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Actinomycetes synthesized silver nanoparticles: characterization and its theraputic uses

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ABSTRACT

Nanoparticles define a major role in plant disease management and therapeutic applications due to their physical attributes. Because of their high surface to bulk silver atom ratio, silver is primarily made up of silver oxide. Strong antibacterial, antifungal, antiviral, and anti-inflammatory properties have been demonstrated by silver nanoparticles. Silver nanoparticles were created using a straightforward, affordable, and environmentally friendly biosynthetic process. The goal of this study was to compare silver nanoparticles made by chemical and microbiological processes and their therapeutic applications by using a pathogenic microorganism. The Actinomycetes sp. was used to create silver nanoparticles by a microbiological process and chemical synthesis by chemical reduction of silver ions (Ag+) with the help of sodium borohydride or sodium citrate. The silver nanoparticles that were produced using Actinomycetes and the obtained silver nanoparticles from chemical synthesis were used for characterization studied using UV-vis spectroscopy, FTIR and SEM. SEM images of the silver nanoparticles had sizes of 70nm -110 nm. In FTIR Analysis all the nanoparticles showed the same functional group of (-OH) ranges between 3000 to 3500 Wavenumber per cm, the Amine(-NH2) functional group attached between the 2000 to 2500 Wavenumber per cm and the carboxyl(-COOH) functional group attached between 1000 to 2000 Wavenumber per cm. In the UV-vis spectroscopy the presence of silver metal was confirmed the range between 200nm to 250nm. The synthesised silver nanoparticles showed the resistance against the pathogens like E. coli, Klebsiella sp., Candida sp., Pseudomonas sp. and Staphylococcus aureus. This antibacterial activity was conducted using Muller Hinton agar media by well diffusion method.

KEY WORDS

Nanoparticles; Actinomycetes; Chemical synthesis; Silver nanoparticles

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Introduction

Actinomycetes are a group of microorganisms which are lies between bacteria and fungi, these are gram positive, filamentous, strong antibiotic producing bacteria. Nearly 90% of actinomycetes genera have been found in soil, and they are beneficial in various areas, including industrial and pharmaceutical applications [1]. Additionally, actinomycetes generate natural pigments in cultures, which can be red, green, yellow, brown, or black. It has been proposed that Streptomyces are the primary organisms responsible for antibiotic production, while Nocardia and Micromonospora are less effective in comparison to Streptomyces in their antibiotic-producing capabilities. The Actinomycetes their high guanine and cytosine (G+C) (i.e.>55%) sequence percentage is high in their DNA [2].

Large amounts of actinomycetes can be found in soil, compost, and both terrestrial and aquatic habitats [3]. The creation of extracellular enzymes, synthesis of secondary metabolites, saprophytic activity, and the production of bioactive compounds make these bacteria significant economically [4]. During the degradation of organic materials, Actinomycetes' colonies frequently generate mycelia, just like fungi [5]. This collection of organisms is referred to as the phylum Actinomycetales (the Actinomycetota). Some soil bacteria like Frankia live symbiotically with plant roots with the roots providing saccharides to the bacteria, and in return, the bacteria help the plant in nitrogen fixation [6].

The demand for new antimicrobial agents is more urgent than ever due to the rise of multidrug-resistant pathogens, the swift emergence of novel infections, and the threat posed using multidrug-resistant bacteria in bioterrorism1. The ability of bacteria to resist the effects of antibiotics presents a significant challenge in the treatment of diseases. Infectious diseases continue to rank as the second leading cause of death globally [7].

The process of using environmentally friendly materials, such as bacteria, fungi, and plants, to produce nanoparticles is referred to as "green synthesis." These attractive biological methods are advantageous for the environment since they lack the disadvantages associated with conventional synthetic approaches [2]. Moreover, synthesis utilizing biologically derived extracts offers several advantages, including rapid production, high yields, and crucially no costly downstream processing required to form particles [8].

One of the most exciting areas in modern medical science is nanotechnology. The physical properties (dimensions and shape) of nanomaterials can be improved for application in both fundamental and practical research sectors. A particularly promising approach to tackle their inherent stability challenge is the development of polymer-metal nanocomposites that incorporate metal nanoparticles [9].

