J Nanostruct 12(3): 675-685, Summer 2022

RESEARCH PAPER

Evaluation of the Antibacterial Inhibitory Activity of Chitosan Nanoparticles Biosynthesized by *Streptococcus thermophilus*

Zahraa Hussein Ali [1] **, *Mohammed Abd Ali Jabber Al-Saady*** [2,3]**, *Nawfal H. Aldujaili*** [4,1]**, *Shiama Rabeea Banoon*** [5]*, ***Aswan Al-Abboodi*** [6]

[1] *Alameen Center for Advanced Research and Biotechnology, Imam Ali Holy Shrine, Najaf, Iraq*

[2] *AL Sader Teaching Hospital, Maysan Health Directorate, Ministry of Health, Maysan, Iraq*

[3] *Department of Mechanical and Aerospace Engineering, Monash University, VIC 3800, Australia*

[4] *Department of Biology, Faculty of Science, University of Kufa, Najaf, Iraq*

[5] *Department of Biology, College of Science, University of Misan, Maysan, Iraq*

[6] *Department of Biology, College of Science, University of Misan, Maysan, Iraq*

ARTICLE INFO

Article History:
Received 04 April 2022
Accepted 16 June 2022
Published 01 July 2022

Keywords:
Antibacterial Activity
Biofilm
Biogenic chitosan
Nanoparticles
Streptococcus thermophilus

ABSTRACT

The nanomaterials field has been promising in recent decades due to its prospective biological applications that may assist overcome many medical difficulties by regulating numerous illnesses. However, there are restrictions on how these materials might perform against microbes including bacteria and viruses. Therefore. This research aims to investigate the nano synthesis of chitosan nanoparticles (NPs) using *Streptococcus thermophilus*, evaluate their characteristics, and analyze their antimicrobial efficiency against pathogenic bacteria associated with diabetic foot, *Aeromonas hydrophila* and *Citrobacter freundii*. By adding precursors to *Streptococcus thermophilus* cell-free supernatant, chitosan nanoparticles were produced. The solution's hue shift showed the production of chitosan NPs. UV-Visible spectrophotometry was utilized to characterize Streptococcus thermophilus' production of chitosan NPs with an absorption peak at 280nm. SEM examination indicated spherical, homogeneous, 29-51nm particles. EDS tests of biogenic chitosan indicated 33.31 % carbon, 10.24 % nitrogen, 50.55 % oxygen, 5.34% phosphate, and 0.55% chloride. Biogenic chitosan NPs were measured using XRD at 20°-26°. Energy-dispersive X-ray spectroscopy was utilized to determine the size, shape (spherical), dispersal (homogenous), and elemental analysis of nanoparticles. Biogenic chitosan NPs inhibit *Aeromonas hydrophila* and *Citrobacter freundii*. Biogenic chitosan NPs at 100, 200, and 400 ug/ml inhibited both tested microorganisms. Biogenic chitosan NPs showed growing antibiofilm activity. DPPH lowers biogenic chitosan nanoparticle activity.

How to cite this article
Ali Z H., Jabber Al-Saady M A A., Aldujaili N H., Banoon S R. Al-Abboodi A Evaluation of the Antibacterial Inhibitory Activity of Chitosan Nanoparticles Biosynthesized by Streptococcus thermophilus. J Nanostruct, 2022; 12(3):675-685. DOI: 10.22052/JNS.2022.03.020

* *Corresponding Author Email: Shimarb@uomisan.edu.iq*

INTRODUCTION

Chitosan is a natural cationic nontoxic biopolymer made from the deacetylation of chitin, which consists of N-acetyl-D-glucosamine and D-glucosamine components crosslinked by -1,4-glycosidic links [1,2]. Many studies have investigated the antibacterial action of Chitosan [1–4], and until now various forms of Chitosan derivates have been fabricated to improve its antibacterial action [5–8]. Chitosan demonstrates further unique biological properties, for instance, biodegradability and biocompatibility that assist in its use in numerous medical applications [2,3,9]. As nanotechnology has recently played a progressively more significant role because it increases the capability to expand antibacterial research to the molecular and atomic levels [2]. Chitosan nanoparticles NPs have exceptional biochemical characterization [10–14] and have been synthesized by several methods [12].

There is strong evidence that the bacterial cell wall or membrane is involved in Chitosan nanoparticles' antibacterial action. Many other ideas have been proposed to try and make sense of this behavior. Electrostatic repulsion between the glucosamine's positively charged amino groups and the bacteria's negatively charged cell membranes is one of them [15]. By causing a shift in membrane permeability, this interaction ultimately causes an osmotic imbalance, followed by the outflow of intracellular chemicals and cytoplasm, and finally cell death [16-18]. Additionally, chitosan nanoparticles can modify the electron transport chain of bacteria [19].

It is generally known that bacteria may fuse and gather molten ions of metals and metalloids. Toxic metal ions can be converted by individual bacteria into safe nanoparticles [20,21]. In this case, some bacteria are used as nano-bioreactors, synthesizing materials with unique properties [22]. since it has low energy consumption and process controllability, the bacterial synthesis of nanoparticles is an extremely promising field [20]. At present, several studies have revealed synthesizing of metal nanoparticles such as zinc oxide ZnO, silver, copper oxide CuO and iron oxide nanoparticles by various types of bacteria [23]. The antimicrobial characteristics of nanoparticles have been known for the most recent periods, which paved the route to be exploited in various contemporary bio-medical applications to terminate or control some microbial infection diseases [22]. *Streptococcus thermophilus* (*S. thermophilus*), one of the most important lactic acid bacteria (LAB), has been extensively applied in combination with other bacteria species in the dairy industry [21]. *S. thermophilus* have antibacterial and antioxidative features and the current study indicated its ability to synthesize the chitosan nanoparticles, therefore the main aim of the current experiment is to produce the chitosan nanoparticles by the *S. thermophilus* and apply these nanoparticles to verify its antimicrobial abilities. To the best of our knowledge, there are no reports about the antibacterial activities of the chitosan nanoparticles from *S. thermophilus* especially the effect on the bacteria isolated from the diabetic feet named *Aeromonas hydrophila* and *Citrobacter freundii*.

