hydroxytyrosol increased the levels both of pro-apoptotic molecules as Bad, Bax, active caspase-3, Htra2/Omi, SMAC/DIABLO and pro-apoptotic death receptor as FAS/TNFRSF6, TRAIL R1/DR4, TRAIL R2/DR5, on the other hand it reduced the level of an inhibitor of apoptosis protein called survivin in MDAH-2774 cells. Moreover, the results using OVAR-3 cells indicated that hydroxytyrosol reduced the levels anti-apoptotic proteins which Bcl-2, pro-caspase-3 and some inhibitor of apoptosis protein as CIAP-1, CIAP-2, XIAP, survivin, livin, and increased the level of cytochrome c. Results of the experiments demonstrated that hydroxytyrosol had apoptotic and cytotoxic effects on ovarian cell lines. Its cytotoxic effect may be through ERK1/2 signalling pathway not PI3K/Akt signaling pathway [44].

Studies about cancerogenic effect of hydroxytyrosol by *in vitro* experiments with different tumor cell lines and *in vivo* experiments on animals demonstrated that hydroxytyrosol induced apoptosis and inhibited proliferation. Further extensive studies must be done to learn possible roles and molecular mechanisms of hydroxytyrosol, and which pathways affected by hydroxytrosol.

Osteoporosis prevention

Coordinated efforts of osteoblasts, osteoclasts and osteocytes are required for bone remodeling. Osteoblasts lay down new bone and synthesize new bone matrix. Osteoclasts which are found in the surface of aged bone absorb bone matrix and break down old bone. Osteocytes descend from osteoblasts and play important roles in paracrine signaling. Imbalance between osteoblasts and osteoclasts causes bone diseases such as osteoporosis. People suffering osteoporosis have bones that fracture easily because of reducing bone density [45].

Bone modeling and remodeling process depend on hormonal, nutritional and mechanical factors as well as heritable traits. Nutrition affects age-related bone loss, bone mass peak and muscle strength. Like cancer incidence, lower osteoporosis incidence was observed in countries where the Mediterranean diet is predominant. Thus, it is possible to say that Mediterranean diet has protective effect about bone health [46].

There are some animal and cell culture studies to investigate the effects of hydroxytyrosol on preventing bone loss. Hagiwara., *et al*. surveyed *in vitro* and *in vivo* effects of major polyphenols in olives on the formation and maintenance of bone using cultured cells (MC3T3-E1 cells) and ovariectomized (OVX) mice. The experiments with MC3T3-E1 osteoblastic cells showed that hydroxytyrosol stimulated the deposition of calcium and decreased H2O2 levels. It has been reported that increased levels of intracellular ROS (reactive oxygen species) due to oxidative stress suppressed bone metabolism. The effect of hydroxytyrosol was dose-dependent manner. They also reported that hydroxytyrosol, oleuropein and tyrosol inhibited the formation of multinucleated osteoclasts in culture. On the other hand both hydroxytyrosol and oleuropein suppressed the bone loss of trabecular bone in femurs in OVX mice but they had no effects on cortical bone [47]. Puel., *et al*. used ovariectomized rats (OVX; an experimental model of postmenopausal osteoporosis) and ovariectomized rats with granulomatosis inflammation (OVX_{inf}; a model created for senile osteoporosis) to determine effects of tyrosol, hydroxytyrosol, olive mill wastewater and olive mill wastewater extracts. They reported these compounds except olive mill wastewater prevented the decline in total femoral, diaphyseal and metaphyseal bone mineral density, and they also found that hydroxytyrosol and olive mill wastewater extracts reduced the level of isoprostane, which is a marker for lipid peroxidation, in OVX_{inf} [48]. Gracia-Martinez., *et al*. investigated potential effects of twelve olive oil phenolic compounds on osteoblast proliferation using MG-63 osteosarcoma cell line. They explained that hydroxytyrosol, apigenin, luteolin, p-coumaric, caffeic acid and ferulic acid increased cell proliferation. On the other hand oleuropein, (+)-pinoresinol, sinapic, vanillic acid and derivative (vanillin) didn't affect cell proliferation. Significant differences were detected in the ability of individual phenolic compounds to stimulate osteoblast proliferation. They also observed that all phenolic extracts induced MG-63 cell proliferation better than individual chemicals. Thus, there could be a synergistic effect among the compunds in olive oil [49].

