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Research Article

Encapsulation of *Coriandrum sativum* Essential Oil in Nanoemulsion and Study Their Antioxidant and Biological Activities Against Food Associated Pathogenic Bacteria

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Foodborne diseases are still a serious global health issue, especially in developing countries, this study aimed to encapsulate the essential oils of coriander (*Coriandrum sativum* L.) in nanoemulsion and explore its efficiency as an antioxidant and antibacterial agent against foodborne pathogens. The *Coriandrum sativum* oil nanoemulsion (CNE) was fabricated using Tween 80 as an emulsifier. Ultrasonication was applied for 8 min to yield a homogenous nanoemulsion. The chemical profile of essential oils was investigated by gas chromatography/mass spectrometry (GC-MS). The physicochemical properties of CNE were examined by dynamic light scattering (DLS), UV-visible (UV-Vis) spectrophotometer, scanning electronic microscopic (SEM), and atomic force microscope (AFM). The CNE was assessed against *Escherichia coli* and *Klebsiella pneumoniae* implicated in foodborne. The GC-MS analysis showed that linalool was the main component in coriander essential oil with 64.06%. The biofabricted CNE had a Z-average of 147.5 nm and a zeta potential of 1.9 mV. SEM images showed that the CNE was spherical. AFM images revealed that the CNE had a moderately flat surface. Interestingly, CNE exhibited high efficiency as an antioxidant and antibacterial agent; this in turn gives it the potential to be a candidate as a natural active bio-based preservative used to tackle food poisoning besides adding value to food.

Keywords: antibacterial; antioxidant; *Coriandrum sativum*; essential oil; foodborne pathogen; nanoemulsion ultrasonication

Summary

- Encapsulation of *Coriandrum sativum* L. essential oils as nanoemulsions to improve their stability and applicability when using this antibacterial and antioxidant compound in food packaging.

1. Introduction

Foodborne diseases are one factor that endangers consumers' health, which can be fatal in some cases [1–3]. In this regard, the World Health Organization [4] reported that approximately one in 10 people worldwide are exposed to foodborne diseases after eating contaminated food. This in turn can lead to over 420,000 deaths every year. Children under five years of age are considered the most at risk of death, as their number reaches 125,000 deaths annually [4]. Pathogenic bacteria are considered dangerous food contaminants that can cause gastrointestinal diseases such as diarrhea [5, 6]. Further serious consequences related to food contamination may occur, such as failure of kidneys and liver, brain and neural disorders, and reactive arthritis, particularly in children, pregnant women, the elderly, and those immunocompromised [7, 8]. Consequently, the discovery of powerful antibacterial agents in the food industry is of great importance in limiting foodborne disease cases.

Essential oils are aromatic and have distinct hydrophobic secondary metabolites produced by different biosynthetic pathways in plants. Essential oils act as insecticidal, antimicrobial, and antioxidant agents [9]. Many essential oils

have promising natural medicinal compounds with generally recognized safe status (GRAS) by the United States Food and Drug Administration (FDA) including *Coriandrum sativum* L oil [10]. However, the main limitations to essential oils include volatility, instability, and hydrophobicity [11].

Another major limitation of essential oil applications is the susceptibility to degradation by volatilization under external factors as oxygen, humidity, light, and temperature [12].

To overcome these obstacles, several studies in medicine and food safety fields have focused on using nanoapproach as a promising new method to improve the stability of essential oils [7, 13–17]. Different methods of nanoencapsulation were developed; the essential oil nanoemulsion can be recommended as an efficient food preservative from microbial deterioration [18, 19]. Nanoemulsion is a colloidal dispersion consisting of a heterogeneous formulation of two different immiscible liquids (oil and water) with droplet sizes in the nanometer scale (10–1000 nm) [20, 21]. The small size in turn can lead to advantageous properties such as increased surface area per unit volume, stability, and tunable rheology [20].

Coriandrum sativum L. (coriander), which belongs to the Apiaceae (Umbelliferae) family, is one of the earliest spices used by humans as an edible herb. Besides its culinary usage, coriander is a natural source of phytochemicals such as essential oils, saponins, terpenoids, flavonoids, coumarin [22], and linalool [23]. *Coriander sativum* has the ability to produce essential oil; essential oils are formed by many biosynthetic cascades in different plant species in different plant organs as a defense mechanism due to response to biotic and abiotic factors. The importance of essential oils is not restricted to the plant protection but also for humans benefit [24]. Coriander essential oil seeds possess medicinal properties including antitumor, antimicrobial, antioxidant, anti-inflammatory, and antiseptic agents [17, 25–31]. Linalool is natural volatile compound and the main component of many fragrant plant essential oils such as basil, mint, and coriander [23], and it has been proved to have an excellent antibacterial action on various pathogens [32].

