

Original Research

Antibacterial activities of essential oils from Iranian medicinal plants on extended-spectrum β -lactamase-producing *Escherichia coli*

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Abstract: The extended-spectrum beta-lactamase (ESBL) -producing *Escherichia coli* strains can lead to various infections particularly urinary tract infections. The main objective of this investigation was to evaluate the antibacterial activities of essential oils (EOs) from different Iranian medicinal plants against TEM gene positive ESBL-producing *E. coli* strains isolated from urine samples of patients with urinary tract infections. EOs were extracted using hydrodistillation method. *E. coli* strains were isolated by different specific Medias. ESBL-producing *E. coli* strains were isolated from urine samples of patients with urinary tract infections in Shiraz hospital, Iran. Then, ESBL-producing strains were identified using double disk synergy test, phenotypic disc confirmatory test and polymerase chain reaction (PCR) for TEM gene detection. The antibacterial activity of the EOs from different plants (*Achillea wilhelmsii* C. Koch, *Echinophora platyloba* DC., *Lallemantia royleana*, *Nepeta persica* Boiss., *Pulicaria vulgaris* Gaertn., *Salvia nemorosa*, and *Satureja intermedia* C.A.Mey) and antibiotics against ESBL-producing strains was studied using the microdilution method for the evaluation of the minimum inhibitory concentration (MIC). The 103 out of 295 *E. coli* strains with 97 (90.65%) TEM gene distributions were identified as ESBL-producing strains. All of the EOs derived from different plants displayed high inhibitory effects against ESBL-producing *E. coli* strains. The results of our investigations may propose a good treatment option against resistant infectious bacteria.

Key words: Essential oils, *Escherichia coli*, antibacterial activities.

Introduction

Escherichia coli is one of the main cause of acquired infections in human community. For about 30 years, β -lactam antibacterial agents were efficiently used for the treatment of infections caused by Gram-negative bacteria especially *E. coli* isolates (1). Although β -lactam antibacterial agents and their extended spectrum types are the suitable choices for the treatment of such infections, *E. coli* isolates produce or gain β -lactamase enzymes and develop resistance to such antibacterial agents (2). Currently, extended-spectrum β -lactamases (ESBLs) are the prevalent kinds of β -lactamases in *E. coli* isolates which can transmit among bacteria and have the ability to inactivate various types of β -lactam agents (3). Nowadays, the risk of ESBL-producing *E. coli* strains among humans, domesticated animals and wildlife represents a great concern in medicine. Therefore their fast discovery and treatments are of outmost importance in these fields (1, 4).

Natural products are among suitable candidates for developing new remedies against resistance bacterial isolates (5). At this time, many researchers focus in their studies on these products and their secondary me-

tabolites which can originate directly or indirectly from plants. It is assumed that the antibacterial effectiveness of plants is due to their secondary metabolites such as essential oils, flavonoids, tannins, saponins and phenols (6). Since many years ago, medicinal herbs have been used because of their health benefits. Due to their complex composition, they are widely used for treatments of many human and animal disorders (7, 8). Essential oils (EOs) are one of the secondary metabolites of plants which are of great interest because of their safety and economic aspects. These derivatives have a vast application in cosmetic, flavor and pharmaceutical fields (9). The antibacterial activities of these volatile compounds have been shown in different studies (10-12). Their biodegradability and producing non-toxic components allowed them to be used safely as antibacterial agents (12).

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Recently, there is a growing trend in reports of the *in vitro* testing of EOs against bacteria, viruses, protozoa and fungi in medical and biological literature (13). It is believed that the antibacterial activity of EOs is largely caused by several components such as monoterpenes, terpenaceous and sesquiterpenes groups (13). Therefore, the objective of our study was to assess the *in vitro* antibacterial activities of EOs derived from aerial parts (leaves, stems and flowers) of some Iranian plants (*Achillea wilhelmsii* C. Koch, *Echinophora platyloba* DC., *Lallemantia royleana*, *Nepeta persica* Boiss., *Pulicaria vulgaris* Gaertn., *Salvia nemorosa*, and *Satureja intermedia* C.A.Mey) against *E. coli* isolates which were collected from urinary tract of infectious patients attended in Hospitals of Shiraz, Iran.

