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Antibacterial Activity of Some Iranian Herbal Essential Oils as Disinfectant Agents on Surfaces Contaminated with Methicillin-resistant-*Staphylococcus Aureus* and Carbapenem-resistant-*Pseudomonas Aeruginosa*

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Abstract

Background & Objectives: Different essential oils (EOs) with antibacterial activities are promising natural sources for providing novel disinfectant agents for hospital surfaces.

Materials & Methods: The component and antibacterial effects of six EOs, including *Cuminum cyminum* (CCEO), *Artemisia sieberi* (ASEO), *Laurus nobilis* (LNEO), *Ferula gummosa* (FGEO), *Lippia citriodora* (LCEO), and *Cymbopogon citratus* (CIEO) were assessed by GC-MS and 96-well micro-plates (IC50), against *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853 and clinical isolates of methicillin-resistant *S. aureus* (MRSA) and metallo-beta-lactamase (MBL)-producing *P. aeruginosa*. Then, the antibacterial effects of FGEO, the most effective EO, were evaluated on the trolley surface in a hospital for 1, 3, 5 and 10 min intervals.

Results: CCEO, ASEO, and FGEO exerted the highest antibacterial activity against *S. aureus*, while CIEO and LNEO inferred the highest activity against *P. aeruginosa*. In addition, FGEO mitigated the growth of *S. aureus* and *P. aeruginosa* on the trolley surface ($P<0.05$).

Conclusion: The studied EOs could be novel encouraging agents to develop further green antimicrobial agents against different infections. In addition, FGEO exhibited considerable antibacterial effects on the surface of the trolley.

Keywords: Nosocomial infections, Antibiotic resistance, Essential oils, Antibacterial activity

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Introduction

Nosocomial infections (NIs), one of leading causes of death in the last decades, are acquired during the healthcare procedure and following hospitalization. In recent years, the prevalence of various NIs has been in great rise in America (3.2%) and Europe (5.7%) (1, 2). Recent reports have revealed that the mean prevalence of NIs in Iranian hospitals (0.71%) is significantly lower than those of worldwide statistics, but according to some reports their prevalence in some

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Iranian hospitals and other Asian clinics reach up to 9.1% (2).

A number of microorganisms can cause NIs, among which, two predominant and miscellaneous bacterial species include *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (3). *S. aureus* is one of the most common causes of surgical infections. Moreover, both *S. aureus* and *P. aeruginosa* play the main role in Central Line-Associated Blood Stream Infections (4).

In recent years, we have faced new difficulties in controlling NIs. Studies on resistant microorganisms have demonstrated that more than 60% of different bacterial pathogens have developed resistance to routine antibiotics (5). From the recent decade, the evolution and spread of resistant *S. aureus* and other agents of NIs, has demanded seeking novel agents or alternatives to survive hospitalized patients (6). Therefore, the efficiency of common antimicrobial agents against NIs has decreased dramatically worldwide in recent years (7).

In the last years, numerous herbal products, essential oils (EOs) and extracts with antibacterial, sedative and antioxidant properties have become available worldwide with low side effects/toxicity (8,9) which can be considered as complementary treatments (10). Moreover, several Iranian herbal species have been introduced as antimicrobial products (11). A large number plant EOs with potential antimicrobial effects have included *Myrtle communis*, *Thymus vulgaris*, *Lavandula angustifolia*, and *Mentha pulegium* (12). Considering the development of various bacterial strains non-susceptibility to chemical disinfectant agents (13), the development of plant EOs and their formulations is warranted. Therefore, the present study aimed to evaluate the antibacterial activity of different Iranian herbal EOs against *S. aureus* and *P. aeruginosa* clinical and standard strains on the hospital contaminated surfaces.

