JJBS

Volume 17, Number 4,December 2024
ISSN 1995-6673
Pages 583 – 591
https://doi.org/10.54319/jjbs/170401

Jordan Journal of Biological Sciences

Synergistic Effect of *Coriander Sativum* Essential Oil and Gentamicin against Biofilm Formation of Some Pathogenic Bacteria

Eliza Hasen[1], Mohammad AA Al-Najjar[1,*], Shaymaa B Abdulrazzaq[1], Amin Omar[1], Wamidh H. Talib[1], Fatma U Afifi[1,2], Jehad almaliti[2,3], Asma Ismail Mahmod[1], Lujain F Alzaghari[1], Muna Barakat[1,*]

[1]*Faculty of Pharmacy, Applied Science Private University, Amman, Jordan;* [2]*Faculty of Pharmacy, University of Jordan, Amman, Jordan;* [3]*Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, San Diego, CA 92093, USA*

Received: January 5, 2024; Revised: March 24, 2024; Accepted: April 25, 2024

Abstract

Background: Essential oils extracted from aromatic plants and spices find diverse applications in food preservation, pharmaceuticals, and natural alternatives in healthcare and therapies. This study aimed to evaluate the antibacterial and antibiofilm of *Coriander sativum* essential oil (CEO) with/without the antibiotic Gentamicin against four bacterial strains: *Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*.

Methods: The Jordanian CEO was isolated using hydrodistillation and then analyzed using gas chromatography-mass spectrophotometry. Antibacterial and antibiofilm activities were assessed using a minimum biofilm eradication concentration assay (MBEC Assay®).

Results: The inhibitory and bactericidal effects of the CEO on all bacterial strains exhibited a concentration-dependent pattern. Among the strains, *P. aeruginosa* demonstrated the least susceptibility to CEO, with the highest minimum inhibitory concentration (MIC) recorded at 25 mg/mL. In contrast, the MIC values for *E. coli, S. aureus,* and *S. epidermidis* were 3.125, 3.125, and 6.25 mg/mL, respectively. Complete eradication was achieved for all tested bacterial strains at concentrations exceeding 50 mg/mL of CEO, except for *P. aeruginosa*. Achieving ~ 3 log reduction for *P. aeruginosa* necessitated a higher concentration of 200 mg/mL of CEO. Notably, the combination of Gentamicin with CEO resulted in either additive or synergistic activity, allowing for the use of lower concentrations of CEO to achieve antibacterial and antibiofilm effects.

Conclusion: The CEO extracted from Jordanian coriander seeds exhibited significant *in vitro* antibacterial and antibiofilm properties against *P. aeruginosa, S. aureus, S. epidermidis*, and *E. coli.* Notably, CEO demonstrated synergistic or additive effects when combined with Gentamicin, suggesting a potential strategy to alleviate antibiotic-associated side effects and enhance their antibacterial efficacy. These findings underscore the promising potential of CEO for future clinical applications, particularly in addressing challenges associated with chronic wounds.

Keywords: Antibacterial, antibiofilm, *Coriander sativum*, essential oil.

1. Introduction

Nowadays, the utilization of essential oils (EO) derived from aromatic plants and spices spans various domains, encompassing applications in food preservation, the pharmaceutical industry, and natural alternatives in medicine and therapies aimed at enhancing healthcare quality (Sulieman *et al.,* 2023). In response to an escalating demand for ingredients sourced from natural origins, essential oils are gaining prevalence in drinks, cosmetics, and toiletries (Cimino *et al.,* 2021). This shift is driven by concerns over the safety of synthetic additives, which have become progressively dubious over time (Aguiar Campolina *et al.,* 2023). Meeting the public need for natural extracts with pleasing sensory attributes and preservative efficacy is essential to thwart lipid deterioration, oxidation, and microbial spoilage. As essential oils primarily revolve around their impact on bacterial cell walls, alternate mechanisms such as disrupting enzymes, membrane proteins, or releasing cellular content subsequent to cytoplasmic membrane breakage are also under exploration (Langeveld *et al.* 2014; Zygadlo *et al.* 2017; Bueno *et al.* 2017; Delaquis *et al.* 2002; Baratta *et al.* 1998; Kačániová *et al.* 2020; Özkinali *et al.* 2017). The study of essential oils was historically dominated by considerations of flavor and fragrance (Fisher *et al.* 2008; Preedy, 2015). This interest is fueled by their perceived safety, consumer acceptance, and multifunctional potential (Ormancey, 2001).

Among plant-derived compounds, EOs from aromatic plants are one of the plant-based secondary metabolites

* Corresponding author. e-mail: moh_alnajjar@asu.edu.jo; m_barakat@asu.edu.jo

that are used as the basis of many modern pharmaceuticals to treat different illnesses (Kabera *et al.*, 2014). The effects of EOs and aroma principles on different body systems, including the respiratory system, gastrointestinal, nervous, and immune systems, as well as their anti-microbial, antifungal, and anticancer activities, have recently been the focus of interest for researchers (Amiri *et al.*, 2016). It is essential to underline that the nature and content of secondary metabolites in medicinal and aromatic plants are widely dependent on environmental factors. The geographic location, seasonal variation, temperature, rainfall, altitude, soil characteristics, the collection time and even the extraction method are known to affect EO's yield and chemical composition (Diao *et al.*, 2014).

Coriandrum sativum L., commonly known as coriander, is a noteworthy medicinal plant rich in essential oils distributed across its flowers, stems, leaves, and fruits/seeds (Klapper *et al.* 2010; Mandal *et al.* 2015). Renowned for its medicinal applications, coriander seeds have been traditionally employed to address a spectrum of health issues, including joint pain, gastrointestinal problems, flatulence, indigestion, insomnia, anxiety, convulsions, and loss of appetite.

Noteworthy, most of the studies focused on several prevalent bacterial pathogens that are known to form persistent biofilm, such as *Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa* (Abdallah *et al.,* 2014). These pathogens are still the vast majority of research interests looking for potential agents with complete eradication processes. Moreover, the dramatic increase in anti-microbial resistance (AMR) for pathogenic bacteria represents a serious problem for human health (ECDC, 2009). In agriculture and veterinary fields, it is crucial to reduce the usage of antibiotics. Instead, it would be more relevant to exploit synergistic interactions with natural products such as essential oil (Carlone *et al.*, 2018). A limited number of studies have investigated the synergistic effects between antibiotics and EOs like coriander essential oil against bacteria (Aljaafari *et al.,* 2019). Accordingly, this study aimed to evaluate the antibacterial and antibiofilm of the seed coriander seed EO with/out the antibiotic Gentamicin against four bacterial strains: *Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*.

