

Antibacterial Activity of Some Iranian Plant Essential Oils as Disinfectant Agents on Surfaces Contaminated with *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*

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Article

Keywords: Essential oil, Antibacterial activity, Microdilution, Disinfectant

Posted Date: January 31st, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2467112/v1>

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Abstract

The component and antibacterial effects of six essential oils (EOs), including *Cuminum cyminum* (CCEO), *Artemisia sieberi* (ASEO), *Laurus nobilis* (LNEO), *Ferula Gummosa essential* (FGEO), *Lippia citriodora* (LCEO), *Cymbopogon citratus* (CIEO) were measured by GC-MS and 96-well micro-plates (IC₅₀), against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Then, the antibacterial effects of FGEO, the most efficient EO, were examined on the trolley surface in hospital for 1, 3, 5 and 10 min intervals. While CCEO, ASEO, and FGEO have the highest effects on the growth of *S. aureus*, CIEO and LNEO showed the best outcomes on *P. aeruginosa*. In addition, our finding showed that FGEO relatively decreases the growth of *S. aureus* and *P. aeruginosa* on the trolley surface ($P < 0.05$). In addition, FGEO, one of the best disinfectant agents of the present study, showed relatively antibacterial effects on the surface of the trolley.

Introduction

Nosocomial infections (NIs) are acquired infections that are present during the healthcare procedure and do not appear before the hospitalization. In recent years, the prevalence of various NIs mostly grew in America (3.2%) and Europe (5.7%)¹. The last reports showed that the mean prevalence of NIs in Iranian hospitals (0.71%) is significantly less than worldwide statistics, but some reports demonstrated that the prevalence of NIs in some Iranian hospitals and other Asian clinics would reach up to 9.1%. On the other hand, NIs became one of the main causes of death in the last decades². Multiple studies reported that different microorganisms would cause NIs; but, among them, some bacterial species, such as *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), as significant part of NIs³. *S. aureus* is one of the most common reasons for surgical infections. Moreover, both *S. aureus* and *P. aeruginosa* would play the main role in Central Line-Associated Blood Stream Infections⁴.

In recent years, we faced new difficulties in controlling NIs. Studies on resistant microorganisms demonstrated that more than 60% of different pathogenic bacteria would present significant resistance to routine antibiotics⁵. In the last 10 years, the increasing growth of resistant *S. aureus* and other microbial agents in NIs cases has demanded new valuable agents to protect hospitalized patients⁶. On the other hand, the resistant serotype of *P. aeruginosa* had increased recently and showed remarkable resistance against gentamicin and ciprofloxacin. Besides, common antimicrobial agents have had low efficiency in controlling the growth of NIs in recent years. The last evaluations revealed that the resistant gram-positive and gram-negative bacterial agents developed in the Iranian hospitals and we require novel agents to prevent NIs morbidity and mortality⁷.

In the last years, numerous herbal products with commercial uses as well as sedative and antioxidant properties have become available worldwide. Therefore, we can use these products without worrying about consumer rejection. On the other hand, herbal extracts show more antimicrobial potentials with the least adverse effects⁸. Studies showed that herbal essential oils (EOs) and extracts with antimicrobial properties would prevent the activities of different bacterial agents⁹.

Recently, novel studies revealed several herbal extracts with promising antimicrobial effects on different microorganisms and provided new complementary treatments with the least side effects¹⁰. Moreover, several recent studies provided multiple antimicrobial products from Iranian herbal species¹¹. Various studies have shown that the use of plant EOs has encouraging antibacterial effects. Today, a large number of plant essential oils with significant antimicrobial effects, including *Myrtle communis*, *Thymus vulgaris*, *Lavandula angustifolia*, and *Mentha pulegium*, are known to have good uses in suppressing bacterial growth¹². On the other hand, although several chemical disinfectant agents have been developed and achieve acceptable level of disinfection, some practices showed that

these agents would show multiple bacterial resistance¹³. Using plant EOs in cleansing gels showed similar effectiveness to previous synthetic products with fewer side effects. Therefore, the present study aimed to evaluate the antibacterial activity of different herbal extracts on *S. aureus* and *P. aeruginosa*. Then, the most effective EOs were applied on the hospital surfaces contaminated with *S. aureus* and *P. aeruginosa* to achieve novel natural agents to restrict NIs in the future.