Nanotechnology is a crucial modern research area that focuses on the design, synthesis, and manipulation of particle structures that range from approximately 1 to 100 nano meters. Nanoparticles (NPs) have a diverse array of applications in fields such as healthcare, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space technology, drug-gene delivery, energy science, optoelectronics, catalysis, single electron transistors, light emitters, nonlinear optical devices, and photo-electrochemical applications. Size ranging

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between 1 x 10 -9 to 1 x 10 -7 m is considered a nanoparticle [10]. Nanoparticles are associated with a broad range of structural morphologies, including nanospheres, nanorods, nanochains, nanoflowers, nano-reefs, nanofibers, and nanoboxes [11,12]. Nanoparticles (NPs) are interesting due to their many medicinal and catalytic applications, as well as their antibacterial, antifungal, cytotoxic, antioxidant, bioremediation, and enzyme inhibitory qualities [13].When comparing bulk materials with the same chemical makeup to nanoparticles, their high surface to volume ratio causes them to exhibit unique and dramatically altered chemical, physical, and biological properties [14,15]. The reliance of NPs on size and form, their usage in chemical sensors, electrometers, wireless electronic logic, computer transistor memory layouts, biosensors, optics, and catalysis are among their intriguing aspects [16].

Nanoparticles (NPs) are gaining more interest in both commercial and applied microbiology sectors because of their antimicrobial properties, excellent electrical conductivity, and optical characteristics. For instance, silver NPs possess a wide-ranging antimicrobial effectiveness against various bacteria and fungi, which makes them perfect for reducing biofouling [17].

The application of silver nanoparticles as an antibacterial agent is a recent development. Thanks to their significant reactivity arising from a high surface-to-volume ratio, nanoparticles are essential in preventing bacterial proliferation in both liquid and solid environments [18]. Materials that contain silver can be utilized to eradicate microorganisms on textile fabrics, or they may be employed in the treatment of water. Unlike the bactericidal properties of ionic silver, the antimicrobial effectiveness of colloidal silver particles is affected by their size; smaller particles exhibit a stronger antimicrobial effect. Consequently, when developing synthesis methods, priority was given to managing the size of silver nanoparticles [19].

Materials and Methods

Soil sample collection and isolation of actinomycetes

The study was conducted at department of studies in Microbiology, Davangere University, Davangere, during August 2024. Various Soil samples were collected from the places of Chitradurga and Davangere districts.

The collected soil samples weighed about 1g, and they were serially diluted for tenfold dilution using 9ml of sterile distilledwater [20]. Starch casein agar media was prepared and sterilized by autoclaving at 121oC at 15lbs pressure for 15 minutes [21]. The sterilized media was poured into sterile Petri plates for solidification. After solidification, 0.1ml of sample was inoculated to the agar plates by spread plate method.

The inoculated plates were incubated at 28oC to 37oC for 5 to 6 days, then the plates were observed for the actinomycetes colony [22,15].

Microbial synthesis of silver nanoparticles

Starch casein broth was prepared and sterilized by autoclave at 121ºC at 15lbs pressure for 15 minutes. The Actinomycetes culture was inoculated into the medium and incubated at 37ºC for 8 to 10 days on rotatory shaker [23]. The mat growth was observed in the medium and then it was harvested by filtration method using whatman filter paper No.1. The harvested mat was used in silver nanoparticles synthesis [24,25], where 1mM of silver nitrate solution was prepared [26]. The mat was treated with a silver nitrate solution in one flask, while another flask contained an equal volume of culture filtrate mixed with 1 mM silver nitrate solution served as the control [27]. These flasks were exposed to sunlight for 24 hours and incubated at 30ºC for 3 days in dark condition on rotatory shakers. The synthesis of silver nanoparticles was indicated by change in the colour of the medium from yellow to brown [28]. The solution which turned into brown colour will be used for the extraction of nanoparticles [29]. The solution was filtered and then dried by using hot air oven, and the powder was obtained and used as a silver nanoparticle for further analysis [30].