2. Materials and Methods

2.1. Plant materials

Coriander seeds were purchased from a farm located in North Jordan. This batch of seeds were harvested in May of 2021. Identification of *C. sativum* species was achieved by the Phytochemistry team, led by Prof. Fatima Afifi, the University of Jordan. The seeds were stored in a dry place, at room temperature in the Faculty of Pharmacy, Applied Science Private University. After which, the essential oil was obtained from the Coriander seeds by hydrodistillation (HD).

2.2. Isolation of the essential oil by hydrodistillation

Half a kilo of Coriander seeds was ground in 1.5L of filtered water using a mixer for 30 seconds and transferred to a round bottom flask of the Clevenger-type apparatus (Borosil, India). HD was conducted for 3 hours using a heating mantle (Electrothermal, UK). The extraction was repeated six times, and the obtained Coriander essential oil was pooled and dried over anhydrous sodium sulphate (Na_2SO_4) (Al-Shuneigat *et al.,* 2015). After dehydration, the 100% stock solution of CEO was stored at 4^o C in amber glass vials prior analysis (Afifi *et al.,* 2015).

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

A sample of the CEO was sent to the University of California- San Diego - USA to be analyzed using Agilent 5977B Gas Chromatograph-Mass Selective Detector (GC-MSD) instrument. This device has electron impact sources coupled with an Agilent 7820A GC system, enabling sample mixtures to be analyzed. This device is particularly useful for routine examination of non-polar small organic compounds with a mass between 50 and 1000 amu. Hits were analyzed against the NIST (National Institute of Standards and Technology) library (> 1 million compounds). Approximately 1 µL aliquot of CEO sample, was diluted in 10 µL of GC grade n-hexane and was then subjected to GC analysis. The analysis was performed using Varian Chrompack CP-3800 GC-MS/MS- 200 (Saturn, Netherlands) equipped with DB-5 (5 % diphenyl, 95 % dimethyl polysiloxane) GC capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses), with helium as a carrier gas (flow rate 0.9 mL/min). The compounds were identified by comparing them to built-in libraries (NIST and Wiley Co. USA).

2.4. Evaluation of the antibacterial and antibiofilm activities

2.4.1. Bacterial strains and growth conditions

All bacterial strains were purchased from the American Type Culture Collection (ATCC). These strains include *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 14169), *Staphylococcus aureus* (ATCC 25923), *and Staphylococcus epidermidis* (ATCC 12228). All bacterial strains were stored at –20±2^o C in cryovials before they were resuspended and then sub-cultured in nutrient broth (NB) (Oxoid, UK) overnight at 37±2^o C. The inoculum of each bacterial strain was assessed in triplicate by measuring optical density (OD). Similarly, the growth of each bacteria was calculated from the measured OD as explained below.

2.4.2. Biofilm formation on the MBEC Assay® plate

Biofilm formation and measurement of antimicrobial sensitivity of the bacterial biofilms were performed using the Minimum Biofilm Eradication Concentration Assay (MBEC Assay®) (ASTM, 2017). Each microtiter plate lid has 96 polystyrene pegs or projections distributed on the lid. Each peg provided the surface for bacteria to adhere to, colonize, and form a uniform mature biofilm (Ceri, 1999). The pegs fit precisely into the wells of a standard 96-well microtiter flat bottom plate that can grow bacterial biofilm, rinsing, anti-microbial challenge and microbial recovery.

Briefly, bacteria were cultured in nutrient broth (NB) test tubes (10 mL) overnight at 37±2^o C where they reached approximately 10^8 CFU/mL. The bacterial suspensions were adjusted to achieve an approximate bacterial density of 10^5 – 10^6 CFU/mL using the optical density where 600 nm was used to measure the planktonic growth (Al-Shuneigat *et al.*. 2014). An inoculum of 150

µL of bacterial culture was dispensed into each well of MBEC bottom plates (except for the sterility control wells) before placing the 96-peg lid back into the inoculated 96 well bottom plate. The inoculated MBEC Assay® plate was incubated for 24 ±2 hours at 37^{o} C on an orbital incubator (Lab Companion SI-600R, Korea) at 110 rpm to allow bacterial biofilm formation on the pegs. The lid with the pegs was transferred to a new sterile 96-well plate (SPL, Korea) containing 200 µL of sterile PBS for rinsing for 30 seconds. Optimizing the growth conditions for biofilm formation was determined in preliminary studies to achieve approximately 10^4 -10^6 CFU/peg.

Following the biofilm formation and the rinse steps, the 96 peg-lid was transferred to the 96-well challenge plate. The challenge plate consisted of different CEO concentrations dissolved in NB with 0.5 % of Dimethyl Sulfoxide (DMSO, AZ Chem for chemicals, Canada). DMSO was used in the untreated growth control wells that were used to calculate the log10 reduction later on. A wide range of concentrations of CEO were used; 300, 200, 100, 50, 25, 6.25, 1.6 and 0.4 mg/mL. All test dilutions were run in triplicates. Gentamicin antibiotic MIC results were obtained from earlier experiments to present as the positive control in this study (0.6, 1.22, 0.15, 0.3 µg/mL) for *P. aeruginosa, S. epidermidis, S. aureus, and E. coli* respectively. The MIC was determined as the least concentration of the antibiotic required to inhibit bacterial growth (Talib *et al.* 2010; Al-Shuneigat *et al.*. 2020). The peg-lid was then removed, rinsed in PBS, then placed over another 96-well microtiter plate containing fresh, sterile broth recovery medium (NB with 0.1% Tween-80). This recovery plate was sonicated using ultrasonic cleaner (ULTRAsonik 104x, USA) for 30±1 minutes. The recovered colony forming unit per 1 milliliter (CFU/mL) of the adhered biomass for all treatment concentrations was determined after serial dilution and spot plating of each well on Nutrient agar plates that were incubated for 24±2 hours. The adhered biomass recovery plates were topped with 100µL of sterile recovery media in each well then incubated for another 24 hours to determine the MBEC breakpoints, which is defined as the lowest concentration of antibiotic or antimicrobial capable of killing biofilm producer bacteria (Al-Ouqaili *et al.*, 2011).

