



Phylogenetic Analysis of *Origanum vulgare* and Its Antioxidant and Antimicrobial Activity

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Highlights

- We carried out the molecular characterization and the phylogenetic analysis of thyme.
- We analyzed the antioxidant activity of the thyme using DPPH method.
- We investigated the antibacterial activity of the thyme using disc diffusion and MIC methods.
- We showed that *O. vulgare* isolate SRY61 has high biological activity compared to the literature.

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Abstract

Our country is home to many endemic plant species including medicinal, aromatic and spice. One of these species is thyme plant used both in daily life and for medical purposes. One of the most popular methods of phylogenetic classification of plants is the internal transcribed spacer (ITS) region which locates between the ITS1 and ITS2 regions that highly conserved. In this paper, a thyme species was used, which grows naturally in Sultan Murat Sarıkaya High-plateau in eastern Black Sea Region (Turkey) and phylogenetic analysis of oregano plant was carried out and antioxidant and antibacterial activity was investigated. The assessment of the antioxidant activity of the plant was researched using DPPH method using plant excretes prepared at various time intervals. The antibacterial activity of plant was studied by using disc diffusion and minimum inhibitory concentration (MIC) methods against Gram (+) and Gram (-) bacteria. The phylogenetic analysis was performed by obtaining the genomic DNA of the plant by the analysis of the specific DNA sequences used in the species identification, and the species of the plant was identified as *Origanum vulgare* and was identified as MH174928.1 (*Origanum vulgare* isolate SRY61) from Gen Bank. *O. vulgare* showed a high rate of antioxidant properties and showed antibacterial effect in accordance with the literature.

1. INTRODUCTION

A species of the genus *Origanum*, a member of the *Lamiaceae* family, *O. vulgare* is a perennial plant growing widely in Europe, North Africa, America and Asia [1]. It is found in areas that are dry and sunny, low humid and streamside [2]. The plant is known for its potent antibacterial, fungicidal and antiviral properties, as well as its nematicidal, antimutagenic and spasmolytic properties [3-5]. It contains various phenolics compounds [6].

It is estimated that there are 300000 plant species on earth. [7]. Today, phylogenetic analysis has become part of the systematics. Studies at the molecular level suggest that the analysis of ribosomal DNA (rDNA) ITS sites can be used to study a genus, species, and even populations because of the evolution of these regions [8]. ITS regions are powerful phylogenetic markers that show species differences at species level [9]. Nuclear ribosomal RNA genes (nrDNA) targeted at molecular classification studies are found in tandemly repeated and several loci [10,11] and nrDNA spacer regions are useful tools to species level in plant systematics.

Reactive oxygen species (ROS) and lipid peroxides are formed as the normal products of aerobic metabolism, but oxidative stress occurs when they are produced at high rates or if their elimination is

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reduced. In this process, ROSs attack and damage macromolecules [12-14]. Some of the products in the structure of the plant are rich in phytochemicals and have strong and diverse biological and pharmacological properties [15,16]. Nowadays, plants with these properties draw interest because they are sources of natural antioxidants and prevent the negative effects of ROS and free radicals which cause cell destruction [17]. Studies showing the antioxidant activity of oregano, which is one of these plants, mainly dedicate this property to carvacrol and thymol [18].

The use of antibiotics, which caused significant reduction of many infectious diseases since their discovery, has become highly risky due to the resistance of microorganisms in which they are effective nowadays and even in 2011, WHO used "Antimicrobial resistance: no action today, no cure tomorrow". This process has led the world of science to produce natural products of plant origin, which contain very few side effects and contain antimicrobial components [19-22]. Especially in some African and Asian countries, the majority of the population benefit from herbal medicinal products in primary health care [23]. Plants oils and extracts with antimicrobial properties have been implicated in many applications, such as pharmaceuticals, alternative medicine and natural treatments, in the preservation of raw and processed foods [24].

