Antibacterial activity of Vitex negundomediated green synthesized silver oxide nanoparticles

Surbhi Dhiman[a] and Gulshan Kumar[b]

[b]Division of Botany, Department of Bio-Sciences, Career Point University, Hamirpur, Himachal Pradesh 176041, INDIA

**Corresponding Authors E-mail: gulshan.botany@cpuh.edu.in(G. Kumar)*

Abstract

This investigation explores the versatile characteristics of environmentally friendly, green-synthesized silver oxide nanoparticles (AgO NPs). The focus is on assessing their efficacy in antibacterial activity. The NPs were produced using a sustainable synthesis method that incorporated phytochemicals derived from *Vitex negundo*. The crystal structure of AgO NPswas confirmed through XRD results.TEM and SEM-EDS examinations unveiled spherical NPs with size 7 nm.Antibacterial assessments highlighted the AgO NPs ability to disrupt *Bacillus subtilis* and *Escherichia coli*. This research's innovation lies in the green synthesis of AgO NPs, leveraging their distinct properties for synergistic applications. The study provides valuable insights into the potential future applications of these NPs, offering novel solutions for environmental remediation and biomedical uses.

Keywords: Antibacterial; Nanoparticles; Silver oxide

1. Introduction

Silver oxide nanoparticles (AgO NPs) have emerged as a promising material in the field of antibacterial applications due to their unique physicochemical properties and potent antimicrobial activity. The increasing resistance of bacteria to conventional antibiotics has driven the search for alternative strategies to combat bacterial infections, and AgO NPs offer a viable solution (Lopez-Carrizales et al., 2022). Physicochemical Properties AgO NPs are characterized by their small size, high surface area-to-volume ratio, and distinctive surface chemistry. These properties enable them to interact more effectively with bacterial cells compared to bulk materials. The NPs are typically synthesized through various methods such as chemical reduction, sol-gel processes, and green synthesis, each method influencing their size, shape, and surface characteristics. The precise control over these parameters is crucial as they significantly affect the NPs' antibacterial efficacy (Islam et al., 2021).

The antibacterial activity of AgO NPs is primarily attributed to several mechanisms. AgO NPs can generate Reactive Oxygen Species (ROS), such as superoxide anions, hydroxyl radicals, and hydrogen peroxide. These ROS cause oxidative stress within bacterial cells, damaging cellular components like proteins, lipids, and DNA, ultimately leading to cell death.

The NPs release Ag^+ ions, which are highly reactive and can interact with thiol groups in bacterial proteins, disrupting their function. Ag ions can also interfere with bacterial DNA, inhibiting replication and transcription processes (Mathimaran et al., 2024). AgO NPs can attach to bacterial cell membranes, increasing membrane permeability and causing structural damage. This disrupts the cell's integrity and can lead to cell lysis. AgO NPs are effective against biofilms, which are protective layers formed by bacterial communities. They can penetrate and disrupt the biofilm matrix, enhancing the susceptibility of bacteria to antibacterial agents (Pourmoslemi, Shokouhiand Mahjub, 2021).

The potent antibacterial properties of AgO NPs make them suitable for various applications in healthcare. Incorporating AgO NPs into wound dressings can prevent infections and promote healing by providing a sustained release of antimicrobial agents at the wound site. Coating medical devices, such as catheters and implants, with AgO NPs can reduce the risk of device-associated infections (Haq et al., 2021). Embedding AgO NPs in textiles and surface coatings can create antimicrobial fabrics and surfaces, useful in hospital settings to minimize the spread of infections. Despite their advantages, there are challenges associated with the use of AgO NPs. These include potential toxicity to human cells and the environment, as well as the need for standardized protocols to assess their safety and efficacy (Suresh et al., 2023). Ongoing research is focused on optimizing the synthesis and functionalization of AgO NPs to enhance their antibacterial activity while minimizing adverse effects. Future directions involve exploring synergistic effects with other antimicrobial agents, understanding the long-term impacts of nanoparticle use, and developing targeted delivery systems to maximize therapeutic outcomes (Minhas et al., 2023).Additionally, advancements in green synthesis methods aim to produce AgO NPs in an eco-friendly and cost-effective manner. In conclusion, AgO NPs represent a powerful tool in the fight against bacterial infections, offering a multifaceted approach to combating antibiotic-resistant bacteria. Continued research and development in this field hold great promise for enhancing antibacterial therapies and improving public health outcomes.

