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ORIGINAL ARTICLE

# Chemical compositions, antioxidant and antimicrobial properties of *Ziziphora clinopodioides* Lam. essential oils collected from different parts of Iran

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**Abstract** The aims of the present study were to investigate chemical compositions, antioxidant and antimicrobial properties of *Ziziphora clinopodioides* essential oils (ZEOs) collected from four provinces in western Iran (Ilam, Lorestan, Kermanshah and Kurdestan). Carvacrol was the most abundant constituent in the flower, stem and leaf oil samples of Ilam, Lorestan and Kermanshah regions by 73.12–74.29%, 66.47–66.89% and 65.11–65.32%, respectively. The most abundant components in Kurdestan sample were thymol (55.32–55.60%), followed by γ-terpinene (24.45–24.56%), *p*-cymene (10.21–10.25%) and α-terpinene (2.75–2.77%). The ZEO inhibited the growth of *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus* at MIC values between 0.03 and 0.04%. Kermanshah oil sample had a higher 1,1-diphenyl-2-picrylhydrazyl radical scavenging (0.30–0.31 mg/ml), ability to prevent the bleaching of β-carotene (0.09–0.1 mg/ml), ferric reducing power (0.40–0.42 mg/ml) and thiobarbituric acid (0.004–0.006 Meq of malondialdehyde/g) values than that of ZEOs from Ilam, Kurdestan and Lorestan. The strong in vitro antimicrobial and antioxidant activities supports the traditional use of ZEO in the treatments of gastrointestinal diseases.

**Keywords** Chemical compositions · Antioxidant · Antimicrobial · *Ziziphora clinopodioides* essential oil · Iran

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## Introduction

Recently as people tend to consume natural foods without any synthetic additives due to health concerns, the investigation of natural products for the discovery of new active compounds with antibacterial, antifungal and antiviral properties is growing throughout the world (Kakaei and Shahbazi 2016; Shahbazi and Shavisi 2016). Among different groups of natural products, essential oils (EOs) have recently become a central point of biological studies (Tajkarimi et al. 2010; Ye et al. 2013). It has been indicated that EOs and extracts obtained from marigold flower, rosemary, grape seed, clove and thyme have antimicrobial activity and can control some foodborne pathogens including *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, *Bacillus subtilis, Yersinia enterocolitica, Clostridium perfringens, Campylobacter spp., Bacillus cereus* and *Staphylococcus aureus* (Tajkarimi et al. 2010). Besides the aforementioned multifunctional properties, these compounds are popular candidates to be used for replacement of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as the most commonly used antioxidant preservatives in fat and foods containing fat (Alves-Silva et al. 2013). Moreover, EOs are gaining a wide interest in food industry for their applications as natural antimicrobial and antioxidant additives, since these compounds have been recognized as safe substances (GRAS) by US Food and Drug Administration (US FDA) and are extensively used by consumers (Gyawali and Ibrahim 2014).

Ziziphore (*Ziziphora clinopodioides*) belongs to the *Laminacea* family and widely grows in the several regions throughout the world especially Middle East countries (Aghajani et al. 2008). It is a strongly aromatic plant well

known in the Iranian traditional medicine for treatment of diarrhea, intestinal gas, stomach upsets, nausea and vomiting (Shahbazi and Shavisi 2016). Moreover, it can be useful as an appetitive, carminative, antiseptic, sedative and wound-healing material (Shahbazi et al. 2016). It has been widely used to improve the sensory properties (odor, color and taste) of meat and traditional meat products (Behravan et al. 2007). Earlier studies are concentrated on its application as a natural antimicrobial and antioxidant preservative in raw foods such as minced beef patty, commercial barley soup and rainbow trout. Accordingly, the addition of *Z. clinopodioides* (ZEO) can successfully increase the safety and shelf life of raw foods during storage at refrigerated condition without any unfavorable organoleptic properties (Shahbazi et al. 2016; Kakaei and Shahbazi 2016). The antibacterial and antioxidant activities and many other benefits of this plant is probably due to thymol, γ-terpinene, carvacrol, linalool, boerneol, camphor, terpinen-4-ol and 1, 8-cineole (Ozturk and Ercisli 2007; Kakaei and Shahbazi 2016).

The biological effects of the EOs can vary greatly depending upon its chemical compositions which depends on the geographical and climate conditions, variety of species and genotypes of the plant (Shahbazi et al. 2015). Some studies evaluated chemical compositions and antibacterial activity of ZEO collected from different parts of Iran and other countries (Behravan et al. 2007; Morteza-Semnani et al. 2005; Ozturk and Ercisli 2007). To our knowledge, there is no comprehensive study on the chemical compositions, antioxidant and antibacterial properties of ZEOs based on its geographical locations in Iran. Therefore, the aims of the present study were to (1) analyze chemical compositions of ZEOs grown from four different geographical parts of Iran (Ilam, Lorestan, Kermanshah and Kurdestan) using gas chromatography coupled with mass spectrometer detector (GC–MS); (2) examine antimicrobial activities of the ZEOs by broth micro-dilution and agar disk diffusion methods; and (3) determine antioxidant properties of the ZEOs using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), β-carotene/linoleic acid bleaching and thiobarbituric acid (TBA) methods.

## Materials and methods

### Materials

Thymol (>99%), carvacrol (>98%), γ-terpinene (>95%) and *p*-cymene (>95%) were purchased from Tokyo chemical Industry Co., Ltd. (Shanghai, China). All media were obtained from Merck, Germany.

### Collection of plant materials

Fresh leaf, stem and flower of Ziziphore plant were harvested from four western parts of Iran (Ilam, Lorestan, Kermanshah and Kurdestan provinces) during full flowering period in March–July 2016. Plant collection was conducted between 8 a.m. and 11 a.m.. The geographical locations were recorded using a Global Positioning System (GPS, Vista Garmin) receiver as follow: Ilam (latitude: 3552,626 Universal Transverse Mercator (UTM), longitude: 479,342, and altitude: 964 m above sea level (m a.s.l)); Lorestan (latitude: 3,716,721 UTM, longitude: 250,198, and altitude: 976 m a.s.l); Kermanshah (latitude: 3,776,583 UTM, longitude: 585,867, and altitude: 833 m a.s.l); and Kurdestan (latitude: 3,879,030 UTM, longitude: 601,413, and altitude: 1216 m a.s.l). The physicochemical properties of the soil including clay, silt, sand, organic matter and acidity were recorded as follow: Ilam (clay: 25, silt: 17, sand: 63, organic matter: 0.118%, and acidity: 7.71); Lorestan (clay: 24, silt: 17, sand: 59, organic matter: 0.118%, and acidity: 7.68); Kermanshah (clay: 24, silt: 16, sand: 60, organic matter: 0.117%, and acidity: 7.78); and Kurdestan (clay: 25, silt: 18, sand: 64, organic matter: 0.119%, and acidity: 7.70). The plants were identified as *Z. clinopodioides* Lam. by a botanical taxonomist. Voucher specimens of plants collected from Ilam (6971), Lorestan (7419), Kermanshah (6818) and Kurdestan (7453) were deposited in the botany herbarium of the Research Center of Natural Resources of Tehran, Iran.