Results

Chemical composition

Table 1. Shows the results of GC-MS analysis of FGEO (the plant with the highest effect). The analysis showed that FGEO mainly contains β -Pinene. It seems that this combination is the most effective one based on the high amount of β -pinene compound and based on the results of other articles.

Table 1. 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	-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	-phellandrene	1036	MH
8	16.74	0.67	trans- -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	-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

Fig. 1a shows the antibacterial effects of various concentrations (0.03-8.00 mg/mL) of *Cuminum cyminum* essential oil (CCEO). The highest effects were reported against standard *S. aureus* (SSA, 32%) and clinical *P. aeruginosa* (CPA, 47%) at 8.00 mg/mL CCEO. In addition, this EO decreased the growth of clinical *S. aureus* (CSA) and standard *P.*

aeruginosa (SPA) with fewer significant antibacterial effects (55% and 56%). The effect of *Artemisia sieberi* essential oil (ASEO) at different concentrations on the targeted bacterial growth is reported in Fig. 1b. ASEO mostly restricted the SSA growth (20%), while other results showed lower antibacterial effects (CSA: 42%; SPA: 38%; CPA: 43%). As shown in Fig. 1c, *Laurus nobilis* essential oils (LNEO) significantly inhibited the growth of SSA (39%), SPA (35%), and CPA (38%). Moreover, LNEO decreased the activity of CSA with fewer effects (51%).

The antibacterial effects of FGEO were observed in Fig. 1d. Totally, FGEO showed one of the best results in the present study and remarkably inhibited all spp. While the growth of CSA was decreased to 17%, the growth of SSA, SPA, and CPA was reduced to 39%, 36%, and 41%.

Lippia citriodora essential oils (LCEO) showed significant inhibitory effects with increasing the concentration (Fig. 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%. On the other hand, the growth restriction of standard spp. higher than in clinical specimens (SPA, 33%; SSA, 37%).

Results of bacterial inhibitory effects of *Cymbopogon citratus* essential oil (CIEO) are shown in Fig. 1f. Mostly, CIEO showed antibacterial activity on SPA (27%), but CIEO totally showed significant inhibitory effects (more than 50% bacterial growth inhibition) on all the other spp. (SSA, 30%; CSA, 32%; CPA, 38%).

Disinfection activity of FGEO on trolley surface

Table 2 shows the antibacterial effects of FGEO on the surface of trolley contaminated with standard and clinical *S. aureus* and *P. aeruginosa*. FGEO significantly decreased the growth of bacterial agents in all samples. In addition, the antibacterial effects were positively related to the concentration and the time of exposure.

Table 2. antibacterial effects of FGEO on trolley surface contaminated with *S. S. aureus*, *C.S.aureus*, *S.P.aeruginosa*, *C.P.aeruginosa*.

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 the various concentrations on standard and clinical species 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. On the other hand, *S. aureus* is one the most opportunistic pathogens that would result in several nosocomial infections ¹⁴. The results showed that ASEO had the best antibacterial effects on SSA among examined EOs. In the following, CCEO and CIEO represented acceptable effects, but the other EOs had the lower inhibitory activity on SSA.

P. aeruginosa is another opportunistic pathogen that belongs to gram-negative strains. The genus *Pseudomonas* is available to survive in various difficult environments and causes life-threatening nosocomial infections. Totally, the experimented EO in the present study showed fewer antibacterial effects against *P. aeruginosa*. The best findings were observed in the case of CIEO, but the other EOs showed significant inhibitory effects, except CCEO ¹⁵.

