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Synthesis and Characterization of Nanoparticles using Actinomycetes and its Evaluation for Antimicrobial Potential

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Author`s Contribution

[SA]Conception and design, Collection and assembly of data, [AA]Analysis and interpretation of the data, Statistical expertise, [SA, R] Final approval and guarantor of the article

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A B S T R A C T

Background: In the field of sustainable nanotechnology, eco-friendly methods of synthesizing nanoparticles are gaining popularity. Various types of microbes and plants have been used to synthesis the nanoparticles.

Objectives: To synthesis of silver nanoparticles (AgNPs) using Actinomycetes isolates and to characterize their antibacterial and antifungal activity.

Methodology: Actinomycetes extract was combined in various ratios with 1 mM AgNO3 to produce AgNPs. Visual examination and UV-V spectrophotometry were employed to confirm production of AgNPs, with an ideal 1:9 ratios. X-ray diffractometry was applied in the structural analysis to determine the cubic, crystalline nature of AgNPs. Aromatic amines were found to be essential components in AgNP synthesis by FT-IR spectroscopy. Atomic Force Microscopy size analysis identified nanoparticles with sizes ranging from 1 nm to 7.5 nm. Using the agar disc diffusion method, nanoparticles were assessed for their antibacterial and antifungal properties.

Results: AgNPs revealed variations in antifungal and bacterial activities. Large sizes zones of inhibition were observed for various bacterial strains and fungi species as AgNP concentrations increased (300 ppm, 400 ppm, and 500 ppm). These results demonstrate the concentration-dependent efficacy of AgNPs in preventing bacterial and mycotic infections.

Conclusions: Actinomycetes extracts allowed for the environmentally friendly synthesis of AgNP, yielding nanoparticles with potent antibacterial and antifungal properties linked to concentration. This highlights the capability of AgNPs for sustainable nanotechnology's biomedical and environmental applications.

Keywords: Silver Nanoparticles, Actinomycetes, Synthesis, Characterization

Introduction

Nanotechnology is a vital and emerging field in biotechnology research. Silver nanoparticles (AgNPs) are of great interest, and their synthesis using environment friendly and cost-effective methods is essential.[1] Biologically synthesized AgNPs play diverse roles as catalysts, electrical batteries, anti-parasitic agents, bio-labeling tools, and optical receptors.[2] Fungi offer an eco-friendly source for extracellular synthesis, while bacterial resources are favored for handling convenience.[3] This paper reviews the latest advancements in the biotechnological applications of AgNPs, highlighting their potential in various scientific fields. The focus is on the advantages of biologically synthesized AgNPs, promoting their use as catalysts, biosensors, conductors, diagnostics, and antimicrobial agents.

Nanoparticles (NPs) exhibit unique properties with applications in biomedical devices, renewable energy, cosmetics, and environmental remediation.[4] Among them, AgNPs stand out for their exceptional biological, chemical, and physical characteristics.[5]AgNPs are advantageous in electronics, medical imaging, and a variety of consumer goods due to their surface-enhanced Raman scattering, high thermal and electrical conductivity, non-linear optical behavior, catalytic activity, and chemical durability.[6] Green nanotechnology employs bacterial species and their extracts to synthesize NPs in an eco-friendly manner.[7] AgNPs demonstrate potent antibacterial, antiviral, and antifungal properties, presenting immense potential for medicinal use and optical sensing. By exploring the green synthesis and multifaceted applications of AgNPs, this paper aims to promote sustainable nanotechnology advancements.[8]

Actinomycetes, a diverse group of bacteria, hold immense biotechnological significance due to their metabolic diversity.[9] Among the various methodologies for their classification, chemotaxonomy, numerical taxonomy, and molecular systematics have gained prominence.[10] Actinomycetes are gram-positive microbes known for their ability to produce reproductive structures and form mycelia organizations.[11] They serve as rich sources of medicinal antibiotics like streptomycin, erythromycin, and gentamycin.[12] In recent times, metal nanoparticles (MNPs) production within solid matrixes has gained attention. Cellulose, owing to its mechanical strength and chemical resistivity, shows promise as a matrix material for MNPs. However, concerns regarding potential toxicity and ecotoxicology of nanotechnology persist.[13] Gold nanoparticles (AuNPs) have been used historically for medicinal and ornamental purposes, while silver nanoparticles (AgNPs) are extensively studied for their antibacterial, antiviral, and antifungal properties. AgNPs have found applications in medicine, agriculture, electronics, and other industries.[14] The research aimed to synthesize AgNPs using actinomycetes from the Peshawar region of Khyber Pakhtunkhwa, employing various methods and characterizing the nanoparticles with advanced techniques.

