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ORIGINAL ARTICLE

Determination of antioxidant and antimicrobial activity of sweetgum (*Liquidambar orientalis*) leaf, a medicinal plant

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Abstract

In the study, sweetgum tree (*Liquidambar orientalis*), which is an endemic species that grows in Mugla, Köyceğiz and is applied for medicinal purposes among the public, its leaves was examined. The antioxidant ability of the extract obtained from dried plant leaves has been evaluated using a variety of methods which are Total Phenolic Substance, Total Flavonoid, FRAP, CUPRAC, DPPH, and $ABTS^+$. Simultaneously, the antimicrobial activity of the plant extract was examined using disk diffusion and microdilution methods to determine the minimum inhibitor concentration (MIC). While the total phenolic content of *Liquidambar orientalis* extract was 96.34 mg GAE/g, the total amount of flavonoid was 2.15 mg QE/g. When the results of the antioxidant analysis were examined, it was observed that it had a good level of antioxidant activity with the results of 49.25 ± 0.54 mmol TEAC/g according to the CUPRAC method, 39.83 ± 0.25 µmol Fe/g according to the FRAP method, 80.34 µg/mL according to the DPPH method and 51.20 µg/mL according to the ABTS+ method. As a result of the antimicrobial analysis, it was indicated that *L. orientalis* extract was more effective on *Staphylococcus aureus* (*S. aureus*), which is a gram-positive bacterium and causes a wide variety of clinical diseases. Even, *L. orientalis* extract with an MIC value of 10 mg/mL has been found to have a higher antibacterial effect than Amoxicillin+Clavulanic acid, which is used as a standard drug in that field. This research is significant because it is the first to report the determination of all biological activities for *L. orientalis*, including total polyphenols, flavonoid contents, antioxidant content, and antimicrobial activity.

Keywords: *sweetgum tree, diary tree, medicinal plant, antioxidant, antimicrobial.*

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1. Introduction

From past to present, people have benefited from plants for nutrition, shelter, heating, healing wounds and curing diseases. It has been determined that there are 250 plants that people used in treatments in the 5000s B.C. Hittites, Egyptians, Sumerians, Assyrians and Mesopotamians have used herbs for years.The introduction of synthetic drugs into production over time has led to a decrease in the use of medicinal and aromatic plants. However, after the 1900s, when people discovered the side effects of synthetic drugs and became aware of the harmful effects of synthetic substances in food and beverages on human health, the demand for natural products increased[1].

Many medicinal plants have been discovered by humankind by trial and error. In Turkey, medicinal aromatic plants are commonly used in daily life to treat a variety of diseases. Turkey is an attractive source of medicinal plants because of its diverse flora and attention to a variety[2]. It is known that plant extracts and components exhibit important biological activities, especially antimicrobial[3], antifungal[4], antibacterial[5], and antioxidant activities[6]. Most medicines are now manufactured pharmacologically, with herbal origins accounting for 25% of them. Free radicals are generated throughout the body by the toxic air we breathe during the day, poisonous compounds in spoiled foods, additives, unconscious eating, and inactivity. Oxygen atoms broken off by these harmful effects from outside circulate freely in the body, breaking down hydrogen atoms and causing tissue damage. Free radicals especially attack the cell and immune system. Molecules that minimize and block the effect of free radicals in the body and prevent chain reactions that may cause many diseases and premature aging are called "antioxidant" substances[7]. Prevention of free radical-mediated reactions that lead to difficult-to-treat problems such as aging, cancer, and diabetes is only possible with the help of antioxidant compounds[8]. As is known, antioxidants are mostly found in green and red-leaved plants. At the same time, vitamins A, C, and E show natural antioxidant properties[7].

Due to the increase of microorganisms with multiple antibiotic resistance in recent years, the treatment of the infection caused by these microbes has become increasingly

intractable. Drug resistance increases and spreads in bacteria that develop resistance to all known antibiotics. As a result, using medicinal plants as an alternative to medications is recommended, and some popular herbs are used as antimicrobials[9]. Many phytochemical compounds are found in vegetable-derived products, and they have been shown to have high antioxidant and antimicrobial properties in the literature[10]. Plant extracts and essential oils have been studied extensively for their antioxidant and antimicrobial properties[11].