The low stability and solubility significantly constrain the application of linalool in the food industry; so, to improve its stability, it could be encapsulated into nanoemulsions using emulsifier agent [33].

In this context, it is assumed that the encapsulation of coriander essential oils as nanoemulsions could offer the potential to improve their applicability in food packaging and protect them from harsh environmental conditions and microbial contamination. From here, the current study aimed to formulate the *Coriandrum sativum* nanoemulsion using high-energy ultrasonication emulsification method to limit food contamination with foodborne bacteria.

The novelty of this research is focused in presenting biosourced strategy for nanoencapsulation of coriander essential oil for biological applications and food safety.

2. Materials and Methods

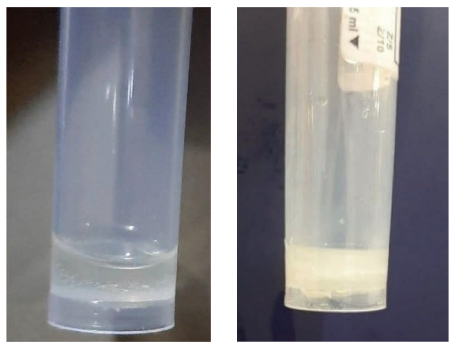
2.1. Coriander Essential Oil Extraction. *Coriandrum sativum* L. essential oils were extracted via the hydrodistillation technique using Clevenger [34]. *Coriandrum sativum* L. seeds were purchased from the local market in Baghdad, Iraq. The seeds were dried over filter paper for 5 days in the shade at room temperature (27°C) and then finely powdered. A total of 100 g of coriander seed powder was mixed with 500 mL of deionized water in a flask for further hydrodistillation. After boiling, the extraction lasted for 5 h. The hydrodistilled oil was then dried over anhydrous sodium sulfate. The essential oil yield was 6.5 g with a milky white appearance. The phytochemical components of *C. sativum* essential oils were analyzed and confirmed by gas chromatography/mass spectrometry (GC-MS) (Shimadzu). Finally, essential oils were kept at 4°C in a sealed and dark container until required for further analysis.

2.2. Coriandrum sativum Nanoemulsion Preparation. To biosynthesized a nanoemulsion, the oil phase: 17% v/v of coriander essential oil and 3%, v/v of biosurfactant Tween 80 representing 20% (v/v) of the total emulsion were mixed by magnetic stirrer for 15 min at 700 rpm; afterward, they were mixed steadily with doubled distilled water (80%) using a magnetic stirrer for 15 min at 700 rpm in room temperature. The resultant mixture was forming the colloidal suspension. The colloidal suspension was sonicated for 8 min using an ultrasonicator with a frequency of 20 kHz and a power output of 750 W. The ultrasonication process was performed by placing the sample container in a bigger beaker containing ice to avoid generated heat, and samples were cooled to room temperature and then stored in dark vials under refrigerated conditions for further analysis [18].

2.3. Physicochemical Characterization of Coriandrum sativum Nanoemulsion. The average droplet size (z-average) and polydispersity index (PDI) of *Coriandrum sativum* oil nanoemulsion (CNE) were analyzed by dynamic light scattering (DLS) (ZEN-3600, Malvern, UK), nanoemulsion was eluted in double distilled water, and three replicates (10–15 cycles) were run. The turbidity of CNE was quantified by UV-visible spectrophotometer (Thermo Scientific, USA) at the absorbance values 200–500 nm.

The morphology of CNE was also revealed using scanning electronic microscopic (SEM) (Hitachi) at 10,000 and 2,000,00 × magnification.

In addition, the surface of the CNE was observed by atomic force microscopy (AFM) (Nanosurf, Switzerland, V3.1R0, BT03710-15) examination was used by dropping CNE steadily on a glass slide then it was dehydrated by air before more analysis. Then, images were captured by applying tapping mode on the CNE samples with a scan rate of 4.80–6.00 μm/s at ambient condition [35].