2. Experimental section

2.1. Chemicals

The substances used in the study included Ethanol (C_2H_5OH), Silver nitrate ($AgNO_3$) and Sodium hydroxide (NaOH).

2.2. Leaves extraction of *Vitex negundo*

The acquisition of *Vitex negundo*leaves transpired in the Hamirpur district of Himachal Pradesh, India. After procurement, a meticulous purification process was applied to the stems, involving thorough washing with distilled water (DW) to eliminate any extraneous impurities. The resulting cleansed leaves, amounting 10 grams, underwent a precise fragmentation process, resulting in smaller, carefully sectioned pieces. To extract plant's constituent, the finely chopped leaves underwent a controlled boiling procedure in 100 ml of DW for an approximate duration of 40 minutes, maintaining a temperature of 60 °C throughout. Following this methodical procedure, the resultant *Vitex negundo*extract was utilized in the synthesis of AgO NPs.

2.3. Green synthesis of AgO NPs

The successful green synthesis of AgO NPswas accomplished using a precipitation method. The synthesis involved combining distilled with a precursor of Ag (0.1 M) and stirring for 60 minutes. Next, 10 mL each of plant extract was introduced into the Ag mixture. Subsequently, NaOH (2 M) solution was added dropwise to maintain the pH at 12 and stirred for 60 minutes at 70 ^{0}C for 12 hours. The obtained NPs were centrifuged in ethanol for three rounds at 4000 rpm for 10 minutes, dried in a vacuum oven at 5 kPa pressure and 50°C for 5 hours. The synthesis yield was 43.81 ± 1.25 % for AgO NPs, indicating consistent results across three replications.

2.4. Characterization methods

A comprehensive characterization of the synthesized NPs was conducted employing a diverse array of analytical methods. X-ray Diffraction (XRD) with CuK radiation (wavelength: 1.5418 Å) was utilized to scrutinize both the crystallite size and crystal structure. UV-visible spectrophotometry was employed to assess the bandgap of the resulting NPs. Fourier-Transform Infrared Spectroscopy (FTIR) was utilized for functional group analysis of the synthesized NPs. The chemical composition and surface morphology of the synthesized NPs were meticulously examined through Energy-Dispersive X-ray Spectroscopy (EDS) and a Scanning Electron Microscope(SEM). Additionally, Transmission Electron Microscopy (TEM) was applied to ascertain the particle size of the synthesized NPs.

2.5. Antibacterial method

The antibacterial potential of AgO NPsagainst both *Bacillus subtilis*(MTCC: 441, Gram-positive) and *Escherichia coli*(MTCC: 739, Gram-negative) bacteriawas evaluated using the well diffusion method (). Colonies isolated from agar plate cultures were collected and transferred to test tubes containing 4-5 mL of autoclaved saline solution (0.85%). The turbidity of the resulting inoculums was standardized against 0.5 McFarland standards. Subsequently, Mueller-Hinton agar (MHA) plates were prepared, and sterilized cotton swabs soaked with the inoculum were evenly streaked across the entire MHA plate surface. Wells were then created at the bottom of the plates, and different concentrations (5, 10, and 20 mg/mL^{-1}) of AgO NPswere introduced into these wells. Further, these plates were incubated at 37 °C for 24 hours, with methanol acting as the negative control. Following the incubation period, the zones of inhibition were measured to assess the antibacterial activity.

3. Result and discussions

3.1. XRD analysis

Green synthesized AgO NPs, along with their diverse structural features, was validated through XRD analysis. **Figure 1** and **Table 1** illustrate peaks of XRD (at angle 2θ) corresponding to Miller indices (hkl) for AgO NPs. The crystallite size was calculated through Debye Scherrer formula as shown in equation (1). XRD analysis, referencing JCPDS-ICDD Card no 14-0191(Ullah et al., 2023), identified face centred cubic crystalline structurein AgO NPs. The crystallite size for AgO NPsis 14.847 nm.