Many phytochemical compounds are found in vegetable-sourced products, and they have been shown to have high antioxidant properties as well as good antimicrobial activity in the literature[10]. There are many studies on the antioxidant and antimicrobial properties of plant extracts and essential oils[11]. There have been studies that show the sweetgum tree, which is an endemic species to the Muğla area, has medicinal properties. *Liquidambar orientalis*, known locally as the sweetgum tree, and its products (leaves, bark, sweetgum oil, etc.) have the natural protective potential[12-14]. *Liquidambar orientalis*[15], one of the four sweetgum tree species present in Turkey today, has been around for about 60 million years. People living in the province of Muğla and its surroundings apply sweet gum trees for shortness of breath, bronchitis, and so on. It is also applied as incense because it helps the healing process of respiratory system diseases. In addition, the essential oil extracted from sweetgum trees is used in the formulation of many natural perfumes, as well as in the soap industry and in the fragrance of gum and tobacco. It is also claimed that it is used in the form of pomade and patch against skin diseases such as scabies, fungus, stomach, and duodenal ulcers, as well as being a healthy antiseptic and parasitic[12-14].

In light of this knowledge, the antioxidant and antimicrobial properties of the leaf of the sweetgum tree (*L. orientalis*), which plays an important role as a medicinal aromatic plant, were investigated in this research.

2. Materials and Methods

2.1 Material (chemicals/plant)

2,4,6-tripyridyl-s-triazine(TPTZ), Folin-Ciocalteu's phenol reagent, Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'-azinobis (3-ethyl-beothiazoline 6 sulfonate) (ABTS) were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Neocuproine (2,9-dimethyl-1,10-phenanthroline), acetic acid, ammonium acetate, aluminium nitrate nonahydrate, potasyum persülfat ($K_2S_20_8$), and sodium carbonate, were bought from Merck Chemical Co. (Darmstadt, Germany). The chemicals were analytical degrees. The subject of the study is *L. orientalis* (sweetgum) leaves and the herbal material was obtained from Muğla-Köyceğiz. *L. Orientalis* plant was authenticated by Prof. Dr. Temel Göktürk, a forest engineer. The dried sweetgum leaves are powdered. Powdered sweetgum leaves were extracted by brewing method with distilled hot water (80 °C). After the mixture had cooled, filtration was carried out with Whatman grade 1 filter paper. The obtained filtrate was then dried under vacuum with a freeze-dryer (lyophilizer) system, model Christ Freeze-Dryer Alpha 1-4 LD, and the crude extract was obtained. Solutions of different concentrations of the extract obtained were prepared and biological activity analyzes such as antioxidant and antimicrobial were tested.

2.2 Total phenolic assay

The total phenolic amount of samples was detected by using the Folin-Ciocalteu test[16]. Gallic acid (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/mL) was used as a standard in this work. Shortly, 400 µL of 0.5 N Folin-Ciocalteu tests, 20 µL methanolic plants (1 mg/mL), 680 µL of distilled water, and 20 µL of different concentrations of gallic acid were mixed and the mixture was vortexed. 400 µL of Na_2CO_3 (10%) solution was added after 3-minute incubation and again vortexed. Then the mixture was incubated for 2 hours. Following the incubation time at room temperature, absorbances of the mixtures were determined at 760 nm. The concentrations of total phenolic compounds were measured as mg of gallic acid equivalents per g of the dry weight of the sample.

2.3 Total flavonoid assay

The total flavonoid amount was determined by using the aluminum chloride test[17]. Quercetin was used as a standard. 4.3 mL methanol, 0.1 mL 1 M NH_4CH_3COO, 0.5 mL of Quercetin (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/mL), and 0.1 mL 10% $Al(NO_3)_3$ were put in the tubes and then they were mixed. Mixtures were incubated for 40 minutes. Following incubation, absorbance was determined at 415 nm. The total flavonoid contents of plants were defined as mg quercetin equivalents per g of dry weight sample.

2.4 The determination of antioxidant activity

The antioxidant activities of the samples were determined using by FRAP and CUPRAC methods. The FRAP method was used for the determination of total antioxidant capacity, based on the reduction of yellow Fe_3^+-TPTZ complex to the blue Fe_2^+-TPTZ complex by electron-donating substance under acidic conditions[18]. The 3 mL of FRAP reagent (containing TPTZ, $FeCl_3$, and acetate buffer) and 100 µL of the test sample or the blank (solvents used for extraction) were added to the test tube and mixed. Maximum absorbance values at 593 nm were recorded for 4 min at 25 °C. The final absorbance was compared with the standard curve (100-1000 µmol/L).