(a) (b)

Figure 1: Biosynthesis of CNE. (a) Coriander essential oil appears a light yellow. (b) Biosynthesized CNE is seen as a milky colloidal solution.

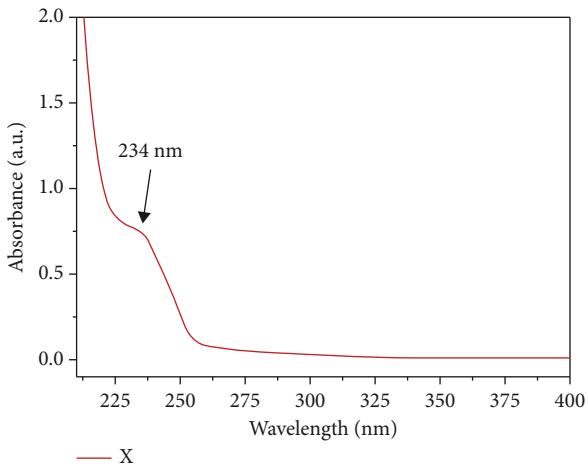


Figure 2: Characterization of biofabricated CNE using a UV-visible technique. UV-visible spectra profile of biofabricated CNE revealed a peak at wavelength 234 nm.

2.4. 2,2-Diphenyl-1-Picrylhydrazyl-Hydrate (DPPH) Assay. The radical scavenging activity for CNE was evaluated using a DPPH assay. The methanolic solution of DPPH was prepared by dissolving 0.025 g of DPPH in 100 mL of methanol. The test was carried out by mixing 12.5, 25, 50, and 100 µg/mL of the biosynthesized CNE with 1 mL of methanolic DPPH solution. All samples were incubated in darkness for 25 min at 27°C. The absorbance was recorded using a UV spectrophotometer at 517 nm. Methanol was used as the blank and ascorbic acid was applied as the positive control with concentration (40 µg/mL). The percent inhibition of the DPPH radical was estimated according to the following equation [36]:

$$\text{Inhibition (\%)} = \frac{Ac - As}{Ac} \times 100, \quad (1)$$

where AC represents the absorbance of the methanolic solution of DPPH. As represents the absorbance of DPPH with CNE samples.

2.5. Antibacterial Activity of Coriandrum sativum Nanoemulsion. The antibacterial assay was carried out using agar well diffusion method with *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) bacteria isolated from contaminated food. Mueller–Hinton agar was used as the base medium for screening of antibacterial activity and Mueller–Hinton broth for preparation of bacterial inoculum. McFarland turbidity standard was used for bacterial density estimation; it was prepared by mixing (9.95) mL of 1% sulfuric acid (H_2SO_4) in Mueller–Hinton broth and (0.05) mL of 1% barium chloride ($BaCl_2$) in distilled water. The tube was sealed and then used for comparison of test bacterial suspension with the control. To prepare inoculum, 4–6 colonies from pure culture of both test organism were transferred to 5 mL of Mueller–Hinton broth and then incubated at 37 C^0 overnight.

The antibacterial activity was performed by the agar well diffusion technique with bacterial density approximately equal to 1.5×10^8 CFU/mL of the bacterial suspension. Mueller–Hinton agar plates were cultured with 0.1 mL of both test organism inoculum; then, inoculum was spread regularly over plate with sterile glass spreader and then incubated for 24 h at 37 C°. Five wells with a diameter of 6 mm were punched at each plate using a cork borer. 100 µL of each CNE concentration (12.5, 25, 50, and 100) µg/mL were added to wells. A total of 100 µL of ampicillin (2 mg/mL) was applied as a reference (positive control). The plates were incubated for 24 h at 37°C. The antibacterial activity of the CNE was recorded as the mean diameter of the resulting inhibition zones of growth measured in millimeters in triplicate [37].

2.6. Statistical Analysis. Data were statically analyzed using the Microsoft Excel program with basic statistical variability indicators. Data were signified as the mean ± SD of antioxidant and antibacterial experiments that were set up in triplicate. To evaluate statistical significance, Student's *t*-test (GraphPad Prism program) was used for the analysis of two groups. Statistical significance was accepted at $p < 0.05$.