Materials and Methods

Bacterial species and herbal essential oils

In this study, standard bacterial strains were obtained from the Microbiology laboratory of the Pasteur Institute of Iran (Standard strains of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853). Moreover, clinical isolates of methicillin-resistant *S. aureus* (MRSA) and metallo-beta-lactamase (MBL)-producing *P. aeruginosa* strains had been collected from wound infections. Also, we utilized the molecular method (PCR test) and the routine culture media (the disk-diffusion method and minimum inhibitory concentration (MIC) tests) to identify phenotypically and genotypically MBL-producing *P. aeruginosa* and MRSA (14). We obtained all EOs from the Tabib Daroo Company (a pharmaceutical company in Kashan). Herbarium specifications of each plant has been included in Table 1.

Table 1. Herbarium specifications of plants used in this study

No	Scientific names (Family)	Part used	Herbarium code	Collector	Place of collection
1	*Cuminum cyminum* (Cuminum Family)	Seed	IAUM-211	Dr. A. Ziaeei	Bajestan, khorasan, Iran
2	*Artemisia sieberi* (Asteraceae Family)	Leave	HUMZ-8629	Dr. A. Taheri	Ghaemshahr, Mazandaran, Iran
3	*Laurus nobilis* (Lauraceae Family)	Leave	IAUGH-1409	Dr. E. Bahrani	Tehran, Tehran, Iran
4	*Ferula gummosa* (Apiaceae Family)	All	UQH-1530	Dr. A. Khani	Arak, Markazi, Iran
5	*Lippia citriodora* (Verbenaceae Family)	Leave	KF-1513	Dr. Mehrabani	Sirch, Kerman, Iran
6	Cymbopogon citratus (Poaceae Family)	Leave	HUI-TB400CI	A. Mazaheri	Kashan, Isfahan, Iran

Bacterial Culture

We used Mueller-Hinton broth, containing 0.5% dimethyl sulfoxide for bacterial growth (Merck Chemicals, Germany) (15).

Gas Chromatography/Mass Spectrometry analysis

Gas Chromatography/Mass Spectrometry (GC-MS) analysis was employed to identify the compounds of the selected herbal EOs. We utilized a 7890A Network GC system alongside a 5975A mass detector (Agilent Technologies, Santa Clara, CA, USA). The compounds of the EOs were separated using fused silica columns of HP-5MS (with a length of 30 meters; internal diameters of 250 µM; film thickness of 25 µM). The temperature was set initially at 50°C, gradually increased to 200°C (5°C per minute), and remained at this temperature for 1 minute. Then, the temperature was elevated up to 250°C (10°C per minute) and again increased to 300°C at a higher rate (25°C per minute). On the other hand, helium was utilized as the carrier gas. Finally, we analyzed the components based on our previously described method (16).

Determination of the antibacterial activity of EOs

We utilized a 96-well plate to provide a proper situation for evaluation of antibacterial effects of EOs against the purposed bacterial species based on standard protocols (17). We achieved 1.5×10^8 CFU/mL of bacterial colonies to reach 0.5 McFarland turbidity. Then a 20 µL suspension was added to all wells. After the preparation of bacterial agents, we dissolved EOs in Mueller Hinton Broth to provide a standard dilution series (8.00, 4.00, 2.00, 1.00, 0.50, 0.25, and 0.13 mg/mL). After the described dilution, we added 80 µL of the broth suspension to all wells and incubated them in a standard set (24 h incubation at 37°C). The turbidity evaluation was performed at 630 nm via a plate reader (Synergy HTX Multi-Mode Reader, USA) and the bacterial growth was assessed through Equation 1.

