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ORIGINAL ARTICLE

Tackling Biofilm Resistance of Gram-Positive and Gram-Negative Bacteria Against Levofloxacin via Nanotechnology and Essential Oils

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Abstract

Introduction The generation of biofilms by bacteria has become a major factor in the rise of antibiotic resistance. Lipid nano-capsules (LNCs) have recently emerged as an innovative platform for drug delivery, due to their unique properties and ability to carry a wide array of therapeutic chemical compounds.

Objectives The objective of this research was to create, optimize, and evaluate the antibiofilm efficacy of a peppermint oil emulsion (o/w) containing levofloxacin against resistant bacteria via biofilm formation.

Methods Essential oils, particularly peppermint oil known for its antifungal properties, were employed instead of traditional medium chain triglycerides to formulate lipid nanocarriers, utilizing alternating surfactant types (Solutol HS 15 and Cremophor EL) and differing oil to surfactant ratios (2:1 and 1:1). The LFX-LNCs formula, with a 2:1 oil to surfactant ratio, was selected for further investigation due to its physical properties, including particle size, zeta potential, transmission electron microscopy, and polydispersity index. The antibacterial efficiency of LFX-LNCs was evaluated, revealing their ability to eradicate established biofilms of Gram-negative pathogens, including Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa), as well as Gram-positive strains such as Staphylococcus aureus (S. aureus).

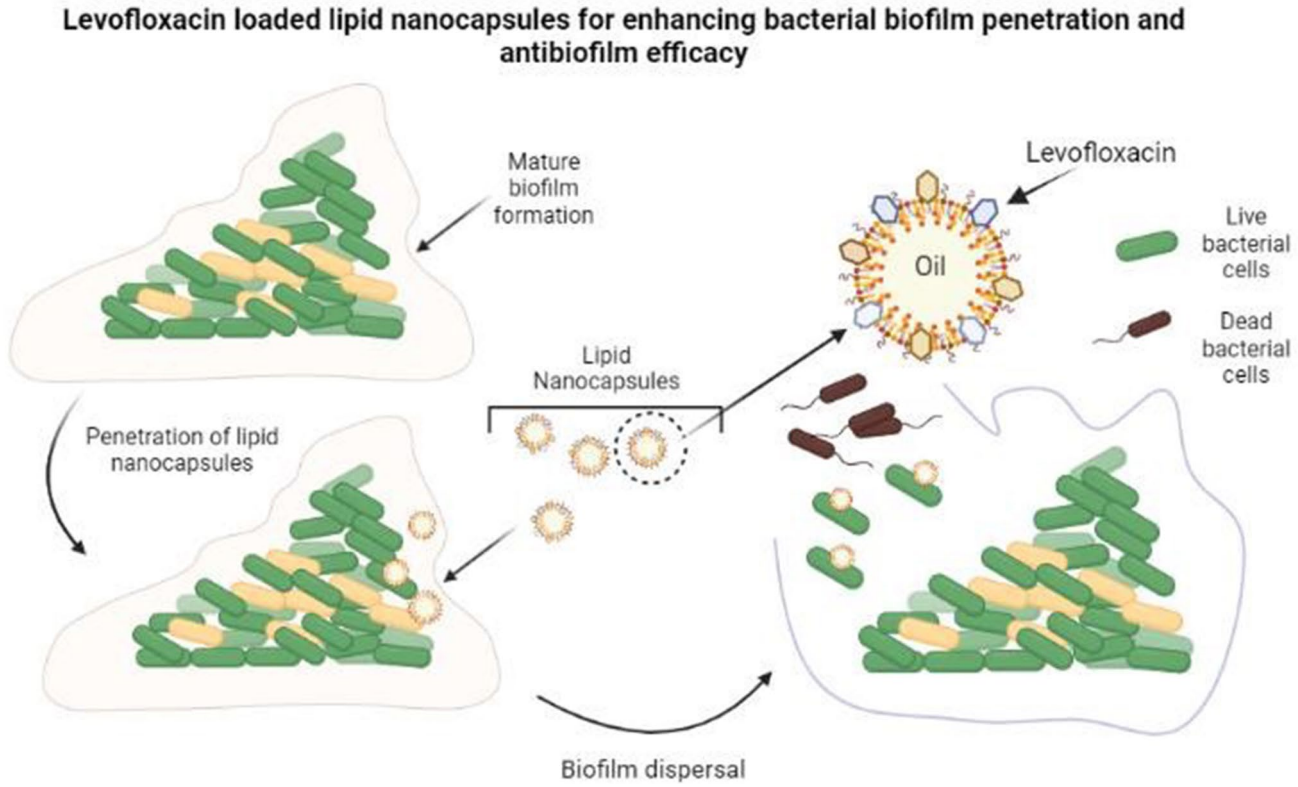
Results The mean particle size of LFX-LNCs varied from 30.86 ± 0.54 nm to 68.36 ± 0.56 nm, demonstrating a narrow size distribution, a negative zeta potential (-1.56 ± 0.24 to -20.2 ± 2.15 mV), and a polydispersity index (PDI) ranging from 0.062 ± 0.006 to 0.26 ± 0.002. Lipid nanocapsules generally exhibit a spherical morphology within the nanometric size range when analyzed by transmission electron microscopy (TEM). The antimicrobial activity assessment revealed that EL 2:1 exhibited the most significant antimicrobial efficacy, characterized by a reduced particle size and an inhibition zone measuring up to (2.43 ± 0.24 cm), demonstrating promising results against several pathogenic strains, including *P. aeruginosa*, *S. aureus*, and *E. coli*.