The genus *Artemisia* is a well-known medicinal plant that grows worldwide ¹⁶. *Artemisia sieberi* is a special species of this large genus usually found in the northern regions of Iran and Saudi Arabia. Chemical analysis revealed that ASEO is mostly made up 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 ¹⁷. On the other hand, a recent GC-MS indicated that Camphor, 1,8-

cineole, and β -Thujone were the first major components of ASEO¹⁸. Therefore, we can conclude that 1,8-cineole is an improved component in the chemical composition of ASEO and would be one of the major antibacterial agents of this EO. Different species of genus *Artemisia* have encouraging antibacterial activities¹⁹. Also, *A. sieberi* products indicated great inhibitory effects on both Gram-negative and Gram-positive strains²⁰. In 2013, Hedayati rad et al. conducted a novel Pullulan film incorporated with ASEO to evaluate its protective effects against different bacterial pathogens. This study revealed that ASEO at low concentrations (5%) has no inhibitory effects on bacterial growth, but with increasing the concentration (at least to 10%) the antibacterial effects would develop. The highest inhibitory zone (18.52 ± 0.73) was observed at 35%²¹. Pure ASEO made large inhibitory zones against *S. aureus* and *P. aeruginosa* in Muller Hinton agar plates. Besides, incorporating basal seed gum films with ASEO resulted in a big inhibitory zone (23.2 ± 0.7 mm) on plates infected with *S. aureus* and a smaller inhibitory zone (10.2 ± 0.6 mm) in the case of *P. aeruginosa*²². Supporting the results of the present study, others also reported that ASEO represented valuable MIC (10 μ L) and minimal bactericidal concentration (MBC) (20 μ L) against *S. aureus*¹⁷. Moreover, recent studies on synergic antibacterial effects of ASEO combined with different routine antibiotics demonstrated that ASEO would increase the inhibitory effects of ampicillin, oxacillin, cephalothin, piperacillin, and vancomycin on *S. aureus*. On the other hand, the combination of various antibiotics, such as piperacillin, gentamicin, ampicillin, and erythromycin with ASEO indicated significant synergic effects on the growth of *P. aeruginosa*²³. Therefore, using pure ASEO and its combination with different products would provide novel antibacterial materials that represent higher inhibitory effects and decrease the difficulties of previous routine antibiotics.

Chemical composition analysis indicated that CCEO mostly contains 1-phenylpropanol and gamma-Terpinene²⁴. Besides, Hajlaoui et al. reported that CCEO mainly comprised cuminaldehyde 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 is believed to have a protective role in food production and storage industries²⁵. Further studies demonstrated that the most protective CCEO concentrations will appear at 0.03% that prevented the *S. aureus* growth in feta cheese and infected hamburgers²⁴. CCEO demonstrated promising MBC and MIC on SSA (2.5 to 5 μ g/mL, and 1.25 to 5 μ g/mL). Moreover, this study showed that CCEO had the same antibacterial effect on SSA as vancomycin, a highly used antibiotic with several adverse effects²⁶. On the other hand, some other studies supported our results. Saei et al. reported that the extract and EO from *C. cyminum* significantly diminished the CPA with higher efficiency than several antibiotics such as gentamicin, amoxicillin, and clotrimazole²⁷. Therefore, utilizing CCEO in preventing and treating procedures would 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 protecting usage in food industries to restrict the activity of common food-borne pathogens. Utilizing CCEO in the preservation of cooked beef patties would save its quality and prevent the growth of pathogenic bacterial strains²⁸. The evaluations showed that neral, geranial, limonene, and geranyl acetate are the major components of CCEO. *C. citratus* products mainly prevent the growth of fungal (*Candida* strains) and Gram-positive bacterial species, such as *S. aureus*, biofilms. Based on the last in vitro trials, the CCEO provides the same antibacterial effects on *S. aureus* as vancomycin and would be a favorable product to prevent the growth of nosocomial pathogens with fewer adverse effects in the future²⁹. In addition, a recent study on EO from the leaves of *C. citratus* indicated a bigger zone of inhibition in the case of *S. aureus* (32.0 ± 0.75 mm) and no antibacterial effects on *P. aeruginosa*. Nevertheless, the other studies showed that *C. citratus* extracts would represent more 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 would be encouraging herbal products against these pathogenic gram-

