



## Original Article

# Antimicrobial and cytotoxic evaluation of some herbal essential oils in comparison with common antibiotics in bioassay condition

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## ARTICLE INFO

## Article history:

Received 3 April 2014

Received in revised form

29 June 2014

Accepted 7 July 2014

Available online 21 July 2014

## Keywords:

antibacterial activity

disk diffusion method

essential oil

minimal bactericidal concentrations

minimal inhibitory concentrations

## ABSTRACT

**Background:** Since ancient times, various infectious diseases have been treated using herbal drugs. Today, efforts regarding the discovery of the effectual components of plants possessing antimicrobial properties are advanced. Herbal essential oils are widely used for treatment of various diseases, and they play an important role in health care considerations. **Methods:** The antibacterial activity of *Artemisia kermanensis*, *Lavandula officinalis*, and *Zataria multiflora* Boiss essential oils against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (PTCC 1310), and *Klebsiella pneumonia* (PTCC 1053) was evaluated using the disk diffusion method as well as determination of the minimal inhibitory concentration and minimal bactericidal concentration. The composition of the three essential oils was determined with gas chromatography-mass spectrometry. Variable amounts of different components (such as oxygenated monoterpenes, thymol, carvacrol, and 1,8-cineol) were found in all three oils. Among the tested bacteria, *S. aureus* was the most sensitive to the three essential oils.

**Results:** The obtained results showed that each of the three essential oils has an inhibitory effect on pathogenic strains. Of these three oils, *Z. multiflora* Boiss essential oil showed the highest inhibitory effect on microbial strains. Furthermore, comparison of the antibacterial effects of these three essential oils with ampicillin and tetracycline revealed that these antibiotics have a better effect in controlling pathogenic strains.

**Conclusion:** The essential oils used in the present study with different components showed antibacterial activity (especially *Z. multiflora* Boiss essential oil), and therefore they can be used as a new antibacterial substance.

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<http://dx.doi.org/10.1016/j.imr.2014.07.001>

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## 1. Introduction

In recent decades, increasingly drug-resistant bacteria have been a major concern. Drug resistance is common among pathogenic staphylococci. *Staphylococcus aureus* is a facultative anaerobic Gram-positive coccobacillus naturally found in parts of the skin and nasal cavity.<sup>1</sup> The inherent virulence of *S. aureus* and its ability to create a diverse array to life-threatening infections and capacity to adapt to different environmental conditions are the main concerns about this pathogen.<sup>2</sup> *Pseudomonas aeruginosa* is Gram-negative aerobic motile basil.<sup>3,4</sup> This bacterium is commonly found in most environments in hospitals. *P. aeruginosa* often exist in small numbers in the normal intestinal flora and on human skin. It becomes pathogenic only when introduced into areas without normal defenses such as the skin and the mucus layer.<sup>3</sup> This bacterium can express a variety of efficient efflux pump and antibiotic inactivating enzymes, so it can be resistant to antibiotics. *Klebsiella pneumonia* (which belongs to the Enterobacteriaceae family) is a nonmobile and encapsulated Gram-negative, facultative anaerobic bacillus, and is found in the normal flora of intestines.<sup>3</sup> This bacterium causes infections in hospitals and communities.<sup>5</sup> The majority of hospital infections caused by *K. pneumonia* are nosocomial pneumonia, urinary tract infections, diarrhea, and intra-abdominal infections.<sup>6</sup> *K. pneumonia* may be attributable to multidrug efflux systems.<sup>7</sup> The mounting concern about drug resistance has led researchers to focus more attention on natural products, including plants, with antimicrobial properties as the future source of antimicrobial agents.<sup>8,9</sup> For thousands of years, humans have been using natural products derived from plants for therapeutic purposes.<sup>9</sup> The World Health Organization reported that in 2008, more than 80% of the world population depended on traditional medicine for their primary health care needs.<sup>10</sup> *Artemisia* is a genus belonging to the Asteraceae family. Many members of this genus are important medicinal plants. *Artemisia kermanensis* is an endemic plant in Iran and important medicinal plant in the south of Kerman Province.<sup>11,12</sup> *Lavandula officinalis* (*L. angustifolia*) is an important species of the family Lamiaceae, and is broadly distributed in the Mediterranean region.<sup>13</sup> In Iranian flora, lavender is chiefly distributed in the northern parts of the country. Lavender oil is known for its excellent aroma and is widely used in flavor, perfume, and cosmetic industries; it is also recommended for its anti-inflammatory and anti-infectious effects.<sup>14</sup> In Europe, lavender is used as an antispasmodic, carminative, and mild tranquilizer for digestive and mild nervous disorders. Lavender oil's antifungal and antibacterial activities oil have been reported.<sup>14,15</sup> Moreover, it has been found that lavender oil is active against many species of bacteria and fungi. For example, *L. angustifolia* oil was indicated to have *in vitro* antibacterial activity against methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus faecalis* at a concentration of < 1%.<sup>16</sup> *Zataria multiflora* Boiss, a member of the Labiatae family, is a native plant of Iran, Pakistan, and Afghanistan. It is traditionally used for anesthetic, antiseptic, and antispasmodic purposes. *Z. multiflora* has also been shown to have anti-inflammatory analgesic effects.<sup>17,18</sup> This plant is also used as a condiment and has

many therapeutic applications in traditional folk medicine (Iranian Herbal Pharmacopoeia). In this study, we examined the antibacterial activity of *A. kermanensis*, *L. officinalis*, and *Z. multiflora* Boiss against three pathogenic bacteria (*P. aeruginosa*, *S. aureus*, and *K. pneumonia*).

## 2. Materials and methods

### 2.1. Origin and isolation of essential oils

Fresh *Z. multiflora*, *L. officinalis*, and *A. kermanensis* plants were gathered from Lorestan and Chaharmahal provinces in Iran (2012). Their scientific names were searched through the Herbarium part of Institution of Traditional Medicine in Iran (nos. 2359, 2360, and 2361, respectively). At first, the aerial parts of the herbs were kept at room temperature for 3 days, and after complete dryness was attained, the parts were powdered by mill. Making of essential oil was done with water using the essential making machine, Clevenger apparatus (model BP, Ashke Shisheh Co., Tehran, Iran & mantle model H610, Fater Electronic, Tehran, Iran) based on boiling point. For each batch, 100 g of the powder was placed in a 1-L balloon of Clevenger, and then water was added. After 5 hours of distillation, the essence—which was a yellow to green liquid with a good smell—was gathered.<sup>19</sup> The oils were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and stored at 4 °C in sealed amber vials until use.<sup>20</sup>

### 2.2. Gas chromatography-mass spectrometry

Analysis was carried out using a GC-mass chromatograph with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of 0.8 mL/minute. The column temperature was kept at 50 °C for 2 minutes, and then it was programmed to 200 °C at a rate of 3 °C/minute and kept constant at 200 °C for 10 minutes. The injection was performed in split mode with ratio of 50:1 at 250 °C. The compounds were identified by comparison of the relative retention indices with those reported in the literature and also by comparison of their mass spectra with published mass spectra.<sup>20,21</sup> The retention indices for all the components were determined according to the Van Den Dool method<sup>22</sup> using *n*-alkanes as standards.

### 2.3. Antimicrobial activities assays

#### 2.3.1. Preparation of bacterial cells

The bacterial species consisted of *S. aureus* (ATCC 25923), *P. aeruginosa* (PTCC 1310), and *K. pneumonia* (PTCC 1053), which were prepared at the Traditional Medicine Institute of Isfahan (Isfahan, Iran). First, the Muller-Hinton agar (MHA) medium was prepared and transferred in sterilized Petri dishes (5 cm thick). Under aseptic conditions, the samples of bacteria were taken from basal culture using an applicator and then inoculated in the medium.

