Screening Chemical Composition and *in Vitro* Antioxidant and Antimicrobial Activities of the Essential Oils from *Origanum syriacum* L. Growing in Turkey

Mehmet Hakki ALMA,^a Ahmet MAVI,^b Ali YILDIRIM,^b Metin DIGRAK,^{*,c} and Toshifumi HIRATA^d

^aDepartment of Industrial Engineering of Forestry, Faculty of Forestry, University of Kahramanmaraş Sutcu Imam; Kahramanmaraş, 46060 Turkey; ^bDepartment of Chemistry, Faculty of Kazım Karabekir Eğitim, University of Atatürk; 25240 Erzurum, Turkey; ^cDepartment of Biology, Faculty of Arts and Science, University of Kahramanmaraş Sutcu Imam; Kahramanmaraş, 46060 Turkey; and ^dLaboratory of Biological Chemistry, Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University; 1–3–1 Kagamiyama, Higashi-Hiroshima 739–8526, Japan.

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In the present study, essential oil from the leaves of Syrian oreganum [*Origanum syriacum* L. (Lauraceae)] grown in Turkish state forests of the Dörtol district, Turkey, was obtained by steam distillation. The chemical composition of oil was analysed by GC and GC-MS, and was found to contain 49.02% monoterpenes, 36.60% oxygenated monoterpenes and 12.59% sesquiterpenes. The major components are as follows: γ -terpinene, carvacrol, *p*-cymene and β -caryophyllene. Subsequently, the reducing power, antioxidant and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities of the essential oil were studied. The reducing power was compared with ascorbic acid, and the other activities were compared with 2,6-di-*tert*-butyl-4-methyl phenol (BHT, butylated hydroxytoluene). The results showed that the activities were concentration dependent. The antioxidant activities of the oil were slightly lower than those of ascorbic acid or BHT, so the oil can be considered an effective natural antioxidant. Antimicrobial activities of the essential oil from the leaves of *Origanum syriacum* was also determined on 16 microorganisms tested using the agar-disc diffusion method, and showed antimicrobial activity against 13 of these.

Key words antimicrobial activity; essential oil; Turkey; antioxidant; chemical composition; *Origanum syriacum*

Essential (volatile) oils of herbs and their components, products from the secondary metabolism of a plant, have many applications in folk medicine, food flavoring and preservation as well as in the fragrance and pharmaceutical industries. The antimicrobial and antioxidant properties of essential oils have been known for a long time, and a number of investigations have been conducted on their antimicrobial activities using various bacteria, viruses and fungi.^{1–6}

Free radicals, *e.g.*, superoxide ions ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$) and non-free radical compounds, can be responsible for lipid peroxidation (deterioration) in foods^{7–9} and for various diseases such as malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer *etc.*^{10,11} It has been reported that some synthetic antioxidant compounds such as butylated hydroxytoluene and butylated hydroxyanisole, commonly used in processed foods, have side effects.^{12,13} Therefore, investigations on identifying the natural antioxidants have become very important issue.^{14–21} In this study, the leaves of Syrian oreganum (*Origanum syriacum* L.) were screened for their antioxidant activity as well as antimicrobial properties and chemical composition.

O. syriacum is native to the eastern Mediterranean, southern Europe and western Asia, and is cultivated in many parts of the world and commonly called Syrian oreganum, bible hyssop or wild marjoram. The descriptions of *O. syriacum* are as follows: Subshrub to 90 cm, hirsute-tomentose. Branches to 10 pairs per stem, to 10 a length of cm. Leaves petiolate or subsessile (petiole to 8 mm), ovate, 5–35×4–23 mm, acute or obtuse, entire or remotely serrulate or crenulate, veins raised on lower surface. Spicules in paniculate inflorescence, c. 5–25×4 mm. Bracts obovate or elliptic, 2–5×1.5–3.5 mm, acute or obtuse, entire or denticulate. Calyx

c. 2.5 mm. Corolla 4.5–7.5 mm. Fl. 5–10. Calcareous rocks and slopes, often in partial shade, 200–2700 m.²²

The leaves of *O. syriacum*, a very popular Arab spice, have been used as an herbal (traditional) medicine, flavour, fragrance and for aromatherapy in the form of bath, massage, steam inhalation, and vaporization.^{23,24} It is used in teas and cooked or baked foods. It has a strong oregano flavor and also is thought to be the “Hyssop” mentioned in the Bible and to have powers of spiritual purification.

