Nanomed J. 11(3): 320-331, Summer 2024

RESEARCH PAPER

# Niosomal tannic acid drug delivery system: An efficient strategy against vancomycin-intermediate *Staphylococcus aureus* (VISA) infections

***Jaber Hemmati [1, 2], Mohsen Chiani [3], Babak Asghari [1], Ghodratollah Roshanaei [4], Sara Soleimani asl [5], Morvarid Shafiei [2]*, Mohammad Reza Arabestani [1, 6]****

*[1] Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*[2] Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran*
*[3]Department of NanoBiotechnology, Pasteur Institute of Iran, Tehran, Iran*
*[4] Department of Biostatistics, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran*
*[5]Anatomy Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*[6]Infectious Disease Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*

## ABSTRACT

***Objective(s):*** Our study aimed to formulate a niosomal system for enhancing tannic acid's antibacterial and antibiofilm activities against vancomycin-intermediate *staphylococcus aureus* (VISA).

***Materials and Methods:*** Niosomal tannic acid was formulated by the thin-film hydration technique, which their physicochemical attributes, including drug loading, particle size, zeta potential, morphology, encapsulation efficiency (EE%), polydispersity index (PDI), release profile, were evaluated. To investigate the cell viability of the prepared niosomes, the cytotoxicity effect was analyzed against the human foreskin fibroblast (HFF) cell line. Finally, the antibacterial and anti-biofilm activities of niosomal formulation were examined against VISA strains and compared tothe free drug.

***Results:*** Scanning electron microscopy images showed that the niosomal formulation incorporated tannic acid was homogeneous, spherical, and identical in size (151.9 nm). EE% and surface charge of the synthesized niosomes were 68.90% and -60 mV, respectively. The tannic acid-encapsulated niosomes showed a significant antibacterial potential in comparison with the free drug. Furthermore, niosomal tannic acid reduced the biofilm formation ability in all VISA strains and efficiently eradicated the formed bacterial biofilms at the same concentrations of the free drug.

***Conclusion:*** Niosomes, as vesicular-based nanoparticles, are known to be potent drug delivery vehicles due to numerous features such as non-toxicity, small size, sustained-release profile, and protection from pharmaceutical degradation. Niosomes have a high capacity to deliver wide range of antimicrobial agents, including natural compounds, which could be presented as a novel approach against bacterial infections, particularly VISA strains.

***Keywords:*** *Biofilm, Drug delivery, Niosome, Tannic acid, Vancomycin-intermediate Staphylococcus aureus (VISA)*

**How to cite this article**

*Hemmati J, Chiani M, Asghari B, Roshanaei GhR, Soleimani asl S, Shafiei M, Arabestani MR. Niosomal tannic acid drug delivery system: An efficient strategy against vancomycin-intermediate Staphylococcus aureus (VISA) infections. Nanomed J 2024; 11(3): 320-331. DOI: 10.22038/NMJ.2024.77016.1873*

## INTRODUCTION

*Staphylococcus aureus* is a prominent pathogen for humans, placing an enormous financial burden on the healthcare system [1-3]. This superbug is one of the seriously life-threatening agents causing various infections with high patient mortality [4-6]. The indiscriminate drug prescription in patients with *S. aureus* infections has contributed to the emergence of resistant strains, creating a major concern for public health. Vancomycin-intermediate *S. aureus* (VISA) strain was first identified in the 1990s in Japan; since then, it has caused significant medical problems, including treatment failure, persistent infections, and prolonged hospitalization [7]. Furthermore, *S. aureus* strains exhibit a high capacity for biofilm formation, causing severe antimicrobial resistance and inefficacy of conventional dosage forms in

* *Corresponding authors: Emails: dr.m.shafiei80@gmail.com; mohammad.arabestani@gmail.com*

Note. This manuscript was submitted on December 20, 2023; approved on February 12, 2024

treating infected patients [8]. Thus, developing a substitute therapeutic solution appears necessary as an emergent approach to tackle the therapeutic challenges caused by VISA infections [9].

Nowadays, the niosomal drug delivery system presents a promising development for preventing and eradicating bacterial antimicrobial resistance [10-12]. The simple handling, biodegradability, bioavailability, acceptable stability, negligible toxicity, cost-effectiveness, are among exceptional properties of these nanoparticles that substantially impact on loaded contents' therapeutic indices [13]. Niosomes, known as a vesicular delivery system, contain nonionic surfactants commonly used in their formulations and could enhance pharmaceutical properties [14]. Furthermore, the niosomes' bilayer structure with amphiphilic molecules can deliver a wide range of materials with various properties [15].

As a water-soluble polyphenol, tannic acid comprises a glucose center in which ten gallic acid molecules are bonded [16]. This natural compound can be commonly extracted from woody and herbaceous plants [17]. Tannic acid is gaining the attention of researchers due to its astounding applications in medicine, food, cosmetics, and other industries [18]. Also, this compound has exhibited antioxidant and antimicrobial activities against some viruses and pathogenic bacteria, including human immunodeficiency virus (HIV) [19], influenza A virus [20], *Helicobacter pylori* [21], *Klebsiella pneumonia*, *Escherichia coli* [22], *Pseudomonas aeruginosa* [23], *and Enterococcus faecalis* [24]. Notably, it has been found that tannic acid has excellent bacteriostatic and bactericidal effects on *S. aureus*, which can be widely applied to eradicate its related infections [25].

Numerous studies have shown the considerable potential of niosomes as an efficient antimicrobial delivery system against a broad range of bacterial pathogens. The experimental results disclose that niosomes can be an efficient drug delivery platform, offering a promising solution for eradicating severe bacterial infections [26-28]. This study synthesized tannic acid-loaded niosomes and analyzed their physicochemical features, including morphology, size, zeta surface potential, loading capacity, cell viability, and release profile. Also, this study aims to propose a novel and effective anti-VISA agent by evaluating the antibacterial and anti-biofilm potentials of tannic acid-containing niosomes.

## MATERIALS AND METHODS

### *Materials*

Span 60 (Sorbitan Monostearate), Tween 60 (Polyoxyethylene Sorbitan Monopalmitate), chloroform, crystal violet, methanol, and all culture media were purchased from Merck Company, Germany. Tannic acid was also supplied from Sigma-Aldrich, India. Spectra/ Por® dialysis membrane (MWCO 12 kDa) was also bought from Sigma-Aldrich, U.S.A. *S. aureus* ATCC 33591, as VISA standard strain, given from microbial collection bank, Pasteur Institute of Iran.