#### 2.3.2. Antibacterial assay

In order to evaluate the antimicrobial effect, the disk diffusion method (which is known as Kirby-Bauer and

is the most common form of antimicrobial assay)<sup>23</sup> and assessment of minimal inhibitory and minimal bactericidal concentrations (MIC and MBC, respectively), were applied. After 18 hours of culture, liquid containing bacteria, with a standard density ( $1 \times 10^6$  CFU/mL) of 0.5 McFarland in MHA, was prepared, and by using Sampler, 500  $\mu$ L of the liquid was transferred to MHA. The liquid was gently distributed on the surface of MHA using sterile loop. There were blank disks with 6 mm in diameter containing 30  $\mu$ L with concentrations of 0.08  $\mu$ g/disk, 0.16  $\mu$ g/disk, 0.31  $\mu$ g/disk, 0.63  $\mu$ g/disk, 1.25  $\mu$ g/disk, 2.5  $\mu$ g/disk, 5  $\mu$ g/disk, 10  $\mu$ g/disk, 20  $\mu$ g/disk, 40  $\mu$ g/disk, 60  $\mu$ g/disk, 80  $\mu$ g/disk, and 100  $\mu$ g/disk on MHA. A disk containing ampicillin, penicillin, and tetracycline was used as positive control, and the diameter of inhibition of zone was measured after 24 hours of incubation at 37 °C at 24 hours, 48 hours, and 72 hours in triplicate. The microwell method<sup>24</sup> was used for determining the MIC value (the lowest concentration of an antibacterial agent that prevents visible bacterial growth after 24 hours of incubation at 37 °C) and MBC value (the lowest concentration required to kill certain bacteria) for *S. aureus*, *P. aeruginosa*, and *K. pneumonia*. The suspension of bacterial strain was prepared from the liquid culture with a standard darkness of 0.5 McFarland. The essential oils were prepared, and different dilutions (6 dilutions) were added to the pipes containing 10 mL liquid culture medium. In this step, in order to determine MIC, the 96-well plate was used. To every well, 95  $\mu$ L Mueller–Hinton broth and 5  $\mu$ L microbial suspension were added. Next, 100  $\mu$ L of the essential oil with a concentration of 500  $\mu$ g/ $\mu$ L was added to the first well. Then, 100  $\mu$ L was taken from the first well and transferred to the next well. This process went on until the sixth well. The last well contained 195  $\mu$ L MHB culture medium and 5  $\mu$ L of microbial suspension without any essential oil) as negative control). In the next step, the ingredients of every well were mixed using a rotary shaker for 20 minutes. Then it was put in an incubator for 24 hours at a suitable temperature (37 °C). The microbial growth was measured at 600 nm.<sup>25</sup>

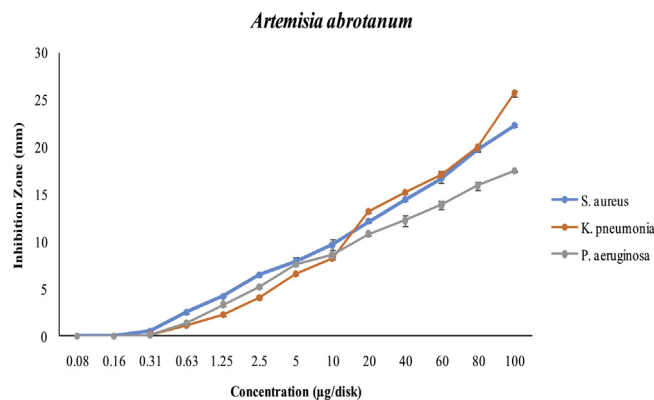
## 2.4. In vitro analyses

### 2.4.1. Cell culture

The L929 fibroblast cell line of mouse (NCBI code 161) (was obtained from the Pasteur Institute of Iran (Tehran, Iran), then grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, 100 U/mL penicillin (Gibco), and 100  $\mu$ g/mL streptomycin (Gibco, Carlsbad, CA, USA), at 37 °C in a humidified atmosphere containing 90% air and 5% CO<sub>2</sub>.

### 2.4.2. Cytotoxicity assay

In order to determine the cytotoxicity effect of essential oils, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used. This method is based on a mitochondrial succinate dehydrogenase activity that changes the yellow dye of MTT to the violet dye of Formosan. Formosan can dissolve in dimethyl sulfoxide, and its optical density (OD) could be measured using enzyme-linked immunosorbent assay.<sup>26,27</sup> The cells were cultured in T25, and after 90% confluency, they were removed from the culture dishes using trypsinization and suspended in 10 mL culture medium. Next, they were seeded with a cell count of  $5 \times 10^3$  for L929



**Fig. 1 – Effect of essential oil of *Artemisia kermanensis* on three species of bacteria.**

cells per well in 96-well plates for 24 hours. After that, the cultured cells were treated with different concentrations of essential oils (0  $\mu$ g/mL, 0.75  $\mu$ g/mL, 1.56  $\mu$ g/mL, 3.12  $\mu$ g/mL, 6.25  $\mu$ g/mL, 12.5  $\mu$ g/mL, 25  $\mu$ g/mL, 50  $\mu$ g/mL, 100  $\mu$ g/mL, 150  $\mu$ g/mL, 200  $\mu$ g/mL, 250  $\mu$ g/mL, 300  $\mu$ g/mL, and 350  $\mu$ g/mL), and the plate was incubated for 48 hours in a CO<sub>2</sub> incubator at 37 °C. Next, 20  $\mu$ L MTT solution was added to the wells and these were incubated for 4 hours. For dissolving Formosan christa, 100  $\mu$ L dimethyl sulfoxide was added. The absorbance of MTT was measured at 560 nm. The vital percent of cells in negative control was considered 100, and it can be obtained using the following equation. A concentration from the essential oil that reduces cells' vitality to half is observed as CC<sub>50</sub>.

$$\text{Percentage of cell survival} = \left[ \frac{\text{Test compound OD} - \text{Blank OD}}{\text{Negative control OD} - \text{Blank OD}} \right] \times 100 \quad [1]$$

## 3. Results

Data analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA), analysis of variance (ANOVA), and Tukey's comparison procedure. After ANOVA showed significantly different values between treatment groups, Turkey's HSD (Honestly Significant Difference) test was performed for all pairwise comparisons which allows to rank means and put them into significant treatment groups, while controlling maximum experiment-wise error rate under null hypothesis. The effects of different concentrations of *A. kermanensis*, *L. officinalis*, and *Z. multiflora* Boiss essential oils were examined against the three bacteria (*S. aureus*, *K. pneumonia*, and *P. aeruginosa*) at 24 hours, 48 hours, and 72 hours using the disk diffusion method (Tables 1–3). The results showed that the concentration of 100  $\mu$ g/disk of each of the three essential oils was more efficient compared with lower concentrations on the bacteria ( $p < 0.0001$ ).