2.5. Combination of Coriander essential oil with Gentamicin concentrations and determination of minimum inhibitory concentration (MIC)

Two-fold serial dilutions of CEO plus Gentamicin were prepared. Gentamicin antibiotic MIC results were calculated using microdilution method (Aboalhaija *et al.* 2021). Concentrations of CEO:Gentamicin studied on *S. aureus*, *S. epidermidis*, and *E. coli* ranged (0.39: 0.094 - 12.5: 3 µl/mL), whereas for *P. aeruginosa*, CEO:Gentamicn concentrations ranged (3.13: 0.047 - 200: 3 µl/mL). The MIC, which represents the concentration of CEO required to inhibit the growth of planktonic bacterial population, was determined using the MBEC Assay®. The MIC was determined by the bacteria shed from biofilms attached to the pegs of the MBEC Assay® plate during the anti-microbial challenge step. The MIC plate wells were spot plated instead of using turbidity reading as the CEO interfered with the absorbance and visual reading at higher concentrations (Ceri, 1999).

2.6. Bacterial Cell Count

Serial dilutions of samples exposed to treatments were prepared in sterile 0.85% (w/v) NaCl solution and plated according to the method described previously (Chen *et al.*2003). The plates were incubated at 37±2 °C for 24±2 hours and the number of colonies was determined. To measure growth inhibition, viability logarithmic reduction was calculated comparing to the untreated growth control sample with 0.5% DMSO (Bouhdid *et al.* 2009). Bacterial inoculums of 5 µL were spot plated on nutrient agar plates, then incubated overnight at 37±2 °C. After 24 ±2 hours.

Equations (1-4) used for the calculations are shown below (Centre 2010):

$$A = \#\ of\ colonies \times Dilution\ factor \quad (1)$$

$$B = CFU/Peg = (A/5mL) \times 200mL \quad (2)$$

$$C = log_{10}(CFU/Peg) = log_{10}(B+1) \quad (3)$$

$$log_{10} Reduction = C - Average\ Growth\ Control \quad (4)$$

2.7. Combination index calculation

The mode of interaction between CEO and Gentamicin antibiotic was determined using the bolographic approach. The combination index (CI) was calculated (equation 5) for combinations of the two treatments against bacterial growth (planktonic/biofilm) (Ichite *et al.*, 2009):

$$CI = (D)1/(Dx)1 + (D)2/(Dx)2 \quad (5)$$

where (Dx) 1 = dose of CEO to produce 3 log reductions (99.9% reduction) in planktonic bacteria and 2 log reduction (99% reduction) in biofilm bacteria; (D) 1 = dose of CEO to produce 99.9% planktonic or 99% biofilm bacterial inhibition in combination with Gentamicin; (Dx) 2 = dose of Gentamicin to produce 99.9% planktonic or 99% biofilm bacteria inhibition alone; (D) 2 = dose of Gentamicin to produce 99.9% planktonic or 99% biofilm bacteria inhibition in combination with CEO. Interpreted as: CI >1.3 antagonism; CI 1.1 to 1.3 moderate antagonism; CI 0.9 to 1.1 additive effect; CI 0.8 to 0.9 slight synergism; CI 0.6 to 0.8 moderate synergism; CI 0.4 to 0.6 synergism; CI <0.4 strong synergism.

In this study, two-fold serial dilutions of CEO, Gentamicin, and CEO + Gentamicin combination were prepared, with final concentration ranging from 0.78 - 25 mg/mL of CEO, 0.18 - 6 µg/mL of Gentamicin, and 0.39:0.094 - 12.5:3 of CEO + Gentamicin (mg/mL:µl/mL) for *S. aureus*, *S. epidermidis* and *E.coli*, and from 3.13 - 200 mg/mL of CEO, 0.09 - 6 µg/mL of Gentamicin, and 3.13:0.047 - 200:3 of CEO + Gentamicin (mg/mL:µl/mL) for *P. aeruginosa*.

2.8. Statistical Analysis

All results are expressed as mean ± SD (n = 4). GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA) was used to perform statistical analyses. Pertaining samples that passed the Shapiro-Wilk normality test, an unpaired t-test was used to compare means in two independent groups, while Mann-Whitney tests were used for samples that did not show normal distribution. One-way analysis of variance (ANOVA) was used to compare means of three groups or more and Tukey's multiple comparison test was used as a post hoc test. A p-value of ≤0.05 was considered a significant difference.

3. Results

3.1. Qualitative analysis of the Coriander essential oil

The amount of the CEO obtained from each 100 g coriander seeds was 0.313 (±0.01) mL, (n=3). GC analysis (Supplementary material: S1) of the hydro-distilled oil from the Coriander seeds revealed 6 major volatile compounds: α-pinene (terpene), limonene (aliphatic hydrocarbon), linalool (terpene alcohol), camphor (terpene ketone), o-cymene and γ-terpinene (hydrocarbons).

3.2. Antibacterial activity of the Coriander essential oil

In general, CEO inhibition and killing effects on the tested bacterial strains in this study were concentration dependent. Table 1 shows the quality control and numeration of average growth of bacteria during study conduction as a negative control.

Table 1. The tested bacteria numeration of average CFU/well of planktonic bacteria and CFU/peg sessile (biofilm).

Test organism	Planktonic concentrations (CFU/well)[a]	Biofilm concentrations (CFU/peg)[b]
Pseudomonas aeruginosa (ATCC 27853)	3.00×10^7	6.30×10^7
Staphylococcus epidermidis (ATCC 12228)	2.95×10^6	5.40×10^6
Staphylococcus aureus (ATCC 25923)	2.00×10^7	9.90×10^6
Escherichia coli (ATCC 14169)	1.45×10^7	3.38×10^7

[a] This column represents the mean number of planktonic bacteria growing in the wells of the MBEC Assay® plate at the same time the peg was sampled.

[b] This column represents the mean number of sessile bacteria on each peg of the MBEC Assay® plate.

