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SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL ACTIVITY OF EXTRACTED CHITOSAN-BASED SILVER NANOPARTICLES

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ABSTRACT

In recent years, nanoparticles of noble metals such as silver have gained enormous attraction owing to their distinct physical, chemical, and biological properties. In the present study, silver nanoparticles (AgNPs) were synthesized from silver nitrate ($AgNO_3$) using extracted chitosan as the reducing agent. The chitosan was extracted from the exoskeleton of the marine crab *Portunus pelagicus*. Three different sample solutions were prepared using 0.02mM AgNO3 in 0.5% (w/v) chitosan, 0.04 mM AgNO3 in 0.5% (w/v) chitosan, 0.06 mM AgNO3 in 0.5% (w/v) chitosan. Preliminary characterization of the formed silver nanoparticles was made based on the measurements of ultraviolet-visible (UV-Vis) spectroscopy and scanning electron microscopy (SEM). The characteristic absorbance peak corresponding to silver nanoparticles was observed at 400nm. The average size of chitosan-based silver nanoparticles was in the range of 160-530nm. The synthesized nanoparticles were further tested for their antimicrobial activity. The synthesized chitosan-based silver nanoparticles exhibited effective antibacterial activity against Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* as well as Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and antifungal activity against *Candida albicans*, *Aspergillus flavus*, *Penicillium chrysogenum*. Among the different concentrations of silver nitrate solutions, a maximum zone of inhibition against all the microbial cultures was observed with chitosan-based silver nanoparticles produced using 0.06mM silver nitrate solution. The synthesized nanoparticles were further incorporated in Cotton Gauze to evaluate its antibacterial activity. It showed higher bactericidal activity against *Pseudomonas aeruginosa* followed by *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* and thus proved to have the potential to be used as wound dressing material.

Keywords: Chitosan, Silver nanoparticles, Antibacterial, Antifungal activity

INTRODUCTION

In recent years, research on nanoparticles has evinced keen interest because of the new opportunities they present in nanocomposites, catalysis, environmental remediation, sensing, and drug delivery **(Lu and Liu., 2007; Lee *et al.*, 2005; Daniel and Astruc, 2004; Zhang, 2003; Fan and Gao, 2006)**. Nanoparticles are tiny solid, colloidal particles that are less than 1000 nm in size **(Medina *et al.*, 2007)**. These nanoparticles embody distinctive physicochemical and biological properties compared to their conventional counterparts, which endow them with their beneficial characteristics **(Gatoo *et al.*, 2014)**. These specific characteristics mainly include size, distribution, and morphology **(Darroudi *et al.*, 2010)**. It is believed that being on the nanoscale, these nanoparticles exhibit a high surface-to-volume ratio. Thus, the small size, customized surface, improved solubility, and multi-functionality of nanoparticles continues to open many doors and have been found to create new biomedical applications **(Singh and Lillard, 2009)**.
Among the different types of nanoparticles, silver nanoparticles (AgNPs) have attracted enormous attention for the creation of novel and advanced functional materials owing to their unique optical and catalytic features as well as antibacterial properties **(Hasse *et al.*, 2012)**. Moreover, silver nanoparticles (AgNPs) have been proven to possess features and impressive potential for the development of novel antimicrobial agents, drug-delivery formulations, detection and diagnosis platforms, biomaterial and medical device coatings, tissue restoration and regeneration materials, complex healthcare condition strategies, and performance-enhanced therapeutic alternatives **(Burdusel *et al.*, 2018)**. As a consequence of their remarkable applicability, the translation of silver-based nanotechnology to clinical applications requires the development of safe, simple, eco-friendly, and cost-effective methods for the synthesis of silver nanoparticles.
Globally, seafood occupies a prodigious market in an extensive range of other food products **(Premasudha *et al.*, 2015).** There are a huge number of seafood processing industries on Rameshwaram island, situated at the southeastern end of the Indian Peninsula, Ramanathapuram District of Tamil Nadu, India. These industries process the seafood during which they generate huge waste, particularly crab shells. The disposal of this waste has become a serious environmental concern since it not only produces an obnoxious odor but also attracts pathogenic microbes, insects, and rodents and creates an unhygienic atmosphere **(Biswas and Gargi, 2013)**. Utilization of these dumped wastes in a beneficial way by producing value-added products is the quick and effective solution to address this problem and it can serve as an additional source of revenue. One such value-added product produced from such waste is chitosan.
Chitosan, the only naturally existing cationic polysaccharide on earth is a copolymer of 1,4 D-glucosamine and N-acetyl glucosamine. It is derived by deacetylation of the N-acetyl glucosamine units of chitin **(Peniche *et al.*, 2008).** The positive facets of this biopolymer such as excellent biocompatibility and tailorable biodegradability with ecological safety, low toxicity with versatile biological activities such as anti-microbial activity, and low immunogenicity have provided ample opportunities for utilization of this polymer for biomedical applications **(Cheung *et al.*, 2015)**. Furthermore, chitosan also has been used to prevent coalescence, acting as an effective stabilizer for silver nanoparticle production **(Regiel-Futyra *et al.*, 2017)**. Thereby, in the present study, the extracted chitosan from the crab shell waste was used as a polymer matrix for the synthesis of silver-based chitosan nanoparticles and also to evaluate the biomedical potential of the synthesized silver nanoparticles.