The data were expressed as µmol $FeSO_4.7H_2O$ equivalents per gram of dry matter. The CUPRAC method is comprised of mixing the antioxidant solution (directly or after acid hydrolysis) with a copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium acetate aqueous buffer at pH 7, and subsequently measuring the developed absorbance at 450 nm after 60 minutes[19] 1 mL 10 mM $CuCl_2$, 1 mL 7.5 mM Neocuproine and 1 mL 1M NH_4Ac were added to test tubes, then 0.2 mL sample and 0.9 mL H_2O were added and mixed. The final volume was 4.1 mL. Then, the final absorbance was measured at 450 nm after incubated 1 h. The test results were evaluated by Trolox® equivalent antioxidant capacity (TEAC).

The scavenging activity of DPPH• radical was determined using the method of Molyneux[20]. Different concentrations

of 0.75 mL of sample extracts were mixed with 0.75 mL of a 0.1 mM of DPPH• solution (dissolved in methanol). Then, extracts were incubated at room temperature in the dark for 50 min. Absorbance was measured by a spectrophotometer at 517 nm. Trolox is used as standards and the values are expressed as IC_{50} (mg sample per mL). The method developed by Re et al.[21] was applied to assess $ABTS^+$ removal activity. This method is based on the principle that the colored $ABTS^{\bullet+}$ cation radical changes color after treatment with the extract. 5 mL $ABTS^{\bullet+}$ cation radical was prepared by mixing ABTS (7 mM) solution with 2.45 mM potassium persulfate ($K_2S_20_8$) solution and incubated for 16 hours in the dark and at room temperature. By diluting 1 mL of this radical solution with ethanol, the absorbance was adjusted. At concentrations ranging from 500 g to 4000 g, 4 mL of ABTS solution prepared in ethanol was applied to samples containing 1 mL of sample. As a control, 1 mL of ethanol was used. At 734 nm, radical scavenging activity was measured after a 10-minute incubation period at room temperature.

2.5 The biological materials

All three microorganisms were used in this work. As bacteria; Escherichia coli (*E. coli*) ATCC 25922, *Staphylococcus aureus* (*S. aureus*) ATCC 6538P, as yeast: Candida albicans (*C. albicans*) ATCC 14053. All test microorganisms were got from the American Type Culture Collection (ATCC), the Faculty of Science of Muğla University, and the commercial culture collections. All microorganisms were stored at -85 °C (Ultrafreezer, New Brunswick) in 15% glycerol and protected on nutrient agar (Merck, 1.05450) and malt extract agar (Merck, 1.05398) slants at 4 °C, respectively. They were subcultured in Petri dishes before use for purity check. The microorganisms chosen for the antibacterial property studies are between significant herb and man pathogens and biofilm giving microorganisms.

2.6 In vitro antimicrobial activity

2.6.1 *Disk diffusion method*

The disk diffusion approach was used to investigate the effects of beef extract on Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 6538P, and *Candida albicans* ATCC 14053[22]. 1000 mL of liquid cultures that had reached 0.5 McFarland normal turbidity were transferred to sterile petri dishes, along with approximately 20 mL of Mueller Hinton Agar (Merck) for bacteria and Sabouraud Dextrose Agar (Merck) for *C. albicans*, and planting was done using disc diffusion method. Then, disks soaked with 20 µL of extract (at 400 mg/mL concentration) were appropriately placed on the agar. Sowed petri dishes were incubated for *S. aureus* and *E. coli* for 24 hours at 37 ± 0.1 °C and for *C. albicans* at 37 ± 0.1 °C for 48 hours. The diameter of the inhibition zones formed around the discs was measured in mm at the end of the incubation. Bacteria, Amoxicillin+Clavulanic acid disc (Oxoid) for *E. coli* and *S. aureus*, and nystatin (Oxoid) for yeast strain *C. albicans* were used as positive controls, as was sterile distilled water in which plant extract was dissolved as a negative control. All three experiments were conducted in parallel.

