Chitosan-Capped Gold Nanostructures: Structure and Morphological Characterization and their Antibacterial Response

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**Abstract:** This study explores the synthesis and biomedical application of chitosan-capped gold nanostructures. Gold nanostructures are valued for their chemical inertness, biocompatibility, and tunable optical properties, while chitosan, a polymer derived from chitin, enhances these structures by providing stability and enabling targeted drug delivery. The synthesis involves dissolving chitosan in acetic acid, adding gold nanoparticles, and stabilizing the mixture with sodium hydroxide. Characterization techniques such as XRD, FTIR, and HRTEM confirm the structural integrity and crystalline nature of the nanostructures. The antibacterial efficacy was evaluated against E. coli and Enterococcus, showing significant inhibition zones, particularly at higher concentrations. The results indicate that chitosan-capped gold nanostructures effectively disrupt bacterial cell membranes and generate reactive oxygen species, making them potent antibacterial agents. Despite their promise, challenges such as stability, scalability, potential immunotoxicity, and limited penetration across biological barriers remain. Addressing these issues through further research could enable the broader application of chitosan-capped gold nanostructures in medicine and other fields.

**Keywords:** Chitosan, nanotechnology, biocompatibility, antibacterial therapy, targeted drug delivery, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, high-resolution transmission electron microscopy (HRTEM)

# INTRODUCTION

In the field of material science and biomedical applications, nanotechnology has significantly transformed it by producing nanostructures with unique properties [(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/ME2hW4/4v77g+qHrQW+mafbD). Gold nanostructures, in particular, have become famous because of their exceptional chemical inertness, biocompatibility as well as adjustable light absorption capabilities amongst other things [(Merchant et al., 2022a; Pandiyan et al., 2022a)](https://paperpile.com/c/ME2hW4/I129O+nqCrW). Integration of chitosan with gold nanostructures presents a promising approach for improving their performance particularly in medical practices such as antibacterial therapy.[(Lewinski et al., 2008)](https://paperpile.com/c/ME2hW4/avEd0) Chitosan is among the biocompatible and promising polymers that can be exploited for fabrication of smart nanoparticles which can ferry chemotherapy drugs to cancerous sites sparing normal cells.[(“Paclitaxel-Loaded Chitosan Oligosaccharide-Stabilized Gold Nanoparticles as Novel Agents for Drug Delivery and Photoacoustic Imaging of Cancer Cells,” 2016)](https://paperpile.com/c/ME2hW4/1byAr)The method of synthesizing nanoparticles is more advantageous if chitosan, which is derived from chitin—a polysaccharides waste product collected from shellfish and is also one of the most plentiful naturally occurring biodegradable compounds– is used than in other biological processes because it can be easily scaled up without complicated steps. Chitin ranks as the second most abundant natural polymer after cellulose.[(Crini & Lichtfouse, 2019)](https://paperpile.com/c/ME2hW4/ifciU)In the event of weak acids, chitosan which is a deacetylated form of chitin can be dissolved; however, the same cannot happen when it comes to strong acids [(Ganapathy, 2021; Merchant et al., 2022b; Pandiyan et al., 2022b)](https://paperpile.com/c/ME2hW4/OFWSc+kGKpH+dvYQg). Chitosan has been examined by several researchers for its application in AuNP synthesis and conservation of fish.[(Gooday et al., 2012)](https://paperpile.com/c/ME2hW4/CYDcD)Chitosan (CS), which is a biopolymer, obtained from natural origin and regarded as one of the most safe and effective biopolymers for stabilizing the nanoparticles in pharmaceutical processing of medication is being used for the preparation of metallic nanoparticles [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/ME2hW4/07HcG+J1b1u). Also, the negative exterior side of the cell membrane might attract persons with a positive surface charge.[(“Chitosan as a Capping Agent: Insights on the Stabilization of Gold Nanoparticles,” 2019)](https://paperpile.com/c/ME2hW4/2zjQg). There would be aggregation because of the transient Van der Waals forces experienced by bare metallic nanoparticles that lack appropriate stabilizers [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/ME2hW4/RrQX3+DpgFg). On the other hand, electrostatic repulsion is very high due to which CS, this is a steric barrier that surrounds the metal with a positive charge to give homogenous nanoparticle solutions.[(Phan et al., 2021)](https://paperpile.com/c/ME2hW4/RFJgw)