MATERIAL AND METHODS

Preparation of chitosan

Shells of *Portunus pelagicus* were collected from the southeast coast of Rameshwaram seashore. The collected shells were packed in plastic bags and stored at -20ºC until it is used for extraction. Chitin and chitosan were prepared from crab shell waste following the method of **Takiguchi (1991)**. The exoskeleton of the crab was washed with tap water followed by demineralization by adding 300ml of 2N hydrochloric acid. Excess acid was drained off and the sample was washed thoroughly with distilled water. It was then dried in a hot air oven at 60°C.

Deproteinisation was carried out by adding 300ml of 1N sodium hydroxide to the filtrate sample at 80 ° C for 24 hrs with constant stirring. After 24 hrs, excess NaOH was removed. The sample was washed with water and filtered till the wash liquid showed neutral pH. The filtrate was dried at 40°C. To obtain chitin, the sample was then deacetylated by adding 250 ml of 40% NaOH, heated under reflux for 6 hrs at 40°C with constant stirring to which 200 ml of 10%acetic acid was added and was kept for 12 hrs at room temperature with constant stirring. The dissolved sample was re-precipitated by adding 40% NaOH and the pH was adjusted to 7. The sample was then centrifuged at 10,000 rpm to obtain chitosan.

Synthesis of chitosan-based silver nanoparticles

In the typical synthesis of silver nanoparticles, 0.5 grams of the extracted chitosan was dissolved in 50 mL of 2 % acetic acid. The mixture was kept at 40°C under vigorous agitation to form a homogeneous solution. After stirring for 15 mins, an aqueous silver nitrate ($AgNO_3$) solution of three different concentrations viz 0.01 mM, 0.04 mM, and 0.06 mM was added. The solution was mixed under stirring for 6 hrs at 90 °C. The color of the solution changed from colorless to light yellow and finally to a brown color indicating the formation of silver nanoparticles (AgNPs) **(Bin Ahamed *et al.*2011)**

Characterization of synthesized silver nanoparticles

UV-Visible (UV-VIS) spectrophotometer analysis

The reduction of silver ions was confirmed by UV–Visible spectrophotometer. The absorbance was measured using UV–Visible spectrophotometer at the 300-700 nm range.

FT-IR [Fourier Transform Infrared Spectroscopy] analysis

FT-IR spectroscopy was used to compare and confirm the chemical conformation of standard chitosan with that of the synthesized chitosan-based silver nanoparticles. The infrared spectra were recorded (Perkin –Elmer STA 5000, USA) from 400cm-1 to 4000cm^{-1} with a resolution of 1 cm^{-1} at room temperature.

Scanning Electron Microscopy (SEM) analysis

The synthesized silver nanoparticles were prepared for the SEM by sputter coating them with gold. It was observed using scanning electron microscopy (SEM), specifically the JEOL 7000 JSM 6390 Japan, with a 15kV applied voltage to analyze the morphology of the synthesized silver nanoparticles.

Antimicrobial activity of the synthesized chitosan-based silver nanoparticles

All the procedures were carried out after ethical approval from the institutional ethics subcommittee.

Antibacterial activity of the synthesized chitosan-based silver nanoparticles

The antibacterial activity of the synthesized chitosan-based silver nanoparticles was evaluated by the disk diffusion method using Mueller Hinton agar. The antibacterial activity was tested against four different bacterial species namely *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus*, obtained from the Institute of Microbial Technology, Chandigarh, India. The plate was incubated at 37°C for 24 hrs and the zone of inhibition was recorded in mm using a standard ruler. Standard Ampicillin disc was used as the positive control and silver nitrate solution was used as the negative control. The experiment was done in triplicates **(Holubnycha *et al.*2018)**

Antifungal activity of the synthesized chitosan-based silver nanoparticles

The antifungal activity of the synthesized AgNPs was determined against three fungal cultures using the disk diffusion assay method. Stock cultures of *Candida albicans Aspergillus flavus, Penicillium chrysogenum*, were obtained from the Institute of Microbial Technology, Chandigarh, India, and maintained in Potato Dextrose Agar (PDA) slants at 4ºC. The fungal plates were examined for evidence of zone of Inhibition after two days of incubation at 37º C. The diameter of such zones of inhibition was measured in mm using a standard ruler. The mean value was calculated by performing the experiments in triplicates **(Erjaee *et al.*2017).**

Antibacterial activity of Cotton Gauze coated with chitosan-based silver nanoparticles

The antimicrobial activity of the synthesized AgNPs coated Cotton Gauze was tested by modifying the Kirby-Bauer diffusion technique. Briefly, A 100 µl of microbial suspension was spread onto agar plates. Plates were inoculated separately with bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis,* and *Staphylococcus aureus*. Cotton Gauze incorporated chitosan-based silver nanoparticles, prepared using 0.06mM $AgNO_3$, were placed on the agar plates and incubated at 37º C for 24-48 hrs. The zone of inhibition was measured in millimeters. Untreated sterile Cotton Gauze was used as a control.