MATERIALS AND METHODS

Identification of the efficient isolate and large-scale production of chitosan NPs

The identification of species sequence was revealed previously via PCR with universal primers [24] for the *S. thermophilus*. The *S. thermophilus* isolate was screened against *E. coli* using the Muller Hinton agar well diffusion assay and incubated for 24 hours at 37ºC. An efficient isolate was selected based on antimicrobial activity, color change, and absorption spectrum efficiency. Preparation of cell-free supernatant of *Streptococcus thermophilus* was inoculated in ready and autoclaved (sterile) broth. After that, bacteria were grown in brain heart infusion broth at 37ºC for 24h. After 15 minutes of incubation, the colloidal suspension was centrifuged at 10,000 rpm to separate the precipitate. The supernatant was then mixed with a solution of distilled water, (chitosan, 2 mg), and (acetic acid, 2ml). After the inoculum was activated, the BHI broth was inoculated (incubation time: 24 hours at 37°C), the culture was incubated for biomass production, and the cell-free supernatant was prepared by centrifugation at 4200xg for 15 minutes at 4°C, after which the pellets of cells of microorganism were discarded and the cell-free supernatants were collected for use in the biosynthesis of chitosan nanoparticles.

The supernatant was collected by centrifuging the broth culture at 4200xg in a large-scale, refrigerated centrifuge that could accommodate 50 ml tubes, thereby pelleting the cells at the bottom of the tubes. To make chitosan

nanoparticles, the supernatant was collected in a clean flask. The strains were inoculated, and then incubated for 24 hours at 37 °C with a shaking incubator at 150 rpm and 400 ml of BHI broth with 4 gm of chitosan as a substrate and 4 ml of acetic acid in each autoclaved flask. This was repeated at different times.

Characterization of biosynthesized chitosan NPs

Physical properties of biosynthetic NPs were studied using XRD, UV visible, and AFM. Detection of the chitosan nanoparticle formation was estimated with UV-Visible spectroscopy (Shimadzu UV-visible1800 spectrophotometer) in the research laboratory of Imam Ali Holy Shrine. While Scanning Electron Microscope (SEM) Analysis, Energy Dispersive X-Ray Spectroscopy (EDS) Analysis, Atomic Force Microscope (AFM), and X-ray Diffraction (XRD) Analysis were examined at the University of Tehran's electron microscopy unit

Antibacterial activity of chitosan NPs

The antibacterial activity of chitosan NPs were examined at various concentrations against diabetic feet bacteria *Aeromonas hydrophila* and *Citrobacter freundii* using the agar diffusion method. With 100 µl of bacterial suspension, the agar plate was inoculated. Bores (6 mm in diameter) were then produced using a sterile borer and filled with 150 µL of chitosan NPs. Subsequently, the Petri dishes were kept at 4 ° C for 2 h, then again incubated at 37 ° C for 24. Antibacterial activities were calculated, and the values were provided as means of triplicate analysis by measuring the growth inhibition zone diameter in millimeters.

Antioxidant Activity of Chitosan NPs

One hundred pure chemical compounds were evaluated using a modified DPPH radical cation technique. DPPH (8 mg) dissolved in MeOH (100 mL) for 80 µl /mL solution. 100 µl DPPH reagent was combined with 100 µl of sample in a 96-well microplate and incubated at room temperature for 30min. After incubation, absorbance was measured at 514 nm using an ELISA reader (TECAN, Gröding, Austria), and 100% methanol was used as a control. Formula for DPPH scavenging effect:

$$\text{DPPH scavenging impact } (\%) = (A_o - A_1) / A_o \times 100$$

where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the chitosan. The real absorption decrease caused by test compounds was compared with the positive controls [25]. We extrapolated the results of regression analysis to determine the IC50 DPPH values (the concentration of sample necessary to block 50% of DPPH radicals). Based on this IC50 value, the antioxidant was rated.

RESULTS AND DISCUSSION

Selection of the efficient isolate that produces chitosan NPs

Twenty-five isolates of various *Streptococcus thermophilus* strains were screened based on the ability of bacteria to synthesize the chitosan NPs depending on the color change from light to dark, UV-Visible Spectroscopy, and the biological activities of the chitosan NPs against *E. coli* to select the efficient strains. The efficient isolate was identified as *Streptococcus thermophilus* based on morphological, biochemical identification, and VITEK 2 compact system.

Diverse microorganisms can synthesize inorganic materials either intracellularly or extracellularly or both ways. The microbial cell has an exceptional ion transfer mechanism in an intracellular way. The negatively charged bacterial cell wall absorbs positively charged metal ions due to electrostatic activity [19]. In addition, enzymes in the bacterial cell wall break down the metal ions into their component nanoparticles. The extracellular technique, on the other hand, involves the bioreduction of metal ions into the corresponding NPs by the secretion of reductases from the microbial cell [22].

Large scale production of chitosan nanoparticles using Streptococcus thermophilus

Streptococcus thermophilus bacteria were subjected to use in the biosynthesis of chitosan NPs, demonstrating the competence in extracellular biosynthesis by using cell-free supernatant after the addition of chitosan as a substrate under previously optimized conditions. The reaction mixture turns from light to dark. The production of chitosan NPs by *Streptococcus thermophilus* was monitored by observing the color change and antibacterial activity after incubation in shaking at 150 rpm. Many techniques exist for the production of chitosan nanoparticles, including ionic gelation, complex coacervation, emulsion cross-linking, spray drying, and a biological method [26]. Manufacturing

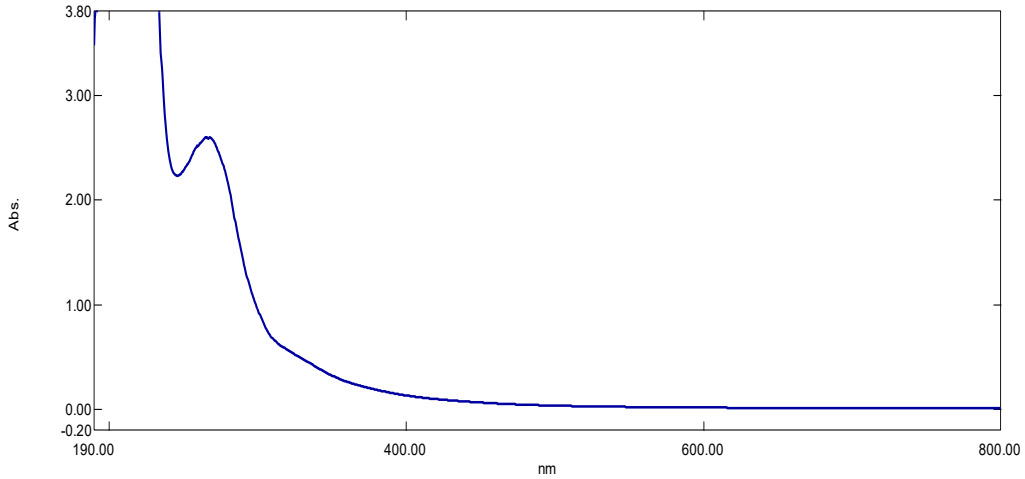

Fig. 1. UV-Visible spectroscopy analysis of chitosan NPS synthesis by *Streptococcus thermophilus*

through biological systems is a relatively new field, and microorganisms are being eyed by scientists as a possible environmentally benign nano-factory [12]. Because of its repeatability and adaptability, the biological method was used. The size and surface charge of the resulting particles may be changed to some extent using this technology, making it a highly manipulable process [26].

