In vitro screening for antioxidant and antimicrobial properties of five commercial *Origanum* species from Turkey

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The present study was conducted to evaluate the antioxidant and antimicrobial properties of methanol extracts of the five *Origanum* species, which are commercial importance in Turkey. Antioxidant activity was measured employing three methods namely, DPPH free radical scavenging activity, ABTS⁺ radical cation scavenging activity and ferric reducing antioxidant/power activity, including total phenolic and total flavonoid contents. In addition, antimicrobial activities of methanol extracts from five *Origanum* species aerial parts were investigated against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Candida albicans* test organisms using the disk diffusion and microdilution methods. Antioxidant studies suggested that methanol extracts behaved as a strong DPPH free radical, ABTS⁺ radical cation scavenging activity and ferric reducing antioxidant/power activity. The antimicrobial test results showed that the methanol extracts of all *Origanum* species had great potential of antibacterial activity against *Staphylococcus aureus*. As a conclusion of this study, the methanol extracts of five *Origanum* species showed strong antioxidant and antibacterial activity and therefore, they can be used as a natural preservative ingredient in food and/or pharmaceutical industry.

**Keywords:** *Origanum* species, Antioxidant, Antimicrobial

**IPC Int. Cl.**¹: A61K 36/00, C07K 15/00, C12Q 1/18, A01P 1/00, A61B 50/39

Free radical formation is controlled naturally by various beneficial compounds known as antioxidants. The terminology describing the actions of antioxidants unfortunately is not completely clear because there are various types of antioxidants. It is known that plant phenolic compounds possess antioxidative properties acting as radical scavengers and chain-breaking antioxidants under certain conditions¹. *Origanum* is an important multipurpose medicinal and spice plant, which belongs to the family Lamiaeae, tribe Menthæ and comprises of 42 species and 18 hybrids widely distributed in Eurasia and North Africa². The genus *Origanum* is represented by 22 species of 25 taxa, 15 being endemic in Turkey³. *Origanum* species are known as *kekik* in Turkey. Aerial parts of *Origanum* species are aromatic and are used as condiment or herbal tea. In recent years, Turkey has become a major supplier of oregano herb of which *Origanum* consists of over 90 % of oregano exports in the world. *Origanum onites* L. (İzmir kekiği, bilyali kekik) is located at the top of the list of commercial *Origanum* species of Turkey. It is obtained from both wild and cultivated plants. The other *Origanum* species collected from wild for commercial use are: *O. majorana* L. (Beyaz kekik, white oregano), *O. syriacum* L. subsp. *bevanii* (Holmes) Greuter & Burdet, *O. vulgare* L. subsp. *hirtum* (Link) Ietsw⁴. *Origanum onites* L. (Lamiaceae) is a steno-endemic taxon of the eastern Mediterranean area. This species is very important from the economic point of view, since it is widely used as a spice along with other taxa of the Lamiaceae, under the popular name of *Oregano*⁷. The essential oil of plant contains linalool, carvacrol and thymol⁷. *Origanum majorana* L. (Marjoram) is widely known and used as Mediterranean spice. The fresh or dried highly aromatic leaves and flowering tops of Marjoram are widely used for flavouring in many foods. Its essential oil and alcoholic extracts are applied in pharmaceuticals, perfumes and cosmetics. The essential oil contains terpinen-4-ol, α and γ-terpinene and terpinolene⁸. *Origanum syriacum* L. subsp. *bevanii* (Holmes) Greuter & Burdet is grow naturally on dry, stony, often chalky soils, in varying

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degrees of shade, from sea level to altitude 2000 m. This species is collected from its natural habitat for commercial in Turkey. Its essential oil contains carvacrol and p-cymene as major components. *Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw. is another commercial species. This plant is a typical East Mediterranean taxon. Ecologically, this species prefers warm, sunny habitats and loose, often rocky, calcareous soils, usually low in moisture content. Though very variable in morphological aspects, it can be distinguished from other *O. vulgare* subspecies by its hairy stems, compact inflorescences, leaves and calyces densely covered with glandular structures, green bracts, which are usually as long as calyces, and white flowers. Its essential oil contains p-cymene and γ-terpinene. *Origanum vulgare* L. subsp. *viridulum* (Martrin-Donos) Nyman is an aromatic, herbaceous and perennial plant. *Origanum vulgare* species enjoy wide industrial, pharmaceutical and traditional usage around the world because of their proven biological (antimicrobial, fungicidal, antioxidant, etc.) properties. The essential oil contains caryophyllene oxide, linalool, 1,8-cineol, caryophyllene, spathulenol, p-cymene and caryophyllenol.

