FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 2, February, 2025, pp 242 - 247
DOI: https://doi.org/10.33003/fjs-2025-0902-3189

SPECTRAL CHARACTERIZATION AND EFFICACY OF BIOGENIC SYNTHESIZED SILVER NANOPARTICLES USING SECONDARY METABOLITE OF *PSEUDOMONAS AERUGINOSA* ON SELECTED PATHOGENS

***Frank Olakunle Otuyelu, Toyin Olayemi Agbabiaka, Ridwan Tope Azeez, Ruth Bukola Tomilayo, Bashirat Abdulkareem Sa'ad and Suad Oluwakemi Katibi**

Department of Microbiology, Faculty of Life Sciences, University of Ilorin, P.M.B 1515, Ilorin, Nigeria.

*Corresponding authors' email: frankotuyelu1@gmail.com Phone: +2347063040682

ABSTRACT

Bacteria resistance to conventional antibiotics has made researchers look for other possible alternatives which include the use of nanoparticles, plant extracts, production of bacteriocin, organic acids etc. This study is focused on biosynthesizing AgNPs using secondary metabolite of *Pseudomonas aeruginosa*, characterize and evaluate its effectiveness against selected bacteria pathogens. FTIR, UV-visible spectroscopy, TEM analyses were used to characterize, agar disk diffusion method was employed for antibacterial sssay. Bacterial pathogens used include *Escherichia coli, Serratia liquefaciens, Bacillus subtilis, Pseudomonas aeruginosa, Citrobacter freundii, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter cloacae, Yersinia enterica* and *K. oxytoca*. Colour change to dark brown indicates AgNPs synthesis. UV-vis spectrophotometer revealed peak absorbance 2.082 A at 410 nm, FTIR analysis revealed highest peak at 3458.58. Synthesized AgNPs size obtained ranged between 10.02 nm and 1.47 nm. Antibacterial assay result showed that AgNPs was effective against seven pathogens with *P. aeruginosa* (21.7 mm) as the most susceptible. *E. coli* and *K. oxytoca* were the most resistant with susceptibility to one antibiotic each while *E. coli* showed little susceptibility to AgNPs. All isolates showed resistance to more than half of the antibiotics used hence making them multidrug-resistant strains. In this study, it was observed that AgNPs were as effective as the antibiotics used.

Keywords: Pathogens, Resistance, Secondary metabolite, Silver nanoparticles, Susceptible

INTRODUCTION

The use of different approaches in nanoparticles synthesis has been outlined severally with numerous physical and chemical techniques available for this process, delivering high rate of production, regulated size and shape of nanoparticles. However, the limitations of these approaches, including high energy and capital loss, unhealthy nature and large bio-waste generation play a key role in scaling up production commercially (Bhardwaj *et al*., 2020), hence elevating the use of biological, ecofriendly and non-toxic greener approach.

Synthesis of nanoparticles through biogenic means can be carried out using sources like plants and animal parts as well as microorganisms. The ability of microorganisms to produce many distinct nanostructures has increased the interest of researchers in utilizing them in nanoparticles synthesis for purposes. Through biologically mediated and induced synthesis, bacteria and fungi can generate inorganic molecules (Moitra *et al*., 2020). This induced synthesis from biological source has also help scientists synthesize inorganic nanomaterials from metallic compounds, while also offering vast range of compositions widely studied with relevance in fields like engineering, medicine, food and agriculture, pharmaceuticals, environmental remediation and Cosmetics. However, biological synthesis is still limited in terms of scalability of the production process, and particle size controllability (Fang *et al*., 2019).

Diverse microorganisms usually interact uniquely with metal compounds during nanoparticles synthesis. For instance, bacteria and fungi have the capability of intra and extracellular production, with both processes having unique pathways. Among the bacteria and fungi genera reportedly used in nanoparticles synthesis include Lactobacillus (Mathivanan *et al*., 2019), Pseudomonas (Tabassum *et al*., 2024), Bacillus (Patil *et al*., 2019), Staphylococcus (Rauf *et al*., 2017), Aspergillus (Otuyelu *et al*., 2025), Fusarium (Soliman *et al*., 2024), Trichoderma (Herrera Pérez *et al*., 2024) etc. Currently, studies are ongoing to further understand the principle behind microbial synthesis of nanoparticles by microbes as it involves dissolvability and toxicity modifications, bioaccumulation, bioadsorption, metals precipitation and metal ion efflux system as compared to physicochemical methods. (Karnwal *et al*., 2024).