Thus, formulated nanoparticles, rods, and cages are gold nanostructures to possess distinctive chemical and physical attributes due to an enhanced surface-volume relationship and quantum confinement [(Wadhwani et al., 2022)](https://paperpile.com/c/ME2hW4/djroF). These characteristics make them suitable for many uses including giving out medication, diagnostic and as antimicrobial agents [(Sreevarun et al., 2023)](https://paperpile.com/c/ME2hW4/AdjGg). Distinctions in the size and shape of gold nanostructures within their constructing are required to create further optical properties such as surface plasmon resonance (SPR) to be used in therapeutic and diagnostic processes.[(Thomas et al., 2014)](https://paperpile.com/c/ME2hW4/v2tAg) Gold nanoparticles (AuNPs) can be synthesized employing physical, chemical, biological methods along with the supercritical fluid technology [(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/ME2hW4/Werjz). Microorganisms, vitamins, enzymes, sugar and biodegradable polymers obtained from plant or animal resources in the biologic methods for preparing nanoparticles. All these methods have been presented as green chemistry and green physical methods which are alternatives to the normal chemical and physical methods.[(Abdullaeva, 2017)](https://paperpile.com/c/ME2hW4/u5zEX)Doping the surface of the average size of the gold nanoparticles (AuNPs) synthesized was in the range of 13–29 nm, depending on the concentration of the reducing agent used is a technique that can change the properties of the material [(Solanki et al., 2023)](https://paperpile.com/c/ME2hW4/zuBNW). When chitosan is employed as the cap for AuNPs, these nanoparticles will be useful in showing signs of abuse in temperature. In these cases, they show expression of colors and intensity in different storage temperatures and storage time [(Ganapathy, 2021)](https://paperpile.com/c/ME2hW4/Vs5dh). Specifically, the color change is ascribed to alterations in size and morphology of the chitosan-capped AuNPs, which can be applied to sensor construction.[(Mohan et al., 2019)](https://paperpile.com/c/ME2hW4/FSZV)As admitted, stability, inertness, non toxicity, and size controllable of AuNPs create a great interest towards using these NPs as antibacterial agents. In particular, increase the production of oxidative stress is not desirable as it leads to the negative impact on the host cells in mammals.[(Kesharwani, 2023)](https://paperpile.com/c/ME2hW4/CjNqe) Chitosan itself as well as its derivatives possess antibacterial effects against fungi, bacteria, and yeasts; the efficacy depends on several factors including the pH of the medium and temperature [(Chokkattu et al., 2023)](https://paperpile.com/c/ME2hW4/KsuqF). Chitosan is known for its antimicrobial activity which in a manner that involves the positively charged sites that are the amino groups of chitosan, interacts with the negative sites of the bacterial cell membrane and attack it leading to the destruction of microorganisms. While gram positive bacteria have layers of peptidoglycan, the gram negative bacteria possess outer membranes with negative charges [(Laghari et al., 2023; Ramakrishnan et al., 2023a)](https://paperpile.com/c/ME2hW4/8vaLw+btZsN). The structures of cationic chitosan-capped composites include protonated amine species. The electrostatic attraction which occurs between the bacterial cells and the cationic composites is the cause of the cells’ demise due to the release of cell contents.[(Kesharwani, 2023; *Website*, n.d.)](https://paperpile.com/c/ME2hW4/CjNqe+zM5a9) It can therefore be deduced from many research works that chitosan exhibits a strong affinity to AuNPs through its amine groups [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023b)](https://paperpile.com/c/ME2hW4/MfOxn+14b3W). Under dilute acidic solution conditions, chitosan has poly-cationic characteristics, and hence it will be positively charged to interact with the negative potential surfaces of the AuNPs via electrostatic forces. Moreover, chitosan increases the immune ability of AuNPs against aggregation owing to presence of hydrophilic groups such as –NH3+, –NH2, and –OH on the composite surface. This stability is advantageous for the different biomedical uses of the compound such as; antimicrobial coatings, sensors and scaffolds.[(Gulati, 2022)](https://paperpile.com/c/ME2hW4/a3p71)

# MATERIALS AND METHOD

## Materials

The materials that were used in synthesizing gold nanostructures capped by chitosan contained 0.1g of chitosan and 0.1g of gold nanoparticles. To dissolve the chitosan, we employed 100ml of a solution having acetic acid present at 10%. Sodium hydroxide (NaOH) was utilized to adjust the pH of the solution to around 10, thereby facilitating stabilization and capping of the nanoparticles. Distilled water was used during initial centrifugation to remove unreacted reactants and other products resulting from reactions. Ethanol and acetone were used in further stages for centrifugation for effective cleaning or purification purposes on nanostructures.

## Synthesis

First, chitosan-capped gold nanostructures were synthesized by dissolving the 0.1g chitosan in 100ml of 10% acetic acid solution and stirring within a magnetic stirrer for 30 minutes to allow it to be completely dissolved. The synthesis was followed by the addition of 0.1g of gold nanoparticles to the chitosan-acetic acid solution. The stabilization of the nanoparticles and facilitation of the capping process was done through the addition of sodium hydroxide NaOH likely during pH ten. An hour at 60°C on a hot plate is allowed to elapse as the mixture is heated up to make chitosan and gold particles interact. Isolation of sample The process used in removing any unreacted materials and byproducts from the sample was heating with a constant temperature of 60°C for one hour, followed by centrifugation using distilled water for about ten minutes under a speed of three thousand rpm (3000rpm). Second centrifugation further required ethanol followed by acetone in order for adequate washing and purification of the structures nanosized ones such that they were finally dried at eighty degrees Celsius within ten hours while still lying within an oven which had become heated earlier on. producing a smooth granule made out of dried structures through grinding with mortar and pestle to be fine enough powder for application tests and other subsequent characterization methods, like those used in test purposes only if uniformity could be preserved, thereby achieving uncontaminated test samples that later do not have traces.