negative strains³⁰. Moreover, the results of our study represented that CIEO mainly decreased the growth of SPA more 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 experimented LCEO³¹. In addition, other studies added terpenic alcohols, monoterpenic hydrocarbons (dl-limonene), and α -curcumene to previously reported chemical components. Several studies reported various medicinal properties, such as sedative, diuretic, and antispasmodic use in recent years. Moreover, the recent papers introduced LCEO as a moderate antibacterial product³². 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 the gram-negative strains more than gram-positive species. On the other hand, some other studies 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* and the MIC and MBC are higher in the case of *E. coli* than *S. aureus*³³. Therefore, the present study and all other studies introduced LCEO as an encouraging antibacterial product, especially for gram-positive strain, but further studies are required to put an end to the controversial results in the case of *P. aeruginosa*.

Laurus nobilis is another aromatic plant with antimicrobial and flavor properties in food industries. According to the 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*, *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 significantly reduces the biofilm activity with acceptable MIC, ranged from 0.3 to 96.4 mg/mL³⁴. Further comparisons showed that gram-positive strains are more susceptible to LNEO than *P. aeruginosa* and *E. coli*. The recent evaluations demonstrated that minimal inhibitory activities of LNEO would be represented at 0.25 mg/mL. On the other hand, LNEO showed remarkable protective effects against common foodborne pathogens, such as *E. coli*³⁵.

The aromatic herbal families, such as *Apiaceae*, are common plants that grow in different regions of Iran. The genus of *Ferula* is a medicinal herb mostly found in EMERO and north Africa floras³⁶. Several studies revealed different protective effects of *F. gummosa* against infection, inflammatory and spasmodic processes. EO from *F. gummosa* seeds mostly showed strong antibacterial effects on gram-positive strains and slight inhibitory activity against gram-negative strains, such as *P. aeruginosa*. Abbaszadegan et al. reported that β -pinene made up more than 50% of the chemical composition of FGEO. Moreover, FGEO at 50 μ g/mL resulted in a big inhibitory zone (22.5 ± 0.82 mm) on plates infected with *S. aureus*³⁷. Considering the antibacterial effects on both *S. aureus* and *P. aeruginosa*, our findings showed that FGEO is the most effective agent among the evaluated EOs in the present study. As a result, we selected FGEO to investigate its disinfection activity on the surface of emergency hospital.

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 (BND) would appropriately clear the contaminated surfaces. Their findings confirmed that phenol-based disinfectants and NBD products disinfect environmental microorganisms equally³⁸. 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 years³⁹. Increasing bacterial resistance to

conventional disinfectants resulted in a great challenge in different healthcare services, especially in dental procedures⁴⁰. In recent years, several observations indicated that green disinfectants would become one of the main agents to provide steel dental practices. In 2019, Nausheen et al. confirmed that aloe vera gel at high concentrations (90%) is a safe disinfectant and shows less poor outcomes than conventional chemical products⁴¹. In addition, our findings supported the previous results. FGEO relatively could decrease the growth of both bacterial agents on the trolley surface. Although the increasing concentration of FGEO developed its antibacterial activity, a significant population of bacterial colonies grew on the evaluated samples of our study. Therefore, the application of FGEO-based disinfectants would not be able to completely clear nosocomial surfaces, similar to conventional and novel green disinfectants⁴².

The present study has several strengths and limitations. The main strength of our study was the comparison of the antibacterial effects of above-mentioned EOs on clinical and standard samples of two major gram-positive and gram-negative strains. Based on our expanded literature review on previous studies, we mainly hypothesized that the gram-negative strains and clinical samples would represent 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 in comparison with previous studies. On the other hand, we just reported the inhibition activity of EOs on bacterial growth in percent. Therefore, further studies with more targeted pathogens and expand measurements would be required to collect more sufficient data and resolve observed controversies.