Nanoparticles are synthesized into different types depending on the metal and metal oxides involved, they include copper oxide nanoparticles (CuONPs), zinc oxide nanoparticles (ZnONPs), silver nanoparticles (AgNPs), iron oxide nanoparticles (FeONPs), gold nanoparticles (AuNPs) among others with some being reportedly good antimicrobial agents (Boroumand *et al*., 2019; Soliman *et al*., 2023). Silver nanoparticles (AgNPs) are a crucial and intriguing nanomaterial among numerous metallic nanomaterials used in biomedical applications (Zhang *et al*., 2016). Its broad antibacterial efficacy has been found against both Gram positive and negative pathogens, even against multidrug-resistant strains. This has led to its extensive use in infection control, wound dressings, catheters and numerous domestic products like topical creams. Also, AgNPs tiny size and huge surface area make them an excellent drug carrier in the field of drug delivery, AgNPs is effective as drug carrier to ensure efficient delivery and can as well as improving the bioavailability of poorly soluble drugs thereby enhancing their therapeutic effects (Chernousova and Epple, 2018).

The biosynthesized AgNPs has been thoroughly studied to understand their mode of action as antimicrobial agents. Microbial synthesis of AgNPs using secondary metabolite of the organism is an easy and cheap method of nanoparticle synthesis. Other microbial products used include enzymes, proteins, and biosurfactants (Otuyelu *et al*., 2025; Tanjung *et al*., 2024; Das *et al*., 2024), all playing key role in nanoparticles stability during the process. This present study investigates the antibacterial efficacy of AgNPs synthesized

from secondary metabolite produced by *P. aeruginosa* against bacterial pathogens and its characterization.

MATERIALS AND METHODS

Collection of Sample and Isolates

Ten (10) clinical pathogens in all were acquired from the University of Ilorin Teaching Hospital's Microbiology Unit in Ilorin, Kwara State. *Escherichia coli, Serratia liquefaciens, Yersinia enterica, Staphylococcus aureus, Citrobacter freundii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis,* and *Enterobacter cloacae* were among the isolates that were utilized.

Pseudomonas aeruginosa with GenBank accession number CP129997.1 reportedly isolated from poultry sawdust was obtained from Environmental and Ecotoxicology Laboratory, Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

Production of secondary metabolite

This was done following the method described by Al-Asbahi *et al*. (2024). Inoculum was developed by inoculating a loopful of pure culture into nutrient broth, incubated at 37 °C for 24 h in a shaker incubator at 160 rpm. The culture broth was then centrifuged at 10000 rpm for 10 min and supernatant was used for AgNPs synthesis.

Extracellular Biogenic synthesis of AgNPs using Culture Supernatant

Synthesis of AgNPs was done using the method described by Tariq *et al*. (2020) with light modification. Nine millilitres (9 mL) of 1mM AgNO3 solution was treated with 1 mL bacterial supernatant solution and mixed thoroughly. The resulting mixture was thereafter placed under light environment at room temperature to gradually reduce the metal ions to atoms. AgNO3 solution without supernatant was also used in a control experiment, which was carried out under the same circumstances. By visually seeing a colour shift from cloudy yellow to dark brown, which may be the result of silver ions being reduced to silver atoms, the extracellular synthesis of AgNPs was monitored.

Characterization of the Synthesized AgNPs

Analysis of the synthesized AgNPs was carried out using fourier transform infrared (FTIR), UV-visible spectrophotometry, and transmission electron microscopy (TEM). To find the optical density of the AgNPs, 5 mL of the reaction mixture were examined using a UV–vis spectrophotometer (Analytika, Specord 200). Using an FTIR Model Nicolet 6700 spectrometer, spectra peaks were examined to determine the presence of functional groups within the 400–4000 cm-1 range with a 4 cm-1 precision. TEM was used to assess the sizes and shape of AgNPs using the Japan (JEM-1010) model (JEMARM200F-G). Using ImageJ 1.45 software (1493), the particle size distribution of AgNPs was assessed.

