Review Article

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Olive leaf: From tradition to clinic

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Abstract

Olive (*Olea europaea* from the Oleaceae family) leaves have had a lot of medicinal applications in traditional and modern medicine. In traditional persian medicine (TPM), it is used as an antiperspirant, has special indications for wound healing and its ash is also used in ophthalmic traditional remedies. Literature survey was performed via electronic search in Pubmed, Scopus and Siencedirect. We found 275 articles related to the topic. Conventional pharmacy texts including Martindale, British pharmacopoeia and Lexi natural products as well as TPM texts were reviewed. Our results showed that olive leaf has antioxidant, antitoxic and anticancer properties. It also has antimicrobial and antiviral effects as well as effects on the nervous, endocrine, gastrointestinal and cardiovascular system. It has therapeutic effects on some metabolic disorders and cutaneous complications and some miscellaneous effects such as anti-arthritis and anti-bone loss activity. Despite the large amount of studies having been done on the possible effects of olive leaf, the number of available products and their therapeutic targets are limited. More extensive studies as clinical trials are expected to be done on olive leaf and considering its dose adjustments and special cautions, it can become a more popular herbal medicine in markets and clinics.

Keywords: Medicinal plant, *Olea europaea*, Olive leaf.

1. Introduction

The olive tree (*Olea europaea* L.) from the Oleaceae family has been a symbol of peace since ancient times and the leaves of this tree have had a lot of medicinal usages in traditional and conventional medicine (1). In current studies olive leaf extract (OLE) is well-known for its antioxidant, antihypertensive, antimicrobial, hypoglycemic, anticancer and antiatherosclerotic properties (2). The main point that is neccessary to notice, is the importance of several therapeutic properties of olive leaves that are demonstrated in *in vivo* and *in vitro* studies and its potential to play role in clinical practices. According to the fact that there has been limited study on olive leaf in recent years and because of its traditional importance and also its availability and cheapness among medicinal plants, this study was done to examine the knowledge of traditional and conventional adresses for olive leaf from manuscripts to clinic.

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2. Materials and methods

Literature survey was performed via electronic search in Pubmed, Scopus and Siencedirect, with keywords 'Olea europaea leaves' and 'Olive leaves' until October 2016. The papers written in English were reviewed in this article. We found 275 articles related to the topic, but some of them had similar final results, therefore we chose about 90 main articles that were more complete and more recently studied. Also the pharmacy books including Martindale 2014, British pharmacopoeia 2015 and lexi natural products 2002 were reviewed. Tra-

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ditional persian medicine (TPM) texts including: *Alhavi of Razi* (865-925 AD), *Canon of Avicenna* (980-1031 AD), *Al-shamel fi senaat tebieh of Ibn Nafis Gharshi* (death 1288 AD)and *Makhzan-al-advieh* of Aghili Alavi Shiazi (death 1771 AD) were reviewed regarding olive leaf monographs by the traditional key words: barg-e-zeitoon and varagh-al-zeitoon.

3. Results

3.1. Active constituents

The main constituents of olive leaf mentioned in the articles can generally be categorized as the followings:

• Poly-phenols (verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside) are an important group of components (1); A research was done on Portuguese olive (*O. europaea*) leaf cultivars and showed that their major phenolic compound is luteolin 4'-O-glucoside (3). Flavonoids (rutin and diosmin) (1) and also lignans (pinoresinol, syringapinoresinol) exist in the olive leaf extract (4).

• Hydroxytyrosol is a minor phenolic constituent in olive leaf (1), also 1,5-anhydroxylitol was obtained from the leaves in 2005 (5).

• Secoiridoids (the main constituent: oleuropein), the content of oleuropein in the leaf is significantly higher than in other parts of the tree (1). Oleuropein is the ester of elenolic acid and 3,4-dihydroxyphenyl ethanol, that has beneficial effects on human health (6). Olive leaf also has unconjugated secoiridoid-type aldehydes (oleacin) (7).

Other compounds in this group are demethyloleoropein and ligstroside (4).

• Triterpenes such as oleanolic acids are its other chemical constituents (1).