Methods

Bacterial species

In this study, we obtained standard bacterial species from the Microbiology laboratory of Pasteur Institute of Tehran (Standard species of *S. aureus*, ATCC 25923; Standard species of *P. aeruginosa*, ATCC 27853). On the other hand, we used clinical isolated Methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* Metallo-beta-lactamase (MBL)-producing from wound infection from previous studies of our researches. Also, we utilized molecular method (PCR test) and the routine culture media (the disk-diffusion method and minimal inhibitory concentration (MIC) test) tests to identify phenotypically and genotypically MBL-producing *P. aeruginosa* and MRSA⁴³. We obtained all EOs from Tabib Daroo Company (a pharmaceutical company in Kashan). Herbarium specifications of each plant are shown in the Table 3.

Table 3. Herbarium specifications of applied plants

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

Bacterial culture

We used Muller Hinton broth, containing 0.5% DMSO for bacterial growth (Merck Chemicals, Germany) ⁴⁴.

Gas Chromatography/Mass Spectrometry analysis

GC-MS analysis was deployed to identify the compounds of the selected herbal EOs. We utilized a 7890A Network GC system alongside a 5975A massive detector (Agilent Technologies, Santa Clara, CA, USA). The compounds of EOs were separated via fused columns of HP-5MS silica (length, 30 meters; internal diameters, 250 μ M; film thickness, 25 μ M). The temperature was set first at 50°C, increased gradually to 200°C (5°C per minute), and remained at this temperature for 1 minute. Then, we elevated the temperature up to 250°C (10°C per minute) and again increased to 300°C with higher rate (25°C per minute). On the other hand, helium was utilized to carry the ingredients. At last, we analyzed the components based on our previously described method ⁴⁵.

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 purposed bacterial species based on standard protocols ⁴⁶. We achieved 1.5×10^8 CFU/mL of bacterial colonies to pass the 0.5 McFarland turbidity. Then 20- μ L suspension was added to all wells. After preparation of bacterial agents, we dissolved EOs in Muller 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⁻¹). After described dilution, we added 80 μ L of broth suspension to all wells and incubated in standard set (24h incubation at 37°C). The turbidity evaluation was performed at 630 nm via plate reader (Synergy HTX Multi-Mode Reader, USA) and the bacterial growth was assessed through Equation 1.

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

Measurement of disinfection properties of *Ferula Gummosa* essential oil (FGEO) FGEO were prepared in two different concentrations (4000 and 8000 µg) to measure its disinfection activity on 72 samples (3 × 3 cm²) from different emergency trolley steel surfaces (three times). First, we cleaned the samples 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 steel swab and they were allowed to drain at room temperature. The bacterial strains were cultured in Muller Hinton Agar (MHA) and suspension of 0.5 McFarland for 24 to 48 hours (approximately 1.5 × 10⁸ 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 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 carried to the laboratory in a freezer to prevent probable growth. Each sample vortexed for 1 minute and 100 µL of the sample was cultured on blood agar. The broths were kept in an incubator for 24 hours at 37°C. Finally, the bacterial colonies of each sample were counted and the mean was reported ¹³.

Statistical methods

We carried out antibacterial tests in triplicates. SPSS software (Version 22, SPSS Inc, USA) was utilized to measure the means ± standard deviations and draw charts. We deployed a regression model (performed in CalcuSyn software, Free version, BIOSOFT, UK) to calculate IC₅₀ of all Eos; then, we compared all IC₅₀ of the Eos together via one-way ANOVA using SPSS software (Version 22, SPSS Inc, USA) independent sample t-test. The confidence interval of this study was set at 95% (CI 95%).

