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Green Synthesis of Silver Nanoparticles, Characterization & Therapeutic Applications using Chitosan Derived from Shrimp Waste

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Abstract: Shrimp compiled a major portion of the processed seafood industry that its exoskeleton (shells) and cephathoraxes consist of about 30 - 40% of raw shrimp weight and is discarded as waste. In this present study, we have collected shrimp waste from markets around Chennai. Decalcification, deproteination, decolourization and deacetylation were carried out to produce chitosan. Chitosan silver nanoparticle were prepared by green synthesis protocol. The synthesized nanoparticles were characterized by UV-Visible spectroscopy, FTIR, Scanning Electron Microscopy (SEM). The result was compared with commercial chitosan and a significant degree of similarities were observed. Size range of chitosan silver nanoparticles were determined by SEM as 10nm-12nm. Antibacterial activity of chitosan and chitosan combined silver nanoparticles as well as commercial antibiotics were analysed. MIC of ciprofloxacin was 40mg/ml whereas, chitosan &ciprofloxacin combination and chitosan silver nanoparticles & ciprofloxacin combinations were 10mg/ml against Shigella sonnei .MIC of Tetracycline was 30mg/ml whereas, chitosan combined tetracycline as well as chitosan silver nanoparticles combined tetracycline was 10mg/ml against Pseudomonas aeruginosa. These findings could also prove to be a promising alternative in the treatment of patients for whom existing antimicrobial treatment fails. Synergistic effect of chitosan and tetracycline & Chitosan ciprofloxacin combinations had showed significant improve in the antibacterial activity.

Keywords: Chitosan, SEM, Silver Nanoparticles, Drug Resistance, Pseudomonas Aeruginosa, FT-IR.

I. INTRODUCTION

Chitosan is a promising candidate for encapsulating and delivering doxycycline or other drugs directly to an infection site. This naturally occurring cationic polysaccharide possesses muco-adhesive properties that enable its transport across the mucosal membrane. In addition, it slowly degrades to nontoxic amino sugars that can be completely absorbed by the body. These properties – biocompatibility and biodegradability – are highly desirable for encapsulation materials. Particle size and co-occurring substances are other important considerations. Encapsulation of doxycycline into chitosan microspheres has been previously accomplished using a water-in-oil emulsion technique, but this approach can introduce oil and other harsh chemicals to the body. Chitosan particles prepared by an alternative method may offer a more benign delivery vehicle. Chitosan can also be used to form nanoparticles, which have a higher cellular uptake than microparticles, thus allowing for greater intracellular delivery of the encapsulated (drug) molecule. Pseudomonas aeruginosa(P. aeruginosa) is a highly drug resistant and opportunistic pathogen. Due to the permeability barrier in the outer membrane it is naturally resistant to many antibiotics. Infections caused by P. aeruginosaare increasing both in hospitals and in general community and it has been reported as one of the principal causes of nosocomial pathogen, particularly among immuno-compromised patients (Lee et al., 2007).

Concurrently, the extensive use of antimicrobial agents and the evolutionary antimicrobial resistance strategies of bacteria have resulted in the emergence of pan-drug resistant bacteria. The efficacy of many antibiotics for treatment of infections has become quite limited due to the development of resistance and the threat from antimicrobial-resistant organisms is accumulating and accelerating.. Also, the development of resistance to monotherapy is a common problem and dual antimicrobial coverage is often a necessity in Pseudomonas infections. Attempts have been made to deal with this problem by using combination therapy. Several studies have reported on the interaction of antimicrobial combinations with multi-resistant planktonic strains of P. aeruginosa. It is also reported that complete eradication of the bacterial cells of Pseudomonas aeruginosa in biofilms is a therapeutic challenge and often results in recurrent and chronic infections. Recently, Černohorska demonstrated the in vitro effect of 8 antibiotic combinations in P. aeruginosa biofilms, using biofilm susceptibility testing. Also, Vancomycin in combination with cephalosporins and penicillins has been shown to synergistically inhibit a number of gram-negative bacilli. However, the threat from antimicrobial-resistant organisms is accumulating and accelerating. With the dearth of new antibiotics coming to the marketplace and the advance of MDR bacteria it is not difficult to see untreatable life-threatening bacterial infection becoming common.

Moreover, it is difficult to identify strategies to prevent or delay the emergence of resistance. Recently, Amyes et al., 2007, discussed on the principles for antibiotic usage to limit resistance development . Thus, there is the need to find new

ways to control P. aeruginosa and embark on the need for a continued search for new antimicrobial compounds. The main aim of this present study is to detect the therapeutic potentials of chitosan derived from marine resource, shrimp shell waste against clinical isolates and the objectives were to collect shrimp shell solid waste from market places around Chennai, to Produce chitosan from shrimp waste, to produce chitosan silver nanoparticles by Green synthesis protocol, to Characterize chitosan silver nanoparticles by UV-Vis spectrometry, FTIR & SEM analysis, to determine antibacterial activity, MIC of chitin, chitosan and chitosan silver nanoparticles against Pseudomonas aeruginosa and Shigella sonnei, to find out synergistic effects of chitosan with commercial antibiotics and to find out the morphological changes of selected bacteria treated with chitosan based silver nanoparticles and tetracycline by SEM analysis.

II. MATERIALS & METHODS

A. Sample Collection& Preparation

Shrimp shells were collected from market places around Chennai. The collected shrimp shells were washed with distilled water, and then the samples were sun light dried for 24 hours and further dried on the furnace at a temperature of 80° C for 24hrs. The dried samples were blended and sieved to 80 meshes. Approximately 20g of shell powders were used for further analysis.