2.6.2 Broth microdilution test for bacteria

The minimum inhibition concentration (MIC) values of the extract on *S. aureus*, which was used in the study and whose inhibition effect was detected by the disk diffusion method, were determined by the broth microdilution method[23]. Serial dilutions of the extract (80, 40, 20, 10, and 5 mg/mL) were performed using (Mueller Hinton Broth (Merck) with a final volume of 2 mL. Bacterial suspensions were prepared at a concentration of 10^6 CFU/mL (using Mac Farland No: 0.5) with turbido for each test bacteria and 20 µL of suspension was added to each test tube. Tubes were incubated at 37 ± 0.1 °C for 48 hours and the lowest concentration without bacterial growth was determined as MIC. Measurements were carried out in triplicate and the results of the antimicrobial test were compared with standard Amoxicillin+ Clavulanic acid as antibacterial agents.

3. Results and Discussions

3.1 Extract (solution) feature

There are numerous diverse antioxidants in plants, and it is very difficult to measure each antioxidant component individually. The chemical complexity of the extracts, often a mixture of dozens of compounds with different functional groups, polarity, and chemical behavior, can lead to varying results depending on the test used. Therefore, it is more informative to utilize diverse tests to assess the antioxidant potential of each test[24,25]. In this research, four fundamental strategies, CUPRAC (copper decreasing control), FRAP (ferric decreasing control), DPPH• and $ABTS^{\bullet+}$ radical scavenging activity strategies were utilized.

At the same time, total phenolic and flavonoid contents were determined for the extract. In these tests, the UV spectrophotometric method was applied. Spectrophotometric strategies are frequently utilized for the standardization of natural raw materials. Total phenolic and total flavonoid contents, FRAP and CUPRAC values are presented in Table 1 and the results shown in the tables refer to the average ± SD of three parallel measurements. IC_{50} values determined from DPPH and $ABTS^+$ analyzes are given in Figure 1 and Figure 2. IC_{50} values were calculated from linear regression analysis (Microsoft Excel, Microsoft Corporation®, USA).

The total polyphenol content of *L. orientalis* leaf extract, which is endemic in the Muğla region, was 96.34 ± 1.75 mg GAE/g, and the total flavonoid content was calculated as 2.15 ± 0.36 mg QE/g. According to the antioxidant activity results, it was found to have 49.25 ± 0.54 mmol TEAC/g according to CUPRAC analysis and 39.83 ± 0.25 µmol Fe/g according to FRAP analysis. According to the antioxidant activity results, it was found to have 49.25 ± 0.54 mmol

Table 1. Results of phenolic contents, flavonoid contents, FRAP and CUPRAC for *L. orientalis* medicinal plant.

Sample	Total phenolics (mg GAE/g)	Total flavonoid (mg QE/g)	CUPRAC (mmol TEAC/g)	FRAP (µmol Fe/g)
L. orientalis	96.34 ± 1.75	2.15 ± 0.36	49.25 ± 0.54	39.83 ± 0.25

TEAC/g according to CUPRAC analysis and 39.83 ± 0.25 µmol Fe/g according to FRAP analysis (Table 1). When DPPH and $ABTS^+$ radical scavenging activity results are examined, it is given that it has IC_{50} concentrations of 80.34 µg/mL for DPPH and 51.20 µg/mL for ABTS+ (Figure 1 and Figure 2). According to these results, it is observed that ABTS+radical scavenging activity of *L. orientalis* leaf is higher than DPPH radical scavenging activity.

In the antimicrobial activity studies of the aqueous extract tested against microorganisms in the current study, the inhibition diameters obtained according to the disk diffusion method are given Figure 3 and Table 2, and the minimum inhibition concentrations (MIC) results are presented in Table 3.

3.1.1 *Staphylococcus aureus* (*S. aureus*) ATCC 6538P

When the antimicrobial activity of *L. orientalis* leaf extracts was evaluated in vitro against three test microorganisms known to cause some diseases in foods. According to the antimicrobial activity results obtained, It has been found, while *L. orientalis* plant extract does not show any activity against *E. coli*, a gram-negative bacterium, and *C.albicans*, a yeast strain, *L. orientalis* plant extract shows very good activity against *S. aureus*, which is a gram-positive bacterium, causing various clinical diseases. While the MIC value obtained for *L. orientalis* has a concentration of 10 mg/mL, the MIC value of Amoxicillin+Clavulanic Acid, which is used as a standard drug in that area, is 40 mg/mL. Therefore, *L. orientalis* extract antibacterial activity is much higher than the standard drug used in that field.

