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Comparative Antimicrobial Analysis of Chitosan Nanoparticles With Gentamicin And Chloramphenicol

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Abstract

Chitosan has attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption and ability to form films and to chelate metal ions. Antibiotics resistance has become rampant and nanoparticles are increasingly used to target microorganisms as an alternative to antibiotics. The research was conducted to produce and synthesize nanoparticles using the extract of chitosan and test the antimicrobial activity against some selected bacteria. Chitosan was produced by demineralization, deproteinization and deacetylation of oyster shell. The silver nanoparticle was synthesized from Chitosan and screened for in vitro antimicrobial activity against selected bacteria using agar well diffusion method. Synthesized nanoparticles were characterized using UV-VIS spectrophotometry, FTIR spectroscopy and Scanning Electron Microscopy. Chitosan nanoparticles showed some inhibiting properties against the growth of most of the organisms tested (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*). Most of the isolates were inhibited by both extracts. The positive control which was chloramphenicol and gentamycin inhibited the growth of all organisms except *Salmonella typhi*.

Keywords: Chitosan, Silver nanoparticles, Agar well-diffusion, SEM, FTIR

Introduction

Metallic nanoparticles have been widely studied in recent years because of their potential use as catalysts (Chimentao *et al.*, 2004; Gong and Mullins 2009; Campelo *et al*. 2009; Li *et al.* 2010). Natural polymers have also been used in the preparation of nanosilver because they are nontoxic and biocompatible. Starch (Hu *et al*., 2008) and chitosan (Hettiarachchi & Wickramarachchi 2011) have been used as stabilizers for the preparation of metal nanoparticles.

Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae (Tolamite *et al*., 2000). Generally, the shell of selected crustacean consists of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin (Knorr, 1984).

Chitosan is a non-toxic, biodegradable polymer of high molecular weight and is very much similar to cellulose, a plant fiber. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility,

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biodegradability, adsorption, and ability to form films, and to chelate metal ions (Rout, 2001).

Many of the nanoparticle synthesis methods, however, involve use of hazardous chemicals, low material conversions and high energy requirements (Venkatesham *et al.*, 2014). Due to the problem encountered, most people abuse antibiotics owing to the general belief that antibiotics can be used in the treatment of all kinds of diseases which result into antibiotics resistance. Chitosan nanoparticles may exhibit potential antibacterial activity.

Chitosan is among the most commonly used natural polymers in nanomedicine and has been proven to be very effective in nanoparticle form. Over past decade, keen interest has been evinced in green synthesis. Green synthesis is cost effective, environment friendly, easily scaled up for largescale synthesis and also there is no need to use toxic chemicals (Venkatesham *et al.*, 2014). Therefore, this study is carried out to investigate the potential of chitosan nanoparticle in inhibiting growth of some bacteria. Hence this study is to evaluate the in-vitro antibacterial activity of chitosan nanoparticles against some microorganisms and also compare the effectiveness of the nanoparticle produced with some reference antibiotics.

Materials and Method

Sample Collection

The shrimp used was obtained from the beach shores, washed, air-dried and crushed with mortar and pestle and stored in a dried container till further use. Pure culture of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella spp.* and *Escherichia coli* were obtained from the Microbiology Laboratory of the Department of Biological Sciences, Fountain University Osogbo.

Demineralization of the Shrimp Shell

The grinded shell was treated with 7% HCl solution at ambient temperature with a solution to solid ratio of 10ml/g. The resulting solid fraction was washed with distilled water until neutral pH was achieved. The de-mineralized samples were dried.

Deproteinization of the Shrimp Shell

Deproteinization of chitin was carried out using 10% NaOH at 60^0C. The treatment was repeated several times. The absence of proteins was indicated by the absence of color of the medium at the last treatment, which was left overnight. Then the resulting solution was washed with water to neutrality and with hot ethanol (10ml/g). Then the purified chitin was dried at 50^0C with constant weight. The chitin content was determined from the weight differences of the raw materials and that of the chitin obtained after acid and alkaline treatment.

Deacetylation of Chitin

The chitin (10g) was put into 50% NaOH at 60^0C for 8h to prepare crude chitosan. After filtration, the residue was washed with hot distilled water at 60^0C for three times. The crude chitosan was obtained by drying in an air oven at 50^0C overnight.