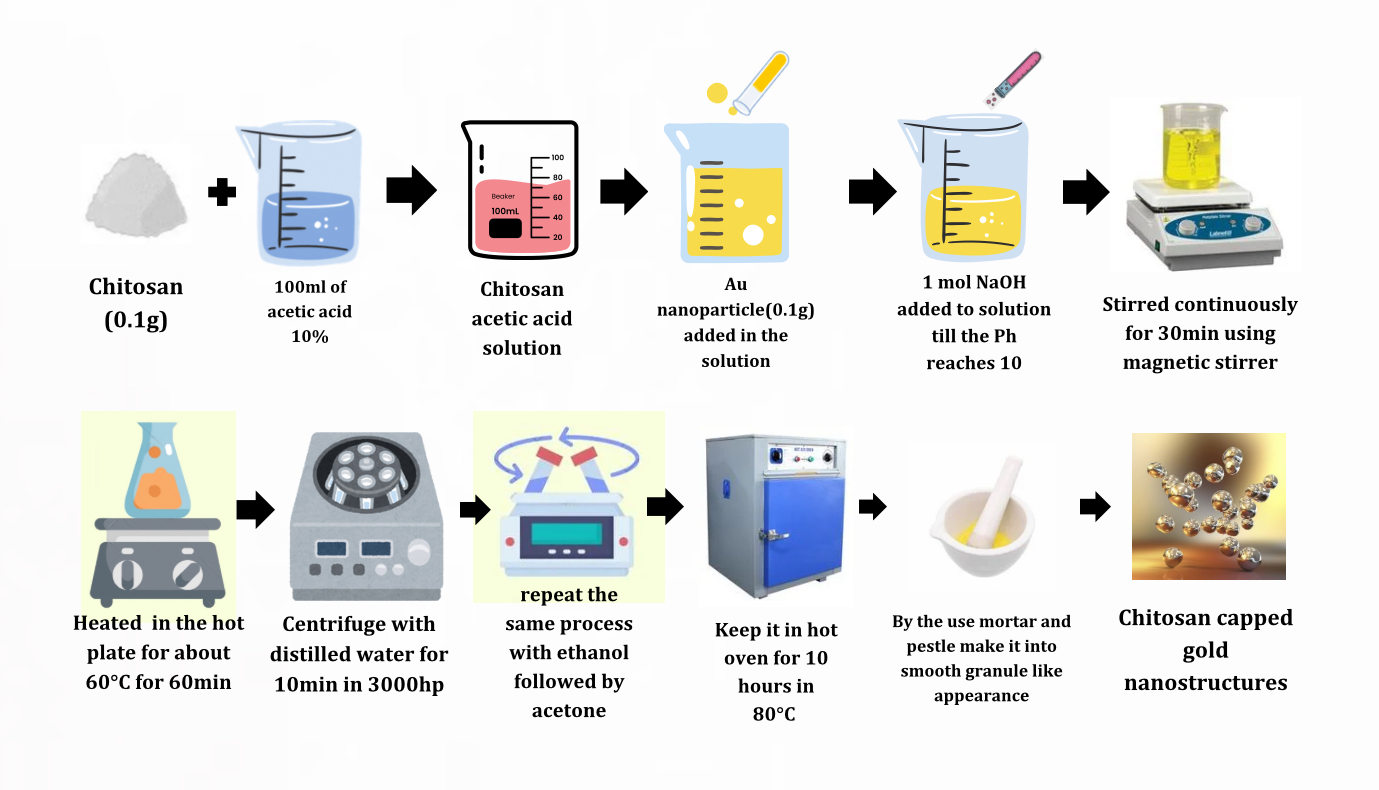


FIGURE 1: Schematic representation of synthesis of Chitosan-capped gold nanostructures

# RESULTS AND DISCUSSION

## XRD analysis

XRD stands for X-Ray Diffraction, a simple and handy technique for the identification and quantitative examination of the composition and structure. The XRD patterns indeed become a fingerprint and can be used to reveal the crystalline structure of gold nanoparticles. All of the XRD patterns that have been recorded up until now have been in full agreement with the FCC structure of the gold.[(Hutchinson et al., 2021)](https://paperpile.com/c/ME2hW4/UrffY) In the realm of materials science and crystallography, X-ray diffraction (XRD) research is employed to investigate both the atomic and molecular structure of the crystalline material. The structural quality of the manufactured Au sample is checked by the XRD analysis method.The characteristics 38.8°, 42.6°, 64.4°, and 77.1° of gold (Au) nanoparticles JCPDS correspond to the (111), (200), (220), and (311) face-centered cubic (fcc) crystal planes. The gold (Au) nanoparticles The characteristics 38.3°, 44.6°, 64.7°, and 77.7° correspond to the (111), (200), (220), and (311) face-centered cubic (fcc) crystal planes.The diffraction pattern of gold (Au) powder is consistent with JCPDS file 04-0784.