In the highest concentration of *A. kermanensis* essential oil (100  $\mu$ g/disk), most of the antibacterial effects was observed against *K. pneumonia*. However, at lower concentrations (from

**Table 1 – Antibacterial activity of different concentrations of *Artemisia kermanensis* essential oil against three bacteria using disk diffusion method (zone of inhibition)<sup>\*</sup>**

<i>Artemisia kermanensis</i> (μg/disk)	<i>Staphylococcus aureus</i>			<i>Klebsiella pneumonia</i>			<i>Pseudomonas aeruginosa</i>		
	24	48	72	24	48	72	24	48	72
0.08	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
0.16	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
0.31	0.50 ± 0.06 <sup>a</sup>	0.63 ± 0.07 <sup>a</sup>	0.63 ± 0.07 <sup>a</sup>	0.17 ± 0.17 <sup>a</sup>	0.27 ± 0.14 <sup>a</sup>	0.27 ± 0.14 <sup>a</sup>	0.10 ± 0.10 <sup>a,b</sup>	0.17 ± 0.17 <sup>a</sup>	0.17 ± 0.17 <sup>a</sup>
0.63	2.49 ± 0.09 <sup>b</sup>	2.67 ± 0.33 <sup>b</sup>	2.67 ± 0.03 <sup>b</sup>	1.20 ± 0.15 <sup>b</sup>	1.37 ± 0.18 <sup>b</sup>	1.50 ± 0.15 <sup>b</sup>	1.33 ± 0.18 <sup>b</sup>	1.60 ± 0.15 <sup>b</sup>	1.60 ± 0.15 <sup>b</sup>
1.25	4.20 ± 0.11 <sup>c</sup>	4.33 ± 0.17 <sup>c</sup>	4.40 ± 0.20 <sup>c</sup>	2.27 ± 0.12 <sup>c</sup>	2.50 ± 0.10 <sup>c</sup>	2.63 ± 0.18 <sup>c</sup>	3.33 ± 0.23 <sup>c</sup>	3.63 ± 0.14 <sup>c</sup>	3.63 ± 0.14 <sup>c</sup>
2.5	6.48 ± 0.02 <sup>d</sup>	6.51 ± 0.01 <sup>d</sup>	6.52 ± 0.01 <sup>d</sup>	4.00 ± 0.06 <sup>d</sup>	4.13 ± 0.13 <sup>d</sup>	4.37 ± 0.14 <sup>d</sup>	5.13 ± 0.09 <sup>d</sup>	5.37 ± 0.09 <sup>d</sup>	5.37 ± 0.09 <sup>d</sup>
5	7.90 ± 0.40 <sup>e</sup>	8.02 ± 0.38 <sup>d</sup>	8.11 ± 0.38 <sup>e</sup>	6.57 ± 0.18 <sup>e</sup>	6.73 ± 0.12 <sup>e</sup>	6.90 ± 0.10 <sup>e</sup>	7.57 ± 0.09 <sup>e</sup>	7.73 ± 0.12 <sup>e</sup>	7.73 ± 0.12 <sup>e</sup>
10	9.64 ± 0.54 <sup>f</sup>	10.02 ± 0.50 <sup>e</sup>	11.16 ± 0.13 <sup>f</sup>	8.27 ± 0.13 <sup>f</sup>	8.70 ± 0.11 <sup>f</sup>	8.70 ± 0.11 <sup>f</sup>	8.53 ± 0.23 <sup>e</sup>	9.13 ± 0.35 <sup>f</sup>	9.13 ± 0.35 <sup>f</sup>
20	12.13 ± 0.13 <sup>g</sup>	13.30 ± 0.21 <sup>f</sup>	13.40 ± 0.15 <sup>g</sup>	13.20 ± 0.11 <sup>g</sup>	13.80 ± 0.11 <sup>g</sup>	13.80 ± 0.11 <sup>g</sup>	10.80 ± 0.15 <sup>f</sup>	11.10 ± 0.15 <sup>g</sup>	11.33 ± 0.17 <sup>g</sup>
40	14.43 ± 0.12 <sup>h</sup>	14.70 ± 0.15 <sup>f</sup>	14.87 ± 0.01 <sup>g</sup>	15.17 ± 0.09 <sup>h</sup>	15.77 ± 0.27 <sup>h</sup>	15.97 ± 0.22 <sup>h</sup>	12.20 ± 0.53 <sup>g</sup>	12.40 ± 0.58 <sup>h</sup>	12.40 ± 0.58 <sup>g</sup>
60	16.70 ± 0.51 <sup>i</sup>	17.23 ± 0.68 <sup>g</sup>	17.50 ± 0.81 <sup>h</sup>	17.13 ± 0.33 <sup>i</sup>	17.57 ± 0.28 <sup>i</sup>	17.57 ± 0.28 <sup>i</sup>	13.83 ± 0.42 <sup>h</sup>	14.20 ± 0.11 <sup>i</sup>	14.20 ± 0.11 <sup>h</sup>
80	19.77 ± 0.23 <sup>j</sup>	20.10 ± 0.49 <sup>h</sup>	20.17 ± 0.44 <sup>i</sup>	19.97 ± 0.32 <sup>j</sup>	20.23 ± 0.18 <sup>j</sup>	20.40 ± 0.15 <sup>j</sup>	15.87 ± 0.47 <sup>i</sup>	16.10 ± 0.40 <sup>j</sup>	16.10 ± 0.40 <sup>i</sup>
100	22.27 ± 0.14 <sup>k</sup>	22.73 ± 0.18 <sup>i</sup>	22.73 ± 0.18 <sup>j</sup>	25.70 ± 0.32 <sup>k</sup>	26.37 ± 0.23 <sup>k</sup>	26.37 ± 0.23 <sup>k</sup>	17.43 ± 0.07 <sup>j</sup>	17.67 ± 0.03 <sup>k</sup>	17.67 ± 0.03 <sup>j</sup>

\* Different letters on every column represent a meaningful difference ( $p < 0.0001$ ).  
SE, standard error.

**Table 2 – Antibacterial activity of different concentrations of *Lavandula officinalis* essential oil against three bacteria using disk diffusion method (zone of inhibition)<sup>\*</sup>**