• Fatty acids: Oleic acid, linoleic acid, linolenic acid (8).

• Mineral elements: Al, Ca, Fe, K, Mg, P,S (8), Zn (9).

It is reported that there are variations between leaf compounds in different seasons (10). Freezing or dehydration of the leaves can influence extract compositions (11). The other conditions that can cause variation in the chemical composition of the leaves are storage conditions, climatic conditions, origin, proportion of branches on the tree, moisture content, and degree of contamination with soil and oils (12).

The olive leaf identified in pharmacopoeias such as the British pharmacopoeia with the minimum content of oleuropein written to be 5.0%, has special properties from organoleptic characteristics of the whole leaf or its powder, and it can be identified by TLC. The loss on drying test and total ash and also the assay test are described instead of control tests in the British pharmacopoeia (13).

3.2. Olive leaf in traditional medicine

Some indications of olive leaf in traditional medicine are the prevention and treatment of hypertension, diabetes and atherosclerosis (12). In TPM it has special indications for wound healing and some cutaneous applications, as antiperspirant, and also its ash is used in ophthalmic remedies instead of *Toutia* as *Kohl* (14) that is a product rich in zinc (15), and this is an important issue because it is proved that zinc plays an integral role in ophthalmic function (16) but it needs further researches to prove whether its benefits are related to the zinc content of the leaves or its other components.

3.3. Pharmacological experiments and clinical trials

An overview of *in vitro* and *in vivo* experiments, and clinical trials carried out for OLE is presented below.

3.3.1. Antioxidant and antitoxic effect

The phenolic content of olive leaf decoction has antioxidant activity and is a potential anticancer agent (17). A study showed that the antioxidant activity of phenolic hydroxyl compounds such as oleuropein, verbascoside, luteolin-7-O-glucoside, apigenin-7-O-glucoside, hydroxytyrosol and tyrosol, Lutein, sesamol and ellagic acid in OLE could be due to the presence of the hydroxyl groups in their structure. (18, 19). OLE has potent antioxidants and prevents $CuSO_4^-$ induced LDL oxidation - *in vitro*, so it may be suitable for use in food and pharmaceutical applications (20). In another research, the antioxidant effect of oleuropein on midbrain and dopaminergic neurons of substantia nigra was studied in aged rats and showed that treatment of the old rats with oleuropein can

reduce the oxidative damage in SNc by increasing the antioxidant enzyme activities (21). OLE is useful in decreasing age-induced oxidative stress in major organs of aged rats without affecting the anti-oxidant system (22).

A study was done among radical scavenging activity of flavonoids in olive leaf polar extracts and supports that the olive leaf, except for oleuropein and its related compounds, is also a stable source for bioactive flavonoids (23). Individual and combined phenolics of OLE have antioxidant and antimicrobial activities (24). Olive leaf supplements are considered as agents effective in promoting health and supporting the body in preventing free radical damage (25). The polyphenolic compounds of *O. europaea* leaves may protect insulin-secreting cells against oxidative stress in H_2O_2 toxicity of insulin secreting β-cells (26). There are other active compounds besides the phenolics in OLE that have antioxidant and aldose reductase inhibition activity (27). A study indicates that OLE inhibits lead poisoning-induced brain injury. Their findings indicate that olive leaf extract can inhibit lead-induced brain injury by increasing antioxidant capacity and reducing apoptosis (28). OLE ameliorates gentamicin nephrotoxicity in rats via its antioxidant activity, increase in renal glutathione content, and increase in renal antioxidant enzymes activity, except for glutathione peroxidase (29). OLE has protective effects against lead-induced neurotoxicity in Wistar rats (30). Oleuropein successfully treated acute doxorubicin cardiotoxicity through suppression of oxidative and nitrosative stress in 50 rats (31). It is reported that olive leaf phenolics can prevent postmenopausal women from age-related and oxidative stress-related processes such as osteoporosis (32).

OLE modulates permethrin induced genetic and oxidative damage and could be able to antagonize permethrin toxicity in rats (33). OLE polyphenolics can protect cytokine-induced β-cell damage by the maintenance of redox homeostasis (34). Dry OLE, has shown to have protective effect on adrenaline induced DNA damage in human peripheral leukocytes in an *in vitro* study (35).