3.3. Antibacterial activity of Coriander essential oil

The effect of CEO was tested using different concentrations and observed onto each bacterial strain as planktonic and biofilm phenotypes, exhibiting different susceptibility effects as shown in Figure 2 and Figure 3. It was shown in Figure 1 that *P. aeruginosa* had the least susceptible tendency to the CEO, as it possessed the highest MIC concentration of CEO (25 mg/mL), whereas the MIC of *E. coli*, *S. epidermidis* and *S. aureus* were 3.125, 6.25 and 3.125mg/mL, respectively. Complete eradication was detected on all tested bacterial strains at 50 mg/mL of CEO, except for *P. aeruginosa*, which needed 200 mg/mL of CEO to reach 2.97 log reductions. CEO showed a comparable antibacterial activity with the MIC of Gentamicin (positive control) against both *P. aeruginosa (*200 mg/mL) *and S. epidermidis*, (12.5 mg/mL). Furthermore, at concentration ≥25 mg/mL CEO, *S. epidermidis, S. aureus and E. coli the* antibacterial activity was significantly higher than MIC-Gentamicin.

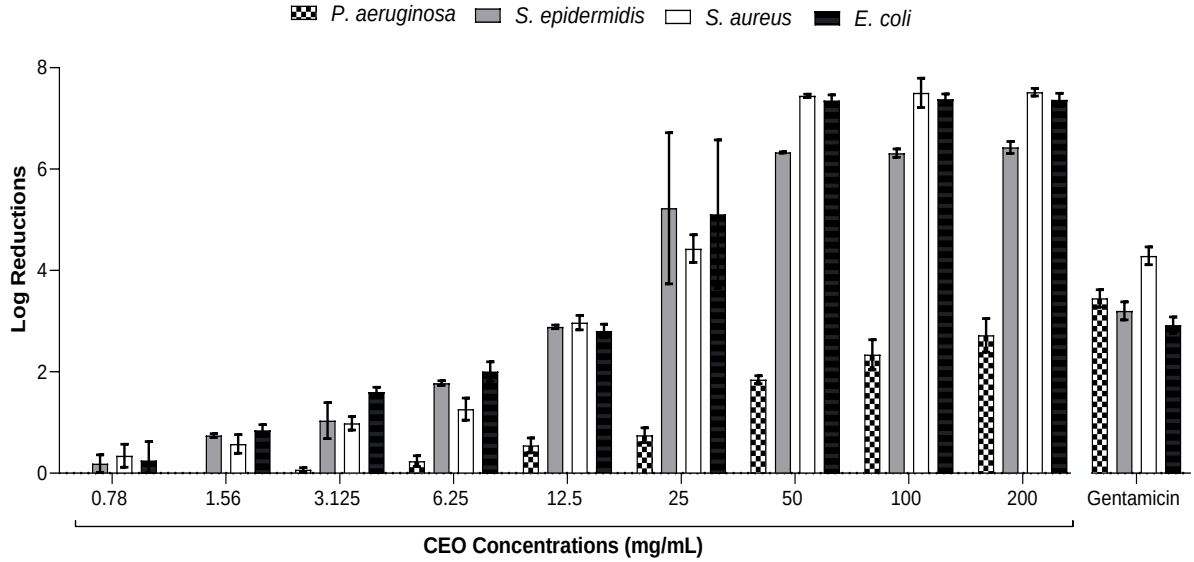

Figure 1. The effect of CEO in mg/mL on planktonic bacteria. Results represented as mean ±SD (n=4). Positive control Gentamicin MIC = 0.75 µL/mL (E. coli), 0.375 µL/mL (P. aeruginosa), 0.188 µL/mL (S. aureus) and 1.5 µL/mL (S. epidermidis).

3.4. Antibiofilm activity of Coriander essential oil (CEO)

The *P. aeruginosa* biofilm was the most resistant among the tested bacterial biofilms. The antibiofilm activity of CEO for *P. aeruginosa* was observed at 200 mg/mL, with 2.18 log reduction (Figure 3). This effect was not significantly different from the positive control-Gentamicin. For the other bacteria, antibiofilm activity of CEO on *S. epidermidis*, *S aureus* and *E. coli* was significantly observed at concentration of >25 mg/mL of CEO, which was associated with more than four log reduction, as shown in Figure 2.

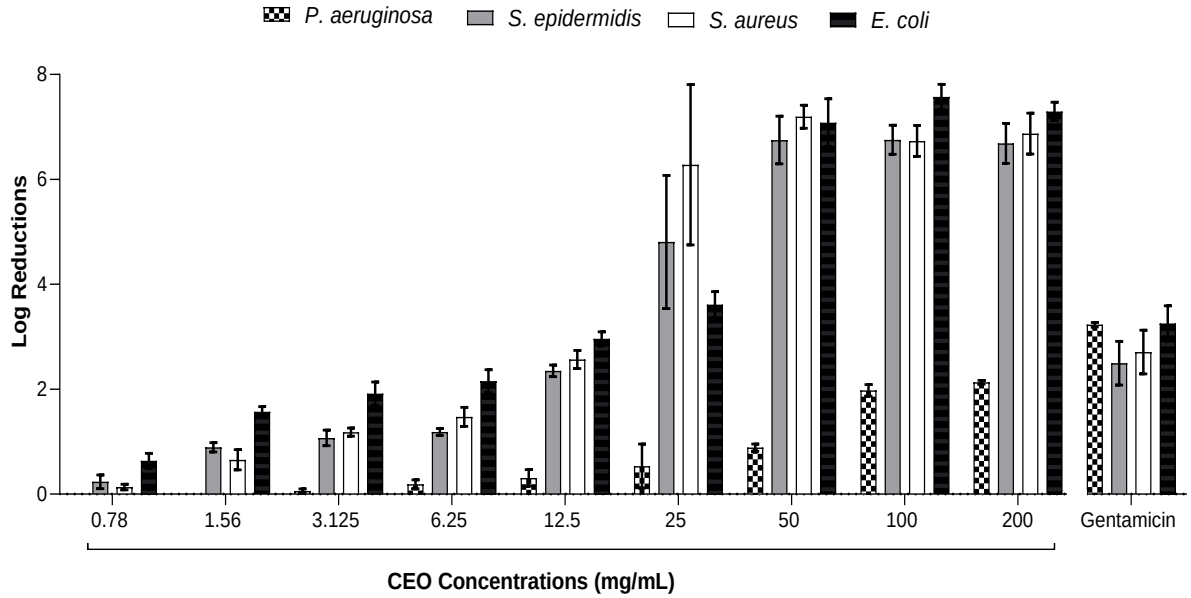

Figure 2. The effect of Coriander essential oil (CEO) in mg/mL on biofilm bacteria. Results represented as mean ±SD (n=4). Positive control Gentamicin MBEC = 6 µL/mL (*E. coli*), >6 µL/mL (*P. aeruginosa*), 3 µL/mL (*S. aureus*) and 3 µL/mL (*S. epidermidis*).

