Original Article

Phenolic Contents and Biological Activity of Endemic *Origanum minutiflorum* Grown in Turkey

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ABSTRACT

Objective: In this study, biological property of *Origanum minutiflorum* O. Schwarz and P. H. Davis (Lamiaceae) was evaluated. **Methods:** The total phenolic and flavonoid amount were detected using Folin-Ciocalteu and aluminum chloride colorimetric assays. The antioxidant activity was assayed with phosphomolybdenum, 2,2- diphenyl-1-picrylhydrazyl radical scavenging (DPPH), hydrogen peroxide scavenging, β-carotene bleaching activity, ferric-ion reducing power (FRAP), reducing power and cupric ions (Cu^{2+}) reducing antioxidant capacity (CUPRAC) methods. Antimicrobial activities of the extract and the essential oil were studied by broth microdilution and agar well-diffusion assays using 12 bacteria, 1 yeast and 2 fungi. **Results:** Twenty nine components were identified by gas chromatography/mass spectrometry (GC/MS), representing 100% of the oil. The main component of *O. minutiflorum* oil was carvacrol (90.87%). *O. minutiflorum* extract exhibited strong DPPH scavenging activity (IC_{50} = 13.68 μg/ml). In addition, *O. minutiflorum* had an effective hydrogen peroxide scavenging and ferric ions (Fe^{3+}) reducing activities. The extract and essential oil had strong antibacterial effects against all bacteria tested. The methanol extract had no activity against yeast and two fungi tested, but essential oil exhibited prominent antifungal activity. **Conclusion:** *O. minutiflorum* extract and essential oil might be alternative agents in vary industries such as food, pharmacy and health.

Key words: Antimicrobial activity, Cuprac, Essential oil, Frap, *Origanum minutiflorum.*

INTRODUCTION

Multiple drug resistance is the main cause of the random use of commercial drugs for the treatment of infectious diseases. Thus, many researchers were worked on finding new antimicrobial sources from plants, especially used by folk for several thousand years.[1] Since plant-derived drugs offer minimum or no side effects, search for such therapeutics has become the goal of phytochemists and microbiologists to reduce the suffering of mankind.[2] In food industry, microbial contamination is responsible for food spoilage and generates risks to consumer.[3] Synthetic antimicrobial and antioxidant agents are commonly used due to increase the product self life.[4] However, there are increasing interests in finding naturally occurring antioxidants to be alternative to synthetic antioxidants. It is known that they have many side effects such as cancer.[1] Reactive oxygen species attack cellular macromolecules and lead to many diseases connected with oxidative stress including cancer, diabetes mellitus and cardiovascular diseases.[5] Plant phenolic have been known to exert strong antioxidant activity and so, can protect cells against free radical-induced oxidative stress. Many medicinal plants attract attention due to their use as food in traditional medicine.[6] Essential oils have long been known to exert biological activity, including antimicrobial and antioxidant activity.[4] Thus, essential oils could be

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promising additive in the food and flavoring manufactures.[7]

Origanum (Lamiaceae) is represented by 24 species and 27 taxa in the flora of Turkey, 16 of which are endemic.[8] They spread in the Mediterranean areas and are commonly used as herbal tea and spices in Anatolia.[9,10] *Origanum* have several biological properties including antimicrobial, antioxidant, antimutagenic,[11-14] antifungal, insecticidal, anticarcinogenic, antispasmodic[11] antiviral, fungicide, nematocide, biocide, growth regulation effects,[13] antithrombin, angiogenic, antiparasetic and antihyperglycaemic activities.[12] *Origanum* has been also used as expectorant, digestive, anti-diabetic, stimulant, tonic, menstrual regulator, sedatives, diuretic, analgesics, carminative, antiparasitic, antihelminthic, for gastrointestinal complaints and for colds, asthma.[12,13,15-17] Results of studies showed that the high activity might be related to the carvacrol and thymol.[11] *O. minutiflorum* is an endemic plant found in the mountains of southern of Turkey. In Anatolia, it is used as a spice for seasoning, as herbal tea and also as a medicinal herb for curing stomach-aches and respiratory colds.[18]

This work was conducted to (i) determine the main constituents of the volatile oil of *Origanum minutiflorum* O. Schwarz and P. H. Davis (Lamiaceae) collected from wild populations in Antalya, Turkey; (ii) examine the antioxidant activity of methanol extract with vary methods: Phosphomolybdenum, 2,2- diphenyl-1-picrylhydrazyl radical scavenging (DPPH), hydrogen peroxide scavenging, β-carotene bleaching activity, ferric-ion reducing power (FRAP), reducing power and cupric ions (Cu^{2+}) reducing antioxidant capacity (CUPRAC) methods; (iii) determine the antimicrobial activity against 15 microorganisms.

MATERIAL AND METHODS

Plant material

Plant was collected from Antalya (Konyaaltı-next to Saklıkent rock resort), Southern Anatolia region of Turkey in July 2015 (39°50'03"K-30°20'41"D, 1815 m), during flowering season. The voucher specimen (Voucher no.: Aksoy 2521) has been deposited at the Herbarium of the Department of Biology, Erciyes University.