Oleuropein and oleacein may impair the biological functions of endothelial progenitor cells via activation of Nrf2/hemeoxygenase-1 pathway (36). Oleuropein has shown anti-aging properties in Human Cells (37).

3.3.2. Cytotoxicity and anticancer properties

The OLE induces apoptosis and monocyte/macrophage differentiation in human chronic myelogenous leukemia K562 cells (38). Phenolic components, which have a role in cancer prevention are rich in olive leaf (17), For example hydroxytyrosol rich extract from olive leaves has *in vitro* anti-tumoral activities and modulates cell cycle progression in MCF-7 human breast cancer cells. Therefore the OLE will be investigated for its probable use as an anticancer food additive (39). Dry OLE has genoprotective and antioxidant properties in adrenaline induced DNA damage according to an *in vitro* comet assay with human peripheral leukocytes (40). A study suggests that OLE may interfere with the pluripotency of GSCs (The stem-like cells of glioblastoma multiforme (GBM) tumors). Further studies are required, but it may have a potential for advanced therapeutic cancer drug studies in GBM by modulating miRNA expression (41).

A pilot study indicates that the mechanism for the cytotoxic effect of OLE, oleuropein and luteolin is the apoptotic pathway, with DNA laddering and cytoplasmic and nuclear changes and suggests OLE, as a nutraceutical compound in the prevention of human cancer (42). Olive leaves can stabilize lysosomal membrane in rat hepatocytes and can be used in case of chemically induced hepatocellular neoplasia (43).

A randomized clinical trial reported that OLE is effective in decreasing the expression of IL-1b and TNF-a levels after chemotherapy and exerts therapeutic effects and prevents the development of severe oral mucositis (44). Oleuropein has anti-breast cancer properties with higher efficiency on ER-negative cells; however, this needs further researches (45). Multiple anti melanoma potential of dry OLE is also studied. The results of this study indicates that dry OLE has strong potential against melanoma. However, in combination with different chemotherapeutics, various outcomes, including synergy and antagonism, were observed. Therefore it is important to be careful

about the use of OLE as a supplementary antitumor therapy (46).

3.3.3. Antimicrobial and antiviral properties

Antimicrobial activities of OLE have been studied and the result shows that it is not broad-spectrum in action, showing good activity only against *H. pylori*, *C. jejuni, S. aureus* and *Methicillin resistant Staphylococcus aureus* (MRSA). Therefore it is concluded that, OLE may have a role in regulating the composition of the gastric flora by selectively reducing levels of *H. pylori* and *C. jejuni* (24). A research has been done to identify the anti-acanthamoeba compounds from Tunisian OLE and concluded that oleanolic, maslinic acids and oleuropein could be used for the development of novel therapeutic agents against acanthamoeba infections (47). OLE could promote hypothiocyanite production by lactoperoxidase that is a key player in the humoral immune response and these results propose a new mode of action about the well-known bacteriostatic and anti-inflammatory properties of OLE (48). Researchers have reported the bindings of OLE to HIV-1 fusion protein gp41 and its anti-HIV activity by blocking the HIV virus entry to host cells (49). Oleuropein has antimycoplasmal activity in an *in vitro* study (50).

Another study found that OLE inhibits acute infection and cell-to-cell transmission of HIV-1. OLE also inhibits HIV-1 replication. it also up-regulates the expression of the apoptosis inhibitor proteins IAP1 and 2, as well as the calcium and protein kinase C pathway signaling molecules IL-2, IL-2Ra, and ornithine decarboxylase ODC1 (51).

OLE shows antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV) (52). The methanolic OLE, has shown positive effect on the management of aflatoxin B1 production by *Aspergillus flavus* (53).

3.3.4. Colds and influenza

OLE can stimulate phagocytosis, therefore it can enhance the immune response to viral infection. Some reports indicate that OLE taken at the onset of cold or flu symptoms can prevent or shorten the duration of the disease. In viral sore throats, gurgling the olive leaf tea may alleviate symptoms by decreasing inflammation and viral infectivity (54).

