Original Article

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Development and Characterization of Cumin Oil-Nanoemulsions and its Enhanced Antibacterial Activity against Some Bacteria

Fatemeh Hosseinali Khani [1], Alireza Yazdinezhad [2], Somayeh Sadighian [3], Mehran Mohseni [1*]

1. *Department of Food and Drug Control, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*
2. *Department of Pharmacognosy, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*
3. *Department of Pharmaceutical Biomaterials, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*

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Corresponding Information:
Mehran Mohseni,
Department of Food and Drug Control, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran
E-Mail: mohsenim@zums.ac.ir

ABSTRACT

Background & Objective: Antibiotic resistance has become a global problem. This study aimed to develop and evaluate nanoemulsions' capacity to improve Cuminum cyminum's antibacterial activity essential oil as an appropriate approach to prevent microbial resistance.

Materials & Methods: Gas chromatography-mass spectrometry was used to determine the main constituent of C. cyminum essential oil. FT-IR was used to investigate the functional groups of chemical composition. A nanoemulsion was generated using high-shear homogenization, followed by several physicochemical characterizations. The antibacterial activity of essential oil in both pure and nanoemulsion forms against important food-borne pathogens, and drug-resistant bacteria was determined by measuring the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC).

Results: Cumin aldehyde with 20.08%w/v is the main constituent of essential oil. The prepared nanoemulsions had an average size of 72 nm, particle distribution of 0.234, and zeta potential of 26 mV. Nanoemulsion formulations of essential oils are more effective compared to pure ones. *Klebsiella pneumonia* and *Pseudomonas aeruginosa* have the highest and the lowest antimicrobial effects of essential oil among all studied bacteria, respectively. In contrast, in resistant samples, the major and minor effects were contributed to Methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus, respectively.

Conclusion: Cumin oil-nanoemulsion had an acceptable function against antibiotic-resistant bacteria. It is advisable to combine this formulation with synthetic antibiotics in order to reduce bacterial resistance and serve as a preservative in the pharmaceutical and food industries.

Keywords: *Cuminum cyminum*. L, Essential oil, Characterization, Nanoemulsion, Antibacterial activity.

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Introduction

An antibiotic is a product or substance taken from a microorganism that can destroy or inhibit other microorganisms (1). The ability of an antibiotic to prevent potential bacterial infections is crucial to the success rate of contemporary medical treatments, including organ transplants, cancer therapy, the care of preterm infants, and major operations like joint replacement, among others (2). Microorganisms have developed mechanisms to circumvent antibiotics via antibiotic resistance, which is categorized as inherent and

acquired resistance. Acquired resistance due to the overuse of antibiotics is the most medically important and dangerous type of resistance that spreads rapidly in the community. In this type of resistance, the bacterium, which was previously sensitive to antibiotics, has become resistant to drugs via mutations or gene transfers in the form of conjugation, transformation, and transduction, all of which cause changes in the bacterial genome.

Nowadays, years after the first treatment of diseases with antibiotics, bacterial infection has once again become a serious threat to human, animal, and environmental health. Reducing the consumption rate of synthetic antibiotics and using herbal medicine against microbes can be a solution to this global problem (2). The antimicrobial activity of natural volatile compounds, including eugenol, thymol, carvacrol, cinnamic aldehyde, and the isothiocyanate allele, was examined by Palaniappan *et al*. in both individual and combination with an antibiotic study. They found that natural antibacterial compounds increased the susceptibility of drug-resistant bacteria. Moreover, most food preservatives are suggested to be carcinogenic and threaten consumer safety. Thus, society's tendency changed to use natural essential oils instead of chemical preservatives (1).