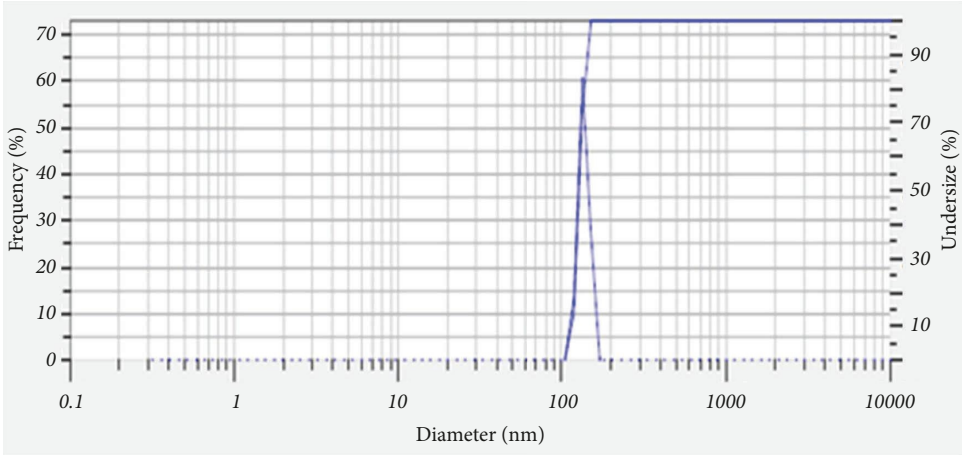


Figure 3: Droplet size distribution of biofabricated CNE. The CNE droplet size was 147.5 nm in diameter.

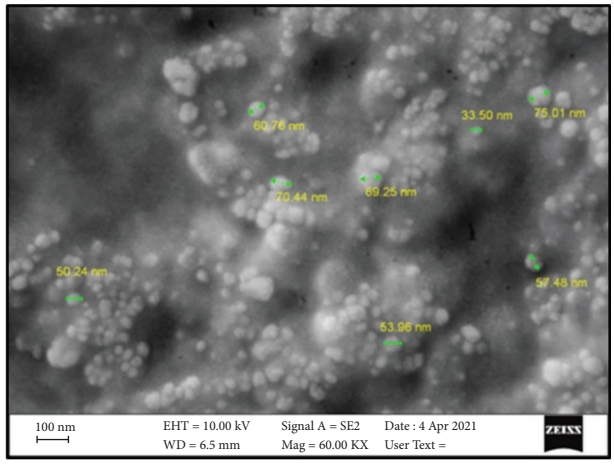


Figure 4: SEM image of CNE droplets. Magnification: 60.00 kx; scale bar: 100 nm.

3. Result and Discussion

Nanoemulsion is the most desirable technique in the encapsulation process due to low-cost fabrication and manageable waste. During the biosynthesis of CNE and as illustrated in Figure 1, it was seen that extracted essential oil from coriander seeds was colorless or light yellow with a distinctive odor, while the biosynthesized CNE was a whitish homogenous colloidal solution, which is an essential feature of oil in water (O/W) nanoemulsion.

Indeed, CNE is the heterogeneous formulation of two different immiscible liquids, resulting from mixing coriander essential oil and water in the presence of surface-active agents and surfactants such as Tween 80. Tween 80 plays a fundamental role in the stability of emulsions. In this regard, the Tween 80 emulsifier is adsorbed on the droplet surface, which can decrease the oil–water interfacial tension. This in turn effectively prevents droplet accumulation. The more systemically the emulsifier is arranged on the surface of the droplets, the greater the stability conferred upon the resulting emulsion [38, 39]. The stability of CNE was clearly observed after both centrifugation at 10,000 rpm for 30 min and storage for a month at 4°C where there was no sign of the nanoemulsion instability including phase separation or creaming.

The chemical profile of coriander essential oil was meticulously analyzed by GC-MS. This comprehensive analysis revealed linalool as the preeminent component by 64.06%. The remaining components involved camphor (1.60%), geraniol (2.09%), γ-terpinene (10.78%), terpinen-4-ol (0.43%), p-cymene (1.22%), tetradecanal (0.6%), neryl acetate (8.03%), limonene (0.3%), 2E-dodecanal (7.43%), and linalyl acetate (2.5%). Collectively, these components constituted a total of 99.04%.