Equation 1: $Growth\ (\%) = \frac{absoprtion\ of\ treated\ wells}{absorption\ of\ control} \times 100$

The disinfection potential of *Ferula Gummosa* essential oil (FGEO)

FGEO was prepared in two different concentrations (4000 and 8000 µg) to measure its disinfection activity on 72 sample areas (3×3 cm^2) from different emergency trolley steel surfaces (three times). Firstly, the samples were cleaned using 70% ethyl alcohol and sterile cotton soaked in sterile water (3 times, each time from the center to the outside). Then, all samples were contaminated with bacterial agents by sterile swab, and were allowed to air-dry at room temperature. The bacterial strains were cultured in MHA and adjusted to a suspension of 0.5 McFarland turbidity standard for 24 to 48 hrs (approximately 1.5×10^8 bacterial colonies). Finally, all surfaces were divided into four groups (alcohol as negative control, untreated surface as positive control, 4000 FGEO, and 8000 FGEO). Each group was divided into four subgroups of bacterial species (*S. aureus* and *P. aeruginosa*). The final samples were gathered after 1, 3, and 5 minutes. The samples were transferred to the laboratory at -20 ºC to prevent potential growth. Each sample was vortexed for 1 minute, and 100 µL of the sample was cultured on blood agar medium. The inoculated broth samples were kept at 37°C for 24 hrs. Finally, the bacterial colonies of each sample were counted, and the mean was reported (13).

Statistical Methods

We carried out antibacterial tests in triplicate. SPSS software (Version 22, SPSS Inc, USA) was utilized to calculate the means ± standard deviations and figures preparation. We employed a regression model (performed in CalcuSyn software, Free version, BIOSOFT, UK) to calculate the IC50 values of all EOs. Then, we compared all IC50 values of the EOs together using one-way analysis of variance (ANOVA) and the independent sample t-test. The confidence interval of this study was set at 95% (CI 95%).

Results

Chemical Composition

Table 2 represents the results of GC-MS analysis of the FGEO (which is the plant with the highest effect). FGEO primarily contained β-pinene (58.70%), followed by bulnesol (6.78%), guaiol (6.52%) and α-pinene (4.08%) as the predominant bioactive compounds.

Table 2. The components of Ferula Gummosa essential oil

No	RT	%	Components	KI	Type
1	11.36	4.08	α-pinene	935	MH
2	13.44	0.44	sabinene	976	MH
3	13.77	58.70	ß-pinene	983	MH
4	14.27	2.14	myrcene	993	MH
5	15.26	1.26	3-carene	1012	MH
6	16.40	0.64	limonene	1034	MH
7	16.51	0.40	ß-phellandrene	1036	MH
8	16.74	0.67	trans- ß-ocimene	1040	MH
9	24.34	0.57	trans-galbanolene	1192	Other
10	37.78	0.82	alloaromadendrene	1492	SH
11	40.52	0.88	elemol	1561	SO
12	42.43	6.52	guaiol	1609	SO
13	44.80	1.10	ß-eudesmol	1673	SO
14	45.09	6.78	bulnesol	1680	SO
		84.98	Total identified		

Abbreviations: RT, Retention time; KI, Kovalts index; MH, Monoterpene Hydrocarbons; SH, Sesquiterpene Hydrocarbons; SO, Oxygenated Sesquiterpenes

Antibacterial Effects

Chart 1A shows the antibacterial effects of various concentrations (0.03-8.00 mg/mL) of *Cuminum cyminum* EO (CCEO). The highest effects were reported against standard *S. aureus* (SSA, 32%) and clinical *P. aeruginosa* (CPA, 47%) at an 8.00 mg/mL concentration. In addition, this EO decreased the growth of clinical *S. aureus* (CSA) and standard *P. aeruginosa* (SPA) with lower antibacterial effects (55% and 56%, respectively). The effect of *Artemisia sieberi* EO (ASEO) at different concentrations on the targeted bacterial growth is reported in Chart 1B. ASEO mostly restricted the growth of SSA (20%), while the other results showed lower antibacterial effects (CSA: 42%; SPA: 38%; CPA: 43%). As shown in Chart 1C, *L. nobilis* EO (LNEO) significantly inhibited the growth of SSA (39%), SPA (35%), and CPA (38%). Moreover, LNEO exhibited lower effects against CSA (51%). The antibacterial effects of FGEO were observed in Chart 1D. While the growth of CSA was decreased to 17%, the growth of SSA, SPA, and CPA was reduced to 39%, 36%, and 41%, respectively. *Lippia citriodora* EO (LCEO) conferred significant inhibitory effects as the concentration increased (Chart 1E). The growth of all targeted bacteria was inhibited. LCEO at a concentration of 8.00 mg/mL reduced the growth of CPA and CSA to 44% and 39%, respectively. On the

