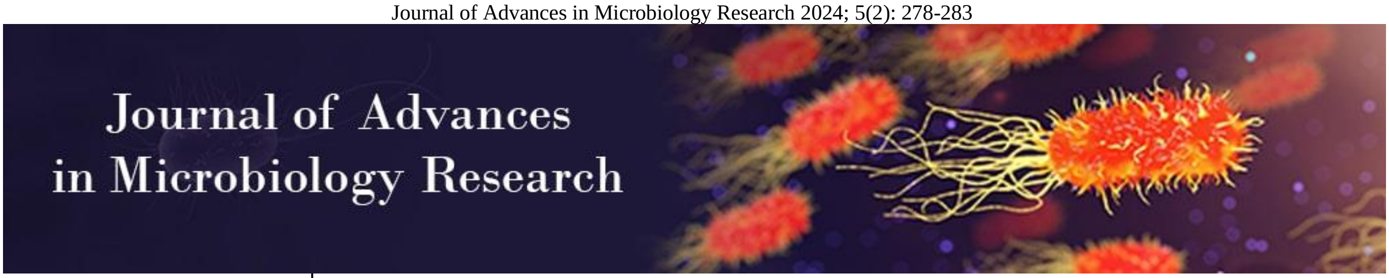

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Silver nanoparticles from chemical synthesis: Assessing their role in antibacterial strategies

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Abstract

Silver nanoparticles (AgNPs) have emerged as potent antibacterial agents due to their unique physicochemical properties and broad-spectrum activity. This study focuses on silver nanoparticles synthesized through chemical methods, evaluating their structural and functional characteristics for antibacterial efficacy. The synthesis process involves chemical reduction techniques, producing nanoparticles with controlled size, shape, and stability. Advanced analytical methods such as UV-Vis spectroscopy, Thermogravimetric Analysis (TGA), Transmission Electron Microscopy (TEM), and X-Ray Diffraction (XRD) were employed to characterize the synthesized nanoparticles. The antibacterial activity was assessed against Gram-positive and Gram-negative bacterial strains using standard antibiotics, revealing significant inhibitory effects attributed to the ability of the nanoparticles to disrupt bacterial membranes and generate Reactive Oxygen Species (ROS). Furthermore, the study examines the influence of nanoparticle concentration, size, and surface modification on their antibacterial potency. The findings highlight the potential of chemically synthesized AgNPs as innovative solutions for combating bacterial infections, offering insights into their integration into biomedical and environmental applications.

Keywords: Nanomedicine, silver nanoparticles, chemical reduction method, antibacterial efficacy

Introduction

The rise in multidrug-resistant bacterial strains has intensified the need for innovative and effective antibacterial agents. Silver nanoparticles (AgNPs) have garnered considerable attention in recent years for their exceptional antibacterial properties, which are attributed to their unique physicochemical characteristics, such as high surface area-to-volume ratio and quantum effects [13, 24, 25]. These properties enable AgNPs to interact with bacterial cells through multiple mechanisms, including disruption of cell membranes, generation of Reactive Oxygen Species (ROS), and interference with DNA replication [11].

Chemical synthesis methods, such as chemical reduction, electrochemical techniques, and solvothermal processes, have been widely employed to produce AgNPs with controlled size, shape, and stability [8]. Among these, chemical reduction is the most common, utilizing reducing agents like trisodium citrate, sodium borohydride, or ascorbic acid to convert silver salts into nanoparticles. These methods allow for fine-tuning of nanoparticle characteristics, which significantly influence their antibacterial efficacy [14].

Studies have demonstrated the broad-spectrum antibacterial activity of chemically synthesized AgNPs against both Gram-positive and Gram-negative bacteria, including resistant strains like *Escherichia coli* and *Staphylococcus aureus* [6]. However, despite extensive research, understanding the precise interactions between AgNPs and bacterial cells remains a challenge. Factors such as nanoparticle size, surface charge, and coating agents play crucial roles in determining their antibacterial activity, antidiabetic activity, antioxidant activity, and cytotoxicity [12, 18, 19, 20, 21, 24, 25, 26, 27].

This study aims to evaluate the antibacterial potential of silver nanoparticles synthesized via chemical reduction. By characterizing the synthesized nanoparticles and assessing their antibacterial activity against selected bacterial strains, this investigation seeks to provide insights into their role in developing effective antibacterial strategies.