In general, similar antibacterial activities were observed for the combination of both CEO and Gentamicin, but at smaller concentrations of CEO. It was seen that the most resistant bacteria studied was *P. aeruginosa*; hence, 100mg/mL of CEO and 1.5 µg/mL Gentamicin exhibited 3 log reductions with combination index (CI)= 1 revealing that the effect was an additive effect. Similar additive effect was observed on *E. coli*, at 6.25mg/mL CEO and 1.5 µg/mL Gentamicin. Substantial synergistic effect was observed upon using this combination on *S. aureus*, as CI=0.5. at 3.123 mg/mL CEO and 0.375µg/mL Gentamicin. In between, a moderate synergism for the combination was reported for *S. epidermidis* at 3.125 mg/mL CEO and 0.75 µg/mL Gentamicin.

Table 2. The concentration values with 3 log reductions effect (99.9% reduce in bacteria), and the combination index for CEO (mg/mL) and Gentamicin (µg/mL) antibiotic against different planktonic bacteria strains.

Tested bacteria (planktonic)	CEO concentrationmg/mL	Gentamicin Concentration µg/mL	CEO concentration in combination mg/mL	Gentamicin concentration in combination µg/mL	Combination Index (CI)	Type of effect
P. aeruginosa	200	3	100	1.5	1	Additive effect
S. epidermidis	12.5	1.5	3.125	0.75	0.75	Moderate synergism
S. aureus	12.5	1.5	3.125	0.375	0.5	Synergism
E. coli	12.5	3	6.25	1.5	1	Additive effect

Furthermore, the combination of CEO-Gentamicin on biofilm of the studied bacteria had a positive effect exhibiting 2 log reductions on all bacteria except for *E. coli*, which had a CI >1.3 and is considered antagonism effect. A strong synergism was observed when using the combination on *S. epidermidis*, as CEO concentration dropped by 8 times and Gentamicin by 4 times. Even the most resistant biofilm of *P. aeruginosa* had a positive effect of the combination. To reach 2 log reductions on *P. aeruginosa* biofilm, a concentration of CEO was 12.5 mg/mL and 0.189 µg/mL of Gentamicin. Moderate synergism was observed when using the combination of CEO and Gentamicin on *S. aureus* biofilm to exhibit 2 log reductions.

Table 3 The concentration values with 2 log reductions effect (99% reduce in bacteria), and the combination index for CEO (mg/mL) and Gentamicin (µg/mL) antibiotic against different biofilm bacteria strains.

Tested bacteria (planktonic)	CEO concentration mg/mL	Gentamicin Concentration µg/mL	CEO concentration in combination mg/mL	Gentamicin Concentration in combination µg/mL	Combination Index (CI)	Type of effect
P. aeruginosa	100	0.75	12.5	0.189	0.502	Synergism
S. epidermidis	12.5	1.5	1.56	0.375	0.3748	Strong synergism
S. aureus	12.5	0.75	3.125	0.375	0.75	Moderate
E. coli	3.125	1.5	3.125	0.75	1.5	Antagonism

4. Discussion

There is a worldwide interest in discovering new and safe antibacterial agents from natural sources. Essential oils (EOs) from natural products are being extensively studied and experimented for their potential as a source of biologically active compounds (Kabera *et al.* 2014; Amiri *et al.* 2016; Diao *et al.* 2014). Most of the studies carried out by researchers of different countries showed that the raw coriander seeds consisted mainly of linalool (72.7%) followed by γ-terpinene (8.8%), α -pinene (5.5%), camphor (3.7%), limonene (2.3%), geranyl acetate (1.9%) and o-cymene (1.5%). Coriander plant exhibits diverse biological activities beyond its medicinal uses, including anti-microbial and food preservative properties (Mandal *et al.,* 2015). It was reported that leaves-coriander essential oil expressed a strong antibacterial activity against Bacillus subtilis followed by *Stenotrophomonas maltophilia* and *Penicillium expansum* (Özkinali *et al.* 2017, Kačániová *et al.* 2020). In this study, CEO's major constituents were linalool, α -pinene, γ-terpinene, camphor, o-Cymene, and Limonene. Compared with other studies in other countries, the same compounds were identified in stage three coriander seed's essential oil. Variations in the concentrations of the constituents will probably be seen when compared to other studies due to different factors, including time of harvest and seasonal conditions (Mandal *et al.,* 2015). Moreover, Linalool also represents the highest peak in the GC-MS in our study, matching the literature; this monoterpene alcohol poses antibacterial properties (Zengin *et al.,* 2014) and anti-inflammatory ones (Peana *et al.* 2002). Therefore, the detected antimicrobial activity in our work could be caused by the presence of linalool in the Jordanian CEO, which is a similar speculation to a study of CEO from Hanus, a.s. (Slovakia) (Kačániová *et al.,* 2020). However, the exact model of inhibitory action of monoterpenes remains unknown and requires further studies. Furthermore, the studies showed that the combination of linalool with α -pinene resulted in an additive antimicrobial effect (Tserennadmid *et al.,* 2011). According to literature, the antimicrobial activity of CEO was higher than that of its main constituent, linalool, suggesting synergistic effect due to the combination of more than one component (Silva *et al.,* 2011). Moreover, fractional distillation of coriander essential oil showed that the fraction that presented as less potent but more effective against tested microorganisms was the one containing a superior concentration of linalool (Delaquis *et al.,* 2002). These findings suggest that the anti-microbial activity is due to complex interactions between individual components that lead to the overall activity and not only to the effects of linalool, as could be expected.

