**Antioxidant activity of *Olea europaea* L. extracts from two different regions of Iran**

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**ABSTRACT:** Olive tree (*Olea europaea* L.) leaves and fruits have been widely used in traditional remedies in European and Mediterranean countries. Olive fruits and leaves were dried in oven at 30-40 °C temperature and dried material crushed into smaller pieces before extraction. Methanolic extracts were used to measure total phenolics, total flavonoids and antioxidant activities. Total phenolic was determined via Folin-Ciocalteu and total flavonoid via AlCl₃ assays. The stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the samples. Total phenolic content of extracts was highest in fruit of Gorgan sample (41.65 mg GAE g⁻¹) and lowest in leaves of Zabol sample extract (14.58 mg GAE g⁻¹). Total flavonoid content of extracts was highest in leaves of Gorgan sample (16.2 mg QUE g⁻¹) and lowest in fruit of Zabol sample (5.57 mg QUE g⁻¹). EC₅₀ for DPPH radical-scavenging activity was lowest in leaves of Gorgan sample (72.38 μg mL⁻¹) and results showed that Antioxidant activities of Zabol samples (both of leaves and fruits) were higher than Gorgan samples. These results show that the combination of olive leaf and fruit extracts, phenolic components possessed antioxidant activity. There was no correlation between antioxidant activity and total phenolic content of samples which is consistent with our results. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases.

**Keywords:** *Olea europaea* L., Antioxidant activity, total phenols, total flavonoids

**INTRODUCTION**

The Olive tree is native to the Mediterranean basin and has been known for its medicinal properties since ancient times. Olive (*Olea europaea* L.) is one of the most remarkable and important among all the trees because of a source of food, hygiene and curative properties [17. Olive tree (*Olea europaea* L.) leaves and fruits have been widely used in traditional remedies in European and Mediterranean countries such as Greece, Spain, Italy, France, Turkey, Israel, Morocco, and Tunisia. They have been used in the human diet as an extract, an herbal tea, and a powder and they contain many potentially bioactive compounds that may have antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic and hypocholesterolemic properties. Bioactive components found in olive include secoiridoid (oleuropein and its derivatives), hydroxytyrosol, polyphenols (verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside), triterpenes including oleanolic acid, flavonoids (rutin and diosmin), phenols and triterpenes [8 and 14]. Olive containing most materials, including carbohydrates, sugars, soluble and insoluble fibers, sodium, vitamins, minerals, fatty acids, amino acids and more regarding olives [17]. Antioxidant activities of Olive extracts were examined in several researches. In 2007, researchers in Australia studying the antioxidant capacity of 55 medicinal herbs found that olive leaf extracts had the highest radical-scavenging activity of all studied herbs – more than twice that of *Camellia sinensis* (green tea) and *Silybum marianum* (milk thistle)[19]. Flavones and flavonols that exist in Olive showed pro-oxidative activity [18]. The purpose of this study was to identify the total phenols and total flavonoids present in olive leaf and fruit extracts. The antioxidant activity of the compounds was also assessed by scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals.
MATERIAL AND METHODS

PLANT MATERIAL

Olea europaea L. fruits and leaves were collected from Nasr Abad village, near the Gorgan city, Golestan province (N: 54° 27', E: 36° 40', Sub-humid) in the North of Iran and Institute of Agricultural Research of University of Zabol, Sistan & Blochestan province (N: 61° 41', E: 30° 54', Sub-tropical), in the South East of Iran.

Chemicals

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH), Potassium ferricyanide were purchased from Sigma Chemicals Co (USA). Gallic acid, Quercetin, Folin-Ciocalteau reagent, sodium carbonate, potassium acetate, aluminum chloride, methanol and ethanol were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

EXTRACTION METHOD

Olea europaea L. fruits and leaves were oven-dried at 30-40°C temperature for 48-72 h and then dried material crushed into smaller pieces using an Electric mills before extraction. 1 gram of plant powder was extracted at room temperature by using 10 mL of methanol-water (80-20 v/v) and was placed in the dark condition for 24 hours on Shaker. Then centrifuged at 3000 rpm for ten minutes and were smoothed using filter paper. These methanolic extracts were used to measure total phenols, total flavonoids and antioxidant activity.

