Adıyaman University Journal of Science

https://dergipark.org.tr/en/pub/adyujsci
DOI: 10.37094/adyujsci.586664

ADYUJSCI

9 (2) (2019)
242-251

Comparison of Walnut (*Juglans regia* L.) and Olive (*Olea europaea* L.) Leaves in Terms of Antioxidant and Anti-inflammatory Activity

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Abstract

Walnut and olive are consumed by people as food for many years. It is seen that not only fruits but also leaves are used among the people. In this study, walnut and olive leaves were compared in terms of antioxidant and anti-inflammatory capacity. $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging activity methods were used for determination antioxidant capacity. For an *in vitro* measure of anti-inflammatory activity, stabilization of human red blood cell membrane by heat induced membrane lysis was done. The results showed that walnut leaf extract had the highest antioxidant and anti-inflammatory activity with IC50 values of 0.0530 mg mL^{-1} for DPPH radical scavenging activity; 0.0421mg mL^{-1} for ABTS radical scavenging activity and 0.3959 mg mL^{-1} for human red blood cell membrane stabilization capacity. It is thought that the difference in the activity levels of extracts may be owing to the several types and amounts of polyphenolic compounds.

Keywords: Antioxidant, Anti-inflammatory, Walnut leaf, Olive leaf

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Received: 04 July 2019 Accepted: 22 November 2019

Ceviz (*Juglans regia* L.) ve Zeytin (*Olea europaea* L.) Yapraklarının Antioksidan ve Antiinflamatuvar Aktivite Açısından Karşılaştırılması

Öz

Ceviz ve zeytin insanlar tarafından uzun yıllar boyunca gıda olarak tüketilmektedir. Sadece meyvelerinin yanı sıra yapraklarının da halk arasında kullanıldığı görülmektedir. Bu çalışmada ceviz ve zeytin yapraklarının antioksidan ve antiinflamatuvar açısından karşılaştırılması yapılmıştır. *In-vitro* antioksidan etkinliğin değerlendirilmesi amacıyla $DPPH^{\bullet}$ ve $ABTS^{\bullet+}$ radikal süpürücü aktivite yöntemleri kullanılmıştır. *In-vitro* antiinflamatuvar aktivitenin belirlenebilmesi amacıyla ise ısının indüklemesi ile hemolize uğrayan kırmızı kan hücrelerinin membran stabilizasyonları değerlendirilmiştir. Çalışmamızın sonucunda, en yüksek antioksidan ve antiinflamatuvar aktivitenin ceviz yaprağı ekstresine ait olduğu bulunmuştur. IC50 değerleri DPPH serbest radikali süpürücü aktivite için 0.0530 mg mL^{-1}; ABTS serbest radikali süpürücü aktivite için 0.0421 mg mL^{-1}; insan kırmızı kan hücrelerinin membran stabilizasyon kapasitesi için 0.3959 mg mL^{-1} bulunmuştur. Aktivite düzeylerindeki farklılığın, ekstrelerin içerdikleri polifenolik bileşiklerin çeşitlerinin ve miktarlarının farklı olmasından kaynaklanabileceği düşünülmektedir.

Anahtar Kelimeler: Antioksidan, Antiinflamatuvar, Ceviz yaprağı, Zeytin yaprağı

1. Introduction

Walnut tree (*Juglans regia* L.) can be grown naturally in every region of Turkey, also is widely distributed throughout the world [1, 2]. In the cosmetic and pharmaceutical industry, green walnut and the walnut's shell, seed, bark, and leaves are used [2-4]. Walnut leaf also is widely used in folk medicine. In Turkish traditional medicine, walnut leaf is used for anti-inflammatory and antinociceptive activity [5]. Dried walnut leaves are used as an infusion. There are many researches on the pharmaceutical effects of walnut leaves and these studies showed that it may be a good candidate to become a new antimicrobial agent due to the antiproliferative effects on respiratory tract infections and human gastrointestinal system [6]. It also has vascular strengthening, anthelmintic, antidiarrheal, antifungal, hypoglycemic, hypotensive and sedative properties. It is used externally as an antiseptic for skin diseases [1, 7]. The phytochemicals contained to

provide a protective effect against degenerative illnesses by reducing oxidative stress and inhibiting macromolecular oxidation [8 ,9]. Many phenolic compounds were found in walnut leaves (3-*o*-caffeoylquinic acid, *p*-coumaric acid, 5-*o*-caffeoylquinic acid, 4-*o*-*p*-coumaroylquinic acid, 3-*o*-coumaroylquinic acid, quercitin 3-*o*-galactoside, quercitin-3-*o*-pentoside, quercitin-3-*o*-arabinoside, quercitin-3-*o*-xyloside, kaempferol-3-*o*-pentoside, ferulic acid, quercitin-3-*o*-ramnoside, coumaric acid, elagic acid, myricetin, vanillic acid, rutin and juglone) [6, 10, 11].