A study observed that alcohols and aldehydes constituents of CEO predominantly inhibited Gram-positive bacteria, while linalool exhibited activity against Gram-negative strains (Delaquis *et al.,* 2002). Another study highlighted the medium to strong anti-microbial activity of aromatic volatiles and essential oils against Gram-positive bacteria such as *S. aureus* and Gram-negative bacteria like *E. coli*, with comparatively weaker effects against *P. aeruginosa* (Prabuseenivasan *et al.,* 2006). The antibacterial prowess of CEO is potentially attributed to its constituents, particularly linalool, which is known to enhance membrane permeability. Thus, it induces structural changes in both Gram-positive and Gram-negative bacteria through interactions with membrane phospholipids, membrane proteins, and specific intracellular targets (Zengin *et al.,* 2014). Additional CEO constituents, including α-pinene, camphor, γ-terpinene, geranyl acetate, and D-limonene, may also contribute to antibacterial effects by disrupting membrane structures, increasing permeability, damaging membrane proteins, and altering respiration and ion transport (Abdi-Moghadam *et al.* 2023; Bunse *et al.* 2022). Furthermore, it was demonstrated that CEO induces membrane damage, permeabilization, loss of membrane potential, and disruption of efflux pump and respiratory activities in both Gram-positive and Gram-negative species (Taiwo *et al.,* 2017).

In biofilms, bacterial cells exhibit increased resistance to antimicrobial agents compared to planktonic cells. Inhibiting biofilm growth poses a greater challenge than impeding cell attachment, a phenomenon consistent with prior findings (Sharma *et al.,* 2023). In this study, the effect of 200 mg/mL CEO on the biofilms of *E. coli, S. aureus, S. epidermidis,* and *P. aeruginosa* yielded log reductions of 7.32, 6.92, 6.68, and 2.13, respectively. A study investigated the anti-biofilm activity of Iranian *C. sativum* on *S. aureus* and *E. coli.* They demonstrated CEO's substantial anti-biofilm activity against both bacteria, with the lowest MIC values recorded at 0.8 µl/mL and 1.6 µl/mL for *S. aureus* and *E. coli*, respectively (Bazargani *et al.,* 2016). Research identified the potential of the CEO as antibacterial against the biofilm produced by Acinetobacter baumannii at 4 µL/mL for MIC and MBC. In our study, we determined the antimicrobial activity with the MBEC® method (Duarte *et al.,* 2012). In a study done in 2016, antiadhesion tests for the evaluation of the reduction in the cell attachments using the crystal violet assay were accomplished for the CEO. They found a variety of effects of the CEO on the development and growth of the biofilm with

at least 50% reduction in cell attachment with a complete inhibition of *S. aureus*. According to this study, the CEO induced the decrease of biofilm formation against *S. aureus* up to 91% (Bazargani *et al.,* 2016). In parallel, Gentamicin possesses bactericidal efficacy specifically targeting aerobic Gram-negative bacteria, making it a valuable treatment option for various common infections and serving as a positive control in our study (Turner *et al.,* 2022). The mechanism of action involves oxygen-dependent active transport through the Gram-negative bacterial membrane. Aminoglycosides, including Gentamicin, are ineffective against anaerobic bacteria due to the oxygen requirement in this process. Upon reaching the cytoplasm, Gentamicin and other aminoglycosides bind to the 16S rRNA at the 30S ribosomal subunit, disrupting mRNA translation and resulting in the generation of truncated or non-functional proteins (Chaves BJ, 2023). A study done in 2018 has explored the combination of Gentamicin with essential oils. Caraway EO, for instance, exhibited synergy with Gentamicin against strains resistant to extended-spectrum beta-lactamases (ESBL) and gentamicin-resistant strains. Similar additive effects were observed when Gentamicin was combined with thyme, fennel, basil, and clary sage (Kwiatkowski *et al.,* 2018). It is noteworthy that the combination of CEOs and antibiotics can impact multiple targets concurrently, as highlighted by a study, confirming that the mode of action of combination differs significantly than that of the same drugs acting individually (Hemaiswarya *et al.,* 2008). In a study, the combination of Gentamicin and coriander essential oil was explored, revealing that CEO can potentially enhance the effectiveness of antibiotics against *Acinto baumannii* (Duarte *et al.,* 2012). This bacterium, characterized as a Gram-negative, nonmotile, non-fermentative, and oxidase-negative bacillus, poses a challenge in treatment (Duarte *et al.,* 2012). The synergistic effect observed between CEO and antibiotics suggests promising avenues for addressing *A. baumannii* infections (Duarte *et al.,* 2012). In our study, similar effects were noted using CEO against both planktonic and biofilm bacteria, aligning with the potential for essential oils to augment antibiotic efficacy.

The broad spectrum of CEO's anti-microbial effects, as reported in the literature, may not be entirely mirrored in the context of our study, potentially limiting the generalizability of our findings. Despite these considerations, our investigation contributes valuable insights into the potential synergy between CEO and antibiotics, offering a basis for further exploration and refinement of anti-microbial strategies. Additionally, the study's focus on planktonic and biofilm bacteria provides valuable insights into the potential applications of CEO. However, the diverse nature of biofilms and their resistance mechanisms may not be fully captured by our experimental design. Biofilm formation is a complex process influenced by numerous factors, and the efficacy of CEO against biofilm-associated bacteria may be subject to variations that require further investigation. Finally, while our study sheds light on the potential synergistic effects of CEO with Gentamicin, acknowledging the limitations related to the choice of positive control, variations in essential oil compositions, and the specificity of interactions with different antibiotics and bacterial forms is crucial for a comprehensive interpretation of our findings. These limitations should be considered in the context of future research endeavors to refine our understanding of the potential applications of CEO in combination with antibiotics.

5. Conclusion

This study presents compelling evidence of the potent antibacterial activity of coriander seed essential oil (CEO) against a range of clinically relevant bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli.* Notably, CEO exhibits synergistic effects when combined with the antibiotic Gentamicin, suggesting a promising avenue for combination therapy. Moreover, CEO demonstrates remarkable efficacy in eradicating biofilms formed by *S. aureus*, *S. epidermidis*, and *E. coli*, with significant impact on pseudomonal biofilm.

The present research is the first to establish CEO action against biofilm in Jordan. The findings hold substantial implications for the management of chronic wounds, where biofilm formation poses a significant challenge. Furthermore, this study sheds light on the potential of CEO as a novel therapeutic approach in the field of infectious diseases, particularly for combating biofilm-associated infections that often exhibit resistance to traditional treatments.

Author contributions

All authors contributed equally to this work.

Funding

This research was generously supported by Applied Science Private University-Amman-Jordan.