It is important to standardize the herbal extracts with protective and therapeutic properties and to protect these plants. It is seen as an important advantage that these naturally sourced compounds reduce the resistance development mechanism in bacteria and target more than one bacterium at the same time. Moreover, some herbal-derived antimicrobials have been accepted as "Generally Recognized as Safe" (GRAS), with a "greener" [25]. Thyme and oregano plants contain bioactive molecules such as thymol and carvacrol in their structures. These molecules, called monoterpenes, have antibacterial effects on bacteria [26]. Carvacrol and thymol have the capacity to break down the outer membrane of G (-) bacteria and disrupt the integrity of the cell membrane structure of G (+) bacteria [27]. ATP synthesis is inhibited because of the change of osmotic pressure due to ion concentration due to the disruption of the membrane structure, followed by cell death [28]. Therefore, thymol and carvacrol are widely used as antibacterial in medicine, veterinary medicine and cosmetic industry [6,29].

In this study, the phylogenetic analysis of the oregano, which grows naturally in Sarıkaya Plateau in the eastern Black Sea Region of Turkey with an altitude of 2100 meters, with the ITS nucleotide sequence was carried out and its antioxidant and antibacterial effects were investigated.

2. MATERIAL METHOD

2.1. Plant Material

The oregano, which grows naturally in Sultan Murat Sarıkaya Plateau located at 40°38'37.2"N 40°09'03.3"E with an altitude of 2100 meters, was collected in August 2017. The oregano was dried in an area with no direct sunlight and light air flow.

2.2. Bacterial Strains

The bacterial strains used in this study were obtained from Prof. İkbâl Agah İNCE. In antibacterial activity assays, eight bacteria were used (see Table 3).

2.3. Ethanol Extraction Procedure

200 mg of dried and powdered plant leaves were weighed and kept for 10 min in 5 mL ethanol (absolute) in ultrasonic bath (Bandelin sonorex, RK 100 H) at various intervals (2, 4, 6, 8, and 10 minutes) and at 35 kHz and 37 °C. Samples were centrifuged at 6000xg for 15 minutes. The supernatant phase was taken and passed through Whatmann paper No: 1. It was put in incubator at 37 °C for complete removal of ethanol.

2.4. Total Genomic DNA Isolation of The Oregano

The oregano leaves were disinfected with 70% ethanol for 3 min to remove the microorganisms that they may contain on their surfaces. The plant leaves were passed through the sterile purified water and then subjected to the bead-beating process of the ZymoBIOMICS DNA Miniprep Kit (D4303). The following steps were carried out according to the defined procedure in the kit. The concentration of the obtained genomic DNA was measured with nanodrop.

2.5. Amplification of Ribosomal RNA (rRNA) Internal Transcribed Spacer (ITS) Regions and Sequence Analysis

ITS DNA regions of oregano were amplified by polymerase chain reaction (PCR) using ITS specific universal primers (Table 1). The 50 μ L of PCR reaction contained 0.2 mM dNTP mix, 0.2 μ M of each primer, 10 ng DNA, 5X Phusion HF buffer (contains 7.5 mM MgCl₂ which provides 1.5 mM MgCl₂ in final concentration) and 0.02 U/ μ L of Phusion DNA polymerase (Thermo Scientific) in the BIO-RAD T100-Thermal Cycler. The PCR conditions were followings; an initial denaturation and enzyme activation step of 30 seconds at 98°C was followed by 35 cycles amplification at the following conditions; 10 seconds at 98 °C, 30 seconds 53 °C and 40 seconds 72 °C and 10 minutes extension at 72 °C completed the protocol. The PCR product was separated in 1% agarose gel at 80 V voltage for 45 min and visualized on BIO-RAD ChemiDoc MP Imaging System. ITS DNA region fragment was excised and purified using the Zymo Gel DNA Recovery Kit (D4007) and purified ITS DNA amplicon concentration was measured using nanodrop and sequenced at the Macrogen Company. The nucleotide sequence data was searched with the GenBank. The ITS DNA sequence of oregano was registered to the GenBank and the accession number was acquired.

Table 1. Primers used in this study

Primers	Sequences (5'-3')
ITS1 Fw	TCCGTAGGTGAACCTGCGG
ITS2 Rv	GCTGCGTTCATCGATGC
ITS3 Fw	GCATCGATGAAGAACGCAGC
ITS4 Rv	TCCTCCGCTTATTGATATGC
ITS5 Fw	GGAAGTAAAAGTCGTAACAAGG

2.6. Phylogenetic Analysis

For the phylogenetic analysis of *Origanum vulgare* isolate SRY61, the ITS1, partial sequence; 5.8S rRNA gene and ITS2, complete sequence, and large subunit rRNA gene, partial sequence of 31 species that are closely related to *Origanum vulgare* isolate SRY61 according to GenBank was used. Phylogenetic analyses were achieved by using the Maximum Likelihood method and Tamura-Nei model and conducted in MEGA X [30-32]. The names and countries of the species and the access numbers of the sequences taken from GenBank used in the dendrogram are shown in Table 2.