Conclusion This study illustrates the efficacy of LFX-LNCs in the treatment of non-healing wounds infected with biofilm-forming bacteria.

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Graphical Abstract



Keywords *Pseudomonas aeruginosa* · *Escherichia coli* · LFX-LNCs · Peppermint oil · Biofilm

Introduction

Antimicrobial resistance (AMR) is considered a dangerous threat to the healthcare system with an economic burden [1, 2]. Since the introduction of the first antibiotic, "Penicillin G," the struggle has been reignited by the emergence of antibiotic resistance. Although there are various processes that contribute to the development of AMR, the most critical one is the creation of biofilm by bacteria. Biofilms are collections of bacteria that lack stalks and can adhere to any biotic or abiotic surface. Proteins, polysaccharides, and extracellular DNAs comprise extracellular polymeric substances (EPS). These compounds create a matrix that encases the bacteria within biofilms, rendering them resistant to antibiotics by up to a thousandfold [3]. When they grow on tissues and implanted devices, diseases such as osteomyelitis, cystic fibrosis, otitis media, and wounds, they affect biofilms to varied degrees [4]. *P. aeruginosa* and *S. aureus* have been associated with a variety of infections due to their ability to build biofilms, which can lead to lengthy infections [5]. Systemic antibiotic treatment is unable to entirely eradicate the wound infection due to the formation of biofilm. Additionally, more potent antibiotic dosages are necessary to eliminate the bacteria present in the biofilm, which may result in adverse systemic effects, including arrhythmia and hepatotoxicity [6, 7]. Consequently, the transdermal delivery of an antibiotic in concert with an antibiofilm agent may be a highly effective and efficient treatment for chronic wounds.

Levofloxacin (LFX) is a third-generation fluoroquinolone that is renowned for its efficacy and broad spectrum of action against both gram-negative and gram-positive bacteria [8]. Furthermore, essential oils were identified as the outcome of a secondary metabolism pathway and were demonstrated to have an impact on yeasts, bacteria, and fungus [9–11]. Peppermint oil is one of the most extensively researched essential oils. It is widely recognised that the presence of limonene, L-menthol, methyl acetate, and menthone in this oil indicates antibacterial activity [12]. Unalan et al. [13] investigated the antibacterial and physical properties of poly fibres infused with peppermint essential oil that were produced using electrospinning for wound healing. Gram-positive and Gram-negative bacteria were examined for antibacterial activity, and it was determined to be a promising option for wound healing. Thosar et al. [14] examined the antibacterial properties of five essential oils,

including peppermint oil, against various bacterial strains. Peppermint oil exhibited the most effective benefits against oral microorganisms, as was shown.

Lipid nano-capsules (LNCs) are emerging nanocarriers that have captured the attention of researchers in the field of medication delivery systems. The oily core of LNCs is predominantly formed of medium-chain triglycerides and is encased in a surfactant shell that is made up of lecithin and surfactant. This structure is a combination of liposomes and polymeric nano-capsules, featuring a lecithin shell and oily core [15, 16]. Further advantages of LNCs include their manufacture and preparation techniques, enhanced physical stability over 18 months, and potent particle size (20–100 nm) [16]. One disadvantage of LNCS is that it is primarily composed of medium-chain triglycerides, which limits the entrance of numerous medicines and the resulting effects. It is possible to anticipate that the inclusion of several medications and the provision of a broader range of applications and activities would be facilitated by the creation of LNCs utilising alternative types of oils [17]. Consequently, it appeared to be extremely promising to enhance the production of LNCs by substituting a portion of these oils for the standard medium-chain triglycerides. A study performed by Tiwari et al. [18] demonstrated the combination efficacy of eugenol and a variety of medications against five gram-negative bacteria. As a result, the medication is administered at a reduced dosage, which results in fewer side effects. The objective of the present study was to combine the potential antibacterial properties of essential oils, such as peppermint oil, with the advantages of LNCs as a very effective drug carrier.

Materials and Methods

Materials

Soybean lecithin was acquired from Caelo in Hilden, Germany. Peppermint oil, Solutol HS, and Cremophor EL were acquired from Sigma Chemical Co., St. Louis, MO, USA. All additional chemicals were acquired from El-Nasr Pharmaceutical Chemicals, Egypt.

Methods

Construction of Calibration Curve for Levofloxacin by Examining its Maximum Solubility in Peppermint oil

The solubility of levofloxacin was used to ascertain the maximum amount that could be dissolved in peppermint oil. The additional quantity of levofloxacin was weighed and blended with 3 g of peppermint oil in a stoppered glass vial. The mixture was at 25 °C and agitated on a magnetic stirrer for 72 h until equilibrium was established. Following this, the solutions were filtered through a 0.45 µ filter, diluted with phosphate buffer solution pH 7.4, and analysed for content at 288 nm using ultraviolet (UV) spectrophotometry [19, 20]. The calibration curves for levofloxacin were produced in ethanol. A stock solution of 0.01 mg/mL was produced by transferring 10 mg of levofloxacin to a 100 mL volumetric flask and thereafter adding 100 mL of ethanol in the right manner. The stock solution was exposed to sonication for a period of 10 min. Serial dilutions were prepared and spectrophotometrically assessed at 288 λ max; the concentrations ranged from 3 to 8 mcg/ml [21].