Materials and Methods

Plant materials

Aerial parts (leaves, stems and flowers) of *Achillea wilhelmsii* C. Koch, *Echinophora platyloba* DC., *Lallemantia royleana*, *Nepeta persica* Boiss., *Pulicaria vulgaris* Gaertn., *Salvia nemorosa*, and *Satureja intermedia* C.A.Mey were collected from different areas in Iran at flowering stage (Table 1). The taxonomic identification of plant materials was confirmed by a botanist at the herbarium affiliated to Shahid Beheshti University of Iran. The collected plant materials were dried in a dark place.

Essential oils extraction

For EOs extraction, 100 g of each dried plants were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus according to the method outlined by the British Pharmacopeia (14). The obtained essential oils for each plant were dried over anhydrous sodium sulfate (Sigma-Aldrich, St. Louis, MO, USA) and kept at 4 °C until further tests.

Escherichia coli isolates

The clinical specimens *E. coli* isolates were collected from urinary tract of infected patients who attended Hospitals in Shiraz, Iran from January to November 2014. Microscopic Gram stain was used for the examination of samples. Then, Gram-negative bacteria were cultured on Nutrient Agar, MacConkey's, Clede Agar, and blood Agar (Merck, Germany) and were incubated for 24 h at 37 °C. Lactose fermenting colonies on Cled and MacConkey agar medias were isolated based on their colors and morphologies (yellow color on Cled agar medium and circular, pink to red colony color on MacConkey agar medium). Biochemical reactions such as potassium hydroxide test, Voges Proskaur reaction, urease and citrate, Indole and methyl red test, H₂S and oxidase test were used for the identification of isolates. A total of 295 strains of *E. coli* were isolated according to the following steps.

Detection extended-spectrum β -lactamase

Double disk synergy test

Ceftazidime (30 μ g) and Ceftriaxone (30 μ g) disks (Patan Teb-Iran), were laid on the surface of the inoculated plates located, 20 mm aside from Augmentin disc. After 24 h incubation at 37 °C, the ESBL producer was

considered in the zone between Cephalosporin disc and Clavulanic acid disc.

Phenotypic disc confirmatory test

Disks were laid on to the surface of Muller Hinton agar. ESBL producing organisms were confirmed according to inhibition diameter of 5 mm for Ceftriaxone (CE) versus Clavulanic acid (CEC) (20-10 μ g) and Ceftazidime disks (CA) (30 μ g) versus Ceftazidime-Clavulanic acid (CAC) (20-10 μ g) or Ceftriaxone (CE) (30 μ g). (*E. coli* ATCC 25922 was used as control and the test was done according to CLSI (Clinical and Laboratory Standards Institute) (15).

DNA extraction and polymerase chain reaction

The whole DNA of isolated ESBL-producing colonies was extracted using boiling method after suspending the isolates in Tris/EDTA. Polymerase chain reaction (PCR) was done for detection and amplification of 857 bp TEM gene by using of (5'- GAGTATTCAA-CATTTCCGTGTC-3') as the forward primer and (5' TAATCAGTGAGGCACCTATCTC-3') as the reverse primer (Gene Gostar- Iran). The PCR reaction was in a total amount of 50 μ L and contained the below items: 1 μ L DNA sample (3 μ g/ μ L), 10 pmol of each forward and reverse primers, 0.2 mM from each dNTP, 1.5 mM MgCl₂, and 5 Unit Taq DNA polymerase (CinnaGen Co, Iran). PCR amplification program was done according to the following platform: initial denaturation (94 °C for 120 seconds), (35 cycles of 60 seconds at 94 °C), (30 seconds at 52 °C) and (60 seconds at 72 °C) and for final extension (300 seconds at 72° C) was considered. After PCR amplification of TEM gene, the agarose gel electrophoresis was used for analysis of products.

