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Antioxidant Potential of *Liquidambar orientalis* Mill. Fruit Extracts: A Total Phenolic Content and Radical Scavenging Evaluation

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Abstract. Antioxidant compounds act as key defenders, preventing the harmful processes associated with oxidative stress by neutralizing free radicals and preserving cellular homeostasis. Synthetic antioxidants are generally more cost-effective than natural ones and are thus widely used across many industries. However, the potential toxicological risks associated with certain commonly used synthetic antioxidants have made the search for natural antioxidant alternatives inevitable. In this study, ethanol and water extracts of *Liquidambar orientalis* Mill. (Anatolian sweetgum tree) fruits were prepared by ultrasound-assisted extraction after the fruits were ground. The results showed that both extracts contained significant amounts of phenolics and flavonoids; however, the water extract exhibited the most notable antioxidant activity. The water extract showed nearly 92 % activity at 100 μg mL^{-1} in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test and 66 % activity at 200 μg mL^{-1} in the N, N-dimethyl-1,4-phenylendiammonium dichloride (DMPD) radical scavenging test. And in the β-carotene assay, the water extract showed results similar to those of butylated hydroxyanisole. Additionally, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, reducing power, metal chelating activity, and total anthocyanin content were determined. The results demonstrated that both extracts exhibited good antioxidant activity and contained natural compounds with promising antioxidant potential, but the water extract showed superior antioxidant performance. This suggests that the fruit of *L. orientalis* may be utilized for its antioxidant potential, thereby increasing its value.

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Resumen. Los compuestos antioxidantes actúan como defensores clave, previniendo los procesos dañinos asociados al estrés oxidativo al neutralizar los radicales libres y preservar la homeostasis celular. Los antioxidantes sintéticos suelen ser más rentables que los naturales y, por lo tanto, se utilizan ampliamente en diversas industrias. Sin embargo, los posibles riesgos toxicológicos asociados a ciertos antioxidantes sintéticos de uso común han hecho inevitable la búsqueda de alternativas antioxidantes naturales. En este estudio, se prepararon extractos en etanol y en agua de frutos de *Liquidambar orientalis* Mill. (árbol liquidámbar de Anatolia) mediante extracción asistida por ultrasonidos tras la molienda de los frutos. Los resultados mostraron que ambos extractos contenían cantidades significativas de fenólicos y flavonoides; sin embargo, el extracto acuoso presentó la actividad antioxidante más notable. El extracto acuoso presentó una actividad cercana al 92 % a 100 μg mL^{-1} en la prueba de eliminación de radicales 2,2-difenil-1-picrilhidrazilo (DPPH) y del 66 % a 200 μg mL^{-1} en la prueba de eliminación de radicales dicloruro de N,N-dimetil-1,4-fenilendiamonio (DMPD). En el ensayo de β-caroteno, el extracto acuoso mostró resultados similares a los del butilhidroxianisol. Además, se determinaron la eliminación de radicales, el poder reductor, la actividad quelante de metales y el contenido total de antocianinas mediante el método de 2,2'-azino-bis(3-etilbenzotiazolin-6-sulfónico) (ABTS). Los resultados demostraron que ambos extractos presentaron una buena actividad antioxidante y contenían compuestos naturales con un potencial antioxidante prometedor, pero el extracto acuoso mostró un rendimiento antioxidante superior. Esto sugiere que el fruto de *L. orientalis* podría utilizarse por su potencial antioxidante, lo que aumentaría su valor.

