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Antimicrobial activity of some plant essential oils and an antimicrobial-peptide against some clinically isolated pathogens

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Abstract

Background: Essential oils and antimicrobial peptides are two well-known safe and natural products that have been considered as alternatives to antibiotics. In the present study, the antibacterial activity of four plant essential oils and one lactoferrin-derived peptide was investigated.

Results: The chemical profile of each essential oil was determined by GC and GC–MS. Antimicrobial activity was shown against seven clinically isolated veterinary pathogens. MIC and MBC assessment of the essential oils and cLFchimera exhibited different antibacterial properties (MIC from a range of 62.5 to 500 µg/mL and 3.5 to 39.0 µg/mL for essential oils and cLFchimera, respectively). Compared to the essential oils, cLFchimera showed more significant antibacterial activity. Among the essential oils, *Vitex agnus-castus* and *Salvia officinalis* showed relatively better antibacterial activity.

Conclusions: The in vitro results reported here suggested that, for animals suffering from these pathogens, cLFchimera and the essential oils particularly *Vitex agnus-castus* could be considered as potential antimicrobial agents.

Keywords: Essential oil, Antimicrobial peptide, Camel, Veterinary pathogens

Background

The uncontrolled use of antibiotics; particularly, in the animal husbandry industry, is one of the global concerns [1]. In recent decades, many studies have been conducted on the replacement of natural and safe compounds with antibiotics in animal feed and veterinary science [2].

Bacterial infections in animals are one of the main problems in the animal husbandry industry [3]. The use of antibiotics to treat these diseases, in addition, to increase concerns of antibiotic resistance, also has huge economic losses to dairy farmers [3, 4]. Hence, extensive research has been done to find a suitable alternative to

antibiotics for the treatment of these diseases [5, 6]. Antimicrobial peptides (AMPs) and plant essential oils (EOs) are two natural components that have been considered as safe alternatives to antibiotics [7, 8].

AMPs are a group of naturally occurring molecules that are produced as the first line of defense by multicellular organisms. AMPs usually contain 12–50 amino acid residues; they have a net positive charge and an amphipathic structure [9, 10]. Many studies have demonstrated the antibacterial, antifungal, antiviral [11], and even anticancer effects of these compounds [12, 13]. Some reports describe cell lysis mechanisms by antimicrobial peptides (AMPs), while others describe the activation of regulated cell death [14–16]. One subgroup of AMPs include antimicrobial peptides derived from large proteins, such as lactoferrampin and lactoferricin [17]. These two AMPs are usually produced by digestion in the gastrointestinal tract [18]. Recently, Tanhaeian et al. showed that cLFchimera (the

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fusion of lactoferrampin and lactoferricin derived from camel lactoferrin) had a broad-spectrum antibacterial activity against some plants, human, and avian bacterial pathogens [19–21].

Essential oils (volatile oils) are complex mixtures of odorous principles stored in special plant cells, glands, glandular hairs, oil ducts, or resin ducts in any part of a plant. They are obtained by pressing and hydro or steam distillation from a whole plant or different parts of plants. They are complex mixtures of components, including terpenic derivatives, with well-known aromatic properties, and contain a range of oxygenated and nonoxygenated terpene hydrocarbons. Essential oils have various biological activities and therapeutic effects, which can be used as safer pest and disease control agents in different industries and treat several disorders in humans, animals, plants, and foods [22, 23].

In the present study, the antibacterial activity of one chimeric peptide derived from camel lactoferrin and four plant essential oils were examined against some clinically isolated veterinary pathogens.

Materials and methods

Plant materials and isolation of essential oils

Medicinal aromatic plants used in this study are listed in Table 1. Fruits of *Vitex agnus-castus* and *Cuminum cyminum* and the aerial parts of *Mentha piperita* and *Salvia officinalis* were harvested from the plants grown in Khorasan province, northeast of Iran. Samples were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The essential oils were collected over water, separated, and dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C until oils analysis and evaluation antibacterial activity test.

GC and GC–MS analysis

The composition of the essential oils was determined by gas chromatography (GC) and mass spectrophotometry (GC–MS). The GC analysis was done on an Agilent Technologies 7890 GC equipped with a single injector and a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 MS column (30 m × 0.25 mm i.d.; film thickness 0.25 µm).

The injector and detector temperatures were kept at 250 and 280 °C, respectively. Carrier gas (He) flow rate was 0.8 mL/min. The oven temperature program was 50–250 °C at the rate of 4 °C/min; the split ratio was 1:50. GC–MS analysis was carried out by the use of Agilent gas chromatograph equipped with fused silica capillary HP-5 MS column (30 m × 0.25 mm i.d.; film thickness 0.25 µm) coupled with a 5975-C mass spectrometer (70 eV) in an *m/z* range of 50–550. The oven temperature program was the same given above for the GC. The constituents of the EOs were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C₈–C₂₅) and the oil on an HP-5 MS column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [24]. The percentage composition was computed from the GC peak areas without using any correction factors.

