

## Assessment the activity of Propolis loaded Nanoparticles against Staphylococcus aureus isolates from tonsillitis Patients

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### Abstract

**Objective:** Tonsillitis is inflammation of the pharyngeal tonsils, predominantly affects the pediatric demographic, with a notably elevated incidence rate observed in children. Methods: 154 samples from pharyngeal tonsils had been collected from patients who had Tonsillitis. The Samples were obtained from Al Zahraa Teaching Hospital 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 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:** Staphylococcus aureus, nanoparticle advantage, Tonsillitis

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### INTRODUCTION

Tonsillitis, defined as the inflammation of the pharyngeal tonsils, predominantly affects the pediatric demographic, with a notably elevated incidence rate observed in children. (1). Inflammation of the tonsils can cause discomfort and difficulty swallowing. These conditions cause disruption to daily activities, the highest incidence of tonsillitis in children is due to the body's imperfect immune response this is supported by children's low knowledge of unhealthy diets such as eating fast food and preservatives (2). The tonsils are located in the lateral oropharynx between the anterior palatoglossal arch and the posterior palatopharyngeal arch. (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 global clone 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 pharyngeal tonsils had been collected from patients who had Tonsillitis. The Samples were obtained from Al Zahraa Teaching Hospital 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:

$$\%EE = (A-B)/A \times 100$$

$$\%LC = (A-B)/C \times 100$$

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 N<sub>2</sub> 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 Tonsillitis

The result of cultural examination for ( 154 ) .samples from pharyngeal tonsils had been collected from patients who had Tonsillitis. The Samples were obtained from Al Zahraa Teaching Hospital in Wasit City, Iraq. During the period from October,2024 to March,2025. revealed 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 Tonsillitis, 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 pharyngeal tonsils *S.aureus* strains can cause antibiotic resistance is widespread and can **Tonsillitis** 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 **Tonsillitis** was 36 (33.8%), followed by tooth decay and dental plaque at 19(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 **Tonsillitis** swabs of 11% in 110 patients attending hospital with a variety of oral illnesses. 13 Salivary carriage of *S. aureus* was detected in 41% of patients with decreased salivary flow rates attending an oral medicine clinic, with concentrations ranging from  $3.7 \times 10^1$  to  $5.2 \times 10^3$  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 Tonsillitis Samples**

patients	Total Samples (N)	<i>S. aureus</i> Positive (%)	MRSA Positive (%) (95% CI)
Tonsillitis	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 medium lrmr7rrrrrrro cvd54

### Preparation and Characteristics of LNPs-PL

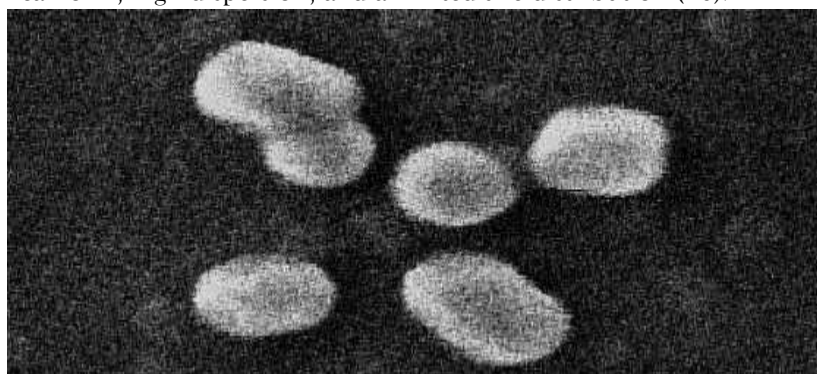
#### DLS analysis

##### 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.

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).