Methodology

This research study was conducted at Abasyn University Peshawar using wholesome bacterial extracts as ligands for NPs production. For the manufacture of AgNPs, silver nitrate (AgNO3) from Merck Pakistan was used. For bacterial growth and antimicrobial studies, nutrient agar, Eosin Methylene Blue media, potato dextrose broth, and lysogeny broth were obtained. Actinomycetes fresh cultures were prepared in the Microbiology laboratory at Abasyn University. UV-V spectrophotometer confirmed the synthesized NPs, while FTIR was used for characterization. Antimicrobial activities were evaluated to assess the nanoparticles' functionality.

Source of Microorganism:

Actinomycetes samples were gathered from a range of soil samples in various Peshawar district locations. The isolates were characterized at the Microbiology and Biotechnology laboratory,Abasyn University Peshawar. Pure cultures were maintained on nutrient broth agar at 27°C and sub-cultured periodically to ensure laboratory feasibility during the study period.

Identification of isolated bacteria

The isolated Actinomycetes were identified using Gram staining and confirmed through various biochemical tests including Coagulase, Oxidase, Indole production, motility, Urease, Catalase, and citrate utilization. The pure cultures were maintained on nutrient broth agar at 27°C and sub-cultured periodically during the study. For the production of biomass, Actinomycetes were cultured in nutrient broth medium at 37°C with agitation at 220 rpm. After 24 hours, the biomass was collected, centrifuged at 10,000 rpm for 15 minutes, and were subjected for the synthesis of nanoparticles following the methods described by.[15]

Production of Silver nanoparticles

AgNPs were produced by reacting 1 mM $AgNO_3$ with Actinomycetes supernatant in various ratios, using pure $AgNO_3$ as a control. Incubation in the dark for 24 hours resulted in color change, indicating AgNPs synthesis. Purification was achieved through 10,000 rpm centrifugation twice for 5 minutes, following.[16] The pellet containing purified AgNPs was used for further characterization.

Assessment of Nanoparticles

The early evidence of AgNPs growth in the mixture was the change in color. When measured, the absorbance of various wavelengths proved that AgNPs were present. FT-IR analysis was performed to confirm the compound responsible for the reduction of Ag ions, following the method described by.[17]

Antibacterial Activity

Actinomycetes bacterial cultures were isolated on nutrient agar petri dishes. For the antibacterial activity study of AgNPs suspension, fresh colonies were suspended in liquid broth (LB) and incubated. The experimental flasks received AgNPs suspension while the control flasks did not. Optical density (OD) of bacterial growth media was measured at regular intervals using UV-Vis Spectroscope at 600 nm. Growth curves were drawn for each bacterial strain, following the method by.[18]

Antifungal Activity (Disc Diffusion Assay)

Fresh fungal cultures were grown on Potato Dextrose Agar (PDA) for evaluation of antifungal activity. Fungi spores were standardized to an OD of 0.1 at 600nm and inoculated onto PDA plates. Actinomycetes extracts were added to 6mm-diameter discs that were placed on the plates. The plates underwent 24 hours of sterile incubation at 25°C. Zone of inhibition was measured to assess antifungal activity. Positive controls included Ciprofloxacin (for bacteria) and Fluconazole (for fungi), while Dimethyl Sulfoxide (DMSO) served as the negative control. The procedure followed the method.[19]

Results

AgNPs (AgNPs) Synthesis

A 1 mM solution of AgNO3 was used to create AgNPs, which were then visually verified. The production was further confirmed by UV-Vis Spectrophotometric measurement, which revealed Surface Plasmon Resonance (SPR) caused by the presence of free electrons in AgNPs. The resonance between the electrons and light wave was detected by the UV-Vis Spectrophotometer.

Visual confirmation of the synthesis

When AgNO3 was introduced to Actinomycetes, the extract's color changed from yellow to dark brown, providing visible proof that AgNPs had been created. The steady construction of NPs was noted as the color of the solution became denser with an increase in the proportion of Actinomycetes and $AgNO_3$, indicating the formation of a higher quantity of NPs.

