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Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey

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Abstract

The present study was conducted to evaluate the antimicrobial activities, antioxidant and properties of essential oils and methanol extracts of *Origanum vulgare* ssp. *vulgare* plants. The chemical composition of a hydrodistilled essential oil of *O. vulgare* ssp. *vulgare* was analyzed by a GC/MS system. A total 62 constituents were identified. Caryophyllene and spathulenol were found to be the main constituents, followed by germacrene-D and α-terpineol. Antioxidant activity was measured employing two methods namely, scavenging of free radical DPPH and the inhibition of linoleic acid oxidation by methanol extracts and the essential oil of *O. vulgare* ssp. *vulgare*. Antioxidant studies suggested that methanol extract behaved as a strong free radical scavenger providing IC_{50} at only 9.9 μg/ml, whereas the oil showed weaker activity with IC_{50} at 8.9 mg/ml. Total phenolic constituents based on gallic acid equivalents revealed the presence of total soluble phenolics in the extract as 220 μg/mg dry extract (22%, w/w) and, most probably, they are responsible for the radical scavenging activity of methanol extracts. Methanol extract was not effectively able to inhibit linoleic acid oxidation and only 32% inhibition was achieved at 2 mg/ml concentration, far below that of the positive control (butylated hyroxytoluene, BHT) at the same concentration. However, 2.2 mg/ml essential oil solutions provided 50% inhibition in the linoleic acid oxidation test system.

The antimicrobial test results showed that the essential oil of *O. vulgare* ssp. *vulgare* had great potential of antimicrobial activity against all 10 bacteria, and 15 fungi and yeast species tested. In contrast, the methanol extract from aerial parts of *O. vulgare* plant showed no antimicrobial activity. The result may suggest that the essential oil *O. vulgare* ssp. *vulgare* possesses compounds with antimicrobial properties as well as antioxidant activity, and therefore can be used as a natural preservative ingredient in food and/or pharmaceutical industry.

Keywords: *Origanum vulgare* ssp. *vulgare*; Essential oil; Antimicrobial activity; Antioxidant activity; DPPH; β-carotene–linoleic acid

1. Introduction

The genus *Origanum* (Labiatae) is an annual, perennial and shrubby herb that is native to the Mediterranean, Euro-Siberian and Irano-Siberian regions (Aligiannis, Kalpoutzakis, Mitaku, & Chinou, 2001). A total 38 *Origanum* species are recognized in the World. Most of the *Origanum* species, over 75%, are concentrated in the East Mediterranean subregion (Ietswaart, 1980). Of them, 16 species are considered as endemic for the flora of Turkey (Guner, Ozhatay, Ekim, & Baser, 2000). *Origanum* species grow abundantly on stony slopes and in rocky mountain areas at a wide range of altitudes (0–4000 m) (Snogerup, 1971). Due to the

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variability in chemical and aroma characteristics, *Origanum* plants belonging to different species and ecotypes (biotypes) are widely used in agriculture and the pharmaceutical and cosmetic industries as a culinary herb, flavouring substances of food products, alcoholic beverages and perfumery for their spicy fragrance (Aligiannis et al., 2001; Novak et al., 2000; Sivropoulou et al., 1996; Vera & Chane-Ming, 1999). It has also used been as a traditional remedy to treat various ailments such as a spasmodic, antimicrobial, expectoran carminative and aromatic for whooping and convulsive coughs, digestive disorders and menstrual problems (Aligiannis et al., 2001; Daferera, Basil, Ziogas, & Polissiou, 2003; Daferera, Ziogas, & Polissiou, 2000; Dmetzos, Perdetzoglou, & Tan, 2001; Dorman & Deans, 2000; Ryman, 1992; Sokovic, Tzakou, Pitarokili, & Couladis, 2002; Tabanca, Demirci, Ozek, Tumen, & Baser, 2001).

