Heliyon 10 (2024) e26486

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Development, physicochemical characterization, and antimicrobial evaluation of niosome-loaded oregano essential oil against fish-borne pathogens

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ARTICLE INFO

Keywords:
Niosome
Oregano essential oil
Antimicrobial
Anti-biofilm

ABSTRACT

Objective: Niosomes have gained attention as a promising drug delivery system for enhancing the antimicrobial and anti-biofilm effects of natural compounds. Oregano essential oil has demonstrated potent antimicrobial and anti-biofilm properties against food-borne pathogens.
Methods: In this study, researchers aimed to explore the use of niosomes as a delivery system to improve the efficacy of oregano essential oil against food-borne pathogens. The structural and morphological properties of different niosome formulations were examined. Different formulations of niosomes were prepared and their structural and morphological properties were examined. The antimicrobial and anti-biofilm effects of niosomes containing oregano essential oil were evaluated using microbroth-dilution and microtiter-plate methods, respectively. The biocompatibility of the synthesized niosomes was assessed using the MTT method on human foreskin fibroblasts normal cell line (HFF).
Results: The optimal formulation of niosomes had an average size of 219 nm and an encapsulation efficiency of 61.22%. The release study demonstrated that 58% of the essential oil was released from niosomes, while 100% was released from free essential oil. Furthermore, the antimicrobial and anti-biofilm effects of the essential oil were found to be 2–4 times higher when loaded in niosomes. The biocompatibility test confirmed that the synthesized empty niosomes had no cytotoxic effects on HFF cell line.
Conclusion: Niosomes encapsulating oregano essential oil demonstrated the capacity to inhibit the activity of genes associated with biofilm formation in pathogenic bacteria. This study highlights the significant antimicrobial and anti-biofilm effects of niosomes containing oregano essential oil, suggesting their potential as a suitable drug delivery system.

1. Introduction

The global population's increasing demand for food has raised concerns about ensuring an adequate food supply. As a result, there is a growing demand for fish and fish products, which are considered a cost-effective and easily accessible source of animal protein.

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https://doi.org/10.1016/j.heliyon.2024.e26486
Received 24 October 2023; Received in revised form 8 February 2024; Accepted 14 February 2024
Available online 19 February 2024

Scientific studies have demonstrated that consuming fish can potentially reduce the risk of cardiovascular diseases [1,2]. However, it is important to recognize that fish, like other animals, can be susceptible to various diseases caused by pathogenic agents, some of which can be transmitted to humans [3]. Additionally, the aquaculture industry utilizes a range of substances for various treatments, including synthetic disinfectants like hydrogen peroxide and malachite green, antibiotics such as sulfonamides and tetracycline, and anthelmintic agents [4–6].

The effects of these chemicals can vary greatly on animals, humans, and the environment. Recently, there has been an increasing interest in exploring the potential of natural substances, including probiotics, essential oils, and plant-based medicine, in aquaculture practices [7]. It is worth noting that although these chemicals may have certain benefits, they can also contribute to the emergence and spread of resistance in fish, which could lead to negative consequences for both fish and humans. Therefore, it is important to use them cautiously and explore alternative approaches to mitigate these risks.

The persistent use of antibiotics has been found to contribute to the emergence of drug-resistant strains, leading to economic losses and the potential transfer of resistance to humans, as reported by Zhang et al. [8]. Extensive research has shown that tetracycline is commonly used in fish farms, and there is a clear link between the excessive use of antibiotics in aquaculture and the development of antibiotic-resistant strains. In particular, certain strains of *Aeromonas hydrophila* found in tilapia fish have exhibited resistance to broad-spectrum antibiotics like streptomycin, tetracycline, and erythromycin.

Antibiotic resistance has been identified in various bacterial species, including *Aeromonas hydrophila*, *Photobacterium damselae*, *Aeromonas salmonicida*, *Yersinia ruckeri*, and *Vibrio* sp [9,10]. Researchers have isolated antibiotic-resistant strains from fish samples and expressed concerns about the potential transmission of these resistant strains to humans [11]. Given the increasing prevalence of drug-resistant infections associated with food consumption, it is crucial for the food industry to address the challenges related to food safety. Adeyemi et al. emphasize the significance of implementing alternative approaches to ensure the safety of food for consumers [12].

In recent decades, the significant increase in antibiotic resistance among pathogenic strains has led scientists to explore natural alternatives that have fewer side effects. Essential oils (EOs) are volatile oils or essences derived from plants, known for their distinct aromas. They are complex mixtures of volatile compounds found in aromatic herbs. While the main components of essential oils are typically mono- and sesquiterpenes, they also contain smaller quantities of alcohols, aldehydes, and esters. These compounds are naturally produced by plants for signaling processes and to attract pollinating insects. However, recent studies have suggested that essential oils may have beneficial effects on human health [13].