Characterization of biosynthesized chitosan NPs
UV-visible Spectroscopy

Nanoparticle biogenesis may be confirmed by seeing their formation and by measuring their absorbance band with UV-visible spectroscopy. Nanoparticles formed from the reaction mixture have a 280 nm peak in their absorption (Fig. 1). This demonstrates the existence of nanoparticle surface plasmon resonance (SPR), and a single SPR band shows that the nanoparticles are spherical in form. The production of nanoparticles was investigated using a UV-Visible spectrophotometer, which indicated a single peak at 280 nm, suggesting the presence of chitosan nanoparticles in the reaction solution. According to a report performed by

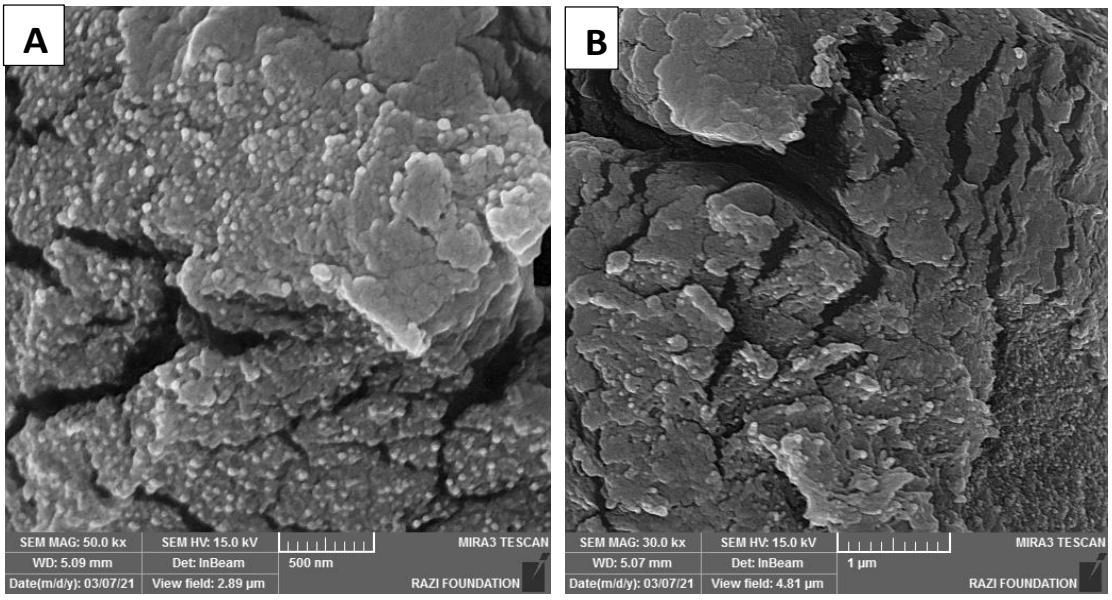

Fig. 2. SEM micrograph of biogenic chitosan NPs synthesize by *Streptococcus thermophilus* demonstrated the chitosan NPs with spherical well dispersed with size (29-51 nm) at A: 500 nm, and B: 1 um

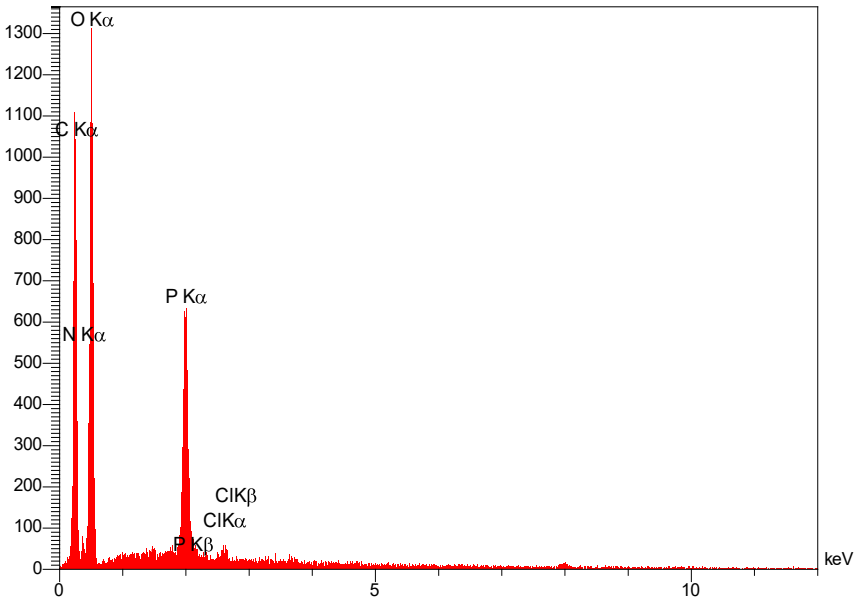

Fig. 3. EDS analysis of chitosan NPs by synthesis using *Streptococcus thermophilus*

Gabriel et al., 2017 [27].

Scanning Electron Microscopy (SEM) Analysis

Biogenic chitosan nanoparticles were approved for morphology and size by scanning electron microscopy (SEM). Chitosan nanoparticles generated by *Streptococcus thermophilus* were well-distributed and spherical in form, with sizes ranging from 29.9 nm to 51.8 nm (Fig. 2).

Nanoparticles (NPs) varied in size from 10 nm to 60 nm. The majority of NPs were the same size, and just a tiny fraction was larger than 60 nm. While prior research has employed various biological chemicals as reducing and stabilizing agents, the approach adopted in the current work allowed for the generation of chitosan NPs of lower size.

EDS analysis of biogenic chitosan NPs

Energy Dispersive Spectroscopy (point and mapping analysis) was used to quantify the presence of chitosan nanoparticles by observing the optical absorption peaks of elements. Elemental analysis exhibited the percentage of elements constituent of CHNPs synthesis by *Streptococcus thermophilus* was 33.31% carbon, 10.24 % nitrogen, 50.55% oxygen, 5.34% phosphate, and 0.55% chloride (Fig. 3). The energy dispersive spectroscopy analysis recognized components, allowing for quantitative and qualitative analysis of the carbon, oxygen, nitrogen, phosphate, and chloride constituents in the formulations produced.