These results are in agreement with a study, indicating that linalool was the major component of essential oil by 63.3% [40]. Another study stated that the seed coriander essential oil contained mostly linalool with 72.7% [41]. The high concentration of the terpenoid molecule, linalool, within the essential oil derived from coriander imparts a profound antioxidant efficacy to this aromatic substance [42, 43].

Linalool contains hydroxyl groups that act as scavengers and inhibitors of radical chain reactions [44–46]. Hence, linalool plays a pivotal role in coriander essential oil's overall biological activity profile.

A biofabricated CNE was characterized using a UV-visible technique, which is a spectroscopic technique for quantitative measurement of bioactive components in the plant [47]. The results revealed the presence of a distinctive peak at wavelength 234 nm, as shown in Figure 2. This suggests the accumulation of bioactive components in CNE. It has been indicated that linalool enhances the nanoemulsion absorption at 284.2 nm because of hyperchromicity [48]. Jaber et al. stated that the linalool shows a peak at 300 nm [49].

The DLS technique exhibited that the droplet size distribution of biofabricated CNE measured 147.5 nm in diameter as shown in Figure 3. The zeta potential of

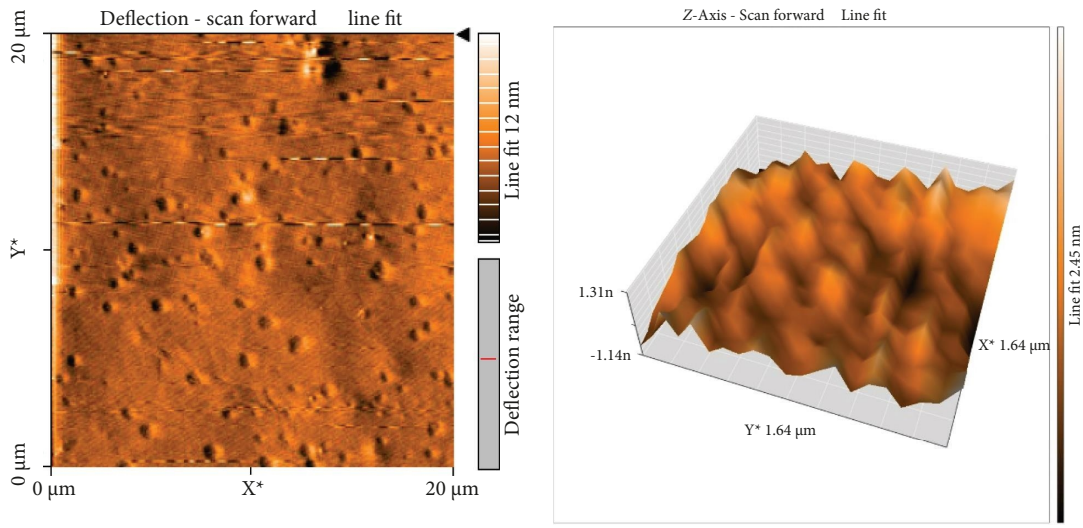


Figure 5: AFM images of biofabricted coriander nanoemulsion: (a) 2D image and (b) 3D image, scale bar 20 µm.

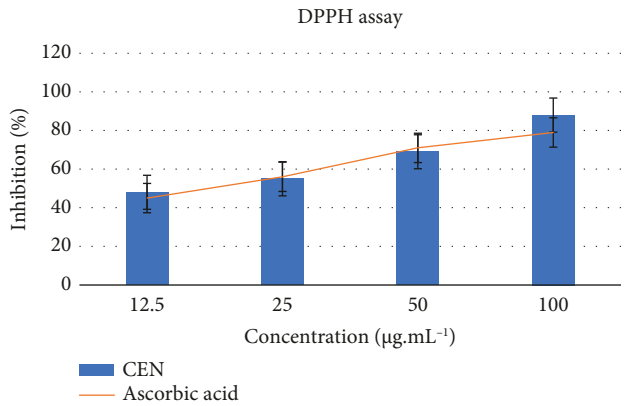


Figure 6: Evaluation of antioxidant activity of CNE by DPPH assay for different concentrations: 12.5, 25, 50, and 100 µg/mL, ascorbic acid was applied as the positive control with concentration (40 µg/mL).

nanoemulsion was a positive charge (1.9 mV). The PDI was also evaluated and it was 0.412. The low PDI value (≤ 0.5) of the formulated CNE refers to a monodisperse particle population, a high uniformity of droplet size, and a narrow size distribution [50]. However, surfactant type was found to be important to get the desired PDI and zeta potential of resultant nanoemulsions [51].

