Olive (Olea europea) oil: physico-chemical characterization and antioxidant activities in vitro and in vivo

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Abstract
Olive oil, especially extra virgin olive oil, gained popularity recently due to its beneficial effects toward human health. Olive oil contained high amounts of monounsaturated fatty acids, especially oleic acid (C18:1) and some minor components such as tyrosol and hydroxytyrosol which are important to human health. Olive oils have been reported to have antioxidant in vitro and in vivo. This article reviewed some physico-chemical properties and antioxidant activities of olive oil either in vitro or in vivo. Olive oil was evaluated in vitro using radical scavenging activities, ferric reducing antioxidant power (FRAP), metal chelating power, beta-carotene bleaching, linoeleic acid-ferric thiocyanate method. In vivo, the antioxidant activities of olive oil were evaluated using glutathione S-transferase, catalase, and superoxide dismutase. Phenolics compounds present in olive oil contribute to antioxidant activities, therefore, olive oil is potential to be used as a food supplement.

1. Introduction
Among edible plant oils, olive oil (OO) has sensory characteristics with high nutritional benefits which make OO to be used as a diet component in the Mediterranean region. OO is a fatty juice and may be consumed after proper processing of olives. OO gained popularity in recent years. It is consumed not only by the people in the Mediterranean countries but also worldwide such as Japan, Indonesia, USA, and South Africa because of its unique flavor (Mannina et al., 2001; Arvanitoyannis et al., 2007). In fats and oils industry, OO commanded high price value compared to other vegetable oils because of its high quality in terms of some aspects including health, sensory, and oxidative stability (Aparicio et al., 2013). These characteristics could be correlated with the composition of fatty acids and also some minor components including phenolic compounds, tocopherols, and squalene (Gomez-Caravaca et al., 2016).

Edible olive oils are graded into six categories, namely (i) extra virgin olive oil (EVOO) with acidity up to 0.8%, calculated as oleic acid; (ii) virgin olive oil acidity about 2.0%; (iii) refined olive oil with acidity of 0.3%, (iv) regular olive oil, which is a mixture of refined olive oil and virgin olive oil with free acidity of 0.1%, (v) refined residue oil, and (vi) olive residue oil, a blend of refined residue oil and virgin olive oil (Boskou, 2009). EVOO is the highest quality of olive oil and accounts for only 10% of the produced oil. Its taste, flavor, and mouthfeel were used by experts to judge EVOO (Huang et al., 2008). The objective of this review was to highlight the physico-chemical properties and antioxidant activities of olive oil using in vitro and in vivo studies.

2. Methods
During performing this review, numerous databases such as PubMed, Scopus, and Google Scholar are used to identify and to download the abstracts, reports, and research papers related to physico-chemical properties, characterization, and antioxidant activities of olive oil. The keywords used were including characterization + olive oil + chemical properties; in vitro or in vivo antioxidant activities + olive oil; nutritional aspects + olive oil + human health in the month of January-March 2019.

3. Composition of olive oils
The extraction methods applied to obtain oil from its fruit significantly affected the chemical compositions of olive oil. The extraction process was affected by crushing olives and separating olive oil from the fruit pulp under accelerated pressure. In addition, olive oil can be extruded, post-pressed, and re-pressed using with or without the use of hot water. Using this process,
Olive oil is typically characterized by the weaker aroma, stronger color intensity, and higher contents of free fatty acids (Gorzynik-Debicka et al., 2018).

The chemical properties and minor components present in EVOO are compiled in Table 1. Olive oil contained a high amount of monounsaturated fatty acids (MUFA) (especially oleic acid) and have a well-balanced of polyunsaturated fatty acids and minor components. Besides, some phenolics compounds which include phenolics acids, simple phenolics such as tyrosol and hydroxytyrosol, derivates of secoiridoid glycosides of oleorupin and ligstroide, lignin, flavonoid, and hydroxy-isochromane are also present in olive oil, especially in high-quality olive oil (virgin olive oil and extra virgin olive oil) (Ballus et al., 2017). These phenolics compounds are believed to contribute to the biological activities of olive oil including antioxidants (Hohmann et al., 2015).