Introduction

Oxidation refers to chemical reactions in which a substance transfers one or more electrons or hydrogen atoms to an oxidizing agent. These reactions can result in the formation of free radicals [1]. The primary mechanism underlying the beneficial effects of antioxidants involves reducing reactive oxygen species (ROS) and oxidative degradation products that arise from oxidative stress in cells and tissues [2]. Free radicals are highly reactive molecules with unpaired electrons that are continuously produced in living organisms during normal metabolic processes and exposure to environmental factors such as air pollution and radiation [3]. These reactive species can directly damage biological macromolecules, such as nucleic acids, proteins, and lipids, which may ultimately lead to cellular dysfunction and death [4]. If such damage occurs within a food system, it leads to food spoilage; if it arises within a biological cellular system, it may result in structural and functional losses, or even cell death. When oxidative degradation occurs in solid and liquid fats, their incorporation into food products can lead to a decline in nutritional quality and the development of off-flavors and unpleasant odors. In humans, such oxidative deterioration is associated with aging, and more critically, the onset of various diseases such as cancer, cardiovascular disorders, weakened immune function, and neurodegenerative conditions like Alzheimer's disease, which is characterized by impaired brain function [5,6]. To prevent these adverse effects, antioxidants play a crucial role. Even at very low concentrations, whether in food matrices or within the human body, antioxidants are compounds that delay, inhibit, or prevent oxidative processes that lead to quality deterioration in foods or to the emergence and progression of degenerative diseases in living organisms. Through this mechanism, antioxidants serve as vital agents in mitigating the harmful effects of oxidative stress [7,8]. The main sources of natural antioxidants are plant-derived phenolic compounds, which can be found in virtually all parts of plants, including fruits, vegetables, seeds, nuts, leaves, roots, flowers, and bark [9]. Due to their potential to prevent cellular damage, delay aging, and inhibit the initiation and progression of cancer and cardiovascular diseases, there is considerable scientific interest in extracting natural antioxidant compounds from medicinal plants, herbal sources, and vegetables [10].

While the research continues for natural antioxidant compounds, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and sodium metabisulfite (SMB) are widely used as synthetic antioxidants in food, cosmetic, and pharmaceutical applications due to their cost-effectiveness and efficiency compared to natural antioxidants [11]. Although synthetic antioxidants have undergone rigorous toxicological evaluation, both BHA and BHT have faced regulatory restrictions due to concerns about their potential toxicity and

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carcinogenicity. In this context, natural antioxidants are increasingly perceived as safer and healthier alternatives, thereby accelerating research efforts to discover and utilize natural antioxidant sources [12].

Liquidambar orientalis Miller, commonly known as the Anatolian sweetgum tree, is an endemic species found predominantly in the southwestern region of Türkiye, especially in Muğla province [13,14]. The most well-known product derived from this species is sweetgum oil. Used for thousands of years, Anatolian sweetgum oil is a valuable raw material across sectors such as cosmetics, pharmaceuticals, and the chemical industry. It is commercially recognized as "Turkish storax" in international markets. Due to its antiseptic properties, it has traditionally been used to treat respiratory and pulmonary conditions [13]. According to the literature, various parts of *L. orientalis*, including its leaves and oils, have been studied for their antioxidant activity [15–18]. In these studies, *L. orientalis* oil [17], extracts prepared from the leaves by Soxhlet extraction using ethanol [15], acetone, ethanol, and methanol [18] as solvents, and also brewing with water [16] methods were used. These studies confirm the presence of antioxidant activity. However, to date, no studies have been found investigating the radical-scavenging activity of the water and ethanol extracts of *L. orientalis* Mill. fruit. Furthermore, ultrasound-assisted extraction has not been employed in previous works [15–18]. While these traditional extraction methods can still be used, they are not fully environmentally friendly because they can require extensive use of solvents and time. Ultrasound-assisted extraction can be a better alternative because it requires less solvent and time and yields a higher extraction rate [19].

This study aims to identify a novel natural alternative to synthetic antioxidants while using a more efficient, environmentally friendly extraction technique. To this end, a series of free radical scavenging assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl), DMPD (N, N-dimethyl-1,4-phenylendiammoniumdichloride), and ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) methods, were conducted. In addition, various chemically-based antioxidant assays were performed, including reducing power, β-carotene bleaching activity, and metal chelating activity. The total phenolic content and total flavonoid content of the extracts, and the anthocyanin content of the fruit of *L. orientalis* were also quantified. The results of this study will determine the antioxidant properties of *L. orientalis* fruits, which have no known use and can be considered natural waste. This will contribute to the literature by revealing whether they can serve as an alternative to synthetic antioxidants, thereby adding a new area of application to *L. orientalis* fruits, which might reduce natural waste and increase their value.

