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Isolation Of Resistant Staphylococcus Aureus (MRSA) From Gingivitis And Assessment The Activity Of Propolis Loaded Lipid Nanoparticles Against Isolates

Ayat Ayid Mahmood Alew [1] , Khairi Jameel AL-Ruaby [2] , Abrar mohammed qneed [3]

[1,2] College of science , Department of Biology , Wasit University, Iraq
[3]Collage of science for women, Baghdad university, Iraq
Corresponding author: std2023204.aalewi@uowasit.edu.iq

Abstract

Objective: *Gingivitis is an inflammatory condition of the gingival tissue or the gums most commonly due to a bacterial infection . Methods: 154 samples from mouth cavity had been collected from patients who had Gingivitis. The Samples were obtained from two sites, Teiba specialized medical center and the specialized center in Wasit City, Iraq. During the period from October ,2024 to March,2025. then methicillin-resistant Staphylococcus aureus were isolated by traditional methods . High-shear homogenization and ultra-sonication methods were used to prepare LNPs-PL. The manufactured formulation's encapsulation efficiency, loading capacity, and physicochemical characteristics were evaluated. Scanning electron microscopy (SEM) was used to analyze the morphology of LNPs-PL (SEM). Using GC/MS analysis, the chemical composition of the tested normal oil and LNPs-PL was assessed. In vitro studies were conducted to compare the antibacterial activity of LNPs-PL and regular PL.*

Results: *the prevalence of MRSA isolates were 37(58.73%) .the result of The antimicrobial activity of LNPs-PL showed increase the level of inhibition on the MRSA bacteria to (36 mm) comparative to (14 mm) with propolis (PL) Highly significant difference (P<0.01). LNPs-PL showed significantly larger inhibition zones vs. PL.*

Conclusion: *Based on the results of the study, modifications should be made in the loading capacity, encapsulation efficiency, and physicochemical properties. Our findings confirmed that SLNs are effective PL carriers for controlling bacterial and fungal diseases and further research is needed on them.*

Keywords: *MRSA, nanoparticle advantage, Gingivitis.*

INTRODUCTION

Gingivitis is an inflammatory condition of the gingival tissue or the gums most commonly due to a bacterial infection. It is inflammation of the gingiva with the attachment of the connective tissue to the tooth remaining at the original level, that is, without attachment loss. The condition is restricted to the soft-tissue area of the gingival epithelium and connective tissue (1). Among all the periodontal diseases, gingivitis is considered to be the commonest. There are various forms of gingivitis based on clinical appearance, duration of infection, severity, and etiology. (2). S. aureus is apresumed pathogen for many oral diseases, including Gingivitis , oral diseases mucositis, periodontitis, endodontic peri-implantitis infections and even teeth decay (3). S. strains of aureus include drug-resistant, developed. Resistant of Methicillin the Staph.aureus (MRSA)(4). the strains of S.aureus that they were immune to antibiotics containing beta-lactam, which Penicillins, Amoxicillin, Methicillin, Ampicillin,Cephalosporins, Oxacillin, etc (5). The inclination of S.The acquisition of antibiotic

resistance by aureus led to a globalclone distribution of distinct antimicrobial resistance expressions. There are several bacterial infections instead of MRSA strains and in the population and clinics,even , leads to death.. S. Infection by aureus, it has long been prevalent, like MRSA strain (6). Propolis is a natural product that is collected by honey bees from parts of plants, buds, and exudates. The general composition of this substance varies in different geographical locations and climates. The biological characteristics of propolis are attributed to its chemical composition especially polyphenols and flavonoids. In recent years, the current trend has been towards the identification of natural products in disinfection. In addition, Nanoparticles are able to penetrate bacteria and bacterial biofilms, so they can be a potential agent for controlling the growth of bacterial infection (6).