In previous studies, it has been demonstrated that the content of essential oil and extracts of medicinal plants like *Origanum* species containing antimicrobial, antioxidant and other biological activities may change based on the differences in cultivation, origin, vegetative stage and growing seasons of the plants (Deans, Svoboda, Gundidza, & Brechany, 1992; Kustrak, Kuftinec, Blazevic, & Maffei, 1996; Leung & Foster, 1996; Milos, Mastelic, & Jerkovic, 2000; Muller-Riebau, Berger, & Yegen, 1995). *Origanum. vulgare* ssp. *vulgare* is one of the most widely distributed subspecies growing in the Eastern Anatolia region of Turkey. However, there have been no attempts to study the chemical composition and biological activities of essential oils and extracts from *O. vulgare* ssp. *vulgare* plants collected from the Eastern Anatolia region of Turkey up to now. In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases (Davis, 1994; Loper et al., 1991; Service, 1995). On the other hand, foodborne diseases are still a major problem in the World, even in well developed countries, like USA (Mead et al., 1999). Food spoilage caused by a variety of microorganisms has often been recognized as inconvenient and one of the most important concern for food industry. So far many bacteria (*Escherichia coli*, *Enterobacter* spp., *Bacillus* spp., *Salmonella* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and *Campylobacter jejuni*), yeast and fungi (*Candida* spp., *Zygosaccharomyces* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp.) species has been reported as the causal agents of foodborne diseases and/or food spoilage (Betts, Linton, & Betteridge, 1999; Deak & Beuchat, 1996; Pitt & Hocking, 1997; Walker, 1988). The contamination of raw and/or processed foods with microflora can take place at various stages from the production to the sale and distribution. (Deak & Beuchat, 1996). Thus, food industry at present uses chemical preservatives to prevent the growth of food spoiling microbes (Sağdıç & Özcan, 2003). Due to the economical impacts of spoiled foods and the consumer's concerns over the safety of foods containing synthetic chemicals, a lot of attention has been paid to naturally derived compounds or natural products (Alzoreky & Nakahara, 2003; Hsieh, Mau, & Huang, 2001). Recently, there has been considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (Alzoreky & Nakahara, 2003; Soliman & Badeaa, 2002; Valero & Salmeron, 2003).

Therefore, the objectives of this study were: (1) to analyze the chemical composition of a hydrodistilled essential oil of *O. vulgare* ssp. *vulgare* collected from the Eastern Anatolia region of Turkey by a GC/MS system in order to be determined the essential oil chemotype; (2) to investigate the antimicrobial and antioxidant activities of essential oil and methanol extracts from *O. vulgare* ssp. *vulgare* plants.

2. Materials and methods

2.1. Plant material

O. vulgare ssp. *vulgare* plants at flowering stage were collected from Oltu valley (1200 m), Erzurum, Turkey. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist, Meryem Şengül, in Department of Biology, Atatürk University, Erzurum, Turkey. Collected plant materials were dried in shadow, and the leaves of plant were separated from the stem, and ground in a grinder with a 2 mm diameter mesh. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Atatürk University, Erzurum, Turkey (ATA. HERB. 9731).

2.2. Preparation of the extracts

2.2.1. Isolation of the essential oil

The air-dried and ground aerial parts of plants collected were submitted for 3 h to water-distillation using a Clevenger-type apparatus (yield 2.31% v/w). The obtained essential oil (EO) was dried over anhydrous sodium sulphate and, after filtration, stored at +4 °C until tested and analyzed.

2.2.2. Preparation of the methanol extracts (MeOH)

Dried and powdered leaves (500 g) were extracted successively with 1 l of methanol by using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent (Lin et al., 1999). The ex-

tracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40 °C using a rotary evaporator. The residues obtained were stored in a freezer at −80 °C until further tests.

2.3. GC–MS analysis conditions

The analysis of the essential oil was performed using a Hewlett Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm) and a HP 5972 mass selective detector. For GC–MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. Column temperature was initially kept at 50 °C for 3 min, then gradually increased to 150 °C at a 3 °C/min rate, held for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, NBS75K library data of the GC–MS system and literature data (Adams, 2001). The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams, 2001).