Peptide

cLFchimera was prepared according to our previous studies [21]. Briefly, transformed *L. lactis* cells containing recombinant expression vector encoding cLFchimera coding sequence were cultured in GM17 medium and incubated at 30 °C to reach proper log-phase. Crude extracts were obtained by disrupting the cells, and the chimeric peptide was purified using Ni–NTA agarose column (for review, please see Tanhaeian et al. [21]). The quality and quantity of purified recombinant cLFchimera were analyzed on SDS-PAGE gel electrophoresis and Bradford method [25], respectively.

Bacterial strains and growth conditions

Seven clinical isolates of bacterial strains (Table 2) were obtained from the Mashhad Medical School laboratory (Mashhad, Iran). All strains were streaked on to an agar plate to obtain single colonies, and then freshly cultured in Mueller–Hinton II medium (Sigma, Germany). All incubation accomplished aerobically at 37 °C, for 24–48 h.

Determination of antimicrobial activity by the agar-well diffusion method

The antibacterial activity was carried out with the agar-well diffusion method [26]. Mueller–Hinton Agar medium was poured into a sterile petri dish (15 cm) and allowed to solidify. Clinically isolated *S. aureus* and *E. coli* (Table 1) were considered as Gram-positive and Gram-negative bacteria candidates, respectively, and

Table 1 Characteristics of plant essential oils which were used in this study

No.	Scientific name	Family	Plant part used
1	<i>Vitex agnus-castus</i>	Verbenaceae	Fruit
2	<i>Cuminum cyminum</i>	Apiaceae	Fruit
3	<i>Mentha piperita</i>	Lamiaceae	Herb
4	<i>Salvia officinalis</i>	Lamiaceae	Herb

Table 2 The list of bacterial strains

Bacterial Strains	Gram reaction	Host	Source	Diseases	Origin
<i>Staphylococcus aureus</i>	+	Bovine	Teat lesion	Mastitis	Mashhad Veterinary Clinical Science
<i>Pseudomonas aeruginosa</i>	—	Bovine	Milk	Mastitis	Mashhad Veterinary Clinical Science
<i>Salmonella typhimurium</i>	—	Calf	Feces	Diarrhea	Mashhad Veterinary Clinical Science
<i>Klebsiella pneumoniae</i>	—	Bovine	Milk	Mastitis	Mashhad Veterinary Clinical Science
<i>Salmonella enteritidis</i>	—	Calf	Feces	Diarrhea	Mashhad Veterinary Clinical Science
<i>Escherichia coli</i>	—	Sheep	Blood	Septicemia	Mashhad Veterinary Clinical Science
<i>Escherichia coli</i> O157	—	Calf	Feces	Diarrhea	Mashhad Veterinary Clinical Science

spread on a plate containing solid Mueller–Hinton agar. Wells, each 6 mm, were cut through the agar using sterile cork borer and the agar removed leaving empty wells that were filled with 100 μ L of each essential oil and peptide. Maintain the plates at room temperature for about 2 h and then incubate the plates at 37 °C for 24.

Evaluation of MIC and MBC in liquid medium

As maintained by the Clinical and Laboratory Standards Institute (CLSI) [27], the minimum inhibitory concentrations (MICs) of the EOs and cLFchimera were measured in Broth microdilution [28, 29]. In summary, Mueller–Hinton broth cation-adjusted that contains increasing concentrations of EOs and cLFchimera were inoculated with specific number of cells (approx. 5×10^5 CFUs/mL) in microtiter plates (polypropylene), while each plate includes a positive (Gentamicin, 50 μ g/mL) and negative (CAMHB without adding EOs or peptide) control. 10 μ L of EOs was added to wells at a range of concentrations, from stock solutions 0.05, 0.10, 0.50, 1.0, 2.5, 5.0, and 10% (w/v). For each dilution, the same volume as the full-strength sample was added. One hundred microliters of bacterial suspension was finally added to each. After incubation, the MIC is defined as the lowest concentration inhibiting the visible growth of bacteria after overnight incubation. All plates were incubated at 37 °C for 18–20 h. The MIC measurements were carried out in triplicate. MBC is the lowest concentration of antibacterial agents that results in microbial death. It was determined by subculturing from wells that exhibited no color change to sterile MHA plates that do not contain the test EOs and peptide. The plates were then incubated at 37 °C for 24 h. This study compared the MIC and MBC results with normal antibiotic suggested according to the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2017) [27].

Statistical analysis

The data are given as means of 3 to 5 experiments \pm one standard deviation (SD). Data were analyzed by statistical

software, GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA), and then were analyzed by one-way ANOVA, with post hoc Tukey's multiple comparisons test. *P*-values of < 0.05 were considered significant.

Results

Essential oils content and composition

The hydrodistillation of *Vitex agnus-castus*, *Cuminum cyminum*, *Mentha piperita*, and *Salvia officinalis* gave essential oils with yield of 0.5, 3.2, 1.5 and 0.4 (% v/w), respectively. Furthermore, the analysis of chemical composition of the essential oil of *V. agnus-castus* and *C. cyminum*, *M. piperita* and *Salvia officinalis* are presented in Table 3.