Chemical synthesis of silver nanoparticles

30ml of 2mM sodium borohydride solution was taken in the beaker. This borohydride solution was continuously agitated vigorously by using a magnetic stirrer with continuous drop wise addition of 1mM silver nitrate solution until the solution appears bright yellow colour up to the volume of 10 ml. The solution reaction mixture was exposed to sunlight then the silver nanoparticles existence was determined by the colour change of the solution [31,32].

Characterization of silver nanoparticles

Ultraviolet visible spectroscopy

The mat growth was observed in the medium was taken and harvested by filtration method using whatman filter paper No.1. that was treated with the silver nitrate solution and incubate for 3 days then powdered was dried that powdered was dissolved in isopropanol that solution was used for UV analyses [24,33].

The harvested mat was used for the silver nanoparticles synthesis in which 1mM of silver nitrate solution was prepared [26,34]. The mat was treated with the silver nitrate solution. UV-vis spectroscopy works on the principle of Beer-Lambert law. Depending on their chemical and physical properties, the molecules in the sample will absorb light at different wavelengths when it passes through them [35]. An excited state typically occurs from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) [36,37]. A portion of the light energy will be absorbed as the electron is moved to a higher energy orbital when sample molecules are exposed to light with an energy that corresponds to a potential electronic transition within the molecule [38]. The wavelength at which absorption takes place and the amount of absorption at each wavelength are both recorded by the spectmolecule38aph of the resultant spectra has displayed [39].

FTIR (Fourier Transform InfraRed spectroscopy)

The mat growth was observed in the medium was taken and harvested by filtration method using whatman filter paper No.1. that was treated with the silver nitrate solution and incubate for 3 days then powdered was dried that powdered was dissolved in isopropanol that solution was used for FTIR analyses [24,40].

A beam splitter is used to split light from a beam source at different infrared wavelengths to two mirrors: one that remains stationary and the other that moves at a consistent speed. Then, with the path difference between the beams, these beams are reflected and recombined to create an

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interference pattern that reflects the constructive and destructive interference of the recombination [41]. Following that, the sample receives this interference pattern, also known as an interferogram, and the transmitted portion of the interferogram is then transferred to a detector [42]. To detect the complete spectrum, a Fourier transform is used in the detector following comparison with a reference sample beam spectrum [43].

SEM (Scanning Electron Microscope)

The mat growth observed in the medium was taken and harvested by filtration method using whatman filter paper No.1. It was treated with the silver nitrate solution and incubated for 3 days. It is then dried, powdered and analyzed under SEM to study its structure [24,44].

The sample is placed on a stage within the chamber, and vacuum is created using number of pumps. The microscope design determines the vacuum level. Variable pumping of certain microscopes enables low-vacuum imaging by maintaining the sample chamber at a lower vacuum level than the other sections of column [45]. Electrons are produced at the top of the column in the electron gun and accelerated through the column at a specified accelerating voltage between 1 kV - 30 kV [46]. Condenser lenses and apertures are used to reduce the beam diameter, and objective lens focuses the beam on the sample surface [47].

Antimicrobial assay

The antibacterial assay of nanoparticles was carried out by agar well diffusion method against E. Coli, Candida sp., Klebsiella sp., Staphylococcus aureus and Pseudomonas sp. (Obtained from JJM Medical college, Davangere). Muller Hinton agar (MHA) media was prepared, and the pathogenic organism was added on the agar medium. Then the wells were made by corkborer, and the nanoparticle samples are filled into the respective wells [48]. Then the plates are incubated at 37°C for 24 hours and later the clear zone around the well was observed and well diameter was measured [49,24].

Results and Discussions

Actinomycetes Isolation

Actinomycetes are isolated from different soil samples. The colonies of Actinomycetes are observed on SCA medium and cfu/g of soil sample was calculated, tabulated in table 1. Figure 1 represents the isolated plates of actinomycetes, and Figure 2 represents the pure cultures of actinomycetes.