<i>Lavandula officinalis</i> (μg/disk)	<i>Staphylococcus aureus</i>			<i>Klebsiella pneumonia</i>			<i>Pseudomonas aeruginosa</i>		
	24	48	72	24	48	72	24	48	72
0.08	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
0.16	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
0.31	0.37 ± 0.18 <sup>a</sup>	0.40 ± 0.20 <sup>a</sup>	0.40 ± 0.20 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.30 ± 0.15 <sup>a</sup>	0.37 ± 0.18 <sup>a,b</sup>	0.50 ± 0.25 <sup>a,b</sup>
0.63	1.50 ± 0.06 <sup>b</sup>	1.70 ± 0.06 <sup>b</sup>	1.70 ± 0.06 <sup>b</sup>	0.20 ± 0.20 <sup>b</sup>	0.23 ± 0.23 <sup>b</sup>	0.23 ± 0.23 <sup>b</sup>	1.30 ± 0.21 <sup>a,b</sup>	1.40 ± 0.15 <sup>b,c</sup>	1.40 ± 0.15 <sup>b</sup>
1.25	3.42 ± 0.21 <sup>c</sup>	3.62 ± 0.21 <sup>c</sup>	3.73 ± 0.09 <sup>c</sup>	1.43 ± 0.23 <sup>c</sup>	1.60 ± 0.21 <sup>c</sup>	1.63 ± 0.23 <sup>c</sup>	2.37 ± 0.18 <sup>b,c</sup>	2.70 ± 0.06 <sup>c,d</sup>	2.77 ± 0.03 <sup>c</sup>
2.5	5.39 ± 0.05 <sup>d</sup>	5.67 ± 0.12 <sup>d</sup>	5.72 ± 0.16 <sup>d</sup>	3.40 ± 0.25 <sup>d</sup>	3.53 ± 0.24 <sup>d</sup>	3.53 ± 0.24 <sup>d</sup>	3.57 ± 0.14 <sup>c</sup>	3.70 ± 0.11 <sup>d</sup>	3.77 ± 0.14 <sup>c</sup>
5	6.63 ± 0.20 <sup>e</sup>	6.77 ± 0.18 <sup>e</sup>	6.77 ± 0.18 <sup>e</sup>	5.23 ± 0.14 <sup>e</sup>	5.47 ± 0.09 <sup>e</sup>	5.57 ± 0.09 <sup>e</sup>	5.97 ± 0.03 <sup>d</sup>	6.23 ± 0.12 <sup>e</sup>	6.47 ± 0.14 <sup>d</sup>
10	8.47 ± 0.12 <sup>f</sup>	8.70 ± 0.12 <sup>f</sup>	8.80 ± 0.15 <sup>f</sup>	7.27 ± 0.14 <sup>f</sup>	7.40 ± 0.11 <sup>f</sup>	7.50 ± 0.11 <sup>f</sup>	7.50 ± 0.11 <sup>e</sup>	7.70 ± 0.06 <sup>f</sup>	7.83 ± 0.03 <sup>e</sup>
20	11.77 ± 0.19 <sup>g</sup>	12.33 ± 0.18 <sup>g</sup>	12.63 ± 0.18 <sup>g</sup>	9.00 ± 0.25 <sup>g</sup>	9.37 ± 0.28 <sup>g</sup>	9.87 ± 0.13 <sup>g</sup>	8.37 ± 0.18 <sup>e</sup>	8.77 ± 0.18 <sup>f</sup>	8.97 ± 0.18 <sup>e</sup>
40	14.67 ± 0.20 <sup>h</sup>	15.13 ± 0.09 <sup>h</sup>	15.13 ± 0.09 <sup>h</sup>	12.53 ± 0.09 <sup>h</sup>	13.03 ± 0.09 <sup>h</sup>	13.27 ± 0.14 <sup>h</sup>	10.43 ± 0.14 <sup>f</sup>	10.67 ± 0.09 <sup>g</sup>	10.73 ± 0.14 <sup>f</sup>
60	16.13 ± 0.07 <sup>i</sup>	16.50 ± 0.06 <sup>i</sup>	16.50 ± 0.06 <sup>i</sup>	15.07 ± 0.18 <sup>i</sup>	16.00 ± 0.40 <sup>j</sup>	16.47 ± 0.34 <sup>i</sup>	12.60 ± 0.17 <sup>g</sup>	12.77 ± 0.14 <sup>h</sup>	12.80 ± 0.17 <sup>g</sup>
80	18.50 ± 0.06 <sup>j</sup>	18.63 ± 0.09 <sup>j</sup>	18.63 ± 0.09 <sup>j</sup>	18.87 ± 0.09 <sup>j</sup>	19.10 ± 0.15 <sup>j</sup>	19.43 ± 0.22 <sup>j</sup>	14.17 ± 0.12 <sup>h</sup>	14.47 ± 0.14 <sup>i</sup>	14.60 ± 0.10 <sup>h</sup>
100	20.17 ± 0.09 <sup>k</sup>	20.47 ± 0.09 <sup>k</sup>	20.47 ± 0.09 <sup>k</sup>	21.23 ± 0.56 <sup>k</sup>	21.93 ± 0.54 <sup>k</sup>	21.93 ± 0.54 <sup>k</sup>	14.80 ± 0.81 <sup>h</sup>	15.03 ± 0.84 <sup>i</sup>	15.13 ± 0.75 <sup>h</sup>

\* Different letters on every column represent meaningful difference ( $p < 0.0001$ ).  
SE, standard error.

**Table 3 – Antibacterial activity of different concentrations of *Zataria multiflora* Boiss essential oil against three bacteria using disk diffusion method (zone of inhibition)\***

<i>Zataria multiflora</i> ( $\mu\text{g}/\text{disk}$ )	<i>Staphylococcus aureus</i>			<i>Klebsiella pneumoniae</i>			<i>Pseudomonas aeruginosa</i>		
	24	48	72	24	48	72	24	48	72
0.08	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
0.16	0.60 $\pm$ 0.23 <sup>a</sup>	0.87 $\pm$ 0.23 <sup>a</sup>	0.87 $\pm$ 0.23 <sup>a</sup>	0.53 $\pm$ 0.14 <sup>a,b</sup>	0.67 $\pm$ 0.14 <sup>a</sup>	0.67 $\pm$ 0.14 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
0.31	1.93 $\pm$ 0.29 <sup>b</sup>	2.37 $\pm$ 0.24 <sup>b</sup>	2.37 $\pm$ 0.24 <sup>b</sup>	1.23 $\pm$ 0.12 <sup>b,c</sup>	1.43 $\pm$ 0.27 <sup>b</sup>	1.67 $\pm$ 0.24 <sup>b</sup>	1.17 $\pm$ 0.17 <sup>b</sup>	1.33 $\pm$ 0.17 <sup>b</sup>	1.33 $\pm$ 0.17 <sup>b</sup>
0.63	3.56 $\pm$ 0.29 <sup>c</sup>	3.73 $\pm$ 0.18 <sup>c</sup>	3.73 $\pm$ 0.18 <sup>c</sup>	2.10 $\pm$ 0.10 <sup>c</sup>	2.40 $\pm$ 0.15 <sup>c</sup>	2.77 $\pm$ 0.14 <sup>c</sup>	2.37 $\pm$ 0.12 <sup>c</sup>	2.53 $\pm$ 0.09 <sup>c</sup>	2.70 $\pm$ 0.06 <sup>c</sup>
1.25	5.62 $\pm$ 0.06 <sup>d</sup>	5.87 $\pm$ 0.09 <sup>d</sup>	5.87 $\pm$ 0.09 <sup>d</sup>	3.40 $\pm$ 0.00 <sup>d</sup>	4.00 $\pm$ 0.00 <sup>d</sup>	4.00 $\pm$ 0.00 <sup>d</sup>	4.27 $\pm$ 0.18 <sup>d</sup>	4.47 $\pm$ 0.22 <sup>d</sup>	4.47 $\pm$ 0.22 <sup>d</sup>
2.5	7.81 $\pm$ 0.13 <sup>e</sup>	7.83 $\pm$ 0.12 <sup>e</sup>	7.87 $\pm$ 0.13 <sup>e</sup>	5.44 $\pm$ 0.02 <sup>e</sup>	6.47 $\pm$ 0.03 <sup>e</sup>	6.77 $\pm$ 0.03 <sup>e</sup>	6.43 $\pm$ 0.12 <sup>e</sup>	6.58 $\pm$ 0.09 <sup>e</sup>	6.65 $\pm$ 0.05 <sup>e</sup>
5	10.10 $\pm$ 0.45 <sup>f</sup>	10.40 $\pm$ 0.45 <sup>f</sup>	8.11 $\pm$ 0.38 <sup>f</sup>	7.90 $\pm$ 0.40 <sup>f</sup>	8.27 $\pm$ 0.37 <sup>f</sup>	8.44 $\pm$ 0.34 <sup>f</sup>	8.56 $\pm$ 0.23 <sup>f</sup>	8.73 $\pm$ 0.12 <sup>f</sup>	8.73 $\pm$ 0.12 <sup>f</sup>
10	11.54 $\pm$ 0.12 <sup>g</sup>	11.64 $\pm$ 0.07 <sup>g</sup>	11.80 $\pm$ 0.06 <sup>g</sup>	10.60 $\pm$ 0.24 <sup>g</sup>	11.29 $\pm$ 0.05 <sup>g</sup>	11.35 $\pm$ 0.05 <sup>g</sup>	10.35 $\pm$ 0.26 <sup>g</sup>	10.51 $\pm$ 0.13 <sup>g</sup>	10.63 $\pm$ 0.12 <sup>g</sup>
20	13.57 $\pm$ 0.12 <sup>h</sup>	14.50 $\pm$ 0.26 <sup>h</sup>	14.73 $\pm$ 0.13 <sup>h</sup>	14.30 $\pm$ 0.26 <sup>h</sup>	14.50 $\pm$ 0.29 <sup>h</sup>	14.70 $\pm$ 0.26 <sup>h</sup>	11.97 $\pm$ 0.20 <sup>h</sup>	12.30 $\pm$ 0.15 <sup>h</sup>	12.30 $\pm$ 0.15 <sup>h</sup>
40	16.43 $\pm$ 0.09 <sup>i</sup>	16.93 $\pm$ 0.12 <sup>i</sup>	17.30 $\pm$ 0.15 <sup>i</sup>	16.70 $\pm$ 0.11 <sup>i</sup>	16.93 $\pm$ 0.07 <sup>i</sup>	17.00 $\pm$ 0.11 <sup>i</sup>	13.27 $\pm$ 0.13 <sup>i</sup>	13.43 $\pm$ 0.18 <sup>i</sup>	13.53 $\pm$ 0.22 <sup>i</sup>
60	20.37 $\pm$ 0.20 <sup>j</sup>	20.93 $\pm$ 0.07 <sup>j</sup>	21.27 $\pm$ 0.14 <sup>j</sup>	20.80 $\pm$ 0.30 <sup>j</sup>	21.67 $\pm$ 0.09 <sup>j</sup>	21.77 $\pm$ 0.09 <sup>j</sup>	15.23 $\pm$ 0.14 <sup>j</sup>	15.63 $\pm$ 0.14 <sup>j</sup>	15.63 $\pm$ 0.14 <sup>j</sup>
80	24.67 $\pm$ 0.03 <sup>k</sup>	24.97 $\pm$ 0.03 <sup>k</sup>	25.20 $\pm$ 0.15 <sup>k</sup>	24.43 $\pm$ 0.12 <sup>j</sup>	25.13 $\pm$ 0.13 <sup>k</sup>	25.33 $\pm$ 0.20 <sup>k</sup>	17.20 $\pm$ 0.11 <sup>k</sup>	17.60 $\pm$ 0.30 <sup>k</sup>	17.83 $\pm$ 0.23 <sup>k</sup>
100	27.80 $\pm$ 0.20 <sup>l</sup>	28.67 $\pm$ 0.33 <sup>l</sup>	28.67 $\pm$ 0.33 <sup>l</sup>	27.83 $\pm$ 0.12 <sup>k</sup>	28.10 $\pm$ 0.21 <sup>l</sup>	28.10 $\pm$ 0.21 <sup>l</sup>	19.90 $\pm$ 0.27 <sup>l</sup>	20.40 $\pm$ 0.23 <sup>l</sup>	20.53 $\pm$ 0.18 <sup>l</sup>