The olive tree (*Olea europaea* L.) is naturally grown in Aegean and Mediterranean region of Turkey, and also is an important fruit tree in Mediterranean countries [12]. Olive and olive oil are important components in people's daily diet [10]. Olive leaves are used in traditional medicine for several thousand years [13]. Dried olive leaves are used as an infusion. Various studies demonstrated that olive leaf extract has hypotensive and hypoglycemic activity. Moreover, it can cause increased blood flow in coronary arteries, relieve the symptoms of arrhythmias. Furthermore; it has spasmolytic, immunostimulatory, anti-inflammatory, antioxidant, cardioprotective and anti-thrombotic effects [10, 13-15]. Olive leaf extract contains many different compounds, which are thought to give the extract these various therapeutic properties [13, 15]. Phenolic compounds found in olive fruits, oil and leaves are mostly associated with antioxidant properties. Many phenolic compounds were found in the olive leaves (verbascoside, oleuropein, rutin, caffeic acid, apigenin-7-*o*-glucoside, luteolin-4'-*o*-glucoside, luteolin-7-*o*-glucoside; hydroxytyrosol, trysol) [10, 16].

Herewith, the aim of the present study is to evaluate and compare the antioxidant and anti-inflammatory activity of walnut leaf and olive leaf are used in traditional medicine in Turkey.

2. Materials and Methods

2.1. Plant Material

Walnut leaves samples were collected from Savaştepe, Balıkesir (Date: 02.09.2017). Olive leaves samples were collected from two different locations Edremit (Balıkesir) (Date: 09.09.2017) and Tirilye (Bursa) (Date:10.09.2017).

2.2. Preparation of Extracts

Walnut and Olive leaves were air dried, then powdered. Powdered leaves were stirred with pure methanol (Merck) for extraction. After filtration, the extracts were concentrated by evaporation at 40 °C and stored at 4 °C. The yield of samples (w/w) were calculated as 7.69% for walnut leaf extract, 8.12% for Olive leaf extract (Savaştepe) and 8.63% for Olive leaf extract (Tirilye).

2.3. Antioxidant Activity

DPPH (2,2-diphenyl-l-picrylhydrazyl) free radical scavenging activity of samples was defined by their ability to whitening DPPH [17]. The reaction mixture comprised 100 μM DPPH (Sigma-Aldrich) in methanol and different concentrations of extracts. After waiting 30 min, samples absorbance was measured at 517 nm. As the percentage of the radical decrease free radical scavenging activity was detected. For each extract, half maximal inhibitory concentrations (IC_{50}) were calculated from a calibration curve. BHT (butylated hydroxytoluene) (Sigma-Aldrich) was used for the reference compound. Each experiment was applied at least in triplicate [18].

$ABTS^{\bullet+}$ (2,2'- azino bis-(3-ethylbenzothiazoline-6-sulphonic acid)) free radical scavenging activity is the other antioxidant activity's procedure [19]. ABTS•+ (Sigma-Aldrich) was made by reacting 7 mM $ABTS^{\bullet+}$ aqueous solution with 2.45 mM potassium persulfate. Before the reaction mixture was usage in the experiment, it was left to keep at darkness place (25 ^{o}C) all night. The resultant $ABTS^{\bullet+}$ radical cation was diluted with methanol, to give an absorbance value of 0.7000±0.02 at 734 nm. The samples were diluted with the $ABTS^{\bullet+}$ solution. Absorbance was measured spectrophotometrically 6 min after addition at 734 nm. After then the percentage of inhibition was calculated. For each extract, IC_{50} was detected from a calibration curve. Trolox (Sigma-Aldrich) was used for reference compound. Each experiment was applied at least in triplicate [18].

2.4. Anti-inflammatory Activity

Fresh whole human blood was collected from a healthy volunteer (Ethics committee number: 16-695-15, Ankara University) and transferred to the centrifuge tubes. They

were centrifuged at 3000 rpm for 10 min. The cells were cleaned with an equal volume of isosaline (0.85%, pH 7.2). The blood was diluted as 10% v/v suspension with isosaline.

Using heat induced human erythrocyte hemolysis was evaluated the membrane stabilizing activity of the extracts. The reaction mixtures occur of an equal volume of samples and 10% RBCs suspension. Acetylsalicylic acid (Sigma-Aldrich) was used for reference compound. All the centrifuge tubes having reaction mixture were incubated in a water bath for 30 min. After incubation, the tubes were cooled. They were centrifuged at 2500 rpm for 5 min. Then the absorbance of the supernatants was measured at 560 nm. The results were expressed as IC_{50}. Each experiment was applied at least in triplicate [18, 20].

3. Result and Discussion

In our research, antioxidant activity was analyzed by DPPH and ABTS free radical scavenging activity tests. The results, which were obtained from DPPH test are presented in Table 1. Walnut leaf extract showed the highest activity (IC_{50}: 0.0530 mg mL^{-1}) among all. Olive leaf extract which was collected from the Tirilye region exhibited higher DPPH free radical scavenging activity than Edremit region with IC_{50} value of 0.0579 and 0.4401 mg mL^{-1}, respectively.