Materials and methods

Chemicals

ABTS, DPPH, and linoleic acid were procured from Sigma-Aldrich, Germany. DMPD and trichloroacetic acid (TCA) were obtained from Merck, Germany. BHA and Ferrozine were sourced from Fluka, Switzerland. All remaining reagents used in the study were of analytical grade and were supplied by either Sigma-Aldrich, Merck, or Fluka.

Preparation of the extracts

The plant material was freshly obtained from Marmaris, Türkiye, thoroughly washed with distilled water, and subsequently dried in a laboratory oven at 30 °C for 4–5 days. The fully dehydrated samples were then sealed in airtight containers and preserved at −20 °C until further analysis. Ultrasound-assisted extraction was employed to obtain both water and ethanolic extracts from pulverized fruits of *L. orientalis* Mill. Briefly, 1 g of fruit was mixed with 10 mL of water or ethanol as the solvent, and extraction was done in an ultrasonic bath (35 kHz, 80/320 W) at 25 °C for 5 min with 1-minute intervals [20]. Following extraction, the mixtures were centrifuged for 15 minutes. After solvent evaporation, the remaining residues were reconstituted in water or ethanol to yield stock solutions with a final concentration of 1 mg/mL, intended for subsequent antioxidant activity assessments. The prepared water and ethanolic extracts were stored at 4 °C until further use. All analyses were done in triplicate, and results were given as mean ± standard deviation. Extraction yield was calculated using Eq. (1).

$$\text{Extraction yield (\%)} = (\text{extract weight}/\ \text{dry plant weight}) \times 100 \quad (1)$$

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DPPH radical scavenging activity

The DPPH method, developed by Brand-Williams et al. (1995), was applied to determine antioxidant capacity [21]. In this method, a 20 mg L^{-1} DPPH solution was prepared. A volume of 0.75 mL of the extract or BHA standard solution, prepared in ethanol at concentrations ranging from 25 to 100 μg mL^{-1}, was mixed with 1.5 mL of the DPPH solution and vigorously vortexed. The mixture was then incubated in the dark at room temperature for 30 minutes. After incubation, absorbance was measured at 517 nm, the wavelength at which DPPH shows maximum absorbance. Ethanol was used as a blank solution and also as a substitute for the sample in the control reaction.

The calculation of the DPPH radical scavenging activity was performed using the following formula:

$$\text{DPPH Radical Scavenging Activity (\%)} = [((A_0 - A_1)/A_0) \times 100] \quad (2)$$

where A_0 indicates the absorbance of the control reaction, while A_1 signifies the absorbance of the sample or standard solution.

DMPD radical scavenging activity

The DMPD free radical scavenging technique was measured using the method described by Fogliano et al. (1999) [22]. In this method, DMPD is converted to its cationic radical form in the presence of an oxidizing agent at acidic pH.

To generate the DMPD radical, 1 mL of 100 mM DMPD solution was mixed with 100 mL of 0.1 M acetate buffer (pH 5.3) and 0.2 mL of 0.05 M $FeCl_3$ solution. From the resulting radical solution, 1 mL was taken and mixed with extracts or BHA (prepared in ethanol) standard solutions at various concentrations (ranging from 50 to 200 μg mL^{-1}). After 10 minutes of incubation, the absorbance was measured at 505 nm using a spectrophotometer. The percentage of DMPD radical scavenging activity was calculated using Eq. (2). A control solution was prepared with distilled water instead of the samples. Measurements were taken by using distilled water as a blank.

ABTS radical scavenging activity

The ABTS radical scavenging assay was performed according to the method described by Arnao et al. (2001) [23]. This method is based on the reduction of the ABTS radical, generated via the oxidation of ABTS with potassium persulfate ($K_2S_2O_8$), upon the addition of an antioxidant-containing sample, resulting in decolorization. To prepare the ABTS, 7.4 mM ABTS solution and 2.6 mM $K_2S_2O_8$ solution were mixed in equal volumes and kept in the dark at room temperature for 12 hours. From the resulting radical solution, 4.5 mL was taken and diluted with 160 mL of methanol until the absorbance at 734 nm was adjusted to 1.1 ± 0.02. Then, 150 μL of sample solutions at various concentrations (25–100 μg mL^{-1}) were mixed with 2850 μL of the ABTS radical solution and incubated in the dark for 2 hours. A control solution was prepared using methanol instead of the sample. At the end of the incubation period, the absorbance was measured at 734 nm using a spectrophotometer. The percentage of ABTS radical scavenging activity was determined using Eq. (2). Methanol was used as a blank solution.