Determination of minimum inhibitory concentration of antibiotic

For the investigation of antibiotic effects, different concentrations (512, 256, 128, 64, 32, and 2 μ g/mL) of antibiotics (Amikacin, Ceftazidime, Ceftriaxone, Gentamicin, ciprofloxacin) (Farabi Pharmaceutical Co, Isfahan, Iran) in Nutrient Broth (Merck, Darmstadt, Germany) with pH= 6.5 were prepared. Nutrient Broth without any antibiotics was used as control media and *E. coli* ATCC 25922 was used as control strain.

Minimum inhibitory concentration of essential oils

The Minimum inhibitory concentration (MIC) was determined using serial dilutions of the EOs (0.125–32 mg/mL) using microdilution test confirmed by Clinical and Laboratory Standards Institute (15). Briefly, serial dilutions of the EOs were prepared in a 96-well microtiter plate ranged from 0.125 to 32 mg/mL. The EO concentrations were dissolved initially in 5% dimethyl sulphoxide (DMSO) (Sigma-Aldrich Corp., St. Louis, MO, USA)/ 95% water. To each well, 50 μ L of each concentration of EOs, 40 μ L of Mueller Hinton Broth (supplemented with Tween 80 at a final concentration of 0.5% ((v/v)) and 10 μ L of bacterial suspension (10⁶ CFU/mL) were added. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and were incubated at 37 °C for 18-24 hours. The color change was assessed visually. The lowest concentration

at which the color change occurred was considered as MIC value. The MIC is defined as the lowest concentration of the EOs at which the microorganism does not demonstrate any visible growth. The microorganism growth was indicated by turbidity.

Results

The chemical composition of the selected plants is shown in Table 1. In this study, 107 (36.27%) out of 295 *E. coli* isolates were ESBL-producing. In PCR test, the distribution of TEM gene in isolated ESBL-producing organisms were 97 (90.65%) (Figure 1). The MICs of different antibiotics and EOs against extended-spectrum β -Lactamase-producing *E. coli* isolates are shown in Table 2 and Table 3, respectively. All antibiotics had good inhibitory effects on *E. coli* isolates but the most effective antibiotics were Gentamicin and Amikacin with MIC values of 0.5 and 4 $\mu\text{g}/\text{mL}$, respectively. Among 107 ESBL-producing *E. coli* isolates, 27 were sensitive to Amikacin (MIC= 4 $\mu\text{g}/\text{mL}$), 31 were sensitive to Gentamicin (MIC = 0.5 $\mu\text{g}/\text{mL}$), 34 were sensitive to Ceftazidime (MIC =32 $\mu\text{g}/\text{mL}$), 45 were sensitive to Ceftriaxone (128 mg/mL) and 22 were sensitive to Ciprofloxacin (MIC= 64 $\mu\text{g}/\text{mL}$). Some isolates were resistant to Ciprofloxacin, Ceftriaxone and Ceftazidime. Regarding the activity of the essential oils, all EOs derived from different plants displayed high inhibitory effects against ESBL-producing *E. coli* isolates. *N. persica*, *P. vulgaris*, *E. platyloba* and *A. wilhelmsii* EOs showed a great effect on isolates at low concentrations (0.5 mg/mL). The *L. royleana* EO showed its best inhibitory effect at a MIC of 1 mg/mL whereas *S. Nemorosa* EO showed its best inhibitory effects at a higher concentration (8 mg/mL).

Discussion

The EOs are a complex mixture of about 100 compounds including predominatory terpenes, phenylpropanoids and other substances which can be extracted from plants for different purposes (16, 17). During the last decades, a large number of studies has been conducted for the evaluation of antimicrobial activity of EOs (11). According to disk diffusion and Agar/Broth dilution methods which are two common tests in this field, both Gram-positive and Gram-negative bacteria are susceptible to plant extracted EOs (18). Although the susceptibility of a wide range of bacteria for EOs has been reported, there is always a great interest in studying the EO effects on bacteria which are an important threat for human health (7, 8, 19-21). In the present study, EOs were extracted from different parts of Iranian plants (*A. wilhelmsii* C. Koch, *E. platyloba* DC., *L. royleana*, *N. persica* Boiss., *P. vulgaris* Gaertn., *S. nemorosa*, and *S. intermedia* C.A.Mey.). The major compounds of the EOs are shown in Table 1. The inhibitory effects of the EOs were tested against ESBL-producing *E. coli* which was isolated from urinary tract of infectious patients. Our results showed that the different EOs had great antibacterial effects against *E. coli* isolates except for the *S. nemorosa* EO which had a high MIC of 8 mg/mL. The other EOs had a good antimicrobial activity with MICs ranging from 0.5 to 4 mg/mL.