Review of Literature

Silver nanoparticles (AgNPs) have been extensively studied for their antimicrobial properties and potential applications in combating drug-resistant bacterial infections. Their broad-spectrum antibacterial activity and multifaceted mechanisms of action have positioned them as promising candidates in nanomedicine [13]. The antibacterial efficacy of AgNPs is primarily attributed to their ability to interact with bacterial membranes, disrupt cellular structures, and generate reactive oxygen species (ROS) that cause oxidative stress [11]. AgNPs can also penetrate bacterial cells, leading to DNA damage and inhibition of essential enzymes [10]. Pal *et al.* (2007) [12] further highlighted that the shape and size of AgNPs significantly influence their antibacterial activity, with smaller nanoparticles exhibiting enhanced efficacy due to higher surface area-to-volume ratios.

The method of synthesis plays a crucial role in determining the physical and chemical properties of AgNPs [22, 23]. Chemical reduction, one of the most common methods, allows for precise control over nanoparticle size and shape, influencing their biological activity [8]. Alternative methods, such as green synthesis and photochemical synthesis, have been explored for eco-friendly production but often face challenges in achieving consistent properties [14].

Several factors influence the antibacterial activity of AgNPs, including size, surface charge, and capping agents. Studies have shown that positively charged AgNPs exhibit stronger interactions with negatively charged bacterial membranes, enhancing antibacterial effects [15]. The type of capping agent used during synthesis can also impact nanoparticle stability and biological compatibility [17].

The integration of AgNPs into biomedical and environmental applications has been widely studied. AgNPs have been incorporated into wound dressings, coatings for medical devices, and water purification systems to leverage their antibacterial properties [6]. Moreover, their use in combination with antibiotics has shown synergistic effects, improving treatment efficacy against resistant bacterial strains [5].

Despite their promising applications, concerns regarding the cytotoxicity and environmental impact of AgNPs persist. AgNPs have been shown to exhibit toxicity toward mammalian cells at higher concentrations, emphasizing the need for careful dose optimization [1, 18]. Additionally, the release of nanoparticles into the environment raises concerns about potential ecological effects, warranting further studies on their long-term impact [4].

This review highlights the significant progress made in understanding the synthesis, antibacterial mechanisms, and applications of AgNPs, while also identifying gaps in addressing toxicity and environmental challenges.

Materials and Methods

Chemical synthesis of silver nanoparticles

An aqueous solution of 0.01 M silver nitrate was heated to boiling, and silver nanoparticles were synthesized using 1% trisodium citrate as a reducing agent. During the process, the solution was vigorously stirred while heating. Upon exposure to 1% trisodium citrate, the aqueous silver ions were reduced, leading to a color change from colorless to pale brown, indicating the formation of silver nanoparticles. The resulting nanoparticle solution was stirred on a magnetic stirrer at 90°C for 20 minutes. The reaction mixture was then centrifuged at 6000 rpm for 15 minutes, and the pellet was collected, rinsed three times with triple-distilled water, and dried in a hot air oven at 80°C [7, 16, 29]. The synthesized silver nanoparticles were subsequently characterized using UV-visible spectroscopy, X-ray Diffraction (XRD), and Transmission Electron Microscopy (TEM).

Characterization of silver nanoparticles

The characterization of silver nanoparticles (AgNPs) synthesized by the chemical reduction method was performed using advanced analytical techniques. UV-VIS spectroscopy (SHIMADZU UV-3150PC) was utilized to monitor the reduction of silver ions within a wavelength range of 200-600 nm, with water as a reference. TEM analysis (JEOL 2000 EX) provided insights into the morphology and structural features of AgNPs, employing a carbon-coated copper grid for sample preparation and operating at 80 keV [9]. X-ray Diffraction (XRD) analysis, conducted using a Rich Seifert P3000 with Cu-Kα1 radiation, was applied to determine crystal structure, phase purity, and nanoparticle size using Bragg's law and the Debye-Scherrer equation [2, 3]. Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) assessed the thermal stability and purity of lyophilized AgNPs (Hitachi STA7200, TA Instruments Q200).

