Research Article

Nanoliposome-encapsulated and biopolymer-coated phlorotannin extract of *Sargassum tenerrimum*: A promising approach for enhanced antibacterial activity against acne-related bacteria

Mohammadi E.[1*]; Shabanpour B.[1]; Pourashouri P.[1]; Payamnoor V.[2]; Sharifian S.[3]; Bita S.[3]

Abstract

Recently, the use of nanoliposome and coated systems has received much attention due to their unique properties for more effective delivery of antibacterial agents. The phenolic extract was co-encapsulated into nanoliposomes and a polyelectrolyte delivery system was obtained by the sequential deposition of positive chitosan (CH) and negative sodium alginate (AL) onto the surface of anionic nanoliposomes (NLs). Phlorotannin compounds were identified by HPLC and the properties of nanoliposomes were evaluated. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of NLs/extracts against acne-related bacteria were determined. The identified compounds of the extract were phloroglucinol, bieckol, dieckol, phlorofucofuroeckol A, and eckol. The mean particle size, zeta potential, and polydispersity index of NLs were 19.77 nm, -13.3 mV, and 0.212, respectively. NLs efficiency on days 0 and 60, at 4°C, was 91.22 and 67.16%, respectively. Release of NLs at pH 3 was higher than at pH 5 and 7. The MIC and MBC values of NLs, AL-CH-NLs, and CH-NLs against all studied bacteria are lower than the extracts. The MIC and MBC activity of the nanoliposomes and coated nanoliposomes decreased over time. The results suggested that NLs, especially CH-NLs and AL-CH-NLs, can be considered an effective carrier system for phenolic compounds.

Keywords: Phenolic extract, Microencapsulation, Coated Nanoliposomes, Antibacterial Activity

1-Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
2-Department of Forestry and Forest Ecology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
3-Department of Fisheries, Chabahar Maritime and Marine University, Chabahar, Iran
*Corresponding author's Email: esmat.mohammadi69@gmail.com

Introduction

Skin disease caused by acne often occurs in adolescents due to the increased sebum secretion, follicle keratinization, bacteria, and inflammation caused by *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria (Lee *et al*., 2014). The use of antibiotics has been instrumental in saving lives and combating bacterial infections, effectively addressing numerous deadly infectious diseases. However, the emergence of antibiotic resistance among microorganisms poses a significant global health threat. Consequently, it is crucial to explore alternative antimicrobial agents or resistance modifiers to overcome this growing antibiotic resistance. While the development of new antibiotics has shown promise (Hassan *et al.,* 2012a, 2012b), enhancing existing antibacterial delivery systems can also play a vital role in facilitating the efficient administration of antibacterial agents, leading to improved therapeutic outcomes (Hallaj-Nezhadi and hassan., 2015).

Brown algae produce a wide range of bioactive compounds, including unique secondary metabolites. Polyphenols in algae include phenolic acids, tannins, flavonoids, catechins, and phlorotannins (Mohammadi *et al*., 2020; Lomartire *et al*., 2021). Phlorotannins are derivatives of tannins that consist of phenolic compounds with oligomeric units of phloroglucinol, these secondary metabolites have a molecular weight ranging from 126 to 650 kDa (Hakim and Patel, 2020). The content of phlorotannins varies from 1 to 14% (based on dry weight) in different types of seaweed (Maheswari and Babu, 2022). Generally, brown algae contain higher amounts of phlorotannins compared to other algae (Lomartire *et al*., 2021). Active compounds isolated from brown algae such as phlorotannins have shown various functional properties such as antioxidant, anti-wrinkle, whitening, anti-inflammatory, anti-diabetic, anti-cancer, antibacterial, antiviral, enzyme inhibition, and anti-allergic properties (Kadam *et al*., 2013). Despite the diverse activities of phlorotannins, the performance of these compounds decreases due to oxidative processes. Therefore, using new delivery systems to protect phenolic compounds can be a suitable solution.

Liposomes are spherical structures formed by the self-assembly of amphiphilic compounds, primarily phospholipids, in aqueous environments. They consist of polar head groups interacting with the surrounding aqueous phases and hydrophobic hydrocarbon tails forming a bilayer and a central aqueous core (Guimarães *et al*., 2021). Nanoliposomes, such as liposomes, are of great interest in developing innovative nanocarrier formulations for controlled delivery of phenolic compounds, particularly hydrophilic molecules (Liu *et al*., 2017). Due to their unique properties, liposomes have found wide application in delivering functional components, including nutraceuticals,