Cyminum cyminum. L, the plant belongs to the Apiaceae family, a fragrant, herbaceous, annual plant (3) whose seeds are elliptical and corneal, greenish-brown, with a striped pattern (4). Cumin aldehydes and terpenoids are the main constituents of its essential oil (5). The essential oil of this plant was attributed with various properties, such as stimulating the urinary and digestive systems, antispasmodic, antifungal, antiseptic, diuretic, antihyperglycemic and dyslipidemia, analgesic, antioxidant, and anti-cancer. Numerous studies confirmed the antimicrobial effectiveness of cumin seed oil on gram-positive bacteria, including S. aureus, S. epidermidis, Bacillus subtilis, etc., gram-negative bacteria, such as Escherichia coli, Listeria monocytogenes, Borrelia burgdorferi (6), etc. and fungi and yeasts, for example, Penicillium nutatum, Aspergillus, Microsporum canis, Candida albicans, Saccharomyces cerevisiae, etc. (7-9). Despite these benefits, using essential oils is often challenging owing to their poor water solubility, high vapor pressure, poor bioavailability, and physical and chemical instability (10). Nanoemulsions are a class of nanomaterials with a particle size of 20 - 200 nanometers (3), which are categorized as water in oil (W/O) and oil in water (O/W) depending on the nature of their constituents (11). Considering chemical and physical stability properties and controllability of the water and oil ratio, these nanoparticles can surmount pure essential oils' inadequacy and antimicrobial properties (10, 12). The production of nanoemulsions requires energy and surfactants as the basic components. The preparation methods for nanoemulsions are categorized into high-energy and low-energy techniques. High-energy methods help distribute the oil phase evenly in the water phase and obtain nanoparticles of selected and uniform size. In high-energy techniques, mechanical tools like homogenizers are often used to generate powerful destructive forces that separate the oil and water phases, and produce nanoemulsion droplets (13). Various medical and food applications of natural products, such as cumin, the spread of microbial resistance to antibiotics in gram-positive and negative bacteria, creating antibiotic-resistant-species, along with the emergence of new technologies, such as nanotechnology, have opened up a new perspective for the researchers to use nanotechnology to deal with harmful microorganisms using natural products. This work developed and characterized a stable and biocompatible cumin oil nanoemulsion. The nanoemulsion was assessed for its antibacterial properties, and it was compared to purified cumin essential oil (CEO) in the presence of common and drug-resistant bacteria that are responsible for food poisoning.

Materials and Methods

Organisms

Food poisoning bacteria, including S. epidermidis (1435 PTCC), Bacillus cereus (1015 PTCC), E. coli (1330 PTCC), *Klebsiella pneumoniae* (1053 PTCC), Salmonella Typhimurium (1709 PTCC), *Pseudomonas aeruginosa* (1310 PTCC), S. aureus resistant to methicillin (1764 PTCC), P. aeruginosa resistant to gentamicin, tetracycline, tobramycin, ceftazidime, ceftriaxone, ciprofloxacin, carbenicillin, imipenem (1811 PTCC) and vancomycin- resistant Enterococcus(1237 PTCC) are donated by Microbial Control Laboratory of Faculty of Pharmacy, Zanjan University of Medical Sciences.

Preparation and characterization of essential oil:

Cumin seeds were procured from a spice store in Zanjan and authenticated by Pharmacognosy Department of the Zanjan School of Pharmacy, bearing herbarium number 4016. These seeds were collected in the spring. Clevenger apparatus extracted the essential oil at 70 °C for a duration of 4 hours. Gas chromatography was performed based on method described (4) to distinguish the chemical compounds in the obtained essential oil, using a 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with 5975 C Mass detector and HP-5ms column (0.25 mmol phenylmethyl siloxane 0.25 mm ×30m, film thickness 0.25 µm), and helium carrier gas by a flow rate of 1 ml/min. The constituent's essential oil was identified using the retention index (5) by Wiley 7n software and the second version of NIST: NIH library and compared to the standard mass spectrometry fragmentation model (14).

Nanoemulsions preparation

The homogenization method, Tween-80 and Spen-60, were used as energy source and surfactants to prepare nanoemulsions following method described by Gharenaghadeh *et al*. with some modifications(15). In brief, Tween 80 and Span 60 were mixed with a specific amount of distilled water in separate

containers; then, both the containers were homogenized by Silent Crusher M equipment (Heidolph, Germany) for 3 min by 10,000 rpm. CEO was then homogenized in the container with a mixture of water and Span 60. The mixture of CEO, Span60, and water was then added dropwise to Tween 80 and water container and homogenized at 13,000 rpm. The harvest nanoemulsions were subjected to a magnetic IKA C-MAG HS 7 stirrer (IKA, Germany) for two hours to stabilize the intended nanoemulsions. The final formulation was maintained at ambient temperature.

Characterization of nanoemulsion

Using Zetasizer Nano-ZS equipment (Malvern, U.K.), the droplet size distributions and mean particle diameters of nanoemulsions were assessed by measuring dynamic light scattering and their zeta potential by assessing their electrophoretic mobility. All readings were done in triplicate at room temperature, and the results were expressed as mean ± standard deviation.