Conclusion

This study indicated that all experimented EOs has encouraging antibacterial effects against targeted pathogens. Some products, such as CCEO, ASEO, and FGEO represented higher inhibitory effects on *S. aureus* and the latter, including CIEO and LNEO, had better effects on *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 nosocomial infections. Since essential oils can have synergistic or antagonistic effects on each other, it is suggested to investigate the effects of the combination of essential oils. Nonetheless, it will be considered in our additional studies in the future.

Declarations

Acknowledgements

The authors wish to acknowledge the support of Fasa University of Medical Sciences for facilitating this research.

Author contributions

All authors contributed to the study conception and design.

Funding

This work was supported by a grant from the Fasa University of Medical Sciences.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Conflict of interest statement

All other authors have no conflicts of interest to declare.

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Figures

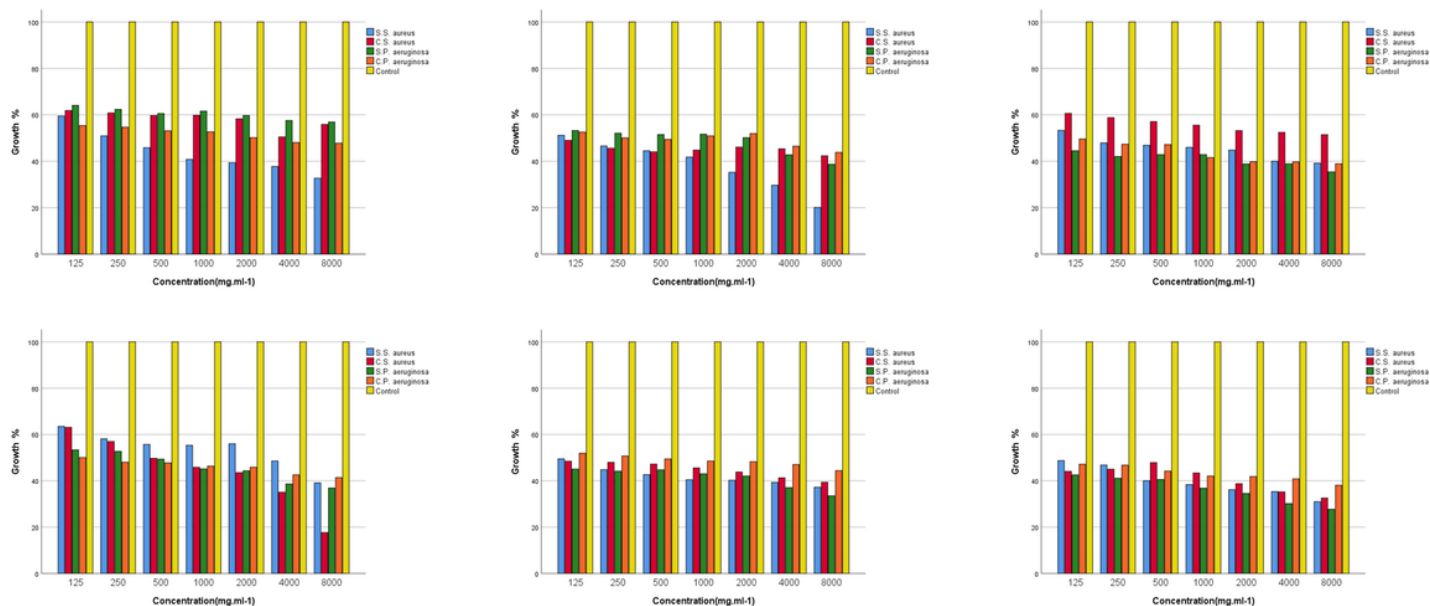


Figure 1

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. aeruginosa*, clinical *pseudomonas aeruginosa*. A) *Cuminum cyminum* essential oil. b) *Artemisia sieberi* essential. c) *Laurus Nobilis* essential oil. d) *Ferula Gummosa* essential oil. e) *Lippia Citriodora* essential oil. f) *Cymbopogon Citratus* essential oil