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ORIGINAL ARTICLE

Biosynthesis of zinc oxide nanoparticles from actinomycetes and its therapeutic applications

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ABSTRACT

This groundbreaking study delves into the biosynthesis of zinc oxide nanoparticles (ZnO NPs) utilizing actinomycetes isolated from soil, shedding light on their remarkable therapeutic potential. Through meticulous screening and optimization, actinomycete strains were identified as efficient producers of ZnO NPs, yielding particles ranging from 70-90 nm in size. Advanced characterization techniques, including scanning electron microscopy (SEM), UV-Visible spectroscopy, and Fourier transform infrared (FTIR) spectroscopy, confirmed the nanoparticles' size and structure. The biosynthesized ZnO NPs demonstrated profound antibacterial activity against multidrug-resistant pathogens, highlighting their promise in combating antimicrobial resistance. This eco-friendly and cost-effective biosynthesis approach presents a sustainable alternative to chemical methods, paving the way for future developments in nanomedicine. By harnessing the potential of actinomycetes, this research offers a viable solution for addressing the growing concern of antimicrobial resistance and advancing healthcare. The therapeutic applications of ZnO NPs are vast, with potential uses in wound healing, targeted drug delivery, cancer therapy, and biosensors. This study validates actinomycete-mediated ZnO NPs biosynthesis as a groundbreaking approach, opening new avenues for nanomedicine innovation. As the world grapples with the challenges of antimicrobial resistance, this research provides a beacon of hope for the development of novel, effective, and sustainable treatments.

KEY WORDS

Nanoparticles; Actinomycetes; Chemical synthesis; Zinc oxide nanoparticles

ARTICLE HISTORY

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Introduction

Actinomycetes is the name of a class of organisms derived from the Greek words "atkis" (ray) and "mykes" (fungus) [1]. However, they possess enough distinct qualities to identify them as belonging to the kingdom of bacteria. Spores are produced by gram-positive, aerobic actinomycetes whose genomes are primarily based on guanine-cytosine (>55%) [2]. They are members of the Actinomycetales group and grow their aerial mycelium on a substrate. Like mushrooms, they are filamentous, free living, feature real aerial hyphae where they are most common in dry and alkaline soil [3,4].

Although research on the antibacterial properties of naturally occurring compounds has been carried out, most efforts to address this have focused on creating nano coatings or nanocomposites with zinc oxide and silver nanoparticulates [5]. There is continuous research into specific applications of nanomaterials, such as how microbial development in food products causes food to degrade and attracts pathogens. Inhibitors in addition to serving as antibiotic carriers and lethal agents [6,7]. The antibacterial activity of zinc oxide increases with decreasing particle size [8].

One of the various types of nanoparticles (NPs) that are gaining popularity is zinc nanoparticles, or zinc oxide nanoparticles [9]. However, there is a paucity of research in this area, therefore to use zinc oxide nanoparticles successfully, it is crucial to understand the mechanisms underlying their influence and significant interactions with the biological and physical environments of the soil [10,11].

In recent years, man-made nanoparticles or nanoparticles have gained popularity. Because many of these NPs are used in agriculture as nano-pesticides and nano-fertilizers, they have the potential to accumulate extensively in soil [12]. Despite being more environmentally beneficial, zinc oxide nanoparticles have a special antibacterial activity that can affect the soil microbiota and, in turn, important microbial processes including mineralization, nitrogen fixation, and activities that stimulate plant development [13]. How they behave and remain in the soil, determined by their chemical composition and the characteristics of the soil [14]. This describes the applications of zinc oxide nanoparticles in soil systems and their effects on various plants and soil microorganisms, especially rhizobacteria that promote plant growth [15]. Zinc oxide nanoparticles stimulate plant morphometric and biochemical characteristics, as well as the activity of the soil microbiota at a relative level [16].

A variety of products and materials, including metals, polymers, glass, ceramics, and pigments, are enhanced using zinc nanoparticles. Such nanoparticles can be synthesized chemically or biologically; however, the later method results in toxic and dangerous consequences [17,18]. Thus, developing nanoparticle production processes that are both ecologically benign and repeatable is crucial19. Therefore, to avoid the accumulation of hazardous substances, it is preferable to use biological processes for the creation of nanoparticles [19,20]. The study was conducted during August 2024 in the department of Microbiology, Davangere University, Davangere.