B. Extraction of Chitin& Chitosan From Shrimp Waste (G.A. Sewvandi And S.U. Adikary, 2012)

- **Phase Deproteination:** A total of 20g samples of shrimp shell powders were added to3.5% sodium hydroxide as much as1:5(w/v). The extract was stirred over heat and left for 1 hour at 90°C.The solution was filtered and the residue was washed with tap water until neutral pH Thereafter, the residue was re-dried in a furnace at a temperature of 60º C for 4 hours and resulted is chitin powder.
- **Stage of Demineralization:** Chitin powder resulted from deproteination step was then added with 2-N HCL in the ratio of 1:5(w/v)and allowed to stand for 1hour at 90° C to separate the residue from the filtrate and the residue was washed with distilled water until neutral pH. Then dried in a furnace at 60° C for 4 hours.
- **Stage of Deacetylation:** The demineralization of chitin was carried out by adding acetone 1:5 (w/v) for 4 hours in soxhlet apparatus and then the residue was washed with distilled water until neutral pH and dried in a furnace at 60 °C 4 hours.

C. Conversion of Chitin into Chitosan

- **Deacetylation of Chitin into Chitosan:** A total of 5g of chitin were reacted with 50ml of 50% sodium hydroxide, then heated using a hot plate at 80°C for 40 minutes, then filtered and the residue was washed until neutral pH and then dried in a furnace at 60^{O} C for 4 hours.
- **Preparation of Silver Nanoparticles (Krishna Rao et al.,2012):** Chitosan (0.5 %) solution was prepared by dissolving chitosan (0.5 g) in acetic acid (100 ml, 2 %) solution and also silver nitrate (0.5 g) in deionized water (100 ml).Chitosan (5 ml, 0.5 %) solution was mixed with silver nitrate (5 ml, 0.5 %) solution in a boiling tube. This mixture was kept in autoclave at 15 psi pressure, at 120ºC for 30 minutes. The resulting solution was clear yellow in colour indicating the formation of silver nanoparticles.
- **Characterization of chitosan Silver Nanoparticles By Uv-Visible Spectro photometry:** Chitosan silver nanoparticles synthesised in the previous step was characterized and the absorption was determined in the range of 410-430nm.The production of nanoparticles from silver nitrate (0.5 %) was monitored with varying concentrations of chitosan (0.1–0.5 %) for 50 min of reaction time and their respective spectra were recorded.
- **Characterization of the Shrimp Shell Chitosan Fitr (Mohammad, 2007):** For FTIR measurements, the Ag nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a JASCO FT/IR-5300 model in (IIT-Chennai) the diffuse reflectance mode operating at a resolution of 4 cm−1.

D. SEM Analysis

Chitosan silver nanoparticles were examined under SEM and their particle size as well as shape were determined using the facility from Anna University, Chennai. Scanning electron microscopy (SEM) specimens of the composites were prepared by casting 5 µl of a water dispersion of the Ag NP/Ch composite, followed by drying at room temperature. Osmium plasma coating was conducted to enhance the conductivity of the specimens. Dried samples were coated using a plasma multi-coater PMC-5000 (Meiwafosis Co., Ltd., Tokyo, Japan). SEM observation was performed using a JSM-6340F (JEOL, Tokyo, Japan) at 5 kV.

E. Anti Bacterial Activity of Chitosan And Chitosan Nanoparticle

Muller Hinton Agar was prepared and 0.5% of chitosan and chitosan silver nanoparticles were prepared. Wells were cut and spread plate method was performed for the clinical isolates Pseudomonas auerginosa and Shigella sonnei. 50 µl of chitosan, chitosan nanoparticles & glacial acetic acid were added on the respective wells. Plates were incubated at 37ºC for 24 hours. Zone of inhibition was measured after the incubation period.

F. Antibacterial Activity of Chitosan and Chitosan Nanoparticles with Tetracyclin And Ciprofloxacin

Commercially available antibiotics tetracycline and ciprofloxacin were procured from the pharmacy. Three formulations were prepared with the stock concentration of 100mg/ml (Formulation I: Tetracycline, Formulation: II- Tetracycline and Chitosan & Formulation: III- Tetracyclineand Chitosan silver nanoparticles) to treat against Pseudomonas auerginosa. .Three more formulations were prepared with the stock concentration of 100mg/ml (Formulation I: ciprofloxacin, Formulation: II- ciprofloxacin and Chitosan & Formulation: III- ciprofloxacin and Chitosan silver nanoparticles)to treat against Shigella sonnei. Muller Hinton Agar was prepared and wells were cut and spread plate method was performed for the clinical isolates Pseudomonas auerginosa and Shigella sonnei. 50 µl of each formulations were added on the respective wells. Plates were incubated at 37ºC for 24 hours. Zone of inhibition was measured after the incubation period.

G. Determination of MIC

The MICs for the chitosan, chitosan silver nanoparticle and chitosan silver nanoparticle with antibiotics were determined .Five ml of sterile Muller Hinton broth was taken in two sets of six test tubes for each clinical isolates. First set was labelled as chitosan, second as chitosan silver nanoparticles and third as chitosan silver nanoparticle antibiotics composite. Various concentrations such as 10mg, 20mg, 30mg, 40mg and 50mg/ml were prepared and 50µl of each concentration were added in corresponding test tubes and control tubes were left without adding anything. Pseudomonas aeruginosa and Shigella sonnei were inoculated in each tube and incubated at 37ºC. OD value was taken after 24hours of incubation at 600nm.