UV-Vis Spectrophotometric confirmation

Surface Plasmon Resonance (SPR), which is triggered by free electrons in AgNPs, is a phenomena. AgNPs' collectively vibrating electrons and the light wave generate an aresonance, which was taken up by the UV-Vis Spectrophotometer's detector. The synthesis of AgNPs from 1mM solution of AgNO3 was monitored and confirmed Figure 1. The sharpness of the peak represents the purity of AgNPs synthesized.

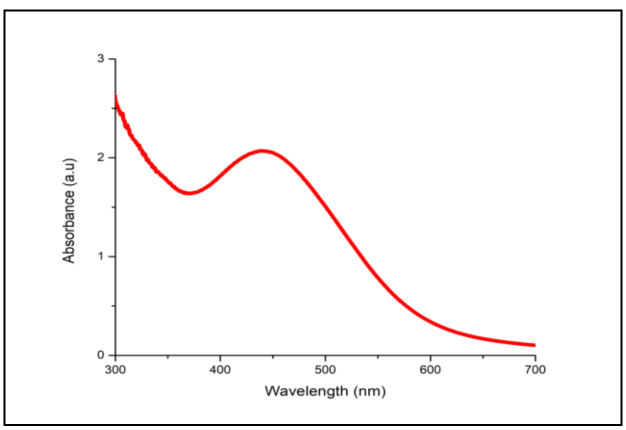


Figure 1: UV-Vis spectrum of AgNPs generated by diluting 9 ml of AgNO3 (1 mM) solution with 1 ml of pure Actinomycetes extract. The purity of the manufactured NPs is indicated by the strong peak of AgNPs at 440.41 nm.

The UV-Vis spectrum shows AgNPs synthesized using pure Actinomycetes extract (1 ml) with various combinationsof 1 mM $AgNO_3$ solution (figure 2). The maximum peak was observed at the ratio of 1:9 (Actinomycetes: $AgNO_3$). This ratio was selected for further studies. It was discovered using UV-visible spectroscopy that the sample had absorbed the greatest amount of energy at 445 nm, which was a typical peak value for AgNPs. Besides this, the absorption peak at 445 nm with no other peak displayed high purity of the nanoparticles as shown in Figure 2.

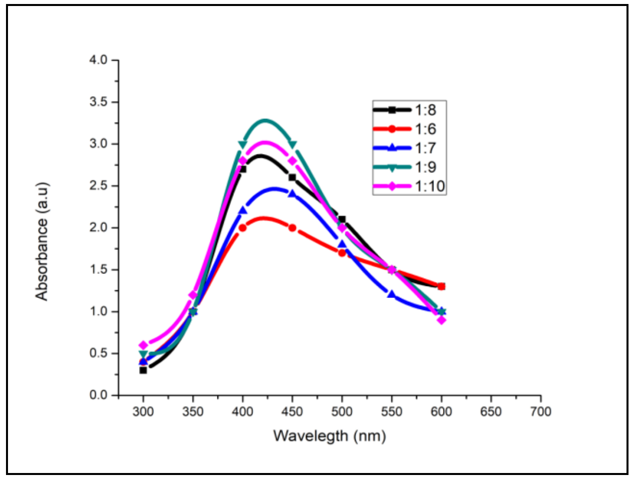


Figure 2:Comparing the UV-Vis spectra of AgNPs created by mixing pure actinomycete extract (1 ml) with various concentrations of AgNO3 (1 mM) solution.

AgNP formation is confirmation by FT-IR

FTIR analysis of Actinomycetes extract and synthesized AgNPs were performed, and different peaks were observed in the FTIR spectrum as shown in Figure 3 representing aromatic amines at 1335.62 cm-1suggesting that −C−N functional group compounds were involved in reducing Ag^{++} of $AgNO_3$ to AgNPs.