According to the results of these studies, particularly hydroxytyrosol may have critical effects on the formation and maintenance of bone and prevented bone loss. It can be used as potent remedies in the prevention and treatment of osteoporosis. But still further studies must be done to find out mechanisms of hydroxytyrosol in bone.

The antioxidant and anti-inflammatory effects

Among the phenolic components in olive leaves, hydroxytyrosol has strongest antioxidant effects [47]. The effect of hydroxytyrosol on the antioxidant property has been tried to be explained by various molecular and biological studies. Visioli., *et al*. researched scavenging actions of hydroxytyrosol with respect to superoxide anion generation, neutrophils respiratory burst, and hypochlorous acid. The data obtained with the study proved that hydroxytyrosol has potent scavenging activity of superoxide formation [50]. It was also reported that hydroxytyrosol prevented the loss in polyunsaturated fatty acids which located in outer layer of LDL (low-density lipoproteins) and completely inhibited CuSO4-induced oxidation. Hydroxytyrosol chelated free metal and scavenged newly formed free radicals. For this reason, it represents antioxidant activity [51]. Salami., *et al*. observed the formation of F2-isoprostanes and production of other markers of lipid peroxidation during in vitro LDL oxidation, and also demonstrated inhibitory effects of hydroxytyrosol [52]. In addition to inhibition of LDL oxidation, hydroxytyrosol inhibited also *in vitro* platelet aggregation and the production of arachidonic acid metabolites [53]. Platelet aggregation is one of the main events in arterial thrombosis, so aggregation prevention is significant in cardiovascular research. Therefore, inflammation plays important role in the development of cardiovascular diseases and also atherosclerosis [54]. Gong., *et al.* analyzed the anti-inflammatory and antinociceptive effects of hyroxytyrosol-20, which is an olive oil extract including 20% of hydroxytyrosol, in a rat model of acute inflammation and pain induced by carrageenan. It was found that hyroxytyrosol-20 inhibited inflammatory swelling and hyperalgesia as well as suppression proinflammatory cytokine [55]. Oxidative modification of LDL is an important indicator for formation of atherosclerotic plaques. So, oxidative stress increases the risk for atherosclerotic damage. Additionally together with dietary antioxidant and the role as free-radical scavengers of hydroxytyrosol could decrease the risk of coronary heart disease by lowering the oxidative status of LDL [52]. Furthermore Zrelli., *et al*. verified the antioxidant effect of hydroxytyrosol on H_2O_2-induced intracellular ROS in porcine pulmonary artery endothelial cells (VECs). It was showed that hydroxytyrosol increased catalase mRNA, protein and activity of nuclear and cytosolic protein levels of FOXO3a (forkhead transcription factor 3a) and also phosphorylation of AMPK (AMP-activated protein kinase). FOXO3a protects quiescent cells from oxidative stress by increasing the expression of antioxidant enzymes while AMPK

appears to activate the transcriptional activity of FOXO3. Findings of the experiments demonstrated that hydroxytyrosol regulated the antioxidant defense system in VECs and could help prevent cardiovascular diseases [56]. The activities of hydroxytyrosol related to cardiovascular protection are that free radical-scavenging property, stimulation of antioxidant transcription and detoxification of defense systems, strong metal chelation, changes in gene expression related to either atherosclerosis development and progression or cardiovascular protection, diminish in homocysteine-induced endothelial dysfunction or cell adhesion improvement of the lipid profile and decline in inflammatory markers such as interleukin-6, thromboxane and leukotriene [57]. A direct correlation between Mediterranean diet and a lower incidence of cardiovascular diseases were detected in various studies [58]. Thus, this diet including high consumption of olive is beneficial for protection from cardiovascular disease.

The antimicrobial effect of hydroxytyrosol

The olive leaf phenolic compounds' *in vitro* antimicrobial activity of has been broadly studied [59-63]. The anti-bacterial effect of olive products is associated with the presence of the different forms of decarboxymethyl elenoic acid such as free, dialdehydic, linked to tyrosol, and linked to hydroxytyrosol. The bactericidal activity of these substances arises from their dialdehydic structure, that is like those of the commercial antiseptics glutaraldehyde and o-phthalaldehyde [59]. The antimicrobial studies have been achieved both for human health, and agricultural pest control [64].