2.4. Antimicrobial activity

2.4.1. Microbial strains

The methanol extracts and the essential oil and its fractions of *O. vulgare* ssp. *vulgare* were individually tested against a range of 40 microorganisms, among them 24 bacteria and 15 fungi and yeast species. The list of microorganisms used is given in Tables 3 and 4. Microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine, and Plant Diagnostic Laboratory, Faculty of Agriculture at Atatürk University, Erzurum, Turkey. Identity of the microorganisms used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Atatürk University.

2.4.2. Disc-diffusion assay

The dried plant extracts were dissolved in the same solvent (methanol) to a final concentration of 30 mg/ml and sterilized through filtration by 0.45 μm Millipore filters. Antimicrobial tests were then carried out by disc diffusion method (Murray, Baron, Pfaller, Tenover, & Yolke, 1995) using 100 μl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast and 10^4 spore/ml of fungi spread on nutrient agar (NA), sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The discs (6 mm in diameter) were impregnated with 10 μl of essential oil or the 30 mg/ml extracts (300 μg/disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (10 μg/disc), sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc) and/or netilmicin (30 μg/disc) were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains, 48 h for yeast and 72 h for fungi isolates. Plant associated microorganisms were incubated at 27 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

2.4.3. Microwell dilution assay

The minimal inhibition concentration (MIC) values were determined for the bacterial strains which were sensitive to the essential oil and/or extracts in disc diffusion assay. The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils and extracts of *O. vulgare* ssp. *vulgare* dissolved in 10% dimethylsulfoxide (DMSO), were first diluted to the highest concentration (500 μg/ml) to be tested, and then serial twofold dilutions were made in order to obtain a concentration range from 7.8 to 500 μg/ml in 10 ml sterile test tubes containing nutrient broth. MIC values of *O. vulgare* ssp. *vulgare* extracts against bacterial strains and *Candida albicans* isolates were determined based on a microwell dilution method (Zgoda & Porter, 2001) with some modifications.

The 96-well plates were prepared by dispensing into each well 95 μl of nutrient broth and 5 μl of the inoculums. A 100 μl from the stock solutions of *O. vulgare* ssp. *vulgare* essential oil and extracts initially prepared at the concentration of 500 μg/ml was added into the first wells. Then, 100 μl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 μl of nutrient broth without compound and 5 μl of the inoculums on each strip was used as negative control. The final volume in each well was 200 μl. Maxipime (Bristol–Myers Squibb) at the concentration range of 500–7.8 μg/ml was prepared in nutrient broth and used as standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the respective absorbance (Abs) at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, VT, USA) and confirmed by plating 5 μl samples from clear wells on nutrient agar medium. The extract tested in this study was screened two times against each organism. The MIC

was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

2.4.4. MIC agar dilution assay

MIC values of the fungal isolates were studied determined using the agar dilution method as, described previously (Gul, Ojanen, & Hanninen, 2002). The essential oils of *O. vulgare* ssp. *vulgare* were added aseptically to sterile molten PDA medium containing Tween 20 (Sigma 0.5%, v/v) at the appropriate volume to produce the concentration range of 7.8–500 μg/ml. The resulting PDA agar solutions were poured into Petri plates immediately after vortexing. The plates were spot inoculated with 5 μl (10^4 spore/ml) of each fungal isolate. Amphotericin B (Sigma A 4888) was used as a reference antifungal drug. The inoculated plates were incubated at 27 and 37 °C for 72 h for plant and clinical fungi isolates, respectively. At the end of incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oil where absence of growth was recorded. Each test repeated at least twice.

2.5. Antioxidant activity

2.5.1. DPPH assay

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical diphenylpicrylhydrazyl (DPPH) as a reagent (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). Fifty microliter of various concentrations of the extracts in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I%) was calculated in the following way:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Synthetic antioxidant reagent butylated hyroxytoluene (BHT) was used as positive control and all tests were carried out in triplicate.