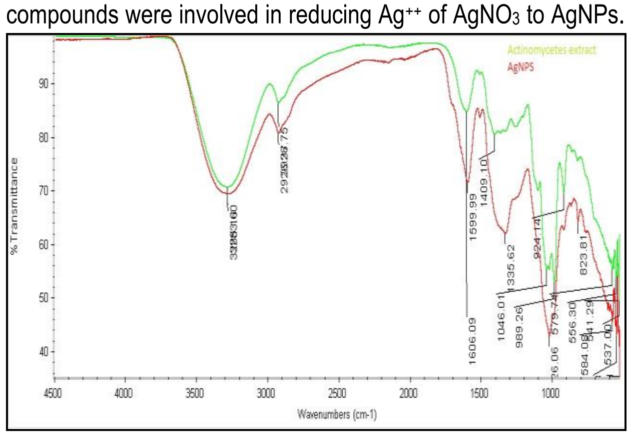


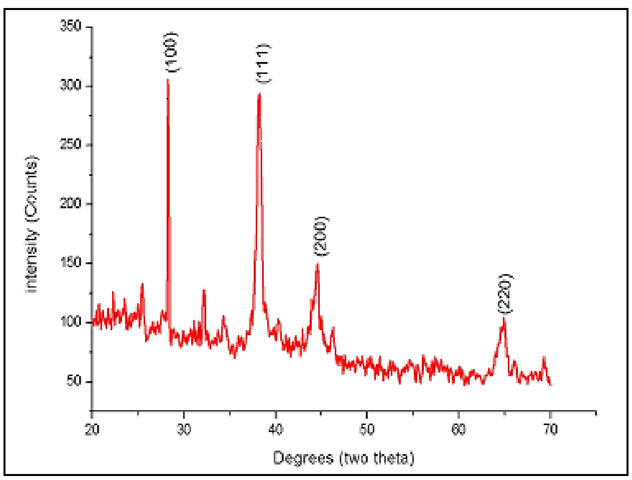
Figure 3:Comparative FT-IR analysis of Actinomycetes extract and synthesized AgNPs. Mainly ring aromatic compounds were responsible for synthesis of AgNPs.

A slight variation of ±1 to 100 wave numbers was observed between the Actinomycetes and AgNPs spectra, indicating the presence of ring aromatic compounds responsible for AgNPs formation. Changes in wave numbers were also observed at 3283.6 cm-1, 2926.23 cm-1, 1599.99 cm-1, 1335.62 cm-1, 1026.06 cm-1, and 823.81 cm-1 in the AgNPs spectra compared to Actinomycetes spectra.

X-Ray Diffraction (XRD) examination of AgNPs

XRD is a swift scientific technique that is mostly used to determine the phase of crystalline materials. In addition, it's utilized to give details about unit cell dimensions. The sample was found to be a finely grounded and homogenized substance on the basis of the XRD spectrum. The XRD examination of AgNPs confirmed their crystal-like nature.

The four district diffraction peaks at 2θ values of. 28.42°, 38.03°, 46.18°, and 63.43°could be indexed to the (100), (111), (200), and (220) reflection planes of cubic structure. The XRD pattern confirms that the AgNPs produced using the bio-inspired method were highly crystalline. The Nano crystallite size of AgNPs was determined to be 7.50 nm using the Sherrer equation, which aligns with AFM data (figure 5). The sharp peaks and absence of crystallographic impurities demonstrate the high purity and nano-sized nature of the synthesized AgNPs. (Figure 4).

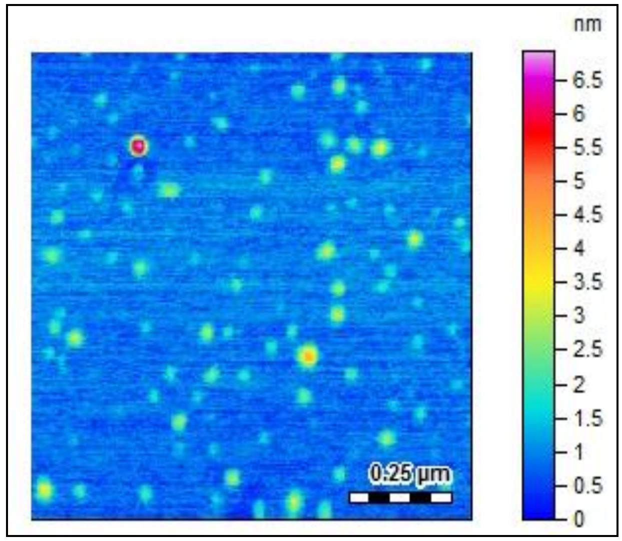


a.