Results

Chemical synthesis of silver nanoparticles

The first visual confirmation of the successful synthesis of silver nanoparticles (AgNPs) was observed during the reaction process. When aqueous silver ions were exposed to a 1% solution of trisodium citrate, a reduction reaction occurred, resulting in a distinct color change from colorless to pale brown (Figure 1). This color change is a clear indication of the formation of silver nanoparticles.

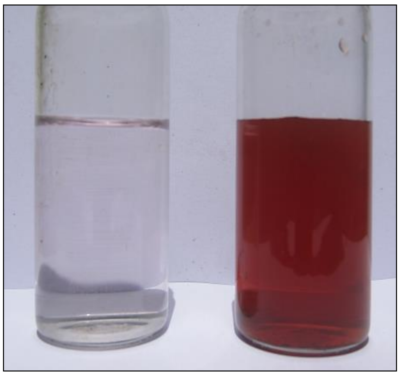

Fig 1: Synthesis of silver nanoparticles by chemical reduction method

Characterization of silver nanoparticles

The chemically synthesized silver nanoparticles (AgNPs) were thoroughly characterized using various analytical techniques. The UV-Vis spectrophotometer analysis revealed a distinct absorption maximum at 420 nm, indicating the formation of polydisperse AgNPs. The peak broadening suggested that the AgNPs had a wide size distribution. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the AgNPs, exhibiting a face-centered cubic (FCC) structure. The XRD pattern showed four distinct diffraction peaks, corresponding to the {111}, {200}, and {220} planes, with a particle size range of 10-50 nm. Thermogravimetric analysis (TGA) was performed to assess the purity and thermal stability of the AgNPs. The results indicated that the AgNPs had a stability of up to 300°C, with a minor coating of chemical molecules on the surface at around 250°C. Transmission Electron Microscopy (TEM) analysis revealed the diverse morphology of the

AgNPs, including spherical, cubic, and hexagonal shapes, with a size range of 5-50 nm. The TEM images also showed the aggregation of the nanoparticles. The comprehensive characterization of the chemically synthesized AgNPs using UV-Vis spectroscopy, XRD, TGA, and TEM provided insights into their physical and chemical properties, including their size, shape, crystallinity, and thermal stability.