antimicrobials, and flavors, to various food products (Jagtiani, 2022). Encapsulating antimicrobial compounds within liposomes and subsequently coating them with chitosan and alginate polysaccharides represents a promising strategy for antimicrobial drug delivery. This approach offers several advantages, including enhanced antibiotic concentrations at the site of infection (passive or active targeting), increased bactericidal efficacy (via fusion with the bacterial membrane), improved drug uptake, and reduced toxicity of potentially harmful antimicrobial agents (Drulis-Kawa *et al*., 2009). Despite extensive investigation into the use of liposomes as carriers for herbal compounds, a major challenge lies in achieving sufficient delivery to the target organ (Zhang *et al*., 2012). Additionally, the preparation of liposomes with high encapsulation efficiency for plant polyphenols is not straightforward due to potential interactions between these compounds and lipid bilayers (Muqbil *et al*., 2011). In this study, we aimed to address these challenges by employing both the liposomal system and a coating system using chitosan and alginate to enhance the delivery system for the phenolic compound derived from the brown algae *Sargassum tenerrimum. S. tenerrimum*, in particular, is abundantly found along the Chabahar Sea coast during winter. Our approach involved encapsulating the phenolic extract of *S. tenerrimum* algae into nanoliposomes and subsequently coating them with chitosan and alginate polysaccharides. The

subsequent sections will describe the physicochemical characterization of the phenolic extract nanoliposomes, including morphology, particle size, polydispersity index, zeta potential, drug entrapment efficiency, and release properties. Furthermore, we evaluated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both the nanoliposomes and the coated nanoliposomes.

Materials and methods
Preparation of algal extract
Brown seaweed *Sargassum tenerrimum* was collected from the coast of Chabahar in February 2020. Extraction was done by the non-ultrasonic and ultrasonic methods. A 100g algal powder was immersed in 400 ml of methanol in a flask and shaken on an orbital shaker at 100 rpm at room temperature for 18 h. Then, 800 ml of chloroform (CHCl3) was added, and the mixture was shaken for 30 min while avoiding exposure to light. The mixture was filtered through Waterman 1 filter paper to obtain algae powder residue and liquid fraction. After filtration, the liquid fraction was separated between the upper and lower layers by adding deionized water (300 mL). The upper layer was extracted twice with ethyl acetate (EtOAc) (300 mL). The EtOAc fraction contained phlorotannins and was evaporated on a rotary vacuum evaporator. The residue was considered crude phlorotannins and kept for further analysis (Shibata *et al*., 2002). In the ultrasonic method, after 2 hours of

shaking, the mixture was homogenized for 10 minutes with an ultrasonic homogenizer (2s on, 2s off) and the process was continued as mentioned above.

Purification and identification of phlorotannin compounds
Crude phlorotannin compounds were measured by high-performance liquid chromatography (HPLC) using an Azura photodiode array detector 6.1 L and a C18 column (particle size 5, diameter and length 4.6 × 250 mm). Fifteen microliters of phlorotannin (100 µg/mL) were separated by a gradient method using phase A (1% formic acid in water) and phase B (acetonitrile) with a flow rate of 1 ml/min. The wavelength was set at 280 nm. The content of phlorotannin compounds was expressed as a percentage of the total extract (Shibata *et al*., 2002).

Preparation of nanoliposomes
Nanoliposomes were obtained by modifying the method of Valencia-Sullca *et al*. (2016). Briefly, 2 g of lecithin was mixed with 2 grams of Tween 80, and the mixture was stirred on a hotplate shaker at room temperature. Then, 40 ml of water containing phenolic extract (100 mg/mL) was added to the mixture. The suspension was mixed for 12 hours on a hotplate shaker at room temperature. To prepare nanoliposomes, the liposome suspension was subjected to sonication (10 minutes, 2s on and 2s off) using the probe sonicator (Sonicator 4,000, 20 kHz, maximum nominal power 400 W)

at 80% of full power under controlled temperature (30 ± 5°C). Nanoliposome samples were stored in a sterilized bottle at 4°C.

Preparation of monolayered and multilayered nanoliposomes based on LbL self-assembly electrostatic deposition
Coating of nanoliposomes enriched with phenolic extract with alginate and chitosan polysaccharides was done according to Liu *et al*. (2017). CH-NLs and AL-CH-NLs were obtained by depositing CH, CH, and AL on the surface of NLs using LbL self-assembly technology, respectively. For the preparation of CH-NLs, positively charged CH (1 g/100 mL, pH 5.5) was deposited on the surface of NLs (negative charge) in a ratio of 1:1 (L: L) under gentle stirring. The obtained cationic solution was incubated at room temperature for 1 hour to form CH-coated NLs. Then, for the preparation of AL-CH-NLs, the first layer (positively charged CH) was deposited on the surface of NLs as mentioned above. For the second layer, the positive particles were titrated into negative AL solution (1 g/100 mL, pH 5.5, 1:1, L: L) and gently mixed again, followed by incubation for another 1 hour. Then, the CH and AL multilayered NLs were centrifuged at 6,000 × g for 15 minutes. Fresh CH and AL solutions were prepared in 1g/100 mL glacial acetic acid aqueous solution (1%) and distilled water, respectively, and their pH was adjusted to 5.5. Both solutions were stirred overnight for complete

dissolution and filtered through a Wattman filter after centrifugation for 10 minutes at 12,000 × rpm.

Particle size and zeta potential
The particle size, polydispersity index (PDI), and zeta potential of the nanoliposome dispersions were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS instrument (Malvern Instruments) as described by Bouarab *et al*. (2014).