FT-IR was used to study the functional groups of pure essential oil, and its nanoemulsion chemical composition using a spectrometer Tensor27 equipment (Bruker, Germany). The morphology and structure of selected fabricated nanoparticles were evaluated by Transmission Electron Microscope (TEM) EM 208s equipment (Philips, Holland). In short, the mixtures were sonicated for one minute. Then, a 5 microliter drop was put to a grid on Whatman paper, naturally dried, and employed in the Transmission Electron Microscope.

Release analysis

An in-vitro release study was performed using dialysis method. Specifically, 2 ml of a selected Cumin oil-nanoemulsion was loaded into a dialysis bag (cellulose acetate membrane with a cutoff of 12000 D). This nanoemulsion contained 24.50% v/v distilled water, 5% v/v essential oil, 0.25% v/v Tween 80, and 0.25% v/v Span 60. The nanoemulsion particles had an average size of 72.60±1.81 nm, a polydispersity index of 0.234±0.08, and a zeta potential of -26.40 mV. To decrease agglomeration and promote more equal oil release, the nanoemulsion was surrounded by 15 mL of PBS containing 0.5% Tween 80 at pH 1.5 to 7.4 in a sink condition. At 37 °C, all sets were incubated. Following immersion, the samples were taken at intervals of 1, 2, 4, 6, 8, 16, 24, and 72 hours. The absorbance of these samples was measured using UV-spectroscopy at a wavelength of 326 nm. This experiment was conducted with equally formulated nanoemulsions and in triplicate, with results expressed as mean ± standard deviation.

The amount of essential oil in the formulation

The formulation was diluted to 100 ml with 40% v/v ethanol from 100 ml. The essential oil concentration was determined by employing a calibration curve to compute UV spectroscopy at a wavelength of 326 nm immediately following the fabrication of the nanoemulsion and two months later. All readings were done in triplicate, and the results were expressed as mean ± standard deviation.

Evaluation of antibacterial activity

The control antibiotics

Positive control of the antibiotic was selected to confirm the microbial susceptibility of samples. Based on CLSI 2018 antibiogram (16), gentamicin and vancomycin were used for the excellent and recurrent effects on the studied bacteria in this research.

The concentrations of gentamicin and vancomycin (10µg, 20µg, 30µg, 40µg, 50µg, 60µg and 100µg in 50µl agar medium) were prepared and tested against *P. aeruginosa* and *S. epidermidis* by Agar well plate method.

The well containing Tween80 and nanoemulsion loaded with 5g paraffin were used as a negative control to confirm the ineffectiveness of these compounds on bacterial growth.

Agar well plate

Agar well plate method assessed the susceptibility of microorganisms to CEO based on the recommended CLSI protocol (16). In summary, the experiment was conducted by adding 200 µl of 0.5 McFarland bacterial suspension to 25 ml of Mueller Hinton Agar (Manufactured by Sigma/America) culture medium. Subsequently, dilutions of CEO with Tween 80 and distilled water were added to the wells at the following concentrations: 1, 2, 6, 8, and 9.5 µg/ml. This experiment was done in triplicate, and the results were expressed as mean ± standard deviation.

Determination of MIC and MBC

The broth microdilution method used the following procedure as CLSI (NCCLS) recommended to evaluate the Minimum Inhibitory Concentration of pure and emulsified essential oils(17). Samples were sequentially diluted in a 96-well plate containing Mueller Hinton Broth (MHB- Manufactured by Sigma/ America) medium due to the sensitivity of microorganisms to CEO to produce a concentration range of 2.3×10^{-5} to 200 mg/ml for pure essential oil and 2.3×10^{-5} to 100 mg/ml for nanoemulsion loaded essential oil. Samples were incubated at 37 ºC for 24 h, and the lowest concentration of additive showing no visible microbial growth was determined.

In order to ascertain the Minimum Bactericidal Concentration (MBC), Mueller Hinton Agar (MHA) was further incubated at 37°C for 24 h with 10 µl of the bouillon medium from the wells that did not exhibit microbial growth. The concentration of MBC that was able to eradicate 99.9% of the microorganisms in the treated wells was the lowest. These experiments were performed in triplicate.

Statistical analysis

All experiments were done in triplicate, and the results were reported as mean ± standard deviation.

One-way analysis of variance was performed, and group means were compared by Tukey post-hoc test. Values were considered statistically significant with a p-value ≤ 0.05.