It includes the relative strengths of diffraction peaks as well as d-spacings.The crystal structure of gold is face-centered cubic (FCC), with an a = 4.0786 Å.Density is calculated to be 19.32 g/cm³.Used in a variety of applications, including as nanoparticles, electrodeposits, and geological samples, to identify gold.[(Ren et al., 2015)](https://paperpile.com/c/ME2hW4/7Kv0)

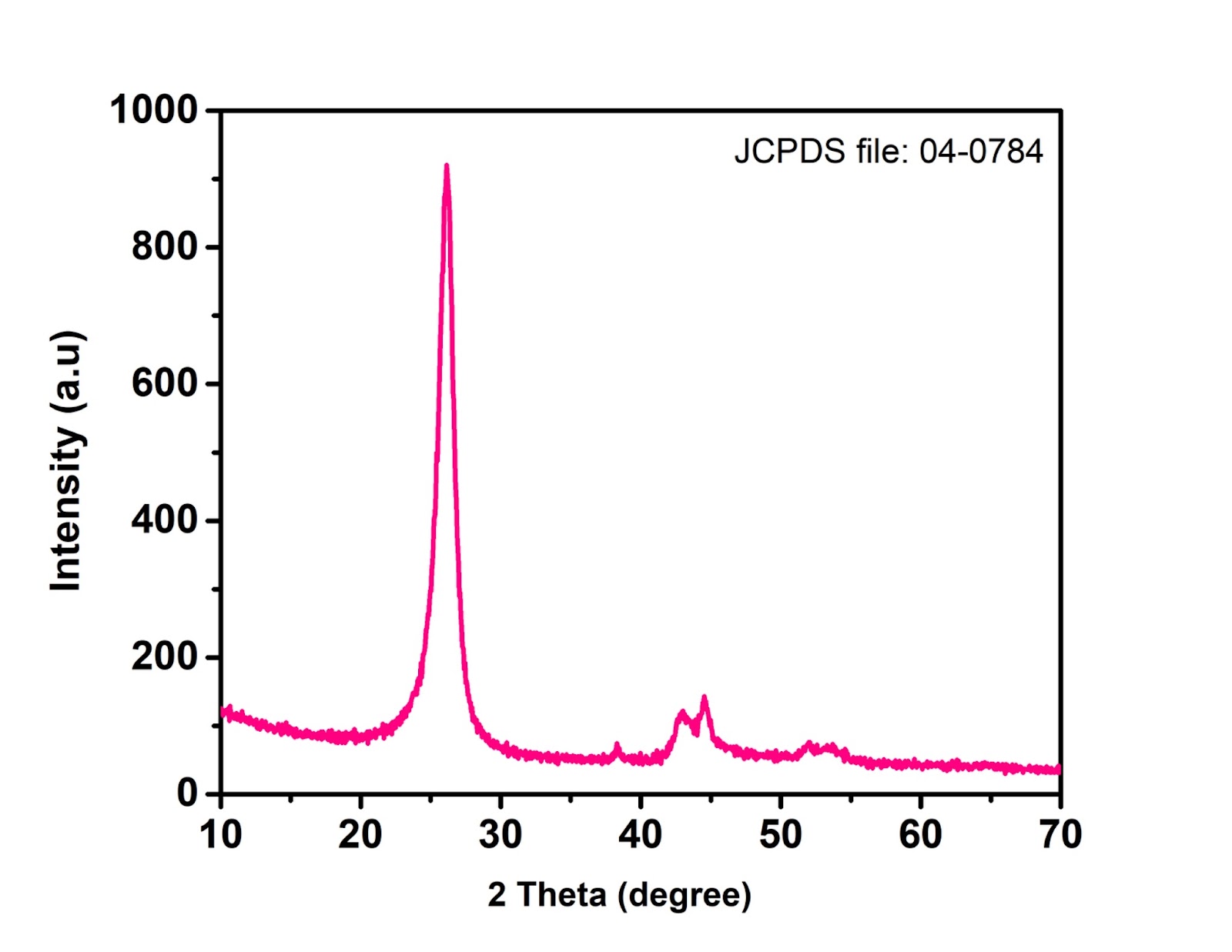
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Fig.2: XRD patterns of Au-Chitosan nanoparticles

## FTIR spectrum

Infrared (IR) or Fourier transform infrared (FTIR) spectroscopy is the technology that can be used for the detection of the smallest of the structures and even at cellular scales, from the molecular analysis of small molecules or molecular complexes to the analysis of cells or tissues. The development of tissue imaging is one of the most recent applications of infrared spectroscopy that uses infrared microscopy and synchrotron IR radiation. It is possible to search or map cellular components which are the carbohydrate, lipid, and protein through the use of this system. The IR spectrometer is also gaining its fame now in the analysis of fungal proteins. They got involved in the process of protein conformation, protein folding and the molecular particulars of active sites from protein enzymes during the reaction by using reaction-induced FTIR difference spectroscopy.