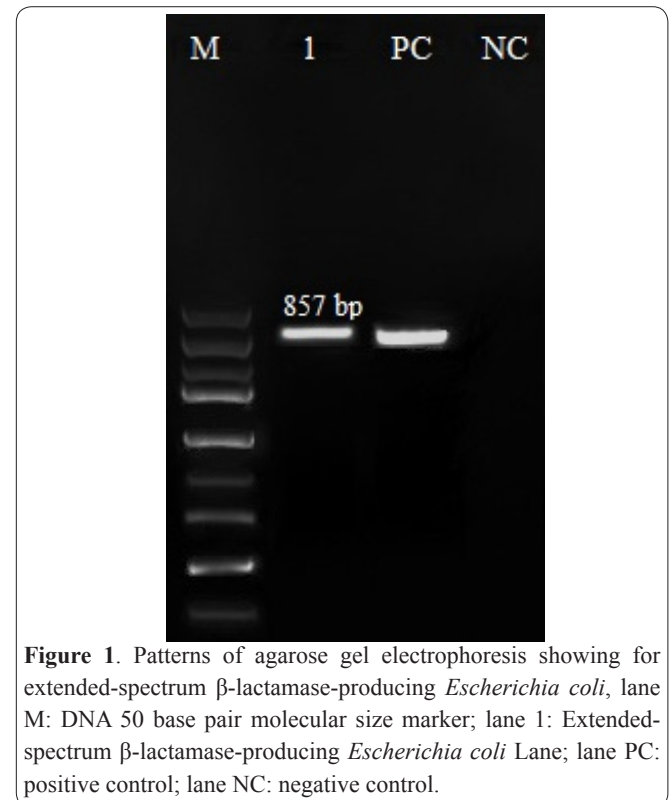


Figure 1. Patterns of agarose gel electrophoresis showing for extended-spectrum β -lactamase-producing *Escherichia coli*, lane M: DNA 50 base pair molecular size marker; lane 1: Extended-spectrum β -lactamase-producing *Escherichia coli* Lane; lane PC: positive control; lane NC: negative control.

N. persica EO has a great inhibitory effect against *E. coli* isolates with MICs ranging from about 0.125 to 16 mg/mL. These results are in accordance with other studies which showed the antibacterial activity of *N. persica* (24-26). Indeed, the antibacterial and anti-anxiety activity of *N. persica* EO against different bacteria especially *E. coli* isolates was confirmed by several studies (24, 25). The antibacterial effects of extracted EO from *N. persica* were evaluated using microbroth dilution assay and MIC values were ranging from 1 to 8 $\mu\text{L}/\text{mL}$ (26). Several investigations show that the major compounds of *N. persica* EOs are 4 α β ,7 α ,7 α -nepetalactone (26 %) and 1,4-hexadiene-2,3,4,5 tetramethyl (10.9%)(22, 23) and it is assumed that the antibacterial effect of *N. persica* EOs originates from these compounds.

Achillea genus have been commonly used as sedative, anti-helminthic, anti-inflammation, and for the treatment of some disorders in folk medicine (27). Our results showed that *A. wilhelmsii* EO which is commonly found in different places of Iran has a good antibacterial effect against ESBL-producing *E. coli* isolates with MICs ranging from 0.5 to 4 mg/mL. The antibacterial effects of extracted oils from *Achillea* species have been shown in several experiments (28-31). The major compounds of EO from *A. wilhelmsii* C. Koch are carvacrol (22.49 %), dihydrocarvone (13.23 %), linalool (12 %), 1,8-cineol (11.42 %), camphene (8.31 %), thymol (5.28 %), camphor (3.71 %), pulegone (2.82 %) α -terpineol (2.11 %), bornyl acetate (1.14 %) and farganol (1.01 %) (20).