H. SEM Analysis to Examine the Morphological Changes Caused by the Antibacterial Formulation

Chitosan silver nanoparticles with antibiotics treated Pseudomonas aeruginosa cells were examined under Scanning electron microscopy Bacteria (overnight bacterial culture diluted to obtain 1×107 CFU/ ml) were cultured for 48 h in MHB containing chitosan with tetracyclin. Primary fixation of samples was done by buffered Glutaraldehyde 2.5% for 1 hour then washed by phosphate buffer (pH = 7.2) and transferred to 1% (w/v) tannic acid in PBS 1 hour, followed by washing in PBS. They were dehydrated by series concentration of ethanol, frozen in a freezer at 65°C and dried at the critical point of vacuum pressure under the following temperature conditions: condenser temperature 53°C and shelf temperature 15°C. Before examination under a scanning electron microscope (SEM) (JEOL, JSM-6060 LV), specimens were coated with 100 Å of a gold– palladium mix in an ion sputter (JEOL JFC 1100) using a voltage of 15–16 kV and a coating time of 30 seconds ,and their morphological changes were determined using the facility from Hindustan University, Chennai.

III. RESULTS & DISCUSSION

A. Determination of Lambda Max for Chitosan Silver Nanoparticles

Chitosan silver nanoparticles were synthesised and the formation of nanoparticles were confirmed by UV-Visible spectrophotometry. Wave length from 310 nm to 500 nm were used and maximum absorption spectrum were determined. Based on this study, the lambda max for the chitosan silver nanoparticles were 430 nm which showed maximum peak. Also UV absorption peak of chitosan-Ag nanoparticles prepared by other researchers was recorded in the range 410–420 nm(Chen et al., 2007). Since F-Calculated Value = 295.7211 < F-Critical Value = 4.747225, we reject the null hypothesis H_0 at 5% level of significance and we conclude that there is a significant difference in the Nanometer and their OD values. Correlation showed that r =0.67756, there was an average relationship between different types of nanometer and their OD value.

B. Charactrization of Chitosan Silver Nanoparticles By Fourier Transforms Infrared Spectrophotometry Spectrum

FTIR result revealed the presence peaks at 1000 cm-1, 3417 cm-1, 2928 cm-1, 2118cm-1, 1629cm-1, 1383cm-1, 1255cm-1, 934cm-1, 900cm-1, 691cm-1 and 622 cm-1. FTIR peaks were in the extract ranging from 1000-4000 cm -1 which confirmed the presence of polyphones with aromatic ring and bound amide region required for the synthesis and stabilization of silver nanoparticles. (Fig.4). Similarly, Solmaz et al., 2013, reported that FTIR spectra of chitosan and chitosan-Ag nanoparticle materials synthesized with 0.02 M, 0.04 M, and 0.06 M $AgNO_3$. The broad absorption peak at 3700–3100 cm^{-1} is the merged characteristic bands for OH and NH2 groups and CONH2 absorption band is also observed at near 1657 cm^{-1} in the FTIR spectrum of chitosan. The shift was observed from 1657 cm^{-1} in the Ag loaded chitosan spectra. This shift may indicate the binding of Ag nanoparticles to N–H bond of chitosan.

C. Scanning Electron Microscopy

SEM imaging was done to analyse arrangement of the nanoparticles. Morphological arrangement and size of the chitosan silver nanoparticles were examined by scanning electron microscope and maximum magnification employed was 22.8Kx. The result showed the presence of silver nanoparticles embedded on a shrimp shell chitosan matrix. Size of the chitosan silver nanoparticles were determined as 10 nm to 12 nm. Based on this study, we could able to interpret that this current green synthesis protocol is one of successful methodology to generate silver nanoparticles without producing any environmental hazards. Chitosan had served as a better reducing agent.(Fig.5). The results of Honary et al., 2011 stating that the silver-chitosan nanoparticles were spherical with particle size in the range of 20 - 120 nm.

D. Antibacterial Activity of Chitin, Chitosan & Chitosan Silver Nanoparticles

Antibacterial activity of chitin, chitosan, chitosan silver nanoparticles and glacial acetic acid which was used as a solvent was examined against Pseudomonas aeruginosa and Shigella sonnei. Glacial acetic acid showed the least

sensitivity against the clinical isolates and which was insignificant. Chitin was also resistant to the clinical isolates. Chitosan showed 13mm zone and 11 mm zone of inhibition against Pseudomonas aeruginosa and Shigella sonnei. Chitosan silver nanoparticles showed little improvement in sensitivity. Maximum activity was shown by the composite Chitosan silver nanoparticle(Fig.6). Pawan et al., 2013, reported that, in terms of surrounding clearing zone, chitosan/silver nanocomposites clearly show greater inhibitory effect against Staphylococcusaureus, Pseudomonas aeruginosa and Salmonella entricai n comparison to chitosan.

E. Antibacterial Activity of Chitosan Tetracyclin Based Formulations Against Pseudomonas Aeruginosa

Antibactericidal activity of chitosan, chitosan silver nanoparticles and chitosan silver nanoparticles with tetracycline were studied against Pseudomonas aeruginosa. Among the three combinations chitosan with tetracycline had showed double fold higher sensitivity. Antibacterial activity of tetracycline(formulation I) and the combination of this antibiotics with chitosan (Formulation II), chitosan silver nanoparticles (Formulation III) were studied against Pseudomonas aeruginosa. Tetracycline was moderately sensitive to the bacteria but the combinations were better active than the additive effect. The zone of inhibition of chitosan and tetracycline combination was 25 mm, which was 75% higher effective than the antibiotics alone. Same way there was a significant improvement with chitosan silver nanoparticles also. (figs. 9&10). San Tin et al., 2009, reported that in P. aeruginosa PT149, the MIC of Sulfamethoxazole was found to be 5 fold higher than the remaining strains suggesting that Sulfamethoxazole might be effluxed by the MexEF-OprN system, thereby reducing the effective concentration of the folic acid biosynthesis inhibitor. Thus, the reduction in drug accumulation is more apparent in the MexEF-OprN overexpressor.