*O. majorana*, *O. onites*, *O. syriacum* subsp. *bevanii*, *O. vulgare* subsp. *hirtum* and *O. vulgare* subsp. *viridulum* are commercial species. They are the major export product for Turkey. Turkey ranks first in the world for exporting Oregano. Also, *Origanum vulgare* subsp. *viridulum* is generally sold in the domestic market. Natural extracts are in increasing demand from the manufacturers of foods, cosmetics and pharmaceuticals.

Therefore, the aim of this study is to reveal antioxidant and antimicrobial activities of five commercial *Origanum* species (O. majorana, O. onites, O. syriacum subsp. bevanii, O. vulgare subsp. hirtum and O. vulgare subsp. viridulum) on the same terms. The antioxidant activities of methanol extracts from five *Origanum* species aerial parts were assayed with DPPH free radical scavenging activity, ABTS$^+$ radical cation scavenging activity and ferric reducing antioxidant/power activity, including total phenolic and total flavonoid contents. In addition, antimicrobial activities of methanol extracts from *Origanum* species aerial parts were investigated against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Candida albicans* test organisms using the disk diffusion and microdilution methods.

### Materials and methods

#### Chemicals

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) and butylated hydroxytoluene was sourced from Fluka. 2,4,6-tripryridyl-s-triazine, 2,2-diphenyl-1-pieryl-hydrazyl (DPPH$^+$), ethylenediaminetetraacetic acid disodium salt dihydrate, Folin Ciocalteu’s phenol reagent, gallic acid, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4’,4”-disulfonic acid sodium salt and ascorbic acid were obtained from Sigma Chemical Co. All other reagents were of analytical grade.

#### Collection of plant material

*O. majorana*, *O. onites*, *O. syriacum* subsp. *bevanii*, *O. vulgare* subsp. *hirtum* and *O. vulgare* subsp. *viridulum* were collected by Narin Sadıkoğlu. Plant materials were identified by Dr. Narin Sadıkoğlu. Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy, İnönü University, herbarium voucher numbers:

- *Origanum majorana* L. (Voucher number: Origanum 2009/019).

#### Preparation of the extracts

The aerial parts of five *Origanum* species (20 g) were extracted with 100 mL portions of methanol by maceration at room temperature for 7 days stirring several times until the extracts remained colorless. The extracts were filtered and evaporated to dryness under reduced pressure at 50 °C in a rotary evaporator. The crude extracts were then transferred to vials and kept at +4 °C.

#### DPPH$^+$ radical scavenging activity

The DPPH$^+$ radical scavenging activity of methanol extracts were measured by the DPPH$^+$ method proposed by Taskin et al. According to the results of scanning the spectrum obtained in this study, DPPH$^+$ exhibited a strong absorption band (λ max) at 517 nM. A solution of DPPH$^+$ in methanol (0.1 mM) was prepared and 3.9 mL of this solution was added to 0.1 mL of extracts in solution across a variety of
concentrations. After 30 min, the absorbance value was read at 517 nM. The IC_{50} value is inversely correlated to antioxidant ability of extracts. A lower IC_{50} value reveals higher antioxidant activity.

**ABTS•⁺ radical cation scavenging activity**

The ABTS•⁺ assay was performed according to the method developed by Siahpoosh & Alikhani\(^\text{15}\). This assay is based on the formation of the free radical cation ABTS•⁺ by reaction of ABTS aqueous solution (7 mM) with K_{2}S_{2}O_{8} (2.45 mM) at room temperature, under darkness, for 12–16 h. This stock solution was diluted with water to an absorbance of 0.700±0.020 at 734 nM. The reaction mixture comprised 3.96 mL of ABTS•⁺ solution and 0.04 mL of the extracts at a variety of concentrations. After 6 min, the absorbance value was read off at 734 nM. The reagent blank was prepared by adding 0.04 mL of water instead of the sample. All measurements were performed in triplicate.