Extraction

Grinded aerial parts of the plant were extracted using methanol by a Soxhlet type extractor. The obtained extract was filtered (Whatman No. 1) and evaporated with a rotary evaporator (T< 40 °C). The extract yield was calculated and stored at 4 °C.

Isolation of Essential Oil

Plant samples were subjected to steam distillation by a Clevenger-type apparatus for 3 h. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4 °C.

Determination of Total Phenolic

Folin-Ciocalteau assay was performed to detect of the total phenolic amount in the extract.[19] forty µL aliquot of the extract was mixed with 200 µL of Folin-Ciocalteu reagent and 600 µL (20% Na_2CO_3) of sodium carbonate. The mixture was vortexed and incubated at room temperature for 2 h. Absorbance was then read at 765 nm by the spectrophotometer. The standard curve was formed with gallic acid. Total phenolic was expressed in terms of milligrams of gallic acid equivalents (mg GAE) /g extract.

Total Flavonoid

Total flavonoid was assessed using Aluminum chloride colorimetric assay according to Pourmorad *et al.*[20] The extract was mixed with methanol (1.5 mL), aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL) and distilled water (2.8 mL). The absorbance of the mixture was read at 415 nm. The result is expressed as mg of quercetin equivalents (QE)/g extract.

Essential Oil Composition

Identification of the essential oil composition was performed using a gas chromatography/mass spectrometry (GC/MS)/quadropole detection analysis using a Shimadzu QP 5050 system fitted with an FFAP (polyethylene glycol+2nitroterephthalate) capillary column (50 m × 0.32 mm i.d., film thickness 1.2 µm). The detector and injector temperatures were set at 250 °C and 240 °C, respectively. The temperature of the column was held at 120 °C for 1 min, then increased at 2 °C min^{-1} to 220 °C and held for 20 min. Helium was used as carrier gas at a flow rate of 10 psi (split 1 : 10). 1 µl of each sample was injected. The ionization energy was set at 70 eV. Qualitative analysis was based on comparison of retention times and mass spectra (Wiley, Nist and Tutor Libraries). The composition (%) of the essential oil was computed from the GC peak areas without using any correction factors.[21]

Antioxidant Activity

Total Antioxidant Activity

Phosphomolybdenum method was used.[22] 4 mL of reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate) was added to the extract solution. The tubes were stored at

95 °C for 90 min. The absorbance was read at 695 nm. The antioxidant activity was expressed as mg of ascorbic acid equivalents (AAE)/g extract.

DPPH Method

The scavenging ability to 2,2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated according to Lee *et al.*[23] 0.1-2 mg/ml concentrations of the extract was mixed with DPPH solution (0.1 mM). The absorbance was read at 517 nm after 30 min incubation period. IC_{50} (concentration required to scavenge 50% DPPH) value was determined. BHT (Butylated hydroxytoluene) was used as control.

Percent Inhibition (%) was calculated in the following way:

$$\text{Inhibition } \% = (A_{\text{absorbance of the control}} - A_{\text{absorbance of the sample}} / A_{\text{absorbance of the control}}) \times 100$$

β-Carotene Bleaching Assay

Lipid peroxidation inhibitory activity in β-carotene bleaching system was assessed.[24] An aliquot of β-carotene in chloroform was mixed with Tween 40 and linoleic acid. The chloroform was evaporated. Then, distilled water saturated with oxygen was added. Theemulsion was added to a tube containing the extraction solution. After the tubes were stored at 50 °C for 2h, the absorbances were read at 470 nm. The same procedure for BHT (Butylated hydroxytoluene) was carried out.

CUPRAC Assay

The cupric ion reducing antioxidant activity of *O. minutiflorum* extract was detected.[25] The extract, Neocuproine (7.5 mM), $CuCl_2$ (10 mM) and NH_4Ac buffer (1 M) solutions were mixed into a test tube. Then, total volume was completed to 4.1 ml and incubated for 30 min. The absorbance of the mixture was read at 450 nm. Trolox was used as the positive control.

FRAP

Ferric ions reducing activity of the extract was examined.[26] FRAP is based on the reduction of ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine [Fe(III)-TPTZ] to the ferrous complex at low pH. FRAP reagent was made by mixing of $FeCl_3$. $6H_2O$ (20 mM), acetate buffer (300 mM) and TPTZ (10 mM). An aliquot of the each plant extract was added to diluted FRAP reagent and stored during 30 min at 37 °C and the absorbance was read at 595 nm. The results were expressed as mmol/l of Fe^{2+}.

H_2O_2 Scavenging Activity

The extract (25-500 µg/ml) was mixed with 43 mM H_2O_2 solution. After 10 min, the absorbance of the solution was read at 230 nm.[27] Gallic acid, BHA and BHT were used as control. The percentages of scavenged hydrogen peroxide of the extract and standards were calculated using the following equation:

$$\text{Scavenged } H_2O_2 \text{ } \% = (A_{\text{absorbance of the control}} - A_{\text{absorbance of the sample}} / A_{\text{absorbance of the control}}) \times 100$$

IC_{50} values was determined graphically.