Synthesis of Chitosan Nanoparticles

Chitosan powder (5g) was weighed into 50ml of distilled water and was heated in a water bath for 10minutes and filtered. Then 1ml of the filtrate was added to 40ml of silver nitrate and was put under the sun and the colour change was observed. The sample was then characterized using UV-Visible spectrophotometer and Fourier Transform Infared (FTIR).

Antibacterial Activity of Chitosan Nanoparticles

Antibacterial activity of chitosan nanoparticles was carried out by agar well diffusion method. Isolates of *S. aureus*, *P. aeruginosa*, *Salmonella spp* and *E. coli* were subcultured on nutrient agar plate and incubated for at 37^0C for 24 hours. Gentamycin and Chloramphenicol were used as positive control. The Isolates used were compared with 0.5 McFarland standard and swabbed onto sterile Muller-Hinton agar plates using sterile cotton swab. Different holes were bore using a sterilized cork borer and 100µl, 50µl, and 25µl concentrations of chitosan nanoparticles were added into each well. The plates were incubated at 37ºC for 24 hours and the inhibitory concentrations were measured.

Results

Synthesis and Characterization

A change in colour was observed during the addition of the extract to silver nitrate from light brown to deep brown due to reduction of silver

ions. Figure 1 shows the chitosan extract and the synthesized silver nanoparticles.

UV-VIS Spectra Analysis

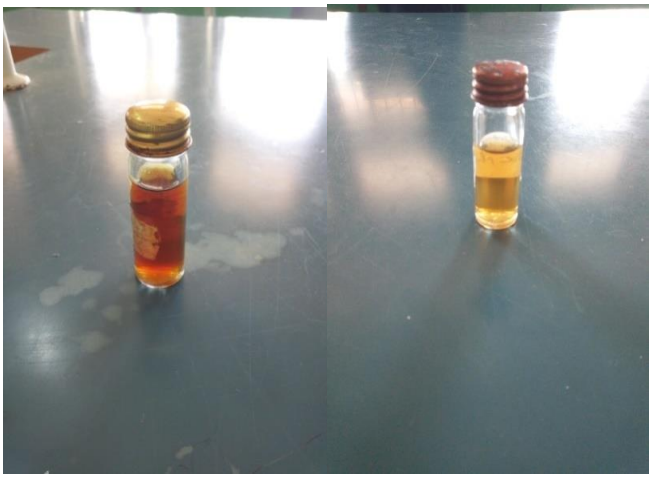
UV-VIS spectroscopy is an important technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. It is also used to check for its maximum absorbance and wavelength. The maximum peak for the plant extract was found to be at 222nm, and the peak for synthesized chitosan nanoparticle was at 414nm. (figure 2 and 3).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR measurement was carried out to identify the possible biomolecules in chitosan extract responsible for capping leading to efficient stabilization of the silver nanoparticles and to determine the chemical functional groups in the samples. The Infrared spectroscopy spectrum of chitosan extract and chitosan nanoparticles manifests prominent adsorption band located at 1645 and 1726 respectively which may be as a result of C=O stretching vibrations. The strong band for the extract is at 3458cm^{-1} while for the nanoparticles is at 2938 respectively and may result from N-H stretching vibration which is similar to the work of Venkatesham *et al.* (2014). These are derived from water soluble compounds such as flavonoids, alkaloids and polyphenols present in leaves. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles (Ganesh *et al*., 2009).

Scanning Electron Microscopic Analysis of the Silver Nanoparticles (SEM)

The silver nanoparticles were further characterized by scanning electron microscope. SEM is a useful tool for studying the size, shape and morphology of silver nanoparticles. Fig 4.8 shows the SEM micrograph of the biosynthesized chitosan nanoparticles by Chitosan Extract of Oyster shell. Chitosan silver nanoparticles was porous spherical in shape and with the size of 20nm.