RESULTS

Synthesis of chitosan-based silver nanoparticles

The formation of chitosan-based silver nanoparticles was preliminarily confirmed by the color change of the solution from colorless (A) to yellowish brown (B, C), or brownish-black color (D and E) **(Fig 1)**. The formation of chitosan-based silver nanoparticles was very fast and was stable in its color for a long time at room temperature.

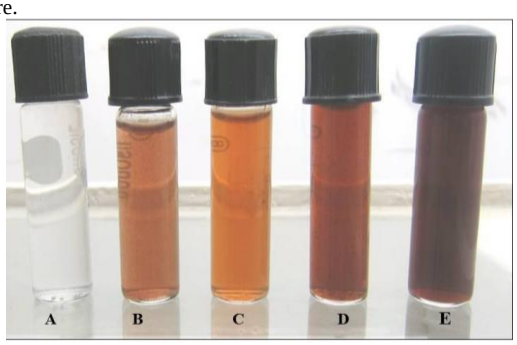

Figure 1 Tubes containing the aqueous solution of $AgNO_3$ and chitosan with colorless at the beginning of reaction (A), with color change to yellowish-brown color (B and C) and then to dark brownish-black color (D and E).

Characterization of synthesized chitosan-based silver nanoparticles

UV-Visible (UV-VIS) spectrophotometric analysis of synthesized chitosan-based silver nanoparticles

Chitosan-based silver nanoparticles were synthesized using aqueous silver nitrate solutions ($AgNO_3$) of three different concentrations viz 0.02 mM, 0.04 mM, 0.06 mM, and 0.5% of extracted chitosan sample. The synthesized chitosan-based silver nanoparticles were characterized by UV–Vis spectroscopy. The characteristic absorbance peak was observed at 400 nm for the silver nanoparticles synthesized using 0.02 mM, 0.04 mM of silver nitrate **(Fig 2A&2B)**, while a sharp absorbance peak was observed at 436 nm for the silver nanoparticles synthesized using 0.06 mM silver nitrate solution **(Fig 2C).**

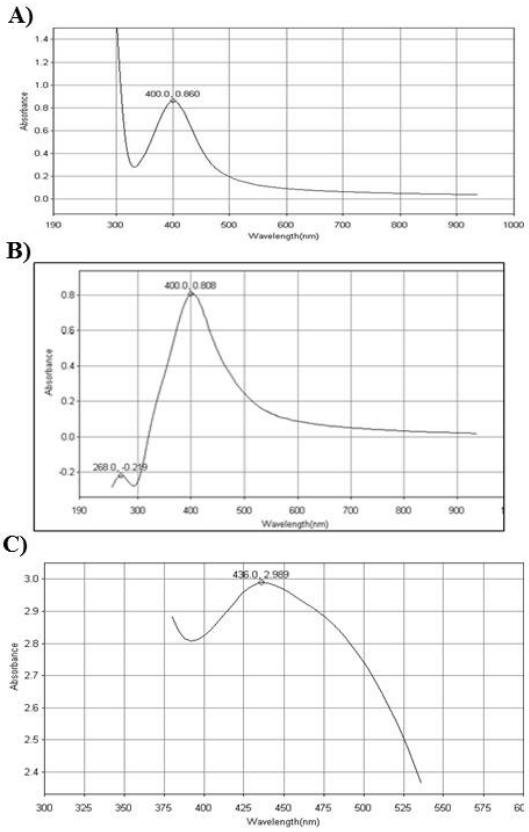

Figure 2 UV–visible range spectra of chitosan-based silver nanoparticles synthesized using **A)** 0.02 mM aqueous silver nitrate and 0.5% of extracted chitosan sample **B)** 0.04 mM aqueous silver nitrate and 0.5% of extracted chitosan sample **C)** 0.06 mM aqueous silver nitrate and 0.5% of extracted chitosan sample