**Ferric reducing antioxidant/power capacity (FRAP)**

The ferric reducing power capacity assay was performed according to the method developed by Attanayake & Jayatilaka\(^\text{16}\). An FRAP working solution was prepared fresh each time: 0.3 M acetate buffer (pH=3.6), 0.01 M TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 M HCl and 0.02 M FeCl\(_3\).6H\(_2\)O were mixed in 10:1:1 (v/v/v) and kept away from light. The mixture was incubated at 37 °C for 30 min away from light. Then 0.2 mL of extract or standard solution were added to 3.8 mL FRAP working solution. After 4 min, the absorbance was measured at 593 nm. A solution of FeSO\(_4\).7H\(_2\)O was used for calibration. The ferric reducing power activity of the extracts was calculated from the linear calibration curve. Results were expressed as mM FeSO\(_4\) equivalents per milligram of extracts. The calibration equation for gallic acid is absorbance = 16.19x–0.066 (R\(^2\) = 0.9950). All the determinations were carried out three times.

**Determination of total flavonoid content**

The total flavonoid content (TFC) of methanol extracts from *Origanum* species aerial parts was determined using the aluminium chloride assay\(^\text{18}\). 0.5 mL of extracts solution were taken in different test tubes then 2 mL of distilled water was added followed by the addition of 0.15 mL of sodium nitrite (5 % NaNO\(_2\), w/v) and allowed to stand for 6 min Later 0.15 mL of aluminium trichloride (10 % AlCl\(_3\)) was added and incubated for 6 min, followed by the addition of 2 mL of sodium hydroxide (NaOH, 4 % w/v) and volume was made up to the 5ml with distilled water. The mixture was left standing at ambient temperature for 15 min. Then, the absorbance was measured at 510 nM against a reagent blank. Results were expressed as milligrams of total flavonoid content per milligram (mg) extract (mg QUE/mg extract). The calibration equation for quercetin is absorbance = 0.927x + 0.01 (R\(^2\) = 0.9940). All the determinations were carried out three times.

**Antimicrobial activity**

Antimicrobial activities against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153 and *Candida albicans* ATCC 10231 were determined by the microbroth dilutions technique using the Clinical and laboratory Standards Institute (CLSI) recommendations\(^\text{19,20}\). Mueller-Hinton broth for bacteria, RPMI-1640 medium buffered to pH 7.0 with morpholinepropanesulfonic acid (MOPS) for yeast strain was used as the test medium. Serial two fold dilutions ranging from 5000 to 4.9 mg/mL were prepared in the medium. As control, antimicrobial effects of the solution were investigated against test microorganisms. The inoculum was prepared using a 4-6 h broth culture of each bacteria and 24 culture of yeast strains adjusted to a turbidity equivalent to a 0.5 McFarland standard, diluted in broth media to give a final concentration of 5x10\(^3\) cfu/mL for bacteria and from 0.5x10\(^3\) to 2.5x10\(^3\) cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. Trays containing Mueller-Hinton broth were incubated at 35 °C for 18-20 h and the trays containing RPMI-1640 medium
were incubated at 35°C for 46-50 h. The experiments were performed three times to minimize the error and the average values were presented. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of compound giving complete inhibition of visible growth. Ciprofloxacin and fluconazole were used as reference antimicrobials for bacteria and yeast, respectively. As control, antimicrobial effects of DMSO were investigated against test microorganisms. According to the values of the controls, the results were evaluated.

Statistical analysis
All the experiments were done in triplicates. All data from the study were shown as mean ± SD and analysed by the Graphpad Prism 5 Demo. Statistical differences between the experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s Multiple Comparison test. Mean values were considered statistically significant when p < 0.05.

Results
Total phenolic and total flavonoid contents
The quantitative analysis of TPC and TFC of methanol extracts from *Origanum* species revealed that the *O. syriacum* subsp. *bevanii* extract contained the highest amount of TPC (8.87 mg GAE/mg extract), whereas moderate amounts were recorded in methanolic extract of *O. vulgare* subsp. *viridulum* (8.43 mg GAE/mg extract) followed by *O. majorana* extract (8.37 mg GAE/mg extract) and *O. vulgare* subsp. *hirtum* extract (8.3 mg GAE/mg extract) and least amount of TFC were found in methanol extract of *O. onites* (8.07 mg GAE/mg extract).