Table 2. The names, countries and the access numbers of the species of *Origanum* and *Micromeria* as an outgroup

Genus	Species	Country	Partial Sequence (bp)	GenBank Accession Number	References
<i>Origanum</i>	<i>Origanum vulgare</i> SRY61	Turkey:Trabzon	668	MH174928.1	Unpublished
<i>Origanum</i>	<i>Origanum vulgare</i> x076	cult-USA	704	DQ667243.1	[33]
<i>Origanum</i>	<i>Origanum vulgare</i> subsp. <i>virens</i> LO414	Morocco	693	MG434484.1	Unpublished

<i>Origanum</i>	<i>Origanum vulgare</i> subsp. <i>virens</i> LO413	Morocco	693	MG434485.1	Unpublished
<i>Origanum</i>	<i>Origanum grosii</i> LO407	Morocco	693	MG434481.1	Unpublished
<i>Origanum</i>	<i>Origanum elongatum</i> LO366	Morocco	695	MG434473.1	Unpublished
<i>Origanum</i>	<i>Origanum onites</i> LR421	Morocco	692	MG434483.1	Unpublished
<i>Origanum</i>	<i>Origanum compactum</i> LO365	Morocco	693	MG434472.1	Unpublished
<i>Origanum</i>	<i>Origanum syriacum</i> RNG:H50	Lebanon	643	JX163016.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> SR562	Malta: Mosta	643	JX162816.1	Unpublished
<i>Origanum</i>	<i>Origanum vulgare</i> 3131	Germany: Bavaria	612	GU381469.1	[34]
<i>Origanum</i>	<i>Origanum syriacum</i> RNG:H50	Lebanon	643	JX163019.1	Unpublished
<i>Origanum</i>	<i>Origanum syriacum</i>	Lebanon	643	JX163018.1	Unpublished
<i>Origanum</i>	<i>Origanum vulgare</i> voucher B. Drew 77	USA	693	JQ669127.1	[35]
<i>Origanum</i>	<i>Origanum syriacum</i> RNG:H50	Lebanon	643	JX163012.1	Unpublished
<i>Origanum</i>	<i>Origanum onites</i> SR777	Turkey: Antalya	643	JX162979.1	Unpublished
<i>Origanum</i>	<i>Origanum grosii</i> LO393	Morocco	693	MG434478.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> SR562	Malta: Mosta	643	JX162818.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> 10659	Turkey: Icel	643	JX162775.1	Unpublished
<i>Origanum</i>	<i>Origanum onites</i> SR777	Turkey: Antalya	642	JX162974.1	Unpublished
<i>Origanum</i>	<i>Origanum grosii</i> LO384	Morocco	693	MG434479.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> SR725	Turkey: Anamur	642	JX162867.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> SR558	Turkey: Antalya	643	JX162856.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> GAZI:H137	Turkey: Akseki	643	JX162789.1	Unpublished

<i>Origanum</i>	<i>Origanum majorana</i> 10659	Turkey: Icel	643	JX162788.1	Unpublished
<i>Origanum</i>	<i>Origanum syriacum</i> RNG:H50	Lebanon	644	JX163021.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> SR1054	Cyprus: Galia	642	JX162938.1	Unpublished
<i>Origanum</i>	<i>Origanum majorana</i> SR725	Turkey: Anamur	643	JX162862.1	Unpublished
<i>Origanum</i>	<i>Origanum syriacum</i>	Lebanon	643	JX163020.1	Unpublished
<i>Origanum</i>	<i>Origanum syriacum</i>	Lebanon	643	JX163015.1	Unpublished
<i>Origanum</i>	<i>Origanum onites</i>	Turkey: Antalya	642	JX162980.1	Unpublished
<i>Origanum</i>	<i>Origanum onites</i> SR777	Turkey: Antalya	642	JX162977.1	Unpublished
<i>Micromeria</i>	<i>Micromeria hyssopifolia</i>	USA	646	AY227142.1	Unpublished