Hydroxytyrosol is a radical scavenger to oleuropein and tyrosol. Oleuropein and hydroxytyrosol have antimicrobial activity on some of the ATCC and clinical bacterial strains [63]. The foremost study concerning the antimicrobial activity of hydroxytyrosol showed that low concentrations of hydroxytyrosol (≤ 8 μg/mL) were potent to inhibit the growth of bacterial reference strains [65]. Bisignano., *et al.* studied the *in vitro* susceptibility of hydroxytyrosol and oleuropein against many bacterial strains that are casual agents of respiratory or intestinal tract infections in humans. It was given that the o-diphenol system in the biophenols is responsible for the olive phenols' antibacterial activity. Also, the diminishment in toxicity of oleuropein was based on its glycosidic group [65].

Hydroxytyrosol, the basic polyphenol isolated from olive brine solutions, shows bactericidal activity against lactic acid bacteria (LAB). Olive oil mill wastewaters and olive leaf extracts has been proved to have antimicrobial activity. The bioactivity of olive oil mill wastewaters has also been associated with the phenolic compounds (oleuropein and hydroxytyrosol). Oleuropein and hydroxytyrosol exerted antimicrobial effects on pathogenic bacteria and viruses [59].

There are several researches about particular phenolic compounds in olive extracts and their antimicrobial activity. These researches suggested that the hydroxytyrosol did not prove strong antimicrobial activity. The olive extracts appear to have more antibacterial activity on Gram positive bacteria compared to the Gram negative bacteria. Moreover, there has been no differences reported for the antibacterial effect of hydroxytyrosol [66].

Furneri., *et al*. showed that mycoplasmas inhibited with hydroxytyrosol at concentrations of 0.03 to 0.5 μg/mL. The MICs (minimum inhibitory concentrations) for *M. hominis, M. pneumoniae* and *M. fermentans*, were 0.03, 0.5, 0.25 μg/ml, respectively [67].

Hydroxytyrosol's antimicrobial activity and its implicit application as a natural preservative have been proved by many studies. The lowest MIC level of hydroxytyrosol was reported as 0.24 μg/mL. The study conducted by Medina-Martínez., *et al.* indicated that addition of 400 μg/mL hydroxytyrosol to the various media considerably changed the growth curve parameters of the *E. coli* strains compared to the control group. Only the highest concentration of hydroxytyrosol (1000 μg/mL) could inhibit growth of *Erwinia carotovora* CECT225, *Klebsiella pneumoniae* CECT143, *Shigella sonnei* CECT457, *Pediococcus acidilactici* CECT98, *Kocuria rhizophila* CECT4070, *Staphylococcus*

aureus CECT794 under many of the analysis conditions. The survival of bacteria was studied for particular combinations of bacterial strains and media, such as *E. coli* CECT533, CECT4972, and CECT679 in LB (Luria Bertani) broth with 1000 µg/mL of hydroxytyrosol and *E. coli* CECT4972 in ISO (Iso-Sensitest) broth with 1000 µg/mL of hydroxytyrosol. Medina-Martínez., *et al*. also stated that they have considered the antimicrobial capacity of hydroxytyrosol as low. Medina-Martínez., *et al*. stated that the antimicrobial activity of hydroxytyrosol depended on selected strain and the media. The stability of hydroxytyrosol through the antimicrobial screening could also be a constituent which supports an antimicrobial function.

Medina., *et al.* proved that hydroxytyrosol has a bactericidal effect against a broad spectrum of bacteria. The bactericidal effect of hydroxytyrosol was stronger against to Gram positive bacteria than Gram negative bacteria. Also, hydroxytyrosol showed bactericidal activity against both harmful (*E. coli* and *Clostridium perfringens*), and beneficial bacteria (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) in the intestinal microbiota [66].

The naturally occurring compounds' antimicrobial activity has been reviewed in the literature, and there are a growing number of studies in the use of these compounds as novel antimicrobial agents in humans. Surely, safety and bioavailability are the main factors for antimicrobial agents to be used for treatment in humans [67].

Conclusions

Hydroxytyrosol is the main phenolic compound found in olive, olive leaf and olive oil and is directly consumed in the human body, thus it has been studied more than the other components of the olive. The studies have shown that hydroxytyrosol, in a dose-dependent manner, has a role in anticancer, antioxidant, anti-inflammatory and antimicrobial effects and osteoporosis prevention. Mediterranean diet is associated with lower incidence of certain cancers, osteoporosis and coronary heart disease. Therefore, it is predicted that consumption of olive oil, which is an important part of this diet, may be effective in preventing these diseases. But still further studies are required to understand the mechanisms of hydroxytyrosol provided these effects. In future, using hydroxytyrosol as a natural drug will provide prevention and treatment of particular diseases.

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