Results

Essential oil characterization

The percentage of essential oil extracted from cumin seeds was calculated as 2.58% g/g based on the density of essential oil (933 mg/ml).

GC-MS essential oil analysis

Regarding the chemical analysis of essential oil, 16 components were detected in Gas-Chromatography, which have formed 92.02% of total essential oil composition, as shown in (Table 1). The main chemical compounds were 20.80% Cumin aldehyde, 16.98% β-Pinene, 16.31% α-Phellandrene, 15.13% α-Terpinene, 11.97% 2-Carene-10-ol, 5.51% Phellandral, respectively.

Table 1. Detected components of Cumin Essential Oil.

Num	Compound	Retention time [a] (min)	Retention time [b] (min)	% Of total compounds
1	α-Thujene	11.93	5.62	0.40
2	α-Pinene	12.27	5.85	1.36
3	β-Pinene	15.49	7.04	16.99
4	β-Myrcene	16.46	7.43	1.11
5	α-Phellandrene	19.04	7.85	16.31
6	α-Terpinene	21.57	8.30	15.13
7	Linalool	27.42	11.32	0.27
8	Trans-Pinocarveol	28.66	12.99	0.43
9	Terpinene-4-ol	29.29	14.66	0.30
10	Trans-Carveol	30.52	16.44	0.89
11	Cumin aldehyde	34.69	17.47	20.08
12	Phellandral	37.48	19.23	5.52
13	2-Carene-10-ol	38.14	21.14	11.97
14	Trans-Caryophyllene	45.68	24.9	0.57
15	β-Farnesene	49.37	26.33	0.47
16	Carotol	60.62	32.67	0.21
17	Total of identification			=92.01

Retention time [a] listed in order of elution from a DB-5 column, and Retention time [b] listed according to literature (Adams, R. P, 2001)

Nanoemulsion Characterization

Particle size and Polydispersity index (PDI)

Different surfactant-to-essential oil ratios were evaluated to achieve a stable nanoparticle with optimal particle size and PDI. In these formulations, the ratio of Tween 80 surfactants, hydrophilic-lipophilic balance (HLB) = 15, and Span 60, HLB = 4.7, were selected based on the following formula, which maintains the balance of hydrophobicity and hydrophilicity and provides correct particle size and stability(18).

$$\text{hydrophilic lipophilic balance } (HLB) = (m_A \times HLB_A + m_B \times HLB_B / m_A + m_B) \times 100$$

Where m_A and m_B indicate the Mass of Tween 80 and Span 60, respectively, moreover, HLB_A and HLB_B are referred to Tween 80 and Span 60 HLB, respectively.

Based on the results, the formulation containing 5g of essential oil, with a mean size of 72.60 ±1.81 nm and -26.4 mV zeta potential, is selected as the optimum formulation.

Stability of nanoemulsions

The stability of the nanoemulsion that was chosen was monitored for a period of three months. (Table 2) illustrates that the particle size did not undergo any substantial modifications (*p-value* >0.05).

Table 2. Stability of selected nanoemulsion (n=3, P-value >0.05).

Time (27)	Mean size (27)	Polydispersity index
0	72.60±1.81	0.234±0.08
7	69.94±1.33	0.220±0.06
14	75.38±1.17	0.302±0.06
28	77.41±1.49	0.256±0.07
60	84.63±1.35	0.289±0.07
90	81.23±0.92	0.224±0.03

Fourier transform infrared spectroscopy

Chemical functional groups were studied using FT-IR technique. The peak of aromatic rings at 1400 cm-1, which is associated with the essential oil, was detected in the nanoemulsion that contained the essential oil, as illustrated in (Figure 1). In (Figure 1), additional typical peaks were shown. Both spectra show the peak at 1600 cm^{-1}, corresponding to the double bond C = O, proving that the essential oil was properly loaded into the nanoemulsion. C-H and O-H bonds are associated with the 2900 cm-1 and 3400 cm^{-1} area maxima, respectively.