Stability of nanoliposomes
The stability of nanoliposomes was evaluated by monitoring changes in particle size and PDI over a period of 30 days. Nanoliposomes were stored at 4°C, and measurements were taken at regular intervals using the DLS technique.

Transmission electron microscopy (TEM)
A drop (20 μL) of the nanoliposome suspension was placed on a Formvar/Carbon grid (TEM Grid Copper, 300 mesh, Electron Microscopy Sciences, USA) for 5 minutes. Then, the sample was negatively stained with one drop (20μL) of 2% uranyl acetate for 30 seconds. The grid was dried using filter paper, and the morphology of the nanoliposomes was evaluated by TEM (Zeiss EM10C) operating at 100 kV (Ruozi *et al*., 2011).

Determination of entrapment efficiency
The entrapment efficiency (EE) of nanoliposomes was determined based on the content of phenolic compounds using the Folin-Ciocalteu method (Madrigal-Carballo *et al*., 2010). Nanoliposomes were sedimented for 30 minutes at 6,000 g. Then, the total phenolic content in the supernatant, containing free (unencapsulated) phenolic compounds, was determined by the Folin-Ciocalteu assay as described in section 2.2. The EE was calculated using the following equation:

$$\text{EE (\%)} = \left(1 - \frac{Phenolic\ content\ in\ the\ supernatant}{Total\ phenolic\ content\ added}\right) \times 100$$

In vitro release
The *in vitro* release of phenolic compounds from nanoliposomes was performed in acetate buffer (pH 3 and 5) and phosphate-buffered saline (pH 7) according to Savaghebi *et al*. (2020). Nanoliposomes containing phenolic compounds were isolated by centrifugation from the nanoliposome suspensions and submerged in each of the above buffers. Sealed tubes containing the samples were kept for 30 days at room temperature under continuous stirring (200 rpm). At predetermined time intervals, the amounts of released phenolic compounds were determined using the Folin-Ciocalteu method, and the cumulative release (CR) percentage of phenolic compounds from nanoliposomes was calculated using the following equation:

$$CR\ (\%) = \frac{\sum_{t=0}^{t} Pt}{P0} \times 100$$

Pt is the amount of released phenolic compounds at time t, and P0 is the total amount of phenolic compounds in the formulation.

Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

The concentrations of phenolic extract, nanoliposomes, and coated nanoliposomes tested ranged from 100 to 400 mg/mL. Each sample was diluted with Mueller-Hinton broth (MHB). To determine the MIC values of the samples, stock solutions of the samples were prepared in the microbial culture medium. Bacterial strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Propionibacterium acnes*, and *Staphylococcus epidermidis* were cultured in MHB and incubated at 37°C for 16 hours. The bacterial suspensions were prepared at a concentration of 10^5 CFU/mL, and the antibacterial properties of the phenolic extract, nanoliposomes, and coated nanoliposomes were evaluated. MIC was defined as the lowest concentration of the samples that inhibited growth after incubating for 24 hours. MICs of the samples were determined by the two-fold serial dilution method in 96-well flat-bottomed microtiter plates at a final concentration of 10^5 CFU/mL. For MBC testing, an aliquot of the inoculum was taken from the well that did not show turbidity at the MIC concentration and was plated onto Mueller-Hinton agar (MHA) plates. The plates were incubated for 2 days at 37 °C. The MBC value was determined as the lowest concentration of the samples at which 99.99% or more of the initial inoculum was killed. For the anaerobic bacterium *Propionibacterium acnes*, an anaerobic jar with a Gas pack was used (Rosenblatt *et al*., 1991).

Results

Extraction and identification of phlorotannin compounds

The extraction of the extract from the brown algae *S. tenerrimum* was carried out using both the usual method and the ultrasonic method. The yield of the extract was found to be 7% in the usual method, while the ultrasonic method yielded a significantly higher extraction efficiency of 15%. The crude phlorotannin compounds present in the *S. tenerimum* algae extract were identified using High-Performance Liquid Chromatography (HPLC). The HPLC analysis revealed the presence of five phlorotannin compounds: Phloroglucinol, Bieckol, Dieckol, Phlorofucofuroeckol A, and Eckol. The respective amounts of these compounds were found to be 32.6%, 6.1%, 15%, 4.7%, and 5.6%, resulting in a total identification of 64% of the compounds present in the extract (Fig. 1).