Determination of Inoculum Size and Antibiotic Susceptibility Test (AST) of test Pathogens

Bacteria pathogens were standardized using 0.5 McFarland standard with an expected approximate cell number of 1.5 x 108 CFU/mL (Agbabiaka *et al*., 2024). Pathogens were tested for their susceptibility to multiple antibiotics following the Kirby-Bauer's disk diffusion method on sterile Mueller Hinton agar (MHA) (Jorgensen and Turnidge, 2007). This was performed by making inoculum lawn on MHA plate using sterile swab stick and then incubated at 37 ˚C for 18- 24 h after careful impregnation with the antibiotics disk. The zones of inhibition for each tested antibiotic were measured to the nearest millimeter. Amoxicillin-clavulanic acid (AUG), Gentamycin (GEN), Ciprofloxacin (CPR), Erythromycin (ERY), Septrin (SEP), Zinnat (ZIN), Ceftriaxone (CAZ), Streptomycin (STR), Ofloxacin (OFL), Cefuroxime (CRX), Nitrofurantoin (NIT), Cefixime (CXM), and Ampiclox (APX) were among the antibiotics utilized.

Antibacterial Screening of Pseudomonas aeruginosa synthesized AgNPs

The synthesized AgNPs was its antibacterial efficacy against the pathogenic bacteria using the Disk diffusion technique (Jorgensen and Turnidge, 2007). A 5 mm diameter sterile paper disk infused in biosynthesized AgNPs was aseptically placed on an MHA plate that had already been coated with a standardized inoculum. It was then incubated for 18 to 24 hours at 37 ºC. The zone of clearance that was attained was measured and noted appropriately.

RESULTS AND DISCUSSION

Biosynthesis of AgNPs

The ability of *P. aeruginosa* to biosynthesis AgNPs was shown in Figure 1. Appearance of dark brown colouration after 48 hours was observed. According to Ahmad et al. (2003), AgNPs formation is observed visually by the appearance of brown color. In agreement with this observation was a study by Akther et al. (2020). who reported synthesis of AgNPs by Pseudomonas aeruginosa with appearance of brown colour. The colour formation is said to be as a result of excitation of surface plasmon vibrations in metal nanoparticles (Wang et al., 2022).

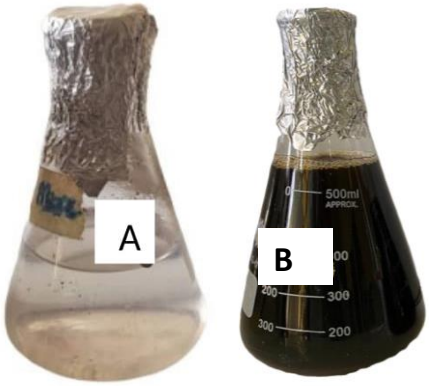

Figure 1: Silver nitrate solution (AgNo3); (b) Synthesized AgNPs from *Pseudomonas aeruginosa*

Characterization of the biosynthesized AgNPs

A UV-visible spectrophotometer was used to measure AgNP production in the 250–700 nm range with an absorbance peak 2.082 A at 410 nm (Figure 2). Similarly, Otuyelu et al. (2025) reported peak absorbance at 440 nm. However, peak at a lower absorbance of 230 nm was reported by Chinnappa et al. (2022) while Sarhan and Fahmy (2021) reported an absorbance peak of 304 nm. The peak absorbance reported in this study is a confirmation of AgNPs synthesis as peak absorbance of AgNPs ranged from 350 - 450 nm (Genc et al., 2020).

FTIR revealed possible biomolecules involved in silver bioreduction and AgNPs stabilization. Figure 3 illustrates that 7 peaks were recorded at 3458.58, 2055.04, 1641.16,1308.71, 1210.55, 689.13 and 415.83 in the band wavelength of 4000-500 cm^{-1} which are due to the existence of –NH or –OH group and some free amides (Shaban et al., 2019; Otuyelu et al., 2025). The peak detected at a wave number of 3458.58 cm^{-1} was ascribed to the O–H stretching of the amide group, the peak at 2055.04 cm^{-1} indicates CH_2 stretching (Otuyelu et al., 2025). Peak at 1641.16 cm^{-1} indicate presence of alkenyl C=C in the proteins (amide I) (Nandiyanto et al., 2019). The stretching vibrations of the carboxyl group C–O showed at peak 1308.71 cm^{-1}, as well as the stretching vibrations of C–O groups in the phenol, ether, or ester group attributed at peak 1210.55 cm^{-1}. The peak at 689.13 cm^{-1} is attributed to the OCN bending in the amide IV band arising, and the C=O of the amide VI bands arising presented at peak 415.83 cm^{-1}. Similar to this result was the study conducted by Abeer Mohammed et al. (2022) who reported peaks at 3431.48, 2926.11, 2862.46, 1648.23, 1397.47, 1110.07, 1076.32 cm^{-1}. The carboxyl group C=O, the hydroxyl group –OH and the free amine–NH of the bacterial protein could be responsible for the AgNPs formation and stability (John et al., 2020)

The TEM image (Figure 4) shows that majority of the nanoparticles are spherical in shape with minimal dispersal with size of the AgNPs ranging from 1.47 nm to 10.02 nm which is in agreement to the TEM study of Debnath et al. (2019) who revealed the formation of dispersed silver nanoparticle in the range of 5–25 nm which are spherical in shape. Wan et al. (2016) synthesized the AgNPs in size of 5–12 nm which is similar to the size obtained in this present study.