### *Bacterial cultures*

In this study, 75 clinical wound specimens were taken using sterile cotton swabs from patients with diabetic and bedsore ulcers hospitalized at Loghman-e Hakim Hospital in Tehran, Iran. The swabs were placed in 1 ml of sterile phosphate-buffered saline (PBS, pH ~ 7.4) and transferred to laboratory of the Department of Bacteriology, Pasteur Institute of Iran for further investigations. The collected samples were examined for *S. aureus* using standard bacteriological and biochemical tests, including Gram staining, culture on Mannitol salt agar (MSA), colonial morphology, coagulase, catalase, and hemolysis [29]. Then, *S. aureus* isolates were diagnosed as methicillin-resistant *Staphylococcus aureus* (MRSA) based on antibiotic susceptibility testing against cefoxitin (30 µg) and oxacillin (1 µg) disks [30]. Polymerase chain reaction (PCR) was also performed with specific primers of the *mecA* gene for the final verification of MRSA isolates [31]. MRSA ATCC 6538 was considered as the control sample in susceptibility experiments and *mecA* gene detection.

For screening of VISA, the MRSA isolates were examined for vancomycin susceptibility using a broth microdilution method according to the recommended guideline by the Clinical and Laboratory Standards Institute (CLSI). In brief, Mueller-Hinton broth (MHB) media containing serial dilution concentrations of vancomycin were added to a sterile 96-well microplate. Afterward, 0.5 McFarland suspensions of the tested isolates were poured into each well, and the microplate was incubated at 37 °C. After 18-24 hr, the minimum vancomycin concentration inhibiting visible bacterial growth was determined as MIC value. According to the CLSI breakpoints, the MICs interpretive criteria for vancomycin were ≤ 2 mg/L for susceptible, 4–8 mg/L for intermediate, and ≥16 mg/L for resistant [28]. Notably, the MIC method was carried out in triplicate, and *S. aureus* ATCC 33591 and uninoculated MHB medium were used as positive and negative controls, respectively.

***Formulation of tannic acid-containing niosomes***

In this study, the thin film hydration technique was applied to preparation the niosomal tannic acid formulation [32]. Initially, a defined amount of Span 60, Tween 60, and cholesterol were dissolved in a molar ratio of 2:2:1 in 20 ml of organic solvent (2:1 v/v solution of chloroform-methanol) at 25 °C using a magnetic stirrer (150 rpm, 50 min) to obtain a completely homogenized suspension. The organic phase was evaporated under a vacuum at 60 °C for 45 min with a rotary evaporation model of WB Eco Laborota 4000 (Heidolph Instruments, Germany). The remaining solvent was purged with nitrogen gas, and then the dried lipid was hydrated for 45 min in 20 ml of PBS (100 mM, pH ~ 7.4) containing tannic acid to obtain a uniform suspension. The molar ratio of lipid to the drug was considered 20:1. Afterward, the niosomal dispersion was sonicated for 10 min with an ultrasonic microprobe (Hielscher UP50H ultrasonic processor, Germany). Notably, the blank niosomes were prepared according to the same protocol, without drug addition to the formulation. The prepared solutions were visually analyzed for flocculation and turbidity and stored at 4° C for further experimentation. Table 1 represents the specific amounts of lipids and the loaded drug in synthesized niosomal formulation.

***Characterization of the prepared niosomes***

*Morphology, size, and surface zeta potential*

Niosomal physicochemical attributes were investigated via field emission scanning electron microscopy (FE-SEM) and dynamic light scattering (DLS). For FE-SEM micrographs, one droplet of niosomal dispersion, diluted at a ratio of 1:100 with deionized water, was fixed onto the plate base and covered with a gold layer. Finally, the taken images were analyzed using ImageJ software bundled with Java 1.8.0_172 [33]. The DLS method was carried out at 625 nm using a Zatasizer instrument (Malvern Instrument Ltd. Malvern, UK). For this purpose, the samples were tested in a polystyrene cuvette at temperature of 25 °C, a concentration of 0.1 mg/ml, and pH 7.4). Notably, the DLS and FE-SEM methods were performed in triplicate, and the average of results was reported.

Table 1. The composition of niosomal tannic acid formulation

| Niosome component | Weight (mg) | Molar ratio |
|---|---|---|
| Span 60 | 40.51 | 2 |
| Tween 60 | 123.40 | 2 |
| Cholesterol | 18.18 | 1 |
| Tannic acid | 20.00 | 0.25 |

*Assessment of entrapped drug in niosomal formulation*

In order to assess the loaded drug into niosomes, tannic acid entrapment efficiency (EE%) was evaluated via the ultra-centrifugation technique [34]. Briefly, 1 ml of the tannic acid-loaded niosomes underwent centrifugation in an Amicon ultrafilter (Merck Millipore Ltd.) with a molecular weight cut-off 50 kDa at 14000 g for 15 min at 4 °C. The amount of drug within the supernatant solution was calculated through standard curve and UV spectrophotometry (Jasco V-530, Japan). The drug's EE% in niosomal formulation was determined as follows: Entrapment Efficiency (EE)% = [(A-B)/A] ×100

Whereas A is the amount of initial drug fed into the niosomal formulation, and B is the amount of free drug in the supernatant solution.

*In vitro release analysis*

The drug release profile from niosomal suspension was studied by dialysis technique [35]. For this purpose, the dialysis bag (molecular weight cut-off 12 kDa) containing 1 ml of niosomal tannic acid was immersed in a 25 ml PBS solution (pH = 7.4, 5 mM) and magnetically stirred at 100 rpm for 24 hr at 37 °C. Afterward, at 1, 2, 4, 8, and 24 hours intervals, 1 ml of samples were aliquoted, and each concentration was measured spectrophotometrically at 280 nm [36]. Notably, the aliquoted samples were substituted with an equal volume of PBS (pH = 7.4, 5 mM) at 37 °C. The sink condition was maintained to improve drug solubility by adding 0.5% sodium dodecyl sulfate (SDS) in the release medium (PBS, pH = 7.4, 5 mM).

