THE EFFECTIVENESS Of SILVER NANOPARTICLES AS ANTIBACTERIAL AGENTS AGAINST MDR BACTERIA

[1]Suman Lata, [2]Kavitha Kumari*, [3]Jasbir Sangwan*, [4]Vijay Kiran

[1,2]Department of Chemistry, Baba Mastnath University, Asthal Bohar, Rohtak, Haryana, India
[3]Department of Chemistry, TDL Govt. College for Women, Murthal, Sonipat, Haryana, India
[4]Department of Chemistry, Chhotu Ram Arya College, Sonipat, Haryana, India

***Email ID**: [2]kavithakumari13@gamil.com, [3]sangwanjasbir@gmail.com*

Accepted: 08.10.2022 **Published**: 01.11.2022

Keywords: Silver Nanoparticles, Acinetobacter Baumannii, Staphylococcus Aureus, and Enterococci, etc

Abstract

The current study aims to explore a novel and cost-effective nano-therapeutic strategy for addressing Acinetobacter baumannii, Staphylococcus aureus, and Enterococci. This study presents a sustainable method for the swift production of silver nanoparticles (Ag-NPs) using an aqueous leaf extract derived from Corchorus Capsularis (CRCP). The production of stable silver nanoparticles (Ag-NPs) at various time intervals predominantly yields spherical particles with sizes ranging from 12 to 60 nm. The produced Ag-NPs were characterized using a variety of methods, TEM, FTIR, and UV-visible spectroscopy. According to the results of extensive investigation, it has been found that Ag-NPs are produced, with an average size of 22.32 nm. The goal of the study was to evaluate the antibacterial effectiveness of the created silver nanoparticles (Ag-NPs) against MDR strains of Acinetobacter baumannii, staphylococcus aureus, and enterococci that were isolated from infections in post-surgical wounds. The current investigation proposes that silver nanoparticles (Ag-NPs) produced using a water-based leaf extract of CRCP exhibit noteworthy antibacterial properties against multi-drug resistant (MDR) strains.

Paper Identification

**Corresponding Author*

Introduction

Postoperative wound infections are a prevalent issue worldwide within the realm of surgical procedures, leading to extended hospitalization, increased healthcare costs, and heightened rates of death and morbidity [1-3]. The majority of surgical wound infections are acquired inside the hospital setting and exhibit variability across different hospitals [4]. Various studies have documented the incidence of post-surgical wound infections, with reported rates ranging from 14.8% to 60%. These infections are mostly attributed to the presence of Acinetobacter baumannii, Staphylococcus aureus, and Enterococci, which are among the most frequently identified pathogens in such cases [3,5]. The absence of standardized criteria for diagnosis is a significant obstacle in tracking the worldwide epidemiology of

surgical site infection. Furthermore, the emergence of significant antimicrobial resistance among bacterial pathogens has posed challenges in effectively managing post-operative wound infections [6].

The potential of microorganisms and vascular plants has been highlighted for use in several biological strategies [7,8,9]. Even at very low concentrations, Ag-NPs have been shown in the literature to be non-toxic and highly effective against bacteria, viruses, and other eukaryotic microorganisms without causing any negative effects [10]. several chemical and physical procedures have been devised to synthesize metal nanoparticles (MNPs) to address practical applications in biomedical fields, catalysis, detection, and storage batteries, among others. The research on magnetic nanoparticles (MNPs) is currently focused on investigating environmentally friendly approaches for their manufacture and utilization in many fields such as antibacterial, antioxidant, and anticancer activities. The utilization of biosynthetic processes has garnered significant interest as a feasible option for the production of MNPs. In this approach, the synthesis of MNPs is achieved using plant extracts, without the incorporation of any chemical components [21-25]. The powerful microbicidal properties of nano-silver against bacteria, fungi, and viruses have received widespread recognition [25]. Silver nanoparticles' (Ag-NPs) substantial antibacterial effectiveness may be related to their highly developed surface area, which allows for the most possible interaction with the microbial environment [27]. In this study, we describe the process of synthesizing silver nanoparticles (Ag-NPs) utilizing leaf extract from the CRCP. The leaf extract reduces and stabilizes the nanoparticles simultaneously. For its stimulant, laxative, appetizer, demulcent, and stomachic qualities, the leaves of CRCP have been widely used. A commercially available mixture that contains CRCP is also used to treat dyspepsia, liver issues, diarrhea, constipation fever, and constipation.

Moreover, the utilization of a decoction derived from nnripe fruits or roots has been documented as a remedy for dysentery [28]. The biologically synthesized silver nanoparticles (Ag-NPs) were subjected to characterization using several analytical techniques, including X-ray diffraction (XRD), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), selected area electron diffraction (SAED), and ultraviolet-visible spectroscopy (UV-vis). Utilizing FT-IR spectroscopy, it was possible to validate the interaction between the biomolecules in the extract and the Ag-NPs. A comprehensive investigation into the antibacterial properties has been conducted, employing disk diffusion and growth kinetics techniques, while varying the concentration of AgNPs. For the growth kinetics test, cytotoxicity assay, and bactericidal activity assay, Acinetobacter baumannii, Staphylococcus aureus, and Enterococci isolates from post-surgical wound infections were used in a liquid culture.

Experimental

Materials

The silver nitrate ($AgNO_3$) used in this study was acquired from Merck (India) and had a purity level exceeding 99.9%. All of the reagents used in this study were of analytical purity and weren't further purified before use. To create Ag-NPs, the leaf of the Indian medicinal plant CRCP was collected on the grounds of MDU, Rohtak. Acinetobacter baumannii, Staphylococcus aureus, and enterococci-resistant bacterial strains were used in the antibacterial research. These strains were procured from CSIR-IMTECH Chandigarh.