Table 1: Miller indices for AgO NPs

hkl	Angle (2θ)
111	38.190
200	44.436
220	64.678
311	77.377

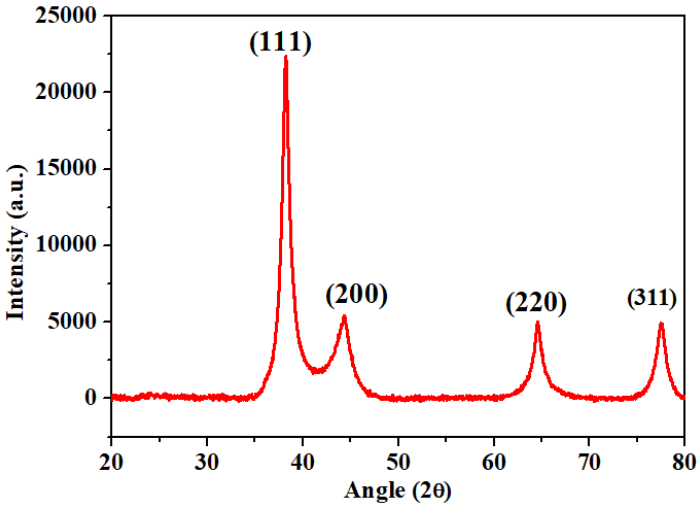

Figure 1: XRD pattern of AgO NPs

32. UV study

In **Figure 2**, the bandgap of AgO NPsis presented using a UV-visible apparatus, covering the wavelength range of 200-800 nm. The bandgap values, extracted from Tauc plots of $(\alpha h\nu)^2$ vs hν (Ernest Ravindran et al., 2022), are depicted in **Figure 3**, indicating values of 3.155 eV for AgO NPs. These results indicate that these NPs exhibit favorable attributes towards generating of electron-hole pairs when bare to the UV light.It is essential to consider various factors, including particle size, oxygen availability, and synthetic material imperfections, as these variables may influence the NPs' substance absorption capabilities. The involvement of OH radicals in generating charges during this process is pivotal for diverse applications (Daoudi et al., 2022).