Preparation of Lipid Nano-Capsules

The solvent-free phase inversion method, which entails the thermal manipulation of oil/water systems, was employed to produce LNCs [22]. The results of preliminary investigations indicate that peppermint oil is an optimal choice for the manufacture of LNCs, as it is known to possess antibacterial properties. Two primary steps comprised the formulation process. In Step I, the oil (peppermint oil) is combined with hydrophilic surfactants (Solutol HS and Cremophor EL) in varying ratios (1:1 and 2:1, respectively). The total proportion of both is 30% w/w of the total weight of the formulation. Then, distilled water (20% w/w) containing NaCl and the lipophilic surfactant lecithin at a constant concentration of 1% for each was added, which contained hydrophilic surfactants and oil [23]. Utilizing magnetic stirring, the components were mixed and heated to 85 °C until a phase inversion to w/o emulsion was seen. Subsequently, the emulsion was refrigerated to 55 °C, which resulted to a second full phase inversion, resulting in an O/W emulsion. This cycle of heating and cooling was thereafter repeated. Sudden cooling was performed in Step II by utilizing an equivalent volume of distilled water at a temperature of 1–4 °C. The stoppage of LNCs was subsequently mixed for 10 min before further research [16, 24].

Determination of Particle Size, Polydispersity Index (PDI) and Zeta Potential

The average volume diameters and polydispersity index of the LNCs were examined in terms of particle size, size distribution, and zeta potential using photon correlation spectroscopy [24, 25]. Before analysis, the LNCs suspensions were diluted in deionized water by a factor of 10. The particle size analyzer Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom) was employed to conduct all measurements in triplicate at 25 °C ± 0.5, at a fixed angle of 173° to the incident beam,

and at a temperature of 25 °C. The laser Doppler Anemometer, in conjunction with the Zetasizer Nano, was employed to determine the zeta potential of the manufactured LNCs in accordance with electrophoretic light scattering technology (model ZS3600, Malvern Instruments Ltd., Worcestershire, UK). The measurements were conducted in triplicate at a temperature of 25 °C ± 0.5 [23].

TEM Observation

Transmission electron microscopy (TEM) is pivotal for elucidating the morphology and structure of lipid nano capsules (LNCs). Samples of lipid nano capsules were prepared by dispersing in an aqueous medium. Then, a drop of the LNC dispersion is placed onto a TEM grid allowing the sample to dry at room temperature [26]. Negative staining with a 1% phosphotungstic acid (PTA) solution is commonly used to enhance contrast [27]. Use a TEM operated at 80–120 kV to visualize the samples. Examine the grid under the electron microscope, focusing on distinct, isolated particles. Capture images at various magnifications to analyze the morphology, size distribution, and potential aggregations [28].

Microbiological Evaluation of Selected LNCs Formulae Using cup Diffusion Agar Method

Standard strains of microorganisms used (*Escherichia coli* ATCC 19433 and *Pseudomonas aeruginosa* ATCC 251312 as Gram-negative strains and *Staphylococcus aureus* ATCC 25922 as Gram-positive strain), were employed as microbiological sources to detect the antibacterial activity of the compounds. The media utilized was Muller Hinton Agar, which was acquired from Oxoid Co., UK. The chemicals utilized to produce a 0.5 Mcfarland standard are Sodium chloride 0.9%, Barium chloride dihydrate, and sulfuric acid (1%) (Normal Saline).

The absorbance value of 0.08–0.12, which is comparable to about 1 × 108 CFU/ml, was obtained by spectrophotometrically adjusting the turbidity of microorganisms at 600 nm to match the 0.5 McFarland standard as indicated by Wikler and Matthew [29]. The concentration of levofloxacin as a standard antibiotic solution was optimized to be 1024 µg\ml which is the highest concentration of any agent to be adjusted before performing the susceptibility testing as reported by De Kievit et al. [30]. The antimicrobial activity was determined qualitatively using Agar cup diffusion method as described by Balouiri et al. [31] to determine the zone diameter after 16 h incubation period at 37 °C for bacteria and 18–20 h at 28 °C for fungi.

Microbiological Evaluation of Selected LNCs Formulae Based on MIC Values

E. coli, *S. aureus*, and *P. aeruginosa* were selected as representative bacterial strains. The strains were cultured in Luria-Bertani (LB) broth at 37 °C with constant shaking. MIC values were determined following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Serial two-fold dilutions of LNCs formulations were prepared in a 96-well microtiter plate. Bacterial inoculum was added to each well, and the plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of LNCs that prevented visible bacterial growth [32, 33].

Qualitative Biofilm Inhibition Assay Using Selected LNCs Formulae

P. aeruginosa is a significant organism in microbiological studies, known for its resilience and adaptability in various environments. This bacterium is often associated with infections in immunocompromised individuals and is a model organism for research due to its complex metabolic pathways and resistance mechanisms. Understanding its behavior and interactions within different ecosystems is crucial for developing effective treatment strategies and managing its impact on human health [34, 35].

A bacterial suspension with a concentration of 1 × 10^8 CFU/mL was prepared in Microtiter plates with 96 wells. 200 µL of the bacterial suspension was introduced into every well of the microtiter plate. The wells were prepared by adding HS 2:1 and EL 2:1 formulae. The plate was then placed in an incubator set to 37 °C for a duration of 24 h. After incubation, it is essential to discard the solution and thoroughly rinse the wells with PBS to eliminate any non-adherent cells.

Adherent cells were incubated for a duration of 15 min using a solution of 0.1% crystal violet. Then, the washing process was continued thoroughly to eliminate any remaining dye. In order to dissolve the dye, 200 µL of ethanol was incorporated into the mixture. The absorbance was determined at a wavelength of 570 nm using a microplate reader [36].