other hand, the growth restriction of the standard species was higher than that of the clinical specimens (SPA, 33%; SSA, 37%).

Results of the bacterial inhibitory effects of *Cymbopogon citratus* EO (CIEO) are shown in Chart 1F. Mostly, CIEO demonstrated antibacterial activity

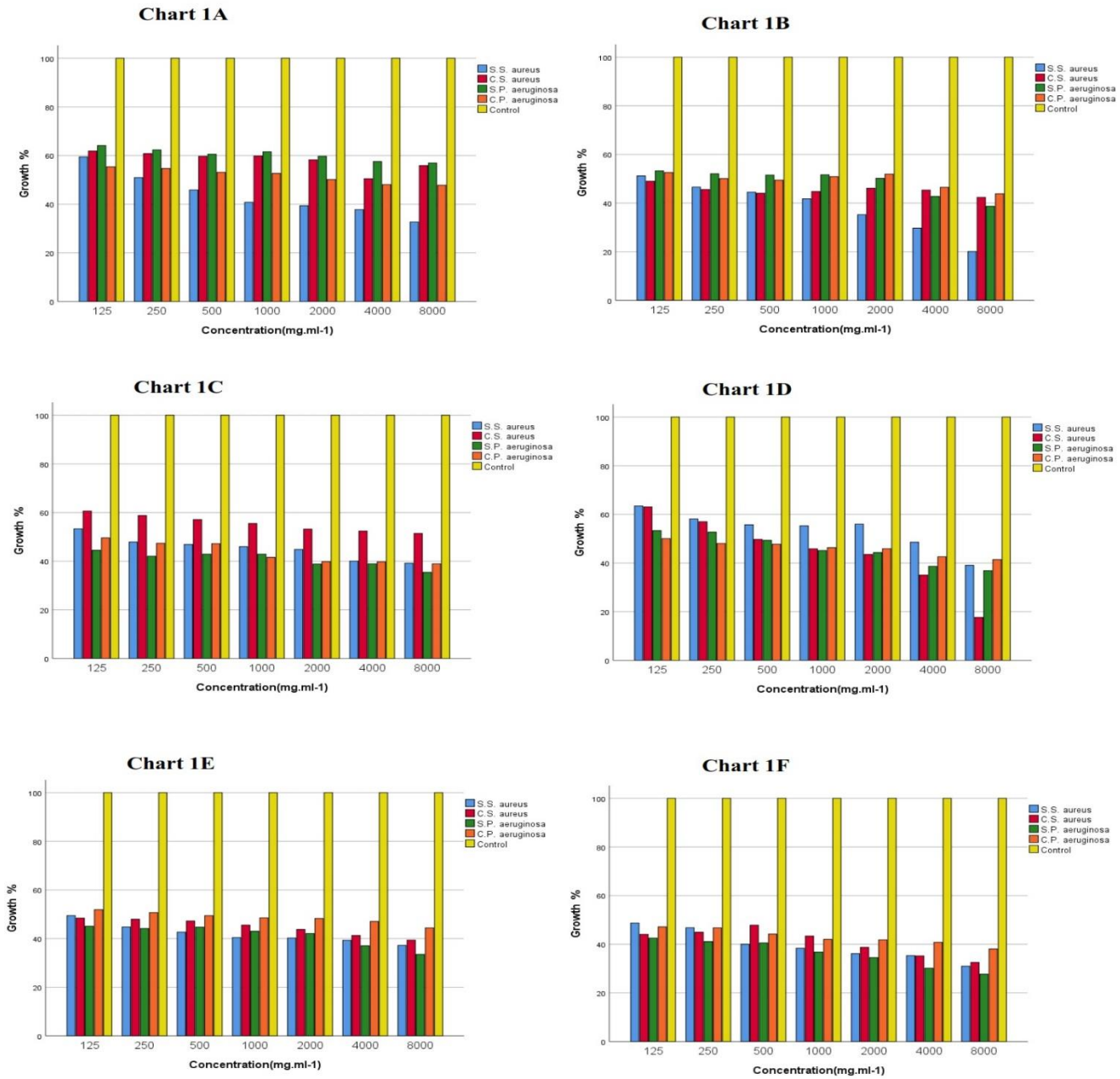


Chart 1. The antibacterial effects off essential oil. S.*S. aureus*, Standard *Staphylococcus aureus*; C.*S. aureus*, clinical *Staphylococcus aureus*; S.*P. aeruginosa*, Standard *pseudomonas aeruginosa*; C.*P. aeroginosa*, clinical *pseudomonas aeruginosa*. (A) *Cuminum cyminum* essential oil. (B) *Artemisia sieberi* essential. (C) *Lauruse Nobilis* essential oil. (D) *Ferula Gummosa* essential oil. (E) *Lippia Citriodora* essential oil. (F) *Cymbopogon Citratus* essential oil

against SPA (27%), but CIEO in total showed significant inhibitory effects (more than 50% bacterial growth inhibition) on all the other species (SSA, 30%; CSA, 32%; CPA, 38%).

Disinfection activity of FGEO on trolley surface

Table 3 outlines the antibacterial effects of FGEO on the surface of a trolley contaminated with standard and clinical strains of *S. aureus* and *P. aeruginosa*. FGEO significantly decreased the growth of these bacterial agents in all samples. In addition, the antibacterial effects were positively related to the concentration and the time of exposure.