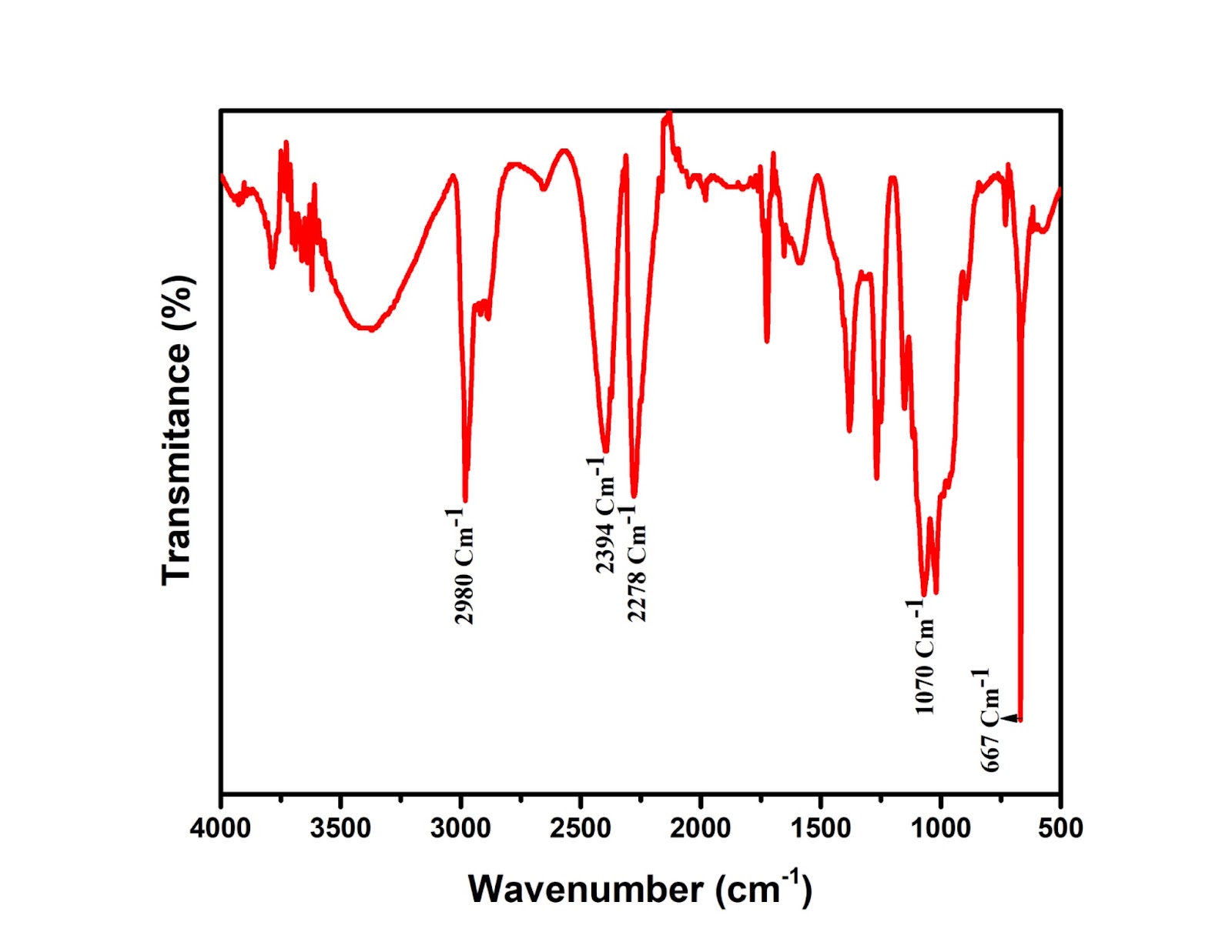
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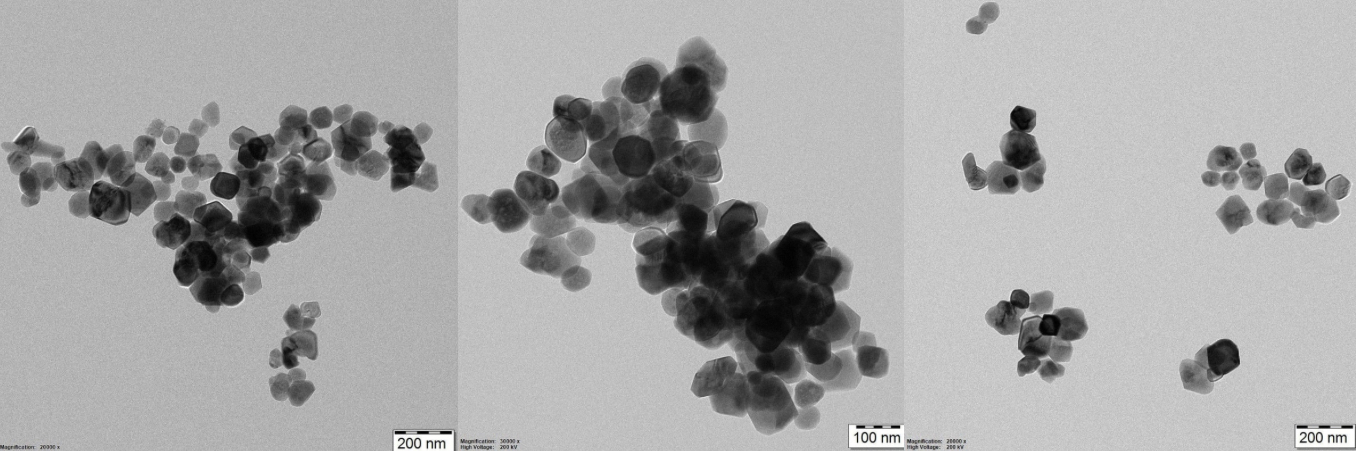
Fig.3: FTIR spectrum of Au-Chitosan nanoparticles

The FTIR spectra of chitosan and Au-chitosan have easily noticeable similarities. The existence of a new peak at 2361 cm−1 in Au-chitosan, which represents the C−N asymmetrical stretching, reveals the interaction of gold nanoparticles with chitosan amino groups. The −OH and −NH stretching vibrations are shifted from 3308 cm−1 to 3216 cm−1, indicating changes in hydrogen bonding due to the incorporation of gold. The 1401 cm−1 band related to the COO− group and 1051 cm−1 band corresponding to C−O−C stretching in the glucose units still stand in position but these two bands have been unexpectedly weakened by the gold nanoparticles, so the gold affects the vibrational environment without impacted the positions of such two units.