Table 1. Results of *DPPH* free radical scavenging activity

Plant extract	**IC_{50} (mg ml -1)±SD**
Walnut leaf (Savaştepe)	0.0530±0.0015*
Olive leaf (Edremit)	0.4401±0.0210*
Olive leaf (Tirilye)	0.0579±0.0002*
Reference: BHT	0.0188±0.001*

(*) Statistically control, $p<0.05$ (one-way ANOVA)

Moreover, all samples also have meaningful free radical scavenging activities as compared to control ($p<0.05$). ABTS values of extracts are presented in Table 2.

Table 2. Results of *ABTS* free radical scavenging activity

Plant extract	**IC_{50} (mg ml -1)±SD**
Walnut leaf (Savaştepe)	0.0421±0.0002*
Olive leaf (Edremit)	0.3597±0.0245*
Olive leaf (Tirilye)	0.0499±0.0344*
Reference: Trolox	0.01503±0.0241*

(*) Statistically control, $p<0.05$ (one-way ANOVA)

Walnut leaf extract was characterized by the greatest ABTS free radical scavenging activity (IC_{50}: 0.0421 mg mL^{-1}) among all extracts. The highest activity among the olive leaf extracts was found in the Tirilye region with IC_{50} values of 0.0499 mg mL^{-1}. On the other hand, all samples have meaningful ABTS free radical scavenging activities as compared to control (p<0.05). As shown in Table 1 and Table 2, both test results indicated a strong resemblance.

Anti-inflammatory test results are shown in Table 3. The results showed that all extracts indicate membrane stabilization effect by inhibiting heat induced lysis of human red blood cell membrane as an indicator of anti-inflammatory function as compared to control (p<0.05). Walnut leaf extract (IC_{50}: 0.3959 mg mL^{-1}) demonstrated the highest anti-inflammatory activity. Olive leaf extracts, which were collected, from Edremit (IC_{50}: 0.4462 mg mL^{-1}) and Tirilye (IC_{50}: 0.4769 mg mL^{-1}) showed similar results.

Table 3. Anti-inflammatory activity results

Plant extract	**IC_{50} (mg ml -1)±SD**
Walnut leaf (Savaştepe)	0.3959±0.0135*
Olive leaf (Edremit)	0.4462±0.0074*
Olive leaf (Tirilye)	0.4769±0.0104*
Reference: Acetylsalicylic acid	0.2910±0.0423*

(*) Statistically control, p<0.05 (one-way ANOVA).

For many years, walnuts and olives are on the daily diet of people [10, 21, 22]. Not only the fruits but also leaves are used by people for various purposes [5, 13]. In Turkish traditional medicine, walnut leaf is used for anti-inflammatory, antinociceptive and hypoglycemic activity and external wound healing [7, 23]; olive leaf is used for aperitive, diuretic, antipyretic, antidiabetic activity and external wound healing [23, 24].

Oxidative stress meaning is an unbalance between free radical molecules in the human body and antioxidant defense systems. Overproduction of free radical molecules can cause oxidative stress and inflammation in human body. Oxidative stress has been a reason to a diversity of diseases including cardiovascular disease, autoimmune disease, neurodegenerative disease, and even various cancer types. Antioxidants, which are produced by the human body cells to neutralize and scavenge the effect of free radicals, are also a group of substances taken as dietary supplements. Antioxidants also inhibit chain reactions that can lead to early aging by neutralizing free radical damaging of the

cells. In order for these molecules to be present at the required levels in the body, it is recommended to take foods such as tea, fruits, and vegetables that contain high levels of antioxidants [24]. Various antioxidants have been widely consumed for their actual or supposed beneficial effects against oxidative stress; including vitamin A, C, E, flavonoids, and polyphenols which may also protect against oxidant-mediated inflammation. Flavonoids and phenolic compounds can scavenge the free radicals. Because of these properties, these compounds possess anti-inflammatory activity and also have strong antioxidant potentials [18].

In our study, the antioxidant and anti-inflammatory activity of walnut leaf and olive leaf which are used in Turkish traditional medicine were evaluated and compared with each other. The human red blood cell membrane is analogous to the lysosomal membrane and the stabilization of the membrane may indicate the anti-inflammatory potential of the extract. Due to the fact that human red blood cell membrane stabilization potentials, ABTS and DPPH free radical scavenging capacities of the extracts were investigated. The conclusion of our research suggested that methanol extracts of walnut leaves possessed the highest antioxidant and anti-inflammatory activity.

There were differences in the antioxidant and anti-inflammatory activity between olive leaves which were collected from different regions of Turkey. This difference may be due to the change in the content and amounts of the polyphenolics. Phytochemical content and biological activities of plants may change according to geographical regions, climatic conditions, and the times of plant harvest [26-28].

4. Conclusions

Our study is about the comparison of walnut leaf and olive leaf which are used in Turkish traditional medicine. Both walnut leaf and olive leaf have high activity in terms of antioxidant and anti-inflammatory properties. It is thought that small differences in activities may be due to changes in the amounts of phenolic compounds. It was shown that phenolic compounds, which are found in extracts, could be the cause of antioxidant and anti-inflammatory effects of extracts [6, 10, 11, 16, 22, 29].

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