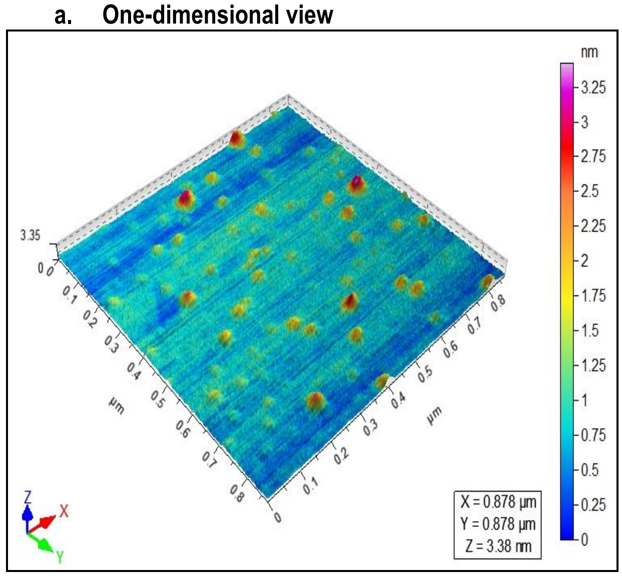
Figure 4. XRD patterns of silver nanoparticles synthesized using Actinomycetes extract displaying the crystal-like nature of AgNPs. The sharp peaks represent purity of AgNPS.

Atomic Force Microscopic analysis of AgNPs synthesized

AFM was used to determine both the particle's dimension and shape. The image in figure 5 demonstrates that the ordered particles were of various sizes. The smallest NPs were first estimated to have a diameter of 1 nm, while the largest NPs had a diameter of 7.5 nm. According to the figure's verified data, 60% of the manufactured particles were between 6-7 nm, 20% were between 3 and 4 nm, 20% were between 1 and 2 nm, and 40% were between 5 and nm, even though 40% of the AgNPs were produced between 5 and nm.



a. One-dimensional view



b. Two-dimensional view

Figure 5. Determination of AgNPs size by Atomic Force Microscope (AFM)

Antibacterial Activity of AgNPs (Concentration in ppm)

AgNPs were tested for their antibacterial efficacy against a variety of bacterial strains at three different concentrations (300 ppm, 400 ppm, and 500 ppm). Greater zones of inhibition typically accompanied higher AgNP concentrations, demonstrating a concentration-dependent interaction and highlighting the enhanced antibacterial activity of AgNPs. In particular, the inhibitory zones for E. coli at the various concentrations were 17±0.7 mm, 19±1 mm, and 22±2 mm. K. pneumonia showed inhibitory zones of 15±1 mm, 16±1.5 mm, and 17.5±1 mm. Additionally displaying different inhibitory zones along the concentration gradient were S. aureus, P.

aeruginosa, B. subtilis, X. campestris, and C. fruendi. The inhibitory zone size of P. aeruginosa impressively increased significantly as the concentration increased, going from 10±1.5 mm to 11±2 mm and then to 21.5±2 mm. The antibacterial action was consistently enhanced by concentration across all bacterial strains. In contrast, the negative control demonstrated no inhibition.

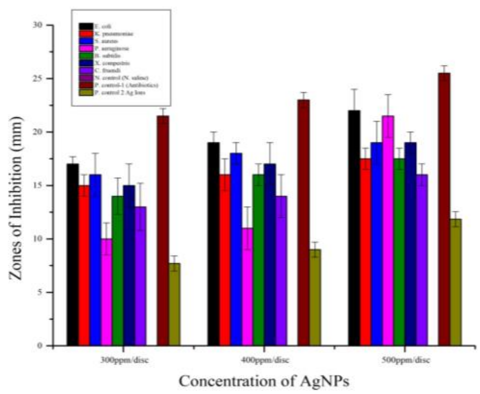


Figure 6: AgNPs of pure Actinomycetes extract exhibit antibacterial action by disc diffusion assay in contrast to several bacterial strains, bars represent the standard deviation.

Antifungal activity of AgNPs

Larger inhibitory zones are consistently associated with increasing AgNPs concentrations, demonstrating a concentration-dependent relationship, and revealing their heightened antifungal activity. Notably, *Penicillium chrysogenum* showed inhibition zones of 14.4±1.5 mm, 15±1 mm, and 16±1.5 mm, while *Candida albicans* showed inhibition zones of 17±2 mm, 19±1 mm, and 21±2 mm across the concentrations. Consistently growing inhibition zones against the investigated fungus strains demonstrate the AgNPs' capacity to perform concentration-associated antifungal action.