2.5.2. β-*Carotene–linoleic acid assay*

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides formation from linoleic acid oxidation (Dapkevicius, Venskutonis, Van Beek, & Linssen, 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 μl linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen (30 min 100 ml/min) was added with a vigorous shaking. 2500 μl of this reaction mixture was dispersed to test tubes and 350 μl portions of the extracts prepared in ethanol at 2 g/l concentrations were added and emulsion system was incubated up to 48 h at room temperature. Same procedure was repeated with positive control BHT and a blank. After this incubation period absorbance of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those BHT at the same concentration and blank consisting only 350 μl ethanol.

2.5.3. Assay for total phenolics

Total phenolic constituent of the methanol extract of *O. vulgare* ssp. *vulgare* was determined by employing the methods given in the literature (Chandler & Dodds, 1983; Slinkard & Singleton, 1997) involving Folin–Ciocalteu reagent and gallic acid as standard. 0.1 ml of extract solution containing 1000 μg extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin–Ciocalteu reagent were added and flask was shaked thoroughly. After 3 min, 3 ml of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions (0–1000 g/0.1 ml^{-1}) and standard curve was obtained that equation given below:

$$\text{Absorbance} : 0.0012 \times \text{Gallic acid } (\mu g) + 0.0033$$

3. Results and discussion

3.1. Chemical composition of the essential oil

The composition of *O. vulgare* ssp. *vulgare* essential oil was analyzed by employing GC–MS, leading to compare the relative retention times and the mass spectra of oil components with those of authentic samples and mass spectra from data library.

As shown in Table 1, GC/MS analysis of the crude oil resulted in the identification of 62 compounds representing about 89% of the oil. Caryophyllene (14.4%) and spathulenol (11.6%) were the most prominent compounds, followed by germacrene-D (8.1%) and α-terpineol (7.5%). As reported previously, two main chemotypes were postulated for the essential oils from several *Origanum* species; one consists mainly monoterpene alcohols, e.g. terpinen-4-ol, either alone or to-

Table 1
Chemical composition of *Origanum vulgare* ssp. *vulgare* essential oil

K.I.	t_R (min)	Components	Composition (%)
975	11.560	Sabinene	1.0
979	12.026	1-Octen-3-ol	0.3
984	12.294	3-Octanone	0.6
991	12.779	3-Octanol	0.3
1017	13.622	α-Terpinene	0.1
1025	14.028	*p*-Cymene	0.7
1030	14.197	β-Phellandrene	0.2
1031	14.355	Eucalyptol	1.5
1037	14.722	(*Z*)β-Ocimene	0.3
1050	15.237	(*E*)β-Ocimene	0.6
1060	15.723	γ-Terpinene	0.3
1070	16.219	*cis*-Sabinene hydrate	tr
1073	16.486	*trans*-Linalool oxide (furanoid)	tr
1097	17.963	Linalool	2.1
1122	18.944	Menth-2-en-1-ol, *cis-para*	0.1
1146	20.035	Camphor	0.7
1153	20.550	Menthone	tr
1163	21.016	Pinocamphone	3.2
1165		Pinocarvone	
1166	21.264	δ-Terpineol	0.3
1175	21.531	Isopinocamphone	0.2
1177	21.799	Terpinen-4-ol	2.4
1189	**22.631**	**α-Terpineol**	**7.5**
1196	23.276	Piperitol	tr
1237	24.832	Pulegone	2.1
1243	24.971	Carvone	0.3
1253	25.536	*Piperitone*	1.2
1265	25.793	*cis*-Chrysanthenyl acetate	0.1
1289	26.963	Bornyl acetate	0.2
1291	27.290	*trans*-Sabinyl acetate	0.1
1290	27.488	Thymol	0.8
1299	27.944	Carvacrol	0.6
1338	29.332	Elemene (delta)	0.1
1343	29.669	Piperitenone	0.9
1351	29.907	α-Cubebene	tr
1369	30.769	Piperitenone oxide	0.2
1377	31.126	α-Copaene	0.2
1388	31.572	β-Bourbonene	1.3
1419	**32.972**	**Caryophyllene**	**14.4**
1432	33.554	β-Copaene	0.5
1435	33.782	Bergamotene (α-*trans*)	0.1
1441	33.961	Aromadendrene	0.1
1455	**34.664**	**α-Caryophyllene**	**2.7**
1460	34.922	Aromadendrene (Allo)	0.6
1480	35.150	γ-Muurolene	0.1
1485	**35.953**	**Germacrene-D**	**8.1**
1496	36.082	Valencene	0.3
1500	36.538	Bicyclogermacrene	2.9
1506	36.974	β-Bisabolene	5.2
1514	37.231	γ-Cadinene	0.5
1520	37.400	Bourbonanol (*endo*-1)	0.2
1523	37.628	δ-Cadinene	1.9
1535	37.985	Cadina-1(2),4-diene (*trans*)	0.1
1539	38.183	α-Cadinene	0.1
1563	39.224	Bourbonanone <1-nor	0.1
1578	**40.235**	**Spathulenol**	**11.6**
1583	40.354	Caryophyllene oxide	5.8
1595	40.601	Salvial-4(14)-en-1-one	0.7
1641	42.098	Caryophylla-4(14),8(15)-dien-5α-ol	0.4
1641	42.276	Caryophylla-4(14),8(15)-dien-5β-ol	0.7
1654	42.970	α-Cadinol	1.0
–	49.076	6,10,14-trimethyl-2-pentadecanone	0.2
		Total	**88.8**