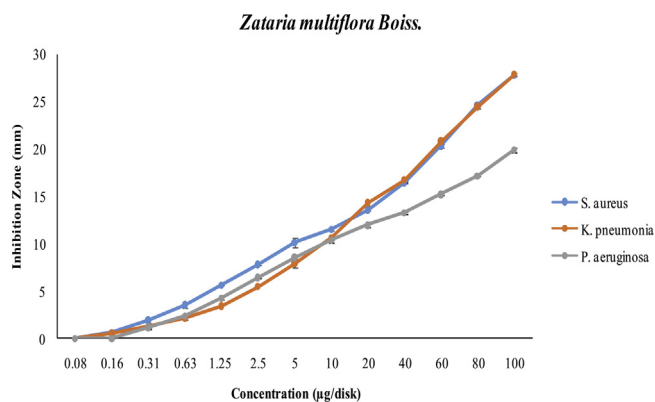
\* Different letters on every column represent meaningful difference ( $p < 0.0001$ ).  
SE, standard error.

**Table 4 – Comparison of different common essential oils concentrations and antibiotics effect on *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa***

Treatment ( $\mu\text{g}/\text{disk}$ )	<i>Staphylococcus aureus</i>			<i>Klebsiella pneumoniae</i>			<i>Pseudomonas aeruginosa</i>		
	24	48	72	24	48	72	24	48	72
<i>Artemisia kermanensis</i> (100)	22.27 $\pm$ 0.14 <sup>c</sup>	22.73 $\pm$ 0.18 <sup>d</sup>	22.73 $\pm$ 0.18 <sup>d</sup>	25.70 $\pm$ 0.32 <sup>d</sup>	26.37 $\pm$ 0.23 <sup>d</sup>	26.37 $\pm$ 0.23 <sup>d</sup>	17.43 $\pm$ 0.07 <sup>c</sup>	17.67 $\pm$ 0.03 <sup>c</sup>	17.67 $\pm$ 0.03 <sup>c</sup>
<i>Lavandula officinalis</i> (100)	20.17 $\pm$ 0.09 <sup>b</sup>	20.47 $\pm$ 0.09 <sup>c</sup>	20.47 $\pm$ 0.09 <sup>c</sup>	21.23 $\pm$ 0.56 <sup>c</sup>	21.93 $\pm$ 0.54 <sup>c</sup>	21.93 $\pm$ 0.54 <sup>c</sup>	14.80 $\pm$ 0.81 <sup>b</sup>	15.03 $\pm$ 0.84 <sup>b</sup>	15.13 $\pm$ 0.75 <sup>b</sup>
<i>Zataria multiflora</i> (100)	27.80 $\pm$ 0.20 <sup>d</sup>	28.67 $\pm$ 0.33 <sup>e</sup>	28.67 $\pm$ 0.33 <sup>e</sup>	27.83 $\pm$ 0.12 <sup>d</sup>	28.10 $\pm$ 0.21 <sup>d</sup>	28.10 $\pm$ 0.21 <sup>d</sup>	19.90 $\pm$ 0.27 <sup>d</sup>	20.40 $\pm$ 0.23 <sup>d</sup>	20.53 $\pm$ 0.18 <sup>d</sup>
Ampicillin (10)	16.44 $\pm$ 0.29 <sup>a</sup>	16.80 $\pm$ 0.25 <sup>a,b</sup>	16.91 $\pm$ 0.31 <sup>a,b</sup>	6.40 $\pm$ 0.61 <sup>a</sup>	6.63 $\pm$ 0.59 <sup>a</sup>	6.63 $\pm$ 0.59 <sup>a</sup>	5.93 $\pm$ 0.35 <sup>a</sup>	6.17 $\pm$ 0.43 <sup>a</sup>	6.17 $\pm$ 0.43 <sup>a</sup>
Penicillin (10)	14.93 $\pm$ 0.52 <sup>a</sup>	14.93 $\pm$ 0.52 <sup>a</sup>	14.93 $\pm$ 0.52 <sup>a</sup>	5.87 $\pm$ 0.41 <sup>a</sup>	6.00 $\pm$ 0.29 <sup>a</sup>	6.00 $\pm$ 0.29 <sup>a</sup>	6.33 $\pm$ 0.67 <sup>a</sup>	6.50 $\pm$ 0.78 <sup>a</sup>	6.50 $\pm$ 0.78 <sup>a</sup>
Tetracycline (30)	19.47 $\pm$ 0.73 <sup>b,c</sup>	19.63 $\pm$ 0.63 <sup>c</sup>	19.63 $\pm$ 0.63 <sup>c</sup>	13.30 $\pm$ 0.70 <sup>b</sup>	13.40 $\pm$ 0.78 <sup>b</sup>	13.40 $\pm$ 0.78 <sup>b</sup>	6.00 $\pm$ 0.52 <sup>a</sup>	6.40 $\pm$ 0.45 <sup>a</sup>	6.40 $\pm$ 0.45 <sup>a</sup>

\* Different letters on every column represent meaningful difference ( $p < 0.0001$ ).  
SE, standard error.