Statistical Analysis

GraphPad Prism 8 was employed to analyze the data. The mean ± standard deviation was used to express the MIC values. The statistical significance was established using a one-way ANOVA followed by Tukey's post hoc test, with a P-value of less than 0.05 being considered significant [37].

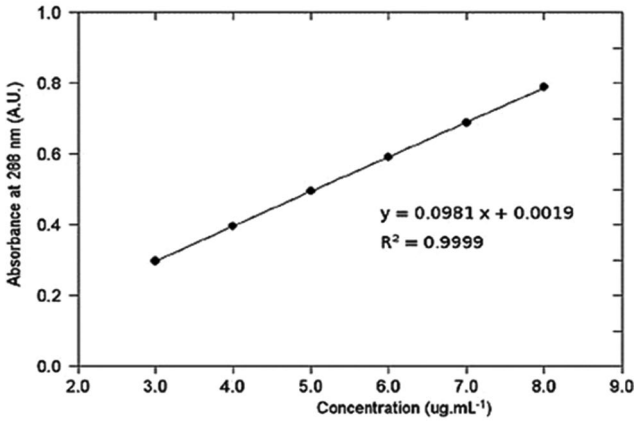


Fig. 1 Calibration curve of levofloxacin

Results and Discussion

The proposed UV spectrophotometric approach was employed to analyse levofloxacin. Linearity was demonstrated in the calibration curve for concentrations ranging from 3.0 to 8.0 µg mL^{-1} (Fig. 1).

Successfully constructed LNCs were utilized to administer levofloxacin. No nano vectors were formed, and phase separation did not describe any structure formation. With a uniform appearance, all of the created formulations were whitish transparent. That is why it is crucial to preserve the high solubility of LFX. LFX's solubility in peppermint oil was 202.8 ± 1.76 mg/ml, which was remarkably high. The investigation employed two surfactants, Solutol HS and Cremophor EL, with variations in the oil-to-surfactant ratios. The choice and ratio of surfactant can significantly influence the physicochemical properties of the nanoparticles. Surfactants play a role in reducing surface tension and stabilizing the nanoparticles, thereby impacting particle size, PDI, and zeta potential. For instance, formulations with Solutol HS showed different characterization outcomes compared to those with Cremophor EL, denoting the impact surfactant type can have on the properties of LFX-LNCs [38–40]. The composition and characterization parameters of LFX-loaded lipid nanocarriers (LFX-LNCs), as illustrated in Table 1; Fig. 2. The table highlights several key parameters such as formulation codes, surfactants used, oil-to-surfactant ratios, drug concentrations, particle sizes, polydispersity indices (PDI), and zeta potentials. It was found that the droplet size was in between 30.86 ± 0.54 nm and 68.36 ± 0.56 nm with narrow size distribution. Nanoparticles within this size range are generally considered suitable for biomedical applications, as they can facilitate enhanced permeability and retention (EPR) effect, thus improving the delivery and efficacy of the encapsulated drug, in this case, LFX. The relatively small size may also help in achieving better cellular uptake and penetration through biological barriers. The polydispersity index (PDI) of the formulations was observed to be between 0.06 and 0.26, which indicates a relatively narrow size distribution and homogenous populations of nanoparticles. It is advisable to ensure consistent performance and reproducibility in drug delivery applications by utilizing a monodispersed system, which is often indicated by a PDI value of less than 0.3. Solutol HS 15 and Cremophor EL are macrogol surfactants that are PEGylated and have a high molecular weight. They prevented oil aggregation by producing more stability in the oil droplets through steric hindrance, which is a result of their long fatty acid chains [41]. Consequently, compact and uniform LNCs were produced. In terms of the impact of the oil surfactant ratio on the particle size of LNCs, the smallest particle size values were observed in LNCs that were synthesized with a 1:1 oil surfactant ratio.

The surfactant's capacity to diminish the interfacial tension between the oil droplets and the aqueous phase is the basis for the interpretation of these results. LFX-LNCs showed smaller particle sizes than the blank corresponding formulations except for one deviation (LFX-LNCs EL 2 − 1) which displayed similar particle sizes for both blank and loaded formulations. The smaller particle sizes of the loaded formulations could refer to the interaction between the drug and the capsule shell which comprised of the hydrophilic surfactants and lecithin leading to reduced size of the outer shell of the lipid nano-capsules. EL1:1 and EL 2:1 showed the lowest particle size in the case of

Table 1 Composition and characterization parameters of LFX-LNCs

Formula code	Surfactant used	Oil: SAA ratio	Drug (LFX) (w/v)	PS (nm)	PDI	ZP (mV)
HS 1:1	Solutol HS	1:1	1	50.63 ± 1.33	0.26 ± 0.002	-15.6 ± 1.63
HS 2:1	Solutol HS	2:1	1	68.36 ± 0.56	0.113 ± 0.011	-1.56 ± 0.24
EL 1:1	Cremophor EL	1:1	1	30.86 ± 0.54	0.119 ± 0.01	-7.59 ± 2.4
EL 2:1	Cremophor EL	2:1	1	48.14 ± 0.06	0.108 ± 0.005	-7.93 ± 0.69
HS' 1:1	Solutol HS	1:1	-	36.69 ± 1.27	0.215 ± 0.036	-20.2 ± 2.15
HS' 2:1	Solutol HS	2:1	-	52.41 ± 0.45	0.062 ± 0.006	-19.4 ± 2.18
EL' 1:1	Cremophor EL	1:1	-	41.94 ± 0.41	0.22 ± 0.001	-3.36 ± 0.16
EL' 2:1	Cremophor EL	2:1	-	47.2 ± 1.35	0.248 ± 0.043	-12.9 ± 2.19