Reducing power

The reducing power of the samples was determined according to the procedure outlined by Oyaizu (1986) [24]. Briefly, 1 mL of each extract or 1 mL of the standard antioxidant solution (BHA in ethanol) at concentrations ranging from 25 to 100 μg mL^{-1} was added to the reaction system. Subsequently, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % (w/v) potassium ferricyanide solution were incorporated. The reaction mixture was incubated at 50 °C for 30 minutes. After incubation, 2.5 mL of 10 % (w/v) trichloroacetic acid (TCA) was added, and the samples were centrifuged at 3000 rpm for 10 minutes. 2.5 mL supernatant was collected, combined with 2.5 mL of distilled water and 0.5 mL of 0.1 % (w/v) ferric chloride ($FeCl_3$) solution. The absorbance of the resulting solution was measured at 700 nm using a UV-Vis spectrophotometer.

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Metal chelating activity

The metal chelating activity was assessed based on the method developed by Decker and Welch (1990) [25]. For this assay, 1 mL of extract at a 1 mg mL^{-1} concentration was mixed with 3.7 mL of distilled water and 0.1 mL of $FeCl_2$ solution. The mixture was left at ambient temperature for 30 minutes. Following this, 0.2 mL of Ferrozine solution was added, and the mixture was incubated for an additional 10 minutes. The absorbance was measured at 562 nm. Ethylenediaminetetraacetic acid (EDTA, 0.1 mM, in distilled water) was used as the reference chelating agent. The inhibition of Ferrozine–Fe^{2+} complex formation was calculated using Eq. (2). Measurements were taken against distilled water as a blank, and distilled water was used instead of the sample for the control reaction.

β-carotene bleaching test

The β-carotene bleaching assay was performed following the methodology described by Bruni et al. (2004) [26]. In this method, 10 mg of trans-β-carotene was dissolved in 10 mL of chloroform. From this solution, 0.4 mL was mixed with 40 mg of linoleic acid and 400 mg of Tween-40. After complete evaporation of chloroform, 100 mL of distilled water was added, and the mixture was homogenized to form a stable emulsion. An aliquot of 5 mL of the emulsion was then combined with 0.2 mL of the extract (1 mg mL^{-1}). The reaction mixture was incubated at 50 °C, and absorbance at 470 nm was measured at 60 and 120 minutes using distilled water as the blank. Butylated hydroxyanisole (BHA, 1 mg mL^{-1} in ethanol) was used as a positive control. The results were given as relative antioxidant activity relative to BHA.

Determination of total phenolic content

Determination of total phenolic content was carried out spectrophotometrically using the Folin–Ciocalteu method, as described by Slinkard and Singleton (1977) [27]. For the assay, 1 mL of the sample or pyrocatechol standard solution was mixed with 45 mL of distilled water and 1 mL of Folin–Ciocalteu reagent, then vigorously shaken. After 3 minutes, 3 mL of 2 % (w/v) sodium carbonate (Na_2CO_3) was added, and the reaction mixture was incubated at room temperature for 2 hours. Absorbance was measured at 760 nm. A calibration curve ($y = 0.0012x + 0.0004$, $R^2 = 0.9954$) was generated using pyrocatechol at concentrations ranging from 10 to 100 µg mL^{-1}, and results were expressed as µg pyrocatechol equivalent (PE) per mg of extract. A solution containing distilled water, rather than the sample, was used as a blank.

Determination of total flavonoid content

Total flavonoid content was determined according to the protocol described by Zhishen et al. (1999) [28]. Initially, 0.25 mL of extract or standard catechin solution was mixed with 1.25 mL of distilled water. Then, 75 µL of 5 % (w/v) sodium nitrite and 150 µL of 10 % (w/v) aluminum chloride solution were sequentially added. After 5 minutes, 0.5 mL of 1 M sodium hydroxide (NaOH) was added, and the total volume was adjusted to 2.5 mL with distilled water. The absorbance was recorded at 510 nm. Results were calculated from the catechin calibration curve ($y = 0.0031x + 0.006$, $R^2 = 0.9983$) prepared at concentrations ranging from 10 to 50 µg mL^{-1} and expressed as µg catechin equivalent (CE) per mg of extract. A solution containing distilled water, rather than the sample, was used as a blank.