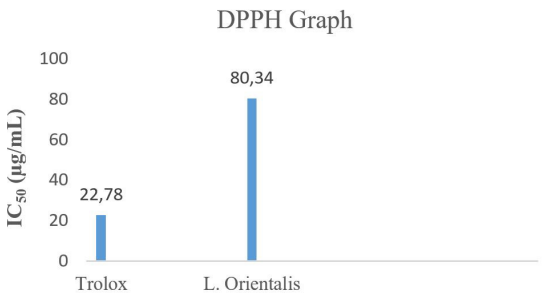

Figure 1. The result of DPPH for *L. Orientalis* extract.

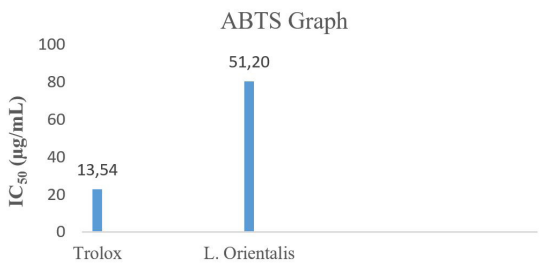

Figure 2. The result of $ABTS^+$ for *L. Orientalis* extract.

Medicinal plants are traditionally applied worldwide for the treatment of various human diseases[26]. Many of these have proven to be abundant sources of biologically active compound ingredients used as a tool to develop new pharmaceutical compounds[27]. Although the antibacterial and antioxidant activities of many plant species have been extensively investigated, the antimicrobial and antioxidant mechanism of the *L. orientalis* plant included in this research has not been reported in detail.

The research conducted in İzmir, Turkey[28], on *L. orientalis* leaves obtained from Mugla like in our study, the total polyphenol amount for the leaves of the plant was found as 0.37 mg GAE/g and this value was found to be much higher as 96.34 ± 1.75 mg GAE/g in our study. Although the plant was obtained from the same region, the extraction solvent was a 70% aqueous ethanol solution in the study conducted in Izmir, while only water was used as the extraction solvent in our research. The number of phenolic compounds found in plants; solvents are affected by many factors such as temperature, extraction time, particle size, sample type, and extraction methods[29]. According to the antimicrobial activity results, while the MIC value against *S. aureus* was 0.4 mg/mL in the literature[28], this value is 10 mg/mL in our study. *L. orientalis* leaf extract has shown the highest antibacterial activity against *S. aureus*, which causes various diseases, both in our study and in the literature. Additionally, according to the study conducted in İzmir, $ABTS^+$ radical removal activity was 8.009 TEAC (mmol/g sample), whereas in our study it was investigated to have antioxidant activity with a value of 51.20 µg/mL.

In a research conducted by Saraç and Şen[30], DPPH radical scavenging activity was investigated to be 3.11 ± 0.024 mg/mL for the ethanol extract of *L. orientalis* leaf obtained from Köyceğiz, Muğla. In our study, for the extract of *L. orientalis* leaf in water, DPPH radical scavenging activity value was measured as 0.080 ± 0.42 mg/mL and it can be accepted as higher DPPH antioxidant activity than the literature.

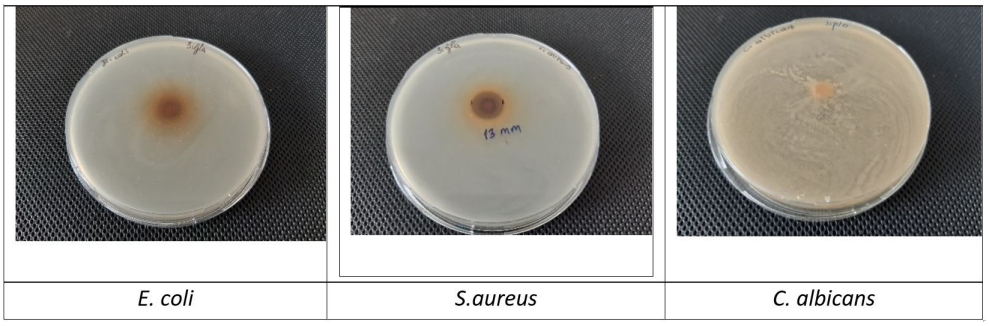

Figure 3. Photos of Inhibition Zone Diameters (mm) for *L. Orientalis* extract.