LFX = Levofloxacin, LNCs = lipid nano capsules, PS = particle size, PDI = polydisperse index, ZP = Zeta potential. Each value represents the mean ± S.D. ($n = 3$)

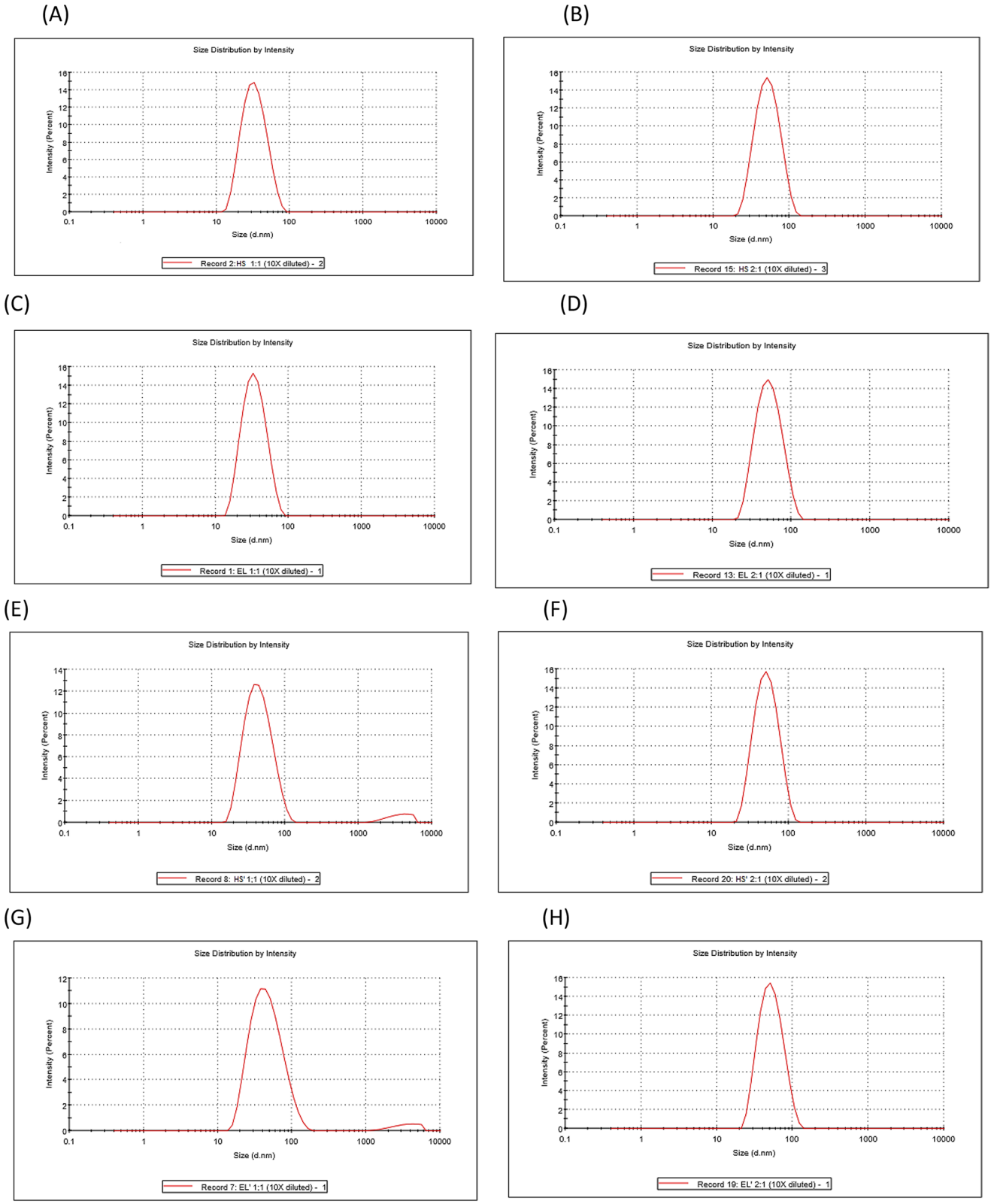


Fig. 2 Size distribution of different LFX-LNCs formulations (**A**) HS 1:1, (**B**) HS 2:1, (**C**) EL 1:1, (**D**) EL 2:1, (**E**) HS' 1:1, (**F**) HS' 2:1, (**G**) EL' 1:1 and (**H**) EL' 2:1. Each value represents the mean ± S.D. ($n = 3$)