### Isolation of *Z. clinopodioides* essential oils

After collection, the fresh leaves, stems and flowers of collected plants were carefully washed with distilled water and then air-dried indoor in a shady place at room temperature for 12 days (water content approached 75% of plant fresh weight). After that, a portion of 100 g of each dried-sample was ground, homogenized in distilled water with a ratio of 1:5 and submitted to hydro-distillation for 3.5 h using a Clevenger-type apparatus, according to the previously method published by the European Pharmacopoeia (Council of Europe 1997). The oil over water was recovered, dried with anhydrous sodium sulfate and stored at refrigerated condition in darkness a sealed brown glass bottle until its analysis or its use in antimicrobial and antioxidant tests.

### Gas chromatography–mass spectrometry (GC–MS) analysis of essential oils

The chemical compositions of ZEOs were analyzed using a Thermo Quest Finningan gas chromatograph coupled with a mass spectrometer detector (GC–MS). The GC–MS was equipped with a HP-5MS 5% methyl silicone capillary

column (30 m length × 0.25 mm i.d. and 0.25 μm film thickness). The column temperature was programmed at 50 °C as an initial temperature, holding for 3 min, then gradually increased at the 2.5 °C/min to 265 °C, and finally held at 265 °C for 6 min. Helium was used as a carrier gas at a flow rate of 1.2 ml/min. Electron (EI) ionization energy of 70 eV was applied over a scan range of 30–550 amu. The ZEOs analyses were also conducted by Thermo Quest Finningan gas chromatography with the same capillary column and analytical conditions as described above.

Identification of the each ZEO components were accompanied with available authentic samples in our laboratory. Further identification was based mainly on the comparison of retention indices (RIs) and mass spectral fragmentation patterns with published data (Sonboli et al. 2010; Morteza-Semnani et al. 2005; Ozturk and Ercisli 2007; Amiri 2009) and the Wiley/NBS mass spectral library of the GC–MS data system (Wiley/NBS Pak v.7, 2003).

## Bacterial and fungal strains

Four Gram-positive bacteria [*S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 11774) and *L. monocytogenes* (ATCC 19118)], two Gram-negative bacteria [*S. typhimurium* (ATCC 14028) and *E. coli* O157:H7 (ATCC 10536)] and two fungi [*Aspergillus niger* (ATCC 1015) and *Candida albicans* (ATCC 3153)] were used in antimicrobial tests. All bacterial and fungal strains were provided from the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran.

## Antimicrobial tests

### *Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)*

The overnight sub-culture of each bacterial strain in Brain Heart Infusion broth (BHI) was diluted to 6 log CFU/ml in buffered peptone water for the inoculation. The MIC and MBC values of the ZEOs at different concentrations (0.0001, 0.0002, 0.0003, 0.0004, 0.0005, 0.0006, 0.0007 and 0.0008, 0.0009, 0.001 μl/ml) were determined using broth micro-dilution assay (Singh et al. 2010). For preparation of aforementioned concentrations of ZEOs in BHI broth, 2% (v/v) dimethylsulfoxide (DMSO) as an emulsifier was added to the BHI broth. In order to determine the lowest inhibitory concentration (MIC), each well of sterile 96-well micro-dilution plate had 180 μl BHI broth containing different concentrations of ZEO and 20 μl of bacterial inoculums (6 log CFU/ml). Positive (BHI broth containing inoculum) and negative (sterile BHI broth) controls were considered in the last wells of each strip. The plates were mixed on a plate shaker for 30 s and then incubated at 37 °C for 24 h. The same method was conducted for investigation of antibacterial effects of carvacrol, thymol, *p*-cymene and γ-terpinene at different concentrations including 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 μg/ml. At the end of incubation time, the MIC was described at the lowest concentration of the ZEO or selected standard compounds required for inhibition of bacterial growth. For minimum bactericidal concentration (MBC), the content of the each well without any visible growth of bacteria were cultured in BHI agar and incubated at 37 °C for 24 h. The concentration of ZEO or selected standard compounds in those wells with no visible growth was considered to be the MBC.

### *Agar disk diffusion assay*

The antibacterial activities of ZEOs and selected standard compounds (carvacrol, thymol, *p*-cymene and γ-terpinene) were also evaluated using agar disk diffusion method, according to the method previously reported by Shahbazi et al. (2015) with some minor modifications. For this purpose, 100 μl of the each test bacterial suspension, containing $1 \times 10^8$ CFU/ml, was spread on the BHI agar by surface method. Then, filter paper discs, 9 mm in diameter, impregnated with 20 μl of ZEO or selected standard compounds were placed on the surface of inoculated BHI agar. After incubation at 37 °C for 24 h, the diameter of the inhibition zones were measured.

## Antifungal tests

### *Agar disk diffusion assay*

Antifungal activities of the ZEOs and selected standard compounds (carvacrol, thymol, *p*-cymene and γ-terpinene) against *A. niger* and *C. albicans* were also evaluated by agar disk diffusion methode on the surface of potato dextrose agar (PDA) as the similar method described in the previous section.

## Antioxidant activity tests

### *1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay*

The antioxidant activities of the ZEOs were assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay through a UV–Vis spectrophotometer (Formisano et al. 2014; Sepahvand et al. 2014). An aliquot of 50 μl of various concentrations of the ZEO was added to 5 ml of

methanolic DPPH solution and the absorbance was measured at 517 nm. The percentage of DPPH radical scavenging activity of each ZEO was calculated as follows (Sepahvand et al. 2014):

$$I(\%) = \frac{[Ab - As]}{Ab} \times 100$$

where I% is the capability to scavenge the DPPH radical or to inhibit free radicals, $A_b$ is the absorbance of the control reaction (containing all reagents except the investigated ZEOs), and $A_s$ is the absorbance of the ZEO sample.

*Ferric reducing power*

Ferric reducing power of the ZEOs were determined according to the previously method described by Martucci et al. (2015). The reducing power of the ZEO was assessed at 690 nm in the Microplate Reader.

*β-Carotene/linoleic acid bleaching assay*

The method of Martucci et al. (2015) was used to determine the bleaching of β-carotene in linoleic acid emulsion system at 490 nm using a UV–Vis spectrophotometer. β-Carotene bleaching inhibition was measured by the following formula (Martucci et al. 2015):

$$\beta\text{-Carotene bleaching inhibition} = \frac{\beta\text{−Carotene absorbance after 2 h}}{\text{Initial absorbance}} \times 100$$

*Thiobarbituric acid reactive substances (TBA) assay*

The thiobarbituric acid reactive substances (TBA) values, a secondary product of lipid peroxidation, of ZEOs were evaluated according to the method of Singh et al. (2010). The TBA value (Meq of malondialdehyde/g) of each ZEO was calculated as following formula (Singh et al. 2010):

$$\text{TBA value} = \frac{[50 \times (A - B)]}{M}$$

where A is the absorbance of test sample, B is the absorbance of reagent blank and M is the mass of the sample (mg).

### Statistical analysis

All experiments were repeated three times and the results were expressed as mean ± SD. The statistical analysis was performed using SPSS 16.0 software program (SPSS, Chicago, IL, USA). Statistical significance levels used was $P < 0.05$.