FT-IR [Fourier Transform Infrared Spectroscopy] analysis

Chitosan, a marine biopolymer that is considered to be responsible for the potential reduction of silver cations as well as stabilization of synthesized silver nanoparticles was scrutinized and confirmed using Fourier-Transform Infrared Spectroscopic (FTIR) technique. The extracted chitosan exhibited the characteristic signature bands of chitosan such as 3429.55 cm^{-1} (O-H stretching overlapping the N-H stretching), 1629.90(amide II band, C-O stretching of the acetyl group), 1589.40 (amide II band, N-H stretching), and 1030.33 cm^{-1} (O bridge stretching) of the glucosamine residue. The characteristic peaks of chitosan-based silver nanoparticles as reported by **Arif *et al.*, 2015** were present at 3454.66 cm^{-1} (O-H stretching vibration), 1558.55 cm^{-1} (C=O stretch, N-H bending for primary amides), 1029.07cm^{-1} (C-N stretching band for all the amines**. (Table 1. Fig 3)**

Table 1 Band assignments of major absorptions peaks in the IR spectra of Extracted chitosan and chitosan based silver nanoparticles

Vibration mode	Extracted chitosan (cm^{-1})	Chitosan-based silver Nanoparticles (cm^{-1})
=C-H	895.00	944.1
NH2	1030.33	1029.07
C-O-C bond	1151.54	1207.49
Amide III	1323.31	1399.42
Amide II	1589.40	1558.55
Amide I	1629.90	1838.24
O-H stretching	3429.55	3454.66
C=O and OH	1419.66	1399.42

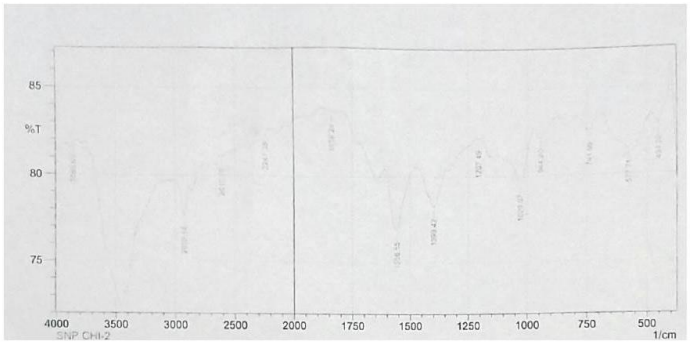

Figure 3 FT-IR spectra of synthesized chitosan based silver nanoparticles

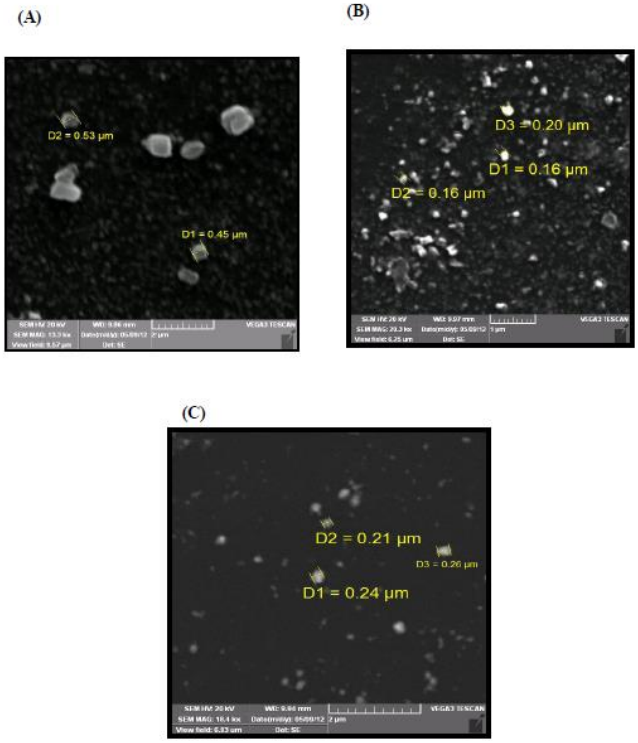

Figure 4 SEM micrograph of chitosan-based silver nanoparticle synthesized using **A)** 0.02 mM aqueous silver nitrate and 0.5% of extracted chitosan sample. **B)** 0.04 mM aqueous silver nitrate and 0.5% of extracted chitosan sample. **C)** 0.06 mM aqueous silver nitrate and 0.5% of extracted chitosan sample.

Scanning electron microscopy (SEM) analysis

SEM analysis was employed to visualize the size and shape of synthesized chitosan-based silver nanoparticles. The morphological dimensions in the SEM study demonstrated that the average size was 450- 530 nm for the silver nanoparticles synthesized using 0.02 mM concentration of silver nitrate and 0.5% of extracted chitosan sample **(Fig 4A)** while the silver nanoparticles were synthesized using 0.04 mM silver nitrate solution had an average size of 210-240 nm **(Fig 4B).** The silver nanoparticles synthesized using 0.5% of extracted chitosan sample and 0.06 mM silver nitrate solution had an average size of 160 -200 nm **(Fig 4C)**. No agglomeration was observed between the synthesized silver nanoparticles.