Acknowledgement

Authors would like to thank Smart Lab and The University of Jordan - Jordan, and California-San Francisco University-USA for their cooperation and support.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdallah, M, Corinne B, Djamel D, Pascal D, and Nour-Eddine Ch. 2014. Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. *Arch Microbiol* , **196**: 453-72.

Abdi-Moghadam Z, Yeganeh M, Alieh RS, Maryam M, Mansour S, Mahnaz M, Samira SH, Ahmad G, Farshid N, and Majid D. 2023. The significance of essential oils and their antifungal properties in the food industry: A systematic review. *Heliyon*, 29; **9**:e21386.

Aboalhaija N, Afifi, FU , Al-Hussaini M, Al-Najjar M, Abu-Dahab R, Hasen E, Rashed M, Abdel-Haq S, and Khalil E.2021. *In vitro and in vivo* evaluation of the wound healing potential of the extracts of Schinus molle L.(Anacardiaceae) grown in Jordan. *Indian J. Pharmaceut. Sci.,* **83**: 261-70.

Afifi, FU, Kasabri V, and Abaza IM. 2015. GC-MS composition and antiproliferative activity of Inula graveolens (L.) Desf. essential oil. *AJMAP* , **1**: 57-66.

Aguiar Campolina G., das Graças Cardoso M., Rodrigues-Silva-Caetano A., Lee Nelson D. and Mendes Ramos E. 2023. Essential oil and plant extracts as preservatives and natural antioxidants applied to meat and meat products: a review. *Food Technol Biotechnol* , **61**: 212-25.

Al-Ouqaili MT, AL-Quhli SQ, and Al-Izzy MY. 2011. The Role of milleri Streptococci in the Formation of Cariogenic Biofilm: Bacteriological Aspects. *Jordan J Biol Sci*, **4:**165-172.

Al-Shuneigat J, Al-Sarayreh S, Al-Saraireh Y, and Al-Qudah M. 2020. Effect of Achillea santolina essential oil on bacterial biofilm and its mode of action. *Curr. Issues Pharm. Med. Sci*, **33**: 83-89.

Al-Shuneigat J, Al-Sarayreh S, Al-Saraireh Y, Al-Qudah M, Al-Tarawneh I, and Al Bataineh E. 2014. Effects of wild Thymus vulgaris essential oil on clinical isolates biofilm-forming bacteria. *IOSR J. Dent. Med. Sci*, **13**: 62-66.

Al-Shuneigat, J, Al-Tarawneh I, Al-Qudah M, Al-Sarayreh S, Al-Saraireh Y, and Alsharafa KH. 2015. The chemical composition and the antibacterial properties of Ruta graveolens L. essential oil grown in Northern Jordan. *Jordan J Biol Sci*, **8**: 139-43.

Aljaafari M, Alhosani MS, Abushelaibi A, Lai KS, and Erin Lim SH. 2019. Essential oils: Partnering with antibiotics. **Essential Oils-Oils of Nature.** IntechOpen Publisher, United Kingdom.

Amiri SH, and Joharchi MR. 2016. Ethnobotanical knowledge of Apiaceae family in Iran: A review. *Avicenna J Phytomed* , **6**: 621.

ASTM. 2017. Standard Test Method for Testing Disinfectant Efficacy against Pseudomonas aeruginosa Biofilm using the MBEC Assay. **E2799-12.** Available from URL: https://www.astm.org/e2799-12.html

Baratta M. Tiziana H J, Dorman D, Deans SG, Biondi DM, and Ruberto G. 1998. Chemical Composition, Antimicrobial and Antioxidative Activity of Laurel, Sage, Rosemary, Oregano and Coriander Essential Oils. *J. Essent. Oil Res.* , **10**: 618-27.

Bazargani MM, and Rohloff J. 2016. Antibiofilm activity of essential oils and plant extracts against Staphylococcus aureus and Escherichia coli biofilms. *Food control*, **61**: 156-64.

Bouhdid S, Abrini, A Zhiri, MJ Espuny, and An Manresa. 2009. Investigation of functional and morphological changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Origanum compactum essential oil. *J Appl Microbiol* , **106**: 1558-68.

Bueno J, Demirci F, and Can Baser KH. 2017. Essential oils against microbial resistance mechanisms, challenges and applications in drug discovery. **Essential oils and nanotechnology for treatment of microbial diseases**, 1ST ed, CRC press, United kingdom 143-58.

B Bunse M, Daniels R, Gründemann C, Heilmann J, Kammerer DR, Keusgen M, Lindequist U, Melzig MF, Morlock GE, Schulz H, Schweiggert R, Simon M, Stintzing FC, and Wink M. 2022. Essential oils as multicomponent mixtures and their potential for human health and well-being.*Front Pharmacol* , **13**: 956541.

Carlone N, Tullio VC, and Cuffini A. 2018. Farmaci antibatterici inMicrobiologia generale e applicata,*Società Editrice Esculapio*. Available from: https://scuolamedicina.unich.it/sites/sc01/files/5_inferm_ass_antibiotici.pdf

The National Science foundation Engineering research Center (NSFERC). 2010. The log reduction (LR) measure of disinfectant efficacy. *MSU Cen for Biofilm Eng*, Monatana State Univeristy, **4**. Available from URL: https://biofilm.montana.edu/documents/KSA-SM-07.pdf

Ceri, H. 1999. The Calgary Biofilm Device: Measurement of antimicrobial sensitivity of bacterial biofilms. *J. Clin. Microbiol.*, **37**: 1771-76.

Chaves BJ, Tadi P. 2023. Gentamicin. **StatPearls [e-Book].** Available from:

https://www.ncbi.nlm.nih.gov/books/NBK557550/.

Chen CY, Nace GW, and Irwin PL. 2003. A 6× 6 drop plate method for simultaneous colony counting and MPN enumeration of Campylobacter jejuni, Listeria monocytogenes, and Escherichia coli. *J Microbiol Methods* , **55**: 475-79.

Cimino C, Maurel OM, Musumeci T, Bonaccorso A, Drago F, Souto EMB, Pignatello R, and Carbone C. 2021. Essential oils: Pharmaceutical and encapsulation strategies into lipid based delivery systems. *Pharmaceutics*, **13**: 327.

de Souza CC, de Souza LZM, Yılmaz M, de Oliveira MA, da Silva Bezerra AC, da Silva EF, Dumont MR and Machado ART. 2022. Activated carbon of Coriandrum sativum for adsorption of methylene blue: Equilibrium and kinetic modeling. *Cleaner Materials*, **3**: 100052.