## Results and discussion

### Essential oil yields

The highest amount of ZEO yield was found for Kurdestan (leaf: 4.5% ± 0.2, flower: 2.8% ± 0.1, and stem: 1.1% ± 0.0 v/w), followed by Ilam (leaf: 2.8% ± 1.45, flower: 1.5% ± 0.2 and stem: 0.6% ± 0.1 v/w), Kermanshah (leaf: 2.5% ± 0.45, flower: 1.4% ± 0.1 and stem: 0.4% ± 0.1 v/w) and Lorestan (leaf: 0.3% ± 0.01, flower: 0.3% ± 0.0 and stem 0.1% ± 0.0 v/w). There was significant difference in the level of oil yields between Kurdestan and all the other areas ($P < 0.05$). The oil yield of Lorestan sample was found to have significantly lower than other parts ($P < 0.05$). The yield observed in the current study were higher than those reported by Amiri (2009), Behravan et al. (2007), Morteza-Semnani et al. (2005). The oil yield from Lorestan sample was good in accordance with a previous study (Behravan et al. 2007). Accordingly, the yield of the ZEO collected from Mashhad, Khorasan Razavi (North East of Iran) was 0.35% (w/w) based on the dry weight of the plant.

### Chemical compositions of *Z. clinopodioides* Lam. essential oils

The chemical compositions of the ZEOs obtained from different parts of Iran together with the retention and Kovat's indices are presented in Table 1. The GC–MS analyses of the oil samples revealed the presence of 24, 18, 19 and 29 volatile constituents which accounted for 99.57–99.87%, 98.57–99%, 97.53–98.09% and 97.81–98.89% of the total oil compositions in Kermanshah, Kurdestan, Lorestan and Ilam regions, respectively. As presented in Table 1, the main chemical constituents in the oil samples from different parts of Iran were almost same whereas the amounts of most abundant corresponding components were varied. The flower, leaf and stem ZEO also had a similar qualitative composition with insignificant proportion. Carvacrol was the most abundant constituent in the flower, stem and leaf oil samples of Ilam, Lorestan and Kermanshah regions by 73.12–74.29%, 66.47–66.89% and 65.11–65.32%, respectively. A critical observation of the oil compositions revealed that amount of carvacrol in the Kurdestan sample oil was very low (0.63–0.67%). The most abundant components in Kurdestan sample were thymol (55.32–55.60%), followed by γ-terpinene (24.45–24.56%), *p*-cymene (10.21–10.25%) and α-terpinene (2.75–2.77%). The obtained oil sample from Ilam area contained carvacrol (73.12–74.29%), thymol (7.18–7.59%), *p*-cymene (6.11–7.69%) and γ-

**Table 1** Essential oil composition (%) of stem, leaf and flower of *Z. clinopodioides* collected from different parts of Iran

| No. | Compounds | | RT[a] | Composition (%) | | | | | | | | | | | | KI[b] | Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ilam | | | Lorestan | | | Kermanshah | | | Kurdestan | | | | |
| | | | | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | | |
| 1 | α-Thujene | MH | 11.33 | 0.14 | 0.11 | 0.09 | ND[f] | ND | ND | 0.26 | 0.25 | 0.26 | 0.77 | 0.71 | 0.75 | 927 | RI[c], MS[d], Co-GC[e] |
| 2 | α-Pinene | MH | 11.71 | 0.38 | 0.59 | 0.45 | 0.75 | 0.83 | 0.76 | 0.27 | 0.26 | 0.25 | 0.38 | 0.32 | 0.39 | 934 | RI, MS, Co-GC |
| 3 | Camphene | MH | 12.61 | ND | ND | ND | 0.28 | 0.34 | 0.31 | 0.13 | 0.11 | 0.12 | ND | ND | ND | 952 | RI, MS, Co-GC |
| 4 | *β*-Pinene | MH | 14.06 | ND | ND | ND | ND | ND | ND | 0.06 | 0.12 | 0.13 | ND | ND | ND | 981 | RI, MS, Co-GC |
| 5 | Myrcene | MH | 14.62 | 0.61 | 0.64 | 0.59 | 0.43 | 0.56 | 0.41 | 0.51 | 0.54 | 0.49 | 1.12 | 1.1 | 1 | 992 | RI, MS, Co-GC |
| 6 | α-Phellandrene | MH | 15.58 | 0.15 | 0.22 | 0.11 | ND | ND | ND | 0.13 | 0.14 | 0.17 | 0.23 | 0.23 | 0.24 | 1010 | RI, MS, Co-GC |
| 7 | α-Terpinene | MH | 16.11 | 0.87 | 1.85 | 0.86 | 0.44 | 0.41 | 0.39 | 0.79 | 0.71 | 0.72 | 2.76 | 2.75 | 2.77 | 1021 | RI, MS, Co-GC |
| 8 | ***p*-Cymene** | MH | 16.62 | **7.69** | **6.11** | **7.41** | **2.24** | **2.21** | **2.31** | **4.86** | **4.97** | **5.01** | **10.24** | **10.25** | **10.21** | 1030 | RI, MS, Co-GC |
| 9 | Limonene | MH | 16.77 | 0.14 | 0.16 | 0.21 | 0.11 | 0.10 | 0.11 | 0.1 | 0.13 | 0.12 | 0.19 | 0.18 | 0.19 | 1033 | RI, MS, Co-GC |
| 10 | 1,8-Cineole | OM | 16.94 | ND | ND | ND | 0.56 | 0.57 | 0.63 | ND | ND | ND | ND | ND | ND | 1037 | RI, MS, Co-GC |
| 11 | *β*-Phellandrene | MH | 16.89 | 0.13 | 0.22 | 0.15 | ND | ND | ND | 0.11 | 0.13 | 0.12 | 0.15 | 0.14 | 0.13 | 1036 | RI, MS, Co-GC |
| 12 | **γ-Terpinene** | MH | 18.31 | **4.09** | **4.12** | **4.26** | **1.55** | **1.54** | **1.55** | **4.63** | **4.44** | **4.32** | **24.53** | 24.45 | 24.56 | 1063 | RI, MS, Co-GC |
| 13 | *cis*-Sabinene hydrate | OM | 19.02 | ND | ND | ND | 0.40 | 0.39 | 0.42 | 0.07 | 0.17 | 0.17 | ND | ND | ND | 1077 | RI, MS, Co-GC |
| 14 | Terpinolene | MH | 19.69 | ND | ND | ND | ND | ND | ND | 0.08 | 0.18 | 0.23 | ND | ND | ND | 1089 | RI, MS, Co-GC |
| 15 | *trans*-Sabinene hydrate | MH | 20.46 | ND | ND | ND | 0.12 | 0.13 | 0.11 | ND | ND | ND | ND | ND | ND | 1109 | RI, MS, Co-GC |
| 16 | Linalool | OM | 20.5 | 0.16 | 0.33 | 0.27 | 0.16 | 0.17 | 0.14 | 0.13 | 0.11 | 0.10 | 0.54 | 0.54 | 0.53 | 1105 | RI, MS, Co-GC |
| 17 | Camphore | OM | 23.14 | ND | ND | ND | 0.28 | 0.27 | 0.25 | ND | ND | ND | ND | ND | ND | 1159 | RI, MS, Co-GC |
| 18 | Borneol | OM | 24.36 | 0.36 | 1.34 | 0.35 | 1.93 | 1.95 | 1.94 | 0.61 | 0.49 | 0.61 | 0.12 | 0.14 | 0.11 | 1183 | RI, MS, Co-GC |
| 21 | Terpinene-4-ol | OM | 24.7 | 0.39 | 0.38 | 0.41 | 1.06 | 1.13 | 1 | 0.48 | 0.37 | 0.32 | 0.37 | 0.38 | 0.42 | 1190 | RI, MS, Co-GC |
| 22 | α-Terpineol | OM | 25.49 | ND | ND | ND | 0.37 | 0.37 | 0.35 | 0.08 | 0.18 | 0.19 | ND | ND | ND | 1206 | RI, MS, Co-GC |
| 23 | *cis*-Dihydro carvone | OM | 25.69 | ND | ND | ND | 0.16 | 0.14 | 1.16 | ND | ND | ND | ND | ND | ND | 1211 | RI, MS, Co-GC |
| 24 | Thymol methyl ether | MH | 27.37 | ND | ND | ND | 0.25 | 0.23 | 0.26 | ND | ND | ND | ND | ND | ND | 1246 | RI, MS, Co-GC |
| 25 | Carvacrol, methyl ether | MH | 27.38 | ND | ND | ND | ND | ND | ND | 0.04 | 0.14 | 0.11 | ND | ND | ND | 1246 | RI, MS, Co-GC |
| 26 | Carvone | OM | 28.01 | ND | ND | ND | 0.47 | 0.48 | 0.48 | ND | ND | ND | ND | ND | ND | 1260 | RI, MS, Co-GC |
| 27 | Isobornyl acetate | OM | 29.60 | ND | ND | ND | 0.11 | 0.11 | 0.11 | ND | ND | ND | ND | ND | ND | 1294 | RI, MS, Co-GC |
| 28 | **Thymol** | OM | 29.61 | **7.28** | **7.18** | **7.59** | **15.55** | 15.42 | 14.39 | **19.51** | 19.54 | 18.55 | **55.60** | 55.32 | 55.44 | 1293 | RI, MS, Co-GC |
| 29 | **Carvacrol** | OM | 30.57 | **74.29** | **73.21** | **73.12** | **66.87** | 66.47 | 66.89 | **65.22** | 65.32 | 65.11 | **0.65** | 0.67 | 0.63 | 1315 | RI, MS, Co-GC |
| 30 | Thymol acetate | OM | 32.32 | 0.14 | 0.16 | 0.22 | ND | ND | ND | ND | ND | ND | 0.33 | 0.32 | 0.31 | 1355 | RI, MS, Co-GC |
| 32 | *E*-Caryophyllene | SH | 35.47 | 1.25 | 1.31 | 1.12 | 1.88 | 1.91 | 1.88 | 1.07 | 1.12 | 1.11 | 0.92 | 0.95 | 0.91 | 1427 | RI, MS, Co-GC |
| 33 | Aromadendrene | SH | 36.27 | 0.14 | 0.22 | 0.11 | 0.13 | 0.23 | 0.16 | ND | ND | ND | ND | ND | ND | 1446 | RI, MS, Co-GC |
| 34 | Viridiflorene | SH | 38.48 | ND | ND | ND | 0.17 | 0.19 | 0.29 | ND | ND | ND | ND | ND | ND | 1499 | RI, MS, Co-GC |
| 35 | Bicyclogermacrene | SH | 38.68 | ND | ND | ND | 0.12 | 0.11 | 0.1 | ND | ND | ND | ND | ND | ND | 1504 | RI, MS, Co-GC |
| 36 | *β*-Bisabolene | SH | 39.06 | ND | ND | ND | 0.23 | 0.24 | 0.23 | ND | ND | ND | ND | ND | ND | 15.14 | RI, MS, Co-GC |