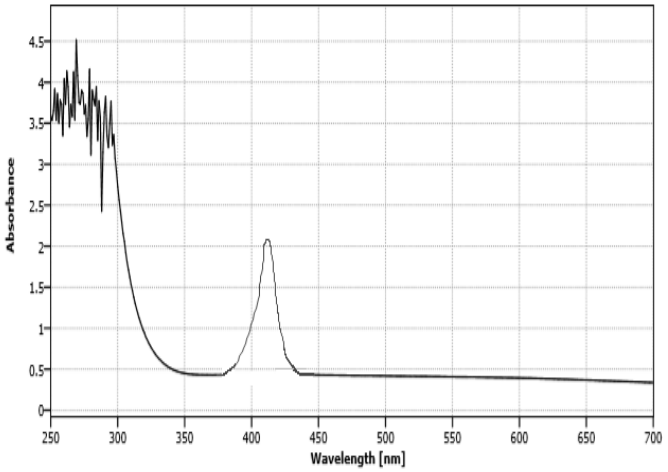

Figure 2: UV-vis spectroscopy of synthesized AgNPs

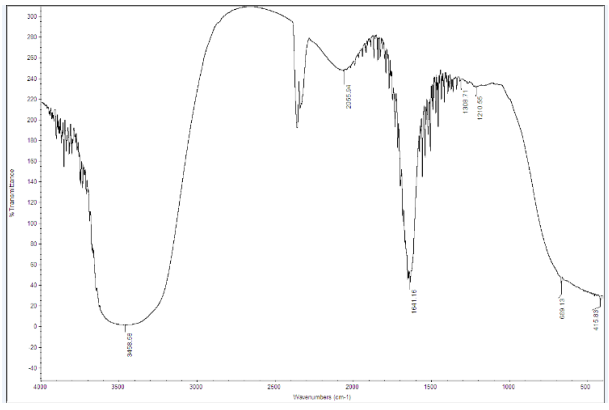

Figure 3: FTIR spectrum of synthesized AgNPs

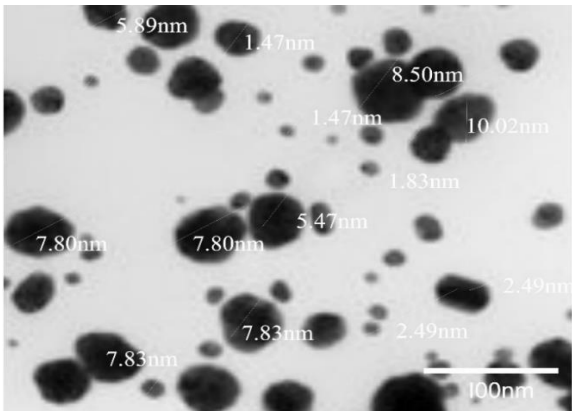

Figure 4: TEM micrograph of synthesized AgNPs

Antimicrobial Assay of AgNPs

Bioactivity of synthesized AgNPs against pathogens was evaluated as shown in Table 1. The results obtained revealed no significant difference between synthesized AgNPs and three other antibiotics (ERY, GEN. CPR) that were effective against *B. subtilis*. AgNPs and four other antibiotics (GEN, CPR, OFL, NIT) were effective against *K. pneumoniae*, however there was significant difference between the activities of AgNPs and two antibiotics (CPR and NIT) with higher effectiveness. S. aureus was susceptible to AgNPs and four other antibiotics (GEN, CPR, OFL, AUG) with no significant difference observed between activities of AgNPs and antibiotics. *P. aeruginosa* was susceptible to AgNPs and three antibiotics (GEN, CPR, OFL) with AgNPs being the most effective with significant difference to the activity displayed by antibiotics. The most resistant bacteria were *Y.*

enterica, E. coli and *K. oxytoca* with susceptibility to two or less agents with no activity obtained for AgNPs against *K. oxytoca*. *S. liquefaciens* and *C. freundii* were found to be susceptible to four antibiotics each, however, they were resistant to AgNPs.