In total, the identified compounds by GC and GC/MS representing about 96.67% of the total detected components of *M. piperita* that oxygenated monoterpenes (84.67%) and monoterpene hydrocarbons (8.54%) were the main compounds. The major compounds of this oil were menthone (35.12%) and menthol (25.93%) (Table 3, Fig. 1).

In fruit essential oil of *C. cyminum*, 97.94% of the components detected which is mainly composed of monoterpene hydrocarbons (57.32%), oxygenated monoterpenes (31.33%), and oxygenated sesquiterpenes (8.92%). The major constituents of the oil were γ -terpinene (28.21%), *p*-cymene (13.25%), cumin aldehyde (12.92%), viridiflorol (8.92%), and β -pinene (6.61%) (Table 3; Fig. 1).

The essential oil extracted from *V. agnus-castus* fruit represented about 99.67% of the total identified constituents which was predominated by oxygenated monoterpenes (29.97%), sesquiterpenes hydrocarbons (29.7%), monoterpene hydrocarbons (25.32%), and oxygenated sesquiterpenes (13.36%). The major compounds were 1,8-cineole (12.7%), caryophyllene oxide (10.72%), *trans*- β -farnesene (9.51%), sabinene (9.12%), β -caryophyllene (7.23%), α -terpinyl acetate (7.22%), α -pinene (7.13%), and β -bicyclogermacrene (6.32%) (Table 3; Fig. 1).

The identified components representing about 99.25% of the total detected compounds of *S. officinalis*. The

Table 3 Chemical composition of essential oils studied in this research

No	Component	RI	GC peak area (%)			
			Mentha piperita	Cuminum cyminum	Vitex agnus-castus	Salvia officinalis
1	cis-Salvene	852	–	–	–	0.33
2	trans-Salvene	859	–	–	–	0.14
3	Tricyclene	915	–	–	–	0.41
4	α -Thujene	929	0.33	0.42	–	0.05
5	α -Pinene	934	1.0	1.12	7.13	3.21
6	Camphene	953	–	–	–	3.52
7	Sabinene	974	0.59	1.3	9.12	0.11
8	β -Pinene	978	1.51	6.61	0.91	2.33
9	1-Octan-3-ol	985	–	–	–	0.12
10	β -Myrcene	990	0.28	0.74	1.33	0.61
11	3-Octanol	995	–	–	–	0.45
12	α -Phellandrene	1006	–	2.41	–	0.13
13	Δ -3-Carene	1010	–	0.08	–	–
14	α -Terpinene	1017	0.32	0.25	–	0.43
15	p-Cymene	1024	0.42	13.25	2.21	1.41
16	Limonene	1031	2.51	1.38	4.12	1.52
17	1,8-Cineole	1033	6.52	0.15	12.7	8.14
18	β -Phellandrene	1034	–	1.22	–	–
19	cis- β -Ocimene	1037	0.21	–	–	0.32
20	γ -Terpinene	1057	0.31	28.21	–	0.23
21	cis-Sabinene hydrate	1066	0.91	–	0.5	0.21
22	α -Terpinolene	1088	0.15	0.33	–	0.11
23	Linalool	1100	0.23	–	0.91	0.44
24	α -Thujone	1112	–	–	–	43.21
25	β -Thujone	1116	–	–	–	7.35
26	trans-Verbenol	1142	–	–	0.34	–
27	Camphor	1145	–	–	0.83	0.12
28	Borneol	1155	–	–	–	8.43
29	Menthone	1158	35.12	–	–	–
30	Isomenthone	1162	1.1	–	–	–
31	δ -Terpineol	1163	–	–	2.52	0.5
32	Menthofuran	1166	10.5	–	–	–
33	Menthol	1178	25.93	–	–	–
34	Terpinene-4-ol	1180	1.33	0.57	2.6	–
35	neo-Menthol	1185	0.45	–	–	–
36	α -Terpineol	1192	0.42	0.12	3.41	0.21
37	trans-Carveol	1217	–	–	0.12	–
38	Cumin aldehyde	1223	–	12.92	0.11	–
39	Pulegone	1240	0.7	–	–	–
40	Piperitone	1255	0.5	–	–	–
41	Safranal	1271	–	4.65	–	–
42	Cuminic alcohol	1279	–	12.92	–	–
43	trans-Sabinyl acetate	1291	–	–	–	0.71
44	Menthyl acetate	1293	2.1	–	–	–
45	Carvacrol	1300	–	–	–	0.65
46	α -Terpinyl acetate	1346	–	–	7.22	–
47	Citronellyl acetate	1351	–	–	0.12	–

Table 3 (continued)