Reducing Power

The reducing power of the methanolic extract was detected.[28] The extracts (0.5-10 mg/ml) were added to 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] and was stored for 20 min at 50°C. Then, the solution was centrifuged at 3,000 rpm for 10 min after trichloroacetic acid (2.5 ml) was added. The upper layer solution was added to $FeCl_3$ (0.5 ml). The absorbance was read at 700 nm. BHT was used as control. The high absorbance value reflects the high reducing power.

Chelating Activity on Ferrous Ion

Ferrous ions (Fe^{2+}) chelating activity of the extract was examined.[27] Aliquots of the extract (1-5 mg/ml) were added to $FeCl_2$ (2 mM, 0.1 ml). After incubation, ferrozine (5 mM and 0.2 ml) was added to the reaction solution. The absorbance was read at 562 nm. The chelating ability was compared with that of ethylene diamine tetra acetic acid (EDTA). Chelating activity was calculated by the following formula:

$$\text{Chelating activity } \% = [1-(\text{Absorbance of sample} / \text{Absorbance of control})] \times 100$$

Determination of Antimicrobial Activity

The following microorganisms were tested: *Aeromonas hydrophila* ATCC 7965, *Yersinia enterocolitica* ATCC 1501, *Salmonella typhimurium* NRRLE 4463, *Listeria monocytogenes* 1/2B, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 13883, *Proteus mirabilis* ATCC 25933, *Bacillus cereus* ATCC 11778, Methicillin resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *Streptococcus pneumoniae* ATCC 10015, *Salmonella enteritidis* ATCC 13076, *Candida albicans* 10231, *Aspergillus parasiticus* DSM 5771 and *Aspergillus flavus* NRRL 3357.

Agar-well diffusion assay was performed to detect of antimicrobial activity.[29] Suspensions of each microorganism were regulated to 10^6-10^7 colony-forming units (cfu)/ml and added to sterile growth medium. The mix was poured into Petri plates (9 cm). The wells (5 mm in

diameter) were cut from the agar. 50 µl of extract (30 mg/ml) was added to the wells. The methanol was used as a control. For antimicrobial activity of essential oil, the disc diffusion assay was used.[30] The disc (6 mm) applied essential oil (10 µl) was placed on the inoculated agar. Then, plates were incubated at 25-37 ˚C for 24-48 h in the inverted position. The growth inhibition zones were measured in millimeters. Inhibition zones of standard antibiotics namely tetracycline (10 mg/ml), natamycin (30 mg/ml), ampicillin (AMP, 10 µg/disk), kanamycin (K, 30 µg/disk) and penicilin (P, 10 µg/disk) were compared.

MIC and MBC

The concentrations of minimal inhibitory (MIC) and minimum bactericidal (MBC) were determined. The inocula of the microorganisms were adjusted to 0.5 McFarland standard turbidity. The extract and essential oil were adjusted at 30 mg/ml and 2000 µg/ml in 10% dimethylsulfoxide (DMSO). Then, two-fold dilutions were made (3.9- 30 mg/ml and 31.25- 2000 µg/ml concentrations, respectively). To determination of MICs were applied to micro-well dilution assay.[31] 95 µL of nutrient broth and 5 µL of the inocula were added into each well. The serial dilutions of the extract and essential oil were prepared. The last well not contain samples as a negative control. The plates were mixed on a shaker and incubated at 25-37 °C for 24 h. The lowest concentration that is not visible growth was identified as MIC. MBC was detected by sub culturing from each negative tube and control. MBC was defined as the lowest concentration that zero or only one colony was observed.

RESULTS

The percent yields of the methanol extract and essential oil obtained from *O. minutiflorum* were 13.63% (w/w) and 1.13% (v/w), respectively. The mean amount of total phenolic was 42.74 ± 0.6 mg GAE/g extract. The mean amount of total flavonoid was 7.94 ± 0.3mg QE/g extract (Table 1).

GC-MS was used for determination of the phenolic composition of the hydrodistillated volatile oil isolated from *O. minutiflorum* (Table 2). 29 compounds constituting 100.00% of the total oil were determined. Carvacrol (90.87%) was found to be predominant component in the oil. It was followed by linalool (2.77%), caryophyllene (1.21%) and *p*-cymene (1.01%). Other compounds were found in minor amounts (0.02-0.8%).

According to literature survey, the phenolic composition of the volatile oils obtained from *Origanum* genus and also *O. minutiflorum* were stated in many studies. In these reports, carvacrol, *p*-cymene and thymol have been identified as major compounds.[8-17,18-32-35] Our results are very similar with these reports. The essential oil content is generally known to be affected by seasonal, climatic, geographic conditions, distillation technique and harvest period.[32]

In phosphomolybdenum assay, Mo (VI) is reduced to Mo (V) by the antioxidant compound. The total antioxidant activity of the extract was found as 252.11 ± 0.6 mg AAE/g dry extract (Table 1).