TFC was found the highest in methanolic extract of *O. majorana* (1.33 mg QUE/mg extract) followed by methanolic extract of *O. vulgare* subsp. *viridulum* (1.32 mg QUE/mg extract), whereas moderate amounts were recorded in methanolic extract of *O. onites* (1.16 mg QUE/mg extract) followed by *O. vulgare* subsp. *hirtum* extract (1.15 mg QUE/mg extract) and least amount of TFC were found in methanolic extract of *O. syriacum* subsp. *bevanii* (0.71 mg QUE/mg extract) (Table 1).

### DPPH• radical scavenging activity
In Table 1, the free radical scavenging effects of methanol extracts of *Origanum* species were demonstrated. The antioxidant activities of the extracts were compared with ascorbic acid and BHT, which are a synthetic antioxidant.

The highest DPPH radical scavenging activity was observed in methanolic extract of *O. vulgare* subsp. *hirtum* (IC₅₀: 0.22 mg/mL) followed by *O. majorana* (IC₅₀: 0.303 mg/mL), *O. vulgare* subsp. *viridulum* (IC₅₀: 0.307 mg/mL), *O. onites* (IC₅₀: 0.32 mg/mL) and *O. syriacum* subsp. *bevanii* (IC₅₀: 0.36 mg/mL). The methanol extracts of *Origanum* species had strong radical scavenging effects. The free radical scavenging effect of the *O. vulgare* subsp. *hirtum*, *O. majorana* and *O. vulgare* subsp. *viridulum* appeared to be stronger than BHT. *O. onites* extract has free radical scavenging effect similar to that of BHT.

### ABTS** radical cation scavenging activity
In Table 1, the ABTS** radical cation scavenging effects of methanol extracts of *Origanum* species were demonstrated. The antioxidant activities of the extracts were compared with ascorbic acid. The methanol extracts of plants had strong ABTS** radical cation scavenging effects at 0.05 mg/mL concentration. All extracts showed a fairly similar degree of scavenging activity to that of ascorbic acid. At a concentration of 0.05 mg/mL, *O. onites* extract showed the highest ABTS** radical cation scavenging

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### Table 1 — DPPH radical scavenging, ABTS radical cation scavenging, ferric reducing antioxidant power activities and the amount of total phenolic (TPC) and total flavonoid (TFC) contents of methanol extracts from *Origanum* species aerial parts.

<table>
<thead>
<tr>
<th>Extracts/Standards</th>
<th>DPPH IC₅₀ mg/mL</th>
<th>ABTS radical cation scavenging activity (%)</th>
<th>FRAP mMFeSO₄/mg extract</th>
<th>TPC mgGAE/mg extract</th>
<th>TFC mgQUE/mg extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. majorana</em></td>
<td>0.30±0.01</td>
<td>95.99±0.73</td>
<td>2.215±0.01</td>
<td>8.37±0.98</td>
<td>1.33±0.09</td>
</tr>
<tr>
<td><em>O. onites</em></td>
<td>0.32±0.01</td>
<td>98.31±0.47</td>
<td>2.216±0.007</td>
<td>8.07±0.94</td>
<td>1.16±0.10</td>
</tr>
<tr>
<td><em>O. syriacum</em> subsp. <em>bevanii</em></td>
<td>0.36±0.06</td>
<td>95.80±0.61</td>
<td>2.207±0.003</td>
<td>8.87±1.0</td>
<td>0.71±0.03</td>
</tr>
<tr>
<td><em>O. vulgare</em> subsp. <em>hirtum</em></td>
<td>0.22±0.004</td>
<td>97.75±0.72</td>
<td>2.199±0.005</td>
<td>8.31±0.92</td>
<td>1.15±0.12</td>
</tr>
<tr>
<td><em>O. vulgare</em> subsp. <em>viridulum</em></td>
<td>0.307±0.01</td>
<td>96.67±0.20</td>
<td>2.198±0.007</td>
<td>8.43±1.03</td>
<td>1.32±0.18</td>
</tr>
<tr>
<td>BHT</td>
<td>0.32±0.03</td>
<td>99.19±0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.09±0.066</td>
<td>99.19±0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These values were the mean values of three replicates ± standard deviation. Different superscript letters in each column exhibit significant differences in mean values at p < 0.05 according to Tukey’s Multiple Comparison test.
effect. The magnitude of the radical scavenging effect at 0.05 mg/mL concentration was ranked as ascorbic acid (99.19 %) > O. onites (98.31 %) > O. vulgare subsp. hirtum (97.75 %) > O. vulgare subsp. viridulum (96.67 %) > O. majorana (95.99 %) > O. syriacum subsp. bevanii (95.80 %), respectively.