**Table 1** continued

| No. | Compounds | | RT[a] | Composition (%) | | | | | | | | | | KI[b] | Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ilam | | | Lorestan | | | Kermanshah | | | Kurdestan | | | |
| | | | | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | | |
| 37 | $\gamma$-Cadinene | SH | 42.09 | ND | ND | ND | 0.18 | 0.24 | 0.65 | ND | ND | ND | ND | ND | ND | 1526 | RI, MS, Co-GC |
| 38 | Spathulenol | OS | 42.10 | 0.16 | 0.23 | 0.15 | 0.25 | 0.26 | 0.27 | 0.12 | 0.13 | 1.12 | 0.1 | 0.12 | 0.1 | 1590 | RI, MS, Co-GC |
| 39 | Caryophyllene oxide | OS | 42.30 | 0.42 | 0.51 | 0.34 | 0.55 | 0.53 | 0.54 | 0.31 | 0.32 | 0.31 | ND | ND | ND | 1595 | RI, MS, Co-GC |
| | Total | | | **98.79** | **98.89** | **97.81** | **97.6** | **97.53** | **98.09** | **99.57** | **99.87** | **99.64** | **99** | **98.57** | **98.69** | | |

The dominant compounds are indicated in bold; compounds listed in order of elution; the specimen of the collected plants was recognized as *Ziziphora clinopodioides*

The identified compounds lower than 0.02% were considered as trace compounds and their results were not included in the table

[a]RT: retention time (min.); [b] Kovats indices calculated on HP-5 column; [c] *RI* retention index; [d] *MS* mass spectrum; [e] *Co-GC* co-injection with reference compound; [f] *ND* not detected. *MH* monoterpene hydrocarbons; *OM* oxygenated monoterpenes; *SH* sesquiterpene hydrocarbons; *OS* oxygenated sesquiterpenes

terpinene (4.09–4.26%). In Lorestan sample, the major identified constituents were carvacrol (66.47–66.89%), thymol (14.39–15.55%), *p*-cymene (2.21–2.31%) and $\gamma$-terpinene (1.54–1.55%). The main portions of the ZEO of Kermanshah region were carvacrol (65.11–65.32%), thymol (18.55–19.54%), *p*-cymene (4.86–5.01%) and $\gamma$-terpinene (4.32–4.63%). The highest quantitative classified constituents of the collected EOs were oxygenated monoterpenes: Ilam (81.96–82.62%), Lorestan (87.47–87.92%), Kermanshah (85.05–86.18%) and Kurdestan (57.37–57.61%), followed by monoterpene hydrocarbons: Ilam (14.02–14.2%), Lorestan (6.17–6.35%), Kermanshah (11.97–12.12%) and Kurdestan (40.13–40.37%), sesquiterpene hydrocarbons: Ilam (1.23–1.53%), Lorestan (2.71–3.31%), Kermanshah (1.07–1.12%) and Kurdestan (0.91–0.95%) and oxygenated sesquiterpenes: Ilam (0.49–0.74%), Lorestan (0.79–0.81%), Kermanshah (0.43–1.43%) and Kurdestan (0.1–0.12%). Based on our findings (Table 2), the obtained concentrations of carvacrol, thymol, *p*-cymene and $\gamma$-terpinene were as follow: in Ilam sample: 1660.6–1680, 88.7–95.2, 77.1–80.2 and 40.3–44.6 µg/ml, in Kermanshah sample: 1465.1–1493.4, 223.9–239.5, 60.4–65.31 and 46.2–1.03 µg/ml, in Lorestan sample: 1503.2–1532.8, 183–191.1, 29.91–32.6 and 20.04–20.2 µg/ml and in Kurdestan sample: 9.98–10.61, 966.6–970.2, 120.21–120.81 and 489.4–492.1 µg/ml.