MATERIALS AND METHODS

Patients Sampling Technique

One hundred fifty four (N =154) samples from mouth cavity had been collected from patients who had Gingivitis. The Samples were obtained from two sites, Teiba specialized medical center and the specialized center in Wasit City, Iraq. During the period from October ,2024 to March,2025. The procedure according to this protocol, standardised in accordance with ICMSF (2002) guidelines, enabled the detection of MRSA prevalence that was statistically significant with control of confounding variables by randomisation and stratification by anatomical cut and source (60% abattoir/40% retail). Strength in the methodology provides confidence in follow-on antimicrobial efficacy testing of propolis nanoparticles on isolated MRSA strains.

Isolation of Staphylococcus aureus.

The bacteria were cultivated on several media, including blood agar, MacConkey agar, and mannitol salt agar, which is specific to Staphylococcus aureus. Isolated bacteria were identified based on colony morphological shape, size, colour, and pigment synthesis after incubating the samples at 37 degrees Celsius for 24 hours.

Propolis exhaustive extraction (PL)

Frozen propolis samples were initially subjected to a drying process in a dark environment maintained at a temperature of 60°C. This drying step was crucial to remove moisture content while preserving the bioactive compounds within the propolis. Following the drying process, the propolis was finely milled to obtain a uniform powder weighing 100 grams. This powder was then homogenized in 100 milliliters of 70% ethanol, ensuring thorough mixing and extraction of the bioactive constituents. The homogenization process was allowed to proceed for 48 hours, facilitating the efficient extraction of bioactive compounds into the ethanol solvent. After the 48-hour extraction period, the homogenized sample was subjected to sonication to further enhance the extraction efficiency by disrupting cell walls and releasing intracellular contents. The sonicated mixture was then centrifuged at 10,000 rpm for 10 minutes to separate the solid residues from the liquid extract. The resulting supernatant was carefully filtered through Whatman No. 2 filter paper to remove any remaining particulate matter. The filtered extract was then concentrated using rotary evaporation at 60°C, a step designed to remove the ethanol solvent and concentrate the bioactive compounds. The concentrated extracts were subsequently lyophilized, a freeze-drying process that removes water content while preserving the integrity and activity of the bioactive compounds. The final lyophilized extract, weighing 20 grams, was carefully weighed and stored at 4°C in a desiccator to prevent moisture absorption and degradation. This meticulously prepared dried extract was then stored under controlled conditions until it was required for further experimental analyses, ensuring the stability and consistency of the bioactive components for subsequent research applications (8).

CHARACTERIZATION OF LNPS-PL

Particle size and zeta potential

The SLNs formulations were assessed for particle size, polydispersity index, and zeta potential using the dynamic light scattering method (ZetaSizer Nano-ZS) (9).

Determination of Encapsulation and Loading Efficiency

The encapsulation efficiency (EE) can be expressed as the percent of the total amount of PL obtained in the formulation at the end of the process. The mass of entrapped GO divided by the entire mass of lipid (stearic acid) is the loading capacity (LC). The EE and LC were determined as described earlier [29,30]. Ten ml of methanol was utilized to dissolve 10 mg of LNPs-PL formulations, which were carefully weighed. After that, the samples were centrifuged for 30 minutes at 9,000 rpm. Utilizing a UV–Vis spectrophotometer (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd.), the quantity of PL in the supernatant was measured at 274 nm. A calibration curve was created using a range of concentrations of pure garlic oil to determine the percentage of PL. Three duplicates of each concentration's measurements were made.

The encapsulation and loading efficiency determined as follows:

%EE= (A-B)/A X 100

%LC= (A-B)/C X 100

where;

A: The total amount of GO (% conc.) added to the formulation.

B: The amount of GO measured in the supernatant.

C: The total weight of lipid (stearic acid, 1% w/w) in the formulation.

Morphology Study

Scanning electron microscopy (SEM) has studied the nanoparticles' morphology. After being sputter-coated with a thin layer of Au-Pd, the nanoparticles were mounted on aluminum stubs and examined with an SEM (SEM XL30, Philips, Netherlands) (10).