2.7. DPPH Radical-Scavenging Assay

DPPH is one kind of the compound that has a proton free radical with a characteristic absorption at 517 nm [36]. The antioxidant activity of the oregano was assessed according to 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical-scavenging assay using the method of Brand-Williams, 1995 [37]. 20 mg/L of the DPPH solution was prepared to be dissolved in ethanol. In the following step, the serial dilutions of the plant extract were prepared as 100, 250, 500, 750 and 1000 µg/mL. 0.75 mL of each plant dilutions were added on 1.5 mL of the DPPH solution. This mixture was incubated in a dark place in the room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm. Ethanol was used as the blank and 100, 250, 500, 750, 1000 µg/mL concentrations of the Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid (AA) solutions were used as the standard antioxidants.

Antioxidant activity of oregano was calculated as:

$$\text{DPPH Scavenging Effect (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100.$$

The experiments were carried out in triplicates. The 50% inhibition (IC₅₀) value of the oregano extract was determined using AAT Bioquest Program [38].

2.8. The Antimicrobial Activity

The antibacterial activities of the oregano extract obtained by ethanol extraction were determined according to literature [39,40]. The oregano extract was dissolved in the DMSO to a final concentration of 30 mg/mL. Eight bacterial species were grown in Luria-Bertani (LB) broth medium at 37 °C for 16 hours at 200 rpm in the dry air incubator. The liquid cultures were used in order to get 10⁸ CFU/mL bacteria [41]. 100 µL of the bacteria suspensions were inoculated on the LB agar with the help of a steril L-spreader. The sterile discs (Whatmann No:1, 6 mm in diameter) were placed on the petri dishes. Then, the disks impregnated with the oregano extracts (300 µg/disc). Appropriate antibiotic disks were used as positive controls for each bacterial strain. For the negative control, the disks impregnated with DMSO were used. Bacteria were incubated at 37 °C for 24 hours and the results of the diameters of inhibition zones were measured in millimeters. The experiments were repeated triplicates.

The MIC of oregano extract against bacteria was studied by broth micro dilution assay using 96-well cell culture plates [42]. 10⁸ CFU/mL of bacteria grown in broth culture for 12 hours were prepared as

suspensions. The plant extract was dissolved in 10% DMSO to a concentration of 500 µg/mL. Into the each well of a 96-well plate, 95 µL of LB broth and serial dilutions of plant extracts (from 500 µg/mL to 7.8125 µg/mL) were placed and 5 µL of bacteria cultures were added. 195 µL of LB broth agar and 5 µL of bacteria were used as the negative control. For the positive control, the standard antibiotics were used. The 96-well plate was put on a shaker at 300 rpm for 20 minutes and incubated at 37 °C for 24 hours. Microbial growth was measured at 600 nm using the nanodrop. The study was repeated twice. MIC was defined as the lowest concentration that inhibits the bacterial growth.

3. THE RESEARCH FINDINGS AND DISCUSSION

3.1. Molecular Identification

Studies in the field of plant identification in the past few years have been conducted to determine the nuclear ribosomal RNA genes (nrDNA), DNA of mitochondria and chloroplast of plant taxonomy and phylogeny [43]. Especially spacer regions of nrDNA provides important information from plant to generic levels in plant systematics [44]. In addition, it is an important reason to design PCR primers suitable for these regions and make it easily in PCR process.

Plants are also found in two different consecutive repeat groups, the first 5S and the second 18S-5.8S-26S, and the 18S-5.8S-26S is used more for plant phylogeny and taxonomy. While the length of the ITS regions amplified using universal primers is approximately 700 bp in angiosperms [43], it can be between 1500 and 3700 bp in gymnosperms [45].

In this study, ITS1 sequence was amplified with ITS1 and ITS2 primer and PCR product of 335 bp length was obtained, ITS2 sequence was amplified with ITS3 and ITS4 primer and PCR product of 420 bp length was obtained, complete ITS sequence was amplified with ITS4 and ITS5 primer and PCR product of 745 bp length was obtained (Figure 1). The analyzed nrDNA length is 668 bp, because the complete ITS sequence PCR product was analyzed without cloning. Sanger dideoxy sequencing method was used in sequence analysis. The nrDNA nucleotide sequences obtained as a result of sequencing were determined to be *O. vulgare* at 99% similarity level compared with different *O. vulgare* species (Accession number: MH645777.1, Accession number: MG434484.1, Accession number: MG434485.1) from NCBI GenBank and the accession number MH174928.1 (*Origanum vulgare* isolate SRY61) was taken from the gene bank.