Green Ag-NP synthesis

In order to get rid of dirt and other impurities, the CRCP leaf was cleaned. The leaves were then dried for an hour at room temperature after being placed on filter paper to absorb moisture. The leaves weighed six

grams and were fragmented. Then, in an Erlenmeyer flask with 300 mL of sterile distilled water, the leaves were cooked for 15 minutes at 90°C. The mixture then cooled until it reached room temperature. The leaf extract was boiled, cooled, and then double-filtered. Green silver nanoparticles were created using the extract of the pale-yellow leaf. A solution of 1.5 mM $AgNO_3$ in 200 mL water was added to an Erlenmeyer flask to create Ag-NPs. In the same flask, 200 mL of CRCP leaf extract was added. The leaf extract becomes brown after 10 minutes. This hue change indicates silver nanoparticles. The tint thickens and stays that way for an hour. Active surface Plasmon vibrations in MNPs cause color changes in aqueous solutions, indicating Ag-NPs. Centrifuging was used to create silver nanoparticles (Ag-NPs) during an 8-minute period at 2500 rpm. The filtrate was repeatedly centrifuged and reconstituted in water to eliminate organic debris. Ag-NPs were centrifuged and then rinsed with deionized water three times. Ag-NPs were then lyophilized after that. Ag-NPs were lyophilized and then kept for analysis and use in a screw-top container [26].

Characterization of synthesized Ag-NPs

Zetasizer Nano ZS-90 was employed to compute the average size range of nanoparticles and their size distribution. The morphology of nanoparticles was examined by transmission electron microscope (TEM: Hitachi H7500). The nanoparticles were loaded on a copper grid and 60,000 magnification factors and 80,000V accelerating voltage were employed to capture the TEM image. The scanning electron microscope (SEM) is capable of generating high-resolution images of individual nanoparticles with dimensions significantly less than 10 nm. The scanning electron microscope (SEM) is capable of generating high-resolution images of individual nanoparticles with dimensions significantly less than 10 nm. The Shimadzu FT-IR-8201 PC was used to do the FT-IR analysis. With the help of a Jasco V-560 double-beam spectrophotometer, the UV-visible spectrum was examined.

Disc diffusion assay

The researchers tested silver nanoparticles (Ag-NPs) against Acinetobacter baumannii, Staphylococcus aureus, and Enterococci using disc diffusion. Aseptic methods were used to create an inoculum for each bacterial isolate to test Ag-NPs' antibacterial properties [27]. Adjusted inoculum cell density met the threshold of 0.5 McFarland turbidity. On Mueller Hinton agar (MHA) plates, the inoculum was then dispersed uniformly. A Whatman 6-mm 1.0 filter paper disc was saturated with silver nanoparticles (Ag-NPs) at 20, 40, and 60 mg/mL. Impregnated discs were air-dried for 30 minutes at ambient temperature under aseptic circumstances. Ag-NPs-impregnated discs were seeded into agar plates and incubated inverted at 37°C for 24 hours. A standard Gentamycin (15 mg) antibiotic disc was the positive control in this study.

Ag-NPs' bactericidal effects on Staphylococcus aureus, enterococci, and Acinetobacter baumannii

To ascertain their bactericidal activity, 106 CFU of Acinetobacter baumannii, Staphylococcus aureus, and Enterococci were individually added to silver nanoparticles (Ag-NPs) at concentrations of 20, 40, 60, 80, and 100 mg/mL. There was also a control group using lyophilized plant extract at 100 mg/mL but no Ag-NPs. The preparations were placed on LB agar plates after an hour of incubation at 37°C. After 24 hours of incubation at 37°C, the plates were counted by an automatic colony counter [28].

Acinetobacter baumannii, Staphylococcus aureus, and enterococci growth kinetics analysis

At Ag-NP concentrations of 20, 40, 60, 80, and 100 mg/mL, the study looked at the kinetics of bacterial growth. In LB broth, the bacterial isolates were cultured and observed. A control group was made by

maintaining a lyophilized plant extract at 100 mg/mL without Ag-NPs. Every three hours, the samples were incubated at 37°C while the optical density (OD) was measured at 660 nm [29].

Anti-Inflammatory Activity

Ag-NPs' anti-inflammatory effects were tested utilizing HRBC (Human red blood cell) membrane stabilization at doses from 0.110 to 0.400 mg/ml [23]. A healthy participant's blood sample was centrifuged at 3000 RPM for 10 minutes. Using a micropipette, packed cells were collected. The method was repeated two to three times to obtain a clear supernatant. The assay mixture contained 1 ml of samples with different concentrations, 1 ml of phosphate buffer, 2 ml of hyposaline solution, and 0.5 ml of HRBCs, carefully mixed. The experiment's control group received distilled water instead of hyposaline.