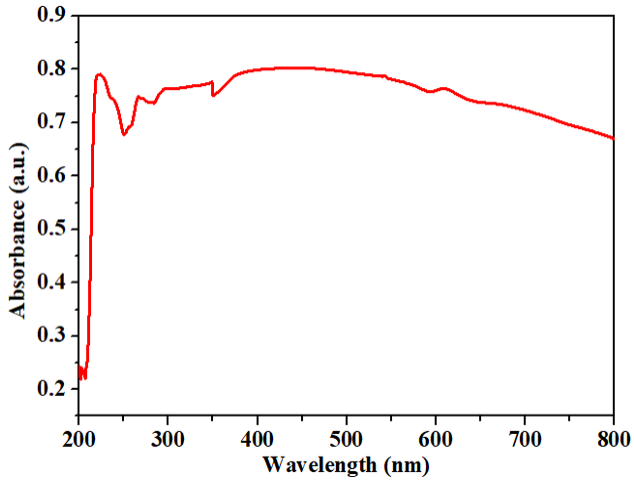

Figure2: Absorbance peaks of AgO NPs

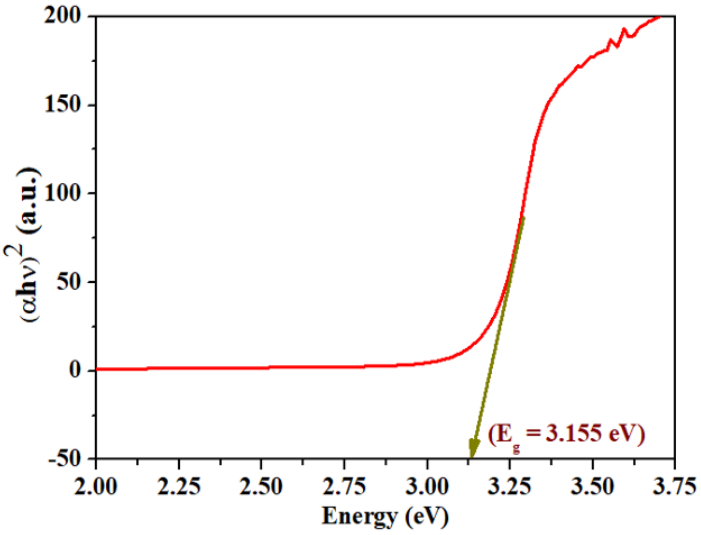

Figure3: Tauc plot of AgO NPs

3.3. FTIR study

The FTIR analysis confirms the distinct vibrational bonds associated with the modification of hydroxyl groups on the AgO surface as shown in **Figure 4**. Notably, vibrations from the carboxyl group are detected, with a C=O stretching mode observed at 1419 cm^{-1}, along with another stretching vibration corresponding to C=C at 1568-1575 cm^{-1}both originating from the Ag salt precursor (Shinde et al., 2023).The spectrum also exhibits bands at 2926-2935 cm^{-1}, indicative of symmetric-asymmetric stretching vibrations of the CH_2 group. Additionally, bands at 3393-3396 cm^{-1} are associated with the O–H mode of vibration (Muhammad et al., 2023). A band below 900 cm^{-1} is attributed to the stretching of metal ions.A noteworthy finding from the experiment is the alteration in the stretching of Ag-O-Ag bonds. The initial cube-like structure supports the presence of the Ag-O-Ag band at 873-880 cm^{-1} (Shahzad Shirazi et al., 2022).

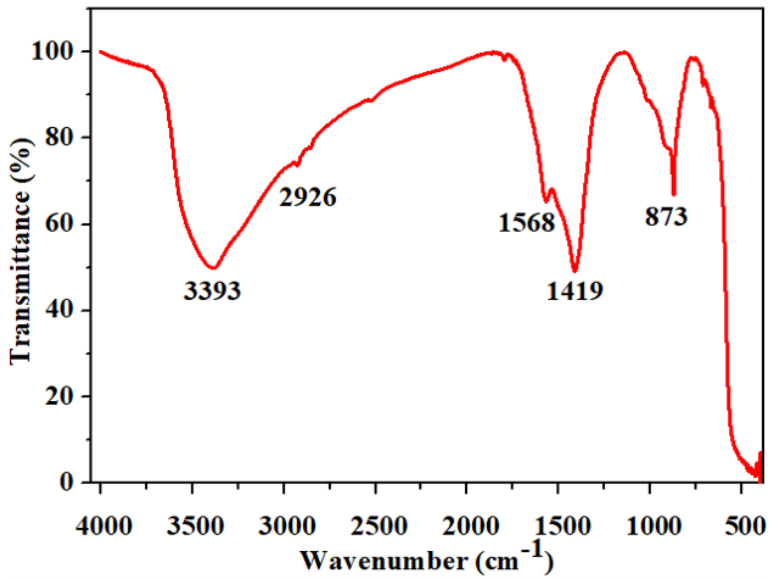

Figure 4:FTIR spectra for AgO NPs

3.4. SEM-EDS analysis

Utilizing SEM-EDS spectroscopy, elemental composition and structural configuration of AgO NPswere investigated. In **Figure 5**, SEM-EDS images provide a detailed representation of the precipitation method employed in synthesizing these NPs. The utilization of this specific precipitation method, carried out under defined temperature and time conditions, demonstrates efficacy in shaping and sizing NPs, concurrently preventing their aggregation (Allaka et al., 2023). SEM analysis approves the spherical shapes of AgO NPs, a morphology advantageous for catalytic activities. The EDS data, reveal that these NPs predominantly consist of Ag and O elements.