The exhibition of antibacterial activity by AgNPs against clinical pathogens as observed in this present study was in agreement with the findings of Chitra and Annadurai (2014). This activity by AgNPs in this study demonstrated the effectiveness of the particles to fight pathogenic microbes in the environment.

According to the result of the antibiotics susceptibility test, all the pathogens were resistant to more than half the antibiotics used in this study making them multi drug resistant isolates. Drug resistant bacteria are emerging pathogens with resistance profiles that pose a major challenge to control their spread and human health impact. More et al., (2023) suggest that silver nanoparticles exhibit their antimicrobial activity by continuously releasing silver ions, which may likely be the mechanism of their ability to kill microbes. Silver ions have a strong affinity for sulfur-containing proteins and are drawn to them by electrostatic forces. They easily adhere to cell walls and the cytoplasm. Ji et al., (2020) reported that silver nanoparticles inhibit the respiratory chain dehydrogenase, thereby inhibiting bacterial growth and metabolic activity of the cell, in bacteria. It was also reported that it affects the integrity of *E. coli* cell through depolarization and destabilization of the cell membrane. The size of metallic nanoparticles ensures that a significantly large surface area of the particles is in contact with the bacterial cells. Such a large contact surface is expected to enhance the extent of bacterial elimination (Hameed et al., 2020). The effectiveness of silver nanoparticles against microbes may be as a result of adhesion to the cell wall and plasma membrane of the microbial cell, resulting in increased cell permeability (Franzolin et al., 2022) or destruction of the plasma membrane and leakage of cell components (Alnahari et al., 2023.). There could be formation of free radicals which can break the membrane lipids, peptidoglycan, DNA, and protein, which can cause damage, dissociation and inhibit the growth of the bacterial cell (Dakal et al., 2016).

Table 1: Antibacterial screening of synthesized silver nanoparticles

Test isolates	AgNPs	CAZ/ ERY	CRX/ SEP	GEN	CPR	OFL	AUG	NIT/ZIN	CXM	STR
B. subtilis	14.3 ± 0.3^{b}	13.3 ±0.3^{b}	0.0 ± 0.0^{a}	15.3 ± 0.7^{b}	15.3 ± 0.7^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	NA	0.0 ± 0.0^{a}
S. liquefaciens	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	15.7 ± 0.3^{b}	18.0 ± 0.0^{b}	16.7 ± 0.3^{b}	0.0 ± 0.0^{a}	18.7 ± 0.3^{b}	0.0 ± 0.0^{a}	NA
K. pneumoniae	18.0 ± 0.0^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	15.7 ± 0.3^{b}	21.7 ± 0.3^{c}	18.7 ± 0.3^{b}	0.0 ± 0.0^{a}	20.3 ± 0.3^{c}	0.0 ± 0.0^{a}	NA
S. aureus	14.0 ± 0.6^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	15.7 ± 0.3^{b}	16.7 ± 0.3^{b}	12.7 ± 0.3^{b}	14.3 ± 0.3^{b}	0.0 ± 0.0^{a}	NA	0.0 ± 0.0^{a}
P. aeruginosa	21.7 ± 2.2^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	14.0 ± 0.0^{b}	16.7 ± 0.3^{b}	17.3 ± 0.7^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	NA
E. coli	9.3 ± 0.2^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	11.0 ± 0.0^{b}	0.0 ± 0.0^{a}	NA
E. cloacae	12.3 ± 0.3^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	23.0 ± 0.6^{c}	21.7 ± 0.3^{c}	24.0 ± 2.0^{d}	0.0 ± 0.0^{a}	27.0 ± 1.2^{d}	0.0 ± 0.0^{a}	NA
Y. enterica	13.3 ± 0.9^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	NA
K. oxytoca	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	17.0 ± 0.0^{b}	0.0 ± 0.0^{a}	NA
C. freundii	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	18.0 ± 1.0^{b}	32.0 ± 1.6^{c}	29.0 ± 0.0^{c}	0.0 ± 0.0^{a}	27.0 ± 0.0^{c}	0.0 ± 0.0^{a}	NA

Key: Ceftriaxone (CAZ), Cefuroxime (CRX), Ciprofloxacin (CPR), Amoxicillin-clavulanic acid (AUG), Gentamycin (GEN), Ofloxacin (OFL), Streptomycin (STR), Cefixime (CXM), Septrin (SEP), Erythromycin (ERY), Nitrofurantoin (NIT), Zinnat (ZIN), Not Applicable (NA)