tr, $\leqslant 0.06\%$.

gether with *cis*- and *trans*-sabinene hydrate and the other rich in phenols, namely thymol and/or carvacrol (Aligiannis et al., 2001; Sarer, Schefler, & Baerheim, 1982; Sivropoulou et al., 1996). Moreover, it is known that the species *O. vulgare* presents great variability in its essential oil composition due to the existence of different subspecies, but also to a numerous of parameters where mainly are the environmental and climatic conditions. In the case of *O. vulgare* ssp. *vulgare*, the known chemotypes of the essential oil are those contain as major components the phenols thymol and/or carvacrol (Fleisher & Fleisher, 1991) and those contain germacrene-D and terpinen-4-ol (Aligiannis et al., 2001; Sivropoulou et al., 1996). Apparently, our findings display a new oil chemotype consists mainly of caryophyllene/spathulenol and secondly germacrene-D. With respect to the essential oil yield, the oregano's essential oils which belong to thymol and/or carvacrol chemotype present high oil yields, in contrast with the others which present much lower values (Kokkini, Karousou, & Vokou, 1994; Sezik, Tümen, Kirimer, Özek, & Baser, 1993). In our case, it is noteworthy the high essential oil yield of *O. vulgare* ssp. *vulgare*, even though the non-phenolic composition of the oil.

3.2. Amount total of phenolic compounds

Based on the absorbance value of the methanol extract solution, reacted with Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents as described above, the amount of total phenolics was estimated as 220 μg/mg dry extract (22%, w/w).

3.3. Antioxidant activity

In the present study, the free radical scavenging activity and lipid oxidation inhibition by *O. vulgare* ssp. *vulgare* extracts and the essential oil were studied in vitro. Both in the extract and the oil cases, the reactions followed a concentration dependent pattern. As shown in Table 2, the DPPH radical scavenging activities of the methanol extract and the reference chemical (BHT) were remarkable, exhibiting ability to reduce the stable radical DPPH to yellow-coloured diphenylpicrylhydrazine with IC_{50}'s at 9.9 ± 0.5 and 19.8 ± 0.5 μg/ml, respectively, whereas that of the oil was insignificant with an IC_{50} at

Table 2
Effects of *Origanum vulgare* ssp. *vulgare* methanol extract, essential oil and positive control BHT on the in vitro free radical (DPPH) scavenging

Sample	DPPH, IC_{50} (μg/ml)
Methanol extract	9.9 ± 0.5
Essential oil	8900 ± 5.0
BHT	19.8 ± 0.5