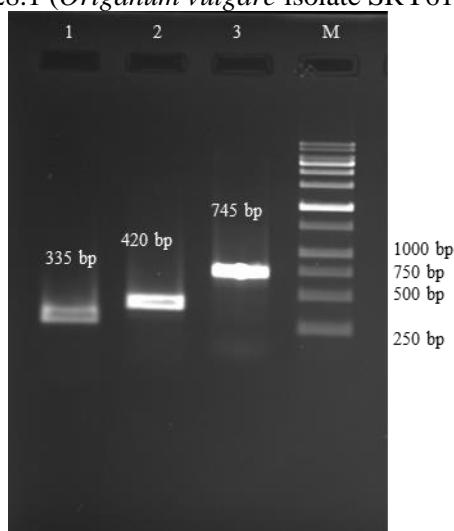


Figure 1. ITS regions of *O. vulgare* amplified by PCR. 1% agarose gel electrophoresis; Well 4 (M) DNA ladder (SiZer-1000 DNA Marker iNtRON Biotechnology)

Based on *O. vulgare*'s nrDNA ITS regions, the degree of relationship between species was determined using MEGA 10.8.1 (Molecular Evolutionary Genetics Analysis) phylogenetic tree program [46]. The evolutionary history was concluded by using the Maximum Likelihood method and Tamura-Nei model [30]. Evolutionary analyses were conducted in MEGA X [31,32]. Phylogenetic analysis grouped the

Origanum vulgare isolate SRY61 (Turkey, MH174928.1) with *Origanum elongatum* (Morocco, MG434473.1), *Origanum compactum* (Morocco, MG434472.1), *Origanum grosii* (Morocco, MG434481.1) and *Origanum vulgare* (Germany: Bavaria GU381469.1) in a phylogenetic clade. *Micromeria hyssopifolia* was used as the outgroup in the phylogenetic tree drawing based on the region of approximately 570-704 bp (Figure 2). It is determined that *O. vulgare*, whose molecular identification was made according to the phylogenetic tree, is in close relationship with other species in the gene bank.