Although, Sulfamethoxazole has high MIC, chitosan and Chitosan oligosaccharide remain effective against this mutant strain either singly (MIC of 32 µg/ml and 4096 µg/ml respectively) or in combination (8 µg/ml of chitosans lower the MIC of sulfamethoxazole to 512µg/ml and 512µg/ml of Chitosan oligosaccharide lower the MIC of sulfamethoxazole to 256µg/ml). These finding provide a strong evidence that chitosans and Chitosan oligosaccharide are promising candidates for combination therapy against multi drug resistant P. aeruginosa infections. Similarly our result with chitosan and tetracycline has shown higher activity when tetracyclin is combined with chitosan and chitosan silvernanoparticles against Pseudomonas aeruginosa. Antibactericidal activity of chitosan, chitosan silver nanoparticles and chitosan silver nanoparticles with ciprofloxacin were studied against Shigella sonnei. Among the three combinations chitosan with ciprofloxacin had showed double fold higher sensitivity. Antibacterial activity of ciprofloxacin (Formulation I), ciprofloxacin with chitosan(Formulation II) and ciprofloxacin with chitosan silver nanoparticles (Formulation III) were studied and the result showed ciprofloxacin was moderately sensitive, and the other two formulations were highly sensitive against Shigella sonnei. There was a 56% increase in the sensitivity of ciprofloxacin and chitosan combination. From the results it could be inferred that the combinatorial therapy is significantly higher than the additive effect. (Figs.11&12).

Minimum inhibitory concentration of formulation I (glacial acetic acid in tetracycline) was studied against pseudomonas aeruginosa. There were four different concentration used and growth rate in each concentration was compared with a control. Maximum growth was attained in a control tube were as there was a 50% reduction in the growth in the tube added with 30 mg /ml tetracycline. Hence the MIC for the formulation I was determined as 30mg/ml (Fig.13). Minimum inhibitory concentration of formulation II (tetracycline & chitosan) was studied against pseudomonas aeruginosa. There were four different concentration used and growth rate in each concentration was compared with a control. Maximum growth was attained in a control tube were as there was a 50% reduction in the growth in the tube added with 10 mg /ml tetracycline. Hence the MIC for the formulation II was determined as 10mg/ml .(Fig.14). Minimum inhibitory concentration of formulation III (tetracycline & CNP) was studied against pseudomonas aeruginosa. There were four different concentration used and growth rate in each concentration was compared with a control. Maximum growth was attained in a control tube were as there was a 50% reduction in the growth in the tube added with 10 mg /ml tetracycline. Hence the MIC for the formulation III was determined as 10mg/ml.(Fig.15). As for ANOVA, Since F – Calculated value = 0.423602< F – Critical value = 3.885294, We Accept the null hypothesis at 5% level of significance and we conclude that there is a significant difference in the three formulations of chitosan. The parameters studied were negatively correlated.

The statistical analysis revealed that as the concentration of antibacterial combination increases there was a decrease in growth rate of Pseudomonas aeruginosa. Minimum inhibitory concentration of formulation I (glacial acidic acid &ciprofloxacin) was studied against shigella sonnei. There were four different concentration used and growth rate in each concentration was compared with a control. Maximum growth was attained in a control tube were as there was a 50% reduction in the growth in the tube added with 30 mg /ml ciprofloxacin. Hence the MIC for the formulation. I was determined as 30mg/ml. (Fig.15). Minimum inhibitory concentration of formulation II (chitosan& ciprofloxacin) was studied against shigella sonnei. There were four different concentration used and growth rate in each concentration was compared with a control. Maximum growth was attained in a control tube were as there was a 50% reduction in the growth in the tube added with 10 mg /ml ciprofloxacin. Hence the MIC for the formulation II. I was determined as 10mg/ml .(Fig. 16), Minimum inhibitory concentration of formulation III (ciprofloxacin & CNP) was studied against shigella sonnei. There were four different concentration used and growth rate in each concentration was compared with a control. Maximum growth was attained in a control tube

were as there was a 50% reduction in the growth in the tube added with 10 mg /ml ciprofloxacin.

Hence the MIC for the formulation III was determined as 10mg/ml.(Fig.17). Hassan Nageh et al., 2014, stated that the antimicrobial activity of the produced nanofiber mats was studied using different strains of human pathogenic bacteria and Fungi. Compared to release profile from Cs/PVA/Ciprofloxacin. HCl nanofibers show faster drug release than that of Cs/PCL/Ciprofloxacin. HCl system, indicating that these nanofiber mats are promising active wound dressing materials for wound dressing applications of variance, F – Calculated value = 0.399484< F – Critical value = 3.885294, hence we accept the null hypothesis at 5% level of significance and we conclude that there is a significant difference in the three formulations of Chitosan. The parameters studied were negatively correlated. The statistical analysis stated that as the concentration of antibacterial combination increases there was a decrease in growth rate of Shigella sonnei.

G. Scanning Electroscopy To Study Pseudomonas Aeruginosa Morphological Changes

The SEM image shows the Psudomonas aeruginosa morphological changes caused by the antibacterial formulation chitosan with tetracycline. There was a severe damaged caused by these combination on the cell wall and plasma membrane.

Fig.1. The Image Shows Shrimp Shell Waste Collected From Market.