3.3.4. Nervous system effects

OLE has the neuroprotective effects, because it reduces infarct volume and brain edema and improves blood-brain barrier permeability and neurologic deficit scores after transient middle cerebral artery occlusion in rats (55). OLE has neuroprotective effect on brain lipidomics of pretreatment in rat stroke model via reduction in the brain ceramide levels and researchers have hypothesized that OLE-induced ischemic tolerance in rats is partly associated with changes in brain lipid level (56). A study indicates that OLE has a potent neuroprotective activity (because of its antioxidant properties) against neuronal damage in hippocampus after transient global cerebral ischemia in *Mongolian gerbils* (30). OLE has analgesic activity, potentiates morphine analgesia and suppresses morphine hyperalgesia in rats, so, it can be used for the treatment and/or management of painful states (57). Dry OLE ameliorates experimental autoimmune encephalomyelitis in rats but further studies are required to investigate if dry OLE could be a useful supplement for the patients suffering from multiple sclerosis and other neuroinflammatory disorders (58). OLE has the neuroprotective effect via improving BBB permeability and brain edema in rat with experimental focal cerebral ischemia (3). Olive leaf is considered to be effective in the treatment of multiple sclerosis (59).

OLE and oleuropein have therapeutic potential in the treatment of Parkinson's disease by the Inhibition of 6-hydroxydopamine-induced PC12 cell apoptosis (60).

OLE with its main component, oleuropein, is responsible for preventing the development of morphine antinociceptive tolerance by inhibition of morphine-induced L-type calcium channel overexpression (61).

3.3.5. Endocrine system effects

Dried OLE ameliorates islet-directed autoimmunity and interferes with the development of autoimmune diabetes by down-regulating production of pro inflammatory and cytotoxic mediators

in mice. Therefore, it has a potential for further investigation of prophylaxis/treatment of human autoimmune diseases (62), and it has been reported to have ameliorative effect on experimental autoimmune encephalomyelitis in rats (58).

The aqueous OLE has effect on the adrenal-kidney-pituitary axis in rats that stimulates the thyroid, unrelated to the pituitary (63).

OLE suppresses mRNA expression of pro inflammatory cytokines and may attenuate insulin resistance and enhance insulin receptor substrate 1 expression in induced diabetes in rats (64). Olive leaf is effective in inhibiting the oxidative stress and immune dysregulation in the mice induced diabetic and may have therapeutic potential (65). In *in vitro* and *in vivo* studies OLE attenuates early diabetic neuropathic pain through the prevention of high glucose-induced apoptosis and suppression of diabetes-induced thermal hyperalgesia (66). Oleuropein has hypoglycemic, hypolipidemic and antiatherogenic effects in alloxan-induced type 1 diabetic rats (67). One of the compounds responsible for hypoglycemic activity is oleuropeoside that may act through two mechanisms: (i) potentiation of glucose-induced insulin release, and (ii) increase in peripheral glucose uptake (68). A randomized, placebo-controlled, cross-over trial, shows that olive leaf polyphenols can improve insulin sensitivity and pancreatic b-cell secretory capacity in middle-aged overweight men (69). OLE is a hypoglycemic agent in human diabetic subjects via improved glucose homeostasis and in rats its mechanism may be through the reduction of starch digestion and absorption (70).

3.3.6. Hepatoprotective and gastrointestinal effects

The oleuropein when given with high-fat diet has hepatoprotective effect, it can reduce the hepatic mRNA level of the genes encoding the key regulators of the hepatic fatty acid uptake and transport. Further, the oleuropein can reduce the expression of a number of hepatic genes involved in the oxidative stress responses and detoxification of lipid peroxidation products and proinflammatory cytokine genes. But the final mechanism may be multifactorial and it is uncovered by gene expression profiling. (71).

A study indicates that OLE possesses gastroprotective activity against cold restraint stress -induced gastric lesions in rats that is possibly related to its antioxidative properties (57). The hydroalcoholic OLE has protective effect on experimental model of colitis in rats and indicates the involvement of nitrergic and opioidergic systems (72).