Furthermore, ultrasonication used in the nanoemulsion preparation can play a crucial role in producing homogeneous and small-sized nanoemulsion [52, 53]. The small size of the obtained CNE in turn gives rise to a suite of exceptional characteristics such as increased surface area and remarkable stability under various conditions [20, 54].

The morphological analysis of CNE was carried out using SEM. SEM images demonstrated that the CNE was spherical droplets with nanometric size of 33.5–75.1 nm (Figure 4). High energy ultrasonication technique uses acoustic energy to generate crumpling droplets in solution which causes a high-pressure gradient with high velocity in the emulsion, creating a shear force that will disperse the coarse emulsion into nanoemulsion [55].

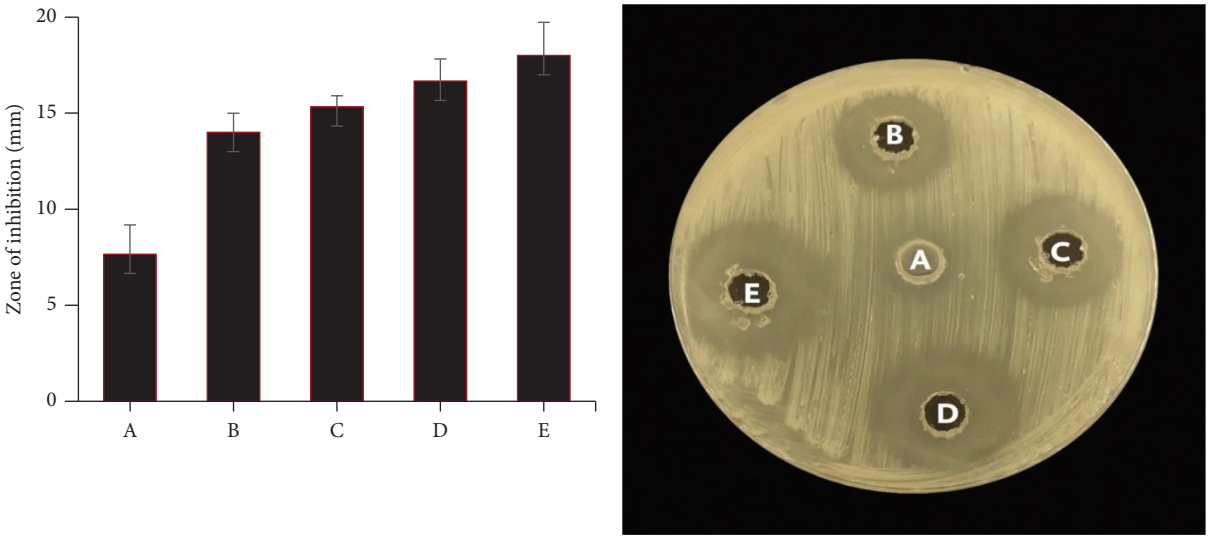
The larger size of the nanoemulsion determined by DLS compared with SEM can be attributed to the hydrophilic portion of the Tween 80, which encapsulates the oil [56]. DLS mostly measures the hydrodynamic diameter of an equivalent sphere of nanoemulsion (not only the electron-rich core). DLS analysis includes the core, organic surface coatings and any solvent molecules tightly bound to the surface coating. This in turn leads to a hydrodynamic diameter typically larger than the particle diameter measured by SEM [57].

Regarding the CNE morphology also, the typical 2D and 3D surface topography of AFM images revealed that the surface of the CNE was moderately flat and with spherical droplets can be seen in the surface of the films with a root mean square (RMS) of 36.62, as displayed in Figure 5.

Dehydration step in sample preparation can result in flattening nanodroplet [58]. Nevertheless, the AFM technique was applied as a complementary technique to study the droplet morphology when create nanometric delivery systems for bioactive compounds in pharmaceutical and food applications [56, 59].