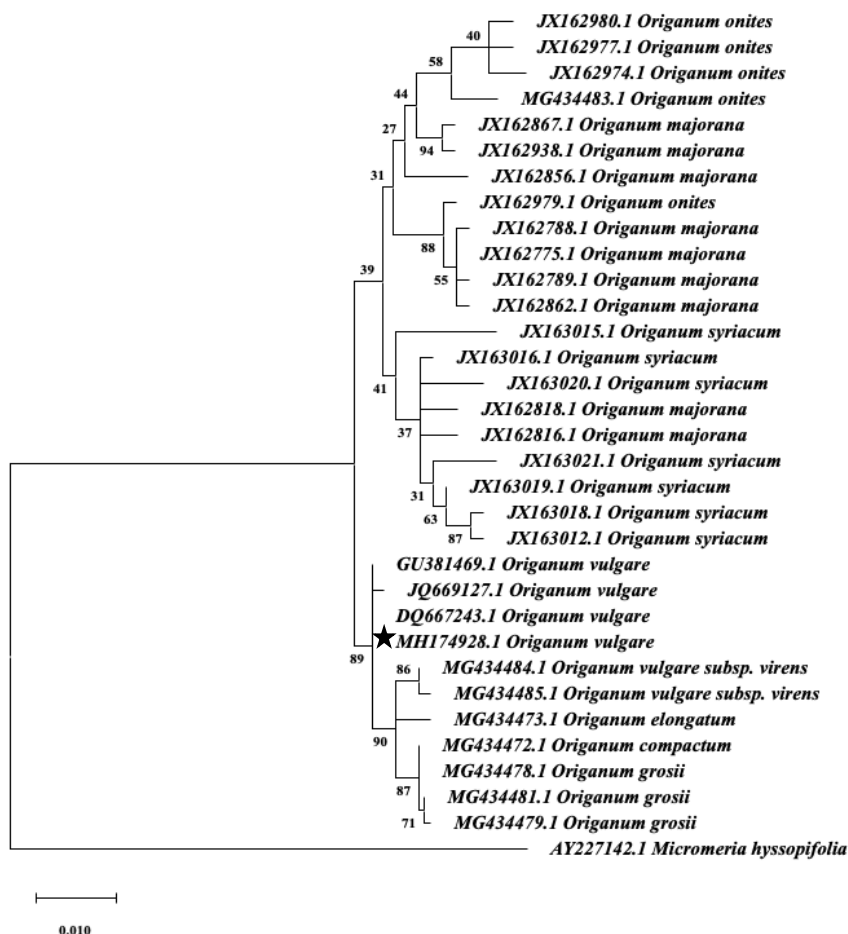


Figure 2. Molecular phylogenetic relationships of the *Origanum* species. *Micromeria hyssopifolia* (AY227142.1) was used as the outgroup

3.2. Antioxidant Activity

Oxidative stress is defined as disruption of the balance between oxidants and antioxidants in the process of detoxifying reactive oxygen species in biological systems [47]. All organisms have cellular defense systems to overcome oxidative stress. Free radicals react with important organic molecules such as lipids, DNA and proteins, and damage these molecules which may result in mutagenesis and carcinogenesis [48].

In recent years, interest and research have been increasing in alternative solutions that closely concern the food industry as well as human health and prevent food spoilage in the food industry or are protective and at the same time natural. Reasons of this include oxidation and microbial pathogen-induced poisoning cases and major economic losses caused by food degradation [49].

The plants under the biotic interactions and the abiotic conditions during their development (ontogenesis)

[50] face a situation that significantly affects metabolism, development and yield. This is considered as stress in the plants and excessive ROS and reactive nitrogen species (RNS) are produced. In response to this increased reactive stress, in other words, to toxic effects, the plants produce antioxidants which are enzymatic Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) [51] and non-enzymatic secondary metabolites such as glutathione, proline, carotenoids, phenolic acids, flavonoids, vitamin C, vitamin E, phenolic acids, terpenes and tannins. These produced molecules act as free radical scavengers, reducing agents and metal chelators [52-54].

Today, there are techniques to assess the antioxidant activity of many plant samples [54]. In studies conducted under the laboratory conditions, plants show strong antioxidant activity due to various compounds found in their structures [55]. HAT-based methods are based on an antioxidant clearing free radicals through hydrogen donation and converting them into stable compounds. Single-electron-transfer (SET)-based methods measure an antioxidant's ability to transfer an electron to reduce any compound, including metals, carbonyls, and free radicals [56,57].

A number of studies have showed differences in the biological activities of extracts prepared using different extraction methods. Ultrasonic assisted solvent extraction (UAE) is a process that uses high intensity, high frequency sound waves and solvents [58]. The standard methods such as soxhlet used for antioxidant extractions are disadvantageous in terms of time, use of large amounts of toxic solvent and cost. UAE has been shown to be a more effective and environmentally safe way of extracting natural antioxidants [59].

IC₅₀ value of DPPH % scavenging activity at the concentrations of 100, 250, 500, 750, 1000 µg/mL was calculated using AAT Bioquest Program as for the 6th minute at various intervals (2, 4, 6, 8, and 10 minutes) prepared from plant extracts 0.0513 ± 0.002 mg/mL. IC₅₀ value for BHA, BHT, and AA solution (100, 250, 500, 750, 1000 µg/mL) was calculated as 0.041 ± 0.002, 0.34 ± 0.002, and 0.23 ± 0.006 mg/mL, respectively [38].

The free radical scavenging activity of extracts are between 84.2-93.36% at a concentration of 250 µg/mL for the 6th minute. It is great importance that these values are very close to the standards values. Positive DPPH test suggested that the extracts were good free radical scavenging. Figure 3 showed thyme extracts had good DPPH scavenging activity with standards and had higher DPPH scavenging activity with the decreasing concentration.