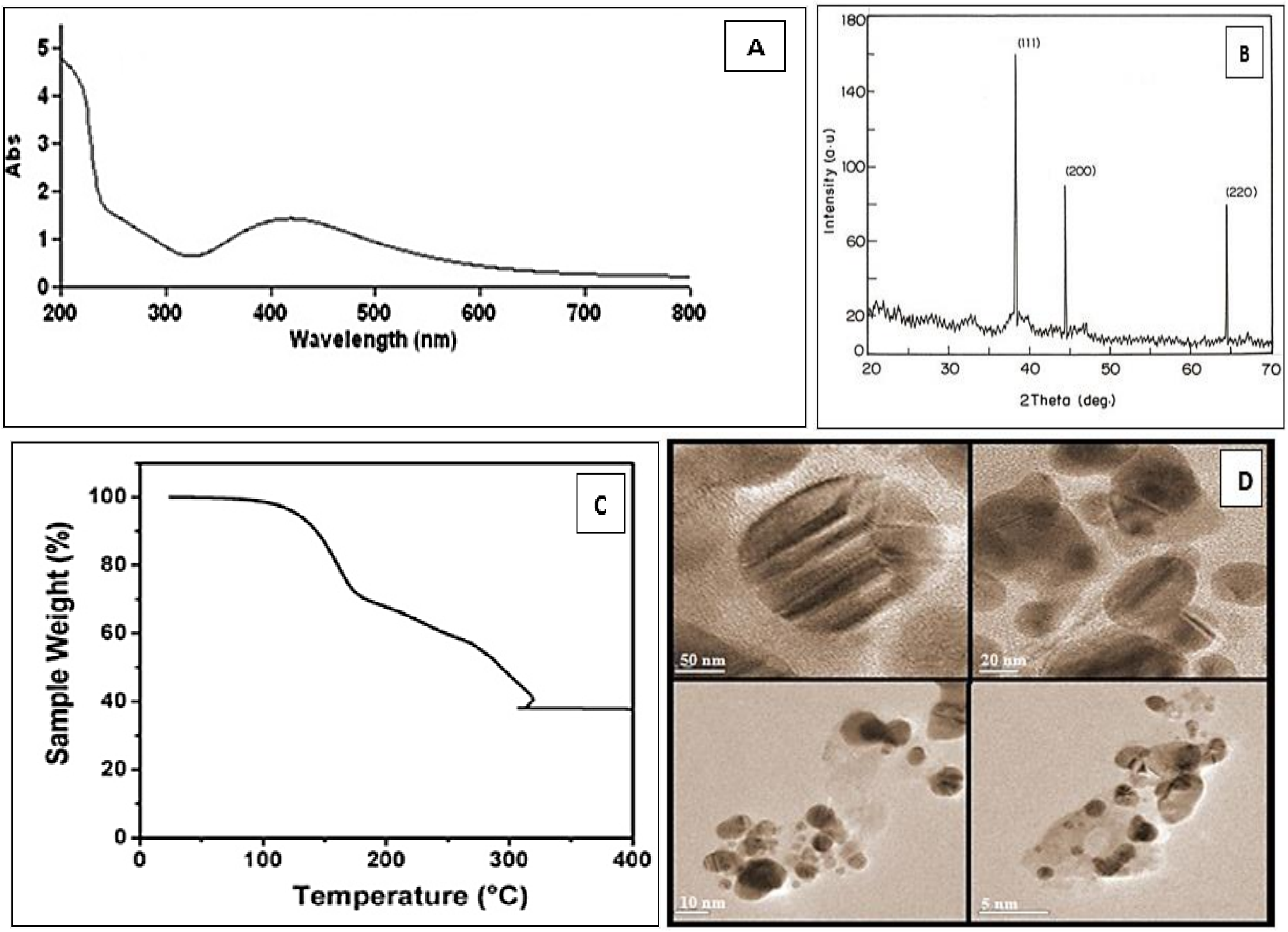

Fig 2: Characterization of silver nanoparticles by chemical reduction method: [A] UV-Vis Spectroscopy analysis, [B] XRD diffraction analysis, [C] Thermogravimetric analysis, [D] Transmission electron microscopic analysis

Antibacterial potential of chemically synthesized silver nanoparticles: A comparative evaluation with standard antibiotics

The antibacterial efficacy of silver nanoparticles (AgNPs) synthesized via chemical reduction was investigated and compared with several standard antibiotics, including erythromycin, polymyxin, streptomycin, and tetracycline. The study was conducted on bacterial cultures of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (S. aureus), and *Proteus vulgaris* (*P. vulgaris*). Antimicrobial susceptibility was assessed using the Kirby-Bauer disc diffusion method, with zones of inhibition measured to determine bacterial growth suppression.

The synthesized AgNPs by the chemical reduction method demonstrated significant antimicrobial activity against all tested organisms. The observed zones of inhibition for AgNPs ranged from 6.5 to 10.5 mm in diameter, indicating notable efficacy. These results, as summarized in Table 1 and illustrated in Figures 3 and 4, were compared against the inhibition zones produced by standard antibiotics.

Among the antibiotics tested, streptomycin exhibited the highest zones of inhibition against both Gram-positive and Gram-negative bacteria, showcasing superior antibacterial activity. In comparison, AgNPs synthesized via the chemical reduction method exhibited moderate to good activity against the tested pathogens. Notably, the AgNPs demonstrated greater antibacterial efficacy against *E. coli* and *P. vulgaris* but were less effective against *S. aureus*.

The findings highlight the potential of AgNPs as a robust alternative to conventional antibiotics, particularly for combating Gram-negative bacterial pathogens. The study underscores the consistent and effective antibacterial properties of AgNPs synthesized chemically, making them a promising candidate for addressing the challenge of antibiotic resistance in human pathogenic bacteria.

These results provide valuable insights into the development of nanomaterial-based antibacterial strategies and emphasize the need for further research into optimizing the synthesis and application of AgNPs for clinical and environmental use.