*Cytotoxicity determination of niosomal formulation*

For the evaluation of the biocompatibility of the synthesized niosomes, the cytotoxicity of tannic acid-loaded niosomes was examined using MTT (dimethylthiazol-2-yl)-2, 5- diphenyl-tetrazolium bromide) method [37]. Briefly, HFF (human foreskin fibroblast) cell line provided from National Cell Bank of Iran (Pasteur Institute of Iran) was cultured into a 96-well polystyrene microtiter plate under sterile condition. The microtiter plate was incubated with 5% $CO_2$ at 37 °C for 24 hr and subsequently the increasing concentrations of niosomal formulation were added to HFF cells. Following 24 h incubation, the

wells were resuspended in 15 µl of 5 mg/ml MTT dye. Following 4 hr incubation at 37 °C, 100 µl of dimethyl sulfoxide (DMSO) solution was added into wells. The absorbance values were measured with a microplate reader (AccuReader, Metertech, Taiwan) at 570 nm wavelength. The cell viability % was calculated using the following criteria: Cell viability (%) = (OD sample / OD control) × 100

***Assessment of antibacterial and anti-biofilm activity***

*Minimum inhibitory and bactericidal concentrations (MIC/MBC)*

The MICs of free and niosomal tannic acid against VISA strains were measured based on the approved CLSI broth microdilution protocol. In brief, MHB, including different concentrations of samples, was included in a sterile 96-well microdilution plate. Then, $1.5\times10^8$ CFU/ml suspensions of the selected bacteria were added into wells and incubated at 37 °C overnight. The minimum concentration of that well inhibiting visible bacterial growth was considered as MIC. The MBCs were assessed as the lowest concentration, resulting in no growth (> 99%) on Mueller-hinton agar (MHA) after overnight incubation. All assays were performed in triplicate, and the uninoculated medium and microbial strains were applied as negative and positive controls, respectively [38].

*Biofilm formation*

In order to evaluate the anti-biofilm ability of niosomal tannic acid in comparison with free form**,** the microtiter plate assay (MTP) was performed. According to this method, 200 µl of 106 CFU/ml bacterial suspension diluted with trypticase soy broth (TSB) medium supplemented with 1% glucose was added into 96-well polystyrene microtiter plate. The isolates were subsequently treated with sub-MIC values of samples for 24 h incubation at 37 °C. In the next step, each well was triplicate rinsed with sterile PBS (pH 7.4) to remove the planktonic cells. Afterward, the formed biofilms were fixed for 15 min using absolute methanol (99.8%). By using methanol as a fixing solvent, the biofilms were strongly attached to the microplate and high reliability results were achieved. After air drying of microplates, the fixed biofilms were dyed with crystal violet solution (1.5%w/v), and wells' optical densities (ODs) were read in triplicate at 570 nm. Finally, the mean ODs of the wells were measured and compared to the acquired absorption of the control [39]. Notably, TSB medium without any inoculation and bacterial culture were considered negative and positive control, respectively.

*Biofilm eradication*

Minimal biofilm eradication concentrations (MBECs) were performed to assess the efficacy of tannic acid-containing niosomes compared tofree drugs. As previously mentioned, the bacterial isolates were allowed to form 1-day, 3-day, and 5-day-old biofilms. Then, the formed biofilms were treated with a sub-MIC concentration of niosomal formulation. Following 24 hr incubation at 37 °C, the contents of each well were inoculated on MHA medium for 48 h at 37 °C. The MBEC values were considered as the lowest concentration killing 100% of the embedded bacteria [40].

***Statistical analysis***

All statistical analyses between investigated parameters were assessed using the t-test. Notably, the statistically significant difference was considered at P-value < 0.05 for all comparisons. Also, GraphPad Prism version 9.0 software was used to design all graphs.

**RESULTS**

***Bacterial isolation***

In our study, 42 *S. aureus* isolates were obtained from 75 clinical wound exudates samples, and four isolates were diagnosed as VISA according to the CLSI vancomycin breakpoints**.**

***Characterization of niosomal tannic acid***

*Evaluation of morphology, size, and surface zeta potential*

According to the micrograph taken from FE-SEM, the tannic acid-loaded niosomes had homogeneous spherical shapes and were almost identical in size (Fig. 1). The mean diameter of niosomal particles determined by FE-SEM was 151.9 nm (Fig. 1.). Also, the mean size measured by DLS method was reported 148.3 nm and size distribution (PDI) of niosomes was measured as 0.508, indicating a uniform dispersion for nanoparticles (Fig. 2). Furthermore, the zeta surface charge of prepared niosomes was reported as -60.0 mV (Fig. 2).

*EE% of niosomal formulation*

Incorporation into the niosomal system is

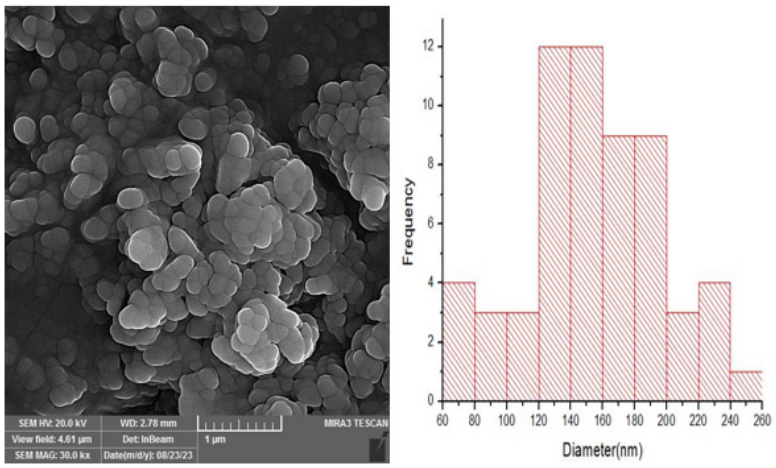


Fig. 1. Spherical morphology (left) and size measurement (right) of the synthesized niosomes according to the taken images by field emission scanning electron microscopy (FE-SEM).