The oxidation of linoleic acid results in the formation of peroxyl radicals in β- carotene bleaching assay. Then, the formed free radical oxidizes and breaks down the β- carotene. It loses its chromophore and characteristic orange colour. The antioxidants in the extract can neutralize hydroperoxides.[24] The extract inhibited the bleaching of β- carotene. The inhibition values of the methanol extract (1 mg/ml), BHT (1 mg/ml) and BHA (1 mg/ml) were found to be 48.61 %, 84.26% and 94.33%, respectively. The β- carotene bleaching effects of the extract was lower than that of BHT and BHA at tested concentrations (Table 1).

DPPH assay to evaluate the free radical scavenging effectiveness of the extracts has been widely used. DPPH assay show the hydrogen donating capacity of the extract. DPPH scavenging capacity was defined as the percentage inhibition of the initial DPPH absorption by extract and is shown in Figure 1. Figure 1 shows an increase in the percentage DPPH inhibition with increasing concentration. The methanol extract had moderate free radical scavenging capacity. The percentage inhibitions of the extract were 33.58%, 41.99%, 53.47%, 62.95 and 79.94% at 6.66, 8.3, 16.6, 33.3 and 66.6 µg/ml concentrations, respectively. DPPH scavenging capacity of the extract was lower than that of the BHT (92.15% at 66.6 µg/ml). IC_{50} value of the extract was 13.68 µg/ml (Table 1). A low IC_{50} value means that antioxidant capacity is high.

Copper (II)-neocuproine reagent was used as the chromogenic oxidizing agent in CUPRAC assay. The absorbance of stable complex formed between neocuproine and copper (I) is measured at 450 nm.[36] Cu^{2+} reducing capability was found to be concentration-dependent. Results showed that *O. minutiflorum* extract had very strong CUPRAC activity. The extract gave CUPRAC values ranging between 0.02 and 2.98 at 0.6-3 mg/ml. The value of the extract (2.73) was very close to that of trolox (2.85) at 1 mg/ml (Figure 2).

The reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants in the extract was measured by FRAP assay. Table 1 shows the FRAP assay results of the *O. minutiflorum* extract compared with ascorbic acid. The

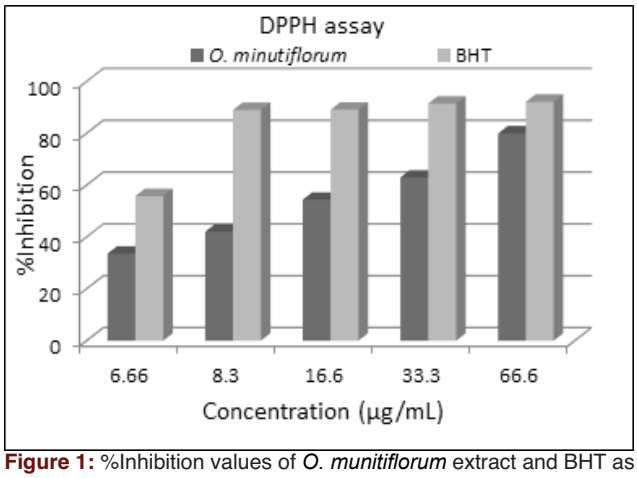

Figure 1: %Inhibition values of *O. munitiflorum* extract and BHT as positive control by DPPH assay.

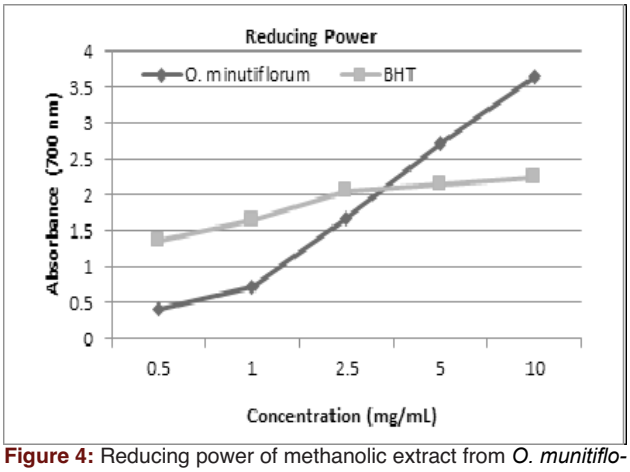

Figure 4: Reducing power of methanolic extract from *O. munitiflorum.*

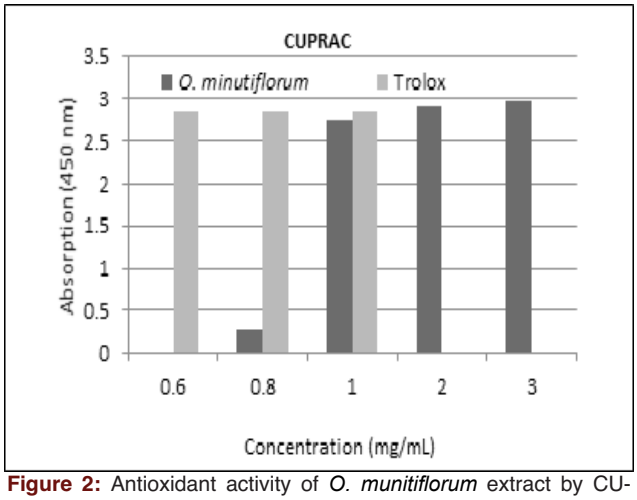

Figure 2: Antioxidant activity of *O. munitiflorum* extract by CUPRAC method.Each value represents the mean ± standard deviation of triplicate experiments.