blank LNCs of 30.86 ± 0.54 nm and 48.14 ± 0.06, and in the case of loaded formulations with sizes of 41.94 ± 0.41 and 47.9 ± 0.26 respectively (Table 1; Fig. 2). This can be attributed to the presence of cremophor El which in addition to its surfactant activity, acts as a formulation vehicle used for various poorly water-soluble drugs. Which in advance managed to distribute LFX all over the formulation [42]. In this context of biofilm and bacteria, particle size is a critical element in determining the antibacterial activity. This diminutive droplet size facilitates the enhanced interaction of NE droplets with the bacterial cell by increasing the surface area [43]. A reduction in particle size was seen as the ratio of surfactant to oil increased (1:1). This is consistent with what was mentioned by B. Heurtault et al. (2002), who explains the motivation for this is the development of micellar systems [22]. Also, the presence of similar concentrations of surfactant and oil helped to produce micelles with characteristic transparency while as the proportion of surfactant decreased, the micelles began to disappear [44, 45]. Zeta potential values ranged from − 20.2 mV to -1.56 mV. Zeta potential is a critical parameter for assessing the stability of colloidal dispersions. Nanoparticles with zeta potentials greater than + 30 mV or less than − 30 mV are generally considered stable due to the electrostatic repulsion between particles preventing aggregation. Although the observed zeta potential values are less than this threshold, formulations with higher negative values (e.g., -20.2 mV) might still possess adequate stability through mechanisms such as steric stabilization imparted by the surfactants used. This decreases the coagulation chances of the system when it comes in contact with the biological environment and during storage. This may be assigned to the negative benefaction of phospholipids molecules [46] (Table 1; Fig. 2). Under TEM (Fig. 3), Lipid nanocapsules typically display a spherical shape with a nanometric size range. TEM images are used to validate the size measurements obtained via other techniques such as Dynamic Light Scattering (DLS) [47]. Figures have demonstrated that LNCs possess uniform distribution and stability, essential for drug delivery applications [48]. To sum up, the results illustrate the influence of surfactant type and oil-to-surfactant ratio on the composition and characterization parameters of LFX-loaded lipid nanocarriers. Such detailed insights are essential for optimizing the design of nanoparticulate systems for enhanced drug delivery applications, ensuring stability, and achieving precise control over the release profiles of the therapeutics. Formulations HS 2:1 and EL 2:1 that displayed a balance between small particle size, high percentage of oil that possesses antibacterial activity, and a lower percentage of surfactant (more biocompatible) were selected for the further microbiological study.

The results of the antimicrobial activity of the two selected formulations against various bacterial strains (Fig. 4) showed that, in contrast to a control, the highest antimicrobial activity was observed in EL 2:1 displaying smaller particle size with inhibition zone up to (2.43 ± 0.24 cm). These results indicated that nanometer sizes of LFX encapsulation into LNCs had significantly accelerated their antimicrobial effects along with the biofilm inhibition activity of peppermint oil itself [49, 50]. Due to their distinctive structure, Gram-negative bacteria are more resistant than Gram-positive bacteria, so the inhibition zone was found to be larger with positive strains [51].

The antimicrobial activity of Levofloxacin (LFX) incorporated into lipid nanocarriers (LNCs) has shown promising results against various pathogenic strains, including *P. aeruginosa*, *S. aureus*, and *E. coli*. *P. aeruginosa* is notorious for its innate resistance to many antibiotics. Studies have demonstrated that LFX LNCs exhibit significantly larger inhibition zones compared to standard LFX formulations [52–54]. The encapsulation of LFX within LNCs enhances its penetration and retention in bacterial cells due to the nanoparticles' ability to bypass efflux pumps and reduce enzymatic degradation [55].

For *S. aureus*, a major cause of hospital-acquired infections, the results indicate that LFX LNCs provide superior inhibition compared to free LFX. The lipophilic nature of LNCs aids in better integration and disruption of the bacterial cell membrane, resulting in improved antimicrobial efficacy [56]. This aligns with previous findings where lipid-based drug delivery systems have been shown to bolster the effects of antibiotics against Gram-positive bacteria [57]. In the case of *E. coli*, the integration of LFX into LNCs has also proven to be advantageous. The nanoscale size of the carriers facilitates greater cellular uptake and sustained release of LFX, thereby maintaining optimal inhibitory concentrations for extended periods [58]. This slow-release mechanism could prove critical in combating the adaptive resistance mechanisms often observed in E. coli [59].

Table 2 illustrates that LNCs formulations significantly enhance the antibacterial activity of the encapsulated agents. In which *E. coli*, shows higher sensitivity to both formulations, requiring 15.6 µg/mL of EL 2:1 and 12.5 µg/mL of HS 2:1 for antibacterial activity. *S. aureus* requires higher concentrations, with 25.0 µg/mL of EL 2:1 and 20.0 µg/mL of HS 2:1. While *P. aeruginosa*, being known for its resistance mechanisms, requires the highest concentration of EL 2:1 at 31.2 µg/mL and 25.0 µg/mL of HS 2:1.

The LNCs formulations (EL 2:1 and HS 2:1) have been shown to significantly enhance the antibacterial activity of the agents they encapsulate with varying degrees of sensitivity and resistance. This performance of LNCs Formulations across all tested strains can be attributed to their optimized