The term “oregano" encompasses a variety of plant species and genera from six different botanical families, known for their distinctive aroma and taste. These plants have gained recognition for their medicinal and biological properties [14–16]. Significant research has been conducted on the composition of oregano essential oil (EO), revealing that it primarily contains carvacrol, thymol, γ-terpinene, and *p*-cymene [17]. The biological properties of essential oils derived from various plants have been extensively studied, and numerous investigations have demonstrated their antiviral, antifungal, and antibacterial activities. These studies are particularly important in light of the emergence of multidrug-resistant (MDR) strains associated with infections. Researchers have focused on examining individual essential oils, combinations of oils, and different extraction methods from diverse plant sources [18]. Among the herbs studied, various species of oregano have received significant attention, with variations in essential oil compositions being explored. However, thymol and carvacrol are the most commonly found compounds in oregano. In the present study, the antimicrobial and antibiofilm activities of oregano extract loaded into noisomes were investigated against MDR fish pathogens.

2. Materials and methods

2.1. Materials

The chemicals and materials used in the study included chloroform, ethanol, Span 80, DCP, DMSO, cholesterol, SDS, and Amicon (Ultra-15-Membrane, MWCO 30000 Da) obtained from Merck, Germany. Gibco, USA provided trypsin-EDTA, Trypan blue, Medium RPMI-1640, DMEM, PBS, FBS, MTT, and Penicillin/Streptomycin 100 X. A dialysis membrane with an MWCO of 12,000 Da was used. Congo red, crystal violet solution, and other necessary chemicals were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). HiMedia Laboratories, India supplied the media, while the control bacterial strain was obtained from the American Type Culture Collection (ATCC). The HFF cell lines were sourced from Pasteur Cell Bank in Iran. For RNA extraction, a kit from Qiagen, United States was used. The cDNA synthesis was performed using the Revert First Strand cDNA Synthesis Kit from Fermentas in Lithuania. Additionally, other chemicals and analytical grade solvents were obtained from Merck (Germany).

Table 1
Different formulations of synthesized niosomes.

Formulation	**Span60/Tween60 (mol ratio)**	**Lipid (μmol)**	Oregano (mg/ml)	**Surf/Chol (mol ratio)**	Sonication time (min)
F1	75:25	200	1	1:1	7
F2	50:50	200	1	1:1	7
F3	25:75	200	1	1:1	7
F4	75:25	200	1	2:1	7
F5	50:50	200	1	2:1	7
F6	25:75	200	1	2:1	7

2.2. Preparation of niosome-loaded oregano essential oil

In order to prepare niosomes that contain essential oil, a solution was created by dissolving a combination of cholesterol, Span 60, and Tween 60 at different concentrations in a mixture of chloroform and methanol solvents (in a 2:1 ratio) (Table 1). The solution was thoroughly stirred until complete dissolution of all components was achieved. Subsequently, the solution was transferred to a flask-shaped container and exposed to vacuum conditions at a temperature of 60 °C and a rotation speed of 150 rpm to facilitate evaporation. After evaporation, 10 ml of essential oil (1 mg/ml) in PBS was added to the solution, and proper hydration was attained by utilizing a rotation machine operating at a speed of 120 revolutions per minute for a duration of 30 min. To further decrease the size of the particles, ultrasound treatment was applied for a period of 7 min and 20 s [18].

2.3. Encapsulation efficiency

A method for determining the drug content in Niosomes involves calculating the Encapsulation Efficiency (EE%). EE% represents the proportion of drug incorporated within the niosome structure relative to the initial concentration of the drug. To calculate EE%, the synthesized niosomes were subjected to centrifugation at 14,000 rpm for 45 min at 4 °C. During centrifugation, Niosome-loaded oregano essential oil precipitates, while free oregano essential oil remains in the supernatant. The absorbance of the supernatant was measured using a spectrophotometer at a wavelength of 265 nm. The amount of free oregano essential oil was determined through calculations and subtracted from the initial amount of oregano essential oil. EE% was then calculated based on this difference (Equation (1)) [19].

$$EE\% = (\text{Amount of free drug} - \text{Amount of primary drug}) / \text{Amount of primary drug} \times 100 \quad \text{(Eq 1)}$$

2.4. Physical and chemical properties of synthesized niosomes

To evaluate the characteristics of the synthesized niosomes, several techniques were employed. The Scanning Electron Microscopy (SEM) method was utilized to examine the visual appearance of the niosomes. The size of the niosomes was determined using the Dynamic Light Scattering (DLS) technique. Additionally, Infrared Spectroscopy (FTIR) was employed to analyze the functional groups present in the niosomes. These techniques yielded valuable information regarding the morphology, size distribution, and chemical composition of the synthesized niosomes [20,21].

2.5. Drug release test

The dynamic evaluation of oregano essential oil release from the niosomes was performed using the following methodology. Two milliliters of niosomes loaded with oregano essential oil were placed inside a dialysis bag, while a separate dialysis bag contained a solution of oregano essential oil. Both bags were then suspended in a beaker containing 50 ml of PBS at a constant temperature of 37 °C. The beakers were positioned on a stirrer to facilitate agitation. Sampling was conducted at various time points over a 72-h period. At each sampling time, 1 ml of PBS from the dialysis bag was withdrawn and replaced with 1 ml of pre-warmed (37 °C) PBS. The sampling intervals included time points at 1, 2, 4, 8, 24, 48, and 72 h. The optical absorbance of the collected samples was measured using a UV spectrophotometer at the appropriate wavelength, employing the spectrophotometer method. This enabled the quantification of the released oregano essential oil from the niosomes. A cumulative release percentage graph was plotted, illustrating the progressive release of oregano essential oil over the 72-h period [22].