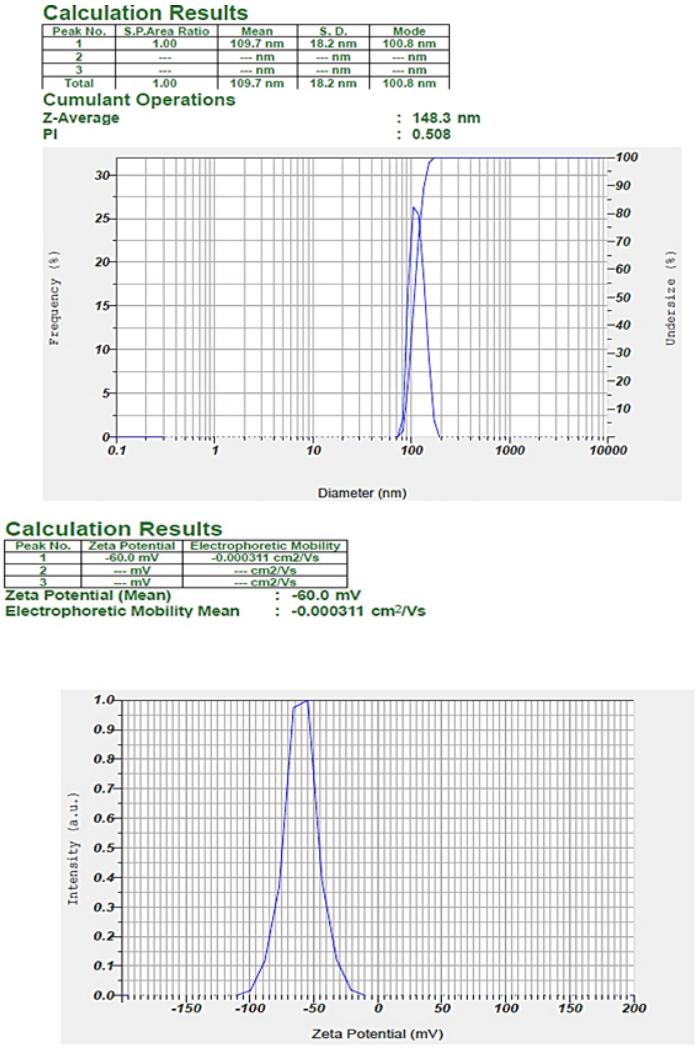


Fig. 2. Investigating the characterization of the prepared niosomes (up) size (down) zeta surface potential obtained from dynamic light scattering (DLS) analysis.

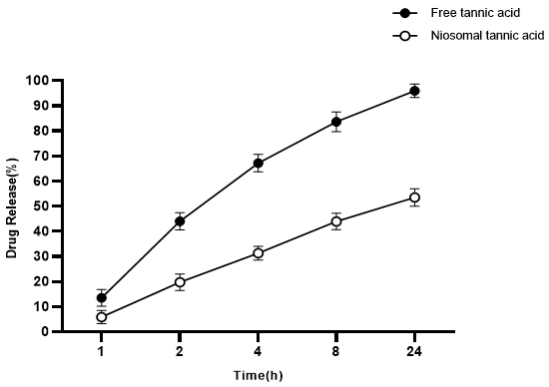


Fig. 3. Comparative release profile of free and niosomal tannic acid at 37 °C

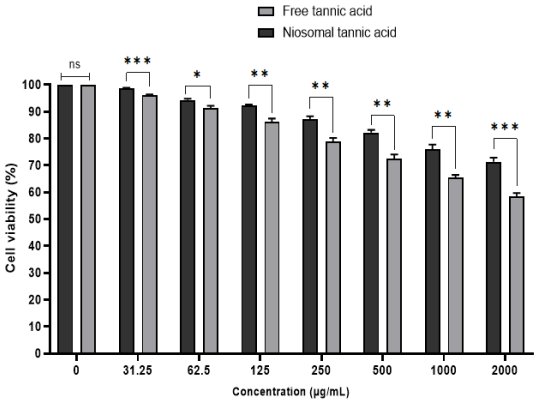


Fig. 4. Cell viability of the free and niosomal formulation on human foreskin fibroblast (HFF) cell line (mean ± SD, n = 3, ns: not significant, *: P< 0.05, **: P< 0.01, ****: P< 0.0001).

recognized to improve the pharmaceutical efficacy of loaded contents, considered a significant factor in developing delivery systems [31]. In our study, the amount of the encapsulated drug in niosomes was 68.90 %, indicating a high yield of drug EE in niosomal suspension.

*Drug release profile*

Fig. 3 compares the tannic acid release profile from the dialysis bag, including the free and niosomal formulation. As shown, approximately 70% of the drug was released from free formulation within four hours, but only 30% of the encapsulated drug was released from niosomal carrier at the same time. Also, in the first 24 hours, around 100% of the drug was released from free formulation, while the highest rate of drug release from the niosomal dispersion was 50%. The comparison release profiles of free and niosomal formulations indicated that the huge drug release could be prevented through encapsulating in niosomal formulation.

*Niosome cytotoxicity*

The viability of different concentrations of niosomal and non-niosomal formulations of tannic acid was investigated on HFF cell lines (Fig. 4). Our results showed the significant lower cytotoxicity effect of tannic acid-containing niosomes in comparison with free tannic acid. The cytotoxicity of the blank niosome was also evaluated, and no significant toxicity was found against HFF cells.

***Assessment of antibacterial and anti-biofilm effect of niosomal tannic acid***

*MIC and MBC*

The MICs and MBCs of the niosomal formulation were measured against VISA strains compared to that of the non-niosomal formulation. According to our results, tannic acid-loaded niosomes significantly had an inhibitory effect, which decreased the MIC values 4-8-fold against all VISA isolates in comparison with free tannic acid. Also, the free formulation exhibited high bactericidal efficacy, and the MBC values of the niosomal formulation were 2-4-fold lower than the free drug (Fig. 5). The antibacterial activity of the blank formulation was also evaluated, which did not show any antibacterial activity against VISA isolates. Notably, the MIC and MBC results of free and niosomal tannic acid against VISA isolates were repeated in triplicate.

*Biofilm formation*

The inhibitory activity of tannic acid-containing niosomes on biofilm formation was investigated and compared to free tannic acid. The antibiofilm efficacy of the blank formulation was also evaluated, which had no inhibitory

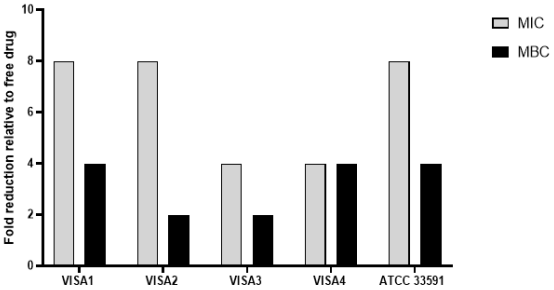


Fig. 5. Comparison of minimum inhibitory and bactericidal concentrations (MICs and MBCs) of free and niosomal tannic acid against vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates

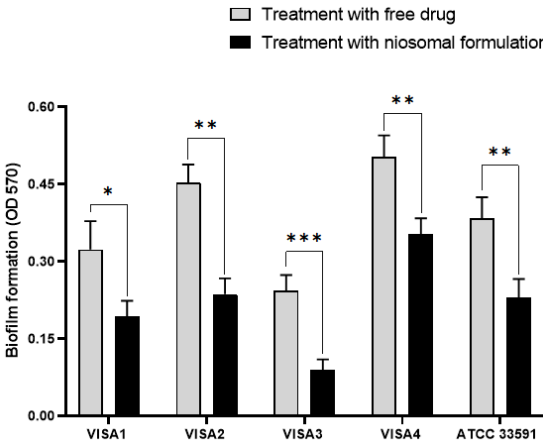


Fig. 6. Comparison of anti-biofilm activity of free and niosomal formulation against vancomycin-intermediate Staphylococcus aureus (VISA) strains (mean ± SD, n = 3, *: P<0.05, **: P<0.01, ***: P< 0.001)

action on biofilm formation against all isolates. Also, the results of MTP assay exhibited that treatment of VISA strains with incorporated tannic acid significantly inhibited biofilm formation, compared to the free drug (Fig. 6). Notably, the inhibitory activity of free and niosomal tannic acid against VISA isolates was carried out in triplicate.