The essential oils from the leaves of Syrian oreganum reportedly have ovicidal,²⁵ bioherbicidal,²⁴ insecticidal,^{26,27} antifungal²⁸ and antimycotic activities.²⁹ The present study was undertaken to verify the chemical composition, antioxidant activities and antimicrobial activities of these oils from leaves of this plant growing in maquis in the southern part of Turkey.

MATERIALS AND METHODS

In this study, the leaves of Syrian oreganum [*Origanum syriacum* L. (Lauraceae); (Syn. *Majorana syriaca* and *Origanum maru*] were collected at the end of June 2002 from maquis in Dörtol–Hatay, in southern Turkey, at an altitude of 200 m. The leaves were air dried and then chopped into small pieces by a mill with 6 rotary knives. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Kahramanmaraş Sutcu Imam University.

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), butylated hydroxytoluene (BHT) and trichloroacetic acid (TCA) were obtained from Sigma. Mueller Hinton agar

* To whom correspondence should be addressed. e-mail: mdigrak@hotmail.com

was obtained from Oxoid. All the other chemicals used were of analytical grade and were obtained from Sigma. Additionally, two reference antibiotics, ampicillin sodium (Ampicillin) and streptomycin sulphate (Streptomycin10) were used as positive control antibactericides while nystatin was used as a positive control fungicide. They were purchased from Eczacibasi Med. Co., Turkey.

Preparation of Essential Oil The air-dried-leaves of Syrian oreganum were steam-distilled for 3 h, and oil with a yield of 2.8% was obtained. Specific gravity of the oil was 0.90 g/cm³ at 25 °C.

Chromatographic Analysis Qualification of the essential oil was analyzed on an MSD 5971A mass spectrometer coupled with a Hewlett-Packard GC-5890II series GC. A DB-1 column (30 m×0.53 mm i.d.) and nitrogen as carrier gas (with a flow rate of 0.5 ml/min) were used. One microliter of oil was injected into the column. The GC oven temperature was programmed from 60 to 220 °C at a rate of 5 °C/min and then kept at 220 °C. MS spectra were taken at EI ion source of 70 eV.

Quantification of the essential oil was conducted by gas chromatography with a flame ionization detector (GC-FID) on a Hewlett-Packard GC-5890II series GC. One microliter of oil was injected into a BPX5 fused silica capillary column (25 m×0.32 mm i.d., 0.5 mm film thickness). The carrier gas (nitrogen) was delivered at a constant pressure of 30 kPa. The initial temperature of the GC oven was 60 °C for 3 min, and then the temperature was increased from 60 to 240 °C at a rate of 4 °C/min.

Bioassay Growth inhibitory activity of the essential oil was tested against 14 bacteria (e.g., *Corynebacterium xerosis* UC9165, *Bacillus brevis* FMC 3, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Mycobacterium smegmatis* RUT, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* Cowan 1, *Klebsiella pneumoniae* FMC 5, *Klebsiella oxytoca* A, *Enterococcus faecalis* CR 25, *Micrococcus luteus* LA 2971, *Escherichia coli* ATCC 25922, *Yersinia enterocolitica* CR 38) and 3 fungi (*Kluyveromyces fragilis*, *Rhodotorula rubra* *Saccharomyces cerevisiae* WET 136). These microorganisms were provided from the Microbiology Laboratory Culture Collection, Department of Biology, Sıtcu Imam University, Turkey.

Antimicrobial activities of the essential oils of the leaves of *O. syriacum* were determined using the agar-disc diffusion method described below. The bacteria were first incubated at 37±0.1 °C for 24 h in nutrient broth (Difco), and the yeasts were incubated in sabouraud dextrose broth (Difco) at 25±0.1 °C for 24 h. After injecting cultures of the bacteria and yeast (prepared as above) into petri dishes (9 cm) in the amount of 0.1 ml (10⁶/ml for the bacteria and 10⁵/ml for the fungi), 15 ml of mueller hinton agar (MHA, Oxoid) and sabouraud dextrose agar (SDA) (sterilized in a flask and cooled to 45–50 °C) were homogenously distributed onto the sterilized petri dishes.

Sterilized blank paper discs 6 mm in diameter were saturated with 2 and 4 µl of essential oil per disc, then placed onto the agar plates which had previously been inoculated with the above organisms. Blank paper discs treated with ampicillin, streptomycin and nystatin-saturated antibiotics were used as positive controls. After the plates combined with the discs were left at 4 °C for 2 h, the plates injected

with yeast were incubated at 25±0.1 °C for 24 h and those injected with bacteria were incubated at 37±0.1 °C for 24 h.^{30,31} At that time, inhibition zones appearing around the discs were measured and recorded in mm.