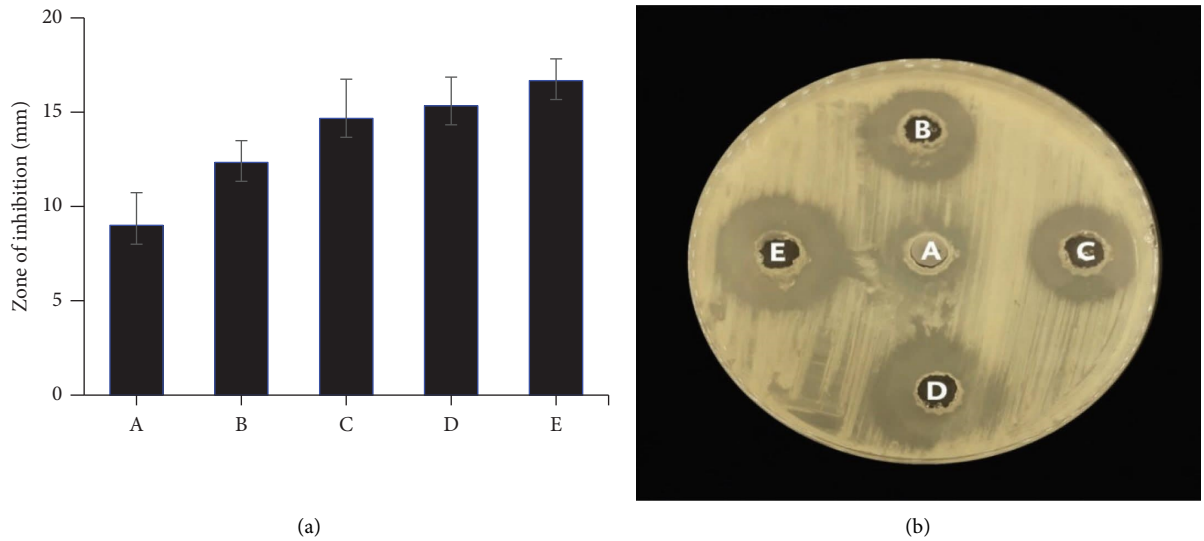
The antioxidant activity of CNE was also assessed using DPPH assay. The results revealed that the antioxidant activity of CNE at 12.5,25,50, and 100 µg/mL was 48 ± 8.5%, 55 ± 8.7%, 69 ± 9.1%, and 88 ± 8.1%, respectively while for ascorbic acid was 45 ± 5.5%, 56 ± 6.2%, 71 ± 7.5%, and 79 ± 6% consecutively (Figure 6). This may be due to the high availability of linalool in CNE [42, 43].

In addition, the antibacterial activity of biosynthesized CNE was studied against pathogenic foodborne bacteria. The CNE was applied against *E. coli* and *K. pneumoniae* implicated in food contamination. The inhibition zone was a visual confirmation of the antibacterial activity of biosynthesized CNE. The results demonstrated that the CNE



(a) (b)

Figure 7: Antibacterial activity of biofabricated CNE against *E. coli*. (a) The data have been represented as a bar graph for an inhibition zone vs CNE concentration. (b) Images of agar well diffusion assay. A represents ampicillin at 2 mg/mL and B, C, D, and E represent CNE at 12.5, 25, 50, and 100 μg/mL, respectively.



(a) (b)

Figure 8: Antibacterial activity of biofabricated CNE against *K. pneumoniae*. (a) The data have been represented as a bar graph for an inhibition zone vs. CNE concentration. (b) Images of agar well diffusion assay. A represents ampicillin at 2 mg/mL and B, C, D, and E represent CNE at 12.5, 25, 50, and 100 μg/mL, respectively.

had activity against *E. coli*. The inhibition zones for *E. coli* were 14 ± 1, 15.3 ± 0.6, 16.6 ± 1.2, and 18 ± 1.7 mm at 12.5, 25, 50, and 100 μg/mL, respectively (Figure 7). The CNE also showed activity against *K. pneumonia*e and the inhibition zones were 12.3 ± 1.2 mm at 12.5 μg/mL, 14.6 ± 2 mm at 25 μg/mL, 15.3 ± 1.5 mm at 50 μg/mL, and 16.6 ± 1.2 mm at 100 μg/mL (Figure 8). The ampicillin at a concentration of 2 mg/mL was used against *E. coli* and *K. pneumoniae* as a positive control and it had antibacterial activity of 7.6 ± 1.2 mm and 9 ± 1.7 mm consecutively (Figures 7 and 8).