*Biofilm eradication*

The anti-biofilm efficacy of tannic acid-containing niosomes against VISA isolates was investigated by evaluating MBECs, which were compared to free tannic acid (Table 2). Based on our results, the encapsulated tannic acid reduced the 1-day-MBEC values by 2–4-fold in all tested isolates in comparison with free tannic acid. Also, the results of MBEC revealed that niosomal formulation eradicated 3-day and 5-day-old VISA biofilms at lower concentrations than non-niosomal formulation. Furthermore, the anti-biofilm of blank niosomes was evaluated, which failed to eradicate biofilms at the same concentrations of niosomal formulation. Notably, the MBEC results of free and niosomal tannic acid against VISA isolates were repeated in triplicate.

## DISCUSSION

As a dangerous human pathogen, VISA strains cause various problems for healthcare systems. Given that conventional treatments failed to effectively control this highly virulent pathogen, there is an immediate need to discover a substitute therapeutic solution against related infections. Moreover, finding the cost-effective and powerful antibacterial agents to fight staphylococcal antibiotic resistance is essential owing to the high prevalence of resistant strains among hospitalized patients [41]. In several studies, researchers have incorporated various antimicrobial agents in niosomal systems, effectively improving their anti-*S. aureus* potential. In this regard, Awate et al. [42] formulated niosomes with berberine, which presented good antimicrobial activities against several pathogens, including *S. aureus*. Furthermore, the results of the conducted research by Pooprommin et al. (15) exhibited that encapsulation of mangosteen extract into niosomes resulted in significant growth inhibition of *S. aureus*. Also, in other experiments, niosomal incorporation was demonstrated to improve the antibacterial potential of conventional anti-*S. aureus* compounds such as amoxicillin [43], vancomycin [44], ciprofloxacin [45], and others. The result of our study is in agreement with the mentioned studies, where tannic acid-loaded niosomes have an excellent bacterial inhibitory potency and can be considered a novel anti-VISA agent.

Natural compounds have attracted the attention of researchers because of their extensive medicinal applications, especially antibacterial potential [46]. Despite their high therapeutic

Table 2. Comparison of minimum eradication concentrations (MBECs) of free and niosomal formulation against vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates.

| Isolates No. | MBEC (µg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Free tannic acid | | | Niosomal tannic acid | | |
| | 1-day biofilm | 3-day biofilm | 5-day biofilm | 1-day biofilm | 3-day biofilm | 5-day biofilm |
| VISA1 | 512 | 1024 | >1024 | 128 | 512 | 1024 |
| VISA2 | 1024 | >1024 | >1024 | 256 | >1024 | >1024 |
| VISA3 | 128 | 512 | 1024 | 32 | 128 | 512 |
| VISA4 | 512 | >1024 | >1024 | 256 | 1024 | >1024 |
| ATCC 33591 | 256 | 1024 | >1024 | 128 | 512 | 1024 |

potential, the natural compounds' bioactivity is restricted due to poor bioavailability, instability during storage, and weak solubility [47]. As a result, the nano-drug delivery system is considered a suitable tool to resolve the disadvantage mentioned above, providing delivery of natural compounds in appropriate dosage form [48]. In this study, the pharmaceutical activities of tannic acid-loaded drugs were increased through encapsulation into the niosomal system, indicating the high capability of niosomes as an efficient drug delivery system. In another study, different niosomal formulations containing essential oils [49, 50], propolis [51], thymol [52], and curcumin [53] were prepared, where niosomes could enhance drug-effective dose of loaded contents. The results of these conducted studies are consistent with our study, suggesting that niosomes could be applied for various therapeutic purposes, especially antibacterial drug delivery.

Niosomes, as a potent vesicular system, could transfer the incorporated loaded contents inside the bacterial cell, increasing the efficacy of antibacterial agents [54, 55]. The present study evaluated the anti-VISA activities of tannic acid-loaded niosomes, and the ability of niosomes as an efficient drug delivery system was demonstrated. According to the present study, encapsulating to niosomal formulation decreased the MICs and MBCs of free drug by 4-8-fold and 2-4-fold, respectively. Our results showed the high potential of niosomes as a powerful antibacterial delivery system against *S. aureus*, which were in confirmatory with other studies. In this regard, in the conducted study by Shadvar et al. [43], the inhibitory effect of drug-loaded niosomes against *S. aureus* was proven, where the encapsulation into niosomal system decreased the MIC of free drug by 2-4-fold. Also, Jastis et al. [54] showed the improved antibacterial activity of antibiotic-loaded niosomes against *S. aureus* strains, where the niosomal formulation had a lower MIC (4-fold) compared to free formulation. Furthermore, Rezaeiroshan et al. [11] found the bacteriostatic and bactericidal behaviors of the niosomal drug system against *S. aureus* strains, which both MIC and MBC values of the free drug were reduced by 2-fold through incorporating to the niosomal formulations. The improvement of antibacterial activity by niosomal encapsulation might be owing to the interaction between bacteria and niosomes [54, 56]. Indeed, niosomes could induce sub-cellular drug release by interacting with the peptidoglycan barrier, creating a concentration gradient [57]. Due to enhancing the drug's effective dose of loaded contents, niosomal encapsulation could be developed for drug delivery against VISA infections.

Management of *S. aureus* biofilm-related infections depends on early inhibition of bacterial surface attachment and biofilm formation, in which the embedded bacteria are more drug-tolerant than planktonic cells [58, 59]. In our study, the inhibitory effect of niosomal entrapment on VISA biofilm was assessed, and it was exhibited that niosomal formulation could increase (almost 2-fold) the anti-biofilm effect of free tannic acid. In this regard, Dwivedi et al. found that the niosomal drug significantly suppressed the attachment of *S. aureus* strains to the abiotic surface by 2-fold [44]. Also, Baraket et al. [60] demonstrated the inhibitory effect of the niosomal drug against bacterial biofilm formation which could drastically be increased (2-8-fold) through encapsulation into the niosomal system. In fact, niosomes cause efficient drug delivery into the embedded bacteria by facilitating their diffusion into the biofilm matrix, inhibiting biofilm formation. The prolonged drug accessibility could improve the drug sustained release from niosomes, inhibiting the development of resistance mechanisms among biofilm-forming bacteria [26]. On the other hand, niosomes, as a physical barrier, could compete with biofilm-forming bacteria for surface attachment, inhibiting biofilm formation by reducing bacterial attachment [61-63].