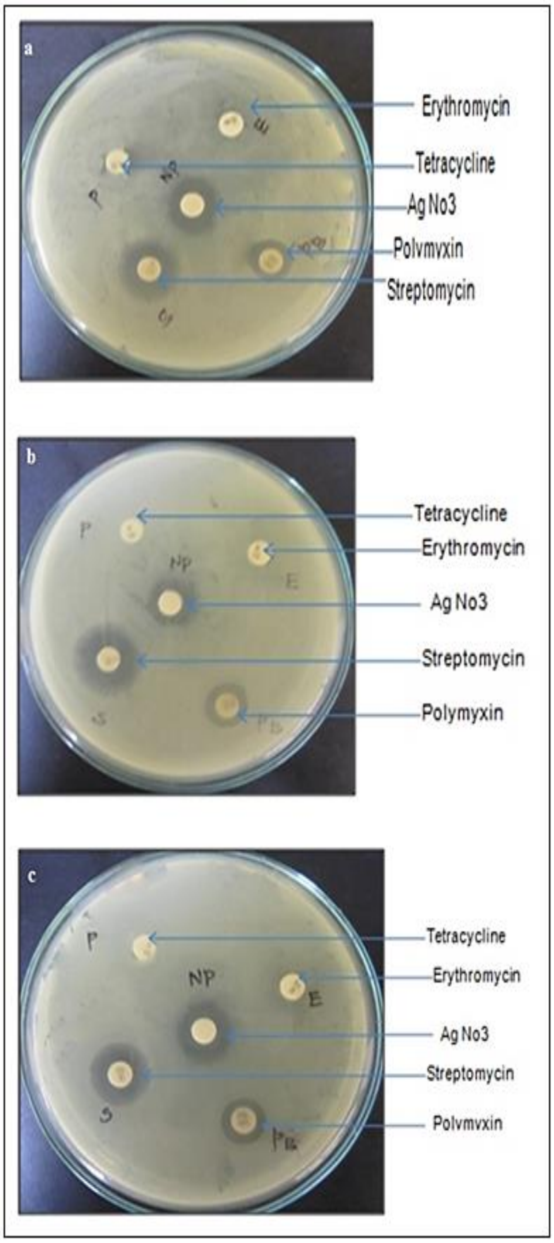

Fig 3: Antimicrobial susceptibility test (Kirby -Bauer method) of silver nanoparticles synthesized by chemical reduction method (a) *Escherichia coli* (b) *Staphylococcus aureus* (c) *Proteus vulgaris*

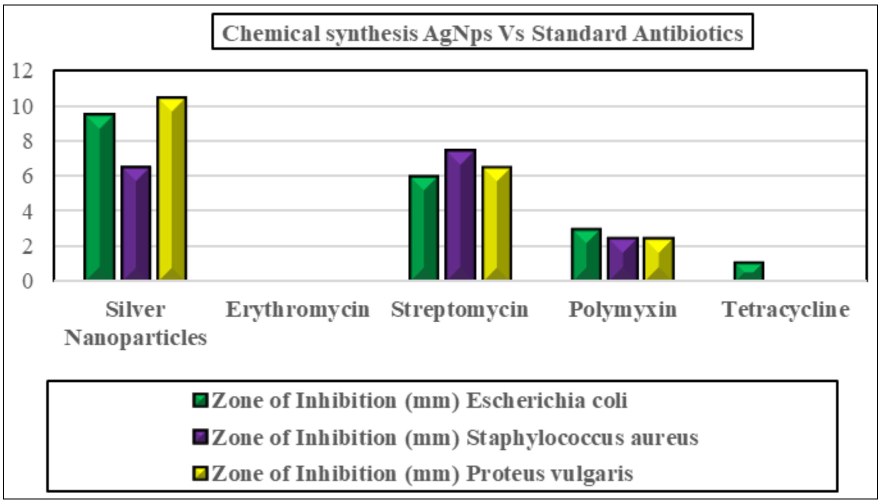

Fig 4: Antimicrobial susceptibility test (Kirby -Bauer method) of silver nanoparticles synthesized by chemical reduction method (a) *Escherichia coli* (b) *Staphylococcus aureus* (c) *Proteus vulgaris*

Table 1: Antimicrobial susceptibility test (Kirby -Bauer method) of silver nanoparticles synthesized by chemical reduction method (a) *Escherichia coli* (b) *Staphylococcus aureus* (c) *Proteus vulgaris*

S. No	AgNPs by Chemical reduction method/ Standard antibiotics	Zone of inhibition (mm)		
		Escherichia coli	***Staphylococcus aureus***	***Proteus vulgaris***
1	Silver Nanoparticles	9.5	6.5	10.5
2	Erythromycin	0	0	0
3	Streptomycin	6	7.5	6.5
4	Polymyxin	3	2.5	2.5
5	Tetracycline	1.1	0	0

Discussion

The emergence of multidrug-resistant bacterial strains poses a significant challenge to global public health, necessitating the development of alternative antimicrobial agents. This study explored the antibacterial potential of chemically synthesized silver nanoparticles (AgNPs) and compared their efficacy with standard antibiotics such as erythromycin, polymyxin, streptomycin, and tetracycline. The findings revealed that AgNPs exhibit promising antibacterial activity, though with varying degrees of effectiveness across different bacterial species.