Ag-NPs are tested for cytotoxicity in vitro (MTT)

Silver nanoparticle cytotoxicity assay in vitro using MTT ((3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide). Ag-NPS which has been purified and lyophilized was examined for cytotoxicity against Hela cells. The cells were grown in a 25 cm2 flask containing DMEM (Dulbecco's Modified Eagle Medium), 10% Fetal Bovine Serum (FBS), 100 IU/ml Penicillin, 100 mg/ml Streptomycin, and 50 mg/ml Gentamycin. The cells were cultivated in monolayers at 37 °C in culture flasks with 5% CO2 in the air in a CO2 incubator. Passage 20 or lower cells were employed in the tests. The serum medium was changed to a serum-free medium containing 1.5 mg/ml lyophilized Ag-BNPs dissolved in DMSO during the experiment. At -20°C, the stock solution was retained. Low 1.0% was the final DMSO concentration. In 96-well plates, 1,105 Hela cell lines were inserted. Cells were treated with various concentrations of lyophilized green AgNPs produced from a plant extract after adhering for 6 hours. From 1.5 to 200 mg/ml was serially diluted in each well. The duration of the therapy was 6, 12, 24, 48, and 72 hours. Each well got 100 cc (50 mg) of MTT dye following treatment. Equal quantities of DMSO were used to maintain the control groups. For 2,000 hours, the plates were in an incubator with 5% carbon dioxide. After a 3-hour incubation to determine the cytotoxicity of silver nanoparticles (Ag-NPS) on Hela cell lines, the dye was removed using dimethyl sulfoxide. A 96-well microplate reader was used to measure the optical density at a wavelength of 570 nm [30].

Results and discussion

Particle size and Zeta potential

The size and zeta (stability) potential of Ag-NPs were assessed using dynamic light scattering. The optimal size of the nanoparticles was determined to be 132.6 nm, as depicted in Figure 1. The zeta potential of Ag-NPs is measured to be -18.1 mV, as depicted in Figure 2. This value indicates the stability of the nanoformulations.

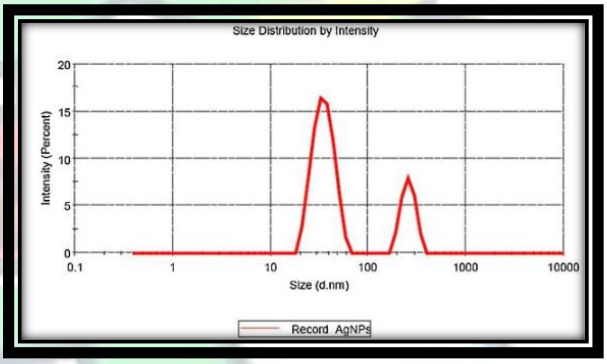

Figure 1. PSA image of Nanoparticles.

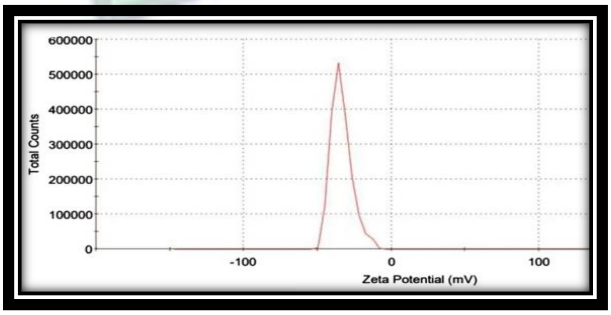

Figure 2. Zeta potential of Nanoparticles.

Resonant surface plasmons

Magnetic nanoparticle structure characterization relies heavily on UV-Vis spectroscopy. By decreasing Ag^+ ions, UV-Vis spectroscopy is used to evaluate Ag-NP production and stability (Fig 3). The surface plasmon resonance of spherical Ag-NPs is represented by a peak at 435 nm [30-34]. This peak represents the presence of Ag-NPs in the reaction mixture. At regular intervals (5 min, 10 min, 20 min, 30 min, 40 min, 50 min, and 1 h), the solution's absorbance was measured as its color changed throughout the synthesis. To test nanoparticle stability, the suspension was maintained for a month. One month later, nanoparticle absorption was unchanged, indicating stability. According to Figure 3, nanoparticles can survive at 28°C for at least one month.

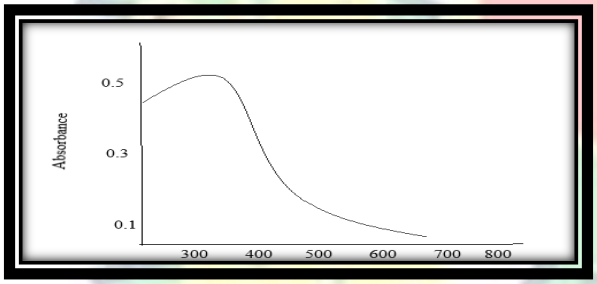

Fig 3 UV Vis absorption spectra of Ag-NPs synthesized from leaf extract of Corchorus Capsularis.

Size and morphology study

Transmission electron microscopy examined Ag-NP particle size and form. Figure 4A shows the synthesized Ag-NPs' TEM image, and frequency distribution histogram. The TEM picture confirms that silver particles are nanoscale and spherical/ellipsoidal. Nanoparticle diameters range from 12 nm to 60 nm. particles, as shown by TEM pictures in Figure 4. Histograms show particle sizes from 12 to 60 nm. Histogram analysis showed that the average nanoparticle size was 22.32 nm. This confirms Ag-NPs' exceptional crystallinity [35]. SEM images showed spherical nanoparticles (Figure 4B) Particle sizes range from 2 nm to 3 mm. Nanoparticles stay stable in aqueous solutions for a month at room temperature. [36-40].