As biofilm is known to be a critical factor in the pathogenesis of *S. aureus*, the destruction of the formed biofilm on abiotic and biotic surfaces has become an essential challenge in medical settings [64]. Our study revealed the significant anti-biofilm potential of the synthesized formulation against VISA biofilms ($P<0.05$), where tannic acid-loaded niosome reduced MBECs by 2-4-fold in all isolates. In a confirmatory study, the eradication effect of the drug-loaded niosomes on formed staphylococcal biofilm was approved, where cefazolin-encapsulated niosomes had a 4-8-fold increase in biofilm elimination rate at the same free drug concentration [31]. Moreover, another study by Kashef et al. [65] approved the effectiveness of niosomal encapsulation against *S. aureus* biofilm, where drug-loaded niosomes could significantly reduce (2-16-fold) the formed biofilm

mass in comparison with the free drug. It could be discussed that niosomes increase the effective dosage of loaded contents into biofilm space, leading to lower drug intake intervals against chronic VISA infections. Therefore, regarding the high potential of VISA isolates in surface adhesion, niosomal drug delivery could serve as a proper solution for solving therapeutic challenges caused by VISA biofilm.

Nowadays, VISA strains present formidable medical challenges in the healthcare system, driving researchers to find novel therapeutic candidates for dealing with threats caused by them [66]. According to the present study, niosomal tannic acid could be developed as a promising approach to effectively target VISA strains and fight conventional drug resistance. The inhibition of enzymatic degradation, controlled release profile, and increased intracellular targeting are among the mechanisms action of the niosomal system against resistant bacteria, providing an effective drug dosage at the infected sites [67]. In addition, the niosomal encapsulation could improve the bacteriostatic and bactericidal abilities of tannic acid, causing the prevention and eradication of chronic VISA infections. Additionally, the significant anti-biofilm activity of tannic acid-loaded niosome could present an ideal way for fighting against bacterial wound infections, particularly those caused by VISA strains. Despite the valuable findings gained from our research, it is important to acknowledge some limitations that may affect the interpretation of our results. Firstly, the limited sample size and inherent biases in the sample selection could impact on the comprehensive and balanced presentation of our research. Moreover, additional investigations into the long-term stability, pharmaceutical behavior, and more physicochemical properties of the prepared formulation could improve the potential therapeutic efficacy of niosomal drug delivery against bacterial infections. Furthermore, in vivo and molecular studies can be used to clarify the exact antibacterial and anti-biofilm mechanisms of niosomal drug delivery system, while the current study only performed *in vitro* experiments.

## CONCLUSION

According to our study, niosomal incorporation could improve the antibacterial ability of tannic acid against VISA strains by 2-8-fold, which can be developed as a novel delivering system for antimicrobial agents. Also, niosomes could be applied as a powerful drug delivery system against VISA strains, which can provide an effective dosage of anti-*S. aureus* agents at the site of actions. Furthermore, the present research revealed that niosomal formulation increased the anti-biofilm effect of tannic acid against VISA strains by 2-4-fold, thereby could reduce the biofilm-associated challenges in medical settings. Additionally, our results proved the high encapsulating potential (68.90%) of niosomal formulation, suggesting a successful carrier for enhancing the pharmacokinetic properties of tannic acid against VISA infections. Moreover, niosomal drug delivery system could prevent the emergence of drug-resistant *S. aureus* strains through improving the therapeutic indices of tannic acid. However, limited sample size, lack of nanoparticle stability analysis, and inherent biases in the sampling process are among the limitations of our study that may impact the interpretation of our findings. The perspective studies could focus on investigation of the exact antibacterial and anti-biofilm mechanisms of niosomal tannic acid through in vivo and molecular analyses. Furthermore, understanding the optimizing formulation techniques, illuminating release kinetics during storage condition, and investigating potential combination therapies with other antimicrobial agents can be used to gain valuable insights into the clinical development of niosomal drug delivery system.

## ACKNOWLEDGMENTS

The authors of this article are grateful to Hamadan University of Medical Sciences for their financial support (grant number 140106084787).

## ETHICAL APPROVAL

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences, Institutional Review Board (IR.UMSHA.REC.1401. 496). The experiments in our study were conducted according to the relevant guidelines and regulations or in accordance to the Declaration of Helsinki. Informed consent was obtained from all patients and from the parents or legal guardians of children participating in the study.

## CONSENT FOR PUBLICATION

Not Applicable.

## AVAILABILITY OF DATA

The datasets used and analyzed during the

current study are available from the corresponding author upon reasonable request.

**FUNDING**

This work was supported by the Research Centre of Hamadan University of Medical Sciences on the grant number 140106084787. This funding has been devoted just to purchase materials used in our study.

**CONFLICTS OF INTEREST**

There is no conflict of interest in this study.