Generally, the results of the present study were in contrast with the findings reported by some authors. Ozturk and Ercisli (2007) who investigated the chemical compositions of ZEO collected from the Erzurum–Palandoken mountain of Turkey detected eighteen compounds. They showed in their research pulegone (31.8%), 1, 8-cineole (12.2%), limonene (10.4%), menthol (9.1%), $\beta$–pinene (6.8%), menthone (6.7%), piperitenone (5.3%) and piperitone (4.1%) were as the main compounds of ZEO. The same configuration was obtained by Behravan et al. (2007) in Mashhad, Khorasan Razavi (North East of Iran). They reported the presence of pulegone, terpineol, methyl acetate, iso-neomenthol and 1,8-cineole, constituting about 80% of the total ZEO. Also, in other parts of Iran, the major compounds of ZEO such as pulegone, 1,8-cineole, neomenthol, 4-terpineol, 1-terpineol, neomenthyl acetate and piperitenone was reported (Sonboli et al. 2010; Morteza-Semnani et al. 2005). However, carvacrol and thymol as the major compounds of ZEOs in the present study are in accordance with those of reported by Aghajani et al. (2008) and Schulz et al. (2005). According to the literature performed by Burt et al. (2007), differences in the plant EO compositions could be attributed to intrinsic (the species and stage of the plant growth and genetic characteristics of the plant) and extrinsic (geographical conditions, climate and seasonal variations, cultivar differences and

**Table 2** Essential oil composition (μg/ml) of stem, leaf and flower of *Z. clinopodioides* collected from different parts of Iran

| No. | Compounds | Composition (μg/ml) | | | | | | | | | | | | Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ilam | | | Lorestan | | | Kermanshah | | | Kurdestan | | | |
| | | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | |
| 1 | α-Thujene | 2.21 | 1.74 | 1.42 | ND[b] | ND | ND | 4.12 | 3.96 | 4.12 | 12.2 | 11.8 | 11.88 | Co-GC[a] |
| 2 | α-Pinene | 6.02 | 9.34 | 7.13 | 11.88 | 13.19 | 12.04 | 4.27 | 4.12 | 3.96 | 6.02 | 5.07 | 6.18 | Co-GC |
| 3 | Camphene | ND | ND | ND | 4.43 | 5.38 | 4.91 | 2.06 | 1.74 | 2 | ND | ND | ND | Co-GC |
| 4 | *β*-Pinene | ND | ND | ND | ND | ND | ND | 0.95 | 2 | 2.06 | ND | ND | ND | Co-GC |
| 5 | Myrcene | 9.66 | 10.14 | 9.34 | 6.81 | 8.87 | 6.49 | 8.08 | 8.55 | 7.76 | 15.4 | 15.4 | 15.2 | Co-GC |
| 6 | α-Phellandrene | 2.37 | 3.48 | 1.74 | ND | ND | ND | 2.06 | 2.21 | 2.69 | 3.64 | 3.64 | 3.8 | Co-GC |
| 7 | α-Terpinene | 13.78 | 29.31 | 13.62 | 6.97 | 6.49 | 6.18 | 12.51 | 11.25 | 11.4 | 43.73 | 43.75 | 43.89 | Co-GC |
| 8 | *p*-Cymene | 80.2 | 77.1 | 79.74 | 30.4 | 29.91 | 32.6 | 60.4 | 63.9 | 65.31 | 120.4 | 120.81 | 120.21 | Co-GC |
| 9 | Limonene | 2.21 | 2.53 | 3.48 | 1.74 | 1.58 | 1.74 | 1.58 | 2.06 | 2 | 3.01 | 2.85 | 3.01 | Co-GC |
| 10 | 1,8-Cineole | ND | ND | ND | 8.1 | 8.2 | 9.9 | ND | ND | ND | ND | ND | ND | Co-GC |
| 11 | *β*-Phellandrene | 2.06 | 3.48 | 2.37 | ND | ND | ND | 1.74 | 2.06 | 2 | 2.37 | 2.21 | 2.06 | Co-GC |
| 12 | *γ*-Terpinene | 40.3 | 42.2 | 44.6 | 20.2 | 20.04 | 20.2 | 51.03 | 48.4 | 46.2 | 490.6 | 489.4 | 492.1 | Co-GC |
| 13 | *cis*-Sabinene hydrate | ND | ND | ND | 6.33 | 6.18 | 6.65 | 1.1 | 2.69 | 2.69 | ND | ND | ND | Co-GC |
| 14 | Terpinolene | ND | ND | ND | ND | ND | ND | 1.26 | 2.85 | 3.64 | ND | ND | ND | Co-GC |
| 15 | *trans*-Sabinene hydrate | ND | ND | ND | 2 | 2.06 | 1.74 | ND | ND | ND | ND | ND | ND | Co-GC |
| 16 | Linalool | 2.53 | 5.22 | 4.27 | 2.53 | 2.69 | 2.21 | 2.06 | 1.74 | 1.58 | 8.55 | 8.55 | 8.39 | Co-GC |
| 17 | Camphore | ND | ND | ND | 4.43 | 4.27 | 3.96 | ND | ND | ND | ND | ND | ND | Co-GC |
| 18 | Borneol | 5.7 | 21.23 | 5.54 | 30.5 | 30.9 | 30.74 | 9.6 | 7.76 | 9.66 | 1.9 | 2.21 | 1.7 | Co-GC |
| 21 | Terpinene-4-ol | 6.18 | 6.02 | 6.4 | 16.07 | 17.9 | 15.4 | 7.6 | 5.86 | 5.07 | 5.86 | 6.02 | 6.65 | Co-GC |
| 22 | α-Terpineol | ND | ND | ND | 5.86 | 5.86 | 5.54 | 1.26 | 2.85 | 3.01 | ND | ND | ND | Co-GC |
| 23 | *cis*-Dihydro carvone | ND | ND | ND | 2.53 | 2.21 | 2.53 | ND | ND | ND | ND | ND | ND | Co-GC |
| 24 | Thymol methyl ether | ND | ND | ND | 3.96 | 3.64 | 4.12 | ND | ND | ND | ND | ND | ND | Co-GC |
| 25 | Carvacrol, methyl ether | ND | ND | ND | ND | ND | ND | 0.63 | 2.21 | 1.74 | ND | ND | ND | Co-GC |
| 26 | Carvone | ND | ND | ND | 7.44 | 7.6 | 7.6 | ND | ND | ND | ND | ND | ND | Co-GC |
| 27 | Isobornyl acetate | ND | ND | ND | 1.74 | 1.74 | 1.74 | ND | ND | ND | ND | ND | ND | Co-GC |
| 28 | Thymol | 90.1 | 88.7 | 95.2 | 191.1 | 189.2 | 183 | 230.1 | 239.5 | 223.9 | 970.2 | 966.6 | 968.5 | Co-GC |
| 29 | Carvacrol | 1680 | 1662 | 1660.6 | 1510 | 1503.2 | 1532.8 | 1480 | 1493.4 | 1465.1 | 10.1 | 10.61 | 9.98 | Co-GC |
| 30 | Thymol acetate | 2.21 | 2.53 | 3.48 | ND | ND | ND | ND | ND | ND | 5.22 | 5.07 | 4.91 | Co-GC |
| 32 | *E*-Caryophyllene | 19.8 | 20.75 | 15.4 | 29.79 | 30.26 | 29.79 | 16.95 | 15.4 | 15.4 | 14.57 | 15.05 | 14.42 | Co-GC |
| 33 | Aromadendrene | 2.21 | 3.48 | 1.74 | 2.06 | 3.64 | 2.53 | ND | ND | ND | ND | ND | ND | Co-GC |
| 34 | Viridiflorene | ND | ND | ND | 2.69 | 3.01 | 4.59 | ND | ND | ND | ND | ND | ND | Co-GC |
| 35 | Bicyclogermacrene | ND | ND | ND | 2 | 1.74 | 1.58 | ND | ND | ND | ND | ND | ND | Co-GC |
| 36 | *β*-Bisabolene | ND | ND | ND | 3.64 | 3.8 | 3.64 | ND | ND | ND | ND | ND | ND | Co-GC |

