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A Phytochemical Study on *Olea europaea* L. Olive Leaf Extract (cv. Koroneiki) growing in Egypt

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ABSTRACT

Olea europaea is an evergreen tree, native to the Mediterranean region and well known for its edible fruits and oil. Recently much focus has been made on the leaves of the trees due to their high antioxidant property in addition to other therapeutic value. The leaves that are considered by-products during olive oil production are now an essential commodity in the nutraceutical industry. Koroneiki (*Olea europaea* L. cv Koroneiki) is one of the well-known Greek olive cultivars as the queen of oil-producing olive trees. Our study focuses on studying this tree growing in Egypt in regards to its total flavonoids (TF) and phenolic content (PPh) as well as antioxidant activity in 2 seasons in a comparative presentation. The average PPh for Koroneiki leaf extract was found to be 116.81 ± 0.97 and 152.98 ± 0.11 mg/g dried extract for autumn and spring respectively while TF was found to be 48.32 ± 0.5 and 82.68 ± 0.71 mg/g respectively. In autumn the oleuropein content was found to be 12.65 ± 0.06 mg/100 g dried extract, while in spring marked an increase in oleuropein content reaching 92.25 ± 0.26 mg/100 g was observed. The % inhibition of the free radical for autumn extract was found to be $86.56 \% \pm 0.39$, while $90.09 \% \pm 1.18$ for spring. The results indicated that spring is the season of choice for leaf collection.

Keywords: *Olea europaea*; Koroneiki; total flavonoids; phenolic content; oleuropein.

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1. INTRODUCTION

Olea europaea is an evergreen tree, well known for its edible fruits and oil. The olive tree is native to Mediterranean countries whose cultivation covers about eight million hectares worldwide [1]. The tree is considered one of the most important crops related to the superior nutritional and medicinal value of its edible oil [2, 3]. Consumption of olive oil is correlated with

a low risk of cancer and cardiovascular diseases [4]. Additionally, several recent studies focused on olive leaf extracts for their antiproliferative, antimicrobial, antioxidant, and antiviral properties [5-7]. Also, the antidiabetic, antihypertensive, anti-inflammatory, and cardioprotective activities of *O. europaea* leaf extract have been proven [8]. The reported

biological activities of the leaf extract have been related to specific phenolic compounds characteristic to *Olea* genus. This includes phenethyl alcohols (tyrosol, hydroxytyrosol), secoiridoids (oleuropein, ligstroside, oleoside, secologanoside), flavonoids (luteolin 7-O-glucoside, rutin, apigenin 7-O-rutinoside, diosmetin) [9] and lignans, in which oleuropein is the major secoiridoid in olive fruit and leaves that account for most of the leaf extract biological activities [10].

The phenolic profile of the leaf extracts varies quantitatively and qualitatively due to several factors. These include sampling time, environmental conditions, geographical area, exposure to light or hydric deficiency in addition to the genotype, being one of the most important factors which contribute to variation in the leaf phytochemical composition. Folin-Ciocalteu reagent is commonly used to determine the total phenolic content of the leaf extract. However, high-performance liquid chromatography coupled to diode-array (HPLC/ DAD) or coupled to mass spectrometry (HPLC/MS) is commonly used to quantify and characterize specific phenolic compounds in the leaf extract [11].

Olive tree presents a vast genetic heritage represented by more than 1200 cultivars [12]. Koroneiki (*Olea europaea* L. cv Koroneiki) is one of the well-known Greek olive cultivars as the queen of oil-producing olive trees. Koroneiki olive tree (Fig. 1), produces a highly fragrant flower, with a huge olive fruit production. It produces fruits weigh between 0.5 and 1.2 grams that yield 20-25% of their weight oil. The leaves (Fig. 2), are short and narrow, with an elliptical-lanceolate shape. The ability of the tree to grow under adverse weather conditions makes it ideal for hot, dry and windy environments. Koroneiki cultivation in Egypt has been successively introduced and used mainly for its superior

quality and low acidity oil production (Monograph on olive cultivars, HRI).