**REFERENCES**

1. Kyaw MH, Kern DM, Zhou S, Tunceli O, Jafri HS, Falloon J. Healthcare utilization and costs associated with S. aureus and P. aeruginosa pneumonia in the intensive care unit: a retrospective observational cohort study in a US claims database. BMC Health Serv Res. 2015;15(1):241.
2. Nasaj M, Farmany A, Shokoohizadeh L, Jalilian FA, Mahjoub R, Roshanaei G, et al. Development of Chitosan-Assisted Fe 3 O 4@ SiO 2 Magnetic Nanostructures Functionalized with Nisin as a Topical Combating System against Vancomycin-Intermediate *Staphylococcus aureus* (VISA) Skin Wound Infection in Mice. J Nanomater. 2022;2022:1-17.
3. Wady AF, Machado AL, Foggi CC, Zamperini CA, Zucolotto V, Moffa EB, et al. Effect of a Silver Nanoparticles Solution on *Staphylococcus aureus* and *Candida* spp. J Nanomater. 2014;2014:1-7.
4. Zhao G HC, Xue Y. *In vitro* evaluation of chitosan-coated liposome containing both coenzyme Q10 and alpha-lipoic acid: Cytotoxicity, antioxidant activity, and antimicrobial activity. J Cosmet Dermatol. 2018;17(2):258-262.
5. Durymanov M KT, Lehmann SE, Reineke J. Exploiting passive nanomedicine accumulation at sites of enhanced vascular permeability for non-cancerous applications. J Control Release. 2017 Sep;261:10-22.
6. Jawad KH, Marzoog TR, Hasoon BA, Sulaiman GM, Jabir MS, Ahmed EM, et al. Antibacterial activity of bismuth oxide nanoparticles compared to amikacin against *Acinetobacter baumannii* and *Staphylococcus aureus*. J Nanomater. 2022;2022:1-11.
7. Zhu X, Liu C, Gao S, Lu Y, Chen Z, Sun Z. Vancomycin intermediate-resistant *Staphylococcus aureus* (VISA) isolated from a patient who never received vancomycin treatment. Int J Infect Dis. 2015;33:185-190.
8. Kranjec C, Morales Angeles D, Torrissen Mårli M, Fernández L, García P, Kjos M, et al. Staphylococcal biofilms: Challenges and novel therapeutic perspectives. Antibiotics. 2021;10(2):131.
9. Kakoullis L, Papachristodoulou E, Chra P, Panos G. Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. Antibiotics. 2021;10(4):415.
10. Raeiszadeh M, Pardakhty A, Sharififar F, Farsinejad A, Mehrabani M, Hosseini-Nave H, et al. Development, physicochemical characterization, and antimicrobial evaluation of niosomal myrtle essential oil. Res Pharm Sci. 2018;13(3):250-261.
11. Rezaeiroshan A, Saeedi M, Morteza-Semnani K, Akbari J, Hedayatizadeh-Omran A, Goli H, et al. Vesicular formation of trans-ferulic acid: an efficient approach to improve the radical scavenging and antimicrobial properties. J Pharm Innov. 2022:1-10.
12. Pardakhty A, Moazeni E. Nano-niosomes in drug, vaccine and gene delivery: a rapid overview. Nanomed J. 2013;1(1):1-12.
13. Lee MK. Liposomes for enhanced bioavailability of water-insoluble drugs: *In vivo* evidence and recent approaches. Pharmaceutics. 2020;12(3):264.
14. Kumar GP, Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta pharmaceutica sinica B. 2011;1(4):208-219.
15. Jain S, Jain V, Mahajan S. Lipid based vesicular drug delivery systems. Adv Pharmacol Sci 2014;2014:1-12.
16. Aelenei N, Popa MI, Novac O, Lisa G, Balaita L. Tannic acid incorporation in chitosan-based microparticles and *in vitro* controlled release. J Mater Sci Mater Med 2009;20:1095-1102.
17. Dabbaghi A, Kabiri K, Ramazani A, Zohuriaan-Mehr MJ, Jahandideh A. Synthesis of bio-based internal and external cross-linkers based on tannic acid for preparation of antibacterial superabsorbents. Polym Adv Technol. 2019;30(11):2894-2905.
18. Lau S, Wahn J, Schulz G, Sommerfeld C, Wahn U. Placebo-controlled study of the mite allergen-reducing effect of tannic acid plus benzyl benzoate on carpets in homes of children with house dust mite sensitization and asthma. Pediatr Allergy Immunol. 2002;13(1):31-36.
19. Nonaka G-i, Nishioka I, Nishizawa M, Yamagishi T, Kashiwada Y, Dutschman GE, et al. Anti-AIDS agents, 2: inhibitory effect of tannins on HIV reverse transcriptase and HIV replication in H9 lymphocyte cells. J Nat Prod. 1990;53(3):587-595.
20. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—A minireview. Materials. 2020;13(14):3224.
21. Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, et al. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against Helicobacter pylori. Microbiol and immunol. 2004;48(4):251-261.
22. Chung KT, Jr SS, Lin WF, Wei C. Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Lett Appl Microbiol. 1993;17(1):29-32.
23. Kyaw BM, Arora S, Lim CS. Anti-Pseudomonal and anti-biofilm activity of tannic acid in combination with antibiotics. Inter J Integr Biol. 2011;11(3):110.
24. Belhaoues S, Amri S, Bensouilah M. Major phenolic compounds, antioxidant and antibacterial activities of Anthemis praecox Link aerial parts. S Afr J Bot. 2020;131:200-205.
25. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 2001;48(4):487-491.
26. Mehrarya M, Gharehchelou B, Haghighi Poodeh S, Jamshidifar E, Karimifard S, Farasati Far B, et al. Niosomal formulation for antibacterial applications. J Drug Target. 2022;30(5):476-943.
27. Ghafelehbashi R, Akbarzadeh I, Tavakkoli Yaraki M, Lajevardi A, Fatemizadeh M, Heidarpoor Saremi L. Preparation, physicochemical properties, *in vitro* evaluation and release behavior of cephalexin-loaded niosomes. Int J Pharm. 2019;569:118580.
28. Hemmati J, Chegini Z, Arabestani MR. Niosomal-based drug delivery platforms: A promising therapeutic approach to fight *Staphylococcus aureus* drug resistance. J Nanomater. 2023;2023:1-18.