Table 3. Antibacterial effects of FGEO on trolley surface contominated with S. S. aureus, C.S.aureus, S.P.aeroginosa, C.P.aeroginosa.

Groups	Positive Control				4000 mg/mL				8000 mg/mL				Negative Control			
Time of exposure to FGEO (minutes)	1	3	5	10	1	3	5	10	1	3	5	10	1	3	5	10
The mean number of S.*S. aureus* colonies	10^6	10^6	10^6	10^6	10^4	10^3	10^3	10^3	10^3	10^2	10^2	10^2	0	0	0	0
The mean number of C.*S. aureus* colonies	10^6	10^6	10^6	10^6	10^4	10^3	10^3	10^3	10^3	10^2	10^2	10^2	0	0	0	0
The mean number of S.*P. aeruginosa* colonies	10^6	10^6	10^6	10^6	10^5	10^4	10^3	10^3	10^4	10^4	10^3	10^2	0	0	0	0
The mean number of C.*P. aeruginosa* colonies	10^6	10^6	10^6	10^6	10^5	10^4	10^3	10^3	10^4	10^4	10^3	10^2	0	0	0	0

Abbreviations: Positive Control, washed with normal saline; Negative Control, washed with alcohol; FGEO, *Ferula Gummosa* essential oil; S.*S. aureus*, Standard *Staphylococcus aureus*; C.*S. aureus*, clinical *Staphylococcus aureus*; S.*P. aeruginosa*, Standard *pseudomonas aeruginosa*; C.*P. aeruginosa*, clinical *pseudomonas aeruginosa*

Discussion

The present study aimed to evaluate the antibacterial effects of six EOs at various concentrations on standard and clinical isolates of two common nosocomial pathogens (*S. aureus* and *P. aeruginosa*). In addition, we selected the most efficient antibacterial EO to investigate its antibacterial effects on nosocomial surfaces.