Ferric reducing antioxidant/power capacity (FRAP)

The ferric reducing antioxidant/ power (FRAP) activity of methanol extracts were shown in Table 1.

The ferric reducing power effects of the extracts are in the following order: O. onites (2.216 mMFeSO₄/mg extract) > O. majorana (2.215 mMFeSO₄/mg extract) > O. syriacum subsp. bevanii (2.207 mMFeSO₄/mg extract) > O. vulgare subsp. hirtum (2.199 mMFeSO₄/mg extract) > O. vulgare subsp. viridulum (2.198 mMFeSO₄/mg extract). The methanol extract of O. onites exhibited the highest ferric reducing antioxidant power. Also, the ferric reducing power effects of the plants is quite close to each other.

Antimicrobial activity

In this study, O. majorana, O. onites, O. syriacum subsp. bevanii, O. vulgare subsp. hirtum and O. vulgare subsp. viridulum showed antibacterial activity against Staphylococcus aureus and Staphylococcus epidermidis. The methanol extracts of O. majorana, O. vulgare subsp. viridulum showed the highest antibacterial activity against Staphylococcus aureus, whereas O. onites and O. vulgare subsp. hirtum extracts showed moderate antibacterial activity against Staphylococcus aureus and O. syriacum subsp. bevanii extract exhibited weak activity against Staphylococcus aureus. Also the O. majorana extract showed weak antifungal activity against Candida albicans. However, the other extracts were found to have no effect against the Candida albicans.

The results of in vitro antimicrobial activities of methanol extracts of plants with MIC values compared to antibiotic and antifungal agents are given in Table 2.

Discussion

The aromatic plants are characterized by the presence of plant phenolic compounds that have been shown to possess multiple pharmacological activities. The extracts of Oregano have the most effective antioxidant activity among aromatic herbs. Different groups of researchers have studied Oregano alcohol extracts. The antioxidant effect of the mentioned extracts is generally attributed to the presence of phenolic compounds. Concerning the bioactivity of Origanum species, recent studies have reported antimicrobial, anti-inflammatory, antispasmodic, antiulcer and antioxidant activities of their polar extracts. Some articles have identified phenolic compounds of Origanum species.

To the best of our knowledge, there are some reports on the antioxidant and antimicrobial potential of Origanum species. The report concerned the antioxidant activity of aqueous tea infusions from O. vulgare subsp. hirtum. Antioxidant activity of aqueous tea infusions was evaluated using four antioxidative methods (the β-carotene bleaching method (BCB), 2,2’-diphenyl-1–picrylhydrazyl (DPPH) radical scavenging method, the thiobarbituric acid reactive species (TBARS assay) and the induction period of lard oxidation (Rancimat assay). The aqueous tea infusion from this plant showed high amount of total phenols and flavonoids. Oregano aqueous tea infusion showed strong antioxidant activity using three methods. According to a study by Vagi et al., antioxidant activity of hexane extract and ethyl acetate extracts from O. majorana were

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.a</td>
<td>S.e</td>
</tr>
<tr>
<td>O. majorana</td>
<td>78</td>
</tr>
<tr>
<td>O. onites</td>
<td>156</td>
</tr>
<tr>
<td>O. syriacum subsp. bevanii</td>
<td>312</td>
</tr>
<tr>
<td>O. vulgare subsp. hirtum</td>
<td>156</td>
</tr>
<tr>
<td>O. vulgare subsp. viridulum</td>
<td>78</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25</td>
</tr>
<tr>
<td>Flucnazole</td>
<td>-</td>
</tr>
</tbody>
</table>