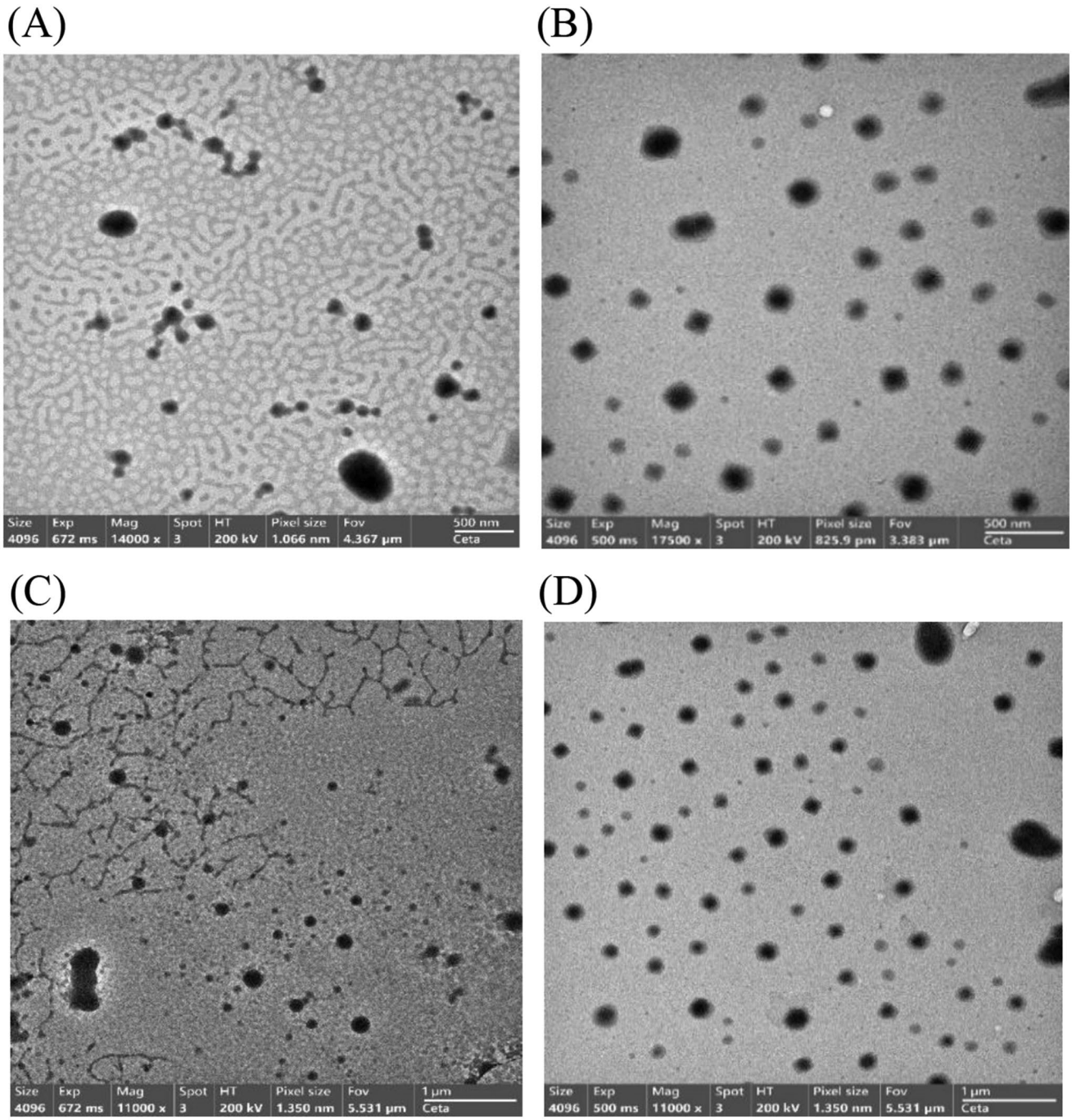


Fig. 3 TEM photographs of different LFX-LNCs formulations. (**A**) HS 1:1, (**B**) HS 2:1, (**C**) EL 1:1, (**D**) EL 2:1

composition, which enhances the stability and release profile of the antimicrobial agent. These findings are consistent with previous studies that have demonstrated the potential of LNCs in improving the therapeutic index of antimicrobial compounds [60]. The statistical analysis confirms that the differences in MIC values between the formulations are significant ($p < 0.05$), underscoring the impact of LNCs composition on antimicrobial efficacy.

Table 3 presents comparative data on Qualitative Biofilm Inhibition expressed as percentage reduction, across different treatments relative to a control. The biofilm inhibition is assessed using two treatments, denoted as HS 2:1 and EL 2:1, alongside a control group. The percentages reflect the extent to which these treatments reduce biofilm formation in comparison to the control. The HS 2:1 treatment shows a substantial inhibitory effect, with 65.5% reduction in biofilm formation while EL 2:1 shows higher inhibitory