Particle size, zeta potential, and polydispersity index analysis of nanoliposomes

The mean particle size, zeta potential, and polydispersity index (PDI) of phenolic extract-loaded nanoliposomes were investigated. In this study, the average particle size of the nanoliposomes was found to be 19.77 nm. The zeta potential of the nanoliposomes was determined to be -13.3 mV. The polydispersity index (PDI) of the phenolic extract-loaded nanoliposomes was measured at 0.212, indicating homogeneous dispersion of the sample (Fig. 2).
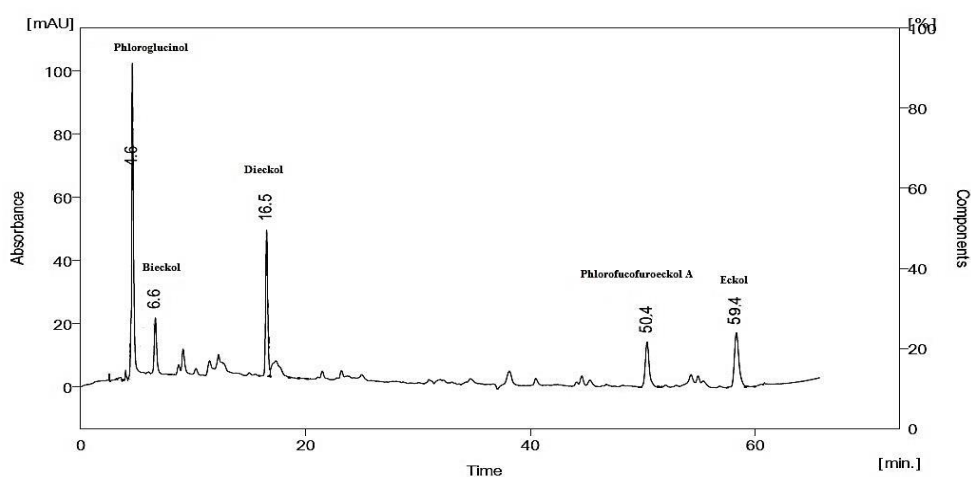

Figure 1: Crude Phlorotannin of *S. tenerrimum* **identified using High-Performance Liquid Chromatography (HPLC).**
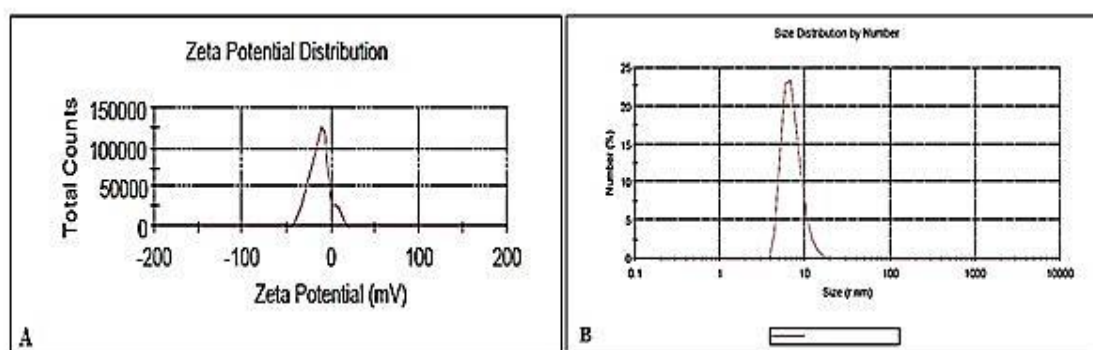

Figure 2: Zeta Potential (A) and Particle Size (B) of Phenolic Extract-Loaded Nanoliposomes of *S. tenerrimum*.

Morphological analysis

The microstructure of the nanoliposomes was investigated using transmission electron microscopy (TEM). The TEM images of the loaded nanoliposomes under the optimized conditions are presented in Figure 3.

Entrapment efficiency

The entrapment efficiency of the nanoliposomes loaded with *S. tenerrimum* phenolic extract was measured over a period of 60 days of refrigeration at 4°C, with measurements taken at 15-day intervals. The results are