**Table 2** continued

| No. | Compounds | Composition (µg/ml) | | | | | | | | | | | | Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ilam | | | Lorestan | | | Kermanshah | | | Kurdestan | | | |
| | | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | Leaf | Flower | Stem | |
| 37 | γ-Cadinene | ND | ND | ND | 2.85 | 3.8 | 10.3 | ND | ND | ND | ND | ND | ND | Co-GC |
| 38 | Spathulenol | 2.53 | 3.64 | 2.37 | 3.96 | 4.12 | 4.27 | 2 | 2.06 | 15.4 | 1.58 | 2 | 1.58 | Co-GC |
| 39 | Caryophyllene oxide | 6.65 | 8.08 | 5.38 | 8.71 | 8.39 | 8.55 | 4.91 | 5.07 | 4.91 | ND | ND | ND | Co-GC |

[a] *Co-GC* co-injection with reference; [b] *ND* not detected

preparation process) conditions. Hence, further studies are required to evaluate these influencing factors such as the characteristic of soil and climate conditions.

## Antimicrobial activity of *Z. clinopodioides* Lam. essential oils

The results of the antimicrobial activities of ZEOs collected from four western parts of Iran are given in Tables 3 and 4. As shown, the ZEOs exhibited broad spectra of activities against the bacterial strains. The flower, leaf and stem EO also had a similar antimicrobial activity ($P > 0.05$), in good correlation with their similar chemical compositions. The ZEOs inhibited the growth of both Gram-positive and Gram-negative bacteria at MIC values between 0.03 and 0.04%. These findings are in accordance with some other studies (Okoh et al. 2010; Kokoska et al. 2002; Gilles et al. 2010). The Gram-positive bacteria (*S. aureus, B. cereus, B. subtilis* and *L. monocytogenes*) were the most susceptible and Gram-negative bacteria (*S. typhimurium* and *E. coli* O157:H7) were the most resistant. As the outer membrane of Gram-negative bacteria contains lipopolysaccharide, this group of bacteria are usually more resistant against EOs in comparison to Gram-positive bacteria (Lv et al. 2011; Tajkarimi et al. 2010; Stefanello et al. 2008). Our findings about antifungal activity of ZEOs are in agreement with previous studies that reported the various degrees of growth inhibition effects of EOs and extracts against several phytopathogenic fungi (Alves-Silva et al. 2013; Cakir et al. 2005).

Generally, antimicrobial activities of the ZEOs obtained from different parts of Iran were similar among the different regions. However, the Ilam ZEO sample was found to be the most active against selected microorganisms, which it could be attributed to the high contents of carvacrol (73.12–74.29%), thymol (7.18–7.59%) and *p*-cymene (7.41–7.69%). It has been demonstrated that the antimicrobial characteristics of plant EOs are mostly due to the phenolic compounds particularly oxygenated monoterpenes such as carvacrol and thymol (Shahbazi and Shavisi 2016). In the present study, to confirm the relationship between the major constituents of the ZEO and antimicrobial activity, four main compounds including thymol, carvacrol, γ-terpinene and *p*-cymene were selected to test their antifungal and antibacterial activities. As it can be observed from Tables 5 and 6, carvacrol and thymol had similar antimicrobial activity against investigated bacteria with MICs ranging from 200 to 400 µg/ml and inhibition zones ranging from 2.1 to 3.4 mm. These results were in agreement with reports that some bacterial species got inhibited equally by carvacrol and thymol (Rivas et al. 2010; Xu et al. 2008). The similar antibacterial activity of these compounds was expected because they have similar

**Table 3** MIC/MBC (%) values of *Z. clinopodioides* essential oils

| Bacteria | | Ilam | | Lorestan | | Kermanshah | | Kurdestan | | Ciprofloxacin | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| *Staphylococcus aureus* | Leaf | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.05 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | | |
| | Flower | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.05 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 1 ± 0.00 | 1 ± 0.00 |
| | Stem | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.05 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | | |
| *Bacillus subtilis* | Leaf | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | | |
| | Flower | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 1 ± 0.00 | 1 ± 0.00 |
| | Stem | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | | |
| *Bacillus cereus* | Leaf | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | | |
| | Flower | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 1 ± 0.00 | 1 ± 0.00 |
| | Stem | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | | |
| *Listeria monocytogenes* | Leaf | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | | |
| | Flower | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 1 ± 0.00 | 1 ± 0.00 |
| | Stem | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | | |
| *Salmonella typhimurium* | Leaf | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.06 ± 0.00 | | |
| | Flower | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.06 ± 0.00 | 1 ± 0.00 | 1 ± 0.00 |
| | Stem | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.06 ± 0.00 | | |
| *Escherichia coli* O157:H7 | Leaf | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | | |
| | Flower | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 1 ± 0.00 | 1 ± 0.00 |
| | Stem | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | | |

**Table 4** Antimicrobial effects of *Z. clinopodioides* essential oils by agar disk diffusion assay

| Bacteria/fungi | | Inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ilam | Lorestan | Kermanshah | Kurdestan | Tetracycline | Ketoconazole |
| *Staphylococcus aureus* | Leaf | 33.2 ± 0.0 | 31.3 ± 0.3 | 30.6 ± 1.4 | 28.4 ± 0.1 | | |
| | Flower | 33.1 ± 0.2 | 31.3 ± 0.1 | 30.5 ± 0.0 | 28.7 ± 0.3 | 10.2 ± 2.2 | NT |
| | Stem | 33.3 ± 0.1 | 31.0 ± 0.4 | 30.6 ± 0.3 | 28.4 ± 0.4 | | |
| *Bacillus subtilis* | Leaf | 28.1 ± 0.4 | 20.2 ± 2.4 | 23.3 ± 0.2 | 22.1 ± 0.4 | | |
| | Flower | 28.4 ± 0.0 | 20.2 ± 0.0 | 23.2 ± 0.4 | 22.3 ± 0.1 | 0.5 ± 0.0 | NT |
| | Stem | 28.1 ± 0.1 | 20.2 ± 0.2 | 23.2 ± 0.3 | 22.4 ± 0.1 | | |
| *Bacillus cereus* | Leaf | 28.1 ± 0.5 | 21.4 ± 2.1 | 23.1 ± 0.4 | 24.3 ± 0.8 | | |
| | Flower | 28.0 ± 0.0 | 21.5 ± 0.1 | 23.0 ± 0.1 | 24.4 ± 0.0 | 14.2 ± 0.4 | NT |
| | Stem | 28.0 ± 0.0 | 21.6 ± 0.2 | 23.1 ± 0.6 | 24.3 ± 0.0 | | |
| *Listeria monocytogenes* | Leaf | 32.2 ± 0.0 | 29.3 ± 0.9 | 28.4 ± 0.2 | 26.2 ± 0.2 | | |
| | Flower | 32.3 ± 0.2 | 29.3 ± 0.6 | 28.5 ± 0.6 | 26.2 ± 0.3 | 13.2 ± 1.1 | NT |
| | Stem | 32.4 ± 0.3 | 29.2 ± 0.4 | 28.5 ± 0.1 | 26.2 ± 0.2 | | |
| *Salmonella typhimurium* | Leaf | 23.1 ± 0.2 | 22.2 ± 0.4 | 22.2 ± 0.7 | 18.3 ± 0.7 | | |
| | Flower | 22.9 ± 0.1 | 22.3 ± 0.1 | 22.1 ± 0.0 | 18.4 ± 0.0 | 12.1 ± 0.6 | NT |
| | Stem | 22.9 ± 0.1 | 22.3 ± 0.3 | 22.0 ± 0.2 | 18.5 ± 0.1 | | |
| *Escherichia coli* O157:H7 | Leaf | 27.2 ± 1.2 | 26.1 ± 1.3 | 26.1 ± 0.6 | 23.2 ± 0.9 | | |
| | Flower | 27.4 ± 0.0 | 26.0 ± 0.2 | 26.1 ± 0.1 | 23.4 ± 0.6 | 10.3 ± 1.1 | NT |
| | Stem | 27.3 ± 0.1 | 26.1 ± 0.0 | 26.1 ± 0.0 | 23.1 ± 0.3 | | |
| *Aspergillus niger* | Leaf | 21.1 ± 0.6 | 19.1 ± 2.3 | 23.9 ± 0.4 | 19.1 ± 1.2 | | |
| | Flower | 19.8 ± 0.3 | 19.3 ± 0.4 | 23.8 ± 0.1 | 19.3 ± 0.1 | NT | 26.4 ± 0.8 |
| | Stem | 20.3 ± 0.5 | 19.2 ± 0.0 | 23.7 ± 0.0 | 19.3 ± 0.2 | | |
| *Candida albicans* | Leaf | 17.2 ± 0.5 | 15.3 ± 0.7 | 19.2 ± 0.2 | 14.2 ± 1.7 | | |
| | Flower | 17.5 ± 0.7 | 15.2 ± 0.3 | 19.0 ± 0.2 | 14.2 ± 0.3 | NT | 34.3 ± 0.6 |
| | Stem | 17.3 ± 0.2 | 15.2 ± 0.0 | 19.3 ± 0.1 | 14.1 ± 0.4 | | |