29. Del AK, Kaboosi H, Jamalli A, Ghadikolaii FP. Prevalence and expression of PSM a gene in biofilm-producing *Staphylococcus aureus* clinical isolates. Jundishapur J Microbiol. 2019;12(8):e89610.
30. Khan R, Irchhaiya R. Niosomes: a potential tool for novel drug delivery. J pharm investig. 2016;46:195-204.
31. Zafari M, Adibi M, Chiani M, Bolourchi N, Barzi SM, Nosrati MSS, et al. Effects of cefazolin-containing niosome nanoparticles against methicillin-resistant *Staphylococcus aureus* biofilm formed on chronic wounds. Biomed Mater. 2021;16(3):035001.
32. Moazeni E, Gilani K, Sotoudegan F, Pardakhty A, Najafabadi AR, Ghalandari R, et al. Formulation and *in vitro* evaluation of ciprofloxacin containing niosomes for pulmonary delivery. J Microencapsul. 2010;27(7):618-627.
33. WS R. Imagej, us national institutes of health, bethesda, maryland, usa. http://imagej nih gov/ij/. 2011.
34. Heidari F, Akbarzadeh I, Nourouzian D, Mirzaie A, Bakhshandeh H. Optimization and characterization of tannic acid loaded niosomes for enhanced antibacterial and anti-biofilm activities. Adv Powder Technol. 2020;31(12):4768-4781.
35. Blainski A, Lopes GC, De Mello JCP. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. Molecules. 2013;18(6):6852-6865.
36. Sahiner N, Sagbas S, Aktas N, Silan C. Inherently antioxidant and antimicrobial tannic acid release from poly (tannic acid) nanoparticles with controllable degradability. Colloids Surf B 2016;142:334-343.
37. Ali H, Shirode AB, Sylvester PW, Nazzal S. Preparation, characterization, and anticancer effects of simvastatin–tocotrienol lipid nanoparticles. Int J Pharm. 2010;389(1-2):223-231.
38. Thornsberry C. Antimicrobial susceptibility testing of anaerobic bacteria: review and update on the role of the National Committee for Clinical Laboratory Standards. Rev infect dis. 1990;1(12):218-222.
39. Barani M, Mirzaei M, Torkzadeh-Mahani M, Adeli-Sardou M. Evaluation of carum-loaded niosomes on breast cancer cells: Physicochemical properties, *in vitro* cytotoxicity, flow cytometric, DNA fragmentation and cell migration assay. Sci Rep. 2019;9(1):7139.
40. Mirzaei R, Alikhani MY, Arciola CR, Sedighi I, Yousefimashouf R, Bagheri KP. Prevention, inhibition, and degradation effects of melittin alone and in combination with vancomycin and rifampin against strong biofilm producer strains of methicillin-resistant Staphylococcus epidermidis. Biomed Pharmacother. 2022;147:112670.
41. Aires-de-Sousa M. Methicillin-resistant *Staphylococcus aureus* among animals: Current overview. Clin Microbiol Infect. 2017;23(6):373-380.
42. Awate PS, Pimple TP, Pananchery JF, Jain AS. Formulation and evaluation of berberine hcl as niosomal drug delivery system. Asian J Pharm Res. 2020;10(3):149-159.
43. Shadvar P, Mirzaie A, Yazdani S. Fabrication and optimization of amoxicillin-loaded niosomes: an appropriate strategy to increase antimicrobial and anti-biofilm effects against multidrug-resistant *Staphylococcus aureus* strains. Drug Dev Ind Pharm. 2021;47(10):1568-1577.
44. Dwivedi A, Mazumder A, Nasongkla N. Layer-by-layer nanocoating of antibacterial niosome on orthopedic implant. Int J Pharm. 2018;547(1-2):235-243.
45. Satish J, Amusa AS, Gopalakrishna P. *In vitro* activities of fluoroquinolones entrapped in non-ionic surfactant vesicles against ciprofloxacin-resistant bacteria strains. J Pharm Technol Drug Res. 2012;1:5.
46. Stan D, Enciu A-M, Mateescu AL, Ion AC, Brezeanu AC, Stan D, et al. Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. Front Pharmacol. 2021;12:723233.
47. Shishir MRI, Taip FS, Saifullah M, Aziz NA, Talib RA. Effect of packaging materials and storage temperature on the retention of physicochemical properties of vacuum packed pink guava powder. Food Packag Shelf Life 2017;12:83-90.
48. Shishir MRI, Xie L, Sun C, Zheng X, Chen W. Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid-based transporters. Trends Food Sci Technol. 2018;78:34-60.
49. Tabatabai MB, Mirjalili M, Yazdiyan F, Hekmatimoghaddam S. Antibacterial activity and cytotoxicity of nanoliposomic and nanoniosomic essential oil of Trachyspermum copticum. Proc Natl Acad Sci India Sect B Biol Sci. 2019;89(3):1109-1116.
50. Yadav, G. and Jain, A. 112 Design and development of tea tree oil niosomal gel for bacterial infection. J Pharm Chem. 2022;18(1):1.
51. Patel J, Ketkar S, Patil S, Fearnley J, Mahadik KR, Paradkar AR. Potentiating antimicrobial efficacy of propolis through niosomal-based system for administration. Integr Med Res. 2015;4(2):94-101.
52. Najafloo R, Baheiraei N, Imani R. Synthesis and characterization of collagen/calcium phosphate scaffolds incorporating antibacterial agent for bone tissue engineering application. J Bioact Compat Polym. 2021;36(1):29-43.
53. Gugleva V, Michailova V, Mihaylova R, Momekov G, Zaharieva MM, Najdenski H, et al. Formulation and Evaluation of Hybrid Niosomal In Situ Gel for Intravesical Co-Delivery of Curcumin and Gentamicin Sulfate. Pharmaceutics. 2022;14(4):747.
54. Satish J, Amusa AS, Gopalakrishna P. *In vitro* activities of fluoroquinolones entrapped in non-ionic surfactant vesicles against ciprofloxacin-resistant bacteria strains. J Pharm Technol Drug Res. 2012;1(1):1-11.
55. Huang C-M, Chen C-H, Pornpattananangkul D, Zhang L, Chan M, Hsieh M-F, et al. Eradication of drug resistant *Staphylococcus aureus* by liposomal oleic acids. Biomaterials. 2011;32(1):214-221.
56. Kopermsub P, Mayen V, Warin C. Potential use of niosomes for encapsulation of nisin and EDTA and their antibacterial activity enhancement. Food Res Int. 2011;44(2):605-612.
57. Furneri PM, Fresta M, Puglisi G, Tempera G. Ofloxacin-loaded liposomes: *in vitro* activity and drug accumulation in bacteria. Antimicrob Agents Chemother. 2000;44(9):2458-2464.
58. Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods. 2000;40(2):175-179.
59. Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. Biomaterials. 2012;33(26):5967-582.
60. Barakat HS, Kassem MA, El-Khordagui LK, Khalafallah NM. Vancomycin-eluting niosomes: a new approach to the inhibition of staphylococcal biofilm on abiotic surfaces. AAPS Pharm Sci Tech. 2014;15:1263-1274.
61. Nejadnik MR, van der Mei HC, Norde W, Busscher HJ. Bacterial adhesion and growth on a polymer brush-coating. Biomaterials. 2008;29(30):4117-4121.

62. Banerjee I, Pangule RC, Kane RS. Antifouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms. Adv Mater. 2011;23(6):690-718.
63. Francolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol. 2010;59(3):227-328.
64. Neopane P, Nepal HP, Shrestha R, Uehara O, Abiko Y. *In vitro* biofilm formation by *Staphylococcus aureus* isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. Int J Gen Med. 2018;18(1):25-32.
65. Kashef MT, Saleh NM, Assar NH, Ramadan MA. The antimicrobial activity of ciprofloxacin-loaded niosomes against ciprofloxacin-resistant and biofilm-forming *Staphylococcus aureus*. Infect Drug Resist. 2020;1(8):1619-1629.
66. Soltani M, Hajikhani B, Zamani S, Haghighi M, Hashemi A, Nasiri MJ, Dadashi M, Pourhossein B, Goudarzi M. Molecular characterization of *Staphylococcus aureus* strains isolated from hospitalized patients based on coagulase gene polymorphism analysis: High frequency of vancomycin-intermediate S. aureus and the emergence of coagulase type II in Iran. Gene Rep.2021;1(23):101078.
67. Hemmati J, Azizi M, Asghari B, Arabestani MR. Multidrug-Resistant Pathogens in Burn Wound, Prevention, Diagnosis, and Therapeutic Approaches (Conventional Antimicrobials and Nanoparticles). Can J Infect Dis Med Microbiol. 2023;2023:8854311.