Table 2. Antimicrobial activity of *L. orientalis* medicinal plant.

Samples	Zone of Inhibition Diameters (mm)		
	E.coli	***S. aureus***	***C. albicans***
L. orientalis	-	13	-
Amoxicillin+Clavulanic Acid	16	20	-
Nystatin	-	-	19

Notemean, Escherichia coli (*E. coli*) *ATCC 25922, Staphylococcus aureus* (*S. aureus*) *ATCC 6538P, as yeast: Candida albicans* (*C. albicans*) ATCC 14053, (-): no activity of test concentrations.

Table 3. MIC values of *L. orientalis* medicinal plant against the bacterial strains tested.

Samples	Minimal Inhibition Concentration Values (mg/mL)
	S. aureus
L. orientalis	10
Amoxicillin+Clavulanic Acid	40

In a study conducted by Sağdıç et al.[31], the antibacterial effect of the extract of the juice obtained from the wood and inner bark of the sweetgum tree in ethanol against various microorganisms was examined according to the disc infusion method and according to the result, *L. orientalis* secretion did not have any activity against *E.coli* while it has good antibacterial activity against *S. aureus* with its inhibition zone diameter of 14 mm. Similarly, in our research, while the extract of *L. orientalis* leaf in water did not show any effect against *E. coli*, it showed a similar amount of activity against *S. aureus* with its inhibition zone diameter of 13 mm.

In another study[32], the antioxidant activities of *L. orientalis* plant extract obtained from Mugla Köyceiz using acetone, ethanol, and methanol solvents, as well as antimicrobial activities against eight test microorganisms, were investigated using the DPPH method, and it was discovered that they had strong antimicrobial and DPPH activity. It is assumed that this difference may be due to the change in extraction solvent and method used[29].

Factors such as biological activity studies, composition and amount of active ingredients available in the plant, genetics (i.e. genus, species, cultivar/genotype) and geographical areas, growth conditions of plant material, climatic factors, ripening stage, harvest time, storage condition and post-harvest management are also affects[33-35].

Although there are antioxidant and antimicrobial studies on strains *Liquidambar styraciflua L*[36-38]. ve *Liquidambar formosana*[39-41], there are few reports on *Liquidambar orientalis*, an endemic species that we investigated. Especially studies on antioxidant activity are very limited, and only a few antioxidant activity studies have been conducted by using DPPH and $ABTS^{+}$ methods.

This study is significant in that it is the first study reporting the determination of both total flavonoid content and antioxidant content of *L. orientalis* plant leaf according to CUPRAC and FRAP methods, unlike the previous studies in the literature. In other words, it will be the first research to report the determination of all biological activities such as total polyphenol, flavonoid contents, antioxidant content, and antimicrobial activity for *L. orientalis* together, and will shed light on the scientists who will work on this species. Phenolic compounds are very essential and significant components of plants and the ability of phenolic compounds to scavenge radicals is due to their hydroxyl groups. Phenolic compounds can directly contribute to the antioxidative effect[42]. Flavonoids are well-known antioxidants and natural phenolic compounds. The antioxidant efficacy of plant extracts rich in flavonoids is very high in various studies[43].

4. Conclusions

This study demonstrated that aqueous extracts of *L. orientalis* have good antioxidant and antimicrobial activity. With a MIC value of 10 mg/mL, it was found to have a higher antibacterial effect on *S. aureus*, a gram-positive bacterium that causes a wide variety of clinical diseases, even than Amoxicillin + Clavulanic acid, which is used as a standard drug in that field. Therefore, *L. orientalis* can be used as sources of natural antimicrobial agents. The antioxidant and antimicrobial properties of extracts obtained from a variety of plants are of great interest to academics as well as the food, cosmetics, and pharmaceutical industries. Since there is a growing trend to substitute synthetic preservatives with natural ones, they can be applied as natural additives. In this respect, it is very critical to work with endemic plant species and to reveal unknown bioactive properties. The results of this study indicate that *L. orientalis* plant leaf, which is an endemic species, contains compounds with antioxidant and antibacterial activity. Due to these activities, the leaf of this plant can be applied in the preparation of medicinal and nutritious products.

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