An *in vivo* study on rats indicates that oleuropein can prevent ethanol-induced gastric ulcers through elevation of antioxidant enzyme activities (73). A study suggests that OLE has the hepatoprotective effect on chemically induced liver cirrhosis in male rats and supposed that it may be attributed to OLE antioxidant activity (74). This compound is used to support normalization of gastrointestinal flora and acts as an immune support in crohn's disease, diarrhea, and ulcerative colitis (59).

3.3.7. Cardiovascular, antihypertensive and platelet aggregation effects

The triterpenoids isolated from *Olea europaea* subsp. *africana*, was studied and showed that it prevents the development of severe hypertension and atherosclerosis and improves insulin resistance of the experimental animals in the study and this indicates the antihypertensive, antiatherosclerotic and antioxidant activities of olive leaves (75). OLE exerts L-type Ca^{2+} channel antagonistic effects and these findings might help to verifying the traditional use of olive leaves in cardiovascular disease (76).

OLE when used 500 mg twice daily by patients with stage-1 hypertension, showed similar effects as captopril 12.5-25 mg twice daily in lowering systolic and diastolic blood pressures (77). The administration of OLE after meal for one month, in a study, could improve the cardiovascular risk markers and oxidative and hepatic parameters in hypercholesterolemic subjects (78). The lyophilized decoction of olive leaf has been studied and the results indicate that oleuropeoside is responsible for vasodilator activity but, it seems that at least one other principle is to be found in the olive leaf which is either a vasodilator itself or else potentiates the relaxant effect of oleuropeoside. Also it is concluded that relaxant activity is independent of the integrity of the vascular endothelium (79). In a clinical trial studying the effect of

a titrated OLE in the treatment of essential arterial hypertension, all patients had a statistically significant decrease in blood pressure while they didn't find any side effect or modification of biological parameters except for a significant decrease in blood sugar and calcium level (80). Oleuropein has also cardioprotective effects (55).

The polyphenols in olive leaves inhibit *in vitro* platelet activation in healthy, non-smoking males. However, further bioavailability studies need to be done to validate this result (81).

3.3.8. Metabolic disorders

Effects of OLE on metabolic disorders and oxidative stress induced by 2.45 GHz WIFI signals was studied and suggested that exposure can induce a diabetes-like status via alteration of oxidative response and OLE was able to correct glucose metabolism disorder by reduction in oxidative stress induced by radio frequency in rat tissues (82).

OLE has anti-atherosclerotic effects due to suppression of inflammatory response in rabbits (83).

In an *in vitro* study kinetic measurements were performed in order to investigate the possible inhibitory effects on xanthine oxidase (XO), the enzyme well known to contribute significantly to this pathological process. The result shows that OLE inhibits the gout-related enzyme (xanthine oxidase) as well as several of its isolated phenolics (84). OLE reduces the incidence of bacterial translocation and liver damage in rats with obstructive jaundice (85).

OLE attenuates obesity in high-fat diet-fed mice by regulating the expression of genes involved in adipogenesis and thermogenesis in their visceral adipose tissue (86).

3.3.9. Cutaneous properties, skin wound healing and anti-leishmania effects

OLE and oleuropein can prevent chronic skin damage and carcinogenesis induced by ultraviolet B radiation in hairless mice (87). Moreover, OLE is effective for healing of experimental cartilaginous injuries in rabbits. It may also be effective for slowing and reducing the pathogenesis of degenerative joint diseases in humans (88). OLE and oleuroepin have preventive effects on acute ultraviolet B irradiation-induced skin damage in C57BL/6J mice; it might be via inhibiting the degradation of extracellular matrixes in the corium, and also by the proliferation of epidermal cells through the inhibition of increases in MMP-13 levels and reactive oxygen species induced by irradiation (89). A study suggests that oleuropein can accelerate skin wound healing in aged male balb/c mice, therefore can be useful in expediting wound healing after surgery (90).