Among the tested antibiotics, streptomycin demonstrated the highest antibacterial activity, producing the largest zones of inhibition against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Proteus vulgaris*) bacterial strains. This aligns with its established efficacy as a broad-spectrum antibiotic. In comparison, the chemically synthesized AgNPs showed moderate to good antibacterial activity, particularly against Gram-negative bacteria such as *E. coli* and *P. vulgaris*. These results highlight the potential of AgNPs as a complementary or alternative antimicrobial agent, especially in cases where conventional antibiotics may fail due to resistance.

The study observed that AgNPs were more effective against Gram-negative bacteria (*E. coli* and *P. vulgaris*) than Gram-positive *S. aureus*. This can be attributed to structural differences between the bacterial cell walls. Gram-negative bacteria possess an outer membrane containing lipopolysaccharides, which AgNPs can penetrate more easily, leading to membrane disruption and oxidative stress. In contrast, the thicker peptidoglycan layer of Gram-positive bacteria may offer a protective barrier, reducing the efficacy of AgNPs. These findings are consistent with prior studies highlighting the preferential action of silver nanoparticles on Gram-negative bacteria.

The antibacterial activity of AgNPs is thought to arise from their multifaceted mechanisms, including the release of silver ions (Ag^+), which interact with bacterial membranes, proteins, and DNA. The generation of Reactive Oxygen Species (ROS) further exacerbates cellular damage, leading to bacterial death. The size and surface properties of the nanoparticles also play a critical role in their activity. Smaller nanoparticles, with higher surface area-to-volume ratios, have greater contact with bacterial cells, enhancing their antimicrobial action.

The moderate to good antibacterial activity of AgNPs against pathogenic bacteria underscores their potential as a robust alternative to traditional antibiotics. AgNPs could be particularly valuable in addressing antibiotic-resistant infections, either as standalone agents or in combination with existing antibiotics to achieve synergistic effects. Moreover, their broad-spectrum activity positions them as promising candidates for use in medical devices, wound dressings, and water purification systems.

Despite their promising potential, the efficacy of AgNPs observed in this study was lower than that of streptomycin, highlighting the need for further optimization. Factors such as nanoparticle size, shape, concentration, and surface functionalization must be explored to enhance their antibacterial properties. Additionally, the observed differences in efficacy against Gram-positive and Gram-negative bacteria call for a deeper investigation into the interaction mechanisms of AgNPs with various bacterial cell wall structures.

Another critical consideration is the potential cytotoxicity and environmental impact of AgNPs. While their antibacterial properties are beneficial, excessive use of silver nanoparticles may pose risks to human health and ecosystems. Future studies should focus on evaluating the biocompatibility of AgNPs and developing eco-friendly synthesis methods to minimize their environmental footprint.

Conclusion

This study demonstrates that chemically synthesized AgNPs exhibit significant antibacterial activity, particularly against Gram-negative bacteria. Although their efficacy does not surpass that of streptomycin, they present a viable alternative for combating antibiotic-resistant pathogens. The findings contribute to the growing body of evidence supporting the potential of nanomaterials in addressing the global challenge of antimicrobial resistance. Further research is warranted to optimize AgNP synthesis, enhance their efficacy, and explore their practical applications in clinical and environmental settings.