Materials and Methods

Isolation and identification of actinomycetes

Soil samples were collected from various places of Honnali, Kotemalluru, Savalanga and Tholhunse in Davangere district, Rajendranagar and Bhadravathi in Shivamogga district and stored in polythene bags. Actinomycetes were isolated from soil samples using the serial dilution method [21]. This method

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is done by weighing 0.5 g of soil and mixing with 9 ml of distilled water in individual test tubes, followed by vigorous shaking to create stock cultures [22]. From these, 1 ml aliquots were aseptically transferred to test tubes containing 9 ml of sterile physiological, each sample was further diluted up to the dilution 10-5 and 0.1 ml was plated on starch casein agar medium [23,24]. After incubating at 30°C for 7 days, actinomycetes colonies with diverse morphologies were obtained, indicating successful isolation [25].

Chemical synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles were synthesized using the coprecipitation method. NaOH (0.48g, 0.1M) and ZnSO4 (0.86g, 0.1M) solutions were prepared in 30ml distilled water [26]. The ZnSO4 solution was gradually added to NaOH over 20-60 minutes, and the mixture was stirred for two hours to maintain temperature. A transparent to white precipitate formed, confirming the synthesis of zinc oxide nanoparticles. The nanoparticles were then separated from the supernatant dispersion. This simple and efficient method allowed for controlled nanoparticle formation [27,28].

Microbial synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles (ZnO NPs) were biosynthesized using actinomycete culture. A starch-casein broth inoculated with a well-grown actinomycete culture was incubated at room temperature for 4-5 days [29]. The culture filtrate (50 mL) was then mixed with equal volumes of zinc sulphate (0.1M) and sodium hydroxide (0.4 M) in an Erlenmeyer flask, shaken, and maintained at 40°C for 15 minutes, allowing zinc oxide nanoparticles to form and settle, confirmed by the emergence of white deposits at the flask bottom [30]. The zinc oxide nanoparticles were washed with deionized water, centrifuged at 3000 rpm for 10 minutes, and repeated until a clear supernatant was obtained [31]. Finally, the pellet was dried in an incubator at 37°C for 24 hours, yielding powdery zinc oxide nanoparticles [32].

Characterization Of Nanoparticles

FTIR (Fourier Transform InfraRed spectroscopy)

Chemically produced zinc oxide nanoparticles are used as standard with microbially produced zinc oxide nanoparticles. The observed mat was filtered and treated with the zinc sulphate solution, incubated for 24 hours, and then dried into a powder [33]. The powder was then dissolved in isopropanol, which was used for FTIR analysis. FTIR spectroscopy works by splitting infrared light into two paths, one stationary and one pattern, which, after passing through the sample, is detected and compared to a reference [34]. Through Fourier transformation, the interferogram is converted into a spectrum, revealing the sample's molecular composition and structure as a function of wavenumber, providing insights into chemical bonding and molecular structure [35].

Ultraviolet visible spectroscopy

Chemically produced zinc oxide nanoparticles are used as standard with microbially produced zinc oxide nanoparticles. The observed mat was filtered and treated with the zinc sulphate solution, incubated for 24 hours, and then dried into a powder [33]. The powder was then dissolved in isopropanol, which was used for UV analysis. UV-Visible spectroscopy measures the absorption of light by molecules at specific wavelengths, driven by electronic transitions from the highestoccupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), resulting in an excited state [36].The energy difference between these orbitals is represented by the absorbed light energy, and the resulting data is displayed as an absorption spectrum graph that shows wavelength and absorption intensity. This process is described by Beer-Lambert's law. Chemically synthesized and biosynthesized samples [37].

SEM (Scanning Electron Microscope)

Chemically produced zinc oxide nanoparticles assumed as standard with microbially produced zinc oxide nanoparticles. The observed mat was filtered and treated with the zinc sulphate solution, incubated for 24 hours, and then dried into a powder [33]. The powder was then dissolved in isopropanol, which was used for SEM analysis. A Scanning Electron Microscope (SEM) analyses samples within a vacuum chamber. Electrons generated from an electron gun are accelerated at 1-30 kV and focused onto the sample surface [38]. The electron beam interaction produces signals revealing detailed information on surface morphology and composition. The microscope's design dictates the required vacuum level, enabling high-resolution imaging [39].