The wide spectrum antibacterial activities of carvacrol, dihydrocarvone, linalool and 1,8-cineol which are the major components of *A. wilhelmsii* C. Koch EOs were shown in many studies (32-37). Their proposed mechanism of actions are the permeabilization and the depolarization of the cytoplasmic membrane of the bacteria (34). Adel *et al.* showed that the minimum concen-

Table 1. Major compounds of the plants essential oils.

Plant Name	Collection area	Growth stages	Plant parts	Major compounds	References
<i>Achillea wilhelmsii</i> C. Koch	Golmakan, Khorasan Razavi, Iran	Flowering	Aerial parts	carvacrol (22.49 %), dihydrocarvone (13.23 %), linalool (12 %), 1,8-cineol (11.42 %), camphene (8.31 %), thymol (5.28 %), camphor (3.71 %), pulegone (2.82 %), α -terpineol (2.11 %), bornyl acetate (1.14 %), farganol (1.01 %)	(19)
<i>Echinophora platyloba</i> DC.	Alvand Mountain, Iran	Flowering	Aerial parts	<i>trans</i> - β -ocimene (67.9%), 2-furanone (6.2%), myrcene (6.0%), linalool (3.1%), and <i>cis</i> - β -ocimene (2.3%)	(38)
<i>Lallemantia royleana</i>	Meymand, Firuzabad County, Fars Province, Iran.	Flowering	Aerial parts	<i>trans</i> -pinocarvyl acetate (26.0%), pinocarvone (20.0%), β -pinene (1.5%), (<i>E</i>)- β -ocimene (4.1%), terpinolene (1.1%), linalool (3.4%), <i>trans</i> - pinocarveol (1.6%), 3-thujen-2-one (5.1%), myrtenal (1.5%), verbenone (7.1%), <i>trans</i> -carveol (5.3%), <i>cis</i> -carveol (3.5%), pulegone (4.4%), carvacrol (1.6%), dihydrocarvyl acetate (2.5%) and β -cubebene (2.1%)	(7)
<i>Nepeta persica</i> Boiss.	Perspolis, Near Shiraz	Flowering	Aerial parts	1,4-hexadiene-2,3,4,5 tetramethyl (10.9%) , 4 β ,7 α , 7 α -nepetalactone (26.5%), <i>cis</i> - β -Farnesene (4.4%), 1-acetyl cyclohexene (3.7%)	(21)
<i>Pulicaria vulgaris</i> Gaertn.	Hamun Lake, Zabol, Iran	Flowering	Aerial parts	thymol (50.22%), <i>p</i> -menth-6-en-2-one (carvotanacetone, 20.2%), thymol isobutyrate (16.88%), menthan-2-one (4.31%), 1-methyl-1,2 propanedione (4.13%), 2,5-dimethoxy- <i>p</i> -cymene (4.01%), myrtenol (1.22%), linalool (1.1%) and β -myrcene (1.9%).	(43)
<i>Salvia nemorosa</i>	Zagruse Mountain, Iran	Flowering	Aerial parts	β -Caryophyllene (18.7%), Isocaryophyllene (6.8%), Caryophyllene oxide (5.2%)	(54)
<i>Satureja intermedia</i> C.A.Mey	Sepidan, Sepidan County, Fars Province, Iran	Flowering	Aerial parts	γ -terpinene (37.1%), thymol (30.2%), <i>p</i> -cymene (16.2%), limonene (3.9%), α -terpinene (3.3%), myrcene (2.5%), germacrene B (1.4%), elemicine (1.1%), carvacrol (0.5%)	(8)

Table 2. Minimum Inhibitory concentration for antibiotics used against extended-spectrum β -Lactamase-producing *E. coli* isolates (%).