summarized in Table 1. It can be observed from Table 1 that the entrapment efficiency of the phenolic compounds in the nanoliposomes decreased over time, reaching 67.16% after 60 days. The highest encapsulation rate of phenolic compounds by the nanoliposome vesicles was observed on day zero, which was significantly different from the other time points ($p<0.05$). The efficiency gradually decreased over time, but a stable trend was observed from day 45 onwards, with no significant difference between the 45 and 60-day time points ($p<0.05$).
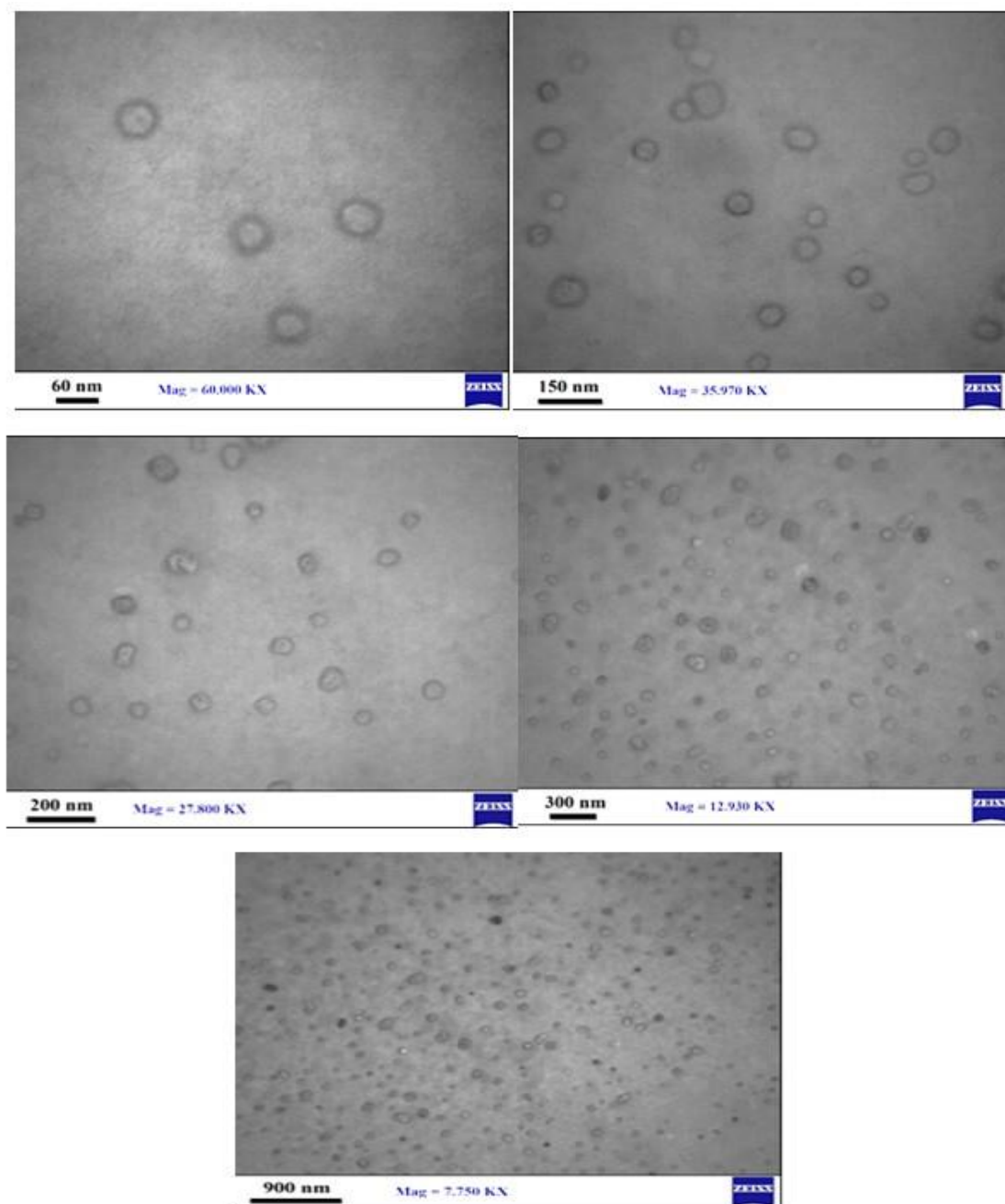

Figure 3: Transmission electron microscopy (TEM) image of phenolic extract-loaded nanoliposomes of *S. tenerrimum*.

In vitro release

In this study, the *in vitro* release of phenolic compounds from the nanoliposomes was investigated over time at 25°C and under different pH conditions (pH 3, 5, 7). The results are presented in Figure 4.

Table 1: The Entrapment Efficiency of Phenolic Extract-Loaded Nanoliposomes of *S. tenerrimum*.

day	Entrapment efficiency
0	91.22±0.18[a]
15	86.37±0.18[b]
30	76.11±1.86[c]
45	67.34±0.19[d]
60	67.16±0.00[d]

Results are expressed as Mean ± SD (n= 3). Different superscript letters in the identical row indicate significant differences (*p*<0.05).

The release rates of phenolic compounds from the nanoliposomes at pH 3, 5, and 7 during days 0-30 ranged from 32.445-47.135, 32.65-47.34, and 32.65-48.97, respectively. The results indicate that the release rate at pH 3 was higher compared to pH 5 and 7.
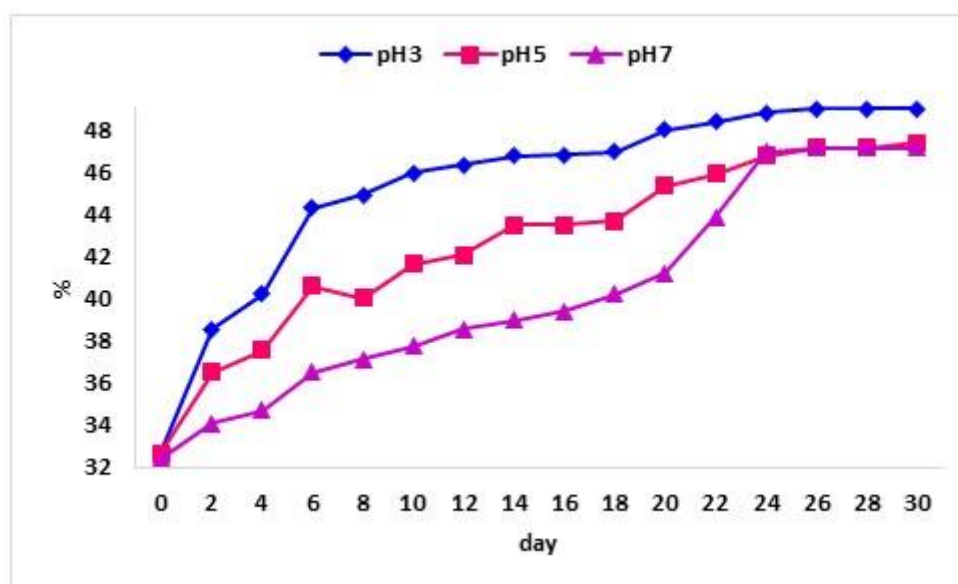

Figure 4: In Vitro Release of Phenolic Extract-loaded Nanoliposomes of *S. tenerrimum*.