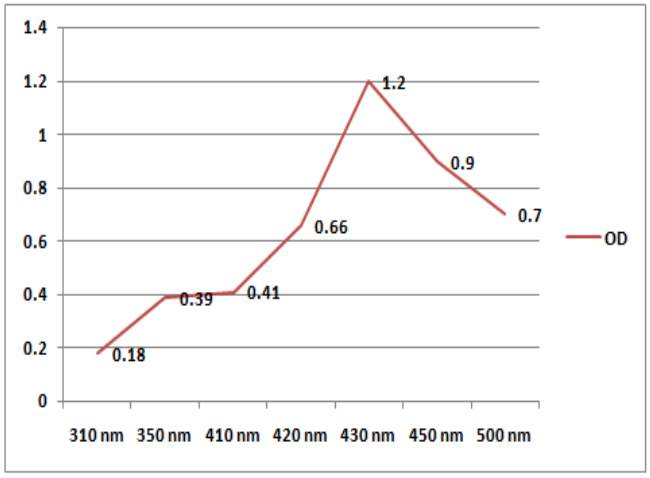

Fig.2.Determination of Lambda Max for Chitosan Silver Nanoparticles.

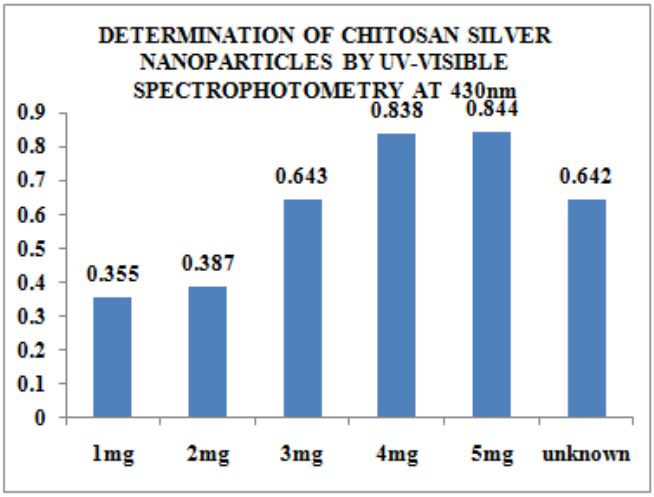

Fig.3.Estimation Ofchitosan Silver Nanoparticles By Uv-Visible Spectrophotometry.

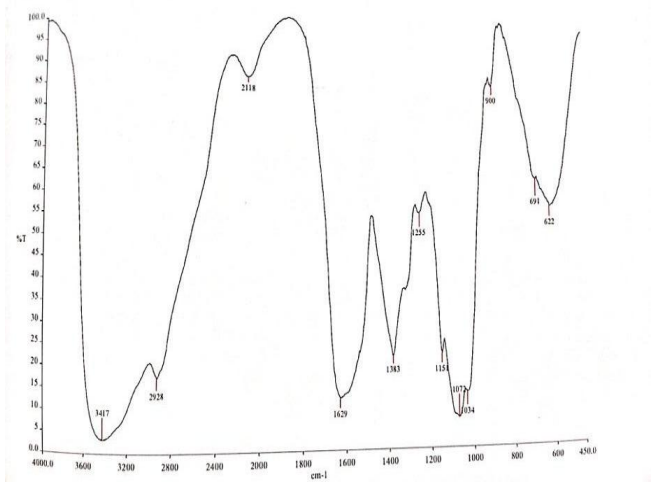

Fig. 4.Charactrization Of Chitosan Silver Nanoparticles For Fourier Transforms Infrared Spectrophotometry Spectrum.

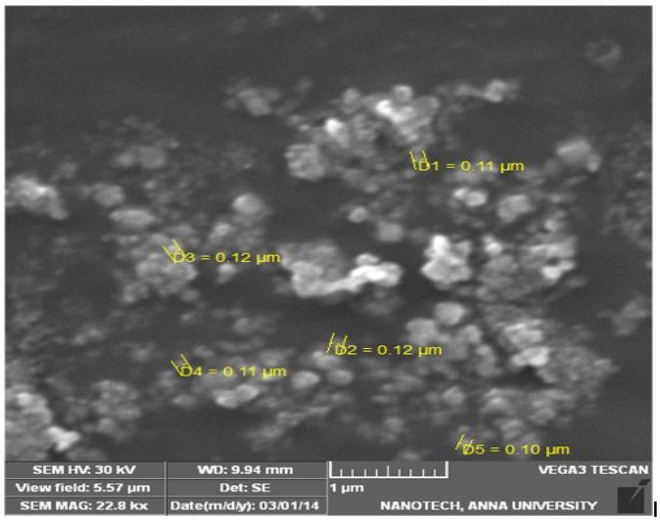

Fig.5. Charactrization of Chitosan Silver Nanoparticals for Scanning Electron Microscopy.

Sem Imaging Was Done To Analyse Size And Arrangement of The Nanoparticles.

Fig.6.Antibacterial Activity of Chitin, Chitosan & Chitosan Silver Nanoparticles.

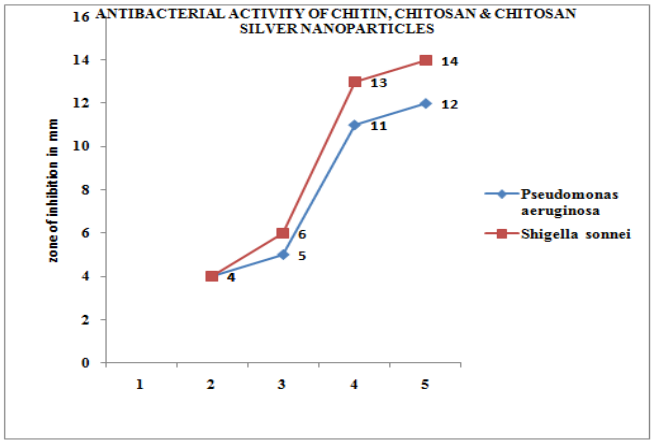

Fig.7.Antibacterial Activity of Chitosan Silver Nano-particles Against Pseudomonas Aeruginosa And Shigellasonnei.