Fig. 1. Koroneiki olive tree grown in Egypt



Fig. 2. Morphology of Koroneiki leaf

During oil production, a large amount of the leaves are accumulated as a waste product. Although several recent studies have proved the importance of the leaf extract as an available rich

source of bioactive components, no particular uses for the leaves were shown until now in Egypt. In this study, the total phenolic and flavonoid contents of the Greek cultivar Koroneiki leaf extract, growing in Egypt, will be quantified in two different seasons (autumn and spring). Moreover, oleuropein content will be assessed in both seasons to detect the effect of seasonal variation on the leaf composition. Also, the radical scavenging ability of the leaf extract will be evaluated in an attempt to highlight its importance as a cheap, readily available antioxidant source. To the best of our knowledge, this is the first study for the antioxidant activity and phytochemical profile of the leaf extract of *O. europaea* L. cv Koroneiki growing in Egypt regarding its total phenolic, flavonoids and oleuropein contents in both flowering and fruiting seasons.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh green olive leaves (*O. europaea* L. cv koroneiki) were collected in autumn (November 2015; during the full fruit maturation) and spring (April 2016; during the flowering stage) from the Horticulture Research Institute (HRI) in Giza, Egypt. The leaves were identified by Prof. Dr. Mohamed El-Sayed, chief researcher in HRI at the olive and semi-arid zone fruits research department. The leaves were collected randomly from multiple trees, subjected to air-drying then ground in a rotor mill to obtain a fine powder stored at 4 °C for extraction process.

2.2. Chemicals and reagents

Organic solvents (ethanol, methanol, and acetonitrile) were obtained from Fisher Scientific, UK in HPLC grade. Folin–Ciocalteu reagent used for polyphenol assay was purchased from Oxford lab chem., India, aluminum chloride (AlCl_3), and sodium bicarbonate (NaHCO_3) were obtained from Al Nasr co., Egypt, while DPPH

(2,2-diphenyl-1-picrylhydrazyl), gallic acid, rutin, and oleuropein were purchased from Sigma–Aldrich (St. Louis, USA).

2.3. Extracts preparation

Extraction was carried out according to Lee et al. (2009), using 70% ethanol at room temperature [13]. The extract was filtered, evaporated using rotary evaporator (Buchi, Switzerland) and completely dried using a lyophilizer (Christ, Alpha 1-2 LD Plus) to obtain the dry powdered extract. The process was repeated for olive leaves collected in spring. The yield % was calculated and found to be 17.6 and 20.32% for autumn and spring respectively. The obtained dried powdered extracts were stored in tightly closed containers at 4 °C.

2.4. Determination of total phenolic content

Total polyphenol content (PPh) was measured according to Singleton et al., (1999); using Folin–Ciocalteu's reagent. A prepared methanolic solution of the leaf extract (1 mg/mL) was mixed with 10% Folin–Ciocalteu's reagent and 7.5% NaHCO_3 [14]. Samples were prepared in triplicate and were allowed to stand in the dark for 30 min. Absorbance was measured at λ_{max} 760 nm using SPECORD 210 plus spectrophotometer (Analytik Jena, Germany). The total polyphenol content was calculated using the standard calibration curve of gallic acid and the results were expressed as mg gallic acid (GA) equivalents (mg GA/g dried leaf extract).

2.5. Determination of total flavonoid content

The total flavonoid content (TF) was determined according to Quettier-Deleu et al. [15]. One mL of 2% AlCl_3 solution was added to the prepared methanolic solutions of the leaf extracts. All samples were prepared in triplicate and the mean absorbance value was obtained at λ_{max} 415 using SPECORD 210 plus spectrophotometer (Analytik Jena, Germany). TF

was calculated using the regression equation obtained from rutin standard calibration curve. The TF content was expressed as rutin equivalent (mg of rutin/g of the dried leaf extract).