In the previous study The FTIR spectrum of Au-chitosan nanoparticles reveals key interactions between gold nanoparticles and chitosan. Notable changes include a shift of O−H and N−H stretching bands to 2992–2901 cm⁻¹ and a new peak at 2361 cm⁻¹ for C−N asymmetrical stretching, indicating gold's interaction with chitosan's amino groups. While the bands at 1401 cm⁻¹ (COO−) and 1051 cm⁻¹ (C−O−C) remain unchanged, they are weakened, suggesting that gold influences the vibrational environment. Overall, these findings underscore the potential of Au-chitosan nanoparticles for biomedical applications.[(Mohan et al., 2019)](https://paperpile.com/c/ME2hW4/FSZV)

## HRTEM images

High-Resolution Transmission Electron Microscopy (HRTEM) is a powerful tool used to obtain detailed images of the internal structure of materials at the atomic level .Gold (Au) nanoparticles come into sharp focus with the help of HRTEM images, which make these particles look like a piece of art and provide a high degree of clarity about their micro-structure. The nanoparticles exist in various sizes, as can be seen in the scale bars, as these dimensions are ranging from 100 nm to 200 nm. The photos demonstrate that the nanoparticles are non-spherical, with some being almost round in the picture while others are more faceted. There is clear evidence of aggregation, which may indicate that the particles have got together either during the synthesizing process or during the sample preparation. At the highest resolution, the crystal structure of the nanoparticles may be visualized by the lattice fringes, which are the clearest illustration of the crystalline phase. Such descriptive information on the structure plays a crucial role in understanding the mechanisms of growth as well as the existence of defects within the gold nanoparticles.[(Mourdikoudis et al., 2018)](https://paperpile.com/c/ME2hW4/PzJ2) In the previous articles HRTEM images of chitosan-gold nanostructures provided valuable insights into their morphology and structure. These images reveal well-defined gold nanoparticles, such as nano raspberry and nanoprism structures, stabilized by chitosan.