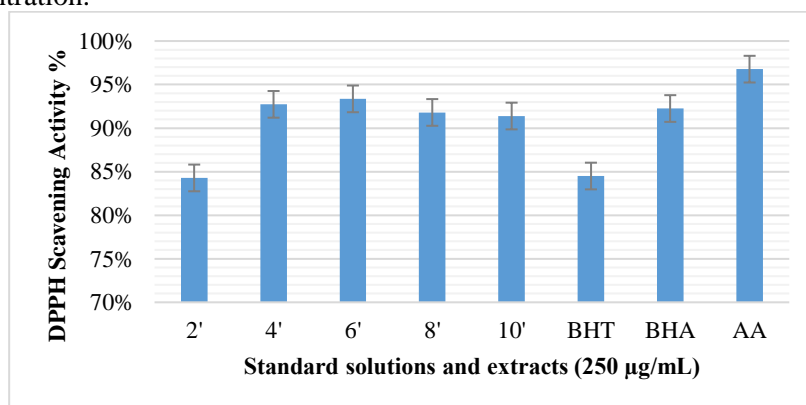


Figure 3. The DPPH scavenging activities of the standard solutions (BHA, BHT and AA) and ethanolic oregano extract

It is clear from results Figure 4 that the highest scavenging activity was viewed 6th extractions minute.

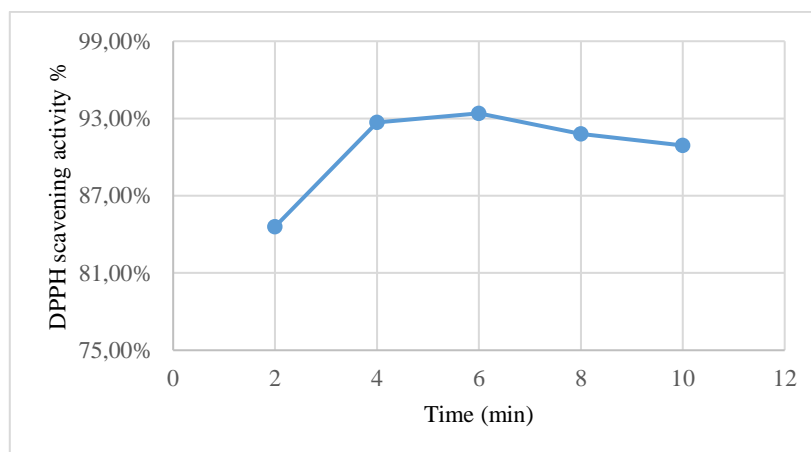


Figure 4. Time related DPPH scavenging activity % of the ethanolic oregano extract (250 µg/mL)

Thus, high radical scavenging effect was recorded for thyme extract in as little as 6 minutes. Major components such as carvacrol and thymol contained by the oregano plant have scavenging activity of free radicals, transition-metal-chelating [60] and single-oxygen-quenching capacity. Many of the free radical scavengers, such as carvacrol and thymol, convert hydrogen radicals into more stable non-radical products [61].

In the previous studies using hydrodistillation extraction method, *O. vulgare* extracts were shown to have high radical scavenging activity. Mechergui et al. in 2010, they examined *O. vulgare* L. subsp. *glandulosum* methanol extract method in different concentrations (12.5 to 500 mg/mL) and determined that IC₅₀ value was 59 to 80 mg/mL [62]. Kaurinovic et al. in 2011, they found the IC₅₀ value of *O. vulgare* methanol extract in different concentrations (2.5 to 50 µg/mL) and determined that IC₅₀ value was 68.17 µg/mL [63]. The ethanol extract in different concentrations (0.10 to 3.20 mg/mL) derived from *O. vulgare* L. was determined that IC₅₀ value 0.332 mg/mL by Han et al. in 2017 [64]. Stanojević et al. in 2016, they showed the IC₅₀ value of *O. vulgare* ethanol extract in different concentrations (0.098 to 12.5 mg/mL) 0.761 to 0.326 mg/mL [65]. Our study compared with the studies in the literature, the results obtained are consistent and it was determined that our oregano extract at lower concentrations has better DPPH radical scavenging activity than some previous papers. It was concluded that these results are due to the ultrasonic assisted solvent extraction method, which prevents possible loss in the content of foods used in the study and provides high efficiency. Therefore, the ultrasonic assisted solvent extraction method, that is used in our study, is more efficient than hydrodistillation extraction method.

3.3. Antibacterial Activity

In this study, we investigated the antibacterial activity of *O. vulgare* obtained by ethanol extraction against eight microorganisms by the disc diffusion and MIC assay. The results are given in Table 3. Based on the results, oregano extract formed a zone of inhibition between 9.5 mm and 11.5 mm on bacteria. In the MIC study, the MIC value of the oregano extract on bacteria species was determined as 15.625 µg/mL and 7.8125 µg/mL.