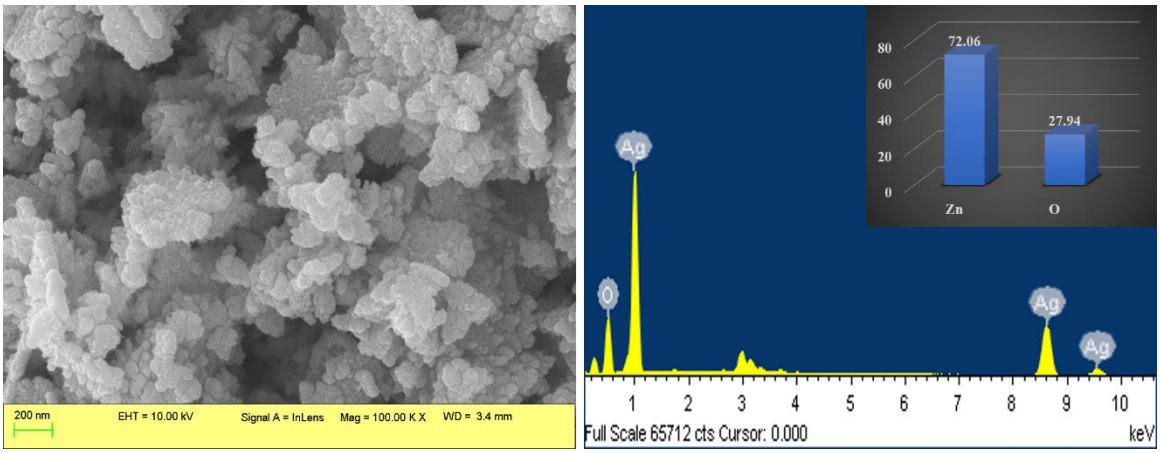

Figure5:SEM-EDS spectra for AgO NPs

3.5. TEM study

TEM analysis was employed to assess the particle size of AgO NPs. Noticeable differences in the size of the synthesized NPs were observed, possibly attributed to the presence of distinct biomolecules in AgO NPs. In **Figure 6**, TEM images of NPs synthesized using a green approach reveal a consistent spherical form. TEM investigations validate the spherical morphology of all synthesized NPs.The mean particle sizes, determined using ImageJ software by evaluating approximately 30-35 particles in a single image, was found to be 7 ± 0.91 nm AgO NPs.

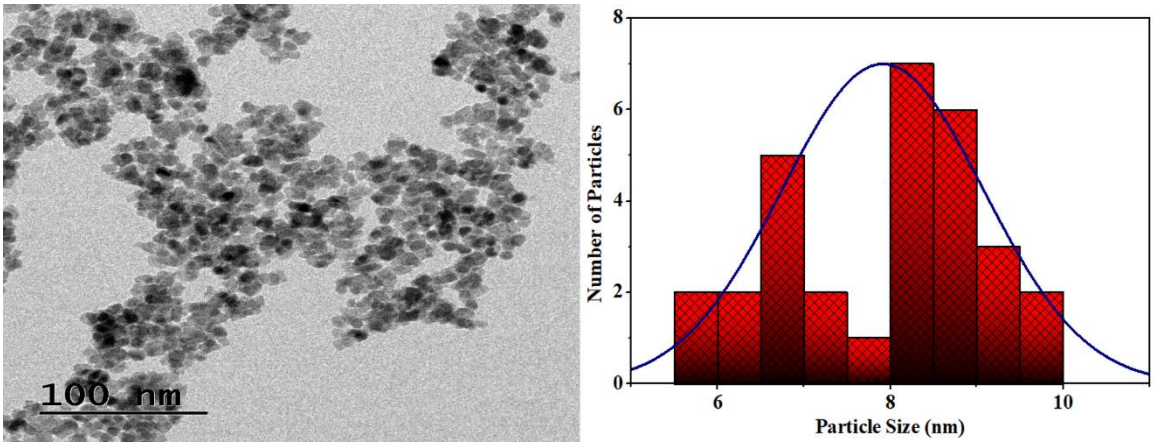

Figure6:TEM images for AgO NPs

3.6. Antibacterial activity

The impact of the varied size and shape, as documented in several studies, significantly influences the antibacterial efficacy of AgO NPs(Mani et al., 2021). These investigations have spurred researchers to synthesize finely tuned NPs with varying sizes to optimize antibacterial outcomes. Numerous studies suggest that smaller NPs can exert a potent effect on microbes, attributed to their increased penetration into bacterial membranes (Dharmaraj et al., 2021). In the present investigation, the synthesis involving a plant extract yielded spheroidal-shaped ZnO NPswith an average size 7 ± 0.91 nm. The evaluation of antibacterial capabilities against *Bacillus subtilis* and *Escherichia coli* was conducted utilizing the well diffusion method (Aisida et al., 2021). Unlike concentrations (5, 10, and 20 mg/mL^{-1}) of samples were prepared, and the corresponding zones of inhibition (ZOI) are illustrated in the provided **Figure7**. The manually measured ZOI in millimetres (mm) is presented in the accompanying **Table 2**. The study revealed that reducing NPs size and increasing concentration positively impacted antibacterial efficacy, with larger ZOIs observed. Additionally, the green synthesis approach contributed to achieving favorable outcomes.