Antioxidant Properties An essential oil solution (1 mg/ml) was prepared by dissolving the oil in ethanol. There was no detectable effect of the ethanol on any measured activity, as established by control experiments in which solvent containing no extract was used in the assays.

Antioxidant Activity Antioxidant activity was determined by to the thiocyanate method.²¹ Briefly, 0.02 M linoleic acid emulsion was prepared by mixing 0.14 g linoleic acid with an equal amount of Tween 20 in 25 ml of 0.02 M, pH 7.4 phosphate buffered saline (PBS). Afterwards, ethanol solution of the oil (final concentration of 20–60 mg/l essential oil) was then mixed with 2.5 ml of the 0.02 M linoleic acid emulsion, the final volume was adjusted to 5 ml with phosphate buffered saline (0.02 M, pH 7.4) and incubated in darkness at 37 °C. The amount of peroxide was determined by reading absorbance at 500 nm after coloring with FeCl₂ and thiocyanate at intervals during incubation.¹⁹ The control was the linoleic acid emulsion without essential oil. As a standard antioxidant, BHT (concentration: 20 mg/l) was used. The results are means of three different experiments in each of which two measurements were made.

Reducing Power This was carried out as described previously.²¹ Briefly, the 1 ml oil solution (final concentration: 100–500 mg/l) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K₃Fe(CN)₆] (10 g/l), then the mixture was incubated at 50 °C for 20 min. Two and one-half, 2.5 ml of trichloroacetic acid (100 g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min (Heraeus Sepetach Suprafuge 22, Germany). Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl₃ (1 g/l) and the absorbance was measured at 700 nm. As a control, ascorbic acid was used (final concentration: 10 mg/l). Increased absorbance of the reaction mixture indicated stronger reducing power.

DPPH Radical-Scavenging Activity This was carried out according to the Blois method with a slight modification.²¹ Briefly, 1 ml of a 1 mM solution of DPPH radical in methanol was mixed with 3 ml of essential oil solution (final concentration of essential oil: 100–500 mg/l), and after 30 min the absorbance was measured at 517 nm. This activity is given as % DPPH radical-scavenging calculated according to the equation:

$$\% \text{ DPPH radical-scavenging} = \frac{(\text{control absorbance} - \text{oil absorbance})}{(\text{control absorbance})} \times 100$$

Control was the DPPH solution without essential oil.

RESULTS AND DISCUSSION

Chemical Composition There are some studies on the essential oils from *S. oreganum* leaves,^{25,32} however, none study is available on GC-MS analysis of oil from the leaves of this plant grown in the Dortyol district (southern Turkey) at an altitude of 200 m. Table 1 represents the chemical composition of the essential oil from laurel leaves. As shown, thirteen components, representing almost 98.21% of the

essential oil of *S. oreganum* leaf, were characterized. The major components were: γ -terpinene (27.79%), carvacrol (26.97%), *p*-cymene (15.69%) and β -caryophyllene (12.59 %).

Baser *et al.*³²⁾ reported that *O. syriacum* var. *bevanii* growing in the Kahramanmaraş region in Turkey contained carvacrol (64.1%) and *p*-cymene (12.3%) as major components. On the other hand, it was reported that while essential oil from *O. syriacum* originating from Israel contained thymol (59.87%) and carvacrol (80.17%) as a major component, the essential oil the same plant originating in Egypt only contained carvacrol as the major constituent (76.7%).³³⁾ It is well known that these sorts of variations are due to geographical origin, harvesting time and growing conditions.

Antimicrobial Activity In this study, the antimicrobial activities of the essential oil from *S. oreganum* leaves, having two different concentrations of 2 and 4 μ l/disc, are compared with those of ampicillin, streptomycin and nystatin used as positive controls as shown in Table 2.