Generally, *S. aureus* is found approximately all over the environment, especially on the skin and nasal cavity of the body and is among opportunistic pathogens causing NIs (18). Our results revealed that ASEO had the highest antibacterial activity against SSA among the examined EOs. Subsequently, CCEO and CIEO re-presented acceptable effects, but the other EOs had lower inhibitory activity against SSA.

P. aeruginosa is another opportunistic pathogen that belongs to Gram-negative bacteria causing NIs. In total, the experimented EOs in the present study exerted lower antibacterial effects against *P. aeruginosa*. The highest effects were observed in the case of CIEO, and the lowest effect was related to CCEO (19).

The genus *Artemisia* is a well-known medicinal plant that grows worldwide (20). Chemical analysis revealed that ASEO is mostly composed of Artemisia ketone and 1,8-cineole. Moreover, Vahdani et al. reported that the main compounds of ASEO were piperitone, camphor, and 1, 8-cineole (21). On the other hand, a recent GC-MS analysis indicated that

Camphor, 1,8-cineole, and β-Thujone were the major components of ASEO (22). Therefore, we can conclude that 1,8-cineole is a prominent component in the chemical composition of ASEO conferring antibacterial effects. Different species of the genus *Artemisia* have promising antibacterial activities (23). Also, *A. sieberi* products indicated significant inhibitory effects on both Gram-negative and Gram-positive strains (24). Hedayati rad et al. developed a novel Pullulan film incorporated with ASEO to evaluate its protective effects against different bacterial pathogens and revealed the ASEO concentration-dependent inhibitory effects (at least to 10%). The highest inhibitory zone (18.52 ± 0.73) was observed at 35% (25). Pure ASEO inferred greater inhibitory zones against *S. aureus* and *P. aeruginosa* in MHA media. Besides, incorporating basal seed gum films with ASEO resulted in a large inhibitory zone (23.2 ± 0.7 mm) on plates containing *S. aureus* and a smaller inhibitory zone (10.2 ± 0.6 mm) in the case of *P. aeruginosa* (26). Supporting the results of the present study, others also reported that ASEO represented valuable (MIC of 10 µg/mL) and minimal bactericidal concentration (MBC) (20 µg/mL) against *S. aureus* (21). Moreover, recent studies on synergistic antibacterial effects of ASEO combined with different routine antibiotics demonstrated that ASEO synergistic effects with ampicillin, oxacillin, cephalothin, piperacillin, and vancomycin against *S. aureus*. On the other hand, the combination of various antibiotics, such as piperacillin, gentamicin, ampicillin, and erythromycin with ASEO indicated significant synergistic effects on the growth of *P. aeruginosa* (27). Therefore, using pure ASEO and its combination with different synthetic antibacterial agents has provided novel antibacterial agents with higher inhibitory effects.

Chemical composition analysis indicated that CCEO mostly contains 1-phenylpropanol and gamma-terpinene (28). CCEO has mainly contained cumin aldehyde and gamma-terpinene. On the other hand, the first evaluations revealed that isobutyl isobutyrate, α-thujene, and α-pinene are the most common compositions in CCEO. CCEO has protective role in food production and storage industries (29). Further studies have demonstrated that the most protective CCEO concentrations include at 0.03% inhibiting *S. aureus* growth in feta cheese and infected hamburgers (28). CCEO demonstrated promising MBC and MIC values on SSA (2.5 to 5 µg/mL, and 1.25 to 5 µg/mL). Moreover, this study unveiled that CCEO had the same antibacterial effect on SSA as vancomycin (30). CCEO has significantly diminished the CPA with higher efficiency than several antibiotics such as gentamicin, amoxicillin, and clotrimazole (31). Therefore, utilizing CCEO in preventing and treating procedures could decrease the limitations of routine antibiotics in the future, but the controversies of its antibacterial effects on Gram-negative strains remained for further studies.