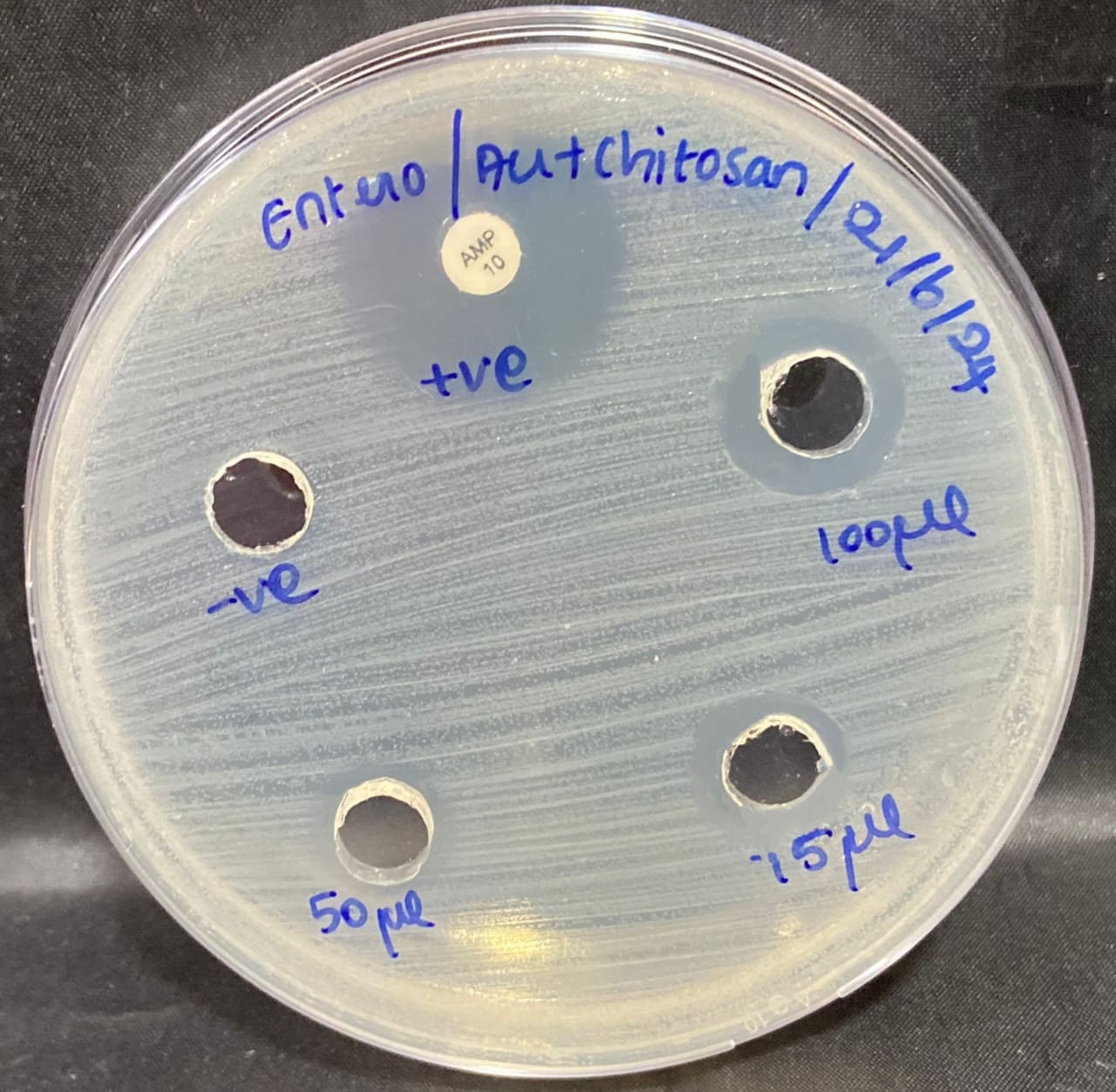
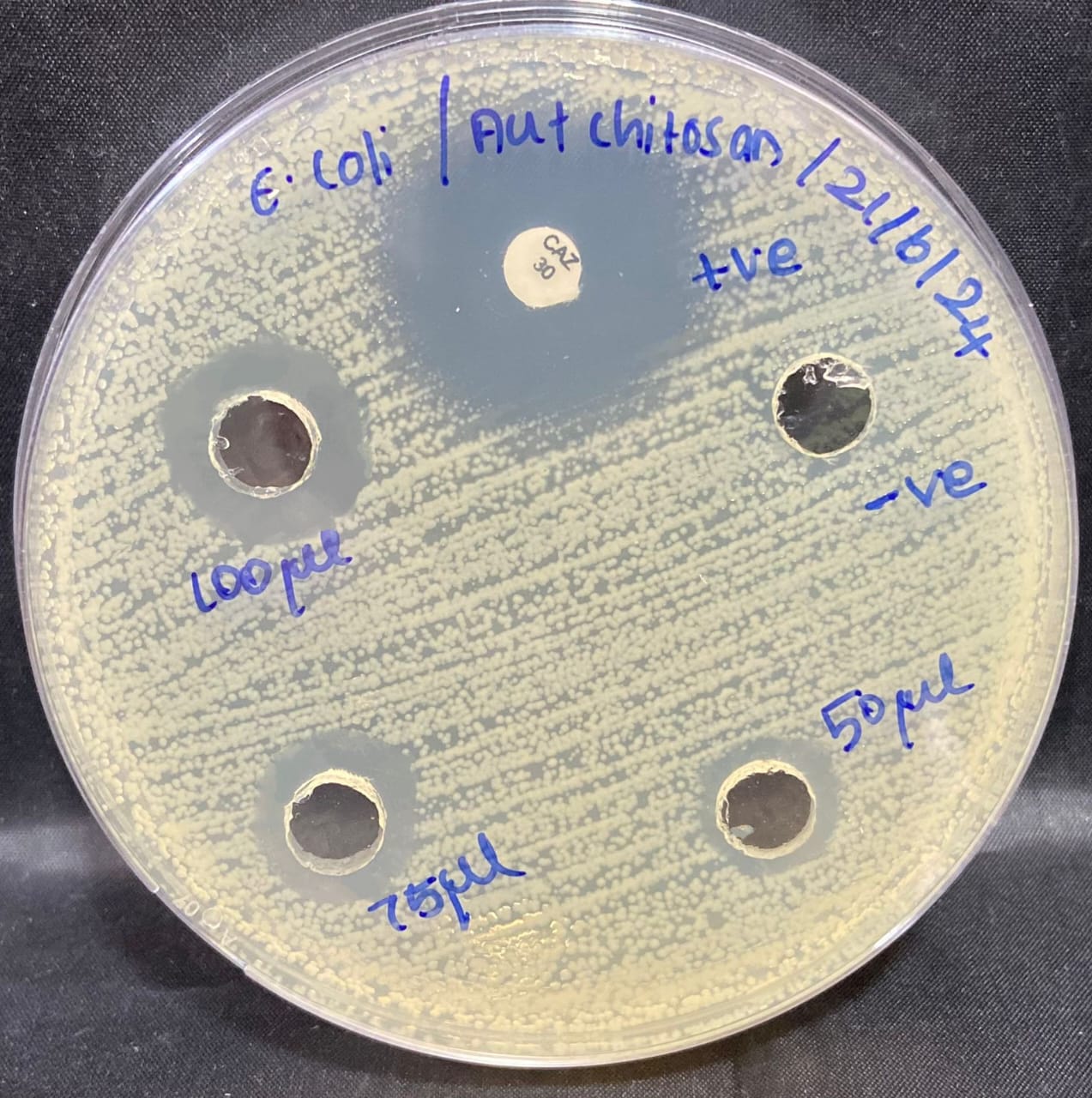
1. (b) (c)