Table 1. Chemical Composition of Essential Oil of *Syrian oreganum* Leaves Originating from Turkey

Compound	Retention time (min)	Yield (%)
Monoterpene hydrocarbons		49.02
α -Pinene	5.20	1.22
Myrcene	6.69	1.38
α -Terpinene	7.30	2.94
<i>p</i> -Cymene	7.50	15.69
γ -Terpinene	8.29	27.79
Oxygenated monoterpenes		36.60
Linalool	9.15	0.89
Terpinen-4-ol	10.70	0.74
α -Terpineol	10.90	0.01
Thymol methyl ether	11.50	1.30
Carvacrol methyl ether	11.76	4.57
Thymol	12.49	2.12
Carvacrol	12.64	26.97
Sesquiterpene hydrocarbons		12.59
β -Caryophyllene	14.70	12.59

It is evident from the table that the antimicrobial (both; antibacterial and antifungal) activities greatly increase with increase of the oil concentration from 2 to 4 μ l/disc. It is also apparent that the oil inhibits the growth of all the bacteria and fungi except for *K. fragilis* and *R. rubra*. Specifically, it is important to note that the growths of *S. aureus* and *E. coli*, two of the most common gram-positive and gram-negative food poisoning bacteria, respectively, are significantly inhibited by the essential oil at both concentrations used. The inhibition zones of the oil (at both concentrations) against *S. aureus* and *E. coli* are determined to be much higher than that of ampicillin (10 μ g/disc) and streptomycin (10 μ g/disc).

On the one hand, the oil having a concentration of 4 μ l/disc gives has a remarkably higher inhibition zone in comparison to ampicillin (10 μ g/disc) or streptomycin (10 μ g/disc) for all the common bacteria studied. On the other hand, except for *K. pneumoniae* and *M. luteus*, the oil with a concentration of 2 μ l/disc has a higher inhibition zone than ampicillin (10 μ g/disc) and streptomycin (10 μ g/disc).

As can be seen from the same table, the oil with a concentration of 2 μ l/disc has an inhibition zone higher than 10 mm, which is considered to be the limit zone for being an efficient antibiotic. The antimicrobial activities of the essential oil from *S. oreganum* leaves against the same microorganisms are found to be much more effective in comparison with those of essential oils of the species *Laurus nobilis*. and *Abies* spp.³⁴⁾ This efficacy is most probably due to the carvacrol and γ -terpinene compounds in the oil.

Also, the oil with the 2 μ l/disc concentration shows comparable antifungal activity to nystatin (30 μ g/disc), the positive control fungicide, under the conditions studied. Even the oil with a 4 μ l/disc concentration gives higher antifungal activity than nystatin (30 μ g/disc).

Antioxidant Properties The antioxidant potential of a compound can be mainly attributed to its reducing power, radical scavenging ability and singlet oxygen quenching ability. Therefore, to be able to evaluate the total antioxidant activity of an essential oil the first two activities were measured.

Table 2. Antimicrobial Activities of the Essential Oil of the *Syrian oreganum* Leaves along with Those of Positive Control Antibiotics

Microorganism	Inhibition zone (mm)				
	OSEO ^{a)} (2 μ l/disc)	OSEO ^{a)} (4 μ l/disc)	Ampicillin (10 μ g/disc)	Streptomycin (10 μ g/disc)	Nystatin (30 μ g/disc)
<i>Corynebacterium xerosis</i>	—	—	12	10	
<i>Bacillus brevis</i>	22	37	14	16	
<i>Bacillus megaterium</i>	25	44	11	17	
<i>Bacillus subtilis</i>	22	39	15	18	
<i>Mycobacterium smegmatis</i>	16	24	19	15	
<i>Pseudomonas aeruginosa</i>	22	34	10	13	
<i>Staphylococcus aureus</i>	20	38	16	21	
<i>Klebsiella pneumoniae</i>	11	19	17	16	
<i>Klebsiella oxytoca</i>	14	21	15	14	
<i>Enterococcus faecalis</i>	20	36	16	17	
<i>Micrococcus luteus</i>	15	26	33	—	
<i>Escherichia coli</i>	16	30	11	—	
<i>Yersinia enterocolitica</i>	14	25	13	17	
<i>Kluyveromyces fragilis</i>	—	—			15
<i>Rhodotorula rubra</i>	—	—			14
<i>Saccharomyces cerevisiae</i>	16	28			18

a) OSEO: Essential oil of *Syrian oreganum* leaf. (—) indicates that no inhibition zone was determined. Blanks mean not investigated.

Table 3. Comparison of Reducing Powers of Essential Oils from Leaves of *Syrian oreganum* with Those of Ascorbic Acid

Sample	Concentration (mg/l)	Absorbance (700 nm)
Control ^{a)}	0	0.07±0.06
<i>Syrian oreganum</i>	100	0.24±0.015
	250	0.58±0.049
	500	0.77±0.07
Ascorbic acid	5	0.52±0.006
	10	0.96±0.006
	15	1.44±0.006

a) The control was a test sample without essential oil. High absorbance indicates high reducing power.