References

1. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009;3(2):279-290.
2. Bragg WL, Bragg WH. The reflection of X-rays by crystals. Proc R Soc A. 1949;88(2):428-438.
3. Das SK, *et al*. Biosynthesis of metallic nanoparticles using microorganisms. Colloids Surf B Biointerfaces. 2009;71(2):198-204.
4. Fabrega J, Luoma SN, Tyler CR, Galloway TS, Lead JR. Silver nanoparticles: Behaviour and effects in the aquatic environment. Environ Int. 2011;37(2):517-531.
5. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against Gram-positive and Gram-negative bacteria. Nanomedicine. 2010;6(1):103-109.
6. Gogoi SK, Gopinath P, Paul A, Ramesh A, Ghosh SS, Chattopadhyay A. Green fluorescent protein-expressing *Escherichia coli* as a model system for investigating the antimicrobial activities of silver nanoparticles. Langmuir. 2006;22(22):9322-9328.
7. Gudikandula K, Maringanti SC. Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. J Exp Nanosci. 2016;11(9):714-721.
8. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles:

Chemical, physical and biological methods. Res Pharm Sci. 2014;9(6):385-406.

9. Lin X, *et al*. Nanoparticles for biomedical applications: Characterization and properties. Nanotechnology. 2014;25(45):4501-4508.
10. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, *et al*. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res. 2006;5(4):916-924.
11. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16(10):2346.
12. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl Environ Microbiol. 2007;73(6):1712-17120.
13. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27(1):76-83.
14. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv Colloid Interface Sci. 2009;145(1-2):83-96.
15. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology. 2007;18(22):225103.
16. Soliwoda KR, Tomaszewska E, Socha E, Krzyczmonik P, Ignaczak A, Orlowski P, Krzyzowska M, Celichowski G, Grobelny J. The role of tannic acid and sodium citrate in the synthesis of silver nanoparticles. J Nanopart Res. 2017;19(273):1-15.
17. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004;275(1):177-182.
18. Manam VK, Subbaiah M. *In vitro* cytotoxic activity of silver nanoparticles biosynthesized from *Colpomenia sinuosa* and *Halymenia porphyroides* using DLA and EAC cell lines. World J Pharm Sci. 2014;2(9):926-930.
19. Manam VK, Subbaiah M. Biochemical investigation of marine seaweeds *Colpomenia sinuosa* and *Halymenia porphyroides* collected along the southeast coast of Tamil Nadu, India. Eur J Biomed Pharm Sci. 2020;4(3):414-717.
20. Manam VK, Subbaiah M. Biogenic silver nanoparticles by *Halymenia porphyroides* and its *in vitro* anti-diabetic efficacy. J Chem Pharm Res. 2013;5(12):1001-1008.
21. Manam VK, Subbaiah M. Biological synthesis of silver nanoparticles from marine alga *Colpomenia sinuosa* and its *in vitro* anti-diabetic activity. Am J Biopharm Biochem Life Sci. 2014;3(1):1-7.
22. Manam VK, Subbaiah M. Biosynthesis and characterization of silver nanoparticles from marine macroscopic red seaweed *Halymenia porphyroides* Boergesen (Crypton). J Nanosci Technol. 2020;6(2):886-890.
23. Manam VK, Subbaiah M. Biosynthesis and characterization of silver nanoparticles from marine macroscopic brown seaweed *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier. J Adv Chem Sci. 2020;6(1):663-666.
24. Manam VK, Subbaiah M. Biosynthesis of silver nanoparticles from marine alga *Halymenia porphyroides* and its antibacterial efficacy. Int J Curr Microbiol Appl Sci. 2014;3(4):96-103.
25. Manam VK, Subbaiah M. Biosynthesis of silver nanoparticles from marine alga *Colpomenia sinuosa* and its antibacterial efficacy. Int J Curr Microbiol Appl Sci. 2014;3(4):887-893.
26. Manam VK, Subbaiah M. *In vitro* antioxidant activity of silver nanoparticles from *Colpomenia sinuosa* and *Halymenia porphyroides*. World J Pharm Sci. 2014;2(8):817-820.
27. Manam VK, Subbaiah M. Phytochemical, amino acid, fatty acid and vitamin investigation of marine seaweeds *Colpomenia sinuosa* and *Halymenia porphyroides* collected along the Southeast coast of Tamil Nadu, India. World J Pharm Res. 2020;9(4):1088-1102.
28. Yerragopu PS, Hiregoudar S, Nidoni U, Ramappa KT, Sreenivas AG, Doddagoudar SR. Chemical synthesis of silver nanoparticles using tri-sodium citrate: Stability study and their characterization. Int Res J Pure Appl Chem. 2020;21(3):37-50.

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