2.6. Stability studies

The stability of the samples in the optimized formulation was assessed by subjecting the Newsome's formulation to two different storage temperatures: 4 °C and room temperature. The samples were stored for duration of one month under these conditions. After the storage period, the samples were evaluated in terms of their encapsulation efficiency (EE) and size to determine any changes or degradation that may have occurred [23].

2.7. Antimicrobial activity

The antimicrobial properties of niosomes containing oregano essential oil and free oregano essential oil were investigated using the microdilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The experimental procedure followed the guidelines established by the CLSI (Clinical and Laboratory Standards Institute) and involved the implementation of serial dilution, which was repeated three times to ensure accuracy and reproducibility. The antimicrobial effects were tested against pathogenic strains, namely *Vibrio vulnificus* ATCC 27562, Listeria monocytogenes ATCC 19115, *Aeromonas hydrophilia* ATCC 35654, and *Yersinia enterocolitica* ATCC 27729. Various concentrations ranging from 3.125 to 100 μg/ml of niosome-loaded oregano essential oil and free oregano essential oil were employed. The MIC represents the lowest concentration of niosomes that inhibits the growth of microorganisms, while the MBC signifies the lowest concentration that is lethal to microorganisms. A negative control was implemented using Mueller Hinton Broth culture medium without bacteria, and a positive control involved a well containing only standard bacteria [24].

2.8. Anti-biofilm effects

The anti-biofilm effects of synthesized niosomes and free oregano essential oil were evaluated using a microtitreplate test with crystal violet staining. Initially, the broth culture of the standard strains was added to each well of a 96-well plate and incubated for 24 h to allow biofilm formation. Subsequently, the wells were washed with PBS to remove any non-adherent cells. Following this, the wells were treated with a concentration below the minimum inhibitory concentration (MIC) of the test compounds and incubated for an additional 24 h at a temperature of 37 °C.

To complete the procedure, the wells were washed with PBS to remove any unattached cells. Subsequently, the wells were fixed by adding methanol and allowing it to dry. A 100 μl solution of 0.1% crystal violet (CV) was then added to each well and incubated at room temperature for 20 min. After the incubation period, 100 μl of 33% acetic acid was added to each well to solubilize the crystal violet dye. The solution in each well was mixed thoroughly, and the optical absorbance was measured at a wavelength of 570 nm. The average absorbance values for each sample were calculated and compared to the average values of the control samples. In this experiment, the control samples consisted of strains that were not treated with niosomes containing oregano essential oil or free oregano essential oil [25].

2.9. Cell toxicity

The cytotoxicity of niosome-loaded oregano essential oil, free oregano essential oil, and free niosome was evaluated using the MTT assay, a colorimetric method. Three breast cancer cell lines and a normal cell line (HFF) were obtained from the Pasteur Institute Iran cell bank in Tehran, Iran. The cells were seeded in a 96-well plate at a density of 104 cells per well and incubated for 24 h to allow attachment. Afterward, the cells were treated with various concentrations (ranging from 3.125 to 100 μg/ml) of niosome-loaded oregano essential oil and free oregano essential oil. Following a 48-h incubation period, a solution of MTT dye (5 mg/ml in PBS) was added to each well and incubated for an additional 3 h. The supernatant was then removed, and 100 μL of dimethyl sulfoxide (DMSO) solution was added to each well to solubilize the formazan crystals formed by viable cells. The absorbance of all wells was measured at a wavelength of 570 nm using a plate reader [26,27].

The survival percentage of the cells was calculated using the following formula (Equation (2)):

$$\text{Survival Percentage } (\%) = (\text{Absorbance of treated cells } / \text{ Absorbance of control cells}) \times 100 \quad \text{(Eq 2)}$$

2.10. Statistical analysis

All the tests conducted in this study were performed in triplicate to ensure the reliability and reproducibility of the results. The obtained data were analyzed using GraphPad Prism software, specifically version 8. A one-way analysis of variance (ANOVA) was employed for the statistical analysis. To determine statistical significance, a significance level of $p < 0.05$ was set. This means that results with a probability of occurrence less than 5% were considered statistically significant.

3. Result and discussion

3.1. Synthesis of niosome-loaded oregano essential oil and their physical and chemical characteristics

The synthesis of niosomes containing oregano essential oil involved the development of an optimal formulation. The optimal formulation was determined by synthesizing different combinations of surfactant/cholesterol and Span 60/Tween 60 ratios while maintaining a concentration of 1 mg/ml of oregano essential oil. Table 1 presents the results of the evaluation of these different formulations. It was observed that formulation F2 exhibited the highest encapsulation efficiency (EE%) and had a smaller size compared to the other formulations. Based on these findings, formulation F2 was selected as the optimal formulation and was used for the remainder of the study.