C. citratus has a valuable preservative usage in food industries to restrict the activity of common food-borne pathogens. Utilizing CIEO in the preservation of cooked beef patties would preserve its quality and prevent the growth of pathogenic bacterial strains (32). The evaluations revealed that neral, geranial, limonene, and geranyl acetate are the major components of CIEO. *C. citratus* products mainly inhibit the growth of fungal (*Candida strains*) and Gram-positive bacterial species, such as *S. aureus* biofilms. Based on the last *in vitro* studies, the CIEO provides the same antibacterial effects on *S. aureus* as vancomycin and can be a favorable product to prevent the growth of nosocomial pathogens with fewer adverse effects in the future (33). In addition, a recent study on EO from the leaves of *C. citratus* indicated a larger zone of inhibition in the case of *S. aureus* (32.0 ± 0.75 mm) and no antibacterial effects on *P. aeruginosa*. Nevertheless, other studies have deciphered that *C. citratus* extracts represent higher antibacterial effects on Gram-negative strains such as *P. aeruginosa*. Subramanian et al. reported that the leaf and root extracts resulted in a very large zone of inhibitions (> 30 mm), and could be encouraging

herbal products against these pathogenic Gram-negative strains (34). Moreover, the results of our study represented that CIEO mainly decreased the growth of SPA higher than the other targeted strains. Therefore, further studies are required to evaluate these controversial results.

L. citriodora and hundreds of other species in the Lippia genus are well-known ancient plants utilized for several decades, especially in Western regions of the world. GC-MS analysis reported that neral and geranial are the major compounds of the experimented LCEO (35). In addition, other studies added terpenic alcohols, monoterpenic hydrocarbons (dl-limonene), and α-curcumene. Several studies reported various medicinal properties, such as sedative, diuretic, and antispasmodic uses in recent years. Moreover, recent papers introduced LCEO as a moderate antibacterial product (36).

Although pure LCEO provided an acceptable zone of inhibition on sterile Whatman paper discs infected with Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) strains, this EO mainly affects Gram-negative strains more than Gram-positive species. On the other hand, some other studies have represented controversial results. Evaluating the effects of LCEO on different pathogens showed that this EO has no significant inhibitory or bactericidal effects on *P. aeruginosa* with higher MIC and MBC values against *E. coli* than *S. aureus* (37). Therefore, the present study and all other studies have introduced LCEO as an encouraging antibacterial product, especially against Gram-positive strains, but further studies are required to put an end to the controversial results in the case of *P. aeruginosa*.

L. nobilis is another aromatic plant with antimicrobial and flavor properties in food industries. According to its high requirement of enough water and humidity, *L. nobilis* usually grows in warmer climate places such as the South of the Mediterranean region. Methyl eugenol, 1,8-cineole, and α-terpinyl acetate are the major compounds of LNEO. LNEO has significant inhibitory effects on all Gram-positive and -negative strains, like *P. aeruginosa*, *S. aureus*, and *E. coli*. The measurements of LNEO inhibition zone showed higher antibacterial effects than tetracycline (8 mg/mL), especially in the case of Gram-positive strains. The evaluations revealed that LNEO has high antibacterial effects on Gram-positive strains such as *S. aureus* (inhibition zone = 13 mm), *S. intermedius*, and *K. pneumoniae*. Also, LNEO has significantly reduced the biofilm production with acceptable MIC, ranging from 0.3 to 96.4 mg/mL (38). Further comparisons have unveiled that Gram-positive strains are more susceptible to LNEO than Gram-negative strains like *P. aeruginosa* and *E. coli*. The recent evaluations demonstrated that MIC of LNEO was equal to 0.25 mg/mL. On the other hand, LNEO has exerted remarkable effects against common foodborne pathogens, such as *E. coli* (39).