8900 μg/ml. In the free radical scavenging activity, superiority of the methanol extract could be attributed to the presence of phenolics as they comprise 22% of the extract. Particularly, synergistic effects of fenolic acids e.g., rosmarinic acid and polyphenols as well as other chemicals such as flavonoids could also be taken into account for the radical scavenging activity observed in the methanol extracts (Choi et al., 2002). DPPH radical scavenging activity of the oil was very low, and this was obviously related to its chemical composition. In several reports, thymol and carvacrol, in particular, were found to be main antioxidant constituents of the oils isolated from several *Origanum* species (Barrata et al., 1998; Milos et al., 2000; Puertes-Mejia, Hillebrand, Stashenko, & Winterhalter, 2002; Ruberto & Barrata, 2000; Ruberto, Barrata, Sari, & Kaabexhe, 2002). Besides, the antioxidative effectiveness of about 100 pure components of essential oils has been studied, and the phenols were confirmed to possess the highest antioxidant activity (Ruberto & Barrata, 2000). Our findings in radical scavenging activity is in accordance with these reports, since the percentage of thymol and carvacrol were remarkably low (0.84% and 0.57%, respectively) in *O. vulgare* ssp. *vulgare* essential oil.

In the case of inhibition of linoleic acid assay, both methanol extract and essential oil were not able to effectively inhibit the linoleic acid oxidation and only 24% and 36% inhibitions were achieved at 2 mg/ml concentrations respectively, which were far below than the positive control BHT at the same concentration (Fig. 1). Activity might be improved at higher concentrations but this was not considered here. These findings are also in

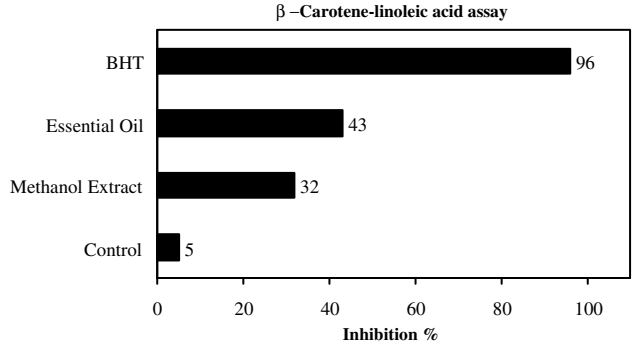

Fig. 1. Antioxidant activity of *O. vulgare* ssp. *vulgare* extract and essential oil defined as inhibition percentage in β-carotene–linoleic acid assay.

Table 3
Antimicrobial activity of *Origanum vulgare* ssp. *vulgare* extract and essential oil against the bacterial strains tested based on disc diffusion method

Bacterial species	Inhibition zone in diameter (mm) around the discs impregnated with 10 μl of essential oil and extracts (300 μg/disc)			
	Essential oil (10 μl/disc)	Plants extracts MeOH	Negative control MeOH	Standard antibiotic discs[a]
Acinetobacter baumanii-A8	18 mm	–	–	18 mm (OFX)
Bacillus macerans-M58	28 mm	–	–	19 mm (OFX)
Bacillus megaterium-M3	–	–	–	9 mm (SCF)
Bacillus subtilis-ATCC-6633	29 mm	–	–	28 mm (OFX)
Bacillus subtilis-A57	12 mm	–	–	28 mm(OFX)
Brucella abortus-A77	–	–	–	12 mm (SCF)
Burkholdria cepacia-A225	–	–	–	22 mm (SCF)
Clavibacter michiganense-A227	33 mm	–	–	25 mm (SCF)
Enterobacter cloacae-A135	–	–	–	20 mm (NET)
Enterococcus faecalis-ATCC-29122	10 mm	–	–	18 mm (SCF)
Escherichia coli-A1	18 mm	–	–	– mm (OFX)
Klebsiella pneumoniae-A137	–	–	–	12 mm (OFX)
Proteus vulgaris-A161	–	–	–	12 mm (OFX)
Proteus vulgaris-Kukem-1329	12 mm	–	–	13 mm (OFX)
Pseudomonas aeruginosa-ATCC-9027	–	–	–	22 mm (NET)
Pseudomonas aeruginosa-ATCC-27859	–	–	–	22 mm (NET)
Pseudomonas syringae pv. *tomato*.-A35	–	–	–	24 mm (OFX)
Salmonella enteritidis-ATCC-13076	–	–	–	27 mm (SCF)
Staphylococcus aureus-A215	10 mm	–	–	22 mm (SCF)
Staphylococcus aureus-ATCC-29213	–	–	–	22 mm (SCF)
Staphylococcus epidermis-A233	–	–	–	– mm (SCF)
Streptococcus pyogenes-ATCC-176	10 mm	–	–	10 mm (OFX)
Streptococcus pyogenes-Kukem-676	–	–	–	13 mm (OFX)
Xanthomonas campestris-A235	–	–	–	20 mm (SCF)
Total 24 bacterial species	10–33 mm	–	–	