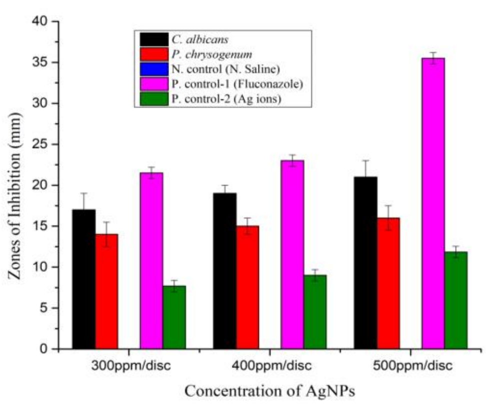


Figure 7. Antibacterial activity of AgNPs of pure extract of the Actinomycetes against two fungal strains by disc diffusion assay, bars signify standard deviation.

Discussion

The present study focused on the bio-inspired synthesis of silver nanoparticles (AgNPs) using eco-friendly methods, which have significant advantages over conventional chemical approaches. Silver nitrate and Actinomycetes extract were used as precursors for the synthesis of AgNPs. The color change of the extract from yellow to dark brown provided visual confirmation of the successful synthesis of AgNPs[20]. UV-Vis Spectrophotometric analysis further supported this observation by detecting the presence of free electrons in the AgNPs, leading to Surface Plasmon Resonance (SPR) and the characteristic absorption peak at 440.41 nm.

FT-IR analysis of the AgNPs and the Actinomycetes extract revealed the absence of certain absorption bands at 1335.62 cm-1, indicating the reduction of Ag++ ions to AgNPs by the compounds present in the extract. This reduction process is likely facilitated by the presence of aromatic amines and ring aromatic compounds.

With distinct Bragg's reflections at 28.42°, 38.03°, 46.18°, and 63.43°, which correspond to the (100), (111), (200), and (220) facets of AgNPs, respectively, the XRD analysis showed that the AgNPs were highly crystalline. AgNPs' calculated Nano crystallite size (7.50 nm) from the XRD data agreed with the AFM findings, confirming the size and shape of the particles.

A dose-dependent antibacterial activity of silver nanoparticles (AgNPs) against various bacterial strains notably, *E. coli*, *Staph* aureus and *K. pneumoniae* displayed a high degree of sensitivity, with significantly increased inhibition zones as AgNPs concentrations escalated from 300 ppm/disc to 400 ppm/disc and further to 500 ppm/disc, suggesting their potential as effective agents against these pathogens. Conversely P. aeruginosa exhibited comparatively lower sensitivity, necessitating higher concentrations or alternative treatments for effective control. *C. freundii*, *B. subtilis* and *X. compestris* demonstrated moderate sensitivity, with increasing zones of inhibition as AgNP concentrations rose. Remarkably, AgNPs consistently exhibited potent antifungal activity against C. albicans regardless of the concentration tested. These findings underscore AgNPs' potential as versatile antimicrobial agents, emphasizing the need for tailored approaches and further research into their mechanisms of action, safety, and potential synergies with existing therapies to enhance their clinical utility in combatting bacterial and fungal infections.

AgNPs' antifungal activity against *C. albicans* was effective at all tested concentrations, with the highest level of inhibition occurring at 500 ppm. Additionally, significant inhibition zones were observed at different concentrations for *P. chrysogynum,* indicating that it was highly susceptible to AgNPs.

The outcomes highlight the potential of bio-inspired techniques for the economical and sustainable synthesis of AgNPs. Additionally, AgNPs' antimicrobial qualities point to their potential use as efficient agents in a number of industries, including water purification, agriculture, and medicine. The study emphasizes the significance of environmentally friendly and economically advantageous methods for synthesizing nanoparticles that are inspired by biological processes. Additional study in this area may result in creative and long-lasting solutions for the many uses of nanoparticles in various industries.

Conclusion

In conclusion, an efficient and environmentally friendly method of producing nanomaterials is provided by the bio-inspired synthesis of AgNPs using Actinomycetes extract. AgNPs can be functionalized for a variety of biomedical applications and exhibit potential as drug transporters for increased antibiotic efficacy. Investigating their numerous attributes, such as antimicrobial, antifungal, antioxidant, phytotoxic, and insecticidal activities, holds promise for improvements in healthcare, agriculture, and the environment. AgNPs are in a position to make a significant contribution to solving current problems and developing numerous fields.

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