No	Component	RI	GC peak area (%)			
			<i>Mentha piperita</i>	<i>Cuminum cyminum</i>	<i>Vitex agnus-castus</i>	<i>Salvia officinalis</i>
48	β -Bourbonene	1387	0.22	–	–	–
49	β -Caryophyllene	1422	1.35	0.12	7.23	1.83
50	β -Gurjunene	1429	–	–	1.11	–
51	<i>trans</i> - α -Bergamotene	1431	–	–	1.12	–
52	<i>cis</i> - β -Farnesene	1438	–	0.11	0.81	–
53	β -Humulene	1441	–	–	0.73	–
54	<i>trans</i> - β -Farnesene	1454	0.31	–	9.51	–
55	α -Humulene	1456	–	–	2.13	2.56
56	Germacrene-D	1483	1.1	0.14	–	–
57	Viridiflorene	1494	–	–	0.21	–
58	Bicyclogermacrene	1496	–	–	6.32	–
59	β -Bisabolene	1505	–	–	0.31	–
60	γ -Cadinene	1512	–	–	0.22	–
61	Myristicin	1519	–	–	0.41	–
62	Spathulenol	1574	–	–	1.12	–
63	Caryophyllene oxide	1577	–	–	10.72	0.64
64	Viridiflorol	1595	0.25	8.92	–	5.87
65	β -Selinene	1604	–	–	–	0.85
66	Humulene epoxide II	1602	–	–	0.31	2.1
67	<i>t</i> -Cadinol	1635	–	–	1.1	–
68	α -Cadinol	1651	–	–	0.11	–
Total			96.67	97.94	99.67	99.25
Monoterpene hydrocarbons			8.54	57.32	25.32	15.7
Oxygenated monoterpenes			84.67	31.33	29.97	69.76
Sesquiterpenes hydrocarbons			2.98	0.37	29.7	5.24
Oxygenated sesquiterpenes			0.25	8.92	13.36	8.61
Aromatic compounds			67.57	82.83	69.95	73.00
Others			0	0	0.41	0.57

chemical composition of the essential oil comprises oxygenated monoterpenes (69.76%), monoterpene hydrocarbons (15.7%), oxygenated sesquiterpenes (8.61%), and sesquiterpenes hydrocarbons (5.24%). The essential oil was composed mainly of α -thujone (43.21%), borneol (8.43%), 1,8-cineole (8.14%), and β -thujone (7.35%) (Table 3; Fig. 1).

Antibacterial activity

To evaluate the antimicrobial properties of essential oils and cLFchimera against tested veterinary pathogens, the determination of MIC and MBC values were necessarily performed. The results showed the variable effects of essential oils and cLFchimera on the tested bacterial strains (Table 4).

cLFchimera showed strong antimicrobial activities in inhibiting the growth of pathogenic bacteria at MIC values ≤ 14.2 $\mu\text{g/mL}$ except for *Klebsiella pneumoniae*.

The bactericidal effect of cLFchimera was determined at a concentration of ≤ 39.0 $\mu\text{g/mL}$ for all tested pathogens. The antibacterial activity of cLFchimera was significantly higher than essential oils, these results conferred by agar-well diffusion method, where the inhibition diameter zones obtained by peptide were higher than EOs as shown in Fig. 2.

All essential oils showed bacteriostatic and bactericidal effects at the concentrations of ≤ 500 $\mu\text{L/mL}$ except for clinically isolated *Escherichia coli*. Among the tested microorganisms *K. pneumoniae* and *P. aeruginosa* were the least sensitive as higher concentrations of EOs were needed with MIC and MBC values ranging from 250 to 500 $\mu\text{g/mL}$. The MIC/MBC values for bacterial strains sensitive to the essential oil of plants were in the range of ND/ND to 500.0/500.0 (Table 4). The essential oils of *V. agnus-castus* and *S. officinalis* had the most bactericidal and bacteriostatic properties