Differential scanning calorimetry (DSC)

DSC scans of PL and LNPs-PL were carried out in a Mettler DSC 821e (Mettler Toledo, Germany). Five mg of the samples were placed into aluminum oxide pans, sealed, and subjected to analysis. As a guide, an empty aluminum pan was used. DSC was done at a 25 to 250 °C temperature range at the rate of 5 °C/min under N2 flow and the melting point of SLN dispersions was compared to the bulk lipid (11).

The antimicrobial activity of LNPs-PL

.Mueller-Hinton agar was the culture medium that was produced for the study's bacterial cultures to grow in. Staphylococcus aureus was cultivated on mannitol salt agar). The well diffusion method was used to assess the antimicrobial activity of the tested compounds on MHA and mannitol salt agar. In the solid agar medium, 8mm-diameter wells were made. 100 µL aliquots of every tested formulation were introduced into the wells. Using a metal caliper, the diameter of the zone of inhibition was measured and recorded in millimeters following a 24-hour stay in the incubator. The experiment was carried out three times for every bacterium .The average zone of inhibition has been determined for each test **LNPs-PL** and the regular **PL**

(12).

Statistical analysis

Data were analyzed using one-way ANOVA with Tukey's post-hoc test for inhibition zones, and unpaired t-tests for microbial counts ($\alpha = 0.05$). Proportions (e.g., MRSA prevalence) included 95% Wilson score confidence intervals. All tests assumed normality ($p > 0.05$, Shapiro-Wilk) and equal variance (Levene's test).

RESULT AND DISSECTION

Prevalence of MRSA among patients of Gingivitis

The result of cultural examination for (154) samples from mouth cavity had been collected from patients who had Gingivitis. The Samples were obtained from two sites, Teiba specialized medical center and the specialized center in Wasit City, Iraqrevealed that (MRSA) isolates, the number and isolation percentage were 63/154 (40.9 %) of S. aureus out of 63 isolates of S. aureus 37(58.73%) were (MRSA) Table (1) ,figure(1) . The present study's findings revealed that S. aureus isolated from oral infections, which may be caused by periodontal disease, can operate as a reservoir for opportunistic microorganisms. If antibiotics are used to treat periodontal disease or other infections, they can lead to an increase in Staphylococcus spp. in the oral cavity. s.aureus strains can cause antibiotic resistance is widespread and can Periodontitis develops as a result of antibiotic therapy. The fact that S. aureus is more prevalent in the oral cavity might result in a more severe illness. The current percentages of isolated S. aureus are consistent with those reported by (13), who found that periodontal disease was 36 (33.8%), followed by tooth decay and dental plaque at19(26.8%) and 12(16.9%), respectively. Also, accord with the findings of (14) who found a prevalence of S.aureus in the saliva of 21% and gingival swabs of 11% in 110 patients attending a dental hospital with a variety of oral illnesses. 13 Salivary carriages of S. aureus was detected in 41% of patients with decreased salivary flow rates attending an oral medicine clinic, with concentrations ranging from 3.7x101 to 5.2x103 cfu ml. Because of the variety of the normal oral flora and the healthy carriage of S. aureus in specific patient groups, the case for S. aureus in the etiology of oral dysaesthesia and mucositis is difficult. However, given the high rates of S. aureus recovery in patients with oral mucosal symptoms such as pain, burning, erythema, and swelling, physicians should consider the potential of this pathogen playing a role in oral mucosal illness.

Table 1: Prevalence of MRSA in Gingivitis Samples

patients	Total Samples (N)	S. aureus Positive (%)	MRSA Positive (%) (95% CI)
Gingivitis	154	63 (40.9 %)	37/63 (58.7 ± 6.9%)

1. Proportions reported as % **± 95% confidence interval** (Wilson score interval).
2. S. aureus prevalence calculated as (positive samples/N) × 100.