Measurement of MIC and MBC values

The antibacterial activity of the phlorotannin extract, nanoliposomes (NLs), chitosan-coated nanoliposomes (CH-NLs), and alginate-chitosan coated nanoliposomes (AL-CH-NLs) was evaluated against six strains of pathogenic bacteria, including *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *P. acnes*, and *S. epidermidis*, using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods. The initial MIC and MBC of the phlorotannin extract were measured on day 0, while the other treatments were

evaluated after 60 days of storage at 4°C. Table 1 presents the MIC and MBC values of the phlorotannin extract, NLs, CH-NLs, and AL-CH-NLs against skin pathogenic microorganisms at day 0.

The results showed that the phenolic extract exhibited the highest MIC and MBC values, indicating the need for higher concentrations to exert its antibacterial activity against pathogenic bacteria. In comparison, nanoliposomes and coated nanoliposomes demonstrated improved antibacterial efficacy at lower concentrations.

Tables 2 to 5 show that MIC and MBC values of the NLs, AL-CH-NLs, and CH-NLs against pathogenic bacteria at days 15, 30, 45, and 60, respectively.

Table 1: MIC and MBC values of the phlorotannin extract, NLs, CH-NLs, and AL-CH-NLs against skin pathogenic microorganisms at day 0.

Bacterial strains	phenolic extract		NLs		AL-CH-NLs		CH-NLs	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus epidermidis	150	300	3.12	25	3.12	25	0.79	6.25
Escherichia coli	200	300	25	50	50	50	50	50
Staphylococcus aureus	150	200	12.5	100	12.5	50	50	50
Pseudomonas aeruginosa	150	200	25	100	50	100	100	100
Bacillus subtilis	200	300	50	100	100	100	50	100
Propionibacterium acnes	150	200	50	>100	50	50	50	50

*All MIC and MBC values are expressed in milligrams per milliliter (mg/mL). NLs: Nanoliposome; AL-CH-NLs: alginate-chitosan coated nanoliposomes; CH-NLs: chitosan-coated nanoliposomes.

Table 2: MIC and MBC values of the NLs, AL-CH-NLs, and CH-NLs against pathogenic microorganisms at day 15.

Bacterial strains	NLs		AL-CH-NLs		CH-NLs	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus epidermidis	3.125	25	3.125	25	0.78125	6.25
Escherichia coli	25	50	50	50	50	50
Staphylococcus aureus	12.5	100	12.5	50	50	50
Pseudomonas aeruginosa	25	100	50	100	100	100
Bacillus subtilis	50	100	100	100	50	100
Propionibacterium acnes	50	>100	50	50	50	50

* All MIC and MBC values are expressed in milligrams per milliliter (mg/mL). NLs: Nanoliposome; AL-CH-NLs: alginate-chitosan coated nanoliposomes; CH-NLs: chitosan-coated nanoliposomes.

Table 3: MIC and MBC values of the NLs, CH-NLs, and AL-CH-NLs against skin pathogenic microorganisms at day 30

Bacterial strains	NLs		AL-CH-NLs		CH-NLs	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus epidermidis	12.5	50	12.5	50	6.25	12.5
Escherichia coli	50	100	50	100	50	100
Staphylococcus aureus	25	100	25	50	50	100
Pseudomonas aeruginosa	50	100	50	100	100	100
Bacillus subtilis	100	100	100	100	50	100
Propionibacterium acnes	50	>100	50	100	50	100

* All MIC and MBC values are expressed in milligrams per milliliter (mg/mL). NLs: Nanoliposome; AL-CH-NLs: alginate-chitosan coated nanoliposomes; CH-NLs: chitosan-coated nanoliposomes.

Table 4: MIC and MBC values of the NLs, CH-NLs, and AL-CH-NLs against skin pathogenic microorganisms at day 45.

Bacterial strains	NLs		AL-CH-NLs		CH-NLs	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus epidermidis	50	100	12.5	50	12.5	25
Escherichia coli	100	100	100	100	100	100
Staphylococcus aureus	50	100	50	50	50	100
Pseudomonas aeruginosa	100	100	50	100	100	100
Bacillus subtilis	100	100	100	100	50	100
Propionibacterium acnes	100	>100	50	100	50	100

* All MIC and MBC values are expressed in milligrams per milliliter (mg/mL). NLs: Nanoliposome; AL-CH-NLs: alginate-chitosan coated nanoliposomes; CH-NLs: chitosan-coated nanoliposomes.