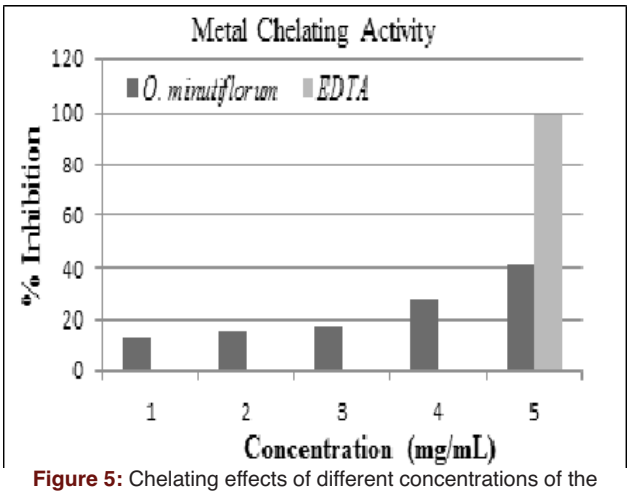

Figure 5: Chelating effects of different concentrations of the methanolic extract on Fe2+ ion.

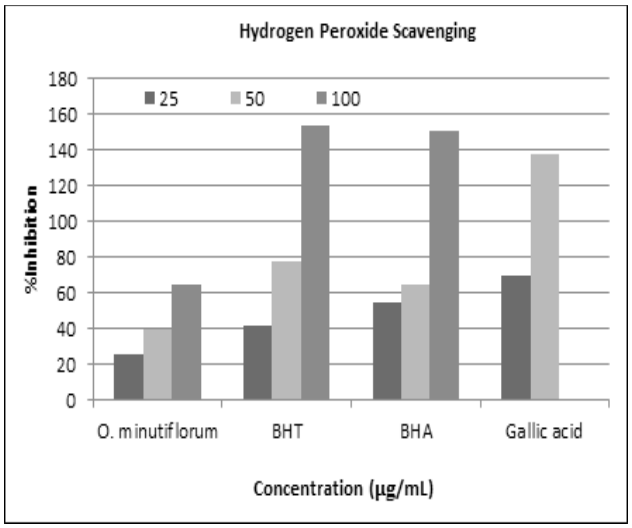

Figure 3: %Inhibition values of the *O. munitiflorum* extract and standards by H2O2 assay.

methanolic extract showed the high antioxidant activity with 3.25 mM/l FRAP value at 2 mg/ml. The FRAP value of ascorbic acid was found as 4.52 mM/l. The reductive ability of the extract was lower than ascorbic acid.

The H_2O_2scavenging capacity of the extract depended on concentration (Figure 3). The scavenging effect of the extract was 39.08% at 50 µg/ml. H_2O_2 scavenging activities of BHT, BHA and gallic acid were 76.97%, 64.73% and 137.61%, respectively at 50 µg/ml. IC_{50} values were 71.56, 31.09, 23.16 and 17.62 µg/ml for *O. minutiflorum* extract, BHT, BHA and gallic acid, respectively (Table 1). The activities were in the following order: gallic acid > BHT > BHA > methanolic extract. According to results, *O. minutiflorum* extract exerted a moderate H_2O_2 scavenging activity.

Table 1: The yields, total phenolic content, total flavonoid content, total antioxidant activities, IC_{50} values and the effects on β-carotene bleaching of *O. minutiflorum*.

	Methanol Extract	BHT	BHA	L-Ascorbic acid	Gallic acid
Yield (%)	13.63*				
Total phenolic content (mg GAE/g extract)	42.74 ± 0.6				
Total flavonoid content (mg QE/g extract)	7.94 ± 0.3				
Total antioxidant activity (mg AAE/g extract)	252,11 ± 0.6				
β-carotene bleaching (I%)	48.61 ± 0.0	84.26± 0.3	94.33 ± 0.6		
DPPH IC_{50} (µg/ml)	13.68	3.35			
FRAP mM Fe(II)/l	3.25			4.52	
H_2O_2 IC_{50} (µg/ml)	71.56	31.09	23.16		17.62

*Values expressed are mean ± standard deviation of three experiments. Total phenolic content expressed as gallic acid equivalent (GAE), total flavonoid content expressed as quercetin equivalent (QE), total antioxidant activity expressed as ascorbic acid equivalent (AAE).
-: not determined