(Figure 1): Methicillin-Resistant Staphylococcus aureus (MRSA) on HiCrome MeReSa Agar Base mediumlrmr7rrrrrrro cvd54

Preparation and Characteristics of LNPs-PL

DLS analysis

Hydrodynamic average size

The observed increase in the size of the lipid nanoparticles from 204.16 nm to 244 nm following the incorporation of propolis extract is indicative of successful loading. This size augmentation implies that the propolis extract has been effectively integrated into the lipid matrix or adsorbed onto the surface of the LNPs. Such an increase is anticipated and confirms the presence of the propolis extract within the nanoparticle system (15).

Morphology Study

The SEM image of LNPs-PL is displayed in Figure 3. The SEM scan revealed that the prepared SLNs had a spherical shape. Particle size distribution was similar to DLS. After loading, the nanocapsules had a spherical form, high dispersion, and a limited size distribution (16).

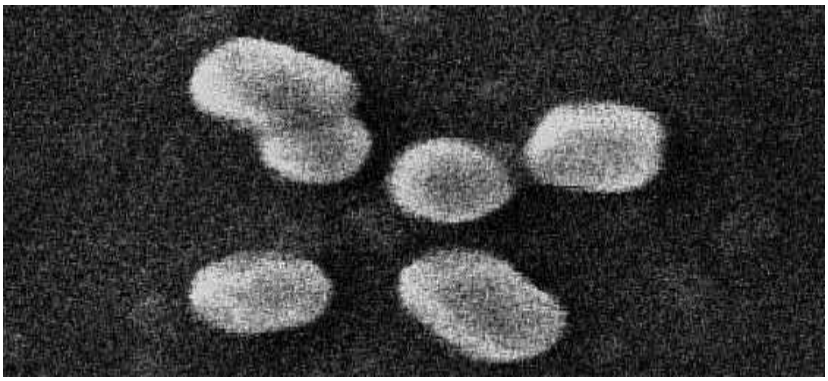

Figure 3: Appearance of LNPs-PL under scanning electron microscopy(SEM image of LNPs-PL at 50,000× magnification. Scale bar: 200 nm)

Polydispersity Index (PDI)

The PDI values provide critical insights into the size distribution and homogeneity of the nanoparticle populations. A PDI of 0.421 for the free LNPs (Fig.3) suggests a moderately broad size distribution, indicating some variability in particle size. Conversely, a PDI of 0.277 for the LNPs-PL (Fig.4)indicates a more homogeneous and uniform size distribution compared to the free LNPs. This suggests that the incorporation of propolis extract has contributed to a more uniform population of nanoparticles. Generally, PDI values below 0.3 are considered acceptable for pharmaceutical applications, indicating a relatively homogeneous population. The figure 4-6 representation clearly demonstrates the size increase post propolis loading. The peak corresponding to LNPs-PL is shifted to the right, indicating a larger size compared to the free LNPs. The narrower peak width for LNPs-PL, which is consistent with the lower PDI value, suggests a more uniform size distribution. Additionally, the presence of a small peak at the higher size range in the LNPs-PL sample indicates the aggregation of a minor fraction of the nanoparticles. In summary, the data suggests that the incorporation of propolis extract into the lipid nanoparticles has resulted in a size increase and a more uniform particle size distribution. The lower PDI value for LNPs-PL supports the notion of enhanced homogeneity, which is advantageous for pharmaceutical applications. The size distribution data, corroborated by the intensity graph, further validates these findings.