Fig.8. Antibacterial Activity of Chitosan Tetracyclin Based Formulations Against Pseudomonas Aeruginosa.

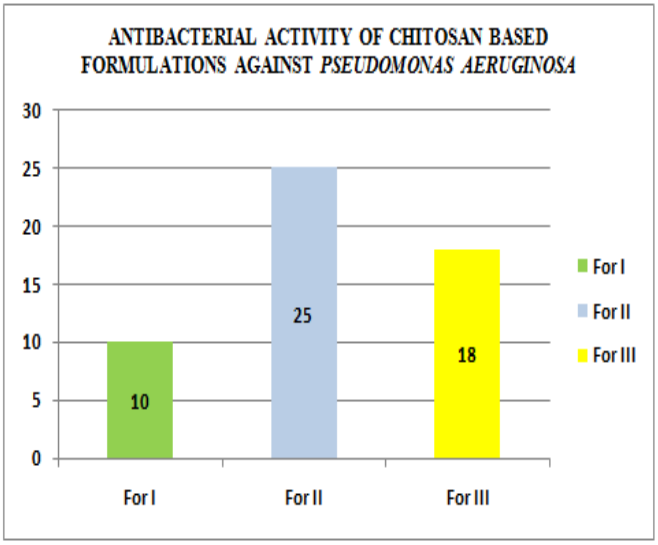

Fig.9. Antibacterial Activity of Chitosan Based Commercial Antibiotics.

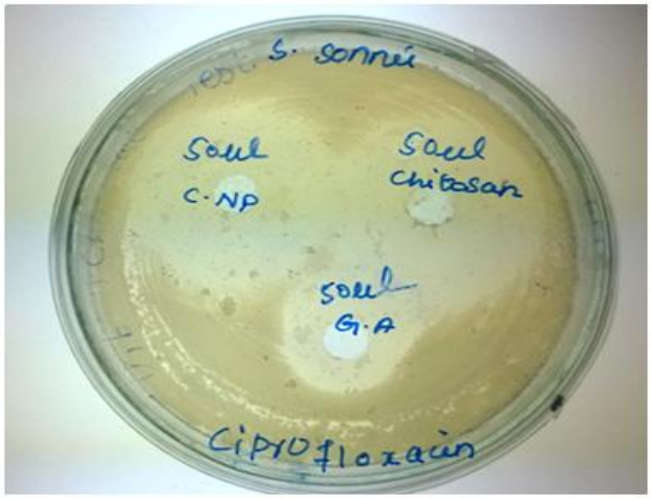

Fig.10. Inhibitory Concentration Plate Shows Antibacterial Sensitivity Against Shigellasonnei.

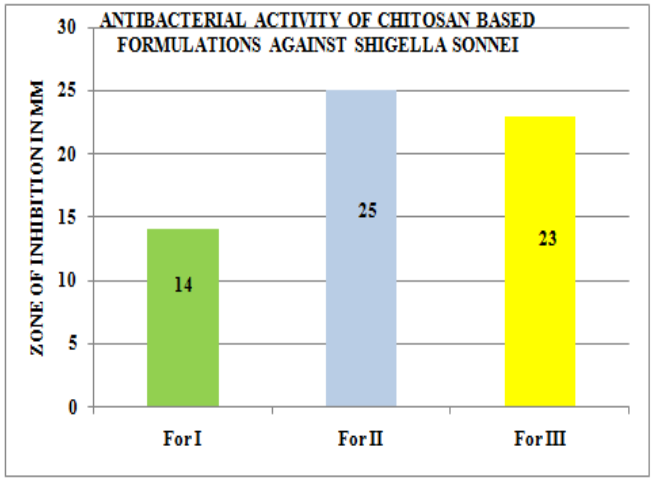

Fig.11. Antibacterial Activity Of Ciprofloxacin BasedFormulations Against Shigella Sonnei.

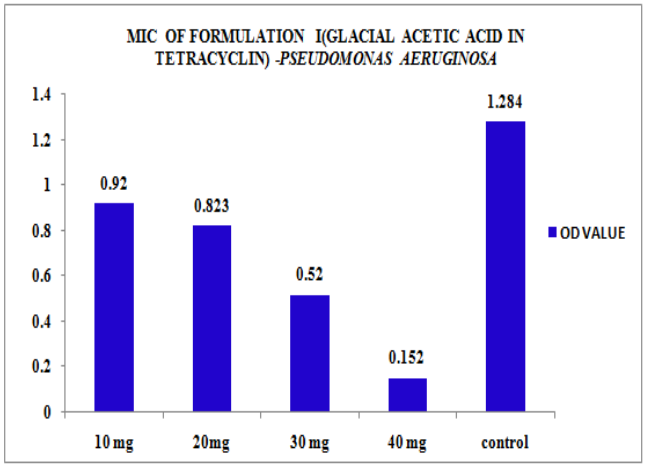

Fig.12. Minimum Inhibitory Concentration Of Formulationi (Glacial Acetic Acid In Tetracyclin) - Pseudomonas Aeruginosa.