Antimicrobial activity

The synthesized chitosan-based silver nanoparticles were tested for their antibacterial activity against two Gram-negative bacteria *E.coli* and *Pseudomonas aeruginosa* and two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The zone of inhibition around the disks with silver nanoparticles prepared using three different concentrations 0.02 mM, 0.04 mM, 0.06 mM, and 0.5% of extracted chitosan is shown in **Fig 5a**. The synthesized silver nanoparticles showed antibacterial activity against both Gram-positive and negative bacteria. Among the different concentrations of silver nitrate solutions tested, the maximum zone of inhibition against all the bacteria was observed with chitosan-based silver nanoparticles produced using 0.06 mM silver nitrate solution followed by 0.04 mM and 0.02 mM silver nitrate **(Fig 5b)**. The highest zone of inhibition was observed for *Pseudomonas aeruginosa* in the case of Gram-negative bacteria and *Bacillus subtilis* in the case of Gram-positive bacteria even at lower concentrations.

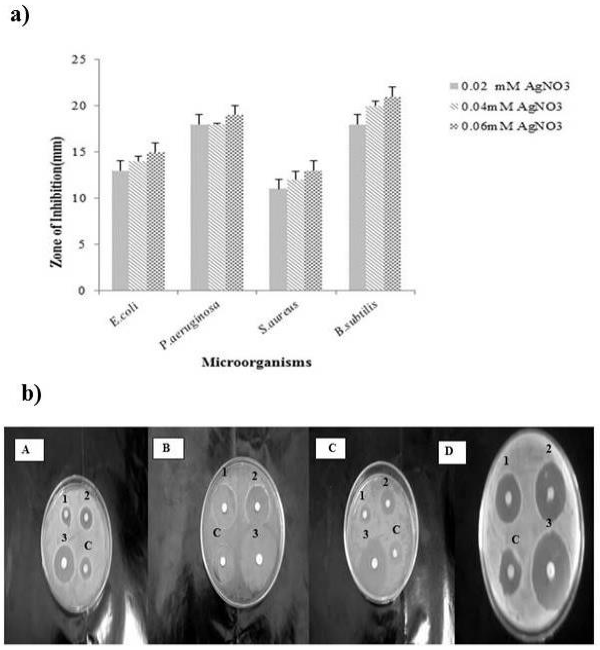

Figure 5 a) Antimicrobial activity of chitosan-based silver nanoparticles against representative microorganisms **b)** Antibacterial activity of chitosan-based silver nanoparticle-containing 1] 0.02 mM $AgNO_3$ 2] 0.04 mM $AgNO_3$ 3] 0.06 mM $AgNO_3$ against gram-negative bacteria A] *E.coli* B] *Pseudomonas aeruginosa* and gram-negative bacteria C] *Staphylococcus aureus* D] *Bacillus subtilis*

Antifungal activity

The antifungal activity of the synthesized chitosan-based nanoparticles was evaluated against three fungal cultures: *Candida albicans*, *Aspergillus flavus*, *Penicillium chrysogenum*. Among the three different organisms tested, *Candida albicans* showed higher sensitivity to chitosan-based silver nanoparticles followed by *Aspergillus flavus* and *Penicillium chrysogenum* **(Fig 6a)**. The values corresponding to the observed zone of inhibition formed while using chitosan-based silver nanoparticles prepared from 0.06mM $AgNO_3$ were found to be 15 ± 0.03 mm for *Candida albicans*,13.8±0.40 mm for *Aspergillus flavus*,11.0±0.06mm for *Penicillium chrysogenum* Among the different concentrations of silver nitrate solutions tested, maximum zone of inhibition against all the fungi was observed with chitosan-based silver nanoparticles produced using 0.06 mM silver nitrate solution followed by 0.04 mM and 0.02 mM silver nitrate **(Fig 6b)**.