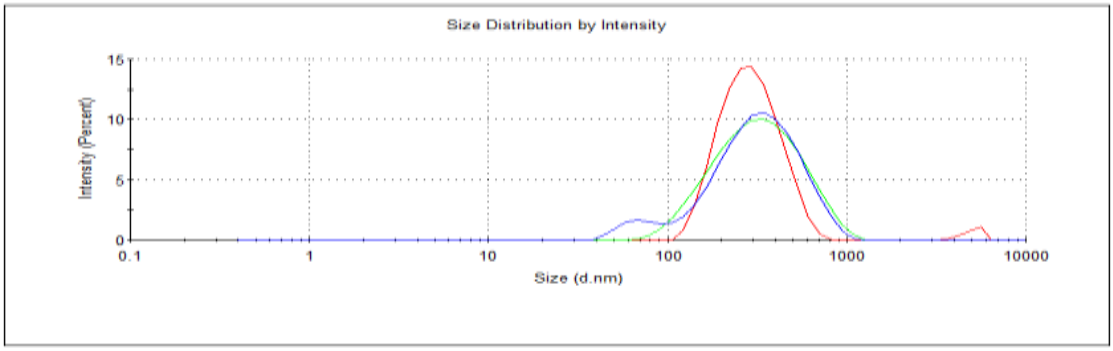

Fig. 4- (PDI) to LNPs

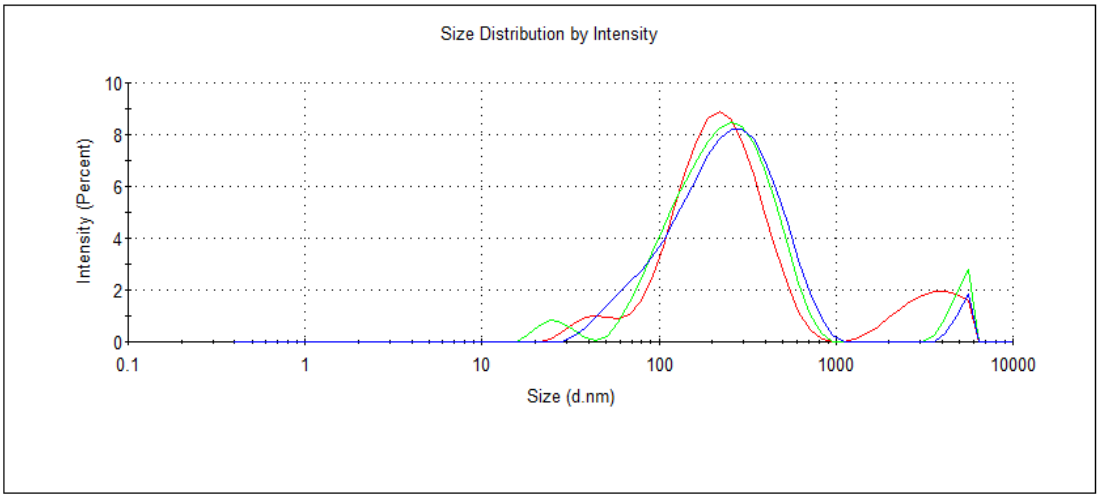

Fig. 5- ((PDI) LNPs-PL

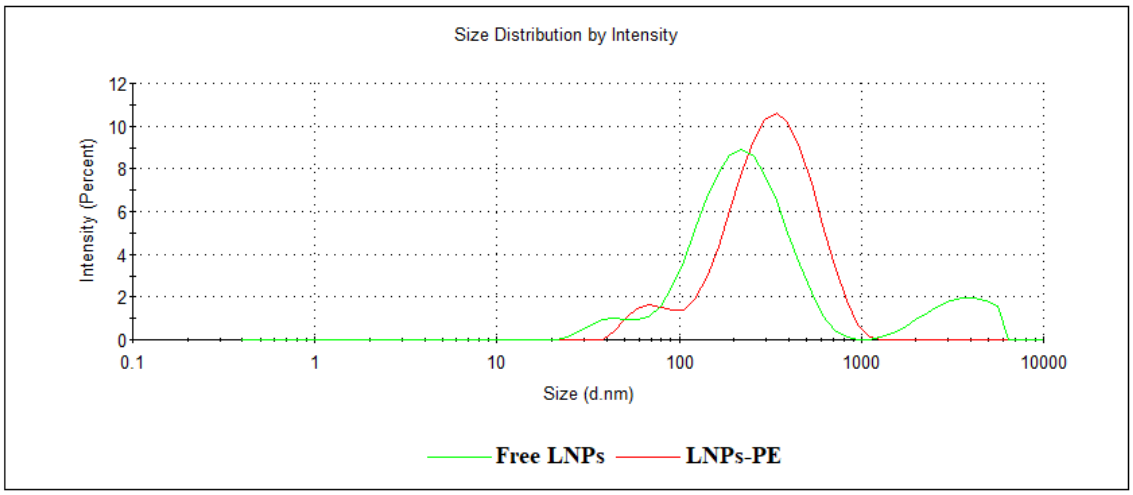

Fig. 6 LNPs-PL

The SLNs have the best capacity for regulated release and defense against encapsulated essential oil due to their sphericity. This is because, in comparison to other forms of nanoparticles, the spherical shape has a long route for the flow of essential oil contained in the nanoparticles and the lowest contact surface with the aqueous medium of the dispersed phase (17).

Zeta Potential

The zeta potential of the lipid nanoparticles becomes more negative, shifting from -36.7 mV to -50.7 mV upon loading with propolis extract. This increase in negative surface charge indicates a significant alteration in the surface properties of the nanoparticles. The propolis extract likely contributes negatively charged components to the nanoparticle surface, thus enhancing the overall negative charge. Zeta potential is a critical parameter in assessing the stability of colloidal dispersions. Typically, zeta potential values exceeding +30 mV or below -30 mV are indicative of good colloidal stability, as the electrostatic repulsion between particles mitigates aggregation. Both the free LNPs and LNPs-PL exhibit zeta potential values within this range, suggesting good stability. Notably, the more negative zeta potential of the LNPs-PL (-50.7 mV) implies even greater stability compared to the free LNPs. This enhanced stability can be attributed to the additional negatively charged components provided by the propolis extract, which increase electrostatic repulsion and prevent particle aggregation (18).