Note: No significant different between values with same superscript across each roll (Duncan test, $p < 0.05$)

CONCLUSION

The present study demonstrated that silver nanoparticles possess significant antibacterial properties against test organisms. The result showed synthesized silver nanoparticles had similar with some standard antibiotics used against some pathogens including B. subtilis, K. pneumoniae and S. aureus while also comparing favourably with standard antibiotics against P. aeruginosa, E. coli and Y. enterica. Therefore, it could be concluded that AgNPs have a vast activity against variety of pathogens. Nonetheless, additional investigation into the possible synergy between silver nanoparticles and antibiotics may be necessary to further reduce resistant pathogens in the environment.

REFERENCES

Abeer Mohammed A.B., Abd Elhamid M.M., Khalil M.K.M., Ali A.S., Abbas R.N. (2022). The potential activity of biosynthesized silver nanoparticles of *Pseudomonas aeruginosa* as an antibacterial agent against multidrug-resistant isolates from intensive care unit and anticancer agent. *Environmental Sciences Europe*, 34(1): 109. https://doi.org/10.1186/s12302-022-00684-2

Agbabiaka T.O., Otuyelu F.O., Abdulsalam Z.B., Mustapha S. (2024). Effect of antioxidant protection against ultraviolet radiation and antibiotic susceptibility of *Escherichia coli*. *Ife Journal of Science*, 26(2): 235-244. https://doi.org/10.4314/ijs.v26i2.2

Ahmad A., Mukherjee P., Senapati S., Mandal D., Khan M.I., Kumar R., Sastry M. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids and surfaces B: Biointerfaces*, 28(4): 313-8. https://doi.org/10.1016/S0927-7765(02)00174-1

Akther T., Khan M.S., Hemalatha S. (2020). Biosynthesis of silver nanoparticles via fungal cell filtrate and their anti-quorum sensing against *Pseudomonas aeruginosa*. *Journal of Environmental Chemical Engineering*, 8(6): 104365. https://doi.org/10.1016/j.jece.2020.104365

Al-Asbahi M.G., Al-Ofiry B.A., Saad F.A., Alnehia A., Al-Gunaid M.Q. (2024). Silver nanoparticles biosynthesis using mixture of *Lactobacillus* sp. and *Bacillus* sp. growth and their antibacterial activity. *Scientific Reports*, 14(1): 10224. https://doi.org/10.1038/s41598-024-59936-1

Alnahari H., Al-Hammadi A.H., Al-Sharabi A., Alnehia A., Al-Odayni A.B. (2023). Structural, morphological, optical, and antibacterial properties of CuO–Fe2O3 –MgO–CuFe2O4 nanocomposite synthesized via auto-combustion route. *J. Mater. Sci. Mater. Electron*. 34(7): 1–12. https://doi.org/10.1007/s10854-023-10120-7

Bhardwaj B., Singh P., Kumar A., Kumar S., Budhwar V. (2020). Eco-friendly greener synthesis of nanoparticles. *Advanced Pharmaceutical Bulletin*, 10(4): 566. https://doi.org/10.34172/apb.2020.067

Boroumand S., Safari M., Shaabani E., Shirzad M., Faridi-Majidi R. (2019). Selenium nanoparticles: synthesis, characterization and study of their cytotoxicity, antioxidant and antibacterial activity. *Materials Research Express*, 6(8): 0850d8. https://doi.org/10.1088/2053-1591/ab2558

Chernousova S., Epple M. (2018). Silver as antibacterial agent: ion, nanoparticle, and metal. Angewandte Chemie *International Edition*, 52(6): 1636-1653. https://doi.org/10.1002/anie.201205923

Chirgadze Y.N., Fedorov O.V., Trushina N.P. (1975). Estimation of amino acid residue side-chain absorption in the infrared spectra of protein solutions in heavy water. *Biopolymers*, 14: 679–694. https://doi.org/10.1002/bip.1975.360140402

Chitra K., Annadurai G. (2014). Antibacterial activity of pH-dependent biosynthesized silver nanoparticles against clinical pathogen. *Biomed Research International*, 2014(1): 725165. https://doi.org/10.1155/2014/725165

Dakal T.C., Kumar A., Majumdar R.S., Yadav V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology*, 7: 1831. https://doi.org/10.3389/fmicb.2016.01831