It is important to note that the impact of cholesterol concentration on drug encapsulation can be influenced by various factors [28]. Increasing the cholesterol concentration in niosomes can improve their stability and reduce their permeability, which facilitates the entrapment of drugs within the niosome bilayers. However, higher cholesterol concentrations can also lead to competition between the drug and cholesterol within the bilayer, potentially resulting in the drug remaining unencapsulated [29]. Cholesterol is commonly employed in niosome synthesis due to its ability to enhance the stability and integrity of the niosome bilayers [30]. This, in turn, can prevent the premature release of encapsulated substances and slow down the penetration of solutes enclosed within the aqueous core of these vesicles [31,32]. The inclusion of cholesterol in niosome formulations can thus play a crucial role in optimizing their performance and desired drug delivery characteristics.

The morphological characteristics of the synthesized niosomes were analyzed using scanning electron microscopy (SEM) and dynamic light scattering (DLS). SEM analysis showed the presence of spherical structures in the synthesized niosomes. DLS measurements were conducted to determine the size of the synthesized niosomes, and the optimal size was found to be 177.9 nm (Table 1, Fig. 1). Fourier-transform infrared spectroscopy (FTIR) was employed to analyze the functional groups present in the niosome compounds. The FTIR results exhibited characteristic peaks corresponding to these functional groups. Specifically, the peak at 1096 cm^{-1} indicated the stretching C–O alcohol bond in the structure of cholesterol and Span 60, while peaks at 1044 cm^{-1} and 1278 cm^{-1} were attributed to oregano essential oil (Fig. 2A and B). Previous studies investigating the loading of essential oils into niosomes have

reported similar findings to those of this study. For instance, Raeiszadeh et al. synthesized niosomes loaded with myrtle essential oil and examined their properties using electron microscopy and DLS. Their results showed the presence of spherical niosomes with sizes ranging from 6.17 ± 0.32 to 7.24 ± 0.61 μm [33]. In another study by Marta García-Díaz et al., the essential oils of *Satureja montana* and *Origanum virens* were encapsulated in niosomes, and the size and morphological features of the synthesized niosomes were investigated using field emission scanning electron microscopy (FE-SEM). Their results revealed that the synthesized niosomes displayed a uniform spherical shape with an average size of 140 nm, a polydispersity index (PDI) of 0.251, and a ζ potential of −14 mV [34].

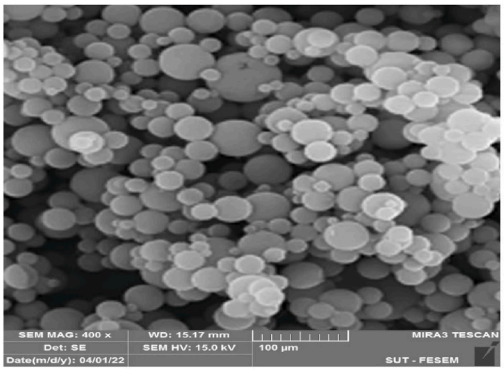
3.2. Drug release pattern

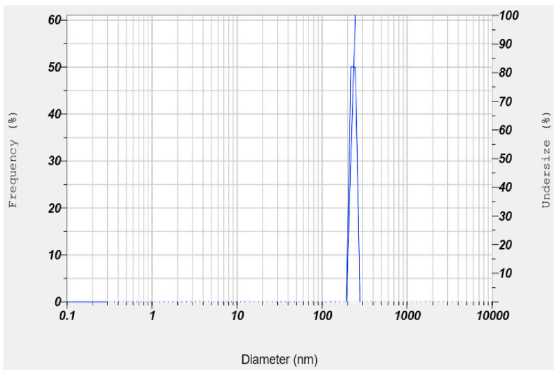
Fig. 3 presents the cumulative release profiles of free oregano essential oil and niosome-loaded oregano essential oil in a PBS release medium over a 72-h period. The use of the PBS release medium aimed to simulate and approximate the ex vivo release environment, mimicking real-life and in vivo conditions. During the 72-h release period, the release of oregano essential oil from the niosomal form (58%) was comparatively lower than that of the free oregano essential oil (100%). In the case of free oregano essential oil, approximately 89% of the oil was released into the medium within the first 8 h. However, for the niosome-loaded oregano essential oil, only 30% of the oil was released from the niosomal structure within the initial 8 h of the release process. These results indicate that the release of oregano essential oil from the niosomal form occurs in two distinct stages. The initial stage (0–8 h) is characterized by rapid and explosive release, where a significant amount of oregano essential oil diffuses into the release medium.

The second stage of the release process involves a slow-release phase, where oregano essential oil gradually diffuses into the release medium over the course of 72 h. This behavior is commonly observed in studies investigating drug release from niosomes. Typically, there is an initial explosive release in the first few hours, followed by a gradual decrease in the release rate over time. The rate of release is influenced by the composition of the niosomes, with stiffer vesicles generally exhibiting slower release kinetics. In the case of the F2 formulation, which primarily consisted of Span 60, Tween 60, and cholesterol, it suggests the formation of a rigid vesicle membrane. Previous research has shown that the incorporation of Tween 80 in niosome preparation can increase membrane fluidity and enhance the release rate. In the context of oregano essential oil-loaded niosomes, the release characteristics align with the requirements of an antimicrobial delivery system. The relatively rapid release of oregano essential oil within the initial 8 h allows for effective concentrations to inhibit the initial colonization by bacteria. Subsequently, the slower release rate enables the maintenance of antimicrobial activity over an extended period, inhibiting microbial growth. This controlled release profile is desirable for sustained antimicrobial efficacy.