Table 5: MIC and MBC values of the NLs, CH-NLs, and AL-CH-NLs against skin pathogenic microorganisms at day 60.

Bacterial strains	NLs		AL-CH-NLs		CH-NLs	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus epidermidis	100	100	25	50	25	50
Escherichia coli	100	100	100	100	100	100
Staphylococcus aureus	100	100	50	100	50	100
Pseudomonas aeruginosa	100	100	100	100	100	100
Bacillus subtilis	100	100	100	100	100	100
Propionibacterium acnes	100	>100	100	100	100	100

* All MIC and MBC values are expressed in milligrams per milliliter (mg/mL). NLs: Nanoliposome; AL-CH-NLs: alginate-chitosan coated nanoliposomes; CH-NLs: chitosan-coated nanoliposomes.

The results indicate that the MIC and MBC values of the coated nanoliposomes (AL-CH-NLs and CH-NLs) were consistently lower than those of the phlorotannin extract, demonstrating their enhanced antibacterial activity. The nanoliposomes (NLs) showed higher MIC and MBC values compared to the coated nanoliposomes, suggesting that the coating process with chitosan and alginate improved the performance of the phenolic compounds. Moreover, the liposomal and coated systems exhibited better antibacterial activity than the phlorotannin extract alone. Among the tested strains, *S. epidermidis* consistently showed the lowest MIC and MBC values for all treatments.

Discussion

The use of ultrasonic resulted in a higher extraction efficiency. The ultrasonic method, which involves breaking the algae cell wall with ultrasound waves, allows for better extraction of bioactive compounds, leading to the higher yield observed in this study. These findings are consistent with similar studies conducted by other authors, including Araujo *et al* (2013) and Dang *et al.* (2017). Identification of extract compounds with HPLC confirmed the presence of 5 phlorotannin compounds. This analysis aligns with the work of

Shibata *et al*. (2002) who identified six phlorotannin compounds, including Phloroglucinol, eckol, phlorofucofuroeckol A, dieckol, and 8,8'-bieckol, in the algae *Eisenia bicyclis* and *Ecklonia kurome*. Other studies on the identification of crude phlorotannin compounds from various algae have also reported similar findings (Shibata *et al*., 2002; Kang *et al*., 2005; 2012; 2013; Nagayama *et al*., 2003; Joe *et al*., 2006; Chowdhury *et al*., 2014). Confirmation of the produced nanoliposome structure was done by measuring particle size, zeta potential, and PDI. The particle size of nanoliposomes is a critical factor affecting their stability, bioavailability, and release behavior. The average particle size of the produced nanoliposome is in the range of nanometers. Such nanometer-sized particles have attractive features for various applications due to their enhanced performance and interactions at the nanoscale (Roostaee *et al*., 2017). Zeta potential is an important parameter that indicates the electrical potential at the interface or particle surface. A higher absolute value of zeta potential typically confers greater stability to colloidal systems, as it prevents aggregation, coagulation, or flocculation of particles due to increased repulsion between them. In this study, the use of anionic phospholipids in the nanoliposome preparation, such as phosphatidylserine, phosphatidylglycerol, phosphatidic acid, and phosphatidylinositol, was found to contribute to the negative surface charge observed (Rafiee *et al*., 2017). The PDI of the produced nanoliposome represents the homogenous dispersion of the sample. A PDI value below 0.3 is generally indicative of a monodispersed sample, suggesting that the phenolic extract was successfully entrapped within the lipid bilayer of the nanoliposomes (Hasan *et al*., 2014). The TEM of nanoliposomes was measured at 60, 150, 200, 300, and 900 nm scales. The images clearly demonstrate that the loaded nanoliposomes had a particle size of less than 100 nm, consistent with the DLS data. The vesicles exhibited a bilayer structure and a spherical shape, confirming that the prepared vesicles were indeed nanoliposomes and not random aggregates of phospholipids. Although the efficacy of nanoliposomes was high after production, it decreased over time. The decrease in entrapment efficiency over time can be attributed to the thermodynamic instability of liposomes and nanoliposomes, leading to the release of active compounds from these lipid vesicles during storage (Amiri *et al*., 2018). The entrapment efficiency of nanoliposomes depends on various factors, including the type of wall material, the ratio of core to wall material, the encapsulation method, particle size, and total solid content (Tavakoli *et al*., 2018). The observed encapsulation efficiency of the nanoliposomes containing phenolic compounds from *S. tenerrimum* extract is consistent with previous reports by Taylor *et al*. (2007), Malheiros *et al*. (2010), ElMeshad *et al*. (2014), Lopes *et al*. (2017), and Bochicchio *et al*. (2020). The release behavior of encapsulated bioactive compounds is a crucial aspect