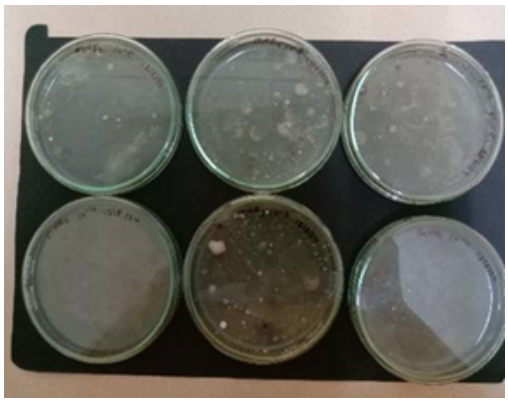

Figure 1. Isolated plates of Actinomycetes.

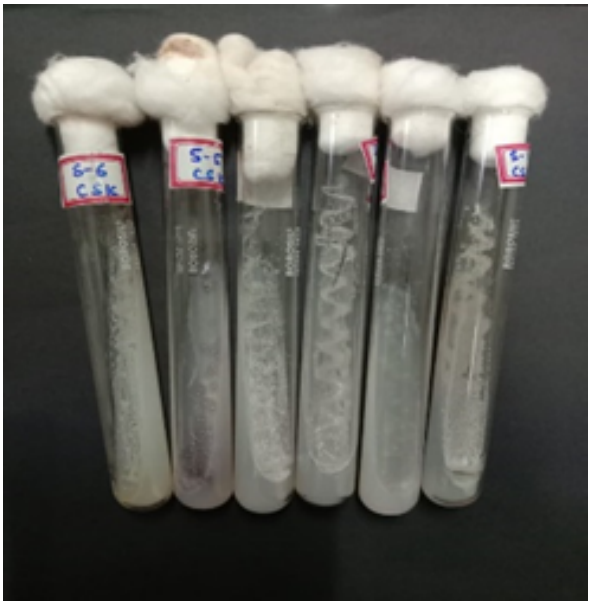

Figure 2. Pure cultures of Actinomycetes.

Table 1. Isolation of Actinomycetes.

Sl.no	Place of collection	Name of the sample	No. of colonies	Cfu/g
1	B G kere	Soil sample1	74	$7.4x10^3$
2	Gowripura	Soil sample2	34	$3.4x10^3$
3	Kurki	Soil sample3	82	$8.2x10^3$
4	Davangere University Campus	Soil sample4	49	$4.2x10^3$

Identification

Biochemical test is conducted to screen the actinomycetes ability to produce the enzymes, utilization of different sugars and production of gas (Ranjith et al., 2017). The all 6 organisms show the gram-positive cocci in grams reaction. These organisms could produce enzymes like citrate permease, urease, gelatinase and hence gives positive result for gelatinase test (3 positive and 3 negative), citrate utilization test (6 positive) and urease test (6 positive). The organism also ability to utilize different sugars which is shown by methyl red test (6 positive) and TSI test (6 positive) and unable to produce butanediol ring hence these organisms give negative result for VP test (6 negative). These have ability to produce H2S gas and gives positive results for H_2S production test (6 positive). The results are represented in table 2.

Table 2. Outcomes of Biochemical tests.

Organism	SS 1	SS 2	SS 3	SS 4	SS 5	SS 6
Gram's staining	+Cocci	+Cocci	+Cocci	+Cocci	+Cocci	+Cocci
Gelatinase test	-	-	+	-	+	+
Citrate utilization test	+	+	+	-	+	+
Urease test	+	+	+	+	+	+
MR test	+	+	+	+	+	+
VP test	-	-	-	-	-	-
Indole test	+	-	+	-	+	-
TSI test	+	+	+	+	+	+
H_2S production test	+	+	+	+	+	+

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Microbial synthesis of silver nanoparticles

The culture filtrate of the organism was treated with equal volume of 1mM silver nitrate solution in one flask is taken as test sample 100ml:100ml in equal portion of culture filtrate and 1mM silver nitrate and in another flask the culture filtrate was not treated with 1mM silver nitrate solution is taken as a blank. After incubation all the tested samples were changed to colour where A represents sample 1 turn to gray colour, B represents sample 2 turn to dark brown colour and C represents sample 3 turn to lite brown colour. Hence all the samples were indicating the production of silver nanoparticles represented in figure 3.