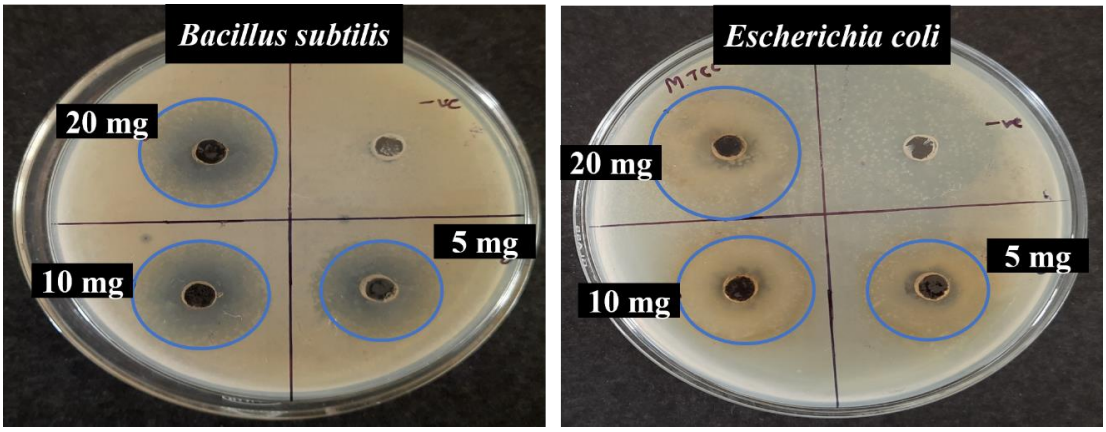

Figure 7: ZOI against *Bacillus subtilis* and *Escherichia coli* bacteria for AgO NPs

Table 2: ZOI values *Bacillus subtilis* and *Escherichia coli* bacteria for AgO NPs

Pathogens	**Concentration of NPs (mg/mL^{-1})**	**ZOI (mm)**
Bacillus subtilis	5	25
	10	28
	20	32
Escherichia coli	5	24
	10	26
	20	29

4. Conclusions

In summary, the eco-friendly synthesis of AgO NPsusing *Murraya koenigii* has showcased significant potential in various domains, encompassingantibacterial activity. The versatile and applicable nature of these NPs, derived from a sustainable approach, underscores their adaptability in tackling intricate challenges. The antibacterial efficacy against *Bacillus subtilis* and *Escherichia coli*, coupled with the adjustability for enhanced antibacterial performance, positions them as promising candidates for applications like antimicrobial coatings, wound dressings, and water purification systems.Delving deeper into optimizing synthesis parameters, such asconcentration and reaction conditions, could fine-tune the properties of these NPs. Additionally, comprehensive studies on the mechanistic aspects of their antibacterialactions will contribute to a more holistic understanding. These green-synthesized NPs exhibit promise for real-world applications, spanning advanced materials for environmental remediation to innovative solutions in healthcare. Continuous research and development in this field will undoubtedly uncover new possibilities and advance the utilization of green-synthesized AgO NPsin addressing contemporary challenges across diverse fields.

5. References

Aisida, S.O., Ugwu, K., Nwanya, A.C., Bashir, A.K.H., Nwankwo, N.U., Ahmed, I. and Ezema, F.I., 2021. Biosynthesis of silver oxide nanoparticles using leave extract of Telfairia Occidentalis and its antibacterial activity. Materials Today: Proceedings, 36, pp.208-213.

Allaka, G., King, M.F.L., Yepuri, V. and Narayana, R.L., 2023. Synthesis of silver oxide nanoparticles using gomutra mediation and their investigations on anti-oxidant property. Materials Today: Proceedings.

Daoudi, H., Bouafia, A., Meneceur, S., Laouini, S.E., Belkhalfa, H., Lebbihi, R. and Selmi, B., 2022. Secondary metabolite from nigella sativa seeds mediated synthesis of silver oxide nanoparticles for efficient antioxidant and antibacterial activity. Journal of Inorganic and Organometallic Polymers and Materials, 32(11), pp.4223-4236.

Dharmaraj, D., Krishnamoorthy, M., Rajendran, K., Karuppiah, K., Annamalai, J., Durairaj, K.R., Santhiyagu, P. and Ethiraj, K., 2021. Antibacterial and cytotoxicity activities of biosynthesized silver oxide (Ag2O) nanoparticles using Bacillus paramycoides. Journal of Drug Delivery Science and Technology, 61, p.102111.

Ernest Ravindran, R.S., Shanmugam, K., Veeramani, S., Ilangovan, R., Ali, D., Almutairi, B.O., Palanivel, H. and Goel, M., 2022. Effect of phytofabricated silver oxide nanoparticles on wound pathogens. Journal of Nanomaterials, 2022(1), p.1760829.

Haq, S., Yasin, K.A., Rehman, W., Waseem, M., Ahmed, M.N., Shahzad, M.I., Shahzad, N., Shah, A., Rehman, M.U. and Khan, B., 2021. Green synthesis of silver oxide nanostructures and investigation of their synergistic effect with moxifloxacin against selected microorganisms. Journal of Inorganic and Organometallic Polymers and Materials, 31, pp.1134-1142.