of their performance (Rodríguez *et al*., 2016). Encapsulation in nanoliposome vesicles and coated nanoliposomes enhances the targeted and controlled release of bioactive substances (Khorasani *et al*., 2018). The release behavior of encapsulated bioactive compounds in food systems depends on their release kinetics. Nanoliposomes are known for their ability to improve the targeted and controlled release of bioactive substances (Savaghebi *et al*., 2020). The release of phenolic compounds from the nanoliposomes at all pH values does not occur at a constant rate, and the release rate decreases over time. The initial burst release observed in the early days may be attributed to the phenolic compounds entrapped in the outer layer of the membrane, which can be released more rapidly from the nanoliposomes. However, the slow release observed over time may be due to the diffusion of entrapped material from the inner layers to the surface of the nanoliposomes and subsequently from the surface to the bulk of the release medium (Lopes *et al*., 2017). Hydrophobic phenolic compounds are entrapped within the non-polar region of the lipid bilayers, while hydrophilic phenolic compounds are entrapped in the aqueous region of the liposomes, which can slow down the release rate (Gosangari and Watkin., 2012). The release of phenolic compounds at pH 3 was faster compared to the other pH conditions. At pH 3, the majority of the phenolic compounds were released from the nanoliposomes within the first 10 days, followed by a slow and steady

release. The release pattern at pH 5 was similar to that at pH 3, with a high release rate observed for the initial 10 days, followed by a decrease. The structure and fluidity of the lipid bilayers are influenced by pH, with acidic pH reducing the surface charge of nanoliposomes and decreasing the repulsion forces between them, leading to an increase in vesicle size. Consequently, the integrity of the phospholipid bilayers is compromised, resulting in increased release of the entrapped material (Lopes *et al*., 2017). At pH 7, the slow release can be attributed to the interaction between the layers of nanoliposomes and the phenolic compounds, facilitated by the formation of hydrogen bonds between the polar region of phospholipids and the -OH groups of the compounds (Rafiee *et al*., 2017). The release behavior observed in this study was consistent with the findings of previous studies, such as Savaghebi *et al*. (2020) on the release of phenolic compounds from *Sargassum boveanum* extract-loaded nanoliposomes, and Mohammadi *et al*. (2016) and Roostaee *et al*. (2017) on the controlled release of phenolic compounds from olive leaf and green pistachio skin-loaded nanoliposomes, respectively. The nanoliposomes (NLs) showed higher MIC and MBC values compared to the coated nanoliposomes, suggesting that the coating process with chitosan and alginate improved the performance of the phenolic compounds. Moreover, the liposomal and coated systems exhibited better antibacterial activity than the

unencapsulated phlorotannin extract. Encapsulation of phenolic compounds in liposome structure protects them from oxidative processes. This causes the higher performance of nanoliposomes compared to the free form of phenolic extract. Also, double-layer biopolymers around the nanoliposome can increase the duration of activity of phenolic compounds and cause a more stable release of phenolic compounds from the nanoliposome structure. These findings suggested that the CH-NLs and AL-CH-NLs systems had the best performance against the studied bacteria. Nanoliposomes and coated nanoliposomes can bind to the bacterial cell membrane and release the phenolic compound directly in the bacterial membrane, leading to bacterial cell destruction. Plant compounds with antibacterial properties are not sensitive to enzymes involved in bacterial resistance. Several mechanisms have been reported regarding the interaction between liposomes and bacteria, including surface adsorption and endocytosis (Gharib *et al*., 2013). It seems that these factors can explain the antibacterial activity of nanoliposomes containing phenolic compounds. The electrostatic interaction between the lipopolysaccharides of the outer membrane of bacteria and nanoliposomes can increase the entry of phenolic compounds into the microorganism cell. In a similar study, the possibility of using liposomes as a carrier for the delivery of lauric acid was investigated by Yang *et al*. (2009). Liposomes containing lauric acid

(LipoLA) with 102 mg/mL LA were able to completely kill *P. acnes*, which confirms the retention of antimicrobial activity of lauric acid after loading into liposomes. The studies of Huang *et al*. (2011) and Obonyo *et al*. (2012) also showed similar results to the present study.

In conclusion, this study demonstrated that the utilization of nanoliposomes and biopolymer coatings, such as chitosan and alginate, significantly enhances the antibacterial activity of phlorotannin extract against acne-related bacteria. The encapsulation of the extract within liposomal vesicles and the application of biopolymer coatings contributed to the improved stability and prolonged release of the phenolic compounds. Consequently, this approach allows for the efficient delivery of antibacterial agents, leading to more effective treatment outcomes. Furthermore, the use of nanoliposomes and their coatings offers the advantage of reducing the required dosage of the phlorotannin extract to achieve the desired antibacterial effect against the studied bacteria. This reduction in dosage not only promotes cost-effectiveness but also helps minimize potential side effects associated with higher concentrations of the extract. Overall, our findings highlight the potential of nanoliposomes and biopolymer coatings as promising strategies for enhancing the efficacy and stability of phlorotannin extract in combating acne-related bacteria. Further research in this area would focus on the development of novel and more efficient

antibacterial treatments for acne and other skin-related diseases.

Acknowledgments

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