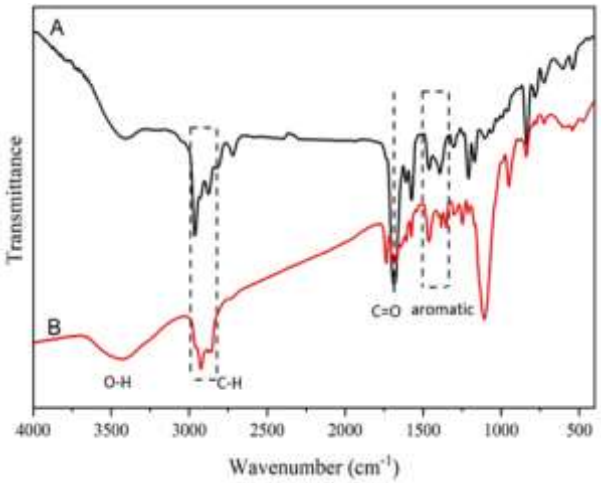


Figure 1. The FTIR spectrum of essential oil (1) and nanoemulsions containing essential oil (1).

Transmission electron microscopy

The formulation containing 5g of essential oil, with a mean size of 72.60 ±1.81 nm and -26.4 mV zeta potential, is selected as the optimum formulation. TEM images of this nanoemulsion, represented in Figure 2, provide a semi-spherical shape of synthesized nanoparticles.

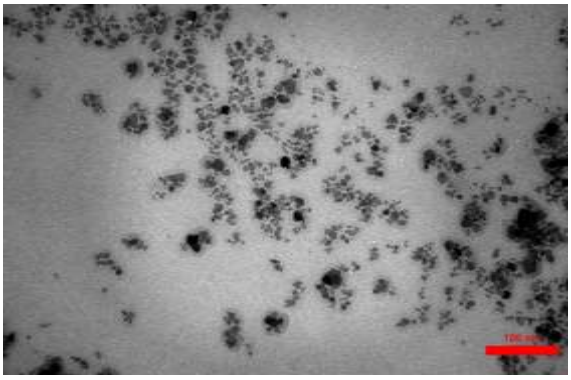


Figure 2. TEM image of nanoemulsion. The depiction of the structure and morphology of the nanoparticles show a relatively symmetrical and spherical morphology with dimensions <100 nm.

The release percentage of the Nanoemulsion

As shown in the cumulative release diagram in (Figure 3), the release percentage of the essential oil decreases by preparing the cumin oil-nanoemulsions. The nanoemulsion form of essential oil releases 50% of the essential oil after 4 and 7 hours at pHs 1.5 and 7.4, respectively, while the unadulterated form releases approximately 50% after 0.5 and 1.5 hours at pH 1.5 and 7.4.Furthermore, it was observed that the release profile at the pH of 7.4 was more sustained than at the pH of 1.5.

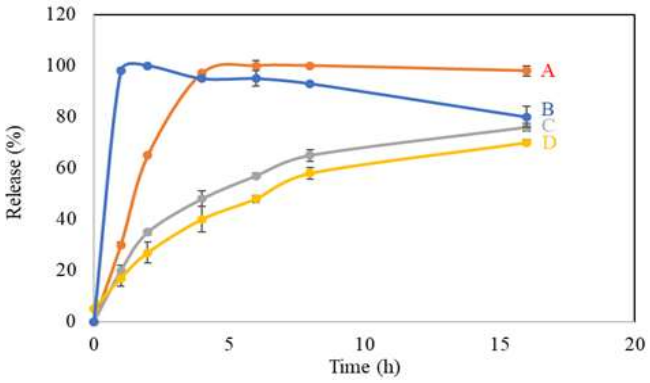


Figure 3. The release profiles for nanoemulsion (C= pH 1.5 and D=pH 7.4) and free essential oil (B= pH 1.5 and A=pH 7.4) (n=3).

Essential oil content evaluation in the nanoemulsion

The essential oil composition of nanoemulsion was researched for two months. After one and two months, the quantity of essential oil dropped to 95.8 and 91.4% of the original value, respectively. On the first day, the amount of essential oil was measured as 100%.