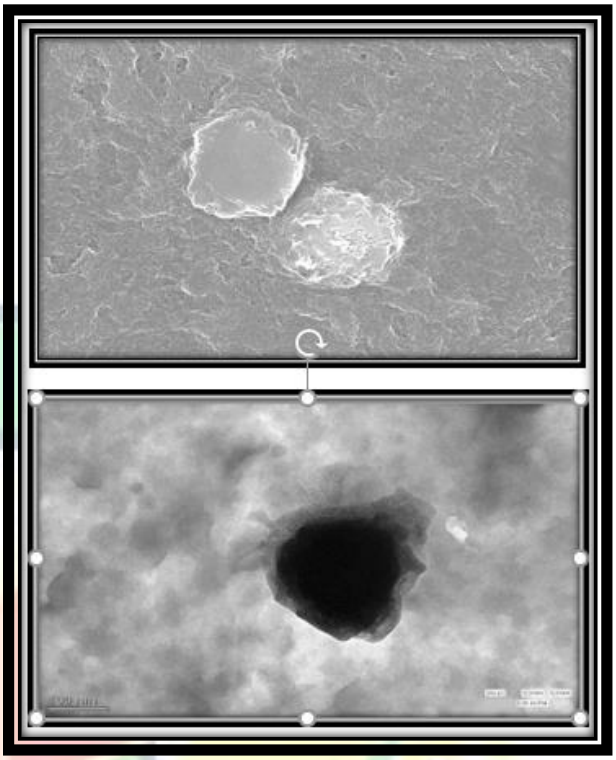

Figure 4: TEM (a), SEM (b) images of Nanoparticles

Potentially implicated reductive functional groups in the synthesis of Ag-NPs

The production of Ag-NP by the CRCP plant was evaluated. Its high phytochemical content made it possible to make Ag-NPs. The phenolic OH, carbonyl, and amino groups in the CRCP extract are confirmed by FT-IR spectroscopy. These groups reduce and stabilize Ag-NP synthesis, preventing agglomeration. Figure 5 shows CRCP functional group band locations from FTIR analysis.

The CRCP leaf extract's amine (N-H), carboxyl (C=O), and hydroxyl (OH) functional groups reduce Ag+ ions to AgO nanoparticles, as seen by FTIR peak location and intensity variations. The FTIR spectroscopic investigation also shows that the phenolic compound's hydroxyl group (OH) and the CRCP extract's protein component lessen and stabilize silver nanoparticles

(Ag-NPs), preventing their aggregation [41]. The significant attraction of amino acid residue carbonyl groups for metal suggests a protective layer around Ag-NPs [28]. This layer stabilizes Ag-NPs in water, preventing agglomeration (Fig 5).

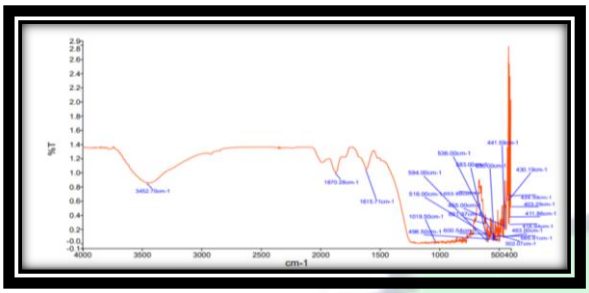

Fig 5: FTIR spectra of Ag-NPs synthesized from leaf extract of Corchorus Capsularis.

Disc diffusion assay

Silver nanoparticles (Ag-NPs) were tested for antibacterial activity against Acinetobacter baumannii, staphylococcus aureus, and Enterococci using the modified Kirby-Bauer disc diffusion assay. A substantial vulnerability to silver nanoparticles' antibacterial efficacy was found. These isolates' zone of inhibition (ZOI) averaged 19 mm, 18 mm, and 14 mm at 50 mg/mL Ag-NPs. The zone of inhibition (ZOI) has a linear relationship with silver nanoparticle (Ag-NP) concentration, consistent with prior research [42]. Compared to Gentamycin, Ag-NPs possess better antimicrobial activity. Ag-exposed clinical bacterial pathogens NPs have reduced cell replication. Due to membrane permeability, Ag-NPs inhibited cellular function. Leaking slows the development of cells. Ag-NPs have an instantaneous impact on a number of bacterial macromolecules, in particular membrane proteins, which can result in cell death and fragmentation. Ag-NPs produced by green synthesis are more bacterially effective and biocompatible and use of biologically active Ag-NPs produced chemically. Abiotically produced Ag-NPs were tested for antibacterial activity and size dependence. The phenomenon under inquiry depends on size, specifically total surface area and bigger surface area.

Growth kinetics assay

In Figure 6, 106 CFU were treated with Ag-NPs at 20, 40, 60, 80, and 100 mg/mL to investigate the kinetics of bacterial growth. By monitoring the optical density (OD) at 600 nm every three hours for 24 hours, the growth kinetics were investigated. According to the graph, bacterial cells get smaller with increasing Ag-NP exposure time and concentration. Furthermore, AgNPs do not reduce bacterial growth in the absence of therapy. Cells survive till decay. Due to their small size, Ag-NPs can easily infiltrate the cell wall, increasing their antibacterial action. Penetration of the bacterial cell wall by Ag-NPs causes structural changes like perforations. This releases internal parts. In the interior, Ag-NPs release silver ions. This release of silver ions generates ROS which influence electron transport via interacting with membrane proteins. ROS are important for antibacterial action [46]. Electrostatic interaction between the negatively charged Ag-NPs' modest positive charge and the cell membrane may explain their antibacterial effect. Bacteria may receive more Ag ions from silver nanoparticles (Ag-NPs). Ag-NPs lower local pH by influencing the bacteria's proton motive force, which improves Ag ion release [47-49]. Silver nanoparticles (Ag-NPs) bind with intracellular enzyme disulfide groups, suppressing metabolic activities. Many Mobile Network Providers (MNPs) are antimicrobial agents, and Ag-NPs are especially important. differs from other micro/nanoparticles in its mechanism. This chemical can damage cellular membranes and interact with disulfide bonds. Internal enzyme intermolecular interactions that damage bacterial cell structure. The organism performs metabolism, cellular uptake, and respiration. Numerous strategies exist for nanoparticles to create antibacterial activity. By causing oxidative stress, these substances prevent bacterial development [50-54].