3.3. Stability study

To evaluate the stability of the synthesized niosome samples, three samples were subjected to temperature conditions of 4 °C and 25 °C. The size and encapsulation efficiency (EE%) of the samples were measured at specific time intervals, and the average results were recorded (Table 2). The results indicate that the samples stored at 4 °C exhibited better stability in terms of size and EE% compared to those stored at 25 °C. Over a period of 30 days, the samples stored in the refrigerator experienced slower changes in size





Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	219.0 nm	13.3 nm	218.6 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	219.0 nm	13.3 nm	218.6 nm

Fig. 1. SEM micrograph and DLS of synthesized noisome.

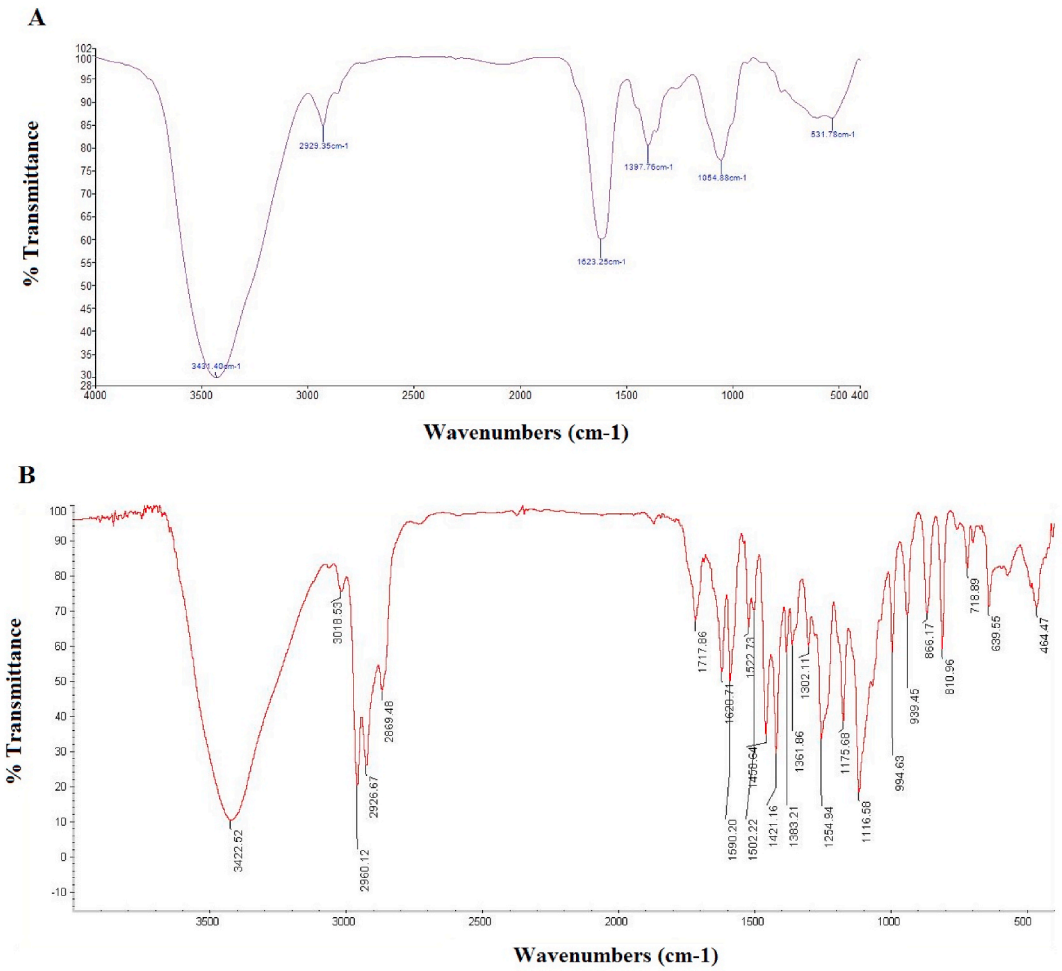


Fig. 2. FTIR analysis of oregano essential oil (A) and niosome-loaded oregano essential oil (B).

and encapsulation efficiency compared to the samples stored at room temperature (Fig. 3A). The study also revealed that the size of the niosomal nanoparticles increased and the EE% decreased with increasing time. Notably, there was a significant difference in size between the samples stored for 14 and 30 days under both temperature conditions being investigated. Additionally, on the 30th day, a significant difference was observed in the EE% between the samples stored in the two studied conditions (Fig. 3B).