Table 2: Composition of *O. minutiflorum* essential oil

Compounds	RT[b]	%
α-thujene[a]	5.593	0.05[c]
α-pinene	5.828	0.05
1 -octen -3 ol	7.334	0.12
Myrcene	7.713	0.17
Phellandrene- α	8.386	0.03
alphga Terpinene	8.844	0.24
p-cymene	9.166	1.01
Limonene	9.385	0.07
1,8-cineole	9.515	0.18
γ-terpinene	10.743	0.72
sabinenehydrate	11.317	0.08
α-terpinolene	12.111	0.07
Linalool	12.934	2.77
Borneol	16.894	0.25
Terpinen-4-ol	17.454	0.80
Thymol	24.659	0.24
Carvacrol	25.724	90.87
Eugenol	28.376	0.04
Caryophyllene	32.510	1.21
Aromadendrene	33.679	0.40
γ-selinene	34.156	0.03
α-humulene	34.734	0.05
Azulene	36.808	0.04
Viridiflorene	37.035	0.30
Germacrene B	37.271	0.02
δ-cadinene	38.804	0.03
Spathulenol	42.081	0.09
Caryophyllene oxide	42.340	0.07
β- humulene	42.619	0.02
Total		100

[a] Compounds listed in order of elution from a FFAP MS column.
[b] Retention time (as min).
[c] The percentage composition was computed from the GC peak areas.

Because a compound exert antioxidant activity, its reducing potential is very significant. In this method, electron donating capacity of phenolic antioxidant is measured.[28] The reducing activity of the extract was higher than that of BHT at 5 and 10 mg/ml. reducing activity increased depend on the increasing concentration (Figure 4). According to observed results, *O. minutiflorum* extract had high electron and hydrogen donating ability.

The ferrous ion (Fe^{2+}) chelating activity of *O. minutiflorum* extract depends on concentration (Figure 5). The methanol extract exhibited 41.20% chelating activity on Fe^{2+} at 5.0 mg/ml. This value was the lower than that of EDTA (99.45%) at same concentration. Obtained data show that *O. minutiflorum* extract have slightly iron binding capacity.

The methanol extract showed good antioxidant activity for all assays tested except for the β- carotene bleaching and H_2O_2 scavenging assay. Literature screening notified that the dominant components such as carvacrol and thymol of the oregano essential oil might be related to its antioxidant effect.[9]

The results obtained in the antimicrobial effect assay of the methanol extract and essential oil of *O. minutiflorum* again55st 12 bacteria, 1 yeast and 2 molds were shown in Table 3. Absolute methanol (control) did not showed any inhibitory effect. The antimicrobial activities of both extract and essential oil are compared with standard antibiotics. The methanol extract was shown to possess a broad spectrum of antibacterial activity. *A. hydrophila* was the most sensitive bacteria to the extract. No activity against yeast and molds tested was exhibited by the methanol extract. The essential oil of *O. minutiflorum* showed high inhibitory effect on all microorganisms tested (Table 3). The inhibition zones were in the range

Table 3: Antimicrobial activity of *O. minutiflorum* extract, essential oil and standard antibiotics (mm, inhibition zones). MIC and MBC concentrations

Bacteria	Extract (30 mg/ml) mm	MIC (mg/ml)	MBC (mg/ml)	Essential oil mm	MIC (mg/ml)	MBC (mg/ml)	Tetracycline mm	MIC (µg/ml)	MBC (µg/ml)	Ampicillin mm	Kanamycin mm	Penicillin mm
A. hydrophila	24	3.13	3.13	26	0.25	0.25	27	<3.9	125	32	19	37
Y. enterocolitica	10	3.13	3.13	35	0.25	0.5	23	<3.9	125	-	10	-
S. thyphimurium	9	3.13	3.13	19	0.25	0.25	15	62.5	125	11	11	10
L. monocytogenes	18	1.56	1.56	35	0.25	0.5	29	<3.9	<3.9	-	-	15
E. coli	10	3.13	3.13	26	0.25	0.25	24	<3.9	125	8	11	7
K. pneumonia	18	0.78	0.78	57	0.25	0.25	48	15.6	15.6	23	7	33
S. aureus (MRSA)	10	3.13	3.13	31	0.25	0.25	25	<3.9	125	-	-	-
P. mirabilis	10	3.13	3.13	22	0.25	0.25	19	31.5	125	7	14	12
B. cereus	17	3.13	1.56	33	0.25	0.25	27	<3.9	<3.9	-	15	11
S. pneumoniae	21	1.56	1.56	25	0.25	0.5	24	7.8	7.8	-	-	13
S. entertidis	10	3.13	3.13	28	0.25	0.25	25	<3.9	62.5	9	10	10
Yeast							Natamycin	MIC (µg/ml)	MBC (µg/ml)			
C. albicans	-	-	-	15	0.25	0.25	23	<3.9	62.5	-	-	-
Molds												
A. flavus	-	-	-	42	0.13	0.13	17	7.8	>250	-	-	-
A. parasiticus	-	-	-	59	0.06	1.00	15	15.6	>250	-	-	-