Determination of anthocyanin

Anthocyanin content was quantified using a modified method described by Padmavati et al. (1997) [29]. The powdered extracts were mixed with acidified methanol and incubated at 4 °C in the dark for 24 hours. After that, the mixture was filtered and centrifuged at 1000 rpm for 15 minutes. Absorbance of the supernatant was recorded at 530 nm and 657 nm. Acidified methanol was used as a blank solution. The anthocyanin concentration was calculated using the following formula:

$$\text{Anthocyanin content (mmol/g)} = [(A_{530} - 0.33 \times A_{657}) / 31.6 \text{ x } l] \times [V \text{ (mL)} / m \text{ (g)}] \quad (3)$$

In Eq. (3), 0.33 was used as a correction factor for chlorophyll interference, and 31.6 M^{-1} x cm^{-1} was the extinction coefficient for anthocyanin. l was the path length of the cuvette, V was used as volume, and m was used as the weight of the sample.

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Results and discussion

DPPH radical scavenging activity

The DPPH radical, due to the presence of an unpaired electron, exhibits a purple color and shows maximum absorbance at 517 nm. When antioxidant compounds interact with DPPH, they donate an electron or a hydrogen atom, reducing the radical and causing a change from purple to yellow. The decrease in absorbance is directly proportional to the radical scavenging capacity of the tested substance [30]. The DPPH radical-scavenging activities of *L. orientalis* Mill. fruit extracts are presented in Fig. 1. The highest activity was observed with the water extract at 100 µg mL^{-1}, with a scavenging rate of 91.84 ± 0.64 %. This activity was comparable to that of BHA, a well-known standard antioxidant, which showed a scavenging rate of 96.32 ± 0.46 %.

In a study by Çetinkaya et al., the fruits of *L. orientalis* Mill. were extracted using hexane, methanol, and ethyl acetate. The antioxidant activity was assessed using the DPPH radical scavenging assay. The reported IC_{50} values were 134.394 ± 1.17 µg mL^{-1} for the hexane extract, 55.264 ± 0.56 µg mL^{-1} for the methanol extract, and 53.394 ± 3.11 µg mL^{-1} for the ethyl acetate extract [31]. Similarly, Okmen et al. [18] investigated the ethanol, methanol, and ethyl acetate extracts of *L. orientalis* Mill. leaves. Using the DPPH method, they evaluated antioxidant activity and found that at 40 mg mL^{-1}, the acetone extract showed 35 % inhibition, the ethanol extract 82 %, and the methanol extract 86 % [18]. These findings regarding antioxidant activities in leaves and fruits extracted with various solvents support the antioxidant capacity demonstrated in the current study.

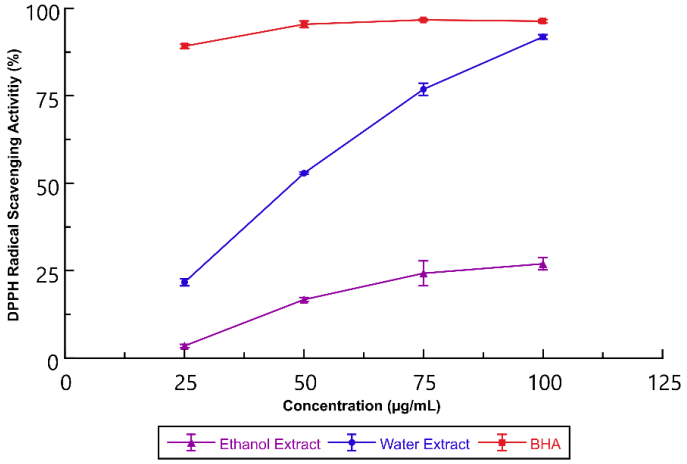

Fig. 1. DPPH radical scavenging activities of extracts from *L. orientalis* Mill. fruits and standard synthetic antioxidant (BHA).