[a] OFX = ofloxacin (10 μg/disc); SCF = sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc); NET = netilmicin (30 μg/disc) were used as positive reference standards antibiotic discs (Oxoid).

accordance with those of the previous reports (Ruberto & Barrata, 2000).

3.4. Antimicrobial activity

The antimicrobial activities of *O. vulgare* ssp. *vulgare* essential oil and extracts against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter, and MIC values. The results were given in Tables 3–6. The results showed that the essential oil of *O. vulgare* ssp. *vulgare* had substantial of antimicrobial activity against 10 bacteria, and 15 fungi and a yeast species tested. On the other hand, the methanol extract from aerial parts of *O. vulgare* ssp. *vulgare* plants showed no antimicrobial activities (Tables 3–5).

The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil of *O. vulgare* ssp. *vulgare*, were in the range of 10–33 mm, and 15.62–125 μl/ml (Tables 3–5). The maximal inhibition zones and MIC values of the yeast and fungi species sensitive to the essential oil of *O. vulgare* ssp. *vulgare*, were 13–35 mm and 31.25–125 μl/ml, respectively (Tables 3 and 5). Based on these results, it is possible to conclude that the essential oil has stronger and broader spectrum of antimicrobial activity as compared to the methanol extract tested. This observation confirmed the evidence in a previous study reported that the essential oil include more antimicrobial substances from medicinal plants than other extracts such as water, methanol, ethanol and hexane (Ahmad, Mehmood, & Mohammad,

Table 5
The MIC values *Origanum vulgare* ssp. *vulgare* of the essential oil against the bacterial strains tested in microdilution assay (μg/ml)

Bacteria species	Essential oil	Standard drug (maxipime)
Acinetobacter baumanii-A8	15.62	31.25
Bacillus macerans-M58	31.25	15.62
Bacillus megaterium-M3	125	15.62
Bacillus substilis-ATCC-6633	31.25	62.50
Bacillus substilis-A57	62.50	125
Brucella abortus-A77	–	62.50
Burkholdria cepacia-A225	–	125
Clavibacter michiganense-A227	15.62	15.62
Enterobacter cloacae-A135	–	31.25
Enterococcus faecalis-ATCC-29122	62.50	31.25
Escherichia coli-A1	31.25	62.50
Klebsiella pneumoniae-A137	–	125
Proteus vulgaris-A161	–	125
Proteus vulgaris-Kukem-1329	62.50	125
Pseudomonas aeruginosa-ATCC-9027	–	31.25
Pseudomonas aeruginosa-ATCC-27859	–	15.62
Pseudomonas syringae pv. *tomato*.-A35	–	125
Salmonella enteritidis-IK27	–	62.50
Staphylococcus aureus-ATCC-29213	–	31.25
Staphylococcus aureus-A215	62.50	62.50
Staphylococcus epidermis-A233	–	15.62
Streptococcus pyogenes-ATCC-176	–	62.50
Streptococcus pyogenes-Kukem-676	–	31.25
Xanthomonas campestris-A235	–	31.25
	15.62–125	15.62–125

Table 4
Antimicrobial activity of *Origanum vulgare* ssp. vulgare extract and essential oil against the yeast and fungi isolates tested based on disc diffusion method