Fig 4:(a) (b) (c) HRTEM images of Au-Chitosan nanoparticles

## Antibacterial Activity

The images and data provided let you see the antibacterial effects of the chitosan-capped nanostructures (Au+Chitosan) against E. coli and Enterococcus. The nanostructures were subjected to three different concentrations 50 µL, 75 µL, and 100 µL, in order to test their effect on the bacteria. The major finding was that a higher inhibition zone was observed in the case of higher drug concentration for both of the bacteria tested. When it comes to E. coli, the sizes of the impact zones were 11 mm, 13 mm, and 15 mm for the drug amounts of 50 µL, 75 µL, and 100 µL. For Enterococcus, the sizes were 11 mm, 13 mm, and 16 mm at the same drug concentrations. The positive controls for each strain, AMP 10 for Enterococcus and CAZ 30 for E. coli, had zone sizes of 22 mm and 25 mm, correspondingly, which is the biggest that was seen and thus the best demonstration of the efficiency of both these standard antibiotics. The negative control displayed minor inhibition and with a zone diameter of 10 mm confirming that the antibacterial action in the test samples was due to the Au+Chitosan. Thus, the outcomes supported the contention that under the correct circumstances, chitosan-capped nanostructures could be employed as a viable treatment to the two pathogens. Much attention is being directed to chitosan-capped gold nanostructures as they hold antibiotic properties that are attractive due to a combination of both chitosan and gold nanoparticles (AuNPs). Chitosan is a natural biopolymer that is known for its capability to break the bacterial cell membrane. This damage happens when chitosan enhances the membrane’s permeability, exposing the essential contents inside the cell and eventually the cell dies(Chehelgerdi et al., 2023). It looks like chitosans can really be used only to fight bacteria as the above mechanism makes them strong.[(And & Kim\*, 2006)](https://paperpile.com/c/ME2hW4/JE1uw)[(“Effect of Chitosan on Epithelial Permeability and Structure,” 1999)](https://paperpile.com/c/ME2hW4/jllAW)

On top of their typical antibacterial characteristics, the nanoparticles of the gold are enhancing the process even more by working through a different way. AuNPs are known for the generation of reactive oxygen species (ROS) through interaction with the bacterial cells. These ROS cause oxidative stress in the bacteria by damaging essential cellular components such as DNA, proteins, and lipids. The damage is significant enough to threaten the bacteria's ability to survive and replicate.[(“Autophagy and Oxidative Stress Associated with Gold Nanoparticles,” 2010)](https://paperpile.com/c/ME2hW4/Zszzh)[(Chompoosor et al., 2010)](https://paperpile.com/c/ME2hW4/HxLuX). One of the most important benefits of chitosan-capped gold nanostructures is the possibility of the inhibition of biofilm formation. Biofilms are jackets that form as a result of the unity of bacterial colonies, making bacteria hard to kill with medicine. The combination of both chitosan's destruction of the membrane and the oxidative stress brought about by AuNPs helps overcome these biofilms and thus aids in the overall antibacterial resistance of the nanoparticles.[(Yu et al., 2016)](https://paperpile.com/c/ME2hW4/RqyQR)[(Walsh et al., 2019)](https://paperpile.com/c/ME2hW4/wImue). In simple words, the study of chitosan followed by the gold nanoparticles at these nanostructures gives a polyhedral approach which is fit to hinder the bacterial disease (Saadh et al., 2024). On one hand, chitosan aims at the disruption of bacterial peace, in the meantime, AuNPs targets oxidative stress and biofilm formation. This free interaction, in turn, produces a profound antibacterial effect. As a result, chitosan-capped gold nanostructures turn up as a good hope for the solution to bacterial infections and the decrease of antibiotic resistance.

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1. **(b)**

Fig.5(a) (b): The antibacterial and anti-microbial effects of Au nanoparticles against Enterococcus bacteria and E.coli bacteria

Table 1: Zone of inhibition values

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Conc** | **Organism** | **E.coli** | **Entero** |
| **Au+chitosan** | 50 |  | 11mm | 11mm |
|  | 75 |  | 13mm | 13mm |
|  | 100 |  | 15mm | 16mm |
|  | Positive C |  | 25mm | 22mm |
|  | Negative C |  | 10mm | 10mm |

# CONCLUSION

In conclusion, This study successfully demonstrates the synthesis and characterization of chitosan-capped gold nanostructures and their significant antibacterial activity. The combination of gold nanoparticles and chitosan offers a promising approach for developing effective antimicrobial materials. Future work should explore the detailed mechanisms of action and potential biomedical applications.Building on the promising results of this study, several avenues for future research are recommended. First, detailed investigations into the mechanisms of antibacterial action at the molecular level are essential. This could involve studying the interactions between chitosan-capped gold nanostructures and bacterial cell membranes using advanced techniques such as atomic force microscopy (AFM) and Raman spectroscopy.

# LIMITATIONS

Gold nanostructures covered with chitosan have a number of drawbacks. The most significant one is stability. The quality and life span of these nanoparticles can be affected by the environment such as acidity and temperature. Another challenge in the industry is the difficulty and costs involved in scaling up the synthesis process for medical or industrial purposes. One important matter arises here-immunotoxicity can be caused by gold nanoparticles and Chitosan safety should be fully evaluated before any adverse effects can be avoided. Also, these nanostructures may face problems in passing across some biological barriers, which may eventually lower their efficiency in treatment or diagnostic applications. Theoretically, adjusting the chitosan coating for properties like imaging and targeting should be possible, however, it is a difficult process needing much research and development.

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