DMPD radical scavenging activity

The DMPD radical-scavenging method is based on the oxidation of DMPD to its cationic radical in the presence of ferric chloride ($FeCl_3$), which yields a purple-red solution. Antioxidant compounds reduce this radical, resulting in a decrease in absorbance, which serves as an indicator of antioxidant capacity [22].

To evaluate the scavenging activity of the DMPD cationic radical, water extract, ethanol extract, and BHA standard were used. The results obtained are shown in Fig. 2. The water extract demonstrated the highest radical scavenging activity, surpassing the BHA standard at all tested concentrations. At a concentration of 200 µg mL^{-1}, BHA exhibited 41.22 ± 0.71 % scavenging activity, while the water extract showed a significantly higher activity of 66.07 ± 1.14 %. The ethanol extract exhibited a comparatively lower activity of 24.73 ± 3.75 %.

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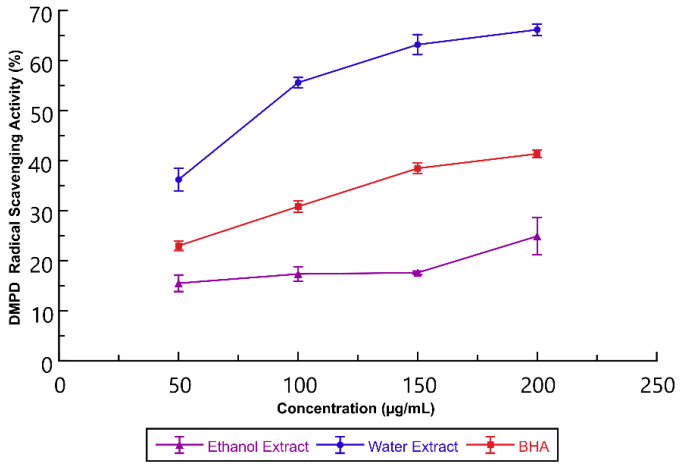

Fig. 2. DMPD radical scavenging activities of extracts from *L. orientalis* Mill. fruits and standard synthetic antioxidant (BHA).

ABTS radical scavenging activity

The ABTS assay relies on the formation of the green-blue ABTS radical cation, which undergoes reduction in the presence of antioxidant compounds. This reduction leads to a measurable decrease in absorbance, and the extent of this change is directly proportional to the antioxidant capacity of the tested sample [32].

The ABTS radical-scavenging activities of the standard solution and the extracts are shown in Fig. 3. Among the tested extracts, the water extract showed the highest activity, with a value of 15.02 ± 0.56 % at 100 µg mL^{-1}. In comparison, the ethanolic extract exhibited a markedly lower activity of 3.69 ± 0.1 % at the same concentration. The activity of the standard antioxidant BHA was calculated as 99.57 ± 0.06 %. For all four tested concentrations, the ABTS scavenging activity of BHA was significantly higher than that of the extracts.

In a study conducted by Baloğlu et al., the antioxidant activity of *L. orientalis* Mill. gum was evaluated using the ABTS radical scavenging method. In this method, the ABTS radical scavenging activity of the gum was reported as 15.56 mg Trolox equivalent (TE) per gram of gum [17].

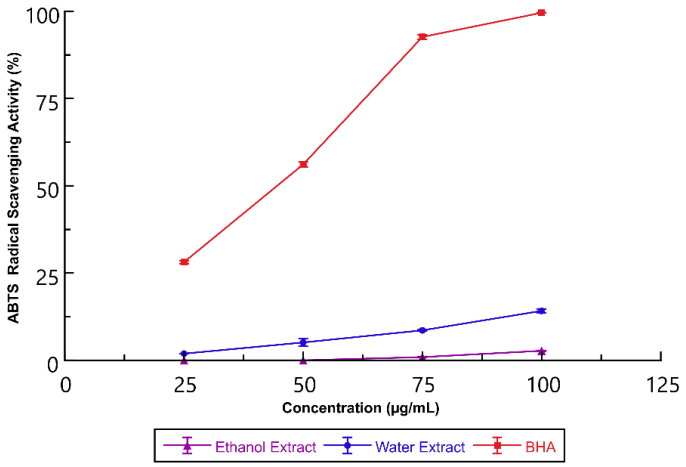

Fig. 3. ABTS radical scavenging activities of extracts from *L. orientalis* Mill. fruits and standard synthetic antioxidant (BHA).