A. Synthesized silver nanoparticles B. Chitosan extract

Figure 1: Chitosan extract, synthesized silver nanoparticles

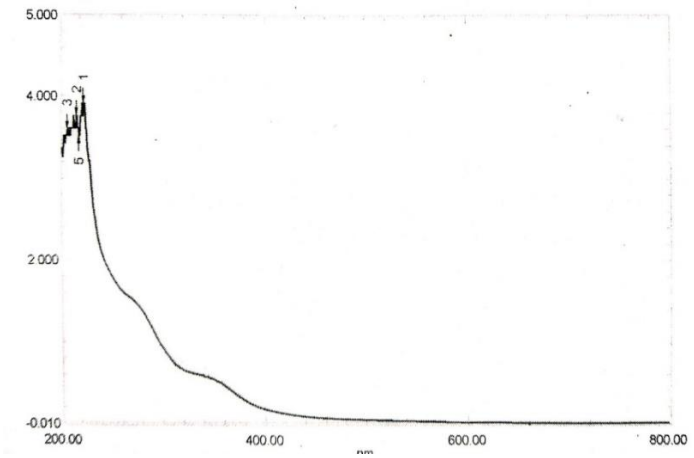


Figure 2: UV-VIS Spectroscopy of Chitosan Extract

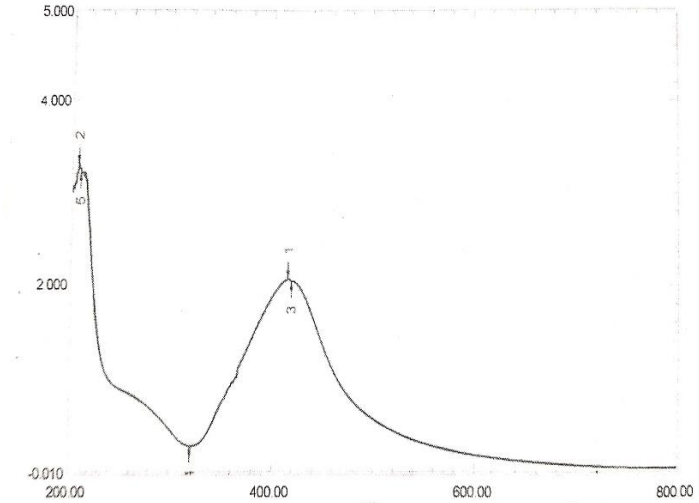


Figure 3: UV-VIS Spectroscopy of Chitosan Nanoparticles

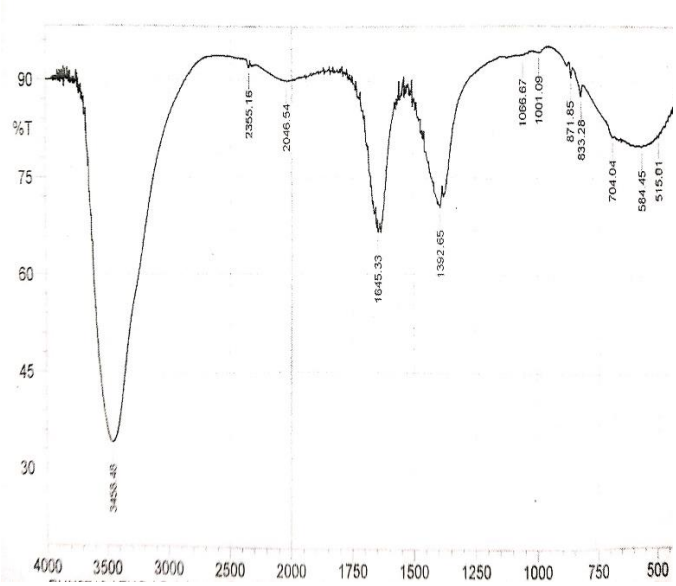


Figure 4: FTIR Analysis of Chitosan Extract

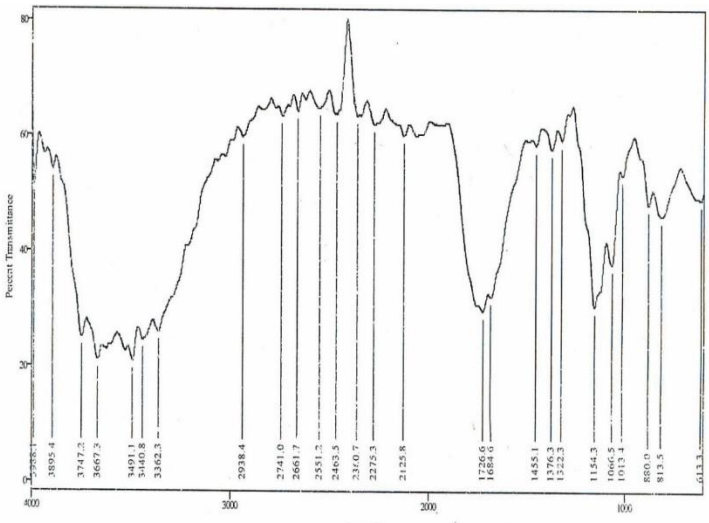