Atomic Force Microscope (AFM)

AFM image of chitosan nanoparticle synthesis by *Streptococcus thermophilus,* gave information on the morphology of biogenic chitosan NPs. The average diameter of chitosan NPs biosynthesis from *Streptococcus thermophilus* was 47.18 (Fig. 4) which showed a three-dimensional image.

AFM's extraordinary resolution allows for precise three-dimensional visualization of molecular structures, as well as atomic-scale strategies. The procedure for preparing samples for AFM is straightforward. Because samples may be seen under circumstances that are almost identical to those found in living organisms, AFM can capture the dynamic processes that occur within molecules, organelles, and other structures in real-time [28].

X-ray Diffraction (XRD) Analysis

The crystalline structure of CHNPs was studied using XRD. The diffraction pattern of the polymorph was assigned to the characteristic peaks of chitosan polymer found at 2Θ=21°. The XRD spectrum of biogenic chitosan nanoparticles seemed at 20° -26°. The sharp peak indicated the presence of bioorganic coupons/proteins in the nanoparticles during the synthesis process (Fig. 5).

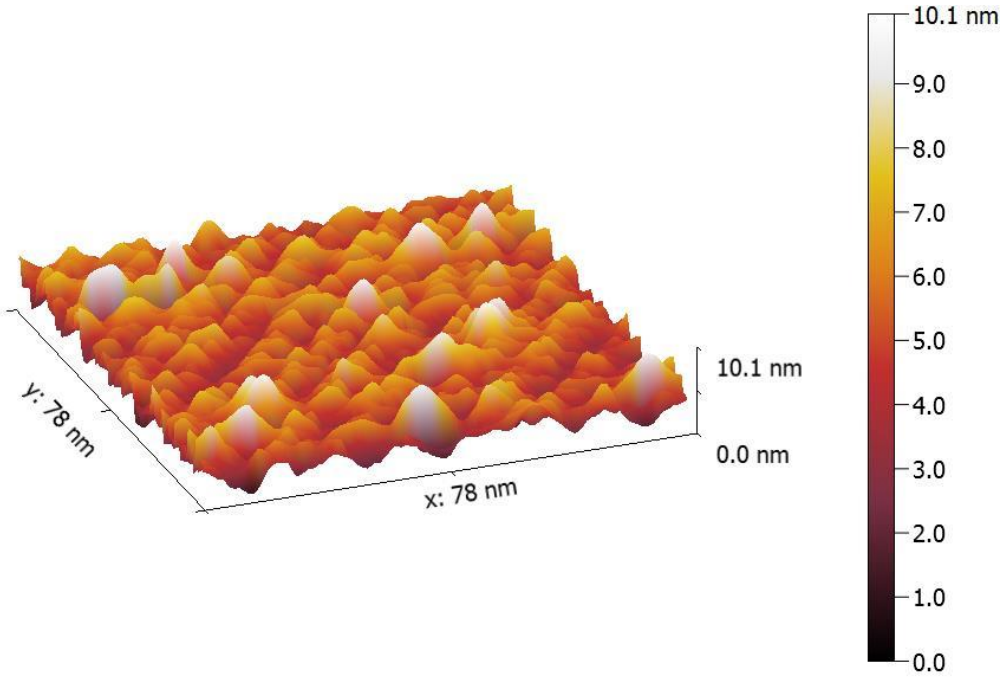

Fig. 4. AFM analysis of biogenic chitosan synthesis by *Streptococcus thermophilus*

X-ray diffraction was used to calculate the typical size of a chitosan nanoparticle. X-ray diffraction (XRD) was used to look into the crystalline structure of CHNPs, and the peak at 2Θ=21° was assigned to the polymorph of chitosan. When X-ray diffraction was applied to CHNPs, the spectra occurred at 20° -26°. Sharp peaking suggested the incorporation of bioorganic couponed/proteins

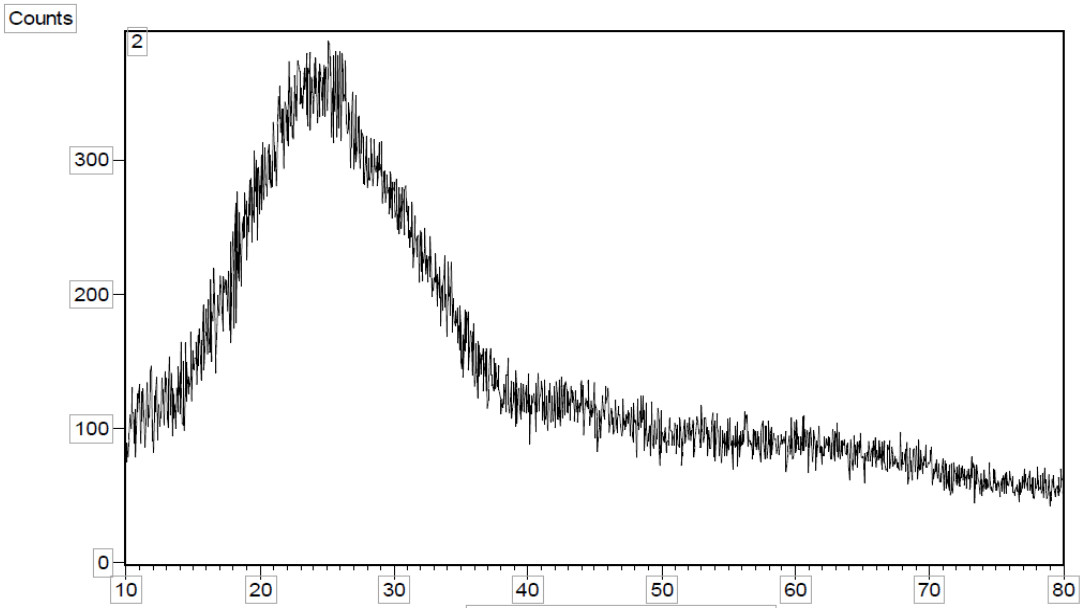

Fig. 5. XRD analysis of biogenic chitosan synthesis by *Streptococcus thermophilus*