Antibiotic sensitivity test

The antibacterial efficacy of nanoparticles was evaluated using the well diffusion method against five pathogenic microorganisms; E. coli, Klebsiella sp., Candida sp., Pseudomonas sp., and Staphylococcus aureus procured from JJM Medical College, Davangere. Muller Hinton agar (MHA) media was prepared, sterilized by autoclaving at 121°C for 15 minutes at 15 lbs pressure, and poured into sterile petri plates [40]. After solidification, the pathogens were inoculated onto the media, and wells were created using a cork borer. The wells were filled with nanoparticle samples and incubated at 37°C for 24 hours. Antibacterial activity was assessed by measuring the zone of inhibition (clear zone) around each well [41].

Results and Discussions

Isolation and identification

Using selective medium, actinomycetes were isolated, but due to their common characteristics, it was difficult to differentiate them from bacterial and fungal colonies. To overcome this, actinomycetes were grown on starch casein agar and distinguished by their unique colony morphology and Gram staining characteristics. The characterization of these colonies as actinomycetes was confirmed by the presence of small to medium-sized, dry, powdery formations combined with filamentous structures that were heavily branched and Gram-positive. Table 1 represents the place of sample collection and number of colonies and CFU/ml of actinomycetes. The maximum actinomycetes colonies were found in the sample collected from Honnali (45 isolates) and least were in the sample collected from Rajendradagar and Tholunse (11 isolates). Further 6 isolates were selected for characterization of actinomycetes. Figure 1 and 2 represents the culture plates and the pure cultures after frequent subcultures.

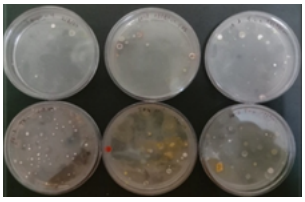

Figure 1. Cultures plates of actinomycetes on SCA media

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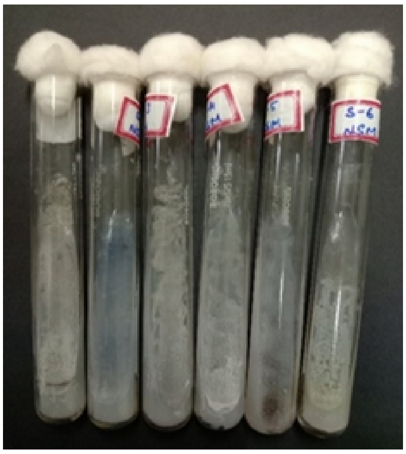

Figure 2. Pure cultures of actinomycetes.

Table 1. Number of colonies were isolated from different soil samples.

Sl.no	Place	Name of the Sample	Number of colonies	Cfu/g
1	Honnali	Soil sample 1	45	$4.5x10^3$
2	Kotemalluru	Soil sample 2	12	$1.2\ x10^3$
3	Savalanga	Soil sample 3	16	$1.6\ x10^3$
4	Tholhunse	Soil sample 4	11	$1.1x10^3$
5	Bhadravathi	Soil sample 5	13	$1.3x10^3$
6	Rajendranagar	Soil sample 6	11	$1.1x10^3$

Biochemical characterization

Biochemical characterization of Actinomycetes is done to identify and analyze their metabolic and enzymatic properties (Ranjith et al., 2017). All 6 organisms show the gram-positive cocci in grams reaction. These organisms could produce enzymes like citrate permease, urease, gelatinase, catalase and hence gives positive result for gelatinase test (5 positive and 1 negative), catalase test (6 positive) citrate utilization test (5 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 H_2S gas and gives positive results for H_2S production test (6 positive) was depicted in Table 2

Table 2. Biochemical Characterization (+ Positive, - Negative).

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

Chemical synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles were produced chemically using the precipitation process. Using a magnetic stirrer, add 30 ml of freshly prepared zinc sulphate solution to the beaker and stir for five minutes. Freshly prepared 30 ml of sodium hydroxide solution needs to be added to another beaker and stirred for five minutes using a magnetic stirrer. Following this, add the sodium hydroxide solution to zinc sulphate and stir for further five minutes. A white precipitate is formed, which can be filtered by washing with 100 ml of ethanol. synthesized ZnO nanoparticles, 10g of 1.0M NaOH was dissolved in 100ml distilled water and heated to 50-90°C with constant stirring, then 17g of 0.5M ZnSo4 in 100ml distilled water was added dropwise over 20-60 minutes, inducing immediate ZnO precipitation. The final ZnO nanoparticles are depicted in Figure 3.