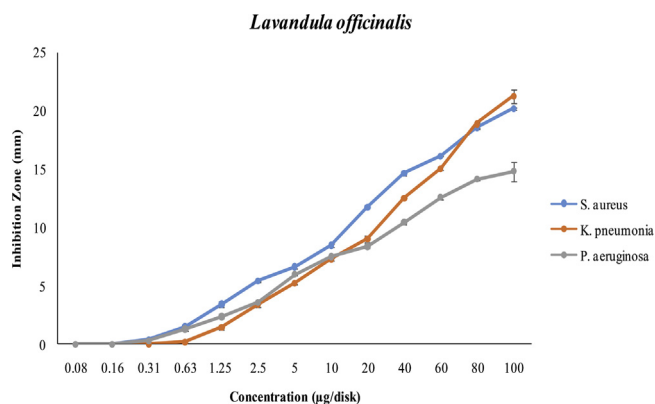


**Fig. 2 – Effect of *Zataria multiflora* Boiss essential oil on three species of bacteria.**

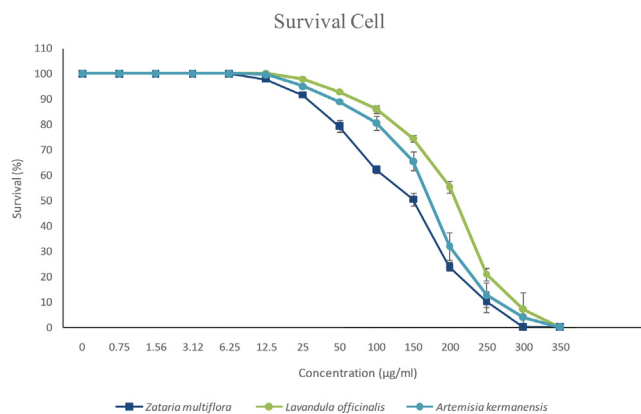
80 µg/disk to 20 µg/disk), a similar effect was observed on both *S. aureus* and *K. pneumoniae*. At concentrations < 20 µg/disk, a minimal antibacterial effect was observed against *K. pneumoniae*. In a broad range of *A. kermanensis* essential oil concentrations used, *P. aeruginosa* (with the smallest inhibition zone compared with other bacteria) showed more resistance toward this oil (Fig. 1).

In concentrations < 20 µg/disk, *Z. multiflora* Boiss essential oil is more effective against *S. aureus* than against the two other bacteria (in these concentrations, the antibacterial effects against *K. pneumoniae* and *P. aeruginosa* were rather similar). But with increasing concentrations (20–100 µg/disk), it had the same effect on both *S. aureus* and *K. pneumoniae*. In a broad range of *Z. multiflora* Boiss essential oil concentrations used, *P. aeruginosa*, which has the smallest inhibition zone compared to other bacteria, showed more resistance toward this oil (Fig. 2).

At high concentrations (80 µg/disk and 100 µg/disk), the essential oil of *L. officinalis* performed more effectively against *K. pneumoniae* compared with the two other bacteria. However, at concentrations < 80 µg/disk, it was found to be more effective against *S. aureus*. In a board range of *L. officinalis* essential oil concentrations used, *P. aeruginosa*, which has the smallest inhibition zone compared to the other bacteria, proved to be more resistant toward this oil (Fig. 3).



**Fig. 3 – Effect of essential oil of *Lavandula officinalis* on three species of bacteria.**



**Fig. 4 – Cytotoxic activity of different concentration of herbal medicine essential oils. Each bar represents the mean±S.D of three independent experiments.**

A comparison between the three plant essential oils (at a concentration of 100 µg/disk) and positive control antibiotics (ampicillin, penicillin, and tetracycline) demonstrated that *Z. multiflora* Boiss essential oil (at all time intervals) had a stronger antibacterial effect (bigger inhibition zone) against the three bacteria (Table 4).

The MIC and MBC results demonstrated that *Z. multiflora* essential oil, with lower MIC and MBC values than *L. officinalis* and *A. kermanensis* essential oils, showed a higher antibacterial activity against the three bacteria (Tables 5–7). Compared with *L. officinalis* essential oil, *A. kermanensis* essential oil had a higher antibacterial activity (lower MIC and MBC values) against the three bacteria (Tables 5–7).

### 3.1. Cytotoxic effects on viability of L929 cells

Results from the cytotoxicity test showed that *Z. multiflora* and *A. kermanensis* essential oils have no cellular toxic effect up to 6.25 µg/mL, and *L. officinalis* oil showed no cellular toxic effect up to 12.5 µg/mL. With the increase in essential oil concentration, cellular resistance is considerably decreased. The CC<sub>50</sub> for *Z. multiflora*, *L. officinalis*, and *A. kermanensis* is 123 µg/mL, 218 µg/mL, and 154 µg/mL, respectively (Fig. 4).

## 4. Discussion

Nowadays, the resistance of bacteria is increasing against antibiotics. Consequently, research on exploration of new materials having antimicrobial properties is growing. As essences and herbal extracts have been historically used in treatment of diseases,<sup>28,29</sup> they can be good candidates in such studies. Herbal extracts possessing antimicrobial effects on a broad range of organisms, nutrient applicability, and fewer side effects (compared with common antibiotics), can be a replacement for antibiotics.<sup>30–32</sup> Various studies have documented the antimicrobial activity of essential oils and plant extracts, such as *A. kermanensis*, *L. officinalis*, and *Z. multiflora* Boiss.<sup>33–35</sup> In this study, we evaluated the antibacterial activity of *A. kermanensis*, *L. officinalis*, *Z. multiflora* Boiss essential oils against *P. aeruginosa*, *S. aureus*, and *K. pneumoniae*. The results

**Table 5 – MIC and MBC of essential oils on *Pseudomonas aeruginosa***

No.	Extract	MIC ( $\mu\text{g/mL}$ )		MBC ( $\mu\text{g/mL}$ )
		MIC 50	MIC 90	
1	<i>Artemisia kermanensis</i>	37	62	71
2	<i>Zataria multiflora</i>	31	58	63
3	<i>Lavandula officinalis</i>	41	75	92
4	Ampicillin	12	21	25
5	Penicillin	12	20	25
6	Tetracycline	6	11	15

MBC, minimal inhibitory concentration; MIC, minimal bactericidal concentration; SE, standard error.

**Table 6 – MIC and MBC of the essential oils on *Klebsiella pneumonia***

No.	Extract	MIC ( $\mu\text{g/mL}$ )		MBC ( $\mu\text{g/mL}$ )
		MIC 50	MIC 90	
1	<i>Artemisia kermanensis</i>	30	54	68
2	<i>Zataria multiflora</i>	25	47	51
3	<i>Lavandula officinalis</i>	39	63	76
4	Ampicillin	10	17	24
5	Penicillin	13	19	26
6	Tetracycline	11	17	22

MBC, minimal inhibitory concentration; MIC, minimal bactericidal concentration.

**Table 7 – MIC and MBC of the essential oils on *Staphylococcus aureus***

No.	Extract	MIC ( $\mu\text{g/mL}$ )		MBC ( $\mu\text{g/mL}$ )
		MIC 50	MIC 90	
1	<i>Artemisia kermanensis</i>	28	48	57
2	<i>Zataria multiflora</i>	18	35	43
3	<i>Lavandula officinalis</i>	32	52	69
4	Ampicillin	4	6	9
5	Penicillin	6	9	10
6	Tetracycline	5	7	10

MBC, minimal inhibitory concentration; MIC, minimal bactericidal concentration.