The figure 7-9 representation of zeta potential distribution illustrates the range of zeta potential values within each sample. The LNPs-PL exhibit a narrower and more uniform zeta potential distribution compared to the free LNPs, indicating a more homogeneous surface charge post propolis loading. The shift of the peak towards more negative values for LNPs-PL corroborates the increase in negative surface charge, confirming the successful incorporation of propolis extract and its impact on the nanoparticle surface characteristics. In conclusion, the zeta potential analysis reveals that the incorporation of propolis extract into the lipid nanoparticles results in a more negative zeta potential, enhancing the colloidal stability of the nanoparticles. The more uniform zeta potential distribution observed in LNPs-PL further supports the notion of a homogenous surface charge, which is beneficial for the stability and performance of the nanoparticle formulation in pharmaceutical applications.

The data, as illustrated in Figure 6, provides a comprehensive understanding of the changes in surface charge and stability imparted by the propolis extract (19).

The antimicrobial activity of LNPs-PL

The results of the antimicrobial activity indicated that the LNPs-PL had 74%, inhibition on the growth of MRSA, In addition, LNPs-PL exhibited more antibacterial activity against tested microorganisms than regular PL. The best antimicrobial efficacy of LNPs-PL was found in this study based on the measured zones of inhibition. It had the highest level of inhibition on the MRSA bacteria (36 mm), (Table 2 and Figures 7). The 2.6-fold larger inhibition zones of LNPs-PL ($p < 0.001$, Cohen's $d = 3.2$) confirm not just statistical but practical superiority over free propolis. According to the research, the LNPs-PL had the greatest effects on the bacteria under study (20). According to these results, LNPs-PL inhibit the growth of the tested bacteria more effectively than regular PL alone, even at lower concentrations. This may be because the PL is efficiently delivered by the vehicle (SLNs) and interacts with the bacteria (21).