Debnath G., Das P., Saha A.K. (2019). Green synthesis of silver nanoparticles using mushroom extract of Pleurotus giganteus: characterization, antimicrobial, and α-amylase inhibitory activity. *Bionanoscience*, 9: 611-619. https://doi.org/10.1007/s12668-019-00650-y

Fang X., Wang Y., Wang Z., Jiang Z., Dong M. (2019). Microorganism assisted synthesized nanoparticles for catalytic applications. *Energies*, 12: 190 https://doi.org/10.3390/en12010190

Franzolin M.R., Courrol D.D., Silva F.R., Courrol L.C. (2022). Antimicrobial activity of silver and gold nanoparticles prepared by photoreduction process with leaves and fruit extracts of *Plinia cauliflora* and *Punica granatum*. *Molecules*, 27(20): 1–16. https://doi.org/10.3390/molecules27206860

Genc, N., Yildiz, I., Chaoui, R., Erenler, R., Temiz, C., & Elmastas, M. (2020). Biosynthesis, characterization and antioxidant activity of oleuropein-mediated silver nanoparticles. *Inorganic and Nano-Metal Chemistry*, 51(3), 411–419. https://doi.org/10.1080/24701556.2020.1792495

Gurunathan S., Park J.H., Han J.W., Kim J.H. (2015). Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by *Bacillus tequilensis* and *Calocybe indica* in MDA-MB-231 human breast cancer cells: Targeting p53 for anticancer therapy. *Int J Nanomed*, 10: 4203–4223. https://doi.org/10.2147/IJN.S83953

Hameed S., Wang Y., Zhao L., Xie L., Ying Y. (2020). Shape-dependent significant physical mutilation and antibacterial mechanisms of gold nanoparticles against foodborne bacterial pathogens (Escherichia coli, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) at lower concentrations. *Materials Science and Engineering*: C, 108: 110338. https://doi.org/10.1016/j.msec.2019.110338

Herrera Pérez G.M., Castellano L.E., Ramírez Valdespino C.A. (2024). Trichoderma and mycosynthesis of metal nanoparticles: role of their secondary metabolites. *Journal of Fungi*, 10(7): 443. https://doi.org/10.3390/jof10070443

Ji H., Zhou S., Fu Y., Wang Y., Mi J., Lu T., Lü C. (2020). Size-controllable preparation and antibacterial mechanism of thermo-responsive copolymer-stabilized silver nanoparticles with high antimicrobial activity. *Materials Science and Engineering*: C, 110: 110735. https://doi.org/10.1016/j.msec.2020.110735

John M.S., Nagoth J.A., Ramasamy K.P., Mancini A., Giuli G., Natalello A., Ballarini P., Miceli C., Pucciarelli S. (2020). Synthesis of bioactive silver nanoparticles by a Pseudomonas strain associated with the antarctic psychrophilic protozoon *Euplotes focardii*. *Marine drugs*,18(1): 38. https://doi.org/10.3390/md18010038

Jorgensen J.H., Turnidge J.D. (2015). Susceptibility test methods: dilution and disk diffusion methods. *Manual of clinical microbiology*, pp 1253-1273. https://doi.org/10.1128/9781555817381.ch71

Karnwal A., Kumar Sachan R.S., Devgon I., Devgon J., Pant G., Panchpuri M., Ahmad A., Alshammari M.B., Hossain K., Kumar G. (2024). Gold nanoparticles in nanobiotechnology: from synthesis to biosensing applications. *ACS omega*, 9(28), 29966-29982. https://doi.org/10.1021/acsomega.3c10352

Mathivanan K., Selva R., Chandirika J.U., Govindarajan R.K., Srinivasan R., Annadurai G., Duc P.A. (2019). Biologically synthesized silver nanoparticles against pathogenic bacteria: Synthesis, calcination and characterization. *Biocatal. Agric. Biotechnol*, 22: 101373. https://doi.org/10.1016/j.bcab.2019.101373

Moitra P., Alafeef M., Dighe K., Frieman M., Pan D. (2020) Selective Naked-Eye Detection of SARS-CoV-2 Mediated by N Gene Targeted Antisense Oligonucleotide Capped Plasmonic Nanoparticles. *ACS Nano* 14: 7617–7627 https://doi.org/10.1021/acsnano.0c03822

More P.R., Pandit S., Filippis A., Franci G., Mijakovic I., Galdiero M. (2023). Silver Nanoparticles: Bactericidal and Mechanistic Approach against Drug Resistant Pathogens. *Microorganisms*, 11(2): 369. https://doi.org/10.3390/microorganisms11020369