3.4. Antimicrobial activity

The objective of the present study was to assess the antibacterial activity of free oregano essential oil, niosomes containing oregano essential oil, and free niosomes against fish-borne pathogens, including *Vibrio vulnificus*, *Listeria monocytogenes*, *Aeromonas hydrophilia*, and *Yersinia enterocolitica*. The microdilution method was employed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values at various concentrations ranging from 3.125 μg/ml to 100 μg/ml. The findings of the study revealed that niosomes containing oregano essential oil exhibited significant antimicrobial effects against the studied pathogens compared to oregano essential oil alone. Specifically, the niosomes containing oregano essential oil displayed the lowest MIC and MBC values against *Vibrio vulnificus*, indicating higher potency against this pathogen. On the other hand, the highest MIC and MBC values were observed against *Yersinia enterocolitica*, suggesting a relatively weaker antimicrobial effect against this particular pathogen. The results demonstrate that the antimicrobial effects were enhanced by 2–4 times when using niosomes containing essential oil, resulting in a 2–4 fold reduction in MIC and MBC values compared to free oregano essential oil alone. This indicates that the encapsulation of oregano essential oil within niosomes improved its antimicrobial activity against the tested fish-borne pathogens. The enhanced efficacy of the niosomal formulation can be attributed to factors such as improved stability, controlled release, and increased bioavailability of the essential oil (Table 3).

Based on the study's findings, it was observed that free niosomes did not possess any antimicrobial activity. This suggests that the antimicrobial effects of niosomes are likely due to their interaction with the bacterial membrane, thereby enhancing the antimicrobial

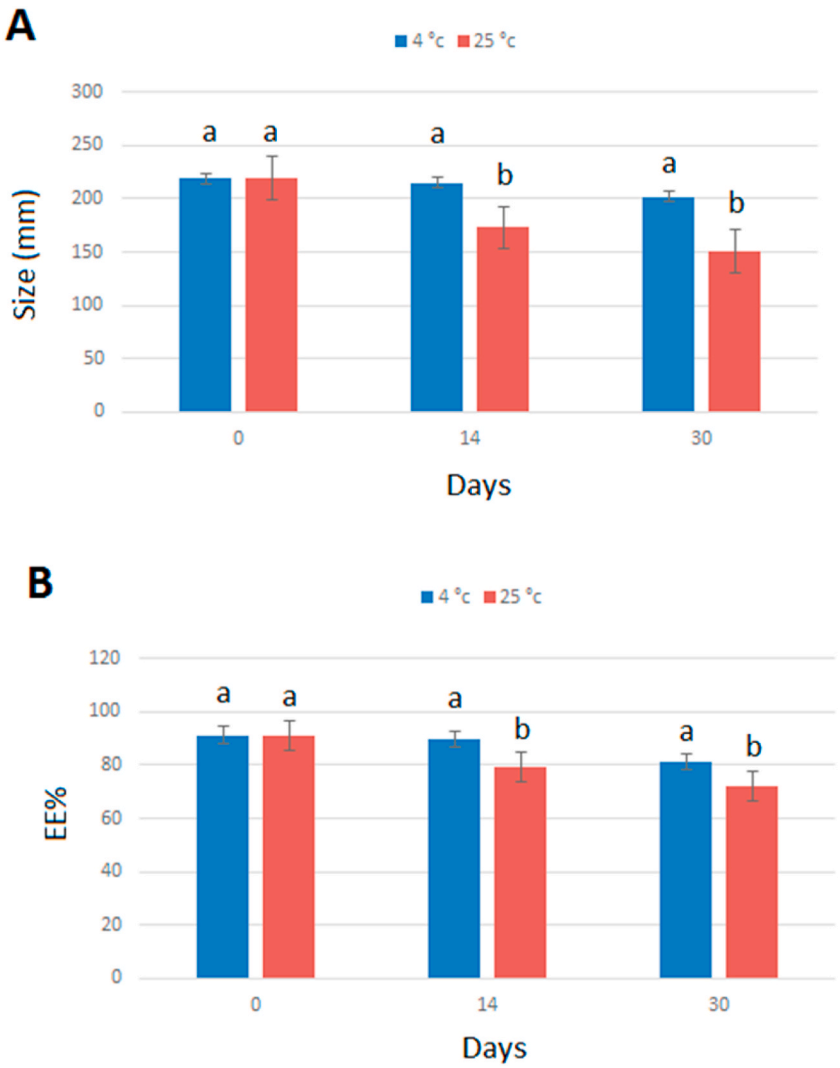


Fig. 3. Size (A) and Encapsulation Efficiency (EE%) (B) Changes of Niosome Samples Stored at 4 °C and 25 °C. All data were calculated as the mean ± SD of three replicates. Data were analyzed using one-way analysis of variance (ANOVA) with tukey's test for multiple group comparisons.

Table 2
The size and encapsulation percentage of organo-containing synthesized niosomes.