Antibacterial properties

The antibacterial effect of agar well plates was assessed by employing varying concentrations of extracted essential oil against microorganisms. CEO was discovered to have an inhibitory effect on all

microorganisms that were examined. However, major and minor effects in standard bacteria were related to *K. pneumoniae and P. aeruginosa*. In contrast, in resistant samples, the major and minor effects were contributed to MRSA and VRE, respectively, as shown in (Table 2). Examination of Tween 80 and nanoemulsion loaded with 5g of paraffin indicates that Tween 80 and Span 60 did not show antibacterial effects. MIC was done to compare the antimicrobial properties of free and Cumin oil-nanoemulsion. Based on microbial dilution results, CEO was effective on bacteria in different dilutions; as such, it had the highest effect on *K. pneumoniae, B. cereus, and S. epidermidis.* CEO *shows* the same effect on methicillin-resistant *S. aureus,* vancomycin-resistant *Enterococcus, E. coli, S.* Typhimurium*, and* MDR *Pseudomonas Bacteria*. Nevertheless, P. aeruginosa was designated as the most resistant to essential oil. The cumin oil-nanoemulsion had the greatest impact on K. pneumoniae and B. cereus, and it affected other bacteria equally. P. aeruginosa was, however, more susceptible to nanoemulsions than to freely available essential oils. As indicated in (Table 3), there was no difference among the antibacterial effects of nanoemulsion on gram-positive and gram-negative bacteria.

Table 3. Mean diameter of inhibition zone of Cumin Essential Oil (CEO), and MIC, and MBC of CEO and its nanoemulsion against standard and resistance bacteria (n=3).

Name	Mean diameter of inhibition zone of CEO	Mean diameter of inhibition zone of positive controls	Essential oil MIC (mg/ml)	Nanoemulsion MIC (mg/ml)	Positive Control MIC (µg/ml)	Essential oil MBC (mg/ml)	Nanoemulsion MBC (mg/ml)	Positive control MBC (µg/ml)
Staphylococcus epidermidis **(1435 PTCC)**	26±0.25	20±1.00 (vancomycin)	6.25	6.25	2 (vancomycin)	12.5	12.5	4 (vancomycin)
Bacillus cereus **(1015 PTCC)**	19±0.50	18±0.25 (vancomycin)	3.125	1.56	2 (vancomycin)	6.25	3.125	8 (vancomycin)
Pseudomonas aeruginosa **(1310 PTCC)**	12±0.25	17±0.50 (gentamicin)	100	25	2-4 (gentamicin)	200	50	4-32 (gentamicin)
Escherichia coli **(1330PTCC)**	13±0.50	15±0.25 (gentamicin)	25	6.25	2-4 (gentamicin)	50	12.5	4-8 (gentamicin)
Salmonella typhi **(1709 PTCC)**	16±0.25	17±1.00 (gentamicin)	25	6.25	0.25 (gentamicin)	50	12.5	0.25 (gentamicin)
Klebsiella pneumoniae **(1053 PTCC)**	32±0.50	22±0.50 (gentamicin)	0.0015	0.049	0.05 (gentamicin)	0.003	0.098	0.05 (gentamicin)
Resistant *Enterococcus* (1237 PTCC)	26±0.25	0.00 (vancomycin)	25	6.25	-	50	12.5	-
MRSA **(1764 PTCC)**	52±0.50	0.00 (Methicillin)	25	6.25	-	50	12.5	-
Multidrug-Resistant *Pseudomonas aeruginosa* (1811 PTCC)	30±0.25	0.00 (gentamicin)	25	6.25	-	50	12.5	

Discussion

Regarding the resistance promotion of microorganisms to antibiotics, various medical agencies, including CDC and WHO, declared antibiotic resistance as a "global public health concern." (19). This problem can be solved by replacing synthetic antibiotics with compounds with robust and limited side effects. Much research was done on the biological effects of secondary metabolites found in plants used as medicines, such as essential oils, particularly when it comes to bacterial control (20). This study aimed to evaluate the antibacterial properties of *C. cyminum* essential oil and its nanoemulsion against various microorganisms.

The amount of volatile oil extracted from the cumin seeds was estimated as 2.58%; other articles have measured it as 1.22%, 1.45% (21), and 2.33% (22)for Tunisian, Iranian, and Indian cumin seeds, respectively, which agrees with this study. Numerous variables affect essential oil production and composition, including geographic location and ecological parameters such as average annual precipitation, height, seasonal temperature variations, and soil organic matter concentration (1, 7). Most of the biological properties of cumin seeds are related to cumin aldehyde and its terpenoids. Hence, like other vegetable oils, the composition and properties of cumin oil vary depending on the extraction method, geographical location, and cultivar type. It was reported that the essential oils of Mexican, Mediterranean, Pakistan, and Indian cumin seeds contain 62.7%, 47.4%, 20%, and 43% cumin aldehyde, respectively, while samples from Iran showed nearly 32.4% cumin aldehyde (5). As showed in (Table 1), the reported percentage of this substance in our study was 20.08%, confirmed by a previous study.