Figure 5: FTIR Analysis Of Synthesized Chitosan Nanoparticles

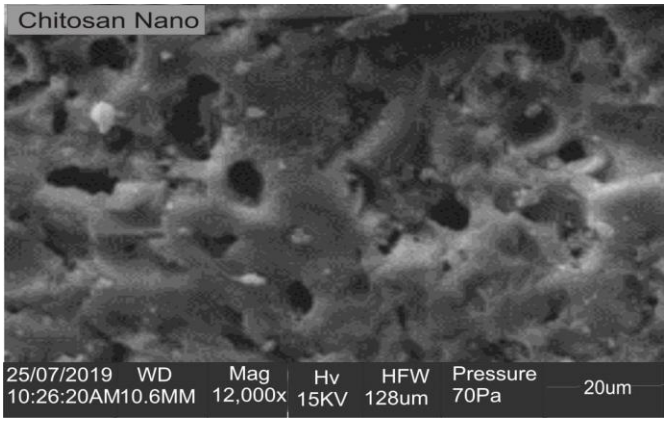


Figure 6: Scanning Electron Microscopic Image of Chitosan Nanoparticles Synthesized Using Chitosan Extracts

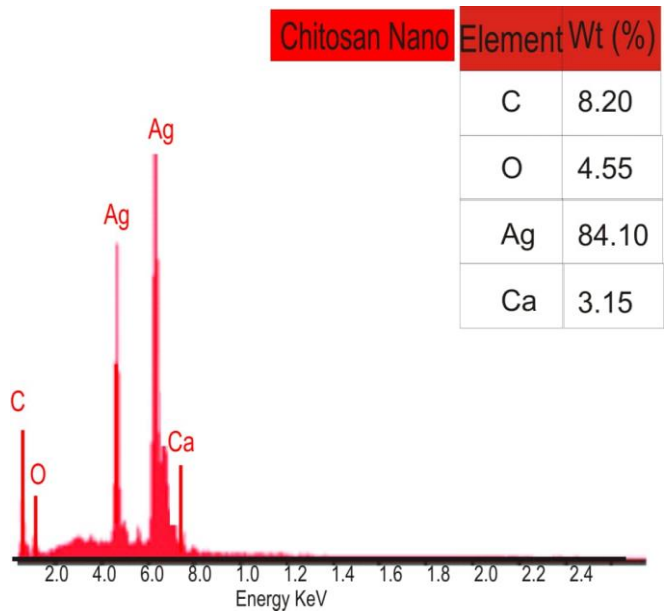


Figure 7: Energy Dispersive X-Ray Signal

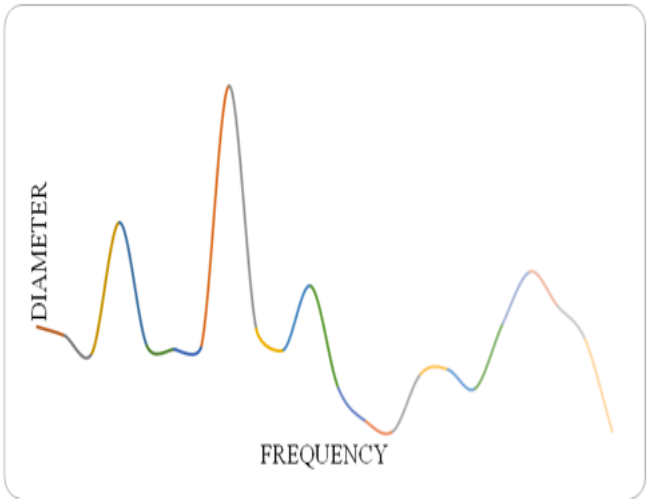


Figure 8: Scanning Electron Micrograph of Chitosan Nanoparticles using Image J.