-: not detected

of 19-59 mm. Both extract and essential oil had significant effect against methicillin resistant *S. aureus*. The essential oil showed also greater antifungal activities against aflatoxigenic *A. parasiticus* and *A. flavus*. The methanol extract showed MIC and MBC in a range of 0.78-3.13 mg/ml. MIC and MBC values of the essential oil for bacteria were in the range of 0.25-0.5 mg/ml. MIC values of the essential oil for *A. flavus* and *A. parasiticus* were 0.13 and 0.06 mg/ml, respectively. MBC values for *A. flavus* and *A. parasiticus* were 0.13 and 1.00 mg/ml, respectively. Least MIC and MBC values of the extract were obtained for *K. pneumonia* (0.78 mg/ml). The essential oil exhibited stronger inhibitory and bactericidal effect than the methanol extract. The essential oil exhibited stronger inhibitory zones against all bacterial strains and molds than tetracycline and natamycin, but their MIC and MBC values were weaker than antibiotics.

DISCUSSION

The total phenolic amounts of *O. vulgare* and *O. heracleoticum* water extracts were found as 91.4 and 27 mg GAE/g.[37] At the other paper, total phenolic amount of *O. vulgare* ethanol extract and its percent inhibition of DPPH was found as 55.35 mg GAE/g and %80-82, respectively.[38] *O. majorana* methanol extract, which had high total phenolic content showed strong DPPH scavenging activity (5.20 mg GAE/g).[39] Şahin *et al.*[15] showed that methanol extract of *O. vulgare* ssp. *vulgare* showed strong free radical scavenging effect (IC_{50} = 9.9 µg/ml), but poor inhibitor effect on linoleic acid oxidation (32%) with 220 µg GAE/mg total phenolic content. The total phenolic amounts of water, ethanol, methanol, acetone extracts of *O. sipyleum* were found in the range of 0.117 to 0.156 µg GAE/ µg extract and their IC_{50} values for DPPH radical scavenging were determined between 0.09 and 1.05 mg/ml.[16] Loizzo *et al.*[17] stated that *O. ehrenbergi* volatile oil, which contains major components such as thymol (19%) and *p*-cymene (16.1%) had DPPH scavenging effect (IC_{50} = 0.99 µg/ml) and *O. syriacum* essential oil which contains thymol (24.7%) and carvacrol (17.6%) as major components prevented oxidation of linoleic acid. Significant antioxidant activities of ethyl acetate extract of *O. majorana* were showed using DPPH (IC_{50}= 2.77 µg/ml) and iron reducing power (0.79 abs at 5 µg/ml) assays.[40] Results in present study were confirmed the findings of some other researchers on antioxidant activity of *O. minutiflorum* as well as those of other *Origanum* species.

In a previous study, Oke and Aslim[10] reported that the total phenolic contents, IC_{50} values for DPPH radical, β-carotene bleaching inhibition rates and metal chelating effects of *O. minutiflorum* extracts collected from Isparta were in the range of 127.47- 137.40 µg/mg GAE, 16.1-42.5 µg/ml, 58.1-98.2% and 21.2-74.8%, respectively. In the same research, inhibition zones and MIC values of these extarcts against *E. coli*, *Shigella sonnei*, *S. aureus*, *L. monocytogenes* and *P. aeruginosa* were found as 5-20.1 mm and 250-2000 µg/ml (n-hexane and acetone extract), respectively. The major components of essential oils for *O. minutiflorum* are carvacrol (% 67.86 -84.95), *p*-cymen (% 5.36 - 8.77) and γ-terpinen (% 0.99-3.46). The antioxidant activity was investigated by using DPPH activity and IC_{50} value was varied between 1.57-5.11 µl.

The strong antimicrobial effects of *O. minutiflorum* volatile oil and carvacrol methyl ether (0.05%) on ciprofloxacin-resistance *Campylobacter* spp. have been reported by Aslim and Yucel.[8] The other authors exhibited that the oil of *O. minutiflorum* showed strong antibacterial effect and their predominant constituent was carvacrol (68.23%). It was followed by *p*-cymene (11.80) similarly to our results.[33] Carvacrol (29.22%) was observed as the predominant compound of *O. minutiflorum* which collected from Isparta and it displayed antibacterial activity against *Helicobacter pylori* in/on infected cells.[18] The major compound of the *O. minutiflorum* essential oil was found as carvacrol (90.78-92.95%) and essential oil showed high antimicrobial effect against S. *aureus, S. faecalis, B. subtilis, E. coli*, and two fungi (C. *albicans, C. tropicalis*).[41]

As mentioned above, antibacterial activities of extract and essential oil obtained from *O. minutiflorum* were reported in many reports. However, their antibacterial effect against wide range of bacteria and antifungal activity against *A. parasiticus* and *A. flavus* were determined here for the first time.

This is the first study to ensure information about antioxidant effect of *O. minutiflorum* extract determined by FRAP, CUPRAC and H_2O_2 scavenging activity assays.