The triterpenic acids (maslinic and oleanolic acids) in olive leaf, were isolated and their *in vitro* activity was studied against the promastigotes stage of *Leishmania infantum* and *Leishmania amazonensis* and both molecules could reduce the mitochondrial membrane potential and also decrease the ATP levels to 15% in parasites treated with IC90 for 24 h. the authors conclude that these triterpenic acids may have potential as future therapeutic alternatives against leishmaniasis (91). Oleuropein also has leishmanicidal and apoptotic activities on leishmania major promastigotes (92).

It can also be mentioned that olive leaf can be effective in treating acne vulgaris, eczema and scleroderma (59).

3.3.10. Anti-arthritis and bone loss

In a murine model with osteoarthritis OLE administration was evaluated and showed to be effective in significantly reducing paw swelling, the paw Evans blue content and the histopathological scores. In the human monocyte cell line, THP-1, OLE reduced the LPS-induced TNF-α production and the reduction was dose dependent. Croton oil-induced ear edema in mice also revealedeness that treatment with OLE suppressed ear edema, myeloperoxidase (MPO) production and was dose dependent. In a study in rat models, antiarthritis mechanism of OLE has been proven to be through an anti-inflammation mechanism (93).

A dose response study on oleuropein effect, was done in an ovariectomy/ inflammation experimental model of bone loss in rats and found that the 4 doses of oleuropein that were tested in the study, could reduce bone loss and improved inflammatory biomarkers except for the dose of 5 mg/kg BW (while 4 groups in the study received

oleuropein at 2.5, 5, 10 or 15 mg/kg BW/ day for 100 days) (94).

4. Pharmacotherapy of OLE

4.1. Dosage

Oral: 250-500 mg 1-3 times/day, standardized to contain 15% to 23% oleuropein per dose (59).

4-2. Adverse effects and theoretical cautions

In a study in rat models, high doses of OLE proved to affect the liver by changing activities of alanine aminotransferase and alkaline phosphatase serum enzymes causing hyperplasia of the bile ducts, cholestasis, hepatocyte necrosis and inflammatory infiltration. Furthermore, the mitochondrial membrane potential, respiratory control ratio and ADP/O of samples were changed (95). The compound must not be used in patients with gallstone and must beused with caution in patients on insulin, hypoglycemic and/or antihypertensive agents. And also must be used with caution in patients with known allergy to herbal materials from the oleacea family (59).

4-3. Interactions

The laboratory reports indicate that there is the risk of interaction between olive leaf and antibacterials, antifungals, antioxidants, antivirals, herbal products, supplements with hypoglycemic potential such as insulin, and supplements with hypotensive effects (59).

4-4. Preparations

There is some tonic compounded herbal medicine from olive leaf, identified in 2014 edition of the reference book of Martindale ; examples are compounded with *Tabebuia avellaneda* and garlic, or with *Echinacea*, *Sambucus*, zinc gluconate, or thyme oil (96).

However, there are various unofficial medication or complementary products from olive leaf for different purposes such as radical scavenging, supporting general well-being (97), immune support, natural antioxidant, coughs and colds, blood sugar support (98), and having been added to *Calendula*, green coffee bean or beetroot, it can act as a natural food supplement (99, 100). It has also been used as an ingredient of a combination effective as a health tonic andimmune support (101) and also for the regulation of cholesterol levels and cardiovascular circulation purposes (102).

5. Discussion

From this review, it is concluded that olive leaf is responsible for some important medicinal properties. The activities that are mentioned in the literature may be summarized into substantial effects such as antioxidant, antitoxic and anticancer properties. Also it has antimicrobial and antiviral effects as well as effects on nervous system, endocrine system, gastrointestinal system and cardiovascular system. It has therapeutic effects on some metabolic disorders and cutaneous complications and some miscellaneous effects such as anti-arthritis and anti-bone loss effects. Despite the large amount of studies on the possible effects of olive leaf having been done and lack of serious adverse effects on its therapeutic doses, the number of available products in markets and therapeutic targets are limited. It is expected that more extensive studies be done on the olive leaf in clinical trials and according to its dose adjustment and special cautions, it has the potential to become a more popular herbal medicine in markets and clinics.

Conflict of Interest

None declared.

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