Antimicrobial Activity

Chitosan nanoparticles showed some inhibiting properties against the growth of most of the organisms tested (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*). Most of the isolates were resistant to aqueous extract. The positive control which was chloramphenicol and gentamycin inhibited the growth of all the organisms. The results are represented in table 1 and the images are shown in figures 9 – 12.

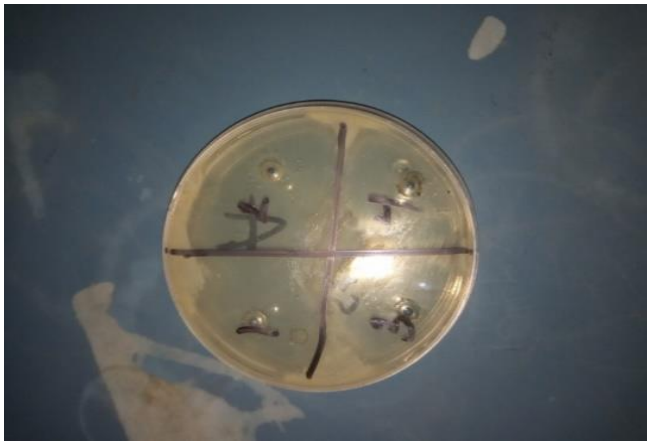


Figure 9: Antimicrobial activity of extract, nanoparticle and antibiotics on *Staphylococcus aureus*

Note: 1-Gentamicin, 2-Chitosan nanoparticles, 3-Chitosan extract, 4-Chloramphenicol

Figure 4.10: Antimicrobial activity of extract, nanoparticle and antibiotics on *Pseudomonas Aeruginosa*

Note: 1-Gentamicin, 2-Chitosan nanoparticles, 3-Chitosan extract, 4-Chloramphenicol

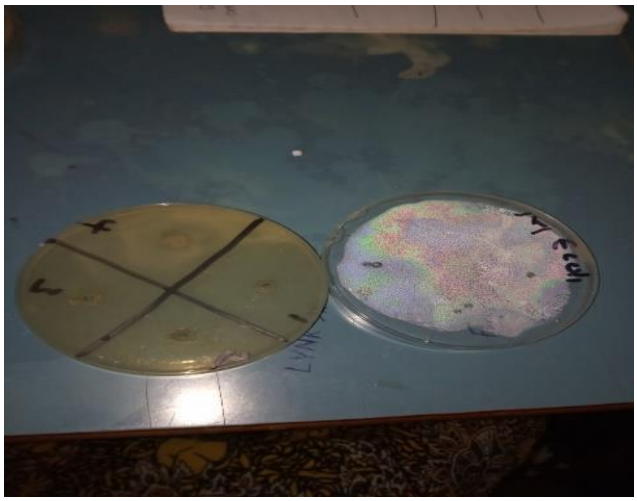


Figure 4.11: Antimicrobial activity of extract, nanoparticle and antibiotics on *Escherichia coli*

Note: 1-Gentamicin, 2-Chitosan nanoparticles, 3-Chitosan extract, 4-Chloramphenicol

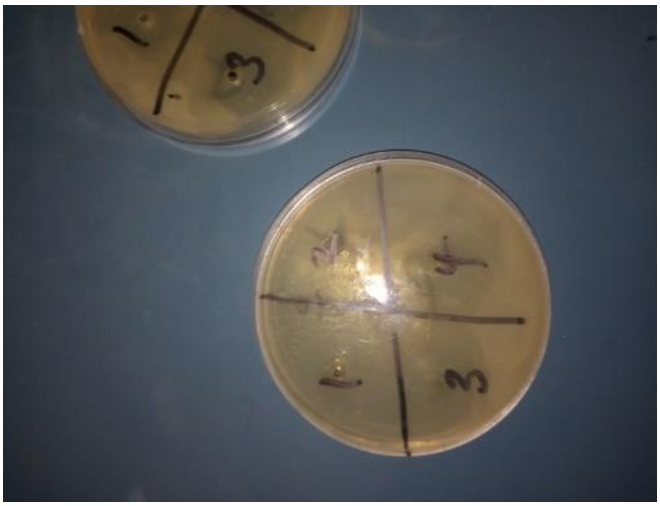


Figure 4.12: Antimicrobial activity of extract, nanoparticle and antibiotics on *Salmonella typhi*