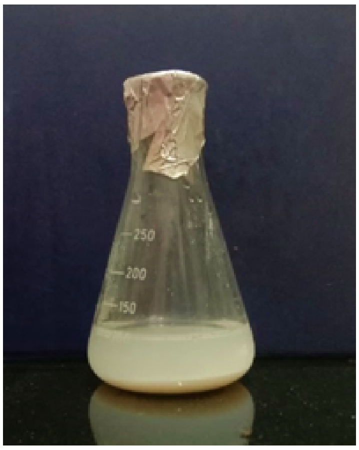

Figure 3. Chemical synthesis of Zinc oxide nanoparticles.

Microbial synthesis of zinc oxide nanoparticles

An equal volume of 0.1M zinc sulphate and 0.1M sodium hydroxide solution was added to the organism's culture filtrate in one flask, which was used as the test sample. In another flask, the culture filtrate was left untreated with 0.1M zinc sulphate, functioning as a blank. On incubation, the colors of all three tested materials turned to white precipitate, indicating the production of zinc oxide nanoparticles by all three Streptomyces SN organisms showed in Figure 4. Cultivated actinomycetes in starch casein nitrate broth to achieve the microbial production of ZnO nanoparticles. Two effective isolates that would produce ZnO nanoparticles were identified and evaluated under a microscope. After being treated with 0.1M zinc sulphate for 96 hours at 37°C, 30 mg of ZnO nanoparticles were produced. By using enzymes and metabolic processes, actinomycetes-mediated synthesis successfully transformed ZnSO4 into nano-sized ZnO. Additionally, compared to its micron-size ZnO, the nanoscale has significantly higher potential.

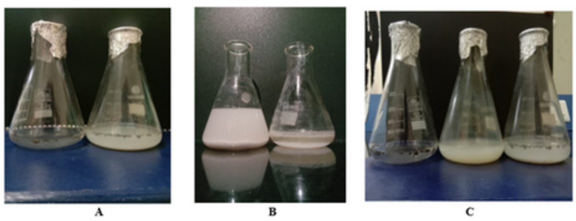

Figure 4. Biosynthesis of zinc oxide nanoparticles by Streptomyces SN organisms A-Streptomyce sSN 1, B- Streptomyces SN 2, C- Streptomyces SN 3.

Characterization of synthesized zinc oxide nanoparticles

FTIR analysis

The FTIR (Module: Alpha 2) analysis was conducted over a spectral range of 3500-1000 cm^{-1}, identified functional

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groups in Actinomycetes culture filtrate with zinc oxide nanoparticles. The analysis revealed characteristic absorption peaks indicative of O-H stretching and deformation, associated with water adsorption on the metal surface, at 3336.10, 3297.80, and 3309.40 cm^{-1}. C-H stretching vibrations were observed at 2183.80, 2110.81, and 2120.52 cm^{-1}, while C=O asymmetric stretching vibrations occurred between 1467.12-1107.50 cm^{-1}, with specific peaks at 1103.19 and 1043.05-1044.00 cm^{-1}. The formation of zinc oxide nanoparticles was confirmed by absorption peaks in the range of 645.29-616.54 cm^{-1} in Figure 5-7. The chemically synthesized zinc oxide nanoparticles exhibited characteristic FTIR absorption peaks at 1044.00 cm^{-1} (C=O stretching), 2122.37 cm^{-1} (C-H stretching), 3317.18 cm^{-1} (O-H stretching), and a distinctive peak at 877.00 cm-1, confirming the successful formation of zinc oxide nanoparticles through chemical synthesis in Figure 8. Further only Streptomyces SN 1 sample was analysed based on the peaks. Similarly, Sheikand et al., reported that The IR spectrum of FTIR showed absorption peaks characteristic of ZnO nanoparticles, including Zn-O stretching at 432 cm^{-1} and oxygen deficiency at 503 cm^{-1}. A broad band at 540-417 cm^{-1} confirmed the presence of ZnO nanoparticles, providing valuable information on their structural characteristics and functional group interactions with biological extracts [42].