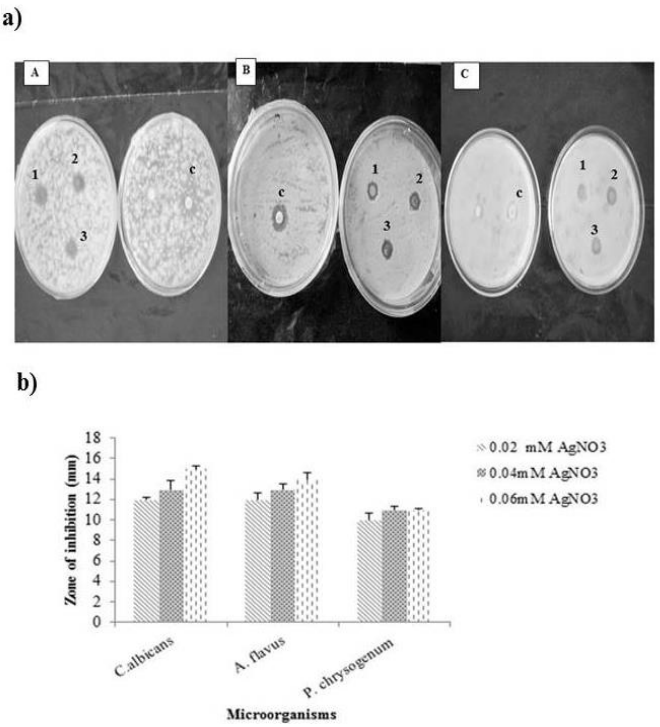

Figure 6 a) Antifungal activity of chitosan-based silver nanoparticle containing 1] 0.02mM $AgNO_3$ 2] 0.04mM $AgNO_3$ 3] 0.06mM $AgNO_3$ against A] *Candida albicans* B] *Aspergillus flavus* C] *Penicillium chrysogenum* **b)** Antifungal activity of chitosan based silver nanoparticles against representative microorganisms.

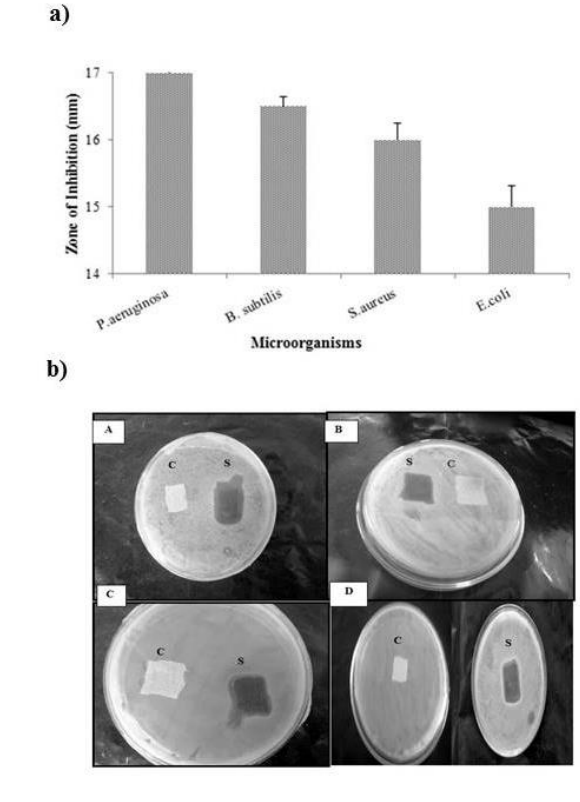

Figure 7 a) Antibacterial activity of Cotton Gauze incorporated with chitosan-based silver nanoparticles against representative Bacteria. **b)** Antibacterial activity of Cotton Gauze incorporated with chitosan-based silver nanoparticle against A] *Pseudomonas aeruginosa* B] *Bacillus subtilis* C] *Staphylococcus aureus* D] *Escherichia coli*

Antibacterial activity of Cotton Gauze coated with chitosan-based silver nanoparticles

The antimicrobial activity of Cotton Gauze coated with chitosan-based silver nanoparticles was studied against bacterial strains, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. The results revealed that the Cotton Gauze coated with chitosan-based silver nanoparticles showed antibacterial activity against all the tested bacteria while the growth of bacterial colonies was observed in the control (untreated Cotton Gauze) **(Fig 7a)**. Among all the tested strains, Cotton Gauze incorporated with chitosan-based silver nanoparticles showed greater bactericidal activity against *Pseudomonas aeruginosa* followed by *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* **(Fig 7b)**. The values corresponding to the observed zone of inhibition formed while using chitosan-based silver nanoparticles prepared from 0.06mM $AgNO_3$ were found to be 17±0.02 mm for *Pseudomonas aeruginosa*,16.5 ± 0.30 mm *Bacillus subtilis*,16.0±0.41mm for *Staphylococcus aureus*, and 15.0 ±0.52 mm for *Escherichia coli*.