Anti-Inflammatory Activity

The anti-inflammatory action of medicines was measured by preventing HRBC membrane lysis. The results showed that membrane stability and % protection increased with concentration from 0.125 to 0.425 mg/ml. The greatest protection was 84.44 % from AgNPs and blank nanoparticles (55.51%), had a much better anti-inflammatory effect than pure blank nanoparticles. Research shows that AgNPs have powerful anti-inflammatory properties.

Cytotoxicity (MTT) assay

Due to their greater surface area, Figure 6 nanoparticles have stronger cytotoxic and anticancer properties [33]. The retention effect and improved vascular penetration of 100-nm nanoparticles let them penetrate cancer cells faster [34]. NPs' effectiveness against cancer cell lines relies on size [56-59]. Nanoparticles reach cancer cells more efficiently. Ag-NPs were more effective than pure active medication against cancer in this investigation. Ag-NPs displayed a significant anticancer effect on MCF-7 cancer cells with an IC50 of 4.45 g/ml (Fig 6).

Figure 6. Optical microscope images of Cytotoxic effect of Ag-NPs against MCF-7 cell lines after 24 h

Assay for bacterial activity

Green silver nanoparticles (Ag-NPs) were examined for their ability to kill the bacteria Acinetobacter baumannii, Staphylococcus aureus, and enterococci. Approximately 106 bacterial isolates were treated with 20–100 mg/mL Ag-NPs (Fig 7). The isolates were grown on MHA plates. After treatment, Acinetobacter baumannii, Staphylococcus aureus, and Enterococci strains have fewer colonies. Even modest Ag-NP concentrations reduce colony numbers. Bactericidal activity is proportional to Ag-NP concentration. At higher concentrations, Ag-NPs completely suppress bacterial cells. When they engage with bacterial cells, Ag-NPs adhere to several cell surface locations and generate membrane perforations. These activities may prompt analysis [41,45].

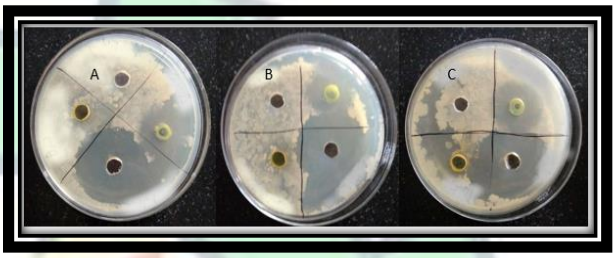

Fig 7. Determination of Antibacterial activity of green synthesized Ag-NPs against Acinetobacter baumannii (A), Staphylococcus aureus (B), and Enterococci (C).

Conclusions

In this study, silver nanoparticles (Ag-NPs) were generated through the utilization of leaf extract of CRCP. The Ag-NPs, which were acquired underwent characterization and were subsequently examined for their efficacy in treating drug-resistant Acinetobacter baumannii, Staphylococcus aureus, and Enterococci. The study observed that all Ag-NPs exhibited a significant level of toxicity towards the bacterial strains. Furthermore, it was observed that the antibacterial effectiveness of Ag-NPs increased proportionally with higher concentrations of Ag-NPs. The potential discrepancy in their antibacterial efficacy may be attributed to the particle density in the Ag-NPs suspension. The results of this work suggest that the disclosed method for creating green silver nanostructures holds promise for potential uses in the field of nanomedicine, particularly for the effective management of wound infections. Future development of wound-healing bandages is anticipated to use the

biocompatible Ag-NPs manufactured sustainably as a nanomaterial.