Yeast and fungi species	Inhibition zone in diameter (mm) around the discs impregnated with 10 μl of essential oil and extracts (300 μg/disc)			
	Essential oil (10 μl/disc)	Plant extracts MeOH	Negative control MeOH	Standard antibiotic discs[a]
Yeast				
Candida albicans-A117	17 mm	–	–	– (NET)
Fungi				
Alternaria alternata	32 mm	–	–	– (NET)
Aspergillus flavus	24 mm	–	–	– (NET)
Aspergillus variecolor	32 mm	–	–	– (NET)
Fusarium acuminatum	27 mm	–	–	– (NET)
Fusarium oxysporum	24 mm	–	–	– (NET)
Fusarium solani	18 mm	–	–	– (NET)
Fusarium tabacinum	35 mm	–	–	– (NET)
Moniliania fructicola	20 mm	–	–	– (NET)
Penicillium spp.	35 mm	–	–	– (NET)
Rhizopus spp.	14 mm	–	–	– (NET)
Rhizoctonia solani	35 mm	–	–	– (NET)
Scloretinia sclerotiorum	13 mm	–	–	– (NET)
Scloretinia minor	27 mm	–	–	– (NET)
Trichophyton mentagrophytes	35 mm	–	–	– (NET)
Trichophyton rubrum	29 mm	–	–	– (NET)
Total 15 isolates	13–35 mm	–	–	

[a] OFX = ofloxacin (10 μg/disc); SCF = sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc); NET = netilmicin (30 μg/disc) were used as positive reference standards antibiotic discs (Oxoid).

Table 6
The MIC values of *Origanum vulgare* ssp. *vulgare* essential oil against the yeast and fungi isolates tested in agar dilution assay (μg/ml)

Yeast and fungi species	Essential oil	Standard drug (amphotericin B)
Yeast		
Candida albicans-A117	125	31.25
Fungi		
Alternaria alternata	125	15.62
Aspergillus flavus	125	15.62
Aspergillus variecolor	31.25	62.50
Fusarium acuminatum	62.50	62.50
Fusarium oxysporum	62.50	62.50
Fusarium tabacinum	31.25	62.50
Moniliania fructicola	62.50	15.62
Penicillium spp.	31.25	31.25
Rhizopus spp.	125	125
Rhizoctonia solani	62.50	31.25
Sclorotinia sclerotiorum	31.25	62.50
Sclorotinia minor	31.25	125
Trichophyton mentagrophytes	31.25	31.25
Trichophyton rubrum	31.25	15.62
	31.25–125	15.62–125

1998; Eloff, 1998). Findings in this study supported the observations of some other researchers about *O. vulgare* ssp. *vulgare* containing some substances with antibacterial properties (Deans & Svoboda, 1990; Leung & Foster, 1996; Zargari, 1990). This is the first study to provide data about the extracts and essential oil of *O. vulgare* ssp. *vulgare* plants evaluated against a wide range of microorganisms possess potential antibacterial, antifungal and anticandidal activities. This result may indicate that essential oil of *O. vulgare* ssp. *vulgare* can be used as natural preservatives in food against the well known causal agents of foodborne diseases and food spoilage such as *E. coli*, *Enterobacter* spp., *Bacillus* spp., *Salmonella* spp., *Staphylococcus aureus*, *Candida* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. isolates.

Our data confirmed the findings of the previous studies reported that caryophyllene and germacrene-D, the main constituents of *O. vulgare*, and some other medicinal plants, have significant antibacterial and antifungal activities (Kalodera, Pepeljnjak, Blazevic, & Petrac, 1997; Kazarinova et al., 2002; Simic, Palic, Vajs, Milosavljevic, & Djkovic, 2002).

Therefore, the results may suggest that *O. vulgare* ssp. *vulgare* possess compounds with antimicrobial and antioxidant properties which can be used for preservation and/or extension the self-life of raw and processed foods as well as pharmaceuticals and natural therapies of infectious diseases in human, and management of plant diseases. In addition, the data in the present study are supporting the use of *O. vulgare* ssp. *vulgare* plants as tea or additive in foods, and traditional remedies for the treatment of infectious diseases.

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