Nandiyanto A.B.D., Oktiani R., Ragadhita R. (2019). How to read and interpret FTIR spectroscope of organic material. Indones. *J. Sci. Technol.* 4(1): 97–118. *https://ejournal.kjpupi.id/index.php/ijost/article/view/189*

Otuyelu F.O., Adebisi O.O., Omojasola P.F., Azeez R.T., Abdulsalam Z.B., Daramola O.B., Akinsanola B.A. (2025). Characterization of Silver Nanoparticles Biosynthesized using Keratinase from *Aspergillus* species and their Antibacterial Activity against Clinical Pathogens. *BioNanoSci*. 15: 136. *https://doi.org/10.1007/s12668-024-01770-w*

Patil M.P., Kang M.J., Niyonizigiye I., Singh A., Kim J.O., Seo Y.B., Kim G.D. (2019). Do Extracellular synthesis of gold nanoparticles using the marine bacterium Paracoccus haeundaensis BC74171T and evaluation of their antioxidant activity and antiproliferative effect on normal and cancer cell lines. *Colloids Surf. B Biointerfaces*, 183: 110455. https://doi.org/10.1016/j.colsurfb.2019.110455

Rauf, M.A., Owais, M., Rajpoot, R., Ahmad, F., Khan, N., Zubair, S. (2017). Biomimetically synthesized ZnO nanoparticles attain potent antibacterial activity against less susceptible: S. aureus skin infection in experimental animals. *RSC Adv.* 7, 36361–36373 https://doi.org/10.1039/C7RA05040B

Sagar P.V., Ramadevi D., Basavaiah K., Botsa S.M. (2024). Green synthesis of silver nanoparticles using aqueous leaf extract of Saussurea obvallata for efficient catalytic reduction of nitrophenol, antioxidant, and antibacterial activity. *Water Science and Engineering*, 17(3): 274-282. https://doi.org/10.1016/j.wse.2023.09.004

Soliman M.K., Abu-Elghait M., Salem S.S., Azab M.S. (2024). Multifunctional properties of silver and gold nanoparticles synthesis by *Fusarium pseudonygamai*. *Biomass Conversion and Biorefinery*, 14(22): 28253-28270. https://doi.org/10.1007/s13399-022-03507-9

Soliman M.K., Salem S.S., Abu-Elghait M., Azab M.S. (2023). Biosynthesis of silver and gold nanoparticles and their efficacy towards antibacterial, antibiofilm, cytotoxicity, and antioxidant activities. *Applied Biochemistry and Biotechnology*, 195(2): 1158-1183. https://doi.org/10.1007/s12010-022-04199-7

Subhani A.A., Irshad M., Ali S., Jawad M., Akhtar M.F., Summer M. (2024). UV-spectrophotometric Optimization of Temperature, pH, Concentration and Time for Eucalyptus Globulus Capped Silver Nanoparticles Synthesis, their Characterization and Evaluation of Biological Applications. *J Fluoresc* 34, 655–666. https://doi.org/10.1007/s10895-023-03260-w

Tabassum N., Khan F., Jeong G.J., Jo D.M., Kim Y.M. (2024). Silver nanoparticles synthesized from *Pseudomonas aeruginosa* pyoverdine: Antibiofilm and antivirulence agents. Biofilm, 7, 100192. https://doi.org/10.1016/j.bioflm.2024.100192

Tanjung Y.P., Dewi M.K., Gatera V.A., Barliana M.I., Joni I.M., Chaerunisaa A.Y. (2024). Factors affecting the synthesis of bovine serum albumin nanoparticles using the desolvation method. *Nanotechnology, Science and Applications*, 17: 21-40. https://doi.org/10.2147/NSA.S441324

Tariq F., Ahmed N., Afzal M., Khan M.A.U., Zeshan B. (2020). Synthesis, Characterization and antimicrobial activity of *Bacillus subtilis*-derived silver nanoparticles against multidrug-resistant bacteria. *Jundishapur Journal of Microbiology*, 13(5): e91934. http://dx.doi.org/10.5812/jjm.91934

Wang Y., Huang W., Lin Y.S., Yang B.R. (2022). A tunable color filter using a hybrid metasurface composed of ZnO nanopillars and Ag nanoholes. *Nanoscale Advances*, 4(17): 3624-3633. https://doi.org/10.1039/D2NA00286H

Zhang X.F., Liu Z.G., Shen W., Gurunathan S. (2016). Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *International journal of molecular sciences*, 17(9): 1534. https://doi.org/10.3390/ijms17091534