Similarly, Bhosale et al., worked on same area that, Production of silver nanoparticles by two distinct Actinomycetes species Streptomyces sp. and Streptomyces verticillium sp [15].

Figure 3. Test organisms treated with 1mM silver nitrate solution; A. Streptomyces SS1, B. Streptomyces SS2, C. Streptomyces SS3.

Chemical synthesis of silver nanoparticles

Figure 4 represents the colour of solution changes to light yellow colour after the reaction complete, this change in colour indicates AgNPs synthesis. In chemical synthesis of silver nanoparticles, the silver nitrate is reduced to silver ion by 2mM sodium borohydride or sodium citrate.But in biological synthesis, actinomycetes are responsible for silver nitrate reduction into silver ion by reductase enzyme. Chemical synthesis was hazardous to the environment compared to biological synthesis. Simultaneously, Wang et al., worked on same area and synthesize the AgNPs by chemical method with the help of silver nitrite and used for anticancer drugs development to improve cancer treatment in clinical settings [50].

Figure 4. Chemically synthesized silver nanoparticles.

Characterization of Silver Nanoparticle

UV visible spectroscopy

Figure 5 represents the presence of silver metal ion in the prepared nanoparticle sample. All the samples were analyzed in UV-Vis spectrophotometer (Module: UH5300) that shows the peak almost at similar wavelength between 200nm to 300nm. The Streptomyces SS organism 1 is which has shown highest peak at 231nm, Streptomyces SS organism 2 is which has shown highest peak at 210nm, and Streptomyces SS organism 3 is which has shown highest peak at 211 nm. These were compared to chemical synthesis which has shown highest peak at 257 nm.

Similarly, Vimala et al., also worked on same area and use the aqueous solution containing silver ions has shown a highest peak in the UV-Vis spectrum between 410 nm to 430nm [51].

Simultaneously Dawadi et al., also worked on same area and treated culture supernatant suggested the formation of AgNPs has shown a highest peak in the UV-Vis spectrum between 420nm [37].

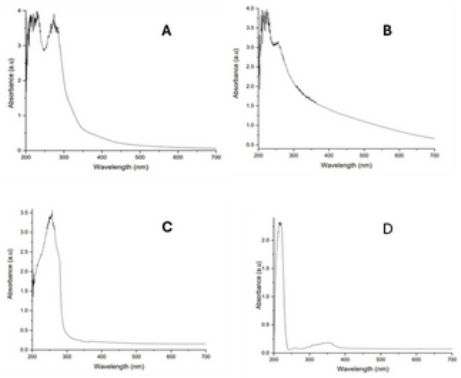

Figure 5.UV Analysis of silver nanoparticles; A. Streptomyces SS1, B. Streptomyces SS2, C. Chemical synthesis, D. Streptomyces SS3.

FTIR

Figure 6 represents the FTIR (Module: Alpha 2) analysis of silver nanoparticles. The AgNPs produced by the Streptomyces SS1, 2, 3 and by chemical synthesis were shows the binding of Hydroxyl(-OH) functional group between 3000 to 3500 Wavenumber per cm, the Amine(-NH2) functional group attached between the 2000 to 2500 Wavenumber per cm and the carboxyl(-COOH) functional group attached between 1000 to 2000 Wavenumber per cm. Here, all the functional groups are attached in between similar range of Wavenumber per cm.

Similarly, Prakash, et al., also worked on same area and they got similar results where, -OH functional group at 3338Wavenumber per cm, -NH2 group at 1338 Wavenumber per cm, C=O group at 1552 Wavenumber per cm, -CH group at 2979 Wavenumber per cm and -CN group at 1100 Wavenumber per cm [52].