Formulation	Vesicle Size (nm)	EE (%)
F1	281 ± 12.1	42.91
F2	265 ± 9.65	61.22
F3	219 ± 13.3	91.32
F4	302.1 ± 15.1	59.45
F5	255 ± 18.7	57.85
F6	289 ± 14.4	49.12

activity of oregano essential oil-loaded niosomes [35]. Several studies have investigated the antimicrobial effects of niosomes containing various antimicrobial compounds. For example, Naresh et al. conducted a study where they synthesized niosomes containing streptomycin and evaluated their antimicrobial effects against microbial pathogens. The results demonstrated that streptomycin-loaded niosomes reduced the minimum inhibitory concentration (MIC) by 4- to 8-fold, indicating a significant antimicrobial effect of streptomycin-loaded niosomes [36]. Similarly, Hedayati et al. prepared niosomes containing tobramycin and examined their antimicrobial effects against *Pseudomonas aeruginosa* strains. Their findings revealed that niosomes containing tobramycin exhibited significant antimicrobial effects compared to free tobramycin, and they also contributed to the reduction of antibiotic resistance [37]. In another study, niosomes loaded with curcumin-Cu and curcumin-Ag were synthesized, and their

Table 3
Antibacterial activity of free oregano essential oil and niosome loaded oregano essential oil against selected pathogenic bacteria.

Bacteria	MIC/MBC of free oregano essential oil (μg/ml)	MIC/MBC of niosome-loaded oregano essential oil (μg/ml)	SubMIC value of free oregano essential oil/noisome- loaded Se oregano essential oil (μg/ml)
Vibrio vulnificus	25/50	6.25/12.5	12.5/3.125
Listeria monocytogenes	50/50	3.125/6.25	25/>3.125
Aeromonas hydrophilia	50/100	3.125/6.25	25/>3.125
Yersinia enterocoltica	100/100	3.125/6.25	50/>3.125

antimicrobial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains were investigated. The results of this study were consistent with our own findings, indicating that the use of niosomes resulted in a significant decrease in the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), thereby enhancing the antimicrobial effects [38]. These consistent findings highlight the potential of niosomes as a promising drug delivery system for enhancing antimicrobial activity when loaded with various antimicrobial compounds.

3.5. Anti-biofilm activity

The study investigated the anti-biofilm effects of niosome-loaded oregano essential oil, free niosome, and free oregano essential oil using the crystal violet method. Fig. 4 depicts that niosome-loaded oregano essential oil exhibited more pronounced anti-biofilm effects compared to free oregano essential oil. The results indicated that niosome-loaded oregano essential oil reduced biofilm formation by 2–4 folds when compared to free oregano essential oil. However, the control group of free niosome did not show any anti-biofilm effects. The enhanced anti-biofilm effects of niosomes can be attributed to their significant antimicrobial activity. This increased antimicrobial activity prevents bacterial growth from reaching levels that can facilitate biofilm formation. As mentioned earlier, the improved antimicrobial effects are likely a result of the fusion between niosomes and the bacterial cell membrane [39].

In a study conducted by Kashef et al. ciprofloxacin was loaded into niosomes, and the anti-biofilm effects of niosome-loaded ciprofloxacin were investigated against biofilm-forming strains of *Staphylococcus aureus*. The results of the study showed that niosome-loaded ciprofloxacin was able to reduce the minimum inhibitory concentration (MIC) value by 2–8 times. Additionally, it significantly reduced biofilm formation at a concentration of 1/8 MIC [40]. This suggests that the incorporation of ciprofloxacin into niosomes enhanced its anti-biofilm activity against Staphylococcus aureus biofilms. In another study by Abu-Elghait et al., a novel tertiary composite comprising cellulose and myco-synthesized selenium nanoparticles was synthesized. The anti-biofilm effects of this composite were investigated against Pseudomonas aeruginosa and *Staphylococcus aureus* strains. The findings of the study demonstrated that the cellulose-based selenium nanoparticle tertiary composite exhibited inhibitory effects on biofilm formation and displayed anti-biofilm activity [41].

3.6. In vitro cytotoxicity

The cytotoxicity assessment of free oregano essential oil, free niosome, and niosome-loaded oregano essential oil on normal HFF cell lines after 24 h demonstrated notable findings. At the highest concentration tested (100 μg/ml), the cell survival rate in HFF cells treated with niosome-loaded oregano essential oil was significantly lower, with values of 23.65 ± 1.21%, 31.25 ± 1.13%, and 23.35 ± 1.36% respectively. In contrast, free oregano essential oil exhibited stronger cytotoxic effects against the HFF normal cell line. This dissimilarity in cytotoxicity may be attributed to the presence of specific herbal compounds found in oregano essential oil. Additionally, the evaluation of free niosome's cytotoxicity on breast cancer and HFF normal cell lines demonstrated no significant harmful effects, indicating its biocompatibility (Fig. 5).

Several studies have been conducted to investigate the potential anti-cancer effects of niosomes loaded with nanoparticles and antimicrobial drugs. For instance, a study by Rezaie Amale et al. focused on loading gold nanoparticles into niosomes and assessing their cytotoxic effects on a human ovarian cancer cell line. The findings of this study revealed that niosomes loaded with gold nanoparticles exhibited dose-dependent cytotoxic effects and demonstrated greater cytotoxicity compared to free gold nanoparticles [42]. Similarly, Xuan et al. developed a co-delivery system comprising liposomes encapsulating selenium nanoparticles (SeNPs@liposome) and investigated its cytotoxic effects on lung cancer (A549) and cervical cancer (HeLa) cell lines using the MTT assay. The results of their study demonstrated that SeNPs@liposome enhanced the cytotoxic effects against the cancer cell lines [43]. These findings are in agreement with other research studies, suggesting that incorporating selenium nanoparticles into a drug delivery system can enhance their cytotoxic effects.