In line with our study, Hajlaoui *et al*. extracted the essential oil of Tunisian cumin seeds and characterized the chemical composition using a GC-MS device. The detected compounds were almost the same as our reported results (7). They studied the antibacterial effects of CEO against several microorganism types, including *S. epidermidis*, *S. aureus*, *B. cereus*, *E. faecalis*, *E. coli*, *S.* Typhimurium, *P.aeruginosa* by microbial dilution method. Their result showed that the antibacterial impact of essential oil was effective by comparing MIC and MBC (7).

As was said in the introduction, there are restrictions on producing pure essential oils. Dima and Dima (2015) and El Asbahani, Miladi *et al*. (2015) assert that nanodispersions represent a promising approach to address the solubility and low bioavailability challenges of these compounds, protecting them from adverse interactions and augmenting their antimicrobial efficacy through enhanced cellular uptake. Hence, we prepared and evaluated the nanoemulsion effects of CEO. Tween 80 and Span 60 surfactants were used to reach an HLB in a clear nanoemulsion via self-assembling and appropriate particle size formation (11, 23). An important characteristic of Tween 80 and Span 60 is that they would not interfere with the antibacterial properties of the essential oils.

The characterization of nanoparticles showed that selected nanoparticles had desired spherical structure with thermodynamic stability in three months at room temperature. Essential oil-related peaks are observed in FT-IR spectrum of cumin oil nanoemulsions, indicating suitable loading of essential oils. The charge of the selected nanoparticles was determined to be -26.4 mV by the zeta-potential measurement. This indicates that the nanoparticles are both physically stable and low-toxic in terms of the magnitude of the charge value and negative electric charge, respectively, as indicated by previous studies (24). Trujillo et al. developed emulsions and nanoemulsions containing clove and lemongrass essential oil using Tween 80 surfactants and sodium alginate. They applied them to *E. coli* bacteria to examine the antimicrobial effects. Obtained zeta potential was equal to -30 mv or higher; the particle size of emulsion and nanoemulsion was 1120 ± 230, 5.5 ± 0.3 nm and 23.23 ± 10.51, and 20.88 ± 5.10 nm for lemon and clove essential oil, respectively. The antibacterial evaluation determined that nanoemulsion was more lethal and faster than common emulsion (25).

The cumin oil-nanoemulsion drug release profile achieved the plateau at a significantly slowed pace. Similar to our investigation, Natrajan *et al*. employed alginate and chitosan surfactants to encapsulate two distinct herbal essential oils and analyzed their release profiles at pH levels of 1.5 and 7.4. Based on the findings of Natrajan *et al*. (2015), essential oil was released from the encapsulated form more slowly (26). Regarding medication fluctuation, a noteworthy drawback of conventional drug administration is that only a tiny portion of the drug is localized in the therapeutic range. This drawback is eliminated by controlled release. However, by controlling drug release, the drug concentration can be maintained in the body for a long time (27, 28).

The mechanism by which essential oils exert their antibacterial effects can vary depending on a variety of factors. Bajpai *et al*. (2013) proposed several antibacterial mechanisms for herbal essential oil based on its composition. First, the membrane in the bacterial well was physically disrupted when sesquiterpenes replaced the membrane lipids, and a different method by changing the fluidity of bacterial membrane's surface. The last one is the binding of sesquiterpenes to active enzymes at the surface of membrane to make a conformational barrier for substrate binding (29).

Based on microbial evaluation, CEO was effective on bacteria in different dilutions. As such, it had the highest effect on *K.pneumoniae, B. cereus,* and *S. epidermidis.* CEO showed the same effect on methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus, E. coli, S.* Typhimurium*, and* MDR *Pseudomonas Bacteria*. However, *P. aeruginosa* was identified as the most resistant to essential oil. It was suggested that the high resistance of *Pseudomonas* is due to the lack of purine gaps (30). In accordance with our research, Derakhshan *et al*. investigated the impact of the sub-MICs concentration of the essential oil of cumin in alcoholic extracts on the formation of biofilms and the integrity of plasmids in K. *pneumoniae*, as illustrated in (Table 3). Based on their findings, cumin may inhibit the development of *K. pneumoniae* by influencing the shape and expression of the capsule and the activity of urease enzyme in the bacterium, even at low levels (31). In another study, Kakarla *et al*. investigated the antimicrobial activity of cumin aldehyde as the main compound of CEO on *S. aureus.* They suggested this essential oil as an inhibitor of bacteria efflux pumps preventing antibiotic resistance.