Note: 1-Gentamicin, 2-Chitosan nanoparticles, 3-Chitosan extract, 4-Chloramphenicol

Table 1: Antimicrobial Activity of Extracts on Bacteria

Organisms	Chloramphenicol (positive control) mm	Gentamycin (positive control) mm	Chitosan Extract (0.1 g/ml) mm	Chitosan Nanoparticles (0.1 g/ml) mm
Staphylococcus aureus	20.00	16.00	24.00	16.00
Escherichia coli	22.00	23.00	25.00	NZ
Pseudomonas aeruginosa	30.00	20.00	15.00	25.00
Salmonella thyphi	NZ	NZ	NZ	26.00

Note: NZ= No zone of inhibition

Discussion

Among the treatments used in the study 3% HCI and 4% NaOH found to be used successfully to extract chitin. Although 60% NaOH treatment yields highest deactivated chitosan with maximum solubility, 50% NaOH treatment could be used to get high quality chitosan of 79.57% degree of deacetylation and 97.02% solubility with minimum chemical utilization.

The colour changed from light brown to dark brown which indicated that there was reduction of silver particles by the microwaves and also indicated the formation of the nanoparticle. The time required for the conventional synthesis of chitosan nanoparticles using chitosan extracts ranged from several minutes to few hours and thus are rather slow. This observation is supported by Nadagouda *et al*. (2011).

The absorption spectrum was observed under the UV-VIS spectroscopy to ascertain the formation and stability of metal nanoparticles in aqueous solution. It is also used to check for its maximum absorbance and wavelength. The maximum peak for the plant extract was found to be at 222nm, and the peak for synthesized chitosan nanoparticle was at 414nm. Honary *et al*. (2011) reported similar results with peaks in the range of 400-420 nm which is typical of surface Plasmon band indicating formation of Silver nanocomposites with Chitosan.

The FTIR spectroscopic studies were carried out to study the plausible mechanism behind the formation of these chitosan nanoparticles and provide information regarding the functional groups present on the surface of the nanoparticles.

The Infrared spectroscopy spectrum of chitosan extract and chitosan nanoparticles manifests prominent adsorption band located at1645 and 1726 respectively which may be as a result of C=O stretching vibrations. The strong band for the extract is at 3458cm^{-1} while for the nanoparticles is at 2938 and may result from N-H stretching vibration. These are derived from water soluble compounds such as flavonoids, alkaloids and polyphenols present in leaves. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles.

The SEM image of the chitosan nanoparticle showed a particle size ranging between 10-50nm and the EDX data confirmed the elemental composition of the nanoparticle. The morphology of the chitosan nanoparticle pictured by SEM appeared to be spherical which is in line with the study of Vaezifar *et al*. (2013).

In this study, chitosan nanoparticles showed a higher antimicrobial activity against *Salmonella typhi* which did showed no zone of inhibition when compared with other antibiotics and chitosan extract. The Chitosan nanoparticle was more effective than Gentamycin against *Pseudomonas aeruginosa,* and it showed the same zone of inhibition with *Staphylococcus aureus.*

Conclusion

Chitin and chitosan extracted from shrimp waste by chemical methods have been characterized in this investigation. Among the treatments used in the study 3% HCI and 4% NaOH found to be used successfully to extract chitin. Although 60% NaOH treatment yields highest deacetylated chitosan with maximum solubility, 50% NaOH treatment could be used to get high quality chitosan of 79.57% degree of deacetylation and 97.02% solubility with minimum chemical utilization. Synthesized Chitosan nanoparticle showed some inhibitory activities against two out of the three Gram negative bacteria used in this study, indicating that chitosan nanoparticles is a good candidate for eco-friendly antibacterial production.

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