References

1. Kasithevar, M., Periakaruppan, P., Muthupandian, S., & Mohan, M. (2017). Antibacterial efficacy of silver nanoparticles against multi-drug resistant clinical isolates from post-surgical wound infections. *Microbial pathogenesis*, *107*, 327-334.
2. Alam, M. M., Rashid, M. M. O., Hossen, F., Akhter, K. N., Nishat, S. A., & Wahab, A. (2017). Antibacterial activity of silver-extract nanoparticles synthesized from the combination of silver nanoparticles and M. charantia Fruit Extract. *Journal of Drug Delivery and Therapeutics*, *7*(3), 112-6.
3. Mousavi, S. M., Hashemi, S. A., Ghasemi, Y., Atapour, A., Amani, A. M., Savar Dashtaki, A., ... & Arjmand, O. (2018). Green synthesis of silver nanoparticles toward bio and medical applications: review study. *Artificial cells, nanomedicine, and biotechnology*, *46*(sup3), 855-872.
4. Patil, R. B., & Chougale, A. D. (2021). On the shape based SPR of silver nanostructures. *International Journal of Nanotechnology*, *18*(11-12), 1015-1027.
5. Nsengumuremyi, D., Adadi, P., Oppong, G. K., Barakova, N. V., & Krivoshapkina, E. F. (2020). The Potential Application of Nanoparticles on Grains during Storage: Part 1–An Overview of Inhibition against Fungi and Mycotoxin Biosynthesis. *Mycotoxins and Food Safety*.
6. Tamilarasi, P., & Meena, P. (2020). Green synthesis of silver nanoparticles (Ag NPs) using Gomphrena globosa (Globe amaranth) leaf extract and their characterization. *Materials Today: Proceedings*, *33*, 2209-2216.
7. Mani, M., Pavithra, S., Mohanraj, K., Kumaresan, S., Alotaibi, S. S., Eraqi, M. M., ... & Kaviyarasu, K. (2021). Studies on the spectrometric analysis of metallic silver nanoparticles (Ag NPs) using Basella alba leaf for the antibacterial activities. *Environmental Research*, *199*, 111274.
8. Sethi, N., Bhardwaj, P., Kumar, S., & Dilbaghi, N. (2019). Development and Evaluation of Ursolic Acid Co-Delivered Tamoxifen Loaded Dammar Gum Nanoparticles to Combat Cancer. Advanced Science, Engineering and Medicine, 11(11), 1115-1124.
9. Sethi, N., Bhardwaj, P., Kumar, S., & Dilbaghi, N. (2019). Development And Evaluation Of Ursolic Acid Loaded Eudragit-E Nanocarrier For Cancer Therapy. *International Journal of Pharmaceutical Research (09752366)*, *11*(2).
10. Vivek, V., Sethi, N., & Kaura, S. (2022). Green synthesis and evaluation of antibacterial activity of zinc nanoparticles from Calotropis procera leaves.
11. Sethi, N. Preparation and evaluation of anticancer drug loaded Ursolic acid Nanoparticulate formulation.
12. Kota, S., Dumpala, P., Anantha, R. K., Verma, M. K., & Kandepu, S. (2017). Evaluation of therapeutic potential of the silver/silver chloride nanoparticles synthesized with the aqueous leaf extract of Rumex acetosa. *Scientific reports*, *7*(1), 11566.
13. Karuppiah, M., & Rajmohan, R. (2013). Green synthesis of silver nanoparticles using

Ixora coccinea leaves extract. *Materials Letters*, *97*, 141-143.

14. Karthik, R., Hou, Y. S., Chen, S. M., Elangovan, A., Ganesan, M., & Muthukrishnan, P. (2016). Eco-friendly synthesis of Ag-NPs using Cerasus serrulata plant extract–Its catalytic, electrochemical reduction of 4-NPh and antibacterial activity. *Journal of Industrial and Engineering Chemistry*, *37*, 330-339.
15. Gopinath, K., Gowri, S., & Arumugam, A. (2013). Phytosynthesis of silver nanoparticles using Pterocarpus santalinus leaf extract and their antibacterial properties. *Journal of nanostructure in chemistry*, *3*, 1-7.
16. Alshameri, A. W., & Owais, M. (2022). Antibacterial and cytotoxic potency of the plant-mediated synthesis of metallic nanoparticles Ag NPs and ZnO NPs: A Review. *OpenNano*, 100077.
17. Parmar, S., Kaur, H., Singh, J., Matharu, A. S., Ramakrishna, S., & Bechelany, M. (2022). Recent advances in green synthesis of Ag NPs for extenuating antimicrobial resistance. *Nanomaterials*, *12*(7), 1115.
18. Poudel, D. K., Niraula, P., Aryal, H., Budhathoki, B., Phuyal, S., Marahatha, R., & Subedi, K. (2022). Plant-mediated green synthesis of Ag NPs and their possible applications: A critical review. *Journal of Nanotechnology*, *2022*.
19. Shukla, S., Masih, A., & Mehata, M. S. (2022). Catalytic activity of silver nanoparticles synthesized using Crinum asiaticum (Sudarshan) leaf extract. *Materials Today: Proceedings*, *56*, 3714-3720.
20. Chen, F., Zheng, Q., Li, X., & Xiong, J. (2022). Citrus sinensis leaf aqueous extract green-synthesized silver nanoparticles: Characterization and cytotoxicity, antioxidant, and anti-human lung carcinoma effects. *Arabian Journal of Chemistry*, *15*(6), 103845.
21. Kocak, Y., Oto, G., Meydan, I., Seckin, H., Gur, T., Aygun, A., & Sen, F. (2022). Assessment of therapeutic potential of silver nanoparticles synthesized by Ferula Pseudalliacea rech. F. plant. *Inorganic Chemistry Communications*, *140*, 109417.
22. Ahmed Naseer, M. I., Ali, S., Nazir, A., Abbas, M., & Ahmad, N. (2022). Green synthesis of silver nanoparticles using Allium cepa extract and their antimicrobial activity evaluation. *Chem. Int*, *8*, 89-94.
23. Al-Hakimi, A. N., Abdulghani, M. A., Alhag, S. K., Aroua, L. M., & Mahyoub, J. A. (2022). Larvicidal activity of leaf extract of Nerium oleander L. and its synthesized metallic nanomaterials on dengue vector, Aedes aegypti. Entomological Research, 52(3), 148-158.
24. Yousaf, A., Waseem, M., Javed, A., Baig, S., Ismail, B., Baig, A., ... & Zaman, I. (2022). Augmented anticancer effect and antibacterial activity of silver nanoparticles synthesized by using Taxus wallichiana leaf extract. *PeerJ*, *10*, e14391.
25. Alhomaidi, E., Jasim, S. A., Amin, H. I. M., Lima Nobre, M. A., Khatami, M., Jalil, A. T., & Hussain Dilfy, S. (2022). Biosynthesis of silver nanoparticles using Lawsonia inermis and their biomedical application. *IET nanobiotechnology*, *16*(7-8), 284-294.
26. Kasithevar, M., Periakaruppan, P., Muthupandian, S., & Mohan, M. (2017). Antibacterial efficacy of silver nanoparticles against multi-drug resistant clinical isolates from post-surgical wound infections. Microbial pathogenesis, 107, 327-334.