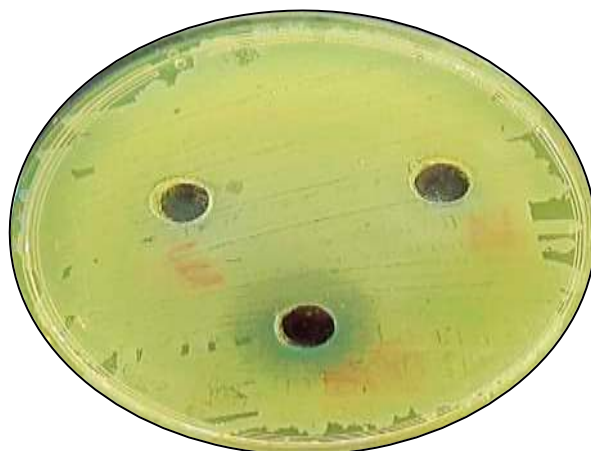
**The antimicrobial activity of LNPs-PL** The results of the antimicrobial activity indicated that the LNPs-PL had 74% inhibition on the growth of MRSA. In addition, LNPs-PL exhibited more antibacterial activity against tested microorganisms than regular PL. The best antimicrobial efficacy of LNPs-PL 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 $\pm$ SD)	p-value (vs. PL)	Statistical Test
LNPs-PL	36.0 $\pm$ 1.2*	<0.001	One-way ANOVA
Propolis (PL)	14.0 $\pm$ 0.8	–	(Tukey's post-hoc)
Empty SLNs	0 (no activity)	–	

1. Data represent mean  $\pm$  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.

## REFERENCE

- 1 Alasmari, N. S. H., et al. (2017). "Causes and treatment of tonsillitis." *The Egyptian Journal of Hospital Medicine* 69(8): 2975-2980.
2. ALKHAFI, A. N. "Bacteriological of Tonsillitis and Evaluation Resistance of Bacterial Isolate among Patients at AL Najaf province, Iraq."
3. Arambula, A., et al. (2021). "Anatomy and physiology of the palatine tonsils, adenoids, and lingual tonsils." *World Journal of Otorhinolaryngology-Head and Neck Surgery* 7(03): 155-160.
4. Loir LY, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research J* 2003; 2(1):63-76. PMID:12917803
5. Faden A. Methicillin-resistant *Staphylococcus aureus* (MRSA) screening of hospital dental clinic surfaces. *Saudi J Biol Sci* 2019; 26(7):1795-1798. <https://doi.org/10.1016/j.sjbs.2018.03.006>
- 6) Rajadurai pandi K, Mani KR, Panneerselvam K, et al. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: A multicentre study. *Indian J Med Microbiol* 2006; 24(1):34-38. <https://doi.org/10.4103/0255-0857.19892>
7. Silva, H. et al. (2021). Te cardiovascular therapeutic potential of propolis—A comprehensive review. *Biology* 10, 27. <https://doi.org/10.3390/biology10010027>.
8. S.-B. Shin, J.-K. Lee, M.-J. Ko, Enhanced extraction of bioactive compounds from propolis (*Apis mellifera* L.) using subcritical water, *Scientific Reports*, 13 (2023) 15038.
- 9- Mader K, Mehnert W. Solid lipid nanoparticles concepts, procedures and physicochemical aspects. In: *Lipospheres in drug targets and delivery*. Ed Nastruzzi C. Florida: CRC press; 2005.p.1-22.
- 10-Parisa Jourghanian<sup>1</sup>, Solmaz Ghaffari, Mehdi Ardjmand, Setareh Haghghat, Mahdieh Mohammadnejad. (2016). Sustained Release Curcumin Loaded Solid Lipid Nanoparticles. *Advanced Pharmaceutical Bulletin*. 6(1), 17-21 | 21.
- 11- Mosallaei N, Jaafari MR, Hanafi-Bojd MY, Golmohammadzadeh S, Malaekheh-Nikouei B. (2013). Docetaxel Loaded solid lipid nanoparticles: preparation, characterization, In vitro, and In vivo Evaluations. *J Pharm Sci*, 102:1994–2004
- 12- Alwan Al-Qushawia, Ali Rassoulia, Fatemeh Atyabib, Seyed Mostafa Peighambaric, Mehdi Esfandiyari-Maneshd, Gholam Reza Shamsa and Azam Yazdanic. (2016). Preparation and Characterization of Three Tilmicosin-loaded Lipid Nanoparticles: Physicochemical Properties and in-vitro Antibacterial Activities. *Iranian Journal of Pharmaceutical Research*. 15 (4): 663-676.