Determination of total phenolic contents

Total phenolic content was determined by the Folin-Ciocalteau method [9]. Briefly, the extract sample (20 µL) was mixed with 1.16 mL distilled water and 100 µL of N Folin-Ciocalteau reagent for 5 min. 300 µL of 1 M sodium carbonate were then added. The tubes were placed in a water bath at 40 °C in the dark condition for 30 min. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as Gallic acid equivalents per gram of the dry weight. Calibration curve were prepared using of different concentrations of Gallic acid (10, 50, 100 and 150 mg L⁻¹). For control sample, pure methanol was used instead of plant extracts.

Determination of total flavonoid contents

Total flavonoids were estimated using the method of Ebrahimzadeh et al [6, 11]. Briefly, 0.5 mL solution of plant extract was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was calculated as Quercetin from a calibration curve. Calibration curve were prepared using of different concentrations of Quercetin (50, 100, 250 and 350 mg L⁻¹). For control sample, pure methanol was used instead of plant extracts.

DPPH radical-scavenging activity

The stable 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the samples [10, 13, 16]. 20 µL of sample were added, at an equal volume, to ethanolic solution of DPPH (100 µM). After 30 min at room temperature, the absorbance was recorded at 517 nm. For control sample, pure methanol was used instead of plant extracts.

Statistical Analysis

All measurements were replicated three times. The data were analyzed by an analysis of variance (p < 0.05) using SPSS software and the means separated by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Total phenol compounds, as determined by Folin-Ciocalteau method, are reported as Gallic acid equivalents by reference to standard curve (y= 0.004 X + 0.1, r² =1). The total phenolic content of extracts was highest in fruit of
Gorgan sample (41.65 mg GAE g\(^{-1}\)) and lowest in leaves of Zabol sample extract (14.51 mg GAE g\(^{-1}\)). Results showed that the total amount of phenols was higher in the fruit organ against of leaf tissues (Fig 1).

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. Flavonoids can interfere with at least three different free-radical producing systems. Due to their lower redox potentials, they are able to reduce highly oxidizing free radicals and so prevent, for example, lipid peroxidation, one of the most important actions of free radicals that leads to cellular membrane damage and, ultimately, to cell death\(^5\). In this study the total flavonoid content of extracts was highest in leaves of Gorgan sample (16.2 mg QUE g\(^{-1}\)) and lowest in fruit of Zabol sample (5.57 mg QUE g\(^{-1}\)), by reference to standard curve (\(y=0.001 \times +0.068, r^2=1\)) (Fig 2). Total flavonoid content was higher in leaf organ against of fruit organ.

\(EC_{50}\) for DPPH radical-scavenging activity was highest in leaves of Zabol sample (87.23 \(\mu g\) mL\(^{-1}\)) and results showed that antioxidant activities of Gorgan samples (both of leaves and fruits) were higher than Gorgan samples (Fig 3) because of higher total phenolic contents and other secondary metabolites which are present in these samples.
This study indicates that olive leaf extract might be a valuable bioactive source, and would seem to be applicable in both the health and medical food. These results show that both of olive leaf and fruit extracts, phenolic components possessed antioxidant activity [4, 10, and 15]. According to our results, Antioxidant activities in olive leaves and fruits collected from Zabol region was lower than leaves and fruit of Gorgan region. The total phenol content of olive extracts was higher than other plants such as Origanum majorana L. [1]. Luis et al. (2012) have demonstrated that, Olea europaea is the tree that has the poorest content in total phenolic against other plants such as Acacia dealbata and Acacia melanoxylon [12]. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases [18]. Polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities, that they are in high value in the olive.

Garcia-Macias et al. (2007) have demonstrated that the increased concentrations of total phenols and the main flavonoids for many plant species was most likely due to the leaves exposure to increased UV levels. Thus, this may confirm that leaf function serves as defense mechanism against UV damage. These secondary metabolites and related phenolic compounds are most likely the major source of UV-B absorption in leaf epidermis [7]. The vegetal material (leaves of Gorgan region) have been shown to possess significant antioxidant activities, it can be considered as green tea. There was no correlation between antioxidant activity and total phenolic content of our samples which is different with other research results [1, 2, and 3]. Plant antioxidants have played an important role in maintaining health and providing protection against coronary heart diseases, cancer, etc. Thus, researches on natural antioxidants of plant origin have attracted scientists, food manufacturers and consumers as potential source of functional foods.

**REFERENCES**