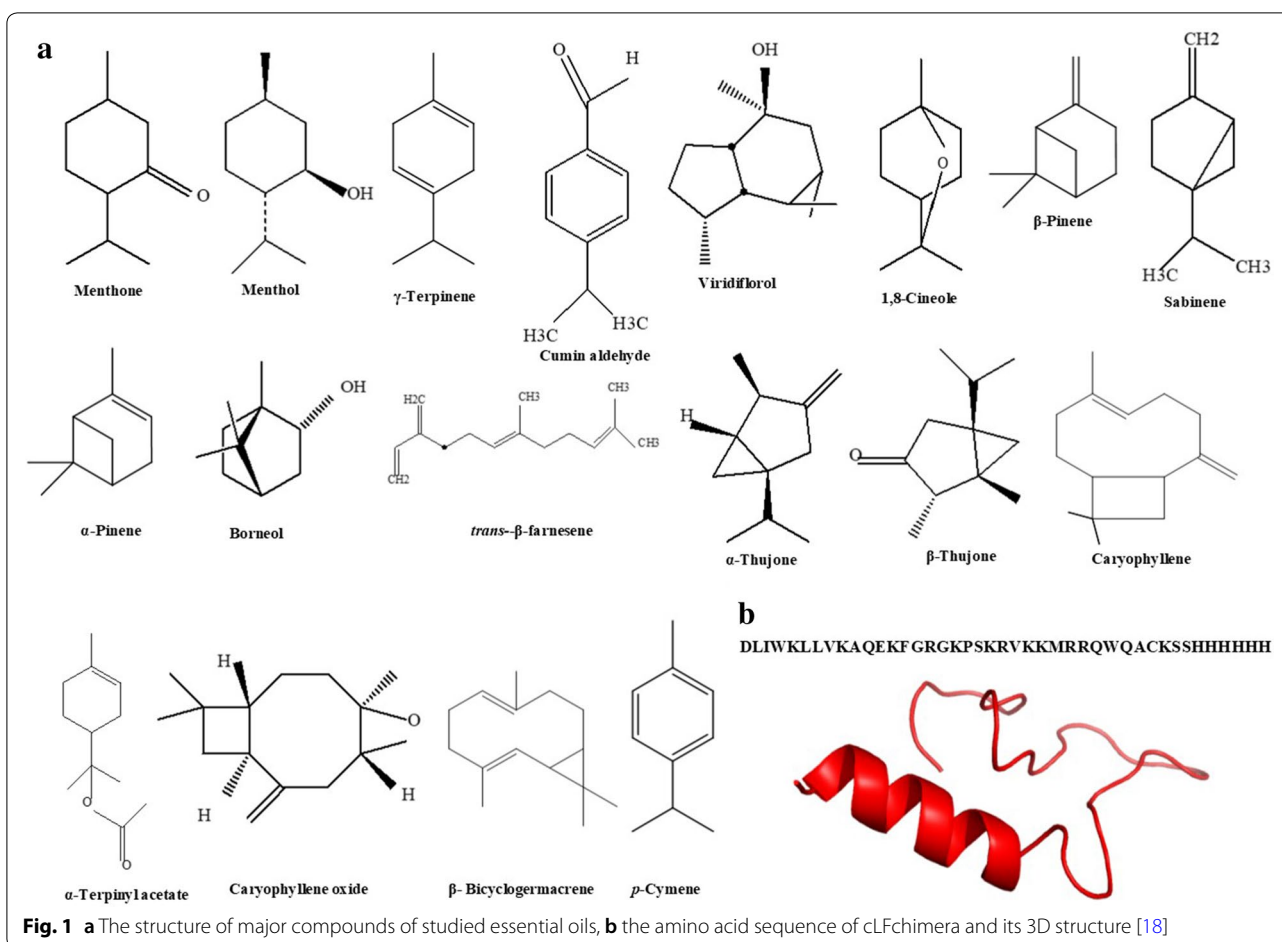


Fig. 1 **a** The structure of major compounds of studied essential oils, **b** the amino acid sequence of cLFchimera and its 3D structure [18]

(62.5/125.0 $\mu\text{g/mL}$) against the Gram-positive bacterium, *S. aureus* (Table 4). Moreover, the essential oil of *S. officinalis* had the lowest MIC and MBC against *E. coli* as a Gram-negative bacterium (Table 4). According to the results, *E. coli* was the most sensitive bacterium to the *S. officinalis* oil. Furthermore, the most resistant bacteria to *Cuminum cyminum* oil were *P. aeruginosa*, *S. typhimurium*, *S. enteritidis*, and *K. pneumonia* (Table 4). In addition, the most resistant bacteria to *V. agnus-castus* oil were *P. aeruginosa* and *S. enteritidis*. On the other hand, *P. aeruginosa*, *E. coli* 0157, and *E. coli* 0157 were the most resistant bacteria to *M. piperita* oil. Moreover, *S. typhimurium* and *E. coli* 0157 were the most resistant bacteria to *S. officinalis* oil (Table 4).

For cLFchimera, *K. pneumoniae* and *Escherichia coli* O157 showed the least sensitivity against this chimeric peptide (Table 4).

Discussion

In veterinary medicine care, antibiotics are used routinely as an essential part of prevention and treatment strategies in bacterial disease. Nevertheless, antibiotic

effectiveness is declining as bacterial development resistance and this fact is considered as one of the most serious health threats. Today, introducing natural components, such as antimicrobial peptides and essential oils as an alternative for antibiotics, are emerging research interest in this field.

In the present study, in vitro antiracial activity of a chimeric peptide which was derived from camel lactoferrin protein and four essential oils were investigated against seven clinically isolated veterinary pathogens. Our results showed that compared to the essential oils, cLFchimera showed more significant antibacterial activity. The high purity of cLFchimera as an effective component compare to these components in EOs could be considered as these significant differences between MIC/MBC values on the test pathogens in the present study.

Supplementation of AMPs as an alternative for antibiotics has been reported to have positive effects on performance, nutrient digestibility, intestinal microflora, intestinal morphology, and immune function in pigs and broilers [30, 31]. Moreover, previous studies have been proved the effects of antibacterial activity of AMPs on

Table 4 Comparative minimum inhibitory concentration (MIC/MBC) of the peptide and essential oils with some antibiotic suggested according to the Clinical and Laboratory Standards Institute (CLSI)