| Table 1. Fatty acid composition and non-triacylglycerol fraction of extra virgin olive oil (Huang et al., 2008; Rohman, 2017). |
|--------------------------------------------------|
| Fatty acids (% w/w)                               |
| Palmitic (C_{16:0})                               |
| Palmitoleic (C_{16:1n-7})                         |
| Stearic (C_{18:0})                                |
| Oleic (C_{18:1n-9})                              |
| Linoleic (C_{18:2n-6})                           |
| α-Linolenic (C_{18:3n-3})                        |
| Nontriacylglycerol fraction component (mg/kg)     |
| C_{18}-C_{30} alcohols                            |
| Triterpene alcohols                              |
| Total sterols                                    |
| Cholesterol                                      |
| Δ5-Avenasterol                                    |
| β-sitosterol                                      |
| Sitostanol                                       |
| Stigmasterol                                      |
| Nonacylglycerol esters                           |
| Waxes                                            |
| Squalene                                         |
| β-carotene                                       |
| α-tocopherols                                    |
| Tocotrienols                                     |
| Protein (µg/kg)                                  |
| ≤ 200                                            |
| 500 – 3,000                                      |
| 1,260.80                                         |
| 1.9                                              |
| 91.5                                             |
| 1,124.40                                         |
| 7.3                                              |
| 8.2                                              |
| 100 – 250                                        |
| ≤ 250                                            |
| 4,277                                            |
| 0.33 – 4.0                                       |
| 300                                              |
| Not detected                                     |
| 1.76                                             |

Free radicals, highly reactive substances, have an ability to damage some molecules like proteins and lipids which cause several degenerative diseases such as diabetes, cancer, coronary heart disease, etc. (Anggraini et al., 2018). Indeed, antioxidants must be used to neutralize free radicals. Antioxidant can be defined as any substances in low concentrations, derived from natural or synthetic, capable of delaying or inhibiting oxidation reaction significantly (Antolovich et al., 2002; Rohman et al., 2006). Based on the reaction mechanism, antioxidant assays can be group into five categories, namely radical scavenging assay, reducing power, chelating agent, lipid peroxidation reaction and synergist. These mechanisms are commonly exploited for assessment of antioxidant from natural sources. The community awareness toward the safety of synthetic antioxidant has attracted scientist of antioxidant coming from plants (Thitilertdecha et al., 2010). One of the plants potential to be used as a natural antioxidant is olive, which yielded olive oil.

Several antioxidant tests have been reported for evaluation of antioxidant capacities in vitro of olive oil from different regions which included radical scavenging activities using 2,2′-diphenyl-1-picrylhydrazil (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺), hydrogen, nitric oxide, and peroxynitrite radicals, ferric reducing antioxidant power (FRAP) and reducing power, metal chelating power, beta-carotene, bleaching beta-carotene linoleic acid method, and cupric ion reducing antioxidant capacity. In vivo, olive oil has been tested for its capability to induce several antioxidant enzymes which include glutathione S-transferase, catalase, and superoxide dismutase (Nur Alam et al., 2013). Furthermore, antioxidants can be also classified based on chemical reaction occurring between the free radicals and antioxidant compounds into two groups, namely hydrogen atom transfer (HAT) based assay and electron transfer (ET)-based assay (Dontha, 2016). ET-based assay included DPPH free radical scavenging assay, Superoxide anion radical scavenging assay, Ferric Ion Reducing Antioxidant Power (FRAP), Trolox equivalence antioxidant capacity, Folin Ciocalteu reagent (FCR), the total phenols assay, reducing power, N,N-dimethyl-p-phenylenediamine assay, nitric oxide radical inhibition activity, and TBARS or thiobarbituric acid reactive substances assay. In addition, HAT included Oxygen Radical Absorbance Capacity (ORAC),
ABTS radical scavenging, total radical-trapping antioxidant parameter, hydroxyl radical scavenging, hydroxyl radical avertion capacity, lipid peroxidation inhibition capacity, scavenging of peroxide radicals, inhibition of oxygen uptake photochemiluminescence assay and β-carotene–linoleic acid (linoleate) assay. Non-enzymatic strategies.