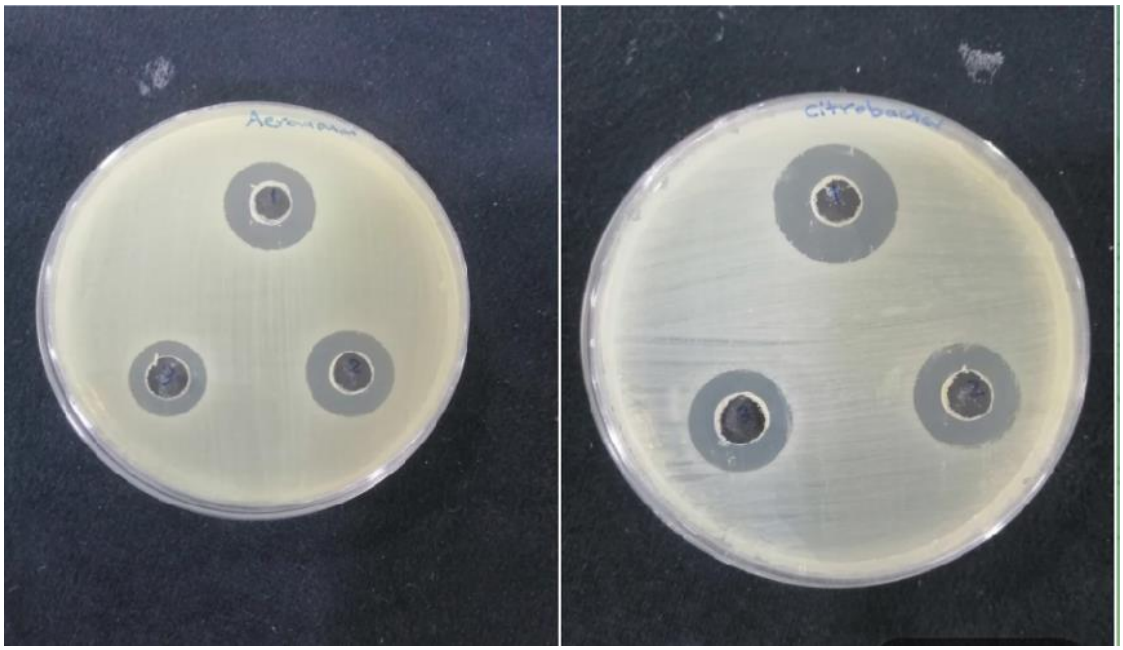

Fig. 6. Anti-bacterial activity of the biogenic Chitosan nanoparticles against some pathogenic bacteria, *Aeromonas hydrophila* (left) and *Citrobacter freundii* (right)

into the nanoparticles.

Antibacterial activity of chitosan NPS

Biogenic chitosan nanoparticles synthesized by *Streptococcus thermophilus* have been evaluated for their antibacterial activity against some multi-drug resistance bacterial pathogens (MDR). The agar well diffusion method was used for detecting the antibacterial activity of biogenic chitosan nanoparticles. Chitosan NPS with different