27. Van Dijk, K., Voets, G. M., Scharringa, J., Voskuil, S., Fluit, A. C., Rottier, W. C., & Stuart, J. C. (2014). A disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in Enterobacteriaceae using phenyl boronic acid, dipicolinic acid and temocillin. Clinical Microbiology and Infection, 20(4), 345-349.
28. Sabir, D. (2018). Synergistic effect of silver nanoparticles combined with different antibiotics against multidrug-resistant Acinetobacter baumannii strain H72721. In ICNS Conference Proceeding (pp. 7-11).
29. Alkhaibari, I., Kc, H. R., Angappulige, D. H., Gilmore, D., & Alam, M. A. (2022). Novel pyrazoles as potent growth inhibitors of staphylococci, enterococci and Acinetobacter baumannii bacteria. Future Medicinal Chemistry, 14(4), 233-244.
30. Supino, R. (1995). MTT assays. In vitro toxicity testing protocols, 137-149.
31. Gulbagça, F., Aygun, A., Altuner, E. E., Bekmezci, M., Gur, T., Sen, F. & Vasseghian, Y. (2022). Facile bio-fabrication of Pd-Ag bimetallic nanoparticles and its performance in catalytic and pharmaceutical applications: Hydrogen production and in-vitro antibacterial, anticancer activities, and model development. *Chemical Engineering Research and Design*, *180*, 254-264.
32. Saravanan, A., Parthasarathy, V., & Kumar, P. S. (2022). Antimicrobial activity and cytotoxicity effect of the prepared bioactive Ag-NPs using Senna alata leaf extract on MCF-7 cancer cell line and brine shrimp. *Journal of Sol-Gel Science and Technology*, *103*(3), 766-776.
33. Aygün, A., Özdemir, S., Gülcan, M., Yalçın, M. S., Uçar, M., & Şen, F. (2022). Characterization and antioxidant-antimicrobial activity of silver nanoparticles synthesized using Punica granatum extract. *International Journal of Environmental Science and Technology*, *19*(4), 2781-2788.
34. Weng, X., Yang, K., Owens, G., & Chen, Z. (2022). Biosynthesis of silver nanoparticles using three different fruit extracts: Characterization, formation mechanism and estrogen removal. *Journal of Environmental Management*, *316*, 115224.
35. Abdelkhalek, A., El-Gendi, H., Alotibi, F. O., Al-Askar, A. A., Elbeaino, T., Behiry, S. I., ... & Moawad, H. (2022). Ocimum basilicum-mediated synthesis of silver nanoparticles induces innate immune responses against cucumber mosaic virus in squash. *Plants*, *11*(20), 2707.
36. Phuyal, S., Lamichhane, G., Gupta, A., Khadayat, K., Adhikari, A., Marahatha, R., ... & Parajuli, N. (2022). Biosynthesis of silver nanoparticles from Rhododendron arboreum for metal sensing, antibacterial assessment, and photocatalytic degradation. *Journal of Nanomaterials*, *2022*.
37. Awad, M. A., Eid, A. M., Elsheikh, T. M., Al-Faifi, Z. E., Saad, N., Sultan, M. H., ... & Fouda, A. (2022). Mycosynthesis, characterization, and mosquitocidal activity of silver nanoparticles fabricated by Aspergillus niger strain. *Journal of Fungi*, *8*(4), 396.
38. Ibrahim, I. A. A., Hussein, A. I., Muter, M. S., Mohammed, A. T., Al-Medhtiy, M. H., Shareef, S. H., ... & Abdulla, M. A. (2022). Effect of nano silver on gastroprotective activity against ethanol-induced stomach ulcer in rats. *Biomedicine & Pharmacotherapy*, *154*, 113550.
39. Subramaniam, S., Kumarasamy, S., Narayanan, M., Ranganathan, M., Rathinavel,

T., Chinnathambi, A., ... & Whangchai, K. (2022). Spectral and structure characterization of Ferula assafoetida fabricated silver nanoparticles and evaluation of its cytotoxic, and photocatalytic competence. *Environmental Research*, *204*, 111987.