4.1 Radical scavenging activities

Among antioxidant assay, radical scavenging methods is the most commonly used for screening or rapid evaluation of antioxidant activities. Two radicals namely DPPH and ABTS are frequently used for this purpose. DPPH radical assay is relied on the scavenging of DPPH radical due to the addition of a radical species coming from hydrogen radical (antioxidants), and the DPPH solution decolorize. The radical scavenging activity is then observed by the absorbance decrease at 515-520 nm (Thitilertdecha et al., 2010). When antioxidants reacted with DPPH, the stable free radical becomes paired off in the presence of a hydrogen donor, while DPPH radicals were reduced into non-radical (DPPH). As a consequence, the absorbances decreased from the DPPH. Radical to the DPPH-H form results in decolorization (yellow) with respect to the number of electrons captured. More the decolorization more is the reducing ability (Bondet et al., 1997; Inoue et al., 2005).

The parameter of IC50 (concentration of sample solution capable of scavenging 50% DPPH radicals) is used to assess the power of antiradical. The lower the IC50 value, the higher the antiradical (Rohman et al., 2006; Permatasari and Rohman, 2016). Methanol is a solvent of choice to dissolve DPPH radical (Sharma and Bhat, 2009). In addition, ABTS+ radicals scavenging assay measured antioxidant capacity as the ability of test samples (as antioxidants) to decrease the intensity of ABTS+ color, and there are two versions of ABTS assay, namely by intercepting initial oxidation and preventing ABTS+ production, or (2) reacting directly with the ABTS+ (Schach and Xie, 2015).

Xiang et al. (2017) have compared DPPH radical assay of four types of olive oil from Liangshan, namely Barnea, Coratina, Koreniki, and Manzanilla. Koreniki oil extract has the highest DPPH radical with an IC50 value of 20.00 µg/mL, over Barnea, Coratina, Koreniki, and Manzanilla oils with IC50 values of 49.66, 21.33, and 25.33 µg/mL, respectively. DPPH radical scavenging activities of EVOO from Southern Italy from five different olive mono varieties namely Coratina, Leccino, Maiatica, Ogliarola del Vulture and Ogliarola del Bradano. The cultivar of Coratina cultivar had the highest DPPH radical-scavenging activity with IC50 of 31.9 µg/mL, over Leccino (IC50 of 40.6 µg/mL), Maiatica (IC50 of 53.46 µg/mL), del Vulture (IC50 of 33.3 µg/mL), and del Bradano (IC50 of 39.9 µg/mL) (Condelli et al., 2015). DPPH antiradical activities of virgin olive oils (VOO) from Croatian varieties of Mašnjaca and Krvavica and VOO variety Leccino from Italy have been investigated. The VOO of Mašnjaca and Krvavica have the same antiradical capacity of 0.5 mmol Trolox equivalent antioxidant capacity (TEAC), while VOO variety Leccino has antiradical power of 1.3 TEAC (Sarolic et al., 2014). VOO varieties of La Pena and Severini from Italian olive oil using the standard active compound in VOO of oleuropein and α-tocopherol have been evaluated as DPPH radical scavengers. VOO La Pena has IC50 of 41.1-43.4 µg/mL, while VOO Severini has IC50 of 40.1-42.3 µg/mL. Both varieties have no significant difference of IC50 values (P>0.05). This difference can be attributed to the phenolics contents contained. VOO La Pena and VOO Severine have phenolics content of 350±4.2 and 343±5.0 mg/kg, respectively. Oleuropein and α-tocopherol have IC50 of 45.1 and 10.1 µg/mL, respectively (Köseoglu et al., 2016).

Olive oils from two different varieties of Memecik and Gemlik have been evaluated for its antiradical activity using ABTS+ radical. The Memecik variety has higher antiradical activity than Gemlik variety significantly (p<0.05) (Cioffi et al., 2010). Kelebek et al. (2015) also reported that ABTS+ radical scavenging activities of Memecik and Gemlik varieties were of 0.83 and 1.31 (µmol Trolox/100 g oil), respectively. These antiradical activities were decreased during ripening of olive fruit. Galvano et al. (2007) and Gorienstien et al. (2003) stated that these antiradical activities are correlated positively with the phenolic and α-tocopherol contents. Two varieties of olive oil (Chetoui and Chemlali) have been evaluated for antiradical activities using DPPH and ABTS+. The variety Chemlali contained less total phenols of 158 mg/kg than variety of Chetoui oil of 395 mg/kg (p<0.05). Olive oil Chemlali also has lower antiradical activities than Chetoui oil (37.23% vs. 78.56%) using DPPH, and 0.61 vs. 2.42 mmol Trolox/kg (Nakbi et al., 2010).