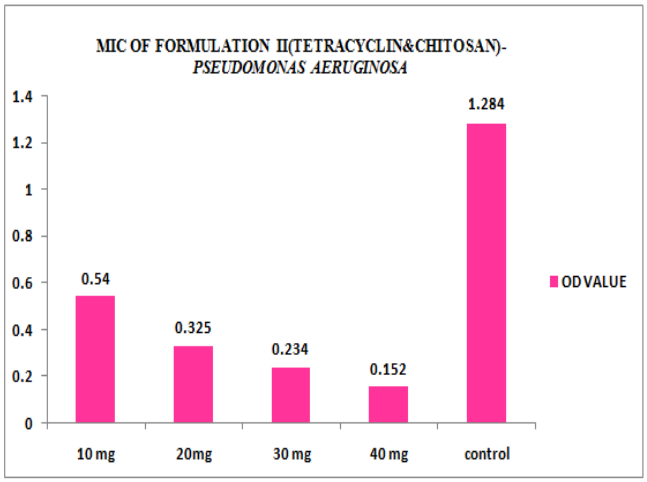

Fig.13. Minimum Inhibitory Concentration of Formulation Ii (Tetracyclin & Chitosan)-Pseudomonas Aeruginosa.

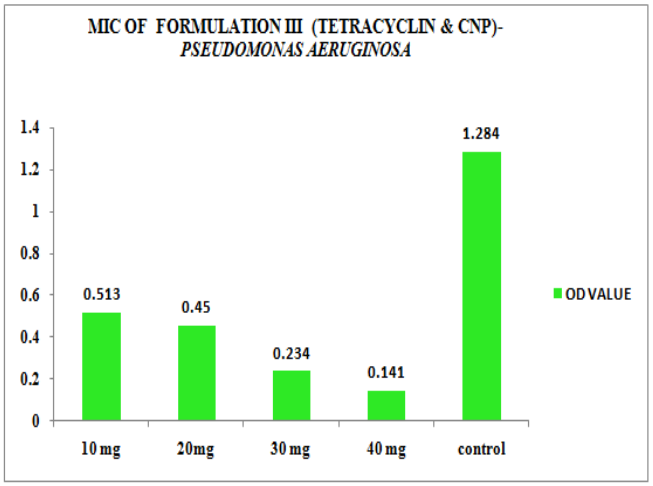

Fig.14. Minimum Inhibitory Concentration of Formulation Iii (Tetracyclin & Cnp)-Pseudomonas Aeruginosa.

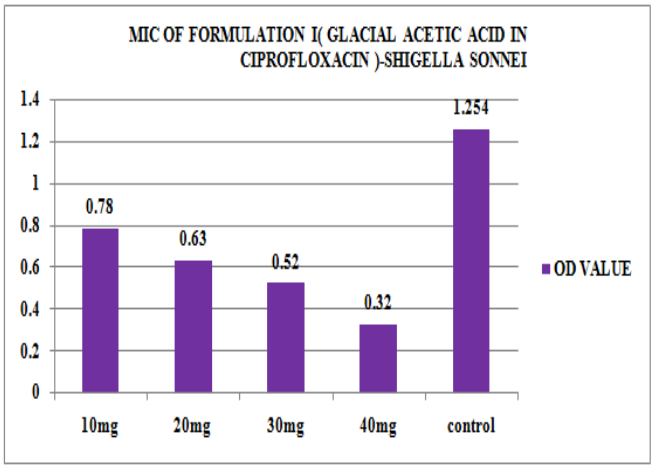

Fig.15. Minimum Inhibitory Concentration of Formulation I (Glacial Acetic Acid In Ciprofloxacin)-Shigella Sonnei.

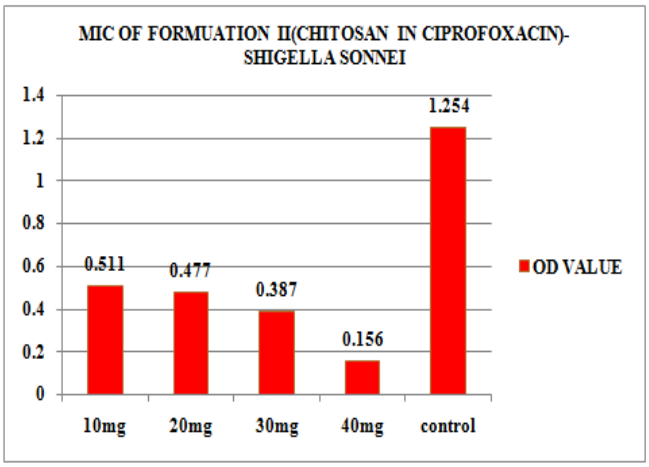

Fig.16. Minimum Inhibitory Concentration Of Formulation Ii (Chitosan In Ciprofloxacin)- Shigella Sonnei.

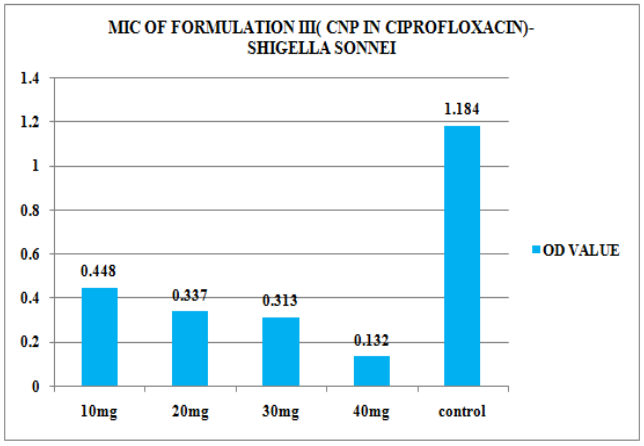

Fig.17. Minimum Inhibitory Concentration Of Formulation Iii (Chitosan Silver Nanoparticle In Ciprofloxaxin)-Shigella Sonnei.