MIC: Minimum Inhibitory Concentration; (-): No inhibition
determined with various methods, including total phenolic compounds, 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) free radical scavenging activity, ABTS cation radical scavenging activity and reducing power. The ethyl acetate extract exhibited strong antioxidant activities and high amount of total phenols contents. The antimicrobial properties of O. majorana ethyl alcohol extract were investigated with microbiological tests against food borne fungi and bacteria strains. In this study, the ethyl alcohol extract showed moderate activity against Aspergillus niger, Penicillium cyclopium, Escherichia coli, Pseudomonas fluorescens and Bacillus cereus. According to a study by Tepe et al., antioxidant activities of hexane, dichloromethane and methanol extracts from O. syriacum subsp. bevanii were determined with two methods, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity and linoleic acid oxidation. In this study, methanol extract showed the highest DPPH radical-scavenging activity. According to a study by Kolda et al., antioxidant activities of ethanol, methanol, ethyl acetate, hexane and water extracts from O. vulgare subsp. viridulum were determined with various methods, reducing power activity, metal-chelating activity, free radical-scavenging activity and hydrogen peroxide-scavenging activity, including total phenolic and flavonoids compounds. The water extract had the highest reducing power, free radical-scavenging activity and hydrogen peroxide-scavenging activity while ethyl acetate extract had the highest metal chelation activity. To the best of our knowledge, there are many reports on the antioxidant and antimicrobial potential of essential oil from Origanum species, which are aromatic plants. According to literature data, generally polar extracts show strong biological activity. We thought that all of the compounds in the plant could play a role in these activities. Therefore, in this study, the methanol extracts were prepared from five Origanum species and evaluated their antioxidant and antimicrobial activity. However, in our current study, antimicrobial activity of methanol extracts from five Origanum species were evaluated with different types of microorganisms (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis and Candida albicans). The methanol extracts of all Origanum species showed strong antibacterial activity against Staphylococcus aureus. It is known that Staphylococcus aureus bacteria reproduce well on food and produce toxin. These toxins are known to cause intoxication by entering the body through the food (cooked meat products, especially prepared to be stored in the refrigerator). Therefore, these species can be used as preservatives in food industry. In addition, in our current study, the antioxidant capacity of methanol extracts of five commercial Origanum species were assayed with various methods; including total phenolic and flavonoids compounds, DPPH free radical scavenging activity, ABTS radical cation scavenging activity and ferric reducing antioxidant/power capacity. In this study, the free radical scavenging effect of O. vulgare subsp. hirtum, O. majorana and O. vulgare subsp. viridulum appeared to be stronger than BHT. O. onites extract has free radical scavenging effect similar to that of BHT. The methanol extract of O. onites exhibited the highest ferric reducing antioxidant power and ABTS•⁺ radical cation scavenging effect. In addition, O. syriacum subsp. bevanii extract contained the highest amount of total phenolic contents whereas O. majorana had the highest total flavonoids contents. Therefore, a linear relationship wasn’t found between the antioxidant activity and phenolic compounds.

Bioactive compounds in methanol extracts were not identified in this study. In further studies, the phenolic compounds in methanol extracts will be identified by ESI-Q-TOF LC/MS.

The methanol extracts of O. vulgare subsp. hirtum, O. majorana, O. vulgare subsp. viridulum and O. onites could be useful as an antioxidant agent in the future. These plants are already used in traditional medicine in particular as analgesic and antispasmodic. In addition, these species are used as a spice in foods, especially meat dishes and salads. It is known to have an important role in food preservation of phenolic compounds such as thymol and cavaracrol. This study has also proven this once more.

Conclusion

Our results showed that Origanum species were rich in phenolic and flavonoid constituents and demonstrated good antioxidant activity measured by different methods. Through our systematically comparative study of five selected Origanum species, especially O. vulgare subsp. hirtum, O. majorana, O. vulgare subsp. viridulum and O. onites were excellent free-radical scavengers and a potnet natural phenolic antioxidant for commercial exploration. Also, the methanol extracts of all Origanum species had great potential of antibacterial activity against Staphylococcus aureus. Therefore, after examining the toxic effects on different normal cell line of
methanol extracts, it is believed that these extracts might be a potential source of antioxidant and antibacterial agents in the future because these species are already used as a spice.

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References