The results also displayed that *K. pneumoniae* tends to be more resistant to CNE than *E. coli* (Figure 9).

Many studies have indicated that *K. pneumoniae* has more multidrug resistance than *E. coli* [58, 60]. The drug resistance in *K. pneumoniae* is frequently caused by the development of biofilm formation, efflux pumps, and the production of β-lactamases [61]. Moreover, the enzymatic modification of the cellular structure and the loss of porins can significantly promote the organism's ability to antimicrobial interventions. This in turn can contribute to the

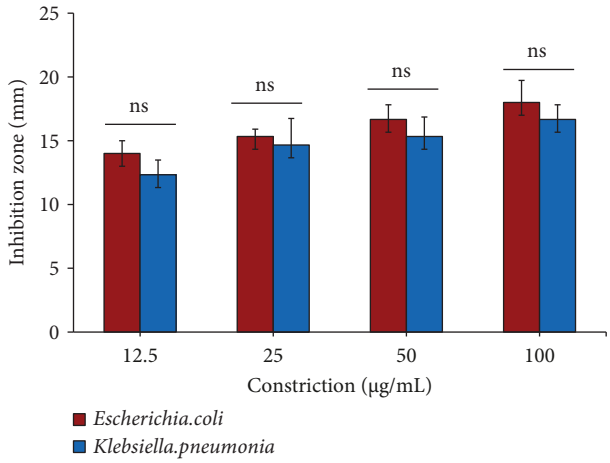


Figure 9: The influence of CNE on *E. coli* and *K. pneumoniae*. Data were expressed as the mean ± standard deviation (S.D). Student's *t*-test was performed to evaluate statistical significance. The values of the CNE effect on *E. coli* were compared with *K. pneumoniae* values in the statistical analysis process. A value of $p < 0.05$ was considered statistically significant and ns was not significant.

exacerbated challenge of treating infections caused by pathogenic bacteria, including *K. pneumoniae* [61–63].

The effectiveness of CNE against pathogenic microbes depends on the phytochemicals and antioxidant properties of the essential oil.

It has been reported that the crude oil extract of coriander has antibacterial activity against *E. coli* and *K. pneumoniae* [64]. Another study indicated that coriander essential oil possesses potent antibacterial activity [65]. This could be due to the ability of coriander essential oil containing linalool to penetrate the bacterial cell, damage the bacterial membrane, inhibit metabolic functions, and ultimately cause bacterial cell death [35]. However, the effectiveness of free essential oils may not meet the ambition.

Nanoemulsion drops act to increase the biological activity of essential oils by activating the passive cell absorption mechanism and the leakage of cellular ions like Na^+ and K^+ accordingly disrupting biomolecules DNA, RNA, and protein biosynthesis that causes eventually to cell death [66, 67]. In this context, several studies have demonstrated that essential oil-based nanoemulsion is more efficient as an antimicrobial agent compared to essential oil alone. It has been shown that the essential oil-based nanoemulsion can disrupt cell integrity and membrane penetrability, inhibit the proton pump with successive loss in the ATP pool, and eventually cell apoptosis [19].

Further study indicated that the essential oil-based nanoemulsion can inactivate ergosterol by destabilizing membrane integrity [68]. The unique properties of biosynthesized CNE such as remarkable biocompatibility, potent antioxidative capabilities, and pronounced antibacterial efficacy present a new approach for encapsulating food products and limiting food contamination with foodborne bacteria. Consequently, significantly mitigating the risks associated with food contamination and enhancing the overall quality and safety profile of the edible items in question.

4. Conclusion and Future Perspectives

This study was performed for presenting biosourced strategy for nanoencapsulation of coriander essential oil for biological applications and food safety using edible plant essential oils through an economically viable, highly scalable, ecofriendly, renewable, and sustainable strategy. The CNE, characterized by their diminutive and uniformly distributed droplets, exhibit profound antioxidant activity and antibacterial efficacy against foodborne pathogens such as *E. coli* and *K. pneumoniae*. These attributes give the possibility for CNE to be a potential candidate as a promising agent for food preservation and safety. Additional investigations of CNE particularly in biological systems and in vivo studies may support pharmaceutical activity; also, the safety of these plant-derived products and their phytocompounds such as linalool need to be considered.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Ethics Statement

Ethics approval was not needed for this work.

Conflicts of Interest

The authors declare no conflicts of interest.

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