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Reducing power

In the reducing power assay, the ability of extracts at different concentrations to reduce Fe^{3+} to Fe^{2+} was investigated by comparing them with the standard antioxidant, BHA. An increase in absorbance is directly proportional to the amount of Fe^{2+} formed in the medium. According to the results, the ethanol and water extracts of *L. orientalis* fruit exhibited the highest reducing power at 100 µg mL^{-1}. The BHA standard demonstrated the highest reducing ability. Although the extracts did not exhibit as strong reducing power as the standard, they were shown to interact with free radicals, transforming them into more stable and inactive compounds.

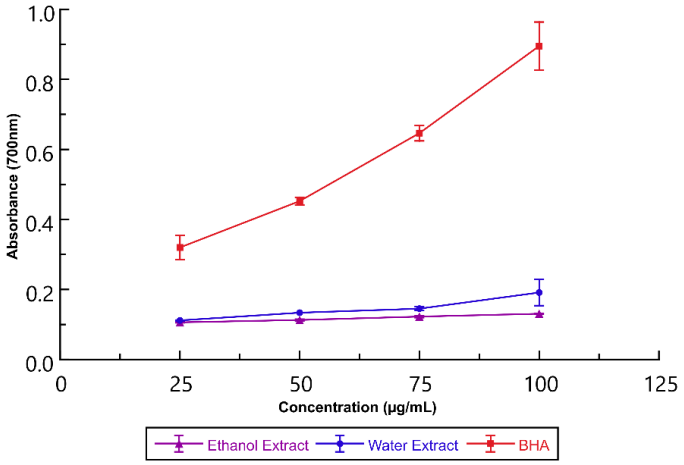

Fig. 4. Reducing power of extracts from *L. orientalis* Mill. fruits and standard synthetic antioxidant (BHA).

Metal chelating activity

Ferrozine is capable of forming complexes with Fe^{2+} ions. However, the presence of chelating agents inhibits complex formation, resulting in a decrease in the complex's purple color intensity. The capacity to chelate metals is crucial because it lowers metal concentrations, thereby preventing lipid peroxidation. Furthermore, metal chelating compounds are classified as secondary antioxidants because they reduce the redox potential and stabilize oxidized metal ions [33]. The effect of chelators was measured using the metal chelating assay, which is based on the reduction in purple color intensity. Compared with EDTA, a well-known strong chelating agent, the ethanol extract showed no measurable chelating activity. However, the water extract demonstrated chelating activity of 7 ± 0.38 %, indicating that it can chelate metal ions.

β-carotene bleaching test

In this method, antioxidant molecules prevent β-carotene from losing its double bonds and color. If antioxidant molecules are not present, free radicals react with β-carotene, and the conjugated double bond system breaks down, ultimately causing β-carotene to lose its color [34]. In the β-carotene bleaching assay, the water extract of the *L. orientalis* fruit demonstrated activity of 0.97 ± 0.01, similar to that of the BHA standard during the first hour. This activity decreased slightly in the second hour, resulting in a value of 0.94 ± 0.003, which was slightly lower than that of BHA. On the other hand, the ethanol extract showed an activity of 0.98 ± 0.01 during the first hour, nearly identical to that of BHA, but this value declined to 0.92 ± 0.01 in the second hour. These findings indicate that the extracts exhibit radical inhibition levels comparable to those of BHA.

In a study by Saraç et al., a β-carotene bleaching assay was performed using the ethanol extract of the leaf, yielding an antioxidant activity of 57.4 ± 3.85 % at 0.5 mg mL^{-1} [15].

Extraction yield

The extraction yields of the water and ethanol extracts were 19.83 ± 1.99 % and 4.37 ± 0.71 %, respectively. Çetinkaya et al. reported the extraction yields of *L. orientalis* fruit with hexane, ethyl acetate, and methanol (6 x 2.5 L each) as 2.83, 0.47, and 10.33 %, respectively [31].