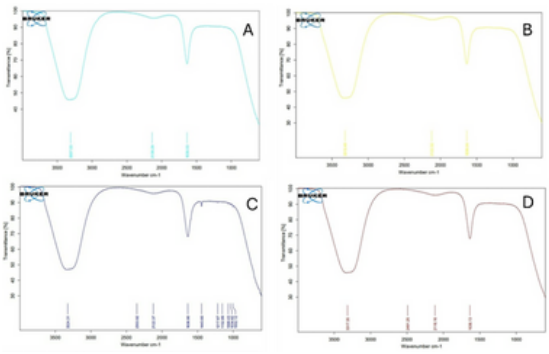

Figure 6.FTIR Analysis of silver nanoparticles; A. Streptomyces SS1, B. Streptomyces SS2, C. Chemical synthesis, D. Streptomyces SS3.

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SEM

Figure 7 presents the SEM analysis of silver nanoparticles (AgNPs). It is the analysis of powder sample where; the sample was mounted on the aluminium holder stubs using a double stickey carbon tape and coated with Au/Pd in a SPI MODULETM High resolution sputter coater and examined in the ZEISS Scanning microscope (Module: EVOLS 15) at 15kV. It determines the size of AgNPs. In this result the size of AgNPs was found to be between 70 nm to 110 nm. The size of the two AgNPs are 71.28nm and 105.5nm. Similarly, Bhosale et al., also worked in the same area where, they synthesized the silver nanoparticles of 1 to 40nm size [15].

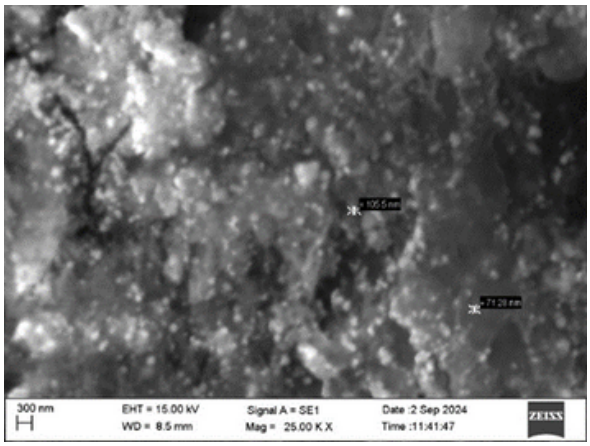

Figure 7.SEM analysis of silver nanoparticles.

Antimicrobial Activity

Table 3 represents antimicrobial activity of culture filtrates and synthesized nanoparticles from Actinomycetes are compared with the chemically synthesized nanoparticles against the pathogenic organisms like E. coli, Klebsiella sp., Candida sp., Pseudomonas sp. and Staphylococcus aureus [Figure 8]. As per the experiment. Petridish A and B have E. coli and Klebsiella sp. colonies. Petridish C and D have Candida sp. and Pseudomonas sp. colonies. Petridish E and F have Staphylococcus aureus sp. colony. The increase of clear zone from 1mm to 5mm indicates that biosynthesized AgNPs are more effective than chemically synthesized AgNPs and antibiotic produced by Actinomycetes.

Similarly, Naganthran et al., also worked on same area where, the synthesized AgNPs showed a strong antibacterial effect on Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, and E. coli. Additionally, the Streptomyces AgNPs that were shown biofilm inhibitory ability against hospital-resistant nosocomial bacteria, like Escherichia coli, Staphylococcus aureus, and Bacillus subtilis [46].