- 11.Scott, R. J., Lian, L. Y., Muharram, S. H., Cockayne, A., Wood, S. J., Bycroft, B. W., ... and Chan, W. C. (2013). Side-chain-to-tail thiolactone peptide inhibitors of the staphylococcal quorum-sensing system. *Bioorganic & medicinal chemistry letters*, 13(15), 2449-2453.
- 12.Waters, A. E., Contente-Cuomo, T., Buchhagen, J., Liu, C. M., Watson, L., Pearce, K., ... and Price, L. B. (2011). Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clinical Infectious Diseases*, 52(10), 1227-1230
13. Porpino, G., Parente, J., and Wansink, B. (2015). Food waste paradox: antecedents of food disposal in low income households. *International journal of consumer studies*, 39(6), 619-629.
14. Mohammed, N. I. and Alwan, M. J. (2017): Isolation and Identification of *Staphylococcus aureus* strain from fresh and frozen meat in karbala province. *International journal of science and nature*, 8(3), 704-709.
15. Parisa Jourghanian1, Solmaz Ghaffari, Mehdi Ardjmand, Setareh Haghighat, Mahdieh Mohammadnejad. (2016). Sustained Release Curcumin Loaded Solid Lipid Nanoparticles. *Advanced Pharmaceutical Bulletin*. 6(1), 17-21 | 21.
16. Samar S. Ibrahim, Nagwa Y. Salem, Shimaa SI Abd ElNaby and Manal M. Adel. (2021). Characterization of Nanoparticles Loaded with Garlic Essential Oil and Their Insecticidal Activity Against *Phthorimaea Operculella* (Zeller) (PTM) (Lepidoptera: Gelechiidae). *Int. J. Nanosci. Nanotechnol.* Vol. 17, No. 3, Sept. 2021, pp. 147-160.
17. Layegh P, Mosallaei N, Bagheri D, Jaafari MR, Golmohammadzadeh S. (2013). The efficacy of Isotretinoin loaded solid lipid nanoparticles in comparison to Isotretin® on acne treatment. *Nanomed J.* 1:41-51.
18. Wencui, Z. QI, W. Ying, W. D. (2015). Preparation of solid lipid nanoparticles loaded with garlic oil and evaluation of their in vitro and in vivo characteristic. *European Review for Medical and Pharmacological Sciences*. 19: 3742-3750.
- 19.Efferth T, Koch E. Complex Interactions between Phytochemicals. (2011). The multi-target therapeutic concept of phytotherapy. *Curr Drug Targets*. 12:122-132.
- 20 .Singh, M.; Singh, S.; Salgar, A.R.; Prathibha, N.; Chandrahari, N.; Swapna, L.A. (2020). An in vitro comparative evaluation of antimicrobial efficacy of propolis, *Morinda citrifolia* Juice, sodium hypochlorite and chlorhexidine on *Enterococcus faecalis* and *Candida albicans*. *J. Contemp. Dent. Pract.* 20, 40–45. [Google Scholar] [PubMed]
- 21.Jaiswal, N.; Sinha, D.-J.; Singh, U.-P.; Singh, K.; Jandial, U.-A.; Goel, S. (2021).Evaluation of antibacterial efficacy of Chitosan, Chlorhexidine, Propolis and Sodium hypochlorite on *Enterococcus faecalis* biofilm: An in vitro study. *J. Clin. Exp. Dent.* 9, e1066–e1074. [Google Scholar] [CrossRef]
22. Valera, M.C.; da Rosa, J.A.; Maekawa, L.E.; de Oliveira, L.D.; Carvalh, O.C.A.; Koga-Ito, C.Y.; Jorge, A.O. (2020).Action of propolis and medications against MRSA bacteria and endotoxin in root canals. *Oral. Surg. Oral. Med. Oral. Pathol. Oral Radiol. Endod.* 110, e70–e74. [CrossRef]
- 23.Park, Y.K.; Alencar, S.M.; Aguiar, C.L.(2020) Botanical Origin and Chemical Composition of Brazilian Propolis. *J. Agric. Food Chem.*, 50 2502–2506. [CrossRef] [PubMed].
24. Awawdeh, L.; Jamleh, A.; Al-Beitawi, M. (2018 ).The Antifungal Effect of Propolis Endodontic Irrigant with Three Other Irrigation So-lutions in Presence and Absence of Smear Layer: An In Vitro Study. *Iran Endod. J.* 2018, 13, 234–239.
25. Johnson M, Olaleye ON, Kolawole OS. (2016). Antimicrobial and Antioxidant Properties of Propolis Extract against *S. aureus* and *Pseudomonas aeruginosa*. *Br Microbiol Res J.* 14: 1–11.
- 26.Bueno-Silva, B.; Marsola, A.; Ikegaki, M.; Alencar, S.M.; Rosalen, P.L. (2017).The effect of seasons on Brazilian red propolis and its botani-cal source: Chemical composition and antibacterial activity. *Nat. Prod. Res.* 31, 1318–1324. [CrossRef].