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Determination of total phenolic content

Polyphenolic compounds, due to their abundance of hydroxyl functional groups, exhibit strong radical-scavenging activity by donating hydrogen atoms or electrons to neutralize ROS. Consequently, they play a critical role in terminating free radical chain reactions and in preventing or attenuating various oxidative stress-related disorders [35]. Based on the Folin–Ciocalteu method, the total phenolic content was determined as 38.81 ± 1.35 μg PE mg^{-1} in the water extract and 28.14 ± 0.97 μg PE mg^{-1} in the ethanolic extract. The higher total phenolic content of the water extract might be the reason for better antioxidant activity.

In a separate study, Saraç and Şen investigated the phenolic constituents of the ethanolic extract of *L. orientalis* Mill. leaves. Their analysis revealed the presence of several key phenolic compounds, including gallic acid, protocatechuic acid, (+)-catechin, caffeic acid, (−)-epicatechin, p-coumaric acid, trans-cinnamic acid, quercetin, kaempferol, and apigenin. The study also reported the total phenolic content of the extract as 333.14 ± 7.96 mg gallic acid equivalent (GAE) per gram [15]. Çetinkaya et al. reported that hexane, ethyl acetate, and methanol extracts of fruits of *L. orientalis* contained 27.71 ± 0.05, 62.41 ± 0.15, and 36.82 ± 0.01mg GAE per gram, respectively. Extracts of leaves contained 25.08 ± 0.08, 47.46 ± 0.11, and 42.30 ± 0.04 mg GAE per gram total phenolic content, respectively [31]. In the study by Ulusoy et al., the total phenolic content of the leaf extract was reported as 96.34 ± 1.75 mg GAE per gram [16].

Determination of total flavonoid content

Flavonoids, which constitute the most important subgroup of polyphenolic compounds found in plants, are known for their high antioxidant activity and serve as major contributors to radical scavenging capacity. The total flavonoid content in plants plays a significant role in achieving elevated antioxidant activity levels. When comparing the flavonoid contents of the extracts, the ethanol extract of *L. orientalis* Mill. fruit contained 44.52 ± 1.28 μg CE mg^{-1}, while the water extract contained 12.11 ± 0.58 μg CE mg^{-1}.

In a related study, Ulusoy et al. examined the ethanol extract of *L. orientalis* leaves and reported a total flavonoid content of 2.15 ± 0.36 mg QE g^{-1}, expressed as quercetin equivalent (QE). This notable flavonoid content in the leaves may be associated with flavonoid compounds in the fruit and with the fruit's strong antioxidant activity [16]. Çetinkaya et al. reported that hexane, ethyl acetate, and methanol extracts of the fruit of *L. orientalis* contained 9.46 ± 0.7, 9.73 ± 0.57, and 8.20 ± 1.92 mg QE per gram. In the same study, the total flavonoid contents of the extracts of leaves were given as 5.50 ± 1.71, 10.06 ± 0.3, and 25.02 ± 0.66 QE per gram for hexane, ethyl acetate, and methanol extracts, respectively [31].

Determination of anthocyanin

Anthocyanins, a major subgroup of flavonoids, are pigments that impart color to plants and help prevent cellular damage by neutralizing free radicals [36]. When the anthocyanin content of the *L. orientalis* fruit was measured, it was found to be 0.18 ± 0.003 mmol g^{-1}.

Conclusions

In this study, ethanol and water extracts of ground and ultrasound-assisted extraction of *L. orientalis* Mill. fruits were used. The antioxidant activities of the extracts were determined using different antioxidant capacity assays, and the values obtained were compared with those of BHA, a widely used synthetic antioxidant. Considering all experimental findings, extracts obtained from *L. orientalis* fruits, especially water extract, exhibited remarkably high antioxidant capacity. This antioxidant capacity may be due to the phenolic content of the water extract. This study highlights the potential of a plant-based material, often regarded as waste, to serve as a natural antioxidant source, especially when processed via the eco-friendly, sustainable ultrasound-assisted extraction method. These findings suggest that such natural extracts not only offer bioactive compounds with significant antioxidant potential but also contribute to waste valorization and sustainable environmental practices. Accordingly, the study presents a promising alternative not only for developing natural antioxidant agents that support public health but also for integrating scientific innovation into waste management strategies.

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