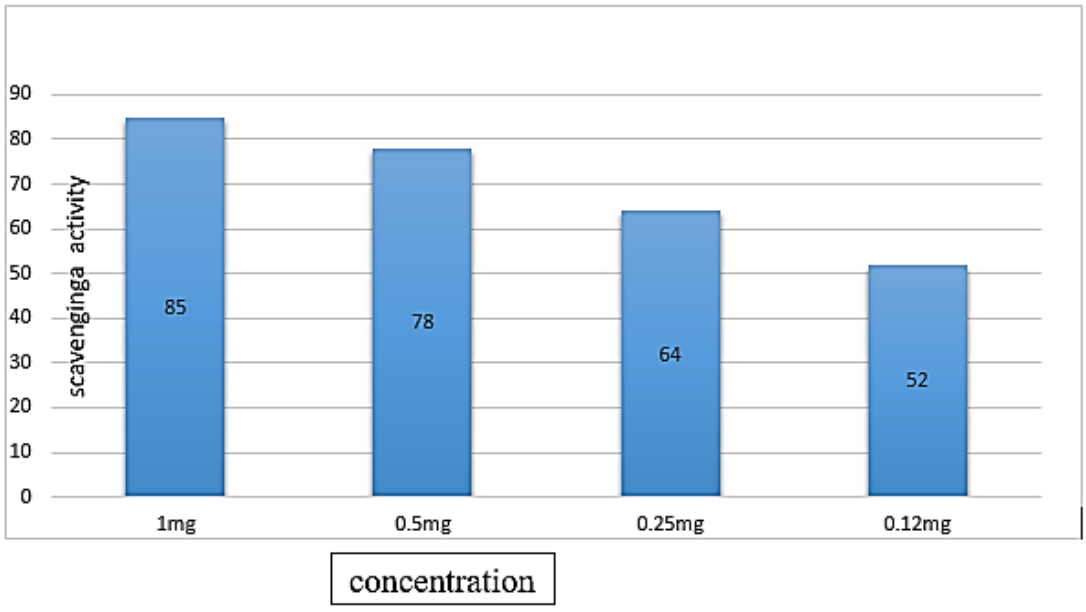

Fig. 7. Antioxidant activity of chitosan NPs by DPPH assay

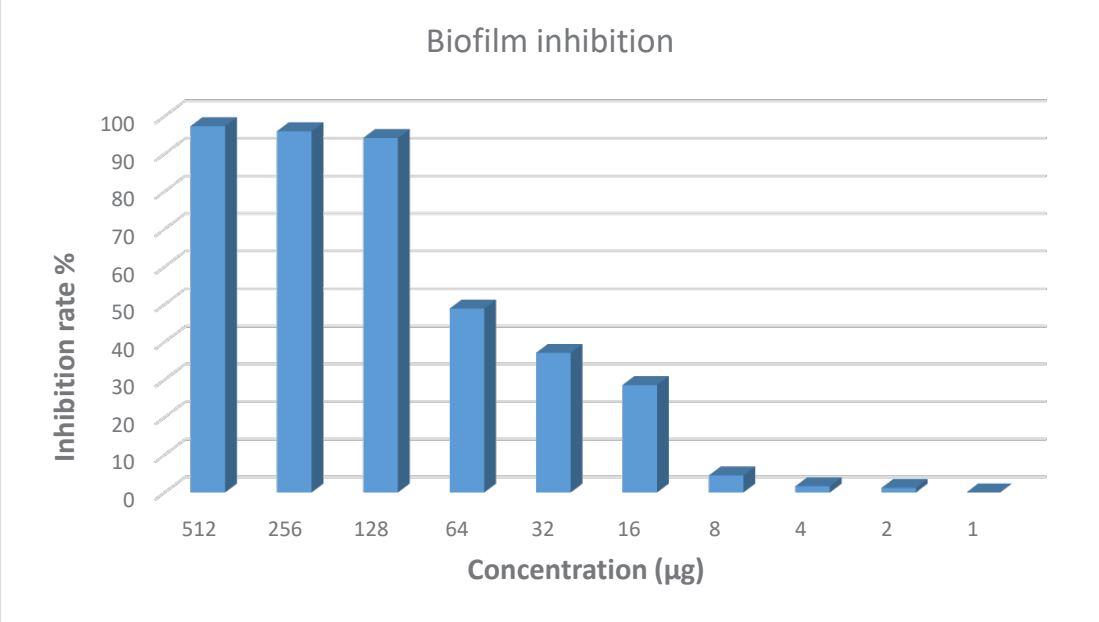

Fig. 8. Anti-biofilm activity of chitosan NPs against *Aeromonas hydrophila*

concentrations (100, 200, 400 ug/ml) showed inhibition activity against all tested bacteria and the highest inhibition zone of Chitosan NPs observed in Gram-Positive bacteria was (26mm) in *Aeromonas hydrophila* and *Citrobacter freundii* with concentration (400 ug/ml), while the lower inhibition zone in Gram-Negative bacteria was (18mm) in *E. coli* with the same concentration. This inhibitory effect increased with increasing concentrations of chitosan NPS as in Fig. 6. Consistent with previous studies [16], we found that high quantities of Chitosan NPs inhibited bacterial growth. Based on the findings, it is clear that nanoparticles may effectively halt the

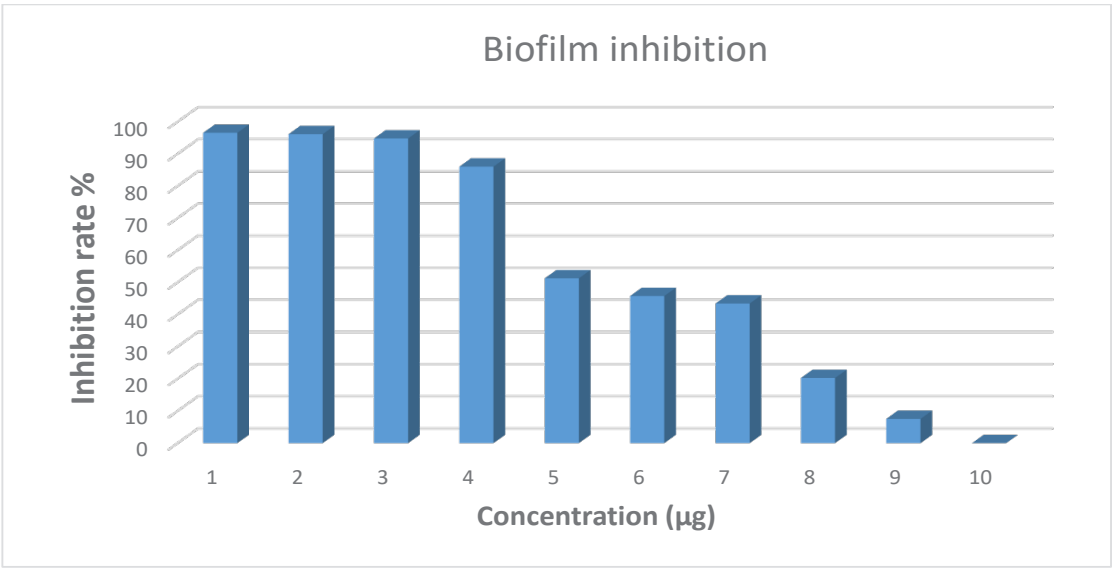

Fig. 9. Anti-biofilm activity of chitosan NPs against *Citrobacter freundii*

proliferation of both gram-positive and gram-negative bacteria. When exposed to biogenic chitosan NPs, gram-positive bacteria were more sensitive than gram-negative bacteria. This agrees with [29] who reported GP bacteria are more susceptible to chitosan's antibacterial effect than GN bacteria. As a result, interpreting bacterial exposure to chitosan is difficult. Since the rising threat of multidrug resistance (MDR), the scientific community has made it their mission to find an effective alternative to replace existing antibiotics that have become resistant. Areas an effective new entry-drug supplement to antibiotics Nanoparticles are now seen as a promising solution to antibiotics and seem to have a high potential to overcome the bacterial MDR problem [21].

Antioxidant Activity of Biogenic Chitosan Nanoparticles

The 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging experiment was performed to assess the antioxidant activity of Streptococcus thermophilus NPs in vitro by decreasing DPPH free radicals. After adding 100, 200, 300, and 400 ug/ml nanoparticles to DPPH solution, absorbance was measured at 517 nm. Color change showed nanoparticles' capacity to scavenge DPPH free radicals. DPPH reduces the activity of biogenic chitosan NPs as their concentration increases. 28% in 100ug/ml, 30% in 200ug/ml, 42% in 300ug/ml, and 54% in 400ug/ml (Fig. 7).

The DPPH scavenging activity of NPs increased as their concentration increased, suggesting that the percentage of inhibition of DPPH increased as the concentration of chitosan NPs increased, indicating that DPPH showed more inhibition due to increased electron donation and agrees. Antioxidants work not only by scavenging free radicals but also by inhibiting free radical formation [30].

Anti-biofilm activity of chitosan

The most crucial characteristic of bacteria is a biofilm, which increases bacterial adherence to surfaces like those found on surgical tools and prostheses. Changes in colony color on congo red agar were recorded as a qualitative measure of biofilm development; Figs (3-8). Chitosan NPs were tested with three different concertation (100,200,400) for antibiofilm activity against two strains of bacteria involving (*Aeromonas hydrophila* and *Citrobacter freundii*). The formation of biofilm in both isolates was prevented by chitosan NPs. The anti-biofilm activity increased with increasing the concentration of biogenic Chitosan NPs (Figs 8 and 9).