Table 3. Antibacterial activity of *O. vulgare* extract against the bacterial strains assayed

Pathogens	Plant Extract (EtOH)		Antibiotics	
	Disc diffusion ^a (300 µg/disc)	Minimal Inhibition Concentration (µg/mL)	Disc diffusion ^b	Minimal Inhibition Concentration (µg/mL)
<i>S. aureus</i> ATCC 25923	10 mm	7.8125	33 mm (AM)	2 (VA)
<i>S. epidermidis</i> ATCC 12228	10.5 mm	15.625	30 mm (VA)	2 (VA)

<i>P. aeruginosa</i> ATCC 27853	9.5 mm	15.625	20 mm (AK)	8 (AK)
<i>K. pneumoniae</i> ATCC 700603	10 mm	15.625	20 mm (FOX)	8 (AMC)
<i>E. coli</i> ATCC 25922	10.5 mm	15.625	20 mm (AMC)	8 (AMC)
<i>P. mirabilis</i> ATCC 27853	10 mm	15.625	20 mm (FOX)	8 (AMC)
<i>S. Typhimurium</i> ATCC 14028	11.5 mm	15.625	23 mm (SXT)	2 (SXT)
<i>A. baumannii</i> ATCC BAA 747	10.5 mm	15.625	24 mm (AM)	2 (SXT)

^a Inhibition Diameter (mm) ² Including Disk Diameter of 6.0 mm²

^b AM: ampicillin (10 µg/mL); VA: vancomycin (30 µg/mL); AK: amikacin (30 µg/mL); FOX: cefoxitin (30 µg/mL); AMC: amoxicillin-clavulanate (20/10 µg/mL); SXT: trimethoprim-sulphamethoxazole (1.25/23.75 µg/mL)

Plant species belonging to *Lamiacea* family are very rich in antimicrobial content [66]. Having a strong antibacterial activity, *O. vulgare* gets this feature from its compounds such as phenolic monoterpenes, carvacrol and thymol [67]. Many existing studies have generally examined the antibacterial effect of *O. vulgare* essential oils (EO). Since EO extract contains carvacrol and thymol more concentrated than oregano plant extract, their toxic effects on bacterial cells will be higher [68].

Santoyo et al. in 2005, they made a solution of oregano extract with a concentration of 40 mg/mL in a volume of 200 µL, using disc diffusion method, 19.2-20.2 mm for *E. coli*, 19.6-20.2 mm for *B. subtilis* and 19 mm for *P. aeruginosa* and they have observed inhibition zones of up to 8-21.8 mm. They determined the minimum bactericidal concentration (MBC) 2.05-2.25 mg/mL for *E. coli*, 1.95-2.25 mg/mL for *B. subtilis* and 1.75-2.12 mg/mL for *P. aeruginosa* [69].

In another study conducted by the company Brđanin et al. in 2015, they found the MIC value of oregano plant for cyclohexane, dichloromethane, methanol extracts 62.5 to 125 µg/mL for *S. aureus* (ATCC 25923), 62.5 to 125 µg/mL for *S. epidermidis* (ATCC 12228), 125 µg/mL for *E. coli* (ATCC 25922), 125 µg/mL for *K. pneumoniae* (NCIMB 9111), 125 µg/mL for *P. aeruginosa* (ATCC 27853) and 62.5 to 125 µg/mL for *S. typhimurium* [70].

In a study conducted by Coccimiglio et al. in 2016, they determined the MIC value by the ethanolic extracts of the oregano plant as 25 µg / mL for *P. aeruginosa* 25619, 25 µg/mL for *E. coli* 25922, 12.5 µg/mL for *E. coli* 700973, 12.5 µg/mL for *A. baumannii* 19606 and 25 µg/mL for *S. aureus* 29213 [15]. In this study, quite good antibacterial activity was observed with the extract obtained by the extraction of the oregano plant with ethanol extraction compared to other studies.

4. CONCLUSION

In this paper, the plant obtained from Trabzon Sultan Murat Sarıkaya Plateau in eastern Black Sea Region has been determined as *O. vulgare* according to phylogenetic analysis and MH174928.1 (*Origanum vulgare* isolate SRY61) has been obtained from Gen Bank as the Accession number. The antioxidant activity of the plant was found to be quite high compared to the standard solutions. In addition, the fact that its antibacterial activity is effective in all eight bacterial species has shown that this plant can be safely used in the preservation of raw and processed foods.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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