Islam, S.N., Naqvi, S.M.A., Parveen, S. and Ahmad, A., 2021. Application of mycogenic silver/silver oxide nanoparticles in electrochemical glucose sensing; alongside their catalytic and antimicrobial activity. 3 Biotech, 11(7), p.342.

Lopez-Carrizales, M., Pérez-Díaz, M.A., Mendoza-Mendoza, E., Peralta-Rodríguez, R.D., Ojeda-Galván, H.J., Portales-Pérez, D., Magaña-Aquino, M., Sánchez-Sánchez, R. and Martinez-Gutierrez, F., 2022. Green, novel, and one-step synthesis of silver oxide nanoparticles: antimicrobial activity, synergism with antibiotics, and cytotoxic studies. New Journal of Chemistry, 46(37), pp.17841-17853.

Mani, M., Harikrishnan, R., Purushothaman, P., Pavithra, S., Rajkumar, P., Kumaresan, S., Al Farraj, D.A., Elshikh, M.S., Balasubramanian, B. and Kaviyarasu, K., 2021. Systematic green synthesis of silver oxide nanoparticles for antimicrobial activity. Environmental research, 202, p.111627.

Mathimaran, A., Pandian, C.J., Sappanimuthu, P., Kirshnakumar, H., Amala, M., Veerapandiyan, M., Kingsly, J., Solomon, A., Sonamuthu, J. and Jeyaraman, J., 2024. Synthesis of multifunctional silver oxide, zinc oxide, copper oxide and gold nanoparticles for enhanced antibacterial activity against ESKAPE pathogens and antioxidant, anticancer activities using Momordica cymbalaria seed extract. Materials Today Communications, 39, p.108838.

Minhas, L.A., Kaleem, M., Jabeen, A., Ullah, N., Farooqi, H.M.U., Kamal, A., Inam, F., Alrefaei, A.F., Almutairi, M.H. and Mumtaz, A.S., 2023. Synthesis of Silver Oxide Nanoparticles: A Novel Approach for Antimicrobial Properties and Biomedical Performance, Featuring Nodulariaharaviana from the Cholistan Desert. Microorganisms, 11(10), p.2544.

Muhammad, S., Ali, A., Shah, J., Hamza, M., Kashif, M., Khel, B.K.A. and Iqbal, A., 2023. Using Moringa oleifera stem extract for green synthesis, characterization, and anti-inflammatory activity of silver oxide nanoparticles. Natural and Applied Sciences International Journal (NASIJ), 4(1), pp.80-97.

Pourmoslemi, S., Shokouhi, S. and Mahjub, R., 2021. Investigation of antibacterial activity of polyvinyl alcohol packaging films composed of silver oxide nanoparticles, graphene oxide and tragacanth gum using Box–Behnken design. Packaging Technology and Science, 34(10), pp.613-622.

Shahzad Shirazi, M., Moridi Farimani, M., Foroumadi, A., Ghanemi, K., Benaglia, M. and Makvandi, P., 2022. Bioengineered synthesis of phytochemical-adorned green silver oxide (Ag2O) nanoparticles via Mentha pulegium and Ficus carica extracts with high antioxidant, antibacterial, and antifungal activities. Scientific Reports, 12(1), p.21509.

Shinde, B.H., Shinde, P.B., Inamdar, A.K., Inamdar, S.N. and Chaudhari, S.B., 2023. Green synthesis of silver/silver oxide composite nanoparticles using Cuscuta Reflexa plant for the insecticidal applications. Materials Today: Proceedings, 92, pp.549-553.

Suresh, R., Yogeshwaran, A., Logababu, P., Sharath, P.S., Aakash, G. and Pugazhendhi, V., 2023. Empowerment the antibacterial activity of Silver Oxide nanoparticles using woodfordia Fruticosa flower extract. International Research Journal of Multidisciplinary Technovation, 5(4), pp.1-11.

Ullah, Z., Gul, F., Iqbal, J., Abbasi, B.A., Kanwal, S., Chalgham, W., El-Sheikh, M.A., Diltemiz, S.E. and Mahmood, T., 2023. Biogenic synthesis of multifunctional silver oxide nanoparticles (Ag2ONPs) using parieterial sinaefoliadelile aqueous extract and assessment of their diverse biological applications. Microorganisms, 11(4), p.1069.