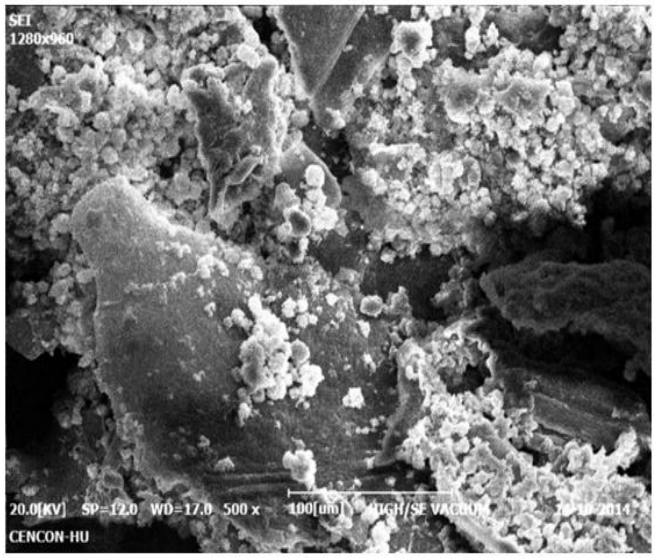

Fig.18. A Sem Image Shows The Psudomonas Aeruginosa Morphological Changes.

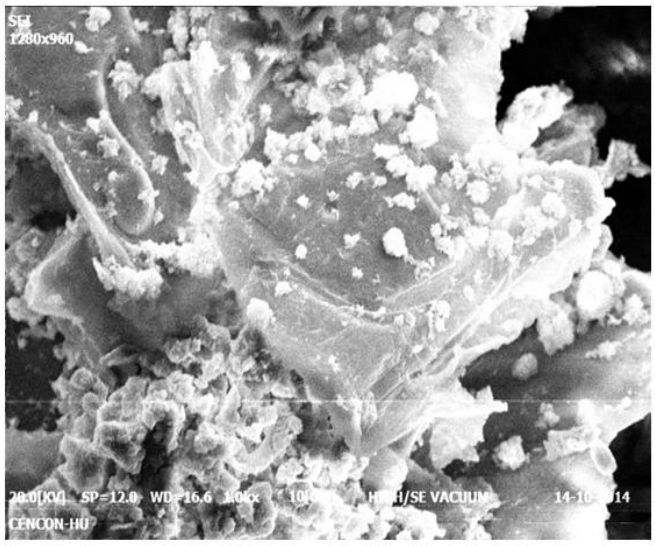

Fig.19.

TABLE I: Anova for Lambda Max of Chitosan

SOURCE OF VARIATION	SS	Df	MS	F	P-value	F – Critical Value
BETWEEN GROUPS	586531	1	586531	295.7211	8.07 E-10	4.747225
WITHIN GROUPS	23800.71	12	1983.393	-	-	-
TOTAL	610331.7	13	-	-	-	-

TABLE II: Anova for Different Formulations of Antibiotics

ANOVA						
SOURCE OF VARIATION	SS	Df	MS	F	P-value	F crit
BETWEEN GROUPS	0.16816	2	0.08408	0.423602	0.664103	3.885294
WITHIN GROUPS	2.38185	12	0.198488	-	-	-
TOTAL	2.55001	14	-	-	-	-

TABLE III: Correlation Analysis for Tetracyclin

	CONCENTRATION OF TETRACYCLINE	OD
CONCENTRATION OF TETRACYCLINE	1	-0.98642
OD	-0.98642	1

TABLE IV:

	CONCENTRATION OF TETRACYCLINE AND CHITOSAN	OD
CONCENTRATION OF TETRACYCLINE AND CHITOSAN	1	-0.88753
OD	-0.88753	1

TABLE V:

	CONCENTRATION OF TETRACYCLINE AND CHITOSAN SILVER NANOPARTICLES	OD
CONCENTRATION OF TETRACYCLINE AND CHITOSAN SILVER NANOPARTICLES	1	-0.89904
OD	-0.89904	1

TABLE VI: Anova Table for Ciprofloxacin and Other Formulations

SOURCE OF VARIATION	SS	df	MS	F	P-value	F crit
BETWEEN GROUPS	0.122847	2	0.061423	0.399484	0.679263	3.885294
WITHIN GROUPS	1.845082	12	0.153757	-	-	-
TOTAL	1.967928	14	-	-	-	-

TABLE VII:

	CONCENTRATION OF CIPROFLOXACIN	OD
CONCENTRATION OF CIPROFLOXACIN	1	-0.95645
OD	-0.95645	1

TABLE VIII:

	CONCENTRATION OF CIPROFLOXACIN AND CHITOSAN	OD
CONCENTRATION OF CIPROFLOXACIN AND CHITOSAN	1	-0.88706
OD	-0.88706	1

TABLE IX:

	CONCENTRATION OF CIPROFLOXACIN AND CNP	OD
CONCENTRATION OF CIPROFLOXACIN AND CNP	1	-0.86757
OD	-0.86757	1

IV. CONCLUSION

Chitosan-silver nanocomposites are ideal alternative for ineffective antibiotics. They exhibit numerous advantages from medical applications point of view. Synergic antibacterial mechanism of chitosan and silver nanoparticles provides efficient bacterial infections control. P. aeruginosa is an important bacterial pathogen most frequently responsible for nosocomial infections. It is often resistant to many antibiotics used in causative therapy. Improving the effectiveness and decreasing the toxicity of antibiotics are the two basic objectives in the development of novel antimicrobial agents. Utilization of combination therapy is one of the contemporary approaches for successful modulation of existent antibiotics. These findings could also prove to be a promising alternative in the treatment of patients for whom existing antimicrobial treatment fails. Synergistic effect of chitosan and tetracycline & Chitosan ciprofloxacin combinations had showed significant improve in the antibacterial activity. Such combinations are clinically huge since it may be able to make some untreatable resistant infections treatable at currently recommended dosages that are often marginally effective against resistant strains when used alone.

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