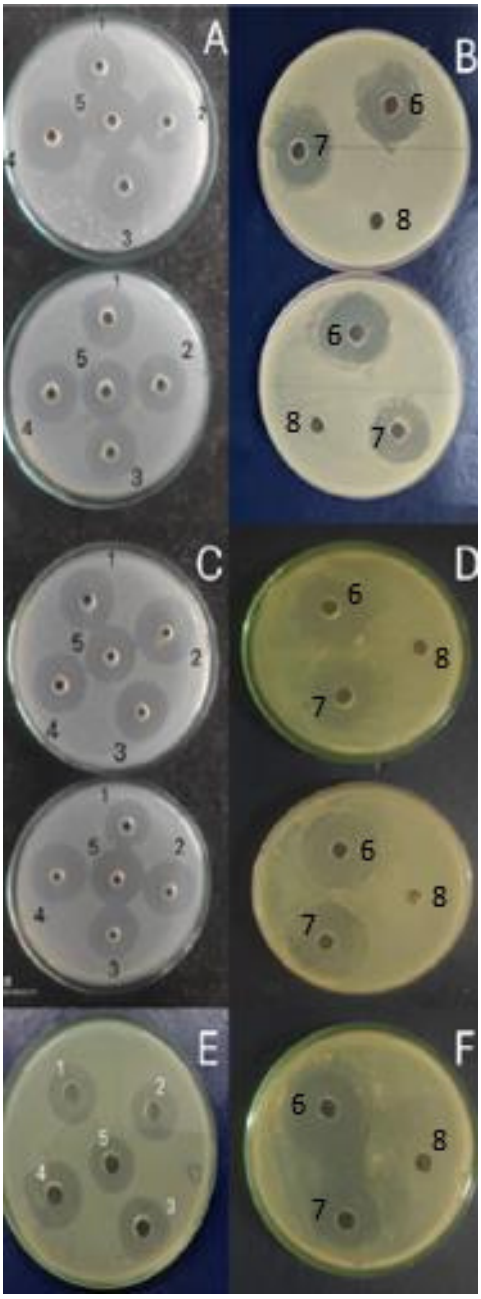

Figure 8. Antimicrobial activity by agar well diffusion method; 1. Culture filtrate 1, 2. Chemical synthesis, 3. Chemical synthesis of AgNPs sample, 4. AgNPs 2, 5. AgNPs3, 6. Culture filtrate 2, 7. Culture filtrate 3, 8. Negative Control.

Table 3: Antimicrobial activity of Silver Nanoparticles.

DIAMETER OF CLEAR ZONE IN mm							
Organisms	**Culture filtrate1**	**Culture filtrate2**	**Culture filtrate3**	**Chemical synthesis**	**AgNPs1**	**AgNPs2**	**AgNPs3**
E. coli	21	23	22	23	23	25	24
Klebsiella sp.	22	21	17	19	24	20	18
Candida sp.	17	22	19	19	20	23	21
Pseudomonas sp.	14	15	17	16	16	19	18
Staphylococcus aureus	17	19	17	15	18	20	19

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Conclusions

This study demonstrates the eco-friendly biosynthesis of silver nanoparticles utilizing Actinomycetes species as an effective biological system. The microbial method demonstrated a sustainable and non-toxic alternative to traditional chemical synthesis, yielding nanoparticles with a well-defined morphology (70–110 nm) and functional stability, as verified by UV–Vis spectroscopy, FTIR, and SEM analyses. Biosynthesized silver nanoparticles demonstrated significant antibacterial activity against various pathogenic organisms, including *E. coli*, *Klebsiella sp.*, *Pseudomonas sp.*, *Candida sp.*, and *Staphylococcus aureus*, outperforming chemically synthesized alternatives.

The improved antimicrobial activity is due to the reduced particle size, greater surface area, and the synergistic interaction between the bioactive metabolites of Actinomycetes and metallic silver. This demonstrates that microbial synthesis is a viable approach for generating high-purity, biologically compatible nanoparticles appropriate for therapeutic and biomedical uses.

Future research should concentrate on optimizing production parameters, investigating the molecular mechanisms of antimicrobial action, and broadening the application of biogenic silver nanoparticles in wound healing, targeted drug delivery, and biofilm inhibition. This study's findings provide significant insights into sustainable nanotechnology and facilitate the translation of Actinomycetes-derived nanoparticles into clinically relevant antimicrobial formulations.

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Conflict of Interest

No potential conflict of interest was reported by the author.

Funding Source

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Ethical Approval

Nonapplicable in this study, because it did not involve any research studies on humans or living animals.

Author's Contributions

Formal analysis, Conceptualization, Methodology, Writing Review and Editing: Niharika S M.; Conceptualization, Methodology, Formal analysis, Revision and Editing: Niharika S M.; Supervision, Conceptualization, Revision and Editing: Dr. Shivaveerakumar S.

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