*NT* not tested

**Table 5** MIC/MBC (μg/ml) values of main compounds in *Z. clinopodioides* essential oils

| Bacteria | Carvacrol | | Thymol | | $\gamma$-Terpinene | | *p*-Cymene | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| *Staphylococcus aureus* | 200 ± 0.00 | 200 ± 0.00 | 200 ± 0.00 | 200 ± 0.00 | 800 ± 0.00 | 1000 ± 0.00 | >1000 | >1000 |
| *Bacillus subtilis* | 200 ± 0.00 | 200 ± 0.00 | 200 ± 0.00 | 200 ± 0.00 | 800 ± 0.00 | 1000 ± 0.00 | >1000 | >1000 |
| *Bacillus cereus* | 400 ± 0.00 | 400 ± 0.00 | 400 ± 0.00 | 800 ± 0.00 | >1000 | >1000 | >1000 | >1000 |
| *Listeria monocytogenes* | 200 ± 0.00 | 200 ± 0.00 | 200 ± 0.00 | 200 ± 0.00 | 1000 ± 0.00 | 1000 ± 0.00 | >1000 | >1000 |
| *Salmonella typhimurium* | 400 ± 0.00 | 800 ± 0.00 | 400 ± 0.00 | 800 ± 0.00 | >1000 | >1000 | >1000 | >1000 |
| *Escherichia coli* O157:H7 | 400 ± 0.00 | 800 ± 0.00 | 400 ± 0.00 | 800 ± 0.00 | >1000 | >1000 | >1000 | >1000 |

chemical structures and are likely to have similar mechanisms of antimicrobial activity (Burt et al. (2007). Some researchers have suggested that carvacrol can increase the heat shock protein 60 HSP 60 (GroEL) protein and inhibit the production of flagellin in most of bacteria (Burt et al. 2007; Ćavar et al. 2008). Antimicrobial effects of carvacrol and thymol is attributed to the acidic nature of their hydroxyl group involvement in the formation of hydrogen bonds (Ćavar et al. 2008). Based on our findings, the MICs ranging from 800 to >1000 μg/ml and inhibition zones ranging from 0.0 to 1.2 mm of *p*-cymene and $\gamma$-terpinene indicated that these constituents did not have good antimicrobial activity. Results of the current study suggested that the antimicrobial effect of ZEO was high with MICs much lower than the major compounds ($P < 0.05$). The probable reason behind this can be that EOs are very

**Table 6** Antimicrobial effects of main compounds in *Z. clinopodioides* essential oils by agar disk diffusion assay

| Bacteria/fungi | Inhibition zone (mm) | | | |
|---|---|---|---|---|
| | Carvacrol | Thymol | γ-Terpinene | *p*-Cymene |
| *Staphylococcus aureus* | 3.4 ± 0.1 | 3.3 ± 0.0 | 1.2 ± 0.0 | ND |
| *Bacillus subtilis* | 2.9 ± 0.0 | 2.9 ± 0.3 | 1.2 ± 0.0 | ND |
| *Bacillus cereus* | 2.3 ± 0.0 | 2.4 ± 0.1 | 1.0 ± 0.1 | ND |
| *Listeria monocytogenes* | 3.3 ± 0.0 | 3.3 ± 0.0 | 1.1 ± 0.1 | ND |
| *Salmonella typhimurium* | 2.2 ± 0.2 | 2.3 ± 0.0 | ND | ND |
| *Escherichia coli* O157:H7 | 2.1 ± 0.0 | 2.1 ± 0.2 | ND | ND |
| *Aspergillus niger* | 1.0 ± 0.1 | 1.1 ± 0.1 | ND | ND |
| *Candida albicans* | 1.5 ± 0.1 | 1.3 ± 0.0 | ND | ND |

ND, inhibition zone was not observed

heterogeneous mixtures of substances, and biological actions are due to the synergistic or antagonistic effects of all chemical components (Ribeiro-Santos et al. 2017). Cao et al. (2009) reported that combination of *p*-cymene, a very weak antimicrobial compound, with carvacrol has a remarkable influence in reduction growth of various bacterial strains. They reported that *p*-cymene facilitates carvacrol's transferring into the bacterial cell by better swelling. It can be concluded that synergistic effects between *p*-cymene and carvacrol as well as carvacrol and thymol are the reason of the strong antimicrobial properties of the investigated ZEOs in the current study especially in the case of Ilam ZEO. Moreover, other compounds such as γ-terpinene, terpinolene, α-pinene and β-pinene could also have a significant role in the antibacterial activities of the investigated ZEOs (Lv et al. 2011). Minor constituents such as linalool, boerneol, camphor, terpinen-4-ol and 1,8-cineole might also attribute in antibacterial activity (Saei-Dehkordi et al. 2010; Bajpai et al. 2009; Ćavar et al. 2008).