2.6. Determination oleuropein content by HPLC/DAD

The chromatographic analysis method described by Al-Rimawi, 2014; was used to determine the oleuropein content in Korneiki leaf extracts collected in autumn and spring [16]. The analysis was performed using HPLC (Agilent 1200 series, Germany) coupled to a diode array detector adjusted at 280 nm. The separation was carried out using a mobile phase system composed of acetonitrile: water: acetic acid (2: 8: 0.01 v/v). A Kromasil C18 column with particle size 5 μm was used and the flow rate was adjusted at 1 mL/min. Quantification of oleuropein was carried out using the standard calibration curve constructed with five concentrations of oleuropein standard.

2.7. Radical scavenging activity of the leaf extracts using DPPH method

The antioxidant activity of the leaf extract was measured based on the percentage inhibition in DPPH radical described by Brahma *et al.* [17]. A solution of 0.1 mM DPPH in ethanol was prepared; and added to 0.1 mL of the methanolic solution of the leaf extract. The absorbance of each extract was measured at 517 nm and the percent inhibition of the free radical was calculated using the following equation

$$I\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.8. Statistical analysis

All the measured variables including total phenolics, total flavonoids, oleuropein contents, and radical scavenging activity were performed

in triplicates. Results presented as mean values \pm Standard error of the mean (SEM). Two-way ANOVA followed by Bonferroni's post hoc test using GraphPad Prism 7.05 software (GraphPad Software, Inc., San Diego, CA, USA) was performed. Differences were considered statistically significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Determination of total phenolic content

Olive leaves are considered a rich source of phenolic compounds [18, 19]. Phenolic content and subsequently antioxidant activities of olive leaves show consistent variations according to their geographical location, type of cultivar and collection time [20]. Total phenolic content was expressed as mg gallic acid /g dried extract. The average PPh for Koroneiki leaf extract was found to be 116.81 ± 0.97 and 152.98 ± 0.11 mg/g dried extract for autumn and spring respectively. The leaf extract collected in spring was shown to have markedly higher PPh content than in autumn ($p < 0.001$).

3.2. Determination of total flavonoid content

Various flavonoids were previously identified in olive leaf extracts, including flavonols (quercetin, rutin), flavones (luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin), and flavan-3-ols (catechin) [21]. The TF in Koroneiki leaf extract was expressed as mg rutin equivalent. It was found to be 48.32 ± 0.5 and 82.68 ± 0.71 mg/g dried extract for autumn and spring, respectively. Here again, the leaf extract exhibited higher TF content during the flowering stage ($p < 0.001$), showing spring the season of higher PPh and TF for Koroneiki leaf extract.

3.3. Determination oleuropein content by HPLC/DAD

Oleuropein is a secoiridoid present abundantly in olive leaves. Several pharmacological effects of olive leaves were shown to be related to

oleuropein [22]. The peak area of oleuropein was detected and its amount was expressed as mg/100 g dried extract. Results showed obvious variation in the oleuropein content between the two seasons. In autumn the oleuropein content was found to be 12.65 ± 0.06 mg/100 g dried extract, while in spring significant increase in oleuropein content reaching 92.25 ± 0.26 mg/100 g dried extract was observed ($p < 0.001$).

3.4. Radical scavenging activity of the leaf extract using DPPH method

To assess the *in vitro* antioxidant activity of olive leaf extract, the DPPH radical scavenging capacity of the leaf extract was used as a common method. It is based on scavenging the stable free radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by antioxidants (including phenolics). These

results in the decolonization of DPPH methanol solution measured at 517 nm. The result was expressed as % inhibition of the free radical for each extract. By measuring the antioxidant activity of the leaf extracts in both seasons, koroneiki leaf extract was shown as a rich source for antioxidants. The % inhibition of the free radical for autumn extract was found to be $86.56\% \pm 0.39$, while for the spring extract, it was found to be $90.09\% \pm 1.18$. The antioxidant activity of the leaf extract was shown to be slightly higher in spring than in autumn ($P < 0.01$). This was in direct correlation with the higher PPh, TF, and oleuropein content during the flowering stage. The total flavonoids, polyphenol, and oleuropein content, in addition to the antioxidant activity of the leaf extracts, were represented in **Fig. 3**