40. Ali, F., Younas, U., Nazir, A., Hassan, F., Iqbal, M., Mukhtar, S., ... & Ishfaq, A. (2022). Biosynthesis and characterization of silver nanoparticles using strawberry seed extract and evaluation of their antibacterial and antioxidant activities. *Journal of Saudi Chemical Society*, *26*(6), 101558.
41. Elnosary, A., Aboelmagd, H., Sofy, M. R., & Sofy, A. (2022). Antiviral properties of synthesis silver nanoparticles with nigella arvensis aqueous extract. *Egyptian Journal of Chemistry*, *66*(7), 209-223.
42. Pasha, A., Kumbhakar, D. V., Sana, S. S., Ravinder, D., Lakshmi, B. V., Kalangi, S. K., & Pawar, S. C. (2022). Role of biosynthesized Ag-NPs using aspergillus niger (MK503444. 1) in antimicrobial, anti-cancer and anti-angiogenic activities. *Frontiers in Pharmacology*, *12*, 812474.
43. Hamida, R. S., Ali, M. A., Almohawes, Z. N., Alahdal, H., Momenah, M. A., & Bin-Meferij, M. M. (2022). Green synthesis of hexagonal silver nanoparticles using a novel microalgae coelastrella aeroterrestrica strain BA_Chlo4 and resulting anticancer, antibacterial, and antioxidant activities. *Pharmaceutics*, *14*(10), 2002.
44. Alhajri, H. M., Aloqaili, S. S., Alterary, S. S., Alqathama, A., Abdalla, A. N., Alzhrani, R. M., ... & Alsaab, H. O. (2022). Olive leaf extracts for a green synthesis of silver-functionalized multi-walled carbon nanotubes. *Journal of Functional Biomaterials*, *13*(4), 224.
45. Abbas, M., Hussain, T., Iqbal, J., Rehman, A. U., Zaman, M. A., Jilani, K., ... & Nazir, A. (2022). Synthesis of silver nanoparticle from Allium sativum as an eco-benign agent for biological applications. *Polish Journal of Environmental Studies*, *31*(1), 533-538.
46. Huang, L., Chen, R., Luo, J., Hasan, M., & Shu, X. (2022). Synthesis of phytonic silver nanoparticles as bacterial and ATP energy silencer. *Journal of Inorganic Biochemistry*, *231*, 111802.
47. Krishnamoorthi, R., Mahalingam, P. U., & Malaikozhundan, B. (2022). Edible mushroom extract engineered Ag NPs as safe antimicrobial and antioxidant agents with no significant cytotoxicity on human dermal fibroblast cells. *Inorganic Chemistry Communications*, *139*, 109362.
48. Askari, S., Khodaei, M. M., Jafarzadeh, M., & Mikaeili, A. (2022). In-situ formation of Ag NPs on the ribonic γ-lactone-modified UiO-66-NH2: An effective catalyst for organic synthesis and antibacterial applications. *Process Biochemistry*, *122*, 149-165.
49. Ivanuša, M., Kumer, B., Petrovčič, E., Štular, D., Zorc, M., Jerman, I., ... & Simončič, B. (2022). Eco-friendly approach to produce durable multifunctional cotton fibres using TiO2, ZnO and Ag nps. *Nanomaterials*, *12*(18), 3140.
50. Fozia, F., Ahmad, N., Buoharee, Z. A., Ahmad, I., Aslam, M., Wahab, A., ... & Tariq, A. (2022). Characterization and evaluation of antimicrobial potential of Trigonella incise (Linn) mediated biosynthesized silver nanoparticles. *Molecules*, *27*(14), 4618.

51. Mohammadzadeh, S. E., Faghiri, F., & Ghorbani, F. (2022). Green synthesis of phenolic capping Ag NPs by green walnut husk extract and its application for colorimetric detection of Cd2+ and Ni2+ ions in environmental samples. *Microchemical Journal*, *179*, 107475.
52. Gul, A., Wahab, A., Fozia, F., Shah, S. M., Gul, R., Ali, J., ... & Sultan, M. A. (2022). Protein kinase inhibition, antibacterial activity, and characterization of phytoextract-mediated silver nanoparticles using aqueous extracts of ifloga spicata. *Journal of Nanomaterials*, *2022*.
53. Jayarambabu, M., Velupla, S., Akshaykranth, A., Anitha, N., & Rao, T. V. (2022). Bambusa arundinacea leaves extract-derived Ag NPs. *Applied Physics A*, *129*(1), 13.
54. Sharwani, A. A., Narayanan, K. B., Khan, M. E., & Han, S. S. (2022). Photocatalytic degradation activity of goji berry extract synthesized silver-loaded mesoporous zinc oxide (Ag@ ZnO) nanocomposites under simulated solar light irradiation. *Scientific reports*, *12*(1), 10017.
55. Cheng, Z., Tang, S., Feng, J., & Wu, Y. (2022). Biosynthesis and antibacterial activity of silver nanoparticles using Flos Sophorae Immaturus extract. *Heliyon*, *8*(8).
56. Changez, M., Anwar, M. F., Al-Ghenaime, S., Kapoor, S., Al Balushi, R., & Chaudhuri, A. (2022). Synergic effect of aqueous extracts of Ocimum sanctum and Trigonella foenum-graecum L on the in situ green synthesis of silver nanoparticles and as a preventative agent against antibiotic-resistant food spoiling organisms. *RSC advances*, *12*(3), 1425-1432.
57. Saratale, R. G., Cho, S. K., Saratale, G. D., Kadam, A. A., Ghodake, G. S., Magotra, V. K., ... & Shin, H. S. (2022). Lignin-mediated silver nanoparticle synthesis for photocatalytic degradation of reactive yellow 4G and in vitro assessment of antioxidant, antidiabetic, and antibacterial activities. *Polymers*, *14*(3), 648.
58. Shi, D., Karmakar, B., Osman, H. E. H., El-kott, A. F., Morsy, K., & Abdel-Daim, M. M. (2022). Design and synthesis of chitosan/agar/Ag NPs: A potent and green bio-nanocomposite for the treatment of glucocorticoid-induced osteoporosis in rats. *Arabian Journal of Chemistry*, *15*(1), 103471.
59. Rajivgandhi, G. N., Ramachandran, G., Kannan, M. R., Velanganni, A. A. J., Siddiqi, M. Z., Alharbi, N. S., ... & Li, W. J. (2022). Photocatalytic degradation and anti-cancer activity of biologically synthesized Ag NPs for inhibit the MCF-7 breast cancer cells. *Journal of King Saud University-Science*, *34*(1), 101725.