Some phenolics components present in or isolated from olive oil were also evaluated for antioxidant activities using DPPH radical assay. At same concentration (218 µg/mL), hydroxytyrosol has the highest antiradical activity with antiradical power (ARP) of 26.23 µmol/g over deacetoxy oleanopren aglycon with ARP of 7.61 µmol/g, oleanopren aglycon with ARP of 6.11 µmol/g, (+)-pinoresinol with ARP of 1.35 µmol/g, ligstrose aglycon with ARP of 0.79 µmol/g, tyrosol with ARP of 0.76 µmol/g, elenolic acid with ARP of 0.69 µmol/g, and (+)-1-acetoxy-pinesinosin with ARP of
4.2 Reducing power

Evaluation of olive oil is based on the electron transfer mechanism. One of the most commonly used methods is Ferric reducing activity power (FRAP). FRAP is relied on the capability of antioxidant to reduce the complex of Fe(III)-TPTZ or 2,4,6-tripyridyl-s-triazine to produce the intensely colored Fe(II)-TPTZ, having maximum absorption at 593 nm (Özyurek et al., 2011). FRAP measures the ability of evaluated samples to reduce complex Fe(III)-TPTZ to produce colored complex Fe(II)-TPTZ. This reduction is monitored by measuring the absorbance change at 593 nm, using UV-vis spectrophotometer. The antioxidant capacity using FRAP method can be evaluated by comparing the absorption change in the test mixture with that obtained from increasing concentrations of Fe(III) and expressed as mM of Fe(II) equivalents per kg or per L sample (Nur Alam et al., 2013). FRAP method has limitations, especially if this method was measured at below pH 3.6. Besides, FRAP is unable to detecting polyphenolic compounds and thiols with slowly-reacting properties (Benzie and Strain, 1999). The compounds with redox potential lower than that of the redox pair Fe(III)/Fe(II) can theoretically reduce Fe(III) to Fe(II), which contributed to an increase in the FRAP value and thus inducing false-positive results (Dinis et al., 1994; Jerkovic et al., 2010).

Bayran et al. (2012) have investigated ferric reducing antioxidant power (FRAP) of 55 mono-varietal and multi-varietal extra virgin olive oil samples from several countries. Olive oil containing the highest contents of phenolics compounds revealed 10 fold higher FRAP values (532±11) than olive oil with the lowest contents of phenolics (45±3.2). FRAP values significantly correlated with total phenolics content with the coefficient of determination (R²) of 0.91. Total reducing capacity also correlated with the individual phenolics of hydroxytyrosol, oleuropein and tyrosol (p values < 0.01). Among these, hydroxytyrosol was reported to have the highest R² with FRAP values.

4.3 Metal chelating activity

Ferro ion (Fe²⁺), as representative of metal, can form a complex with chelates of ferrozine to obtain red color having maximum absorption at 562 nm. This reaction is limited in the presence of other chelating agents and results in a decrease of the red color of the ferrozine-Fe²⁺ complexes. Measurement of the color reduction due to the addition of evaluated samples for inhibition of complexation reaction Fe²⁺-ferrozine determines the chelating activity (Soler-Rivas et al., 2000). Ethylenediamine tetraacetate (EDTA) or citric acid can be used as a positive control (Jagtap et al., 2010).

Four varieties of olive oil (Barnea, Coratina, Koreniki, and Manzanilla) from Liangshan, China have been studied in terms of its capability to provide chelating activities toward iron. Four olive oils exhibited obviously Fe-chelating activity in the manner of concentration-dependent. The effective concentration 50% (EC₅₀) of these four olive oils were 39.00±7.00 µg/mL (Barnea), 19.00±1.00 (Coratina), 83.00±9.64 (Koreniki), and 16.66±3.78 µg/mL for Manzanilla (Bondet et al., 1997). Ziogas et al. (2010) also reported that the metal chelating activities of polyphenol extracted from olive oils with different varieties were significantly different. The authors also stated that there was a correlation between the IC₅₀ values of chelating activities with total polyphenols as determined using the Folin-Ciocalteu method.