In the study of Baydar *et al.*[32] stated that the major constituent of the *O. onites* and *O. minutiflorum* oils was cavracrol (86.9% and 84.6%, respectively) and they had inhibitory activities against many bacteria. The antibacterial effects of *O. vulgare* oil against some food-borne bacteria tested by Boskovic *et al.*[35] and reported that essential oil had antibacterial effect on all tested bacteria (MIC= 160-640 µg/ml and MBC= 320- ≥2560 µg/ml). Stefanakis *et al.*[42] showed that the oils from*O. vulgare* subsp. *hirtum*, *O. onites* and *O. marjorana* posses strong antimicrobial activity against *E. coli*, *S. cerevisiae*, *Listonella anguillarum*, *Vibrio splendidus*, *Vibrio alginolyticus* and *Vibrio* sp. (5.4-18.8 mm inhibition zones). Similarly to our results, Özkalp *et al.*[43] showed that MIC values of essential oil of *O. vulgare* for *E. coli*, *K. pneumonia*, *L. monocytogenes* and MRSA *S. aureus* was 250 µg/ml.

The moderate antibacterial activity of *O. vulgare* methanol extract on bacteria (MIC = 62.5- 250 µg/ml) between have been described.[44] Antimicrobial effects of the water, ethanol and methanol extracts of *O. vulgare* subsp. *hirtum* against *E. coli* and *S. aureus* (MIC =1000 µg/ml) were determined. Also, their antioxidant activities were tested by DPPH (IC_{50}= 3.80-8.10 µg/ml), metal chelating assay (IC_{50}= 4.80-6.0 mg/ml) and their total phenolic and flavonoid contents were 217.12- 361.98 mg GAE/g and 38.32-111.90 mg QE/g, respectively.[13] *O. vulgare* subsp. *hirtum* and *O. vulgare* subsp. *vulgare* essential oils posses moderate antibacterial and antifungal activities (MIC = 85.3 -426.7µg/ml).[14]

Carvacrol was dominant compound in *O. syriacum* essential oil and it showed antibacterial activity against *S. aureus* (32 mm, inhibition zone) and also antifungal activity with MIC 0.25-5 mg/l values against *Aspergillus fumigates*, *A. flavus* and *A. niger*.[45] The antifungal effect of oregano essential oil is mainly attributable to phenols such as thymol and carvacrol in the oil. It has been proposed that possible action mechanisms are reduction or inhibition of mycelia growth. Phenolics could alter microbial cell permeability and interact with membrane proteins.[45]

In the many reports, strong antimicrobial effects of this oils could be attributed to present phenolic compounds *i.e.* carvacrol, thymol and linalool.[8-44,45] These observations confirm antimicrobial activity results of *O. minutiflorum* which obtained in present study.

CONCLUSION

The findings obtained from various antioxidant activity assays showed that methanol extract of *O. minutiflorum* has potential antioxidant activity. The main constituent of *O. minutiflorum* essential oil was determined as carvacrol. Both extract and essential oil demonstrated high antibacterial activity. *O. minutiflorum* essential oil was also active against aflatoxigenic fungi because of its high carvacrol content. Thus, *O. minutiflorum* can serve as natural antimicrobial and antioxidant additive to extend the shelf life of foods. It is possible to conclude that this herb was beneficial to human health. However, further investigations are needed for clinical, nutritional or pharmacological applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATION

FRAP: Ferric-ion Reducing Power, **CUPRAC:** Cupric ions Reducing Antioxidant Capacity, **DPPH:** 2,2- diphenyl-1-picrylhydrazyl, **IC_{50}:** Concentration Required to Scavenge 50%, **BHT:** Butylated hydroxytoluene, **BHA:** Butylated hydroxyanisole, **EDTA:** Ethylenediamine tetraacetic acid, **MIC:** Minimum Inhibitory Concentration; **MBC:** Minimum bactericidal concentration.

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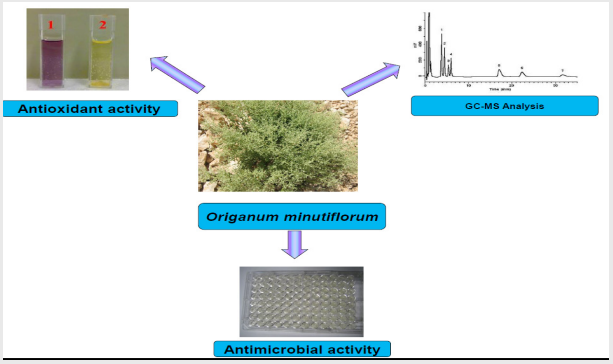
PICTORIAL ABSTRACT



SUMMARY

• This study was performed to determine of phenolic compositions, *in vitro* antioxidant and antimicrobial activity of Origanum minuiflorum, which are endemic, belongs to Turkish flora.
• Carvacrol in the essential oil has been identified as main constituent.
• The methanol extract has potential antioxidant activity.
• Essential oil and extract showed strong antibacterial activity.
• They may be potential sources of preservatives for use in the food and/or pharmaceutical industries.

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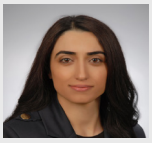

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