Biofilms are multicellular bacterial communities that may evade antibiotics and the body's immune system. Because there are currently no antibiotics on the market that are capable of killing off biofilms. This issue was fixed by using nanoparticles. The

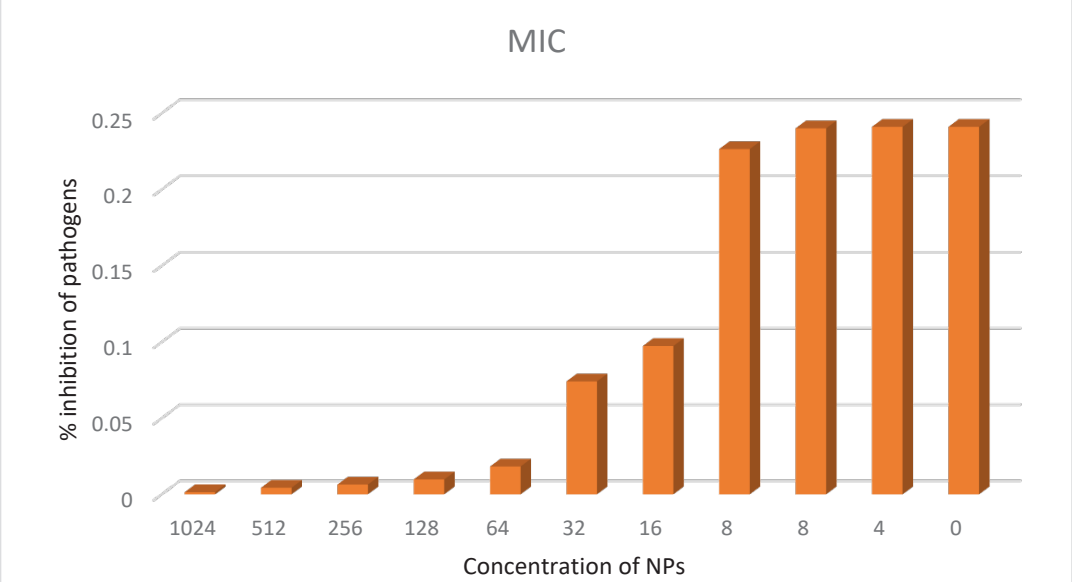

Fig. 10. MIC of chitosan NPs against *Aeromonas hydrophila*

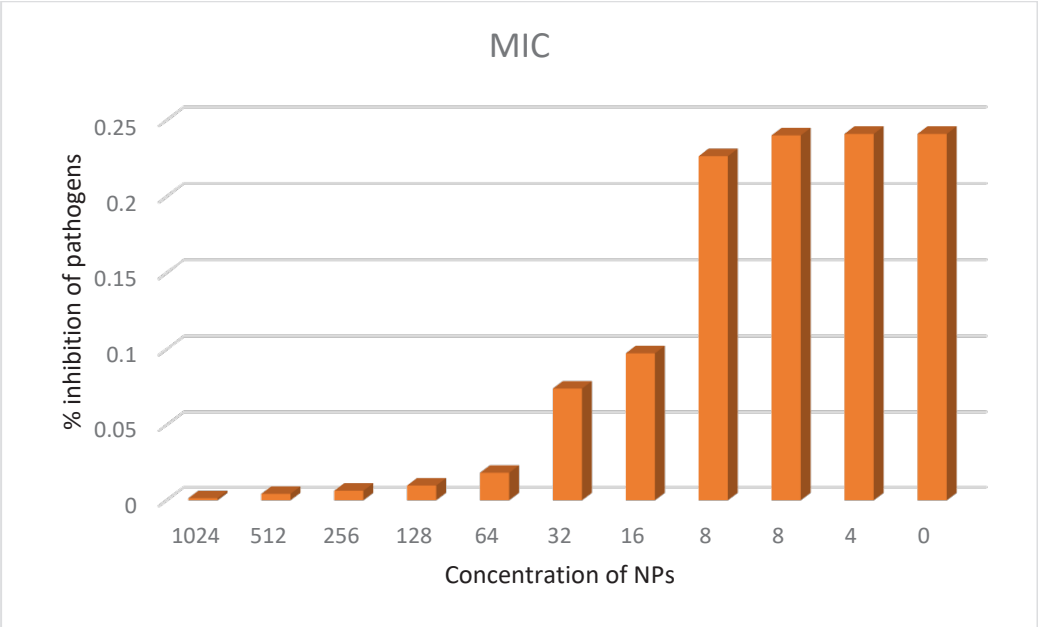

Fig. 11. MIC of chitosan NPs against *Citrobacter freundii*

antibiofilm activity shown in NPs suggests that they might be a promising candidate therapy. The concentration of antibiotics needed to destroy bacteria in biofilms is substantially greater than the MIC of planktonic cells, hence antibiotics typically fail to remove these cells [31].

Determination of minimal inhibitory concentration (MIC)

The MIC values were defined as the lowest concentration of the chitosan NPs able to inhibit visible growth of bacteria, while the MBC values are defined as the lowest concentration of the chitosan NPs at which 99.9 % of the inoculated microorganisms are killed (32).

A broth microdilution protocol was performed according to CLSI guidelines in flat bottom 96-well microtiter plates. Cultures of each pathogen were taken with an inoculating needle after 7 days and suspended in sterile saline containing 0.1% Tween 80. Bacteria were put to 5 ml of RPMI1640 once settling time had passed for the heavier particles. First, 100 µl of the medium was poured into each well, then 90 l in Column 3 wells, and 80 l in Column 4 wells. Then, a chitosan NPs suspension, in volumes of 10 µl and 20 µl, respectively, was added to each well in Columns 3 and 4. In order to get the final NPs concentrations, which ranged from 2.5 mg/ml (4th well) to 0.020 mg/ml (11th well), we did serial dilutions from Column 4 to Column 11. (11th well). Each well had 100 µl of bacterial inoculum put to it, with the exception of all wells in Columns 2 and 3, for a total of 200 µl. As a growth control, we used a well (Column 12) without any NPs that contained media and inoculum.

The microtiter plates were inoculated, then placed in a moist chamber and incubated at 37°C in a B.O.D. incubator to see whether any infections will thrive. We used spectrophotometry to take readings of the optical density ($OD_{530\ nm}$) every 24 hours, 48 hours, and 72 hours (3 times), and the findings are depicted in Figs 10 and 11. no visible signs of growth or a reduction in the growth of 80% compared to the drug-free well. The IC_{50} value was calculated as the concentration at which growth was inhibited by 50% relative to that in the drug-free well.