Table 4. Comparison of DPPH Radical-Scavenging Activity of BHT with Essential Oil from the Leaves of the Essential Oil of *Syrian oreganum* Leaf

Sample	Concentration (mg/l)	% DPPH radical scavenging
Essential oil of <i>Syrian oreganum</i>	100	07.01±2.48
	250	12.88±2.61
	500	17.12±1.21
BHT	20	54.80±1.94
	40	76.60±1.87
	100	82.10±2.44

Reducing Power Table 3 shows the reducing power of essential oil along with that of ascorbic acid at concentrations between 100–500 mg/ml. The reducing power of the essential oil was determined by the method of Oyaizu.³⁵⁾ High absorbance indicates high reducing power. The reducing power of the essential oil of *S. oreganum* leaf increases as the amount of oil increases (Table 3). However, this reducing power is lower than that of ascorbic acid which was used as positive control. Therefore, the absorbance of ascorbic acid in a sample was (10 mg/l) 0.96, while at the 500 mg/l essential oil concentration it was 0.77. Nevertheless, the reducing power of essential oil of *S. oreganum* was considerably higher than those of essential oils from the leaves of *L. nobilis* and the gum of *Abies cilicia*.³⁴⁾

DPPH Radical Scavenging Activity The reducing power and radical-scavenging activity of a substance may be an indicator of its antioxidant activity.³⁶⁾ DPPH is stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.¹⁶⁾ The %DPPH radical scavenging activity is presented in Table 4. Like reducing power, the DPPH radical scavenging activity of the oil increases with increasing concentration (Table 4). However, at the 500 mg/l essential oil concentration, only 17.12% DPPH was scavenging. Nevertheless, it was 82.1% in the presence of 100 mg/l BHT. Although this oil shows lower DPPH radical scavenging activity in comparison to BHT, it is markedly higher than those of essential oils from the leaves of *L. nobilis* and the gum of *A. cilicia*.³⁴⁾

Antioxidant Activity In the present study, the antioxidant activities of the essential oil from *S. oreganum* leaves were determined using the thiocyanate method in which the amount of peroxides formed in emulsion during incubation was determined spectrophotometrically by measuring the absorbance at 500 nm. High absorbance is an indication of a

Fig. 1. Antioxidant Activities of Essential Oil from the Leaves from *Syrian oreganum*, along with Those of Control and BHT (20 mg/l)

high concentration of formed peroxides and *vice versa*.

Figure 1 demonstrates the antioxidant activities of the essential oils from *S. oreganum* leaves along with those of control (linoleic acid without essential oil) and BHT (a known antioxidant). The data points without error bars indicate that the standard deviation was too small to show. The antioxidant activities of the essential oil applied at three concentrations, 20, 40 and 60 mg/l, are determined to be comparable to that of BHT (20 mg/l) at all the incubation times studied. It is obvious from the figure that the essential oil used at all the concentrations showed an antioxidative effect in comparison to control. Therefore, essential oil concentration above 60 mg/l was used. However, on reducing power and %DPPH radical scavenging studies higher concentrations were used (100–500 mg/l, Tables 3, 4) due to lower absorbance values.

The antioxidant activities of the oil from *S. oreganum* leaves obviously increase with increasing concentration of oil (Fig. 1). Although at the same concentration (20 mg/l) the antioxidant activities of the oil were somewhat lower than that of BHT, they were higher than those of essential oils from leaves of *L. nobilis* and gum of *A. cilicia*.³⁴⁾

The antioxidant activity, like reducing power and DPPH radical-scavenging activity, was concentration dependent. However, in comparison to BHT, these antioxidant activities were lower. The reducing power and radical-scavenging activity of a substance may be an indicator of its antioxidant activity³⁴⁾; the present study supports this point. The reducing power and DPPH radical scavenging activity of essential oil were relatively lower than the compared compounds (ascorbic acid and BHT). Thus, its antioxidant activity was also lower than BHT. Nevertheless, even at the concentration of 20 mg/l essential oil concentration high antioxidant activity was observed. This activity may be attributed primarily to the high content of phenolic components of the essential oils.³⁷⁾ The chemical composition of essential oil of *Syrian oreganum* indicates that the phenolic compounds (carvacrol, carvacrol methyl ether and thymol methyl ether) are relatively rich in the essential oil (Table 1). In fact, recently it has been reported these compounds actually have antioxidant ability.³⁸⁾ Therefore, we can suggest that this essential oil can serve as a good antioxidant.

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