Delaquis PJ, Stanich K, Girard B, and Mazza G.. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int J Food Microbiol* , **74**: 101-09.

Diao WR, Hu QP, Zhang H, and Xu JG. 2014. Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.). *Food control*, **35**: 109-16.

Duarte A, Ferreira S, Silva F, and Domingues FC. 2012. Synergistic activity of coriander oil and conventional antibiotics against Acinetobacter baumannii. *Phytomedicine*, **19**: 236-38.

European center for disease prevention and control (ECDC). 2009. The bacterial challenge–time to react a call to narrow the gap between multidrug-resistant bacteria in the eu and development of new antibacterial agents. *Solna: ECDC & EMEA Joint Press Release*. avaioable from : https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf

Fisher K and Phillips C. 2008. Potential antimicrobial uses of essential oils in food: is citrus the answer?. *Trends Food Sci Technol* , **19**: 156-64.

Hemaiswarya SH, Kruthiventi AK, and Doble M. 2008. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*, **15**: 639-52.

Ichite N, Chougule MB, Jackson T, Fulzele S, Safe S, and Singh M. 2009. Enhancement of docetaxel anticancer activity by a novel diindolylmethane compound in human non–small cell lung cancer. *Clin Cancer Res*, 15: 543-52.

Kabera JS, Semana E, Mussa AR, and He X. 2014. Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J. Pharm. Pharmacol*, **2**: 377-92.

Kačániová M, Galovičová L, Ivanišová E, Vukovic NL, Štefániková J, Valková V, Borotová P, Žiarovská J, Terentjeva M, Felšöciová S, and Tvrdá E. 2020. Antioxidant, Antimicrobial and Antibiofilm Activity of Coriander (Coriandrum sativum L.) Essential Oil for Its Application in Foods. *Foods*, **9**: 282.

Klapper I and Dockery J. 2010. Mathematical description of microbial biofilms. *SIAM review*, **52**: 221-65.

Kwiatkowski P, Pruss A, Grygorcewicz B, Wojciuk B, Dołęgowska B, Giedrys-Kalemba S, Kochan E, and Sienkiewicz M. 2018. Preliminary study on the antibacterial activity of essential oils alone and in combination with gentamicin against extended-spectrum β-lactamase-producing and New Delhi

metallo-β-lactamase-1-producing Klebsiella pneumoniae isolates. *Microb Drug Resist* , **24**: 1368-75.

Langeveld W, Veldhuizen E, and Burt S. 2014. Synergy between essential oil components and antibiotics: a review. *Crit Rev Microbiol* , **40**: 76-94.

Mandal S and Mandal M. 2015. Coriander (Coriandrum sativum L.) essential oil: Chemistry and biological activity. *Asian Pac J Trop Biomed* , **5**: 421-28.

Ormancey X. 2001. Formulation of essential oils in functional perfumery. *PCA*, **157**: 30-40.

Özkinali S, Şener N, Gür M, Güney K, and Olgun Ç. 2017. Antimicrobial activity and chemical composition of coriander & galangal essential oil. *Indian J. Pharm. Educ. Res*, **51**: 221-24.

Peana T, Panin F, Serra G, Pippia P, and Moretti MD. 2002. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine*, **9**: 721-26.

Prabuseenivasan S, Jayakumar M, and Ignacimuthu S. 2006. In vitro antibacterial activity of some plant essential oils. *BMC Complement Altern Med* , **6**: 1-8.

Preedy VR. 2015. **Essential oils in food preservation, flavor and safety**, Academic press, United Kingdom.

Said, Hakim Mohammad, and Aftab Saeed. 1996. **Medicinal herbal: a textbook for medical students and doctors**, Hamdard Foundation, Pakistan.

Shahrajabian MH . 2021. Medicinal herbs with anti-inflammatory activities for natural and organic healing. *Curr Org Chem* **25**: 2885-901.

Shahrajabian MH, Sun W, and Cheng Q. 2022. The importance of flavonoids and phytochemicals of medicinal plants with antiviral activities. *Mini Rev Org Chem*, **19**: 293-318.

Sharma S, Mohler J, Mahajan SD, Schwartz S, Bruggemann L, and Aalinkeel R. 2023. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. *Microorganisms*, **11**: 1614.

Silva F, Ferreira S, Queiroz JA, and Domingues FC. 2011. Coriander (Coriandrum sativum L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry. *J. Med. Microbiol.* , **60**: 1479-86.

Sulieman A, Abdallah E, Alanazi N, Ed-Dra A, Jamal A, Idriss H, Alshammari AS, and Shommo S. 2023. Spices as Sustainable Food Preservatives: A Comprehensive Review of Their Antimicrobial Potential.*Pharmaceuticals*, **16**: 1451.

Taiwo MO and Adebayo OS. 2017. Plant essential oil: an alternative to emerging multidrug resistant pathogens. *J Microbiol Exp*, **5**: 00163.

Talib W H, and Mahasneh A. 2010. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine.*Molecules*, **15**: 1811-24.

Tserennadmid R , Takó M, Galgóczy L, Papp T, Pesti M, Vágvölgyi C, Almássy K, and Krisch J. 2011. Anti yeast activities of some essential oils in growth medium, fruit juices and milk. *Int J Food Microbiol* , **144**: 480-86.

Turner J, Muraoka A, Bedenbaugh M, Childress B, Pernot L, Wiencek M, and Peterson YK. 2022. The chemical relationship among beta-lactam antibiotics and potential impacts on reactivity and decomposition. *Front Microbiol* **13**: 807955.

Wei S, Lyu J, Wei L, Xie B, Wei J, Zhang G, Li J, Gao CH, Xiao X, and Yu J. 2022. Chemometric approaches for the optimization of headspace-solid phase microextraction to analyze volatile compounds in coriander (Coriandrum sativum L.). *LWT*, **167**: 113842.

Zengin H and Baysal A. 2014. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules*, **19**: 17773-98.

Zygadlo J, Zunino M, Pizzolitto R, Merlo C, Omarini A, and Dambolena J. 2017. Antibacterial and anti-biofilm activities of essential oils and their components including modes of action.' in, **Essential oils and nanotechnology for treatment of microbial diseases,** CRC Press, United Kingdom.