CONCLUSION

Streptococcus thermophilus has the capability to synthesize Chitosan NPs through biological techniques and the nanoparticle's characterizations showed optimal properties by conducting AFM, XRD, SEM, and EDS examinations. It showed a great inhibition activity

against pathogenic bacteria related to diabetic feet (*Aeromonas hydrophila* and *Citrobacter freundii*) at a concentration of 400 ug/ml. Current biosynthesized Chitosan NPs exhibited antioxidant activity and antibiofilm inhibition activity which made it suitable for various medical applications in regards of fighting diseases that emerge by microorganisms.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Goy RC, Britto Dd, Assis OBG. A review of the antimicrobial activity of chitosan. Polímeros. 2009;19(3):241-247.
2. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: A state of the art review. Int J Food Microbiol. 2010;144(1):51-63.
3. Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W. Chitosan as Antimicrobial Agent: Applications and Mode of Action. Biomacromolecules. 2003;4(6):1457-1465.
4. Li B, Wang X, Chen R, Huangfu W, Xie G. Antibacterial activity of chitosan solution against Xanthomonas pathogenic bacteria isolated from Euphorbia pulcherrima. Carbohydr Polym. 2008;72(2):287-292.
5. Jia Z, shen D, Xu W. Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydr Res. 2001;333(1):1-6.
6. Vinsova J, Vavrikova E. Chitosan Derivatives with Antimicrobial, Antitumour and Antioxidant Activities - a Review. Curr Pharm Des. 2011;17(32):3596-3607.
7. Wang X, Du Y, Fan L, Liu H, Hu Y. Chitosan- metal complexes as antimicrobial agent: Synthesis, characterization and Structure-activity study. Polym Bull. 2005;55(1-2):105-113.
8. Yang T-C, Chou C-C, Li C-F. Antibacterial activity of N-alkylated disaccharide chitosan derivatives. Int J Food Microbiol. 2005;97(3):237-245.
9. Kammoun M, Haddar M, Kallel TK, Dammak M, Sayari A. Biological properties and biodegradation studies of chitosan biofilms plasticized with PEG and glycerol. Int J Biol Macromol. 2013;62:433-438.
10. Ma Z, Garrido-Maestu A, Jeong KC. Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles as antimicrobial agents: A review. Carbohydr Polym. 2017;176:257-265.
11. Divya K, Vijayan S, George TK, Jisha MS. Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. Fibers and Polymers. 2017;18(2):221-230.
12. Divya K, Jisha MS. Chitosan nanoparticles preparation and applications. Environ Chem Lett. 2017;16(1):101-112.
13. Kassem A, Ayoub GM, Malaeb L. Antibacterial activity of chitosan nano-composites and carbon nanotubes: A review. Science of The Total Environment. 2019;668:566-576.
14. Rozman NAS, Tong WY, Leong CR, Tan WN, Hasanolbasori MA, Abdullah SZ. Potential Antimicrobial Applications of Chitosan Nanoparticles (ChNP). Journal of Microbiology and Biotechnology. 2019;29(7):1009-1013.
15. Tsai G-J, Su W-H. Antibacterial Activity of Shrimp Chitosan against Escherichia coli. J Food Prot. 1999;62(3):239-243.
16. Sudarshan NR, Hoover DG, Knorr D. Antibacterial action of chitosan. Food Biotechnol. 1992;6(3):257-272.
17. Shahidi F, Arachchi JKV, Jeon Y-J. Food applications of chitin and chitosans. Trends in Food Science & Technology. 1999;10(2):37-51.
18. Raafat D, von Bargen K, Haas A, Sahl H-G. Insights into the Mode of Action of Chitosan as an Antibacterial Compound. Applied and Environmental Microbiology. 2008;74(12):3764-3773.
19. Birsoy K, Wang T, Chen Walter W, Freinkman E, Abu-Remaileh M, Sabatini David M. An Essential Role of the Mitochondrial Electron Transport Chain in Cell Proliferation Is to Enable Aspartate Synthesis. Cell. 2015;162(3):540-551.
20. Fang X, Wang Y, Wang Z, Jiang Z, Dong M. Microorganism Assisted Synthesized Nanoparticles for Catalytic Applications. Energies. 2019;12(1):190.
21. Garole DJ, Choudhary BC, Paul D, Borse AU. Sorption and recovery of platinum from simulated spent catalyst solution and refinery wastewater using chemically modified biomass as a novel sorbent. Environmental Science and Pollution Research. 2018;25(11):10911-10925.
22. Rautela A, Rani J, Debnath M. Green synthesis of silver nanoparticles from Tectona grandis seeds extract: characterization and mechanism of antimicrobial action on different microorganisms. Journal of Analytical Science and Technology. 2019;10(1).
23. Fatemi M, Mollania N, Momeni-Moghaddam M, Sadeghifar F. Extracellular biosynthesis of magnetic iron oxide nanoparticles by Bacillus cereus strain HMH1: Characterization and in vitro cytotoxicity analysis on MCF-7 and 3T3 cell lines. J Biotechnol. 2018;270:1-11.
24. Aldujaili NH, Banoon SR. ANTIBACTERIAL CHARACTERIZATION OF TITANIUM NANOPARTICLES NANOSYNTHESIZED BY STREPTOCOCCUS THERMOPHILUS. Periódico Tchê Química. 2020;17(34):311-320.
25. Arnab A, Goyal A, Middha S. Evaluation of the DPPH radical scavenging activity, total phenols and antioxidant activities in Indian wild Bambusa vulgaris “Vittata” methanolic leaf extract. Journal of Natural Pharmaceuticals. 2010;1(1):40.
26. Ing LY, Zin NM, Sarwar A, Katas H. Antifungal Activity of Chitosan Nanoparticles and Correlation with Their Physical Properties. International Journal of Biomaterials. 2012;2012:1-9.
27. Gabriel JS, Gonzaga VAM, Poli AL, Schmitt CC. Photochemical synthesis of silver nanoparticles on chitosans/montmorillonite nanocomposite films and antibacterial activity. Carbohydr Polym. 2017;171:202-210.
28. Deng X, Xiong F, Li X, Xiang B, Li Z, Wu X, et al. Application of atomic force microscopy in cancer research. Journal of Nanobiotechnology. 2018;16(1).
29. Alishahi A. Chitosan: A Bioactive Polysaccharide in Marine-Based Foods. The Complex World of Polysaccharides: InTech; 2012.
30. Huang T-W, Ho Y-C, Tsai T-N, Tseng C-L, Lin C, Mi F-L. Enhancement of the permeability and activities of epigallocatechin gallate by quaternary ammonium chitosan/fucoidan nanoparticles. Carbohydr Polym. 2020;242:116312.
31. Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence. 2018;9(1):522-554.
32. Mihajilov-Krstev T, Radnović D, Kitić D, Zlatković B, Ristić M, Branković S. Chemical composition and antimicrobial activity of Satureja hortensis L. essential oil. Open Life Sciences. 2009;4(3):411-416.