Nevertheless, the more notable effect of *C. cyminum* essential oil was on *K. pneumoniae,* a gram-negative bacterium, and was slightly better on gram-positive bacteria than gram-negative bacteria. The high resistance of gram-negative bacteria is due to its lipopolysaccharide (LPS) wall, which has low permeability to lipophilic compounds such as essential oils. The phospholipid wall of gram-positive bacteria lacks this protective coating, which renders it more vulnerable to and permeable to essential oil. Except for *K. pneumoniae*, the antibacterial effects of nanoemulsions containing essential oil are more potent in gram-negative bacteria than in pure essential oil. The ineffectiveness of cumin oil-nanoemulsions against *K. pneumoniae* bacteria compared to pure essential oil might be the bacterium's structure or the presence of surfactants. Some studies reported an antagonistic relationship between the effects of these compounds and surfactants, despite the fact that surfactants could conceivably increase the antimicrobial activity of essential oil compounds. Based on the findings of Trujillo *et al*. on the nanoencapsulation of 10 different herbal essential oils, our results can be interpreted that improving the antimicrobial effects of CEO by nanoencapsulation is due to increasing the penetration of these compounds in gram-positive and negative bacteria. The entrance of the nanoemulsion in gram-negative bacteria, such as *Escherichia coli,* is mediated by the purine channels in the bacterial membrane. This mechanism is constrained by the size of particles. Consequently, in order to load lipophilic compounds, such as essential oils, it is necessary to take into account a variety of factors, including the presence of hydrophilic groups in emulsion molecules, the expansion of the surface area, and the selection of suitable particle sizes (25, 32). Therefore, no significant difference has been observed comparing the antibacterial activity of nanoemulsion containing essential oil against gram-positive and negative bacteria. Moreover, Anand Prakash *et al*. created nanoemulsions of Eos, such as pepper, fennel, lemongrass, coriander, and cumin and tested their antibacterial and anti-biofilm effects on *S.* Typhimurium. Nanoemulsion of lemongrass EO showed a twofold enhanced antibacterial efficacy (MIC 0.156 mg/ml and MBC 0.312 mg/ml). The superior activity of this lemongrass EO nanoemulsion could be due to its smaller particle size (20.79 nm) and, therefore, easy passive transport via the bacterial cell membrane (33, 34).

Based on the observations of this study and previous studies, nanoemulsion probably improves the antibacterial activity of CEO via multiple different mechanisms. First, Nanoemulsion increases the solubility of essential oil in the aqueous phase compared to when the essential oil itself is used alone; therefore, at least the inhibitory concentration of the essential oil is reduced. Thus, this drug delivery system increases the contact of the essential oil with the cytoplasmic membrane by increasing the level and inactive transfer. Moreover, the active transport of essential oils via membrane proteins, such as purines, facilitates the targeting of essential oil molecules to their binding sites on microbes. Also, the release of essential oil continuously increases its activity. Finally, the electrostatic effect of nanoparticles that have a positive charge to the negative charge of the cell membrane increases the activity of essential oil at the effect site (11).

Conclusion

Cuminum cyminum. L pure essential oil and its nanoemulsions showed significant antimicrobial properties. The produced nanoemulsion showed sufficient thermodynamic stability and appropriate particle size. A pH-sensitive release profile was observed for cumin oil nanoemulsions, with a release percentage in the circulatory system that reaches its maximum at 7.4 and a release profile that is more persistent than that of free essential oil. Nanoemulsion formulations of essential oils have shown to be more effective compared to the pure oil form; it can be deduced that *K.pneumoniae* had the highest sensitivity to the antimicrobial effects of *C. cyminum* among all studied food-poisoning-causing bacteria. However, cumin oil-nanoemulsion had an acceptable function against antibiotic-resistant bacteria, which can be associated with the simultaneous oral administration of this formulation with synthetic antibiotics to reduce the resistance of bacteria.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethics approval and consent to participate

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Authors' Contribution

Conceptualization, M.M. and F.H.K.; methodology, M.M and A.Y..; investigation, M.M., A.Y., S.S and FHK.; writing manuscript and editing, M.M. and

F.H.K.; supervision, M.M., A.Y., and S.S.; project administration, M.M.; All authors have read and agreed to the published version of manuscript.

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