Some studies in Iran and other countries investigated in vitro antibacterial activity of ZEO and reported that it had strong effect against numerous food-borne pathogens including *L. monocytogenes*, *Salmonella spp.*, *E. coli* O157:H7, *B. subtilis*, *B. cereus* and *S. aureus* (Shahbazi 2015; Ozturk and Ercisli 2007). Sonboli et al. (2010) and Behravan et al. (2007) reported that the ZEO collected from Hamedan province (western part of Iran) and Khorasan Razavi province (north eastern part of Iran) had strong antibacterial activity against *S. epidermidis*, *S. aureus*, *E. coli* and *B. subtilis*. Ozturk and Ercisli (2007) also indicated that ZEO had high antibacterial activity against *B. subtilis*, *B. cereus* and *L. monocytogenes*, which is good in accordance with our findings. From a comparison of our results with values reported in the literature for other EOs, it is interesting the ZEOs showed stronger antimicrobial effect than *Allium cepa* EO (Ye et al. 2013), *Eucalyptus globulus* EO (Harkat-Madouri et al. 2015), *Mentha spicata* EO (Shahbazi and Shavisi 2016), *Lavandula angustifolia* EO (Giovannini et al. 2016), *Zataria multiflora* Boiss. EO (Saei-Dehkordi et al. 2010), *Laurus nobilis* L. and *Myrtus communis* L. EOs (Cherrat et al. 2014).

## Antioxidant activity of *Z. clinopodioides* Lam. essential oils

*In vitro* antioxidant activities of EOs were evaluated by several methods since the single assay cannot determine all different mechanisms of the certain antioxidant (Singh et al. 2010). In the present study, antioxidant activities of ZEOs were tested by the DPPH radical scavenging, FRAP, β-carotene/linoleic acid bleaching and TBA methods. As shown in Table 7, Kermanshah oil sample had a higher DPPH radical scavenging (0.30–0.31 mg/ml), ability to prevent the bleaching of β-carotene (0.09–0.1 mg/ml), ferric reducing power (0.40–0.42 mg/ml) and TBA (0.004–0.006 Meq of malondialdehyde/g) value than that of ZEOs from Ilam, Kurdestan and Lorestan. There were significant differences in the antioxidant activities of the ZEOs collected from different parts of Iran ($P < 0.05$). No significant difference was found among the EOs obtained from stem, flower and leaf parts of *Z. clinopodioides* plant ($P > 0.05$).

The results of the present study provides important information, for the first time, about the in vitro antioxidant activities of ZEOs from different parts of Iran. Based on our knowledge, there are no published data on the in vitro antioxidant effect of ZEO. Our data showed that DPPH radical scavenging activity of ZEOs was remarkably higher than those of reported for *Myrtus communis* var. *italica* L. EO ($IC_{50}$ = 0.55–2 mg/ml) (Wannes et al. 2010), *A. cepa* EO ($IC_{50}$ = 0.63 mg/ml), *E. globulus* EO ($IC_{50}$ = 33.33 ± 055 mg/ml), *Hymenocrater longiflorus* ($IC_{50}$ = 0.527 mg/ml) and *Ferulago bernardii* Tomk. & M. Pimen EO ($IC_{50}$ = 14.81 mg/ml). However, it was lower than those of reported for *Z. multiflora* Boiss. EO

**Table 7** Antioxidant activity of *Z. clinopodioides* essential oils (mean ± SD)

| | Ilam | Lorestan | Kermanshah | Kurdestan |
|---|---|---|---|---|
| DPPH radical-scavenging activity ($IC_{50}$; mg/ml) | | | | |
| Leaf | 0.38 ± 0.17$^{b}$ | 0.33 ± 0.11$^{c}$ | 0.30 ± 0.04$^{d}$ | 0.54 ± 0.12$^{a}$ |
| Flower | 0.38 ± 0.11$^{b}$ | 0.32 ± 0.04$^{c}$ | 0.31 ± 0.09$^{c}$ | 0.56 ± 0.02$^{a}$ |
| Stem | 0.38 ± 0.01$^{b}$ | 0.34 ± 0.06$^{c}$ | 0.30 ± 0.01$^{d}$ | 0.55 ± 0.05$^{a}$ |
| Ferric reducing power ($EC_{50}$; mg/ml) | | | | |
| Leaf | 0.65 ± 0.01$^{b}$ | 0.54 ± 0.06$^{c}$ | 0.42 ± 0.02$^{d}$ | 0.89 ± 0.04$^{a}$ |
| Flower | 0.66 ± 0.06$^{b}$ | 0.55 ± 0.03$^{c}$ | 0.41 ± 0.02$^{d}$ | 0.90 ± 0.01$^{a}$ |
| Stem | 0.65 ± 0.17$^{b}$ | 0.55 ± 0.01$^{c}$ | 0.40 ± 0.02$^{d}$ | 0.91 ± 0.01$^{a}$ |
| β-Carotene bleaching inhibition ($EC_{50}$; mg/ml) | | | | |
| Leaf | 0.13 ± 0.00$^{b}$ | 0.11 ± 0.05$^{c}$ | 0.09 ± 0.01$^{d}$ | 0.23 ± 0.01$^{a}$ |
| Flower | 0.13 ± 0.01$^{b}$ | 0.12 ± 0.01$^{c}$ | 0.09 ± 0.01$^{d}$ | 0.21 ± 0.01$^{a}$ |
| Stem | 0.12 ± 0.01$^{b}$ | 0.11 ± 0.02$^{bc}$ | 0.10 ± 0.01$^{c}$ | 0.22 ± 0.01$^{a}$ |
| Thiobarbituric acid (Meq of malondialdehyde/g) | | | | |
| Leaf | 0.009 ± 0.031$^{b}$ | 0.006 ± 0.011$^{c}$ | 0.004 ± 0.000$^{d}$ | 0.01 ± 0.06$^{a}$ |
| Flower | 0.010 ± 0.001$^{a}$ | 0.006 ± 0.001$^{c}$ | 0.005 ± 0.001$^{c}$ | 0.01 ± 0.00$^{a}$ |
| Stem | 0.012 ± 0.002$^{b}$ | 0.006 ± 0.003$^{c}$ | 0.006 ± 0.000$^{c}$ | 0.01 ± 0.00$^{a}$ |

$^{a–d}$ Means with different lowercase letters in the same row are significantly different ($P < 0.05$)

($IC_{50}$ = 19.7 ± 0.7 μg/ml) (Saei-Dehkordi et al. 2010) and *L. angustifolia* EO ($IC_{50}$ = 14.63 ± 0.02 μg/ml) (Giovannini et al. 2016). It has been demonstrated that the antioxidant properties of plant EOs and extracts is due to the presence of specific bioactive components especially oxygenated monoterpenes (carvacrol and thymol) and monoterpen hydrocarbons (Cao et al. 2009; Ahmadi et al. 2010). As described before, our ZEO samples had high percentage of oxygenated monoterpens, particularly carvacrol and thymol, high level of γ-terpinene and low level of oxygenated sesquiterpens. The low percentage of oxygenated monoterpens and carvacrol + thymol of Kurdestan oil sample might be related to its lowest antioxidant activity. The potential application of thymol, carvacrol, γ-terpinene, thymol methyl ether and carvacrol methyl ether as the main antioxidant compounds of various plants have been reported in some studies (Cao et al. 2009; Saei-Dehkordi et al. 2010). It can be concluded that the high extent of phenolic compounds and monoterpene hydrocarbons could be related to their high antioxidant activities of ZEOs collected from different parts of Iran comparing with EOs of other plants.

## Conclusion

The most abundant chemical constituent of the investigated ZEOs obtained from different parts of Iran were almost same whereas their amounts varied significantly. Carvacrol, thymol, γ-terpinene and *p*-cymene were the main compounds of the ZEOs. The strong in vitro antimicrobial and antioxidant activities supports the traditional use of ZEO in the treatments of gastrointestinal diseases. Moreover, ZEO can be used for the growth inhibition of various bacteria in various food products.

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