Antibiotic	Concentration ($\mu\text{g/mL}$)												
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Amikacin	0	0	8 (8.24) [§]	6 (6.18)	8 (8.24)	27 (27.83)	6 (6.18)	9 (9.27)	11 (11.34)	19 (19.58)	2 (2.06)	1 (1.03)	0
Gentamicin	0	0	31 (31.95)	11 (11.34)	11 (11.34)	8 (8.24)	3 (3.09)	8 (8.24)	2 (2.06)	1 (1.03)	7 (7.21)	5 (5.15)	9 (9.27)
Ceftazidime	0	0	0	0	0	0	0	29 (29.89)	34 (35.05)	9 (9.27)	10 (10.3)	8 (8.24)	7 (7.21)
Ceftriaxone	0	0	0	0	0	0	0	0	0	11 (11.34)	45 (46.39)	25 (25.77)	16 (16.49)
Ciprofloxacin	0	0	0	0	0	0	3 (3.09)	6 (6.18)	8 (8.24)	22 (22.68)	18 (18.55)	23 (23.71)	17 (17.52)

[§]Data are presented as Number (%).

Table 3. Minimum Inhibitory concentration for various essential oils used against extended-spectrum β -Lactamase-producing *E. coli* isolates.

Essential oil plant	Concentration (mg/mL)												
	0.125	0.25	0.5	1	2	4	8	16	32				
<i>Achillea wilhelmsii</i> C. Koch	0	0	24 (24.74)	20 (20.61)	15 (15.46)	34 (35.05)	3 (3.09)	1 (1.03)	0				
<i>Echinophora platyloba</i> DC.	0	5 (5.15)	53 (54.63)	3 (3.03)	8 (8.24)	9 (9.27)	8 (8.24)	8 (8.24)	3 (3.09)				
<i>Lallemantia royleana</i>	0	2 (2.06)	3 (3.09)	56 (57.73)	20 (20.61)	8 (8.24)	5 (5.15)	3 (3.09)	0				
<i>Nepeta persica</i> Boiss.	1 (1.03)	9 (9.27)	32 (32.98)	33 (34.02)	11 (11.34)	4 (4.12)	4 (4.12)	3 (3.09)	0				
<i>Pulicaria vulgaris</i> Gaertn.	1 (1.03)	8 (8.24)	31 (31.95)	5 (5.15)	39 (40.2)	6 (6.18)	7 (7.21)	0	0				
<i>Salvia nemorosa</i>	0	0	2 (2.06)	3 (3.09)	8 (8.24)	3 (3.09)	66 (68.04)	9 (9.27)	6 (6.18)				
<i>Satureja intermedia</i> C.A.Mey	1 (1.03)	3 (3.09)	6 (6.18)	22 (22.68)	31 (31.95)	11 (11.34)	22 (22.68)	1 (1.03)	0				

[§]Data are presented as Number (%).

trations of *A. wilhelmsii* C. Koch EO (125 to 800 µg/L) could prevent the growth of several bacteria (38). In our previous study, the antibacterial activity of *A. wilhelmsii* C. Koch EO was also shown against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* species (20).

Among the EOs studied for their inhibitory effects, *E. platyloba* D.C. EO exerted a good antibacterial effect against *E. coli* isolates with a wide MIC range from 0.25 to 32 mg/mL. The main components of *E. platyloba* were reported by Asghari *et al.* as follows: *trans*-β-ocimene (67.9%), 2-furanone (6.2%), myrcene (6.0%), linalool (3.1%), and *cis*-β-ocimene (2.3%) (39). The antibacterial effect of *E. platyloba* D.C was demonstrated in several studies (40-42). Hashemi *et al.* showed that *E. platyloba* D.C EO had a powerful antimicrobial activity against *L. monocytogenes* and *S. aureus* with MIC values of 6250 and 12500 ppm, respectively (42). Concerning the activity of *L. royleana* EO, our study showed that it had a great antibacterial effect on ESBL-producing *E. coli* isolates with a MIC of 1 mg/mL. In fact, *L. royleana* EO was also evaluated in our previous examination against bacteria and fungi by disk diffusion and micro broth dilution method. Results showed that the EO have significant inhibition effects on several bacterial and fungi species such as *S. aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* (7). The two major components of *L. royleana* EO are *trans*-pinocarvyl acetate (26.0%) and pinocarvone (20.0%). It is assumed that these two components are the main antibacterial agents of *L. royleana* EO (43).