DISCUSSION

In recent years, though several approaches are probed for efficient synthesis of silver nanoparticles, the chemical approach is considered to be the most appealing choice for their production wherein the synthesis of silver nanoparticles occurs through chemical reduction **(Iravani *et al.*, 2014)**. In the present investigation, chitosan, a marine biopolymer was explored as a reducing agent for the production of silver nanoparticles. Silver nanoparticles were synthesized using aqueous silver nitrate solution ($AgNO_3$) of three different concentrations viz 0.02 mM, 0.04 mM, 0.06 mM, and 0.5% of extracted chitosan sample. The formation of chitosan-based silver nanoparticles was qualitatively confirmed by the formation of a brownish-black color solution **(Fig 1)**. It is reported that the formation of brown color is due to the excitation of surface plasmon resonance (SPR) of silver nitrate and provides the signature for the formation of silver nanoparticles **(Mulvaney, 1996)**. Upon further investigation of the formed silver nanoparticles by UV-Vis spectroscopic analysis, a sharp absorption peak was observed at 400 nm for the silver nanoparticles synthesized using 0.02 mM, 0.04 mM concentration of silver nitrate **(Fig 2A,2B)**, while it was observed at 436 nm for the silver nanoparticles synthesized using 0.06mM silver nitrate solution **(Fig 2C)**. These results are in accordance with previous reports which suggest that noble metal nanoparticles exhibit a specific surface plasmon resonance (SPR) characteristic to each metal and the SPR peak corresponding to silver nanoparticles varies from 410 to 440 nm, which is in the range of Mie scattering and confirms the formation of silver nanoparticles **(Daniel *et al*. 2014)**. An increase in the intensity of the SPR peak of silver nanoparticles synthesized using 0.06 mM silver nitrate solution is attributed to the fact that the efficiency of silver nanoparticle synthesis increases with an increase in the concentration of silver nitrate, due to an enhancement in the oxidation of hydroxyl groups of chitosan by silver ions **(Venkatesham *et al*. 2014)**. The chemical conformation of the synthesized chitosan-based silver nanoparticles was confirmed by FT-IR spectroscopic analysis. The extracted chitosan exhibited the characteristic signature bands of chitosan such as 3429.55 cm^{-1} (O-H stretching overlapping the N-H stretching), 1629.90(amide II band, C-O stretching of the acetyl group), 1589.40 (amide II band, N-H stretching), and 1030.33 cm^{-1} (O bridge stretching) of the glucosamine residue. The characteristic peaks of chitosan-based silver nanoparticles as reported by **Arif *et al.*, 2015** were present at 3454.66 cm^{-1} (O-H stretching vibration), 1558.55 cm^{-1} (C=O stretch, N-H bending for primary amides), 1029.07cm^{-1} (C-N stretching band for all amines **(Fig 3)**. In context to the FTIR spectrum of chitosan-based silver nanoparticles, the shift in position and reduction in the intensity of some bands such as is C=O and N–H was observed when compared to the extracted chitosan. These results are in accordance with **Rezazadeh *et al.*, (2020)** who suggested that the reduction in the intensity of some bands such as C=O, N–H indicates effective electronic interaction between silver ions with nitrogen and oxygen-containing biomolecules in the chitosan and further helps in the nucleation process and aggregation of metal nanoparticle during the synthesis process. Furthermore, the FTIR spectrum of chitosan-based silver nanoparticles indicates a few alterations when compared to chitosan, a new shoulder peak of the amide B band appeared at 2926 cm^{-1} which is due to the interaction of primary amino and amide groups between chitosan and silver nanoparticles **(Dara *et al*., 2020).**

The morphology of the synthesized nanoparticles was done by SEM analysis. The average size of chitosan-based silver nanoparticles is found to be in the range of 160-530 nm **(Fig 4A, 4B, 4C)**. No agglomeration is observed in the synthesized silver nanoparticles. A possible reason is the presence of polymer chitosan **(Futyra *et al.*, 2017)**. It is reported that the potential migration and agglomeration of nanoparticles are prevented because of the dispersion of silver ions in chitosan **(Govindan *et al*., 2012)**. The antibacterial activity of the synthesized chitosan-based silver nanoparticle was tested against Gram-positive and Gram-negative bacteria **(Fig 5a)**. In the case of Gram-negative bacteria, the silver nanoparticles bring about antibacterial activity by binding to their cell membranes and increasing permeability due to structural changes that result in cell lysis **(Salomoni *et al.*, 2017)**. Moreover, it is demonstrated that the electrostatic attraction between bacterial cells (negative) and silver nanoparticles (positive) increases the susceptibility of these bacteria to silver ions released by chitosan-based silver nanoparticles **(Yun an Qing *et al*., 2018)**. While in Gram-positive bacteria silver nanoparticles adversely affect the cell morphology and DNA integrity **(Hsueh *et al*., 2015)**. Among the different concentrations of silver nitrate solutions tested, a maximum zone of inhibition against all the bacteria was observed with chitosan-

based silver nanoparticles produced using 0.06 mM silver nitrate solution **(Figure 5b)**. This is ascertained by the fact that the nanoparticle was of smaller size owing to which they have a large contact area, thus providing better contact with the microorganisms by binding to the cell membrane and also by penetrating inside the cells **(Wang *et al*., 2017)**. Thus smaller sized nanoparticles display greater antibacterial activity. Moreover, chitosan exhibit significant antibacterial activity against a broad spectrum of bacteria **(Goy *et al*., 2009)**. Several reports suggest that various combinations of chitosan and silver show improved antimicrobial properties **(Huang *et al*., 2011)**.