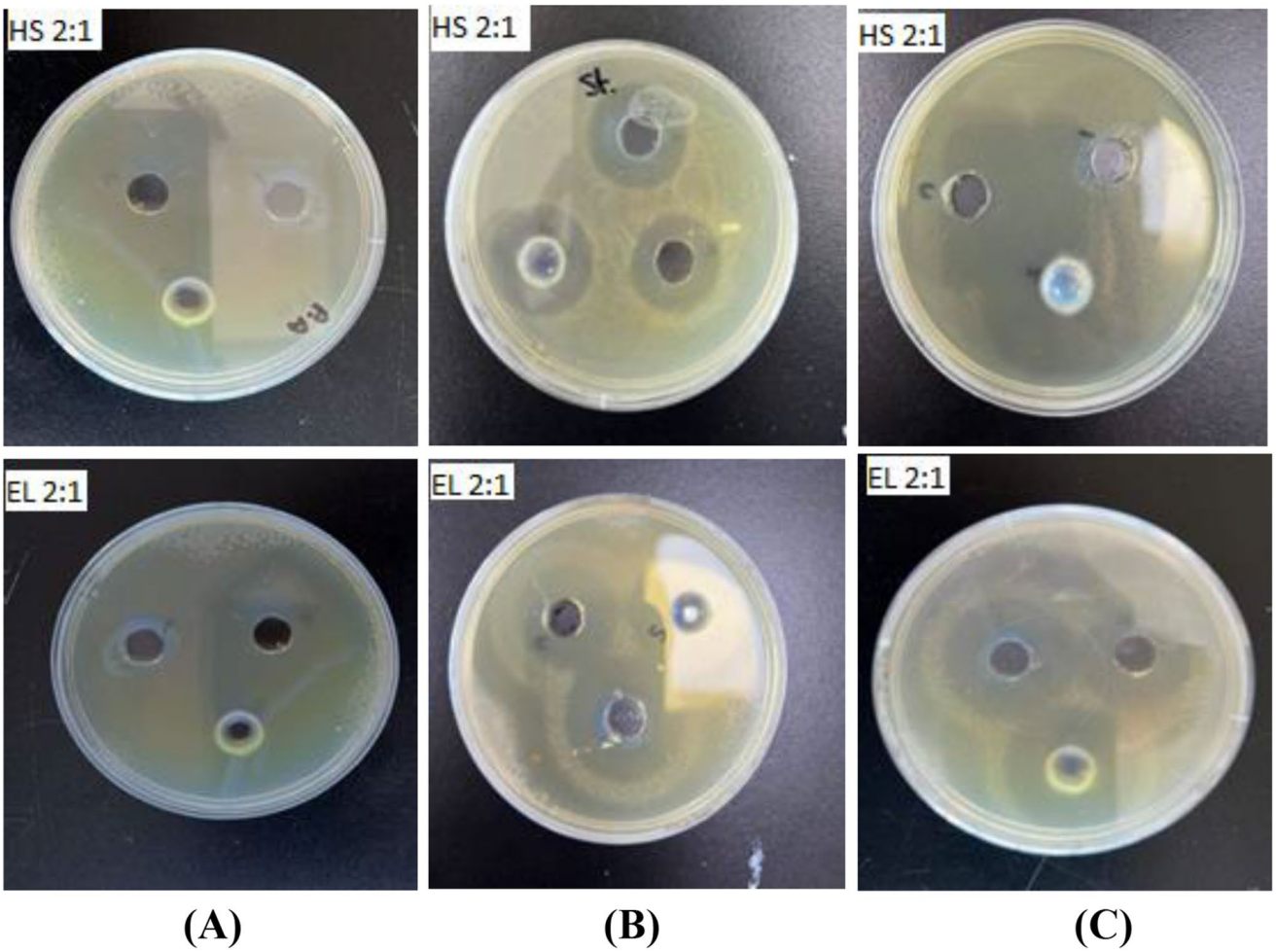


Fig. 4 Inhibition zones produced by antimicrobial activity of LFX LNCs formulations against different strains. (**A**) *Pseudomonas aeruginosa*, (**B**) *Staphylococcus aureus*, (**C**) *Escherichia coli*

Table 2 Antibacterial efficacy of different LFX LNCs formulations against various bacterial strains

Bacterial strain	LNCs formula (EL 2:1) (µg/mL)	LNCs formula (HS 2:1) (µg/mL)
E. coli	15.6**	12.5**
S. aureus	25.0*	20.0*
P. aeruginosa	31.2	25.0*

LNCs = lipid nano capsules, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, P. aeruginosa = Pseudomonas aeruginosa, * (one star) for $p < 0.05$, ** (two stars) for $p < 0.01$. Each value represents the mean ± S.D. ($n = 3$)

Table 3 Qualitative biofilm inhibition of different LFX LNCs formulations against S. aureus strains

Formula	Qualitative biofilm inhibition (% reduction)
Control	0
HS 2:1	65.5**
EL 2:1	72.3**

LNCs = lipid nano capsules, S. aureus = Staphylococcus aureus, ** (two stars) for $p < 0.01$. Each value represents the mean ± S.D. ($n = 3$)

effect with a 72.3% reduction. The EL 2:1 treatment surpasses the HS 2:1 treatment in terms of biofilm inhibition, suggesting possible differences in the mechanisms of action or the constituents of the two treatments [36, 61, 62]. This finding warrants further investigation into the components and methods by which these treatments inhibit biofilm formation, potentially leading to more effective antimicrobial strategies.

Conclusion

The previous work documented the successful utilisation of essential oils, such as peppermint oil, for the manufacture of LNCs due to its antibacterial properties, as an alternative to conventional medium-chain triglycerides. LNCs that were produced were discovered to be thermodynamically stable and exhibit an incredibly small nanoscale size. Furthermore, peppermint oil exhibits a synergistic impact by functioning as a vehicle for the loading of levofloxacin, which is able

to overcome the resistance generated by biofilm formation in many bacterial strains. Moreover, peppermint oil has biofilm inhibition and antibacterial properties. such as *P. aeruginosa*, *S. aureus* and *E. coli*. These findings verify that Levofloxacin's antibacterial efficacy against both gram-positive and gram-negative bacteria is substantially enhanced by LFX-loaded LNCs. These LNC formulations generated inhibition zones that were significantly larger than those generated by free LFX, suggesting that the efficacy of the medication was successfully improved. This increase in antibacterial activity can be related to the nano-sized carriers' capacity to enhance medication solubility, stability, and targeted delivery. As a result of their innate antimicrobial activity and their substantial drug encapsulating capability, these LFX LNCs offer a highly promising drug delivery system that is capable of controlling the susceptibility of bacterial biofilms.

Future Prospective

Future studies should focus on elucidating the exact mechanisms by which LNCs enhance the antimicrobial properties of LFX, including detailed pharmacokinetic and pharmacodynamic studies. Additionally, in vivo experiments are necessary to validate these findings and explore the therapeutic potential of LFX LNCs in clinical settings. Addressing potential toxicity and ensuring biocompatibility will also be critical steps towards developing LFX LNCs as a viable treatment option for bacterial infections, especially those involving multi-drug resistant pathogens.

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Data availability Data and materials related to this article are available from the author upon reasonable request.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest All authors have no conflicts of interest to disclose with regard to the content of this article.

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