4.4 β-carotene bleaching

β-carotene bleaching (BCB) method is the rapid method used for antioxidants screening, mainly relied on the principle that an unsaturated fatty acid as represented by linoleic acid (C18: 2) was oxidized by reactive oxygen species, produced by oxygenated water. The oxidized products formed radicals which then oxidized β-carotene to yield discoloration of beta-carotene. The antioxidants components decrease the extent of discoloration, as measured at 434 nm (Kabouche et al., 2007). In the absence of an antioxidant rapidly bleaches the typically orange of β-carotene which is monitored spectrophotometrically at 450 nm (Miller, 1971).

Condelli et al. (2015) have investigated the antioxidant activities of five different olive mono varieties (Coratina, Leccino, Maiatica, Ogliarola del Vulture and Ogliarola del Bradano) from Southern Italy. The cultivar Coratina cultivar has the highest antioxidant activity of 30.50±4.09%, while others have antioxidant activity via bleaching of β-carotene of 12.30±2.44% (Maiatica), 10.60±1.61% (O. vultura), 22.80±2.12% (Ogliarola del Bradano) and 24.10±2.26% (Leccino). Bouarroudj et al. (2016) have evaluated antioxidant activity through BCB of extra virgin olive oil (EVOO) from Algerian region. EVOO has antioxidant of 53.04%, lower than oleaster oils as a comparison. There were positive correlations (p < 0.05) between antioxidant activity with the contents of total polyphenol and ortho-diphenols with correlation coefficients obtained of 0.79 and 0.76, respectively.

5. Antioxidant activities in vivo

The diet of olive oil was associated with a lower incidence of degenerative diseases such as coronary heart disease, atherosclerosis, Alzheimer’s disease and heart disease, atherosclerosis, Alzheimer’s disease and...
some types of cancers. These diseases are especially due to the imbalance between radicals and antioxidants in the human body, therefore the consumption of olive oil having some phenolics compounds contributes to antioxidants activities in vitro and in vivo (Cicerale et al., 2012). Low-density lipoprotein (LDL) oxidation is taken into account as major causes for the development of atherosclerosis and coronary disease by inducing the formation of plaque within the arterial wall. Several studies revealed that phenolics compounds can decrease LDL oxidation in vivo (Marrugat et al., 2004; De la Torre-Carbot et al., 2007). The oxidative damage to DNA is believed as a precursor in human carcinogenesis. A study by Cooke et al. (2003) showed that the intake of phenol-rich olive oil (up to 592 mg/kg) decreased the damage of oxidative DNA in humans by up to 30% in vivo. The in vitro evaluation and pre-clinical study using animal model also supported the clinical study using human model (Jacomelli et al., 2010).

Farras et al. (2018) reported that individuals with hypercholesterolemia in a randomized, double-blind, controlled, crossover trial who ingested 25 mL virgin olive oil (VOO) for 3 weeks at concentration 80 ppm have beneficial effects on high-density lipoprotein (HDL)-related markers. HDL antioxidant compounds were increased. The study indicated that long-term consumption of olive oils rich in phenolics compound induced HDL antioxidant levels. The phenolics compounds and vitamin E contained in EVOO also inhibited the formation H2O2 significantly in healthy subjects, and the production of catalase was increased. The authors concluded that olive oil possesses antioxidant activities in human (Roberto Carnevale et al., 2018).

6. Conclusion

Olive oil is one of the edible oils having a high price in fats and oils industry. Several antioxidant assays either in vitro or in vivo revealed that olive oil good sources of antioxidant. Based on this fact, olive oil can be the function as functional oils due to its beneficial effects as an antioxidant and is good components to be used in pharmaceuticals formulation.

Conflicts of Interests

The author reported no conflict of interests and is responsible for the contents and writing of this review article.

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