The aromatic herbal families, such as *Apiaceae*, are common plants growing in different regions of Iran. The genus of *Ferula* is a medicinal herb mostly found in the Eastern Mediterranean region and north Africa (40). EO from *F. gummosa* seeds mostly showed strong antibacterial effects on Gram-positive strains and low inhibitory activity against Gram-negative strains, such as *P. aeruginosa*. Abbaszadegan et al. reported that β-pinene account for > 50% of the chemical composition of FGEO. Moreover, FGEO at 50 µg/mL resulted in **a** large inhibitory zone (22.5 ± 0.82 mm) culture medium containing *S. aureus* (41). Considering the antibacterial effects on both *S. aureus* and *P. aeruginosa*, our findings inferred that FGEO is the most effective agent among the evaluated EOs. As a result, we selected FGEO to investigate its disinfection activity on the surface of an emergency hospital ward.

In the last two decades, several studies have attempted to use EO-based disinfectants instead of conventional disinfectants. Sharma et al. indicated that a novel green disinfectant, named Neutral Biodegradable Disinfectant (NBD), would appropriately clean the contaminated

surfaces. Their findings confirmed that phenol-based disinfectants and NBD products disinfect environmental microorganisms equally (42). As a result, given that natural products provide much less resistance and reduce the population of microorganisms evenly, many of these products are expected to replace conventional phenol-based disinfectants in the coming years (43). The increasing bacterial resistance to conventional disinfectants has resulted in a great challenge in different healthcare services, especially in dental procedures (44). In recent years, several observations have demonstrated that green disinfectants would become one of the main agents to provide sterile dental practices. Aloe vera gel at high concentrations (90%) is a safe disinfectant and shows fewer poor outcomes than conventional chemical products (45). In addition, our findings supported the previous results. FGEO relatively could decrease the growth of both bacterial agents on the trolley surface. FGEO developed its antibacterial activity in a concentration-dependent manner. Therefore, the application of FGEO-based disinfectants would not be able to completely clean nosocomial surfaces, similar to conventional and novel green disinfectants (46).

The present study had several strengths and limitations. The main strength of our study was the comparison of the antibacterial effects of several EOs on clinical and standard strains of two major Gram-positive and Gram-negative strains. Based on our expanded literature review of previous studies, we mainly hypothesized that the Gram-negative strains and clinical samples would exhibit more resistance against different antibacterial EOs. Generally, the findings supported these hypotheses with little controversy (especially in the case of CIEO and LNEO). Moreover, we faced some conflicts compared to previous studies. On the other hand, we just reported the inhibitory activity of EOs on bacterial growth in percent. Therefore, further studies with more targeted pathogens and expanded measurements are required to collect sufficient data and resolve the observed controversies.

Conclusion

This study indicated that all the assessed EOs have encouraging antibacterial effects against the targeted pathogens. Some products, such as CCEO, ASEO, and FGEO exhibited higher inhibitory effects against *S. aureus*, and CIEO and LNEO had higher inhibitory effects against *P. aeruginosa*. Therefore, further studies are required to evaluate the antibacterial effects of the combination of these EOs and utilize novel techniques, such as nanoparticles, to develop more efficient natural products and prevent life-threatening NIs. Since EOs can have synergistic or antagonistic effects on each other, it is suggested to investigate the effects of the combination of EOs.

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Conflict of Interest

The authors declare no competing interest.

Funding

This study was approved by Fasa University of Medical Sciences with 99174 code.

Ethical Considerations

This study was conducted in accordance with ethical considerations.

Code of Ethics

IR.FUMS.REC.1400.004

Authors' Contributions

This study was designed and supervised by Z.B. and A.A. L.N. and Z.B. A.A. participated in sample collection and conducting experiments. Statistical analysis, interpretation, and drafting of the paper were conducted by A.A. L.N. and Z.B. revised the paper.

All authors have read and approved the manuscript for publication.

Data Availability Statement

The data that support the findings of this study are available via corresponding author, [Abbas Abdollahi], upon reasonable request.

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