4. Conclusion

The study findings underscore the efficacy of niosomes incorporating biosynthesized oregano essential oil. This research marks the successful encapsulation of oregano essential oil within niosomes, and characterization of these niosomes was conducted using techniques such as FTIR (Fourier Transform Infrared Spectroscopy), SEM (Scanning Electron Microscopy), and DLSEM (Differential

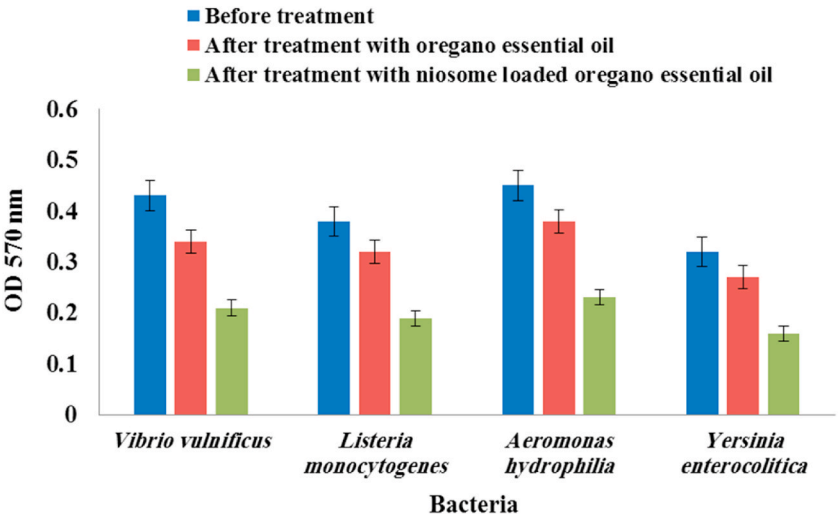


Fig. 4. Anti-biofilm activity of free Oregano essential oil and noisome loaded Oregano.

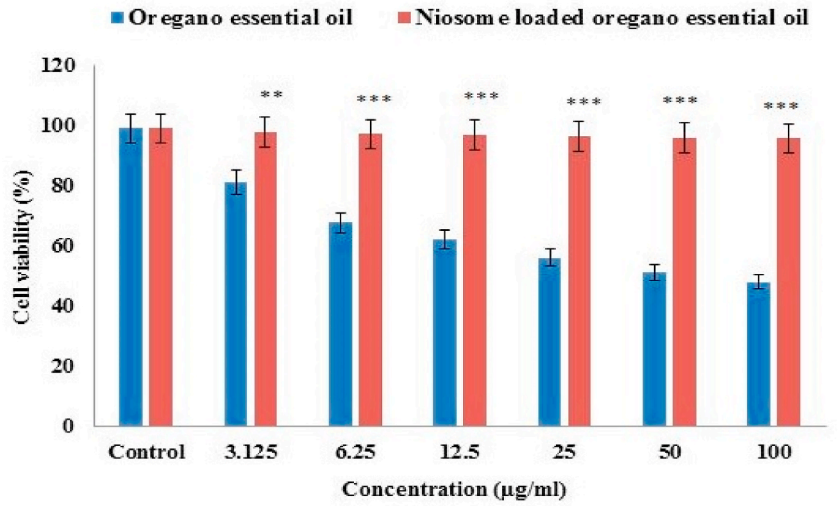


Fig. 5. Cytotoxicity of noisome and free Oregano essential oil. All data were calculated as the mean ± SD of three replicates. Data were analyzed using one-way analysis of variance (ANOVA) with tukey's test for multiple group comparisons.

Scanning Calorimetry). Moreover, the study investigated the impact of niosome-loaded oregano essential oil on antimicrobial activity, anti-biofilm properties, and cytotoxicity against normal HFF cell lines. The results revealed significant antibacterial and anti-biofilm effects against pathogenic bacteria. Furthermore, niosomes loaded with oregano essential oil effectively down-regulated the expression of biofilm-related genes in pathogenic bacteria. These findings support the conclusion that niosomes present a promising drug delivery system for therapeutic applications, particularly in enhancing the antimicrobial and anti-biofilm effects of oregano essential oil.

Additional information

No additional information is available for this paper.

Data availability statement

Data included in article/supp. Material/referenced in article.

CRediT authorship contribution statement

Rameen Sirati: Writing – original draft, Investigation, Data curation, Conceptualization. **Amir Eghbal Khajehrahimi:** Writing – review & editing, Software, Resources, Formal analysis. **Reza Kazempoor:** Supervision, Methodology, Data curation. **Shapoor Kakoolaki:** Supervision, Methodology. **Arman Ghorbanzadeh:** Writing – review & editing, Validation, Formal analysis, Data curation.

Declaration of competing interest

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

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