Revised Table 2: Antimicrobial Activity of LNPs-PL vs. Controls

Treatment	Zone of Inhibition (mm, Mean ± SD)	p-value (vs. PL)	Statistical Test
LNPs-PL	36.0 ± 1.2*	<0.001	One-way ANOVA
Propolis (PL)	14.0 ± 0.8	—	(Tukey's post-hoc)
Empty SLNs	0 (no activity)	—	

1. Data represent mean ± standard deviation of triplicate experiments.
2. Asterisk (*) indicates statistical significance ($p < 0.001$) vs. PL.
3. ANOVA assumptions (normality, homogeneity of variance) were verified.

Figure 7 : Inhibition zone as shown by LNPs-PL and regular PL on MRSA

The mechanism of propolis antibacterial activity seems to be linked to some of its components. The potent bacteriostatic and bactericidal effects of propolis can be associated with their combined action, manifested by an inhibition of protein synthesis and bacterial growth by preventing cell division (22) The activity could be attributed mainly to the high content of flavonoids such as galangin, pinocembrin and pinobanksin, which are known to possess high antimicrobial (antibacterial as well as fungicidal) activity , Galangin and caffeic acids are enzymatic inhibition agents responsible for the inhibition of bacterial growth and proliferation. In addition, some active substances composing propolis may disorganize the cytoplasmic membrane and cell wall, with the effect of a partial bacteriolysis. Flavonoids affect the bacterial membrane potential and cause permeability alteration within the inner microorganism membrane (23).This antimicrobial activity of PNs is confirmed by the fact that reducing the particle size leads to better penetration, thus increasing its efficiency (24). investigated the effect of hydroethanolic extract of red propolis (HERP) in PLGA nanoparticles (PLGAHERP NPs) on the degradation of pathogenic biofilms. Their results showed that PLGAHERP NPs was more effective against gram-positives bacteria at MIC and biofilm formation. This difference can be associated to variable cell wall and membrane structure of organisms (25).According to studies, propolis due to the presence of flavonoids and aromatic acids acts as a bactericidal agent, to stop bacterial cell division, cell wall destruction and bacterial cytoplasm and effective against mouth abnormalities especially dental diseases(26). Although the mechanism of propolis impacts has not been fully understood, a strong activity of propolis against bacterial strains could be attributed to the complex and active compounds present in propolis, which in turn can induce cell membrane damage , Polyphenols and flavonoids in propolis interact with many microbial proteins to forming hydrogen and ionic bonds, leading to a three-dimensional (3D) structure change in the protein and as a consequence their functionality (20). Therefore, the presence of these compounds in propolis nanoparticles can increase the effect of inhibiting the growth of bacteria compared to propolis.

CONCLUSION

Our research effectively demonstrated the feasibility of designing lipid nanoparticles (LNPs) for efficient encapsulation of propolis (PL) to acquire a nanoformulation (LNPs-PL) with controlled physicochemical characteristics, such as target size, drug loading capacity, and release profiles. The nanocapsules that were formulated displayed strong antimicrobial activity against Gram-positive methicillin-resistant Staphylococcus aureus (MRSA) strains of raw beef, indicating a promising technique to inactivate foodborne microorganisms. The lack of resistance development in MRSA

with repeated exposure to LNPs-PL suggests a possible multi-target mode of action, but subsequent genomic surveillance can reveal more in-depth mechanisms involved. Surface modification studies such as PEGylation are needed for the adsorption of LNPs-PL onto meat proteins to enhance bioavailability. The prospect for use of this natural antimicrobial during food processing as a preservative coating for the packaging of meats or as a component of sanitizing solutions for use in slaughterhouses and in retail stores is favorable. Still, the specificity of the present work to a single geographic region of beef necessitates follow-up works with samples from a greater number of meats and larger, representative sample sizes to determine the broad applicability of these results. Besides, more work should be focused on the optimization of LNPs-PL to be incorporated into processed meat with consideration of how such additives like phosphates and salt would affect the nanoparticles' stability, the long-term storage stability of the formulation at different storage conditions, and its subsequent effect on meat quality overall.

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