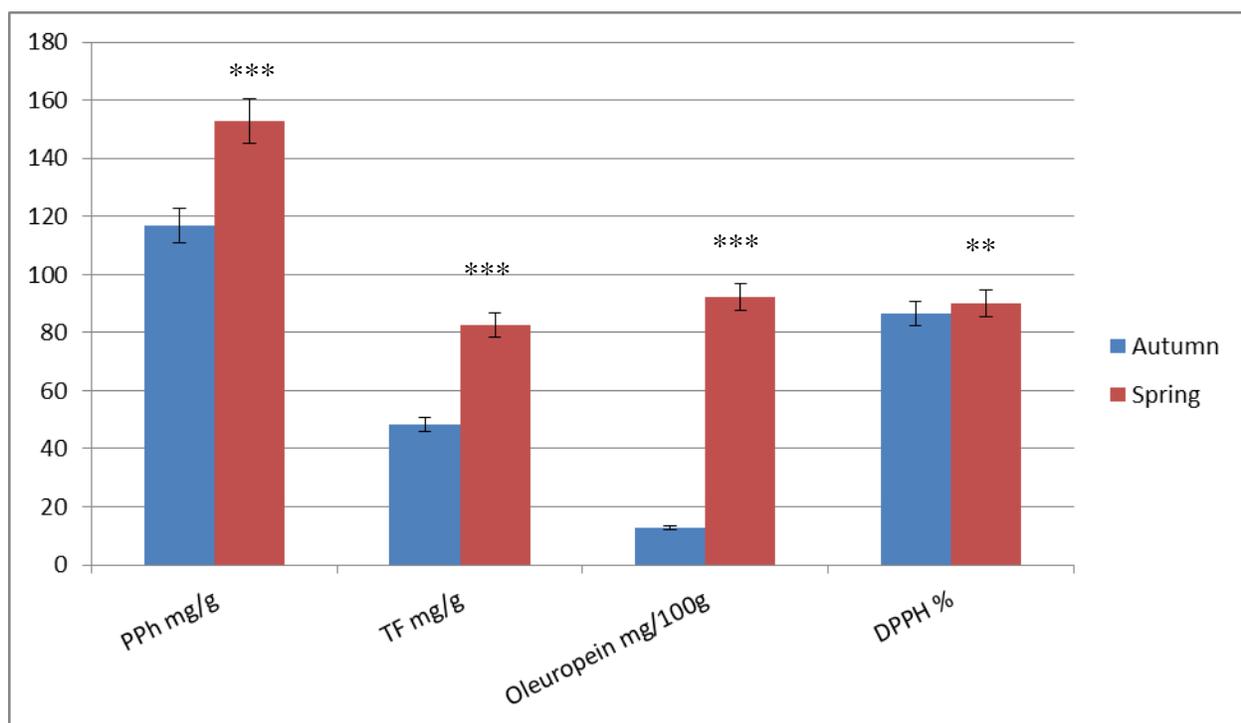


Fig. 3. Koroneiki leaf extracts PPh (mg/g dried extract), TF (mg/g dried extract), oleuropein (mg/100 g dried extract) and antioxidant activity (% inhibition of the free radical) in autumn and spring. Statistical analyses were performed using two-way ANOVA followed by Bonferroni's posthoc test. Differences were considered statistically significant where ** $P < 0.01$; ***, $P < 0.001$

Conclusion

Koroneiki leaf extract can be considered as a cheap readily available antioxidant source. Results showed the richness of the leaf extract in phenolic constituents as well as high oleuropein content. Moreover, the effect of sampling time on the leaf composition was studied herein, where spring was superior to autumn in terms of higher TF, PPh, and oleuropein content. Our results highlight the importance of the Greek cultivar koronreiki; not only for oil production but also for its valuable leaf extract.

Recommendations

Although olive is well-known as an economically important crop in Egypt; the leaves are considered waste by-products. Owing to the increasing demand for antioxidants consumption nowadays, we recommend the introduction of olive leaves in pharmaceutical products and industries as an available rich antioxidant source. The richness of olive leaves in phytochemical constituents favors the collection of the leaves in spring during the flowering stage for the studied cultivar. Expanding the data to include more olive leaf cultivars and to further investigate different biological activities of the leaf extract are recommended for future work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Not applicable

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