showed that the extract of *Z. multiflora* has the highest effect on all species of bacteria used. The highest effect was observed against *S. aureus*, with an MBC of 43  $\mu\text{g/mL}$  (Table 7). Motevasel and coworkers<sup>36</sup> reported that the high concentration of *Zataria* extract showed the best antimicrobial activities and killed numerous types of bacteria with no difference between pathogens and nonpathogens. In a research by Sharififar and coworkers,<sup>37</sup> the effect of this extract was tested on *K. pneumoniae* and *S. aureus*, in which MIC was measured as 30  $\mu\text{g/mL}$  and 21  $\mu\text{g/mL}$ , respectively. In our study, the MIC<sub>90</sub> of *Z. multiflora* against *K. pneumoniae* and *S. aureus* was measured as 25  $\mu\text{g/mL}$  and 18  $\mu\text{g/mL}$ , respectively. Our results are compatible with those obtained by Sharififar et al.<sup>37</sup> Owlia and coworkers<sup>38</sup> tested the effect of the extract on *P. aeruginosa*, and their results showed an MIC of 64  $\mu\text{g/mL}$  and an MBC of 128  $\mu\text{g/mL}$ . In our study, the MIC and MBC of *Z. multiflora* essential oil on *P. aeruginosa* were measured as 31  $\mu\text{g/mL}$  and 63  $\mu\text{g/mL}$ , respectively. This indicates that the essential oil used in our study has a better effect. Moreover, in another study by El-Shouny and coworkers,<sup>39</sup> the researchers determined the effect of *Thymus vulgaris* essential oil on *P. aeruginosa*, which is resistant to common antibiotics. The MIC was measured as 0.32 mg/mL (320  $\mu\text{g/mL}$ ). Another experiment reported the

effect of *T. vulgaris* essential oil on *S. aureus* ATCC 25923, and yielded an MIC of 1.33 mg/mL.<sup>38,40</sup> Another experiment in 2008 reported the effect of *Z. multiflora* on *K. pneumoniae*. The MIC was reported to be between 312  $\mu\text{g/mL}$  and 624  $\mu\text{g/mL}$ .<sup>41</sup> Results of the study indicated that the essential oils possess a better effect on pathogens. The analysis of *Z. multiflora* Boiss essential oil with gas chromatography–mass spectrometry (GC-MS) showed 34 constituents representing 96.94% of the total oil. The major components consisted of thymol (33.05%), carvacrol (25.88%), and *p*-cymene (11.34%) (Table 8). Govaris and coworkers<sup>42</sup> reported that carvacrol (80.15%) and thymol (4.82%) were the major components of *Z. multiflora*.<sup>37</sup> Different studies indicated that essential oils containing thymol, carvacrol, or eugenol possess the highest antimicrobial properties.<sup>43</sup> The antimicrobial effect of essential oil components such as thymol, menthol, and linalyl acetate may be caused by a perturbation of the lipid fractions of bacterial plasma membranes, which might be influenced by the membrane permeability and leakage of intracellular materials. *Z. multiflora* essential oil, with a high percentage of thymol and carvacrol, has a considerable antimicrobial activity.<sup>44</sup> According to our results and previous research, thymol is the major compound of *Z. multiflora* oil. Sharif Roohani and coworkers

**Table 8 – Chemical composition of the essential oil of *Artemisia kermanensis***

No	Compositions	%	RI
1	Artemisiatriene	0.41	926
2	$\alpha$ -Pinene	0.54	934
3	Camphene	0.93	949
4	Verbenene	1.88	954
5	Benzaldehyde	0.11	960
6	$\beta$ -Pinene	0.08	977
7	<i>p</i> -Menthatriene	0.57	993
8	Yomogi alcohol	2.67	1001
9	$\alpha$ -Terpinene	0.2	1016
10	<i>p</i> -Cymene	1.88	1024
11	1,8-Cineole	1.82	1030
12	Artemisia ketone	0.11	1032
13	<i>trans</i> -Carane	0.13	1050
14	$\gamma$ -Terpinene		1056
15	Artemisia alcohol	1.48	1082
16	Styrene	0.82	1087
17	$\alpha$ -Thujone	13.83	1108
18	$\beta$ -Thujone	6.23	1117
19	<i>trans</i> -Pinocarveol	1.39	1138
20	Camphen	4.13	1142
21	Camphore	10.23	1144
22	<i>p</i> -Menth-1,5-dien-8-ol	2.04	1147
23	1-Menthene	0.49	1156
24	Pinocarvone	1.37	1160
25	Borneol	1.97	1164
26	<i>p</i> -Mentha-1,5-dien-8-ol	4.38	1166
27	Terpinene-4-ol	1.01	1175
28	Naphthalene	0.73	1178
29	<i>p</i> -Cymen-3-ol	1.26	1182
30	$\alpha$ -Terpineol	0.72	1188
31	Verbenone	1.53	1206
32	Norbornane	0.36	1215
33	Cuminic aldehyde	1.1	1235
34	(+)-Carvone	0.48	1239
35	Carvotanacetone	0.28	1243
36	<i>cis</i> -Myrtanol	0.15	1247
37	Carvenone	0.12	1253
38	Chrysanthenyl acetate	1	1256
39	Cinnamic aldehyde-E	0.16	1264
40	Bornyl acetate	2.3	1280
41	Thymol	1.29	1286
42	Carvacrol	1.78	1297
43	$\alpha$ -Copaene	0.23	1368
44	Methyl cinnamate	0.15	1375.7
45	( <i>Z</i> )-Jasmone	0.22	1393.1
46	Methyleugenol	0.15	1399.3
47	<i>trans</i> -Caryophyllene	0.3	1395.6
48	$\alpha$ -Curcumen	0.15	1475.4
49	Spathulenol	0.25	1569
50	Caryophyllene	0.07	1644.5
Total	75.84		

(2007) (39.65%), Mahboubi and GhazianBidgoli<sup>45</sup> (38.7%), and Saei-Dehkordi and coworkers<sup>46</sup> (47.46%) reported thymol as the major component of *Z. multiflora* oil. The European Chemicals Agency reported that thymol has not shown chronic side effects or teratogenic effects in previous studies, and it can be considered a safe compound.<sup>35,45</sup>

Orhan and coworkers<sup>47</sup> observed the effect of *L. officinalis* essential oil against *P. aeruginosa*, *K. pneumonia*, and *S. aureus*, and reported an MIC of 32  $\mu$ g/mL, 128  $\mu$ g/mL, and 128  $\mu$ g/mL, respectively. In our study, the MIC of *L. officinalis* essential oil

on the bacteria was 41  $\mu$ g/mL, 63  $\mu$ g/mL, and 52  $\mu$ g/mL, respectively. The comparison between our study and that of Orhan et al<sup>47</sup> indicated that in our study *L. officinalis* essential oil was more effective in terms of its inhibitory effect on *K. pneumonia* and *S. aureus*. Changes in the antimicrobial properties of the essential oil in different concentrations can be attributable to the different amounts of flavonoid compositions or different active forms of flavonoids.<sup>48</sup> According to GC-MS results, 69 constituents were identified in *L. officinalis* essential oil, representing 83.99% of the total oil and major components, and these included 1,8-cineol (12.01%), camphore (9.16%), verbenone (8.47%), alpha-pinene (7.58%), thymol (6.23%) (Table 9). Soković and coworkers<sup>50</sup> reported that linalyl acetate (27.54%) and linalool (27.21%) are the most abundant components in *L. angustifolia* (*L. officinalis*) oil. Meanwhile, Hamad and coworkers<sup>13</sup> reported that the major components of *L. langustifolia* oil are linalool (24.63%) and camphor (13.58%). 1,8-Cineole and camphor, which are used as useful substances in producing numerous drugs, have antiseptic properties.<sup>49,50</sup>