Bacterial strains	Antimicrobial Activity MIC/MBC ($\mu\text{g/mL}$)					MIC
	cLFchimera Gentamicin	<i>Vitex agnus-castus</i>	<i>Cuminum cyminum</i>	<i>Mentha piperita</i>	<i>Salvia officinalis</i>	
Gram-positive						
<i>Staphylococcus aureus</i>	14.2 \pm 0.3/28.4 \pm 0.3*	62.5 \pm 4.0/125.0 \pm 8.0	125.0 \pm 13.0/250.0 \pm 23.0	125.0 \pm 16.0/250.0 \pm 25.0	62.5 \pm 10.0/125.0 \pm 18.0	0.12–1
Gram-negative						
<i>Klebsiella pneumoniae</i>	ND/ND	250.0 \pm 11.0/500.0 \pm 13.0	500.0 \pm 33.0/500.0 \pm 35.0	250.0 \pm 17.0/250.0 \pm 23.0	250.0 \pm 18.0/500.0 \pm 32.0	ND
<i>Salmonella typhimurium</i>	3.5 \pm 0.1/28.4 \pm 0.4	125.0 \pm 11.0/250.0 \pm 16.0	500.0 \pm 27.0/500.0 \pm 28.0	250.0 \pm 25.0/250.0 \pm 17.0	500.0 \pm 36.0/500.0 \pm 21.0	≥ 16
<i>Pseudomonas aeruginosa</i>	9.7 \pm 0.3/39.0 \pm 0.9	500.0 \pm 23.0/ND	500.0 \pm 35.0/ND	500.0 \pm 36.0/ND	250.0 \pm 24.0/500.0 \pm 28.0	0.5–2
<i>Salmonella enteritidis</i>	7.0 \pm 0.5/28.4 \pm 0.8	500.0 \pm 24.0/500.0 \pm 19.0	500.0 \pm 26.0/500.0 \pm 30.0	250.0 \pm 19.0/250.0 \pm 21.0	125.0 \pm 17.0/250.0 \pm 24.0	≥ 16
<i>Escherichia coli</i>	9.7 \pm 0.1/19.5 \pm 0.6	250.0 \pm 17.0/500.0 \pm 30.0	125.0 \pm 15.0/250.0 \pm 19.0	500.0 \pm 35.0/ND	ND/ND	0.25–1
<i>Escherichia coli</i> 0157	39 \pm 0.5/39 \pm 1.0	125.0 \pm 11.0/250.0 \pm 18.0	125.0 \pm 16.0/250.0 \pm 21.0	500.0 \pm 20.0/500.0 \pm 20.0	500.0 \pm 37.0/ND	0.25–1

Values are results of four replicates. Data are collected as MICs according to the CLSI. ND not determined

* Data are mean \pm SD (standard deviation)

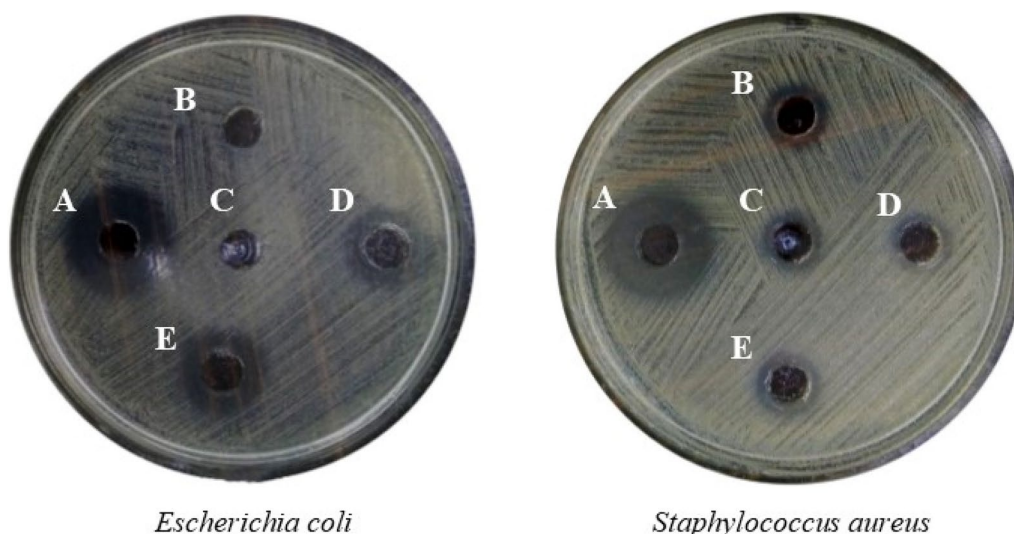


Fig. 2 Inhibition diameter zones obtained by well diffusion method for *E. coli* and *S. aureus* as two Gram-negative and Gram-positive clinically isolated photogenes in this study. **A**: cLFchimera, **B**: *Mentha piperita*, **C**: *Salvia officinalis*, **D**: *Vitex agnus-castus*, and **E**: *Cuminum*

the incidence of mastitis [5], diarrhea [32, 33], and septic shock [34]. Tomasinsig et al. [5] reported cathelicidin peptides BMAP-27, BMAP-28, Bac5, and indolicidin showed a variably broad spectrum of activity against 28 bacterial isolates from bovine mastitis, some of which were antibiotic resistant. In our study, cLFchimera

showed in vitro activity against *S. aureus* and *P. aeruginosa* which isolated from teat lesion and milk from cows suffering from mastitis. However, this chimeric peptide did not show antibacterial activity against *K. pneumoniae* isolated from milk of bovine mastitis. In addition, using a synthetic fusion peptide lactoferricin–lactoferrampin

improved growth performance and decreased diarrhea incidence in weaning pigs [32, 33]. These results are consistent with the antibacterial activity of cLFchimera against *S. typhimurium*, *S. enteritidis*, *E. coli* O157 which were isolated from feces of calf suffering from diarrhea. Interestingly, Giacometti et al. [34] showed that a sheep myeloid antimicrobial peptide (SMAP)-29 had considerable activity against *E. coli* 0111 as a known cause of septic shock both in vitro and in vivo. They showed that SMAP-29 completely inhibited the LPS procoagulant activity and reduced the lethality rate in LPS-induced septic rats when compared with control animals [34]. These results are consistent with the antibacterial activity of cLFchimera against *E. coli* O157 which was isolated from the blood of sheep suffering from septicemia.