Few researches reported the antimicrobial activities of *P. vulgaris*. Our study showed that *P. vulgaris* EO had a great inhibitory effect on the growth of ESBL-producing *E. coli* isolates with a MIC value of 2 mg/mL. Thymol (50.22%), *p*-menth-6-en-2-one (carvotanacetone, 20.2%) and thymol isobutyrate (16.88%) are considered the major components of *P. vulgaris* EO (44). The antibacterial effects of thymol as the main component of *P. vulgaris* EO was shown in different experiments (34, 45, 46). Its inhibition activity is related to its ability to permeabilize and depolarize the bacterial membrane (34). Our recent investigation is also in accordance with these findings since the EO extracted from *P. vulgaris* leaves exerted great antimicrobial activity on several Gram-positive and Gram-negative bacteria such as *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa* (44).

Salvia nemorosa which belongs to *Salvia* genus plants is rich of secondary metabolites with antimicrobial properties and is well used for the treatment of several disorders in the folk medicine. To best of our knowledge, our study reported for the first time the inhibitory effect of *S. nemorosa* EO against *E. coli* especially ESBL-producing *E. coli* isolates. Our results showed a good antibacterial effect with MIC value of 8 mg/mL. Methanol extracts from different types of these plants were examined and showed antimicrobial effects against some bacterial strains (47). β-Caryophyllene (18.7%), Isocaryophyllene (6.8%), Caryophyllene oxide (5.2%) were reported as major components of *S. nemorosa* EO. The antibacterial effects of β-Caryophyllene considered as the major secondary component of different plants was proved in some investigations (48, 49).

According to Dahham *et al.* β-Caryophyllene is a potential candidate that can be used for further developments as a promising antibacterial agent (48). This component may act by leaking potassium and phosphate ions from bacterial cell (50). Božin *et al.* showed that the EO of woodland sage (*Salvia nemorosa* subsp. *nemorosa* L., *Lamiaceae*) exerted a noteworthy antibacterial activity against *Salmonella typhi*, *S. epidermidis* and *Shigella sonnei* (51). Concerning *S. intermedia* EO, it showed a good antibacterial effect against ESBL-producing *E. coli* isolates with MIC ranging from 1 to 8 mg/mL. Shahnazi *et al.* was demonstrated the high antibacterial activities of *S. intermedia* EO against Gram-positive and Gram-negative bacteria (52). Our previous experiment also showed the good antimicrobial activities of *S. intermedia* C.A.Mey EO against oral pathogens (8). The major components of *S. intermedia* C.A.Mey EO are γ-terpinene (37.1%), thymol (30.2%) and *p*-cymene (16.2%) (8). The antibacterial activity of such components are proved and their proposed mechanisms of antibacterial effects were evaluated in several investigations; they cause damages in cell wall and membrane of Gram-positive and Gram-negative bacteria (34, 53).

Some plant essential oils have a vast activity against different pathogenic bacteria. Such properties and potentials of EOs allow them to be attractive secondary metabolites in various contexts. Plants EO can be promising platforms for producing safe, biocompatible, economical and highly efficient therapeutic agents. According to results of this study, the antimicrobial effects of EOs from different plants against resistant and multi-resistant bacteria such as ESBL-producing isolates are clear. Moreover, the relatively low prices of obtaining EOs from plants would raise some suggestions and possibilities for producing and applying such materials in a good quantity and stability.

In conclusion, the EOs derived from aerial parts of different plants showed high inhibitory effects against ESBL-producing *E. coli* strains which were previously isolated from urinary tract of infectious patients. The results of our investigations may propose a good treatment option against resistant infectious bacteria.

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