Derakhshan and coworkers<sup>51</sup> investigated the effect of *Artemisia turcomanica*, *Artemisia khorassanica*, *Artemisia kopetdaghensis*, and *Artemisia ciniformis* extracts against *S. aureus* ATCC 25923, and their results showed MIC to be 3 mg/mL, 2 mg/mL, 2 mg/mL, and 1.5 mg/mL, respectively. Another study reported on the effect of *Artemisia absinthium* extract on *S. aureus*, and reported an MIC of 52  $\mu$ g/mL.<sup>52</sup> We also investigated the effect of *A. kermanensis* extract on *S. aureus*. Our results yielded an MIC of 48  $\mu$ g/mL (Table 7), which is compatible with that obtained by Blagojevic et al<sup>52</sup> in 2006. Konatchiev and coworkers<sup>53</sup> examined the effect of *Artemisia distans* extract on *S. aureus*. They reported an MIC of 20  $\mu$ g/mL. A comparison between our results with those of Konatchiev et al<sup>53</sup> shows that the *A. distans* extract possesses a better inhibitory effect on *S. aureus* compared with the *A. kermanensis* extract. Generally, in reference to different studies, it is revealed that the essential oil and extract of different species of *Artemisia* have an inhibitory effect against *P. aeruginosa*, *K. kermanensis*, and *S. aureus*.<sup>52</sup> Generally, Gram-positive bacteria are more sensitive to herbal extracts when compared with Gram-negative ones. This can be attributed to their different cell wall structures. Gram-positive bacteria have mucopeptide compositions, whereas Gram-negative bacteria just have a thin layer of mucopeptide and most of their cell wall is made of lipoprotein and lipopolysaccharide. Hence, they are more resistant against antibacterial materials.<sup>54,55</sup> In *A. kermanensis* oil, 50 constituents were identified representing 75.84% of the total oil. The major components were alpha-thujone (13.83%), camphor (10.23%), and *p*-mentha-1,5-dien-8-ol (4.38%) (Table 10). Kazemi and coworkers<sup>56</sup> determined the constituents of *A. kermanensis* oil using GC-flame ionization detection and GC-MS methods. They reported that the major components of this oil were isoborneol (21.5%) and camphor (9.8%). Sardashti and Pourramazani Harati<sup>49</sup> also analyzed *A. kermanensis* oil with GC-MS, and reported the following results: in 1,8-cineole (56.55%), borneol (5.28%), and camphene (4.48%). Oxygenated monoterpenes (examples of this substance include linalool,  $\alpha$ -terpineol, 1,8-cineole, borneol, camphor, and alpha, beta-thujone) are prevalent components of essential oils.<sup>49</sup> Oxygenated monoterpenes have shown variable antibacterial activities.<sup>34</sup> Based on



**Table 9 – Chemical composition of the essential oil of *Lavandula officinalis***

No	Compositions	%	RI
1	$\alpha$ -Pinene	7.58	938
2	Camphene	4.51	952
3	Verbenene	0.64	956
4	1,3,5-Cycloheptatriene	0.03	972
5	$\beta$ -Pinene	0.49	979
6	3-Octanone	2.19	988
7	$\beta$ -Myrcene	1.18	993
8	3-Octanol	0.36	997
9	$\alpha$ -Phellandrene	0.05	1007
10	o-Isopropenyltoluene	0.08	1014
11	$\alpha$ -Terpinene	0.12	1017
12	p-Cymene	2.96	1025
13	1,8-Cineol	12.01	1033
14	gamma-Terpinene	0.08	1057
15	Linalool oxide	0.06	1072
16	Methyl benzoate	0.99	1088
17	Linalool	2.45	1100
18	Thujancis	0.81	1103
19	D-Fenchyl alcohol	0.28	1112
20	Pinocarveol	0.12	1138
21	Camphore	9.16	1144
22	Isopinocampnone	1.72	1158
23	Pinocarvone	0.13	1160
24	Pinocampnone	0.39	1171
25	Terpene-4-ol	1.27	1174
26	naphthalene	0.08	1177
27	p-Cymen-8-ol	0.23	1183
28	$\alpha$ -Terpineol	2.31	1188
29	Myrtenol	0.35	1194
30	No pol (terpene)	1.11	1203
31	Verbenone	8.47	1209
32	trans-Carveol	0.13	1215
33	$\beta$ -Citronellol	0.1	1225
34	Pulegone	0.09	1235
35	Piperitone	0.04	1250
36	Cinnamaldehyde	0.05	1265
37	Borneol acetate	2.41	1281
38	Thymol	6.23	1288
39	2-Hydroxy-4-isopropyl-1-methylbenzene	0.17	1290
40	Carvacrol	4.14	1297
41	$\alpha$ -Terpinene	0.15	1329
42	Piperitenone	0.37	1335
43	$\alpha$ -Cubebene	0.06	1343
44	Thymyl acetate	0.06	1349
45	$\alpha$ -Copaene	0.43	1369
46	trans-Caryophyllene	0.47	1412
47	$\alpha$ -Humulene	0.22	1446
48	Farnesene	0.08	1451
49	$\beta$ -Acoradiene	0.09	1460
50	gamma-Cadinene	0.16	1473
51	Zingiberene	0.1	1488
52	$\beta$ -Himachalene	0.28	1492
53	delta-Cadinene	0.27	1516
54	$\alpha$ -Cedrene	0.15	1524
55	Germacrene B	0.06	1548
56	spathulenol	0.26	1567
57	Caryophyllene oxide	0.29	1572
58	$\alpha$ -Farnesene	0.06	1587
59	Butylidene-phthalide	0.15	1642
60	3N Butylphthalide	4.62	1687
61	Butylidene dihydro-phthalide	0.09	1720
	Total	83.99	

**Table 10 – Chemical composition of the essential oil of *Zataria multiflora* Boiss**

No	Compositions	%	RI
1	$\alpha$ -Thujene	0.34	931
2	$\alpha$ -Pinene	3.88	937
3	Camphene	0.18	951
4	Verbenene	0.02	956
5	Sabinene	0.02	974
6	$\beta$ -Pinene	0.68	979
7	$\beta$ -Myrcene	0.68	993
8	$\alpha$ -Phellandrene	0.11	1007
9	$\Delta$ -3-Carene	0.04	1012
10	$\alpha$ -Terpinene	1.32	1016
11	p-Cymene	11.34	1025
12	Limonene	0.67	1032
13	1,8-Cineole	0.55	1030
14	gamma-Terpinene	4.73	1057
15	trans-Sabinene hydrate	0.27	1087
16	Linalool	1.46	1098
17	Borneol	0.37	1162
18	Terpinen-4-ol	0.82	1186
19	$\alpha$ -Terpineol	0.67	1191
20	Carvacrol methyl ether	0.77	1239
21	Carvol	0.77	1239
22	trans-Anethole	2.46	1281
23	Thymol	33.05	1285
24	Carvacrol	25.88	1297
25	Thymyl acetate	1.03	1311
26	Carvacryl acetate	0.69	1371
27	$\beta$ -Caryophyllene	1.83	1412
28	Aromadendrene	0.84	1437
29	$\alpha$ -Humulene	0.09	1443
30	Germacrene-D	0.13	1473
31	Ledene	0.77	1491
32	cis- $\alpha$ -Bisabolene	0.09	1537
33	(+)-Spathulenol	0.24	1579
34	Caryophyllene oxide	0.15	1589
	Total	96.94	

season, geographical location, and the location of plants, the overall quality and quantity of the essential oil of species vary. Climate and soil conditions can also affect the composition of the oil, and differences in the major constituents of the different compounds of the essential oils can likely be attributable to differences in habitat conditions.<sup>57,58</sup>

## 5. Conclusion

Essential oils possess a range of volatile molecules such as terpenes and terpenoids, and phenol-derived aromatic and aliphatic compounds, which might have bactericidal, virucidal, and fungicidal consequences. Essential oils directly affect the cell membrane of the pathogenic microorganism by causing an increase in permeability and leakage of vital intracellular elements, and finally disorder the cell respiration and microbial enzyme system.<sup>59,60</sup> In this study, three essential oils showed antibacterial effects against tested bacterial strains. *Z. multiflora* Boiss essential oil showed stronger antibacterial effects than the other essential oils. *Z. multiflora* oil has also been found to be active against clinical isolates of a broad spectrum of beta-lactamase-producing *K. pneumoniae*.<sup>61</sup> It has been shown that *Z. multiflora* extract can inhibit the release of the deoxyribonuclease (DNase) enzyme and the

making of enterotoxin in *S. aureus*.<sup>62</sup> Due to the increasing problem of antibiotic resistance in bacteria, using these essential oils as natural and new antimicrobial substances can be useful.

## Conflicts of interest

The authors declare no conflict of interest.

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