The antifungal activity of the synthesized chitosan-based nanoparticles was evaluated against three fungal organisms, *Candida albicans, Aspergillus flavus, and Penicillium chrysogenum* **(Fig 6a)**. Among the three different organisms tested, *Candida albicans* showed high sensitivity to chitosan-based silver nanoparticles followed by *Aspergillus flavus* and *Penicillium chrysogenum* **(Fig 6b)**. The proposed inhibition mechanism of chitosan-based silver nanoparticles against *C. Albicans* was due to the diffusion of nanoparticles into the fungal cells, followed by inhibition of DNA or RNA synthesis, subsequently causing direct cell death**.** Furthermore, it is also reported that DNA loses its ability to duplicate when the fungal culture is treated with Ag^{+}, which leads to a deactivated expression of ribosomal subunit proteins and the synthesis of disabled enzymes and cellular proteins, important for the adenosine triphosphate production. It is also proposed that the silver ions mostly affect the function of the membrane-bound enzymes such as those in the respiratory chain (**Bragg and Rainnie, 1974**; **McDonnell and Russell**, **1999; Uchida *et al*., 2003)**. Moreover, the higher sensitivity of *C. albicans* to chitosan-based silver nanoparticles is attributed to the fact that *C. Albicans* is more susceptible to being inhibited by chitosan-based nanoparticles when compared with other types of fungi due to the presence of anionic-charged sialic acid in the cell wall constituent. Nevertheless, the polycationic polymer chitosan strongly binds and permeates the cell wall of the *C. Albicans* yeast by reducing the negative surface charge of the cell wall, this permeabilization enables silver nanoparticles to enter the yeast in excess **(Pena *et al*., 2013; Dai *et al*., 2001)**. This leads to loss of cellular content thereby causing cell death. Fungi that have chitin as one of the components in the cell wall are more resistant to externally amended chitosan. This fact, therefore, explains The high inhibition of *C. albicans* by Chitosan-based silver nanoparticles when compared to other fungi cultures used in the study**(Yien *et al*., 2012).**

In this study, conventional cotton gauze was incorporated with chitosan-based silver nanoparticles to benefit from the supreme properties of both silver ions and chitosan that will improve its wound care ability when used as wound dressings. Among all the tested strains, Cotton Gauze incorporated with chitosan-bas silver nanoparticles showed higher bactericidal activity against *Pseudomonas aeruginosa* followed by *Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli* **(Fig 7a,7b).** These results are in harmony with previous reports which advertise that the silver-impregnated gauzes demonstrate a high reduction in the viability and proliferation of bacteria adhered to the surface of the Gauze thus providing the bacteriostatic and bactericidal effect of the silver coating on the Cotton Gauze **(Paladini *et al*., 2016)**. Among the tested microbes, *Pseudomonas aeruginosa* is inhibited more by chitosan-based silver nanoparticles than the other three microbes. This is a significant outcome of the study considering the threat posed by *P. Aeruginosa* as an opportunistic pathogen that easily infects patients with wound infection and is a predominant colonized bacterium in implanted medical devices, such as a catheter. Furthermore, infection and spread of this multi-drug-resistant microbe have made them the "culprits" for high morbidity and mortality of patients owing to hospital infections **(Chen *et al.*, 2018). Liao *et al.*, (2019)** proposed that the higher inhibitory effect of silver nanoparticles on *P. aeruginosa* is accounted for by the fact that upon contact with the silver nanoparticles the cell membrane of the microbe becomes shriveled and fractured; and the cell constituents leaked out, owing to which, the REDOX homeostasis was thrown off and the oxidative stress response is promoted in the bacteria. Silver nanoparticles inhibit the activity of CAT and POD, and hence the excessive ROS is not timely eliminated, which results in impaired DNA and ribosome and declined synthesis of the macromolecules. Moreover, chitosan also possesses superior antibacterial activity against Gram-negative *P. aeruginosa* **(Madhi *et al.*, 2020)** thereby the synergistic events have worked together toward bacterial death.

CONCLUSION

In this study, chitosan, a cationic polysaccharide extracted from the exoskeleton of blue swimming crab *Portunus pelagicus* was used in the preparation of silver nanoparticles. The synthesized chitosan-based silver nanoparticle was qualitatively confirmed by the formation of brown color followed by UV-Vis spectroscopic analysis wherein a sharp absorption peak was observed around 400 nm for the synthesized silver nanoparticle. The morphology of silver nanoparticles was determined by SEM analysis and it was found that there was no visible agglomeration and the average size of chitosan-based silver nanoparticles was observed to be in the range 160-530 nm. Furthermore, the synthesized nanoparticles exhibited significant antibacterial and antifungal activity. The bactericidal properties of cotton gauze were greatly improved by the incorporation of chitosan-based silver nanoparticles. Thus these results provide baseline information on how chitosan-based silver nanoparticles can be used for biomedical applications.

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