Essential oils (EOs) as alternative treatments to bovine mastitis have been previously described [35, 36]. Dal Pozzo et al. [37] demonstrated that essential oils of oregano, thyme, Mexican oregano, as well as the major constituent's thymol and carvacrol showed antimicrobial activity (range of 400–1600 µg/mL) against *Staphylococcus spp.* The EOs which were used in this study showed antimicrobial activity in the lower range (62.5–250 µg/mL) against clinically isolated *S. aureus*.

Recently, a study was investigated to evaluate the effects of oregano essential oil on the severity of neonatal diarrhea syndrome in calves aged less than 15 days. The results of their study indicated that daily administration of oregano essential oil in calves for the first 10 days of their life effectively diminishes the severity of naturally acquired diarrhea under field conditions and, under certain hygiene practices, possess a preventive effect against neonatal diarrhea syndrome [38]. These results are in conformity with the antibacterial activity of all EOs used in the present study against *S. typhimurium*, *S. enteritidis*, *E. coli* O157 which were isolated from feces of calf suffering from diarrhea. Bachir and Benali indicated that the addition of essential oil leaves in broth culture inoculated with *S. aureus* and *E. coli* inhibited the growth of these organisms. The rate of inhibition was greater on Gram-negative bacteria (*E. coli*) than that observed on Gram-positive bacterium (*S. aureus*) [39].

Based on the previous study, a broad variation in antibacterial activity of EOs from aromatic plants, such as *Origanum minutiflorum* and *Sideritis erythraea* subsp. *erythraea* [40]; *Origanum compactum*, *Cymbopogon flexuosus*, *Cinnamomum cassia*, *Thymus vulgaris* and *T. capitatus* [41], *Ocimum ciliatum* [42], *Ferulago angulata* [43] *Origanum vulgare* subsp. *viride* [44] against different tested Gram-negative and Gram-positive bacteria were observed [45]. The antibacterial activity of EOs is not attributable to one specific mechanism and has not been well comprehended yet [46]. The main influence of

essential oils related to the available naturally compounds in essential oil of aromatic plants [47]. Therefore, chemical composition of EOs is of great importance to explain their antibacterial activity [48].

According to the results of the previous researches, biological activities of EOs are first ascribed to its major compounds [49, 50]. However, the antibacterial activity of EOs cannot be attributed to a single component because they are a complex mixture [51]. In addition, minor constituents of essential oils may have synergistic influences with other compounds because of antibacterial properties [46]. Therefore, the relation between chemical constituents and antibacterial activity may be imputable to their major components as well as the minor constituents present in EOs. They act together to give the activity of the whole tested EOs [51]. Referring to another study, the major constituents of EOs include monoterpene or sesquiterpene hydrocarbons, and their oxygenated derivatives demonstrate antibacterial activity [52].

The antibacterial activity of EOs may be due to monoterpene hydrocarbons. In this research, essential oil of *Vitex agnus-castus* is mainly composed of the oxygenated monoterpenes (1,8-cineole, α -terpinyl acetate). In addition, another main compound in this oil is caryophyllene oxide (oxygenated sesquiterpenes) and also several monoterpene hydrocarbons (sabinene, α -pinene) are highly present in this oil. The oxygenated groups present in 1,8-cineole are known to increase the antimicrobial properties of this terpenoid [53]. Therefore, in this experiment, *V. agnus-castus*, which contained 1,8-cineole as a main compound showed the highest antibacterial activity among the other studied essential oils. Moreover, oxygenated monoterpenes (α -thujone, borneol, β -thujone) are the main compounds of *S. officinalis* oil. Therefore, the results of our study showed that the synergistic phenomenon between oxygenated monoterpenes, oxygenated sesquiterpenes, and monoterpene hydrocarbons cause to indicate antibacterial activity. On the other hand, our results, as well as previous research, allow us to suggest that constituents, such as 1,8-cineole, α -terpinyl acetate, α -thujone, borneol, β -thujone, alongside sabinene, α -pinene, and caryophyllene oxide, might contribute to antibacterial activity in conformity with earlier studies [54–56].

The antibacterial activity of *C. cyminum* essential oil is attributable to the present of cuminaldehyde, a constituent with well-known antibacterial properties [52] and α -pinene, the other main compound of cuminal oil which inhibited the growth of bacteria [52]. The mechanism of antibacterial activity of peppermint oil and especially menthol is not clearly elucidated, even though it was reported in some previous studies [57, 58]. According to the results of previous studies, menthol as a

monoterpene, showed the antibacterial activity because of the interruption of the lipid fraction of the plasma membrane, causes to alter the permeability and leakage of intracellular materials [57].

It is necessary to indicate that other constituents can cause to improve this activity. Even the components are not abundant in essential oil, their activity is important. The antibacterial activity of α -pinene, β -pinene, 4-terpineol, α -terpineol, and caryophyllene oxide has been reported earlier [59]. Antibacterial activity of the essential oil is likely due to the presence of above-mentioned components.

The exact mechanism of antibacterial effect of the combined application of the tested constituents is still unknown. Based on the literature, the percentage of chemical constituents and the major compounds of the essential oil controlled the antibacterial activity [60]. Some researchers reported that the maximum antibacterial activity is caused by chemical compounds of essential oils containing hetero atoms, such as oxygen [61, 62]. Generally, several accepted mechanisms of antibacterial interaction, which produced synergism are available that included the inhibition of protective enzymes, the sequential inhibition of common biochemical pathways, and the enhancement of the uptake of antibacterial by agents that influence the cell wall [63]. There is synergistic interaction of the essential oil compounds against the bacterium and a marked inhibition of bacterial growth in vegetable matrices. The antibacterial properties of essential oils have been attributed to terpene compounds, including a terpenic oxide, as the major compounds found in the studied essential oils [64].

Our findings strengthen the consistency of the low MIC value found for *S. officinalis* oil, as oxygenated monoterpenes (like α -thujone, β -thujone 1,8-cineole, and borneol) were found to be the main components of the oil.

On the other hand, variation between antibacterial activities of EOs may be connecting to the distinct interactions with the target cell structures [59, 65]. There are different mechanisms in the microbial cells that think to be the location of essential oil action. The antibacterial activity of EOs perhaps affects their potential to bacterial membranes and shows the inhibitory activity on the functional characteristics of the cell [48]. EOs can break down the cell wall, interrupt the phospholipid bilayer of the cytoplasmic membrane, and injury the membrane proteins causing to enhance the permeability of the cell membrane and loss of cellular components. They can further disrupt the proton motive force, electron flow and active transport, and coagulate the cell contents [46]. Then, leakage of intracellular compounds and impairment of microbial enzyme systems happen [48] and

extensive losses of the cell contents will bring about cell death [64].

Overall, our results showed that both cLFchimera and EOs used in this study had considerable activity against the clinically isolated veterinary pathogens. Although the antibacterial activity of the chimeric peptide was significantly higher than EOs, the production of AMPs is usually expensive compared to EOs [66]. Based on the results of some previous studies, essential oils are more active against Gram-positive bacteria than Gram-negative bacteria [43]. On the other hand, some authors have reported that Gram-negative microorganisms are slightly more sensitive to essential oils when compared with Gram-positive microorganisms [67]. The variation between the antibacterial activity of essential oils against Gram-negative and Gram-positive bacteria [39] depend on several factors, such as tested bacterial strains and different concentrations [68], as well as the main constituents of essential oils [69]. Furthermore, trace components in the oil play an important role in the inhibition of microorganisms because of the synergistic effects of all chemical compounds of the oil [70]. In addition, monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives showed antibacterial activity [52]. Also, this differentiation may be due to the variation in cell structure or external lipopolysaccharide structure, which may retain the entry of hydrophobic constituents in the cell of the Gram-negative and Gram-positive bacteria, which were more resistant and sensitive to essential oils [46, 47].

However, previously published papers indicated that AMPs that were derived from lactoferrin were more effective toward Gram-positive bacteria [17, 71]. In light of the above facts, it seems a combination of peptides and EOs could be effective in decreasing the cost and increasing the spectrum of antibacterial activity, and maybe synergistic effects that must be evaluated in future studies.

Conclusion

Since the use of synthetic antibiotics in animal husbandry and veterinary fields is being increasingly discouraged due to their presence of animal products and subsequently effects on population health; and introduction of safe and natural components seems to be critical. Our results showed that cLFchimera in comparison to EOs which was used in this study was more effective against clinically isolated veterinary pathogens. In the future study, to lessen the cost production of AMPs, the chimeric peptide could be combined with EOs for evaluating the synergistic effects.

Abbreviations

MIC: Minimum inhibitory concentrations; MBC: Minimum bactericidal concentration; AMPs: Antimicrobial peptides; EOs: Essential oils; GC: Gas

chromatography; MS: Mass spectrophotometry; FID: Flame ionization detector; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; CSLI: Clinical and laboratory standards institute; SD: Standard deviation.

Acknowledgements

The authors would also like to express their gratitude to the Ferdowsi University of Mashhad for their support.

Authors' contributions

MHS designed, supervised the study and wrote the manuscript. AT undertook all of the sample analysis and contributed to the writing of the manuscript. MM contributed to the design and writing of this manuscript. All authors read and approved the final manuscript.

Funding

The present study was funded by Ferdowsi University of Mashhad with grant No. 38736.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have no conflict of interest to declare.

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Received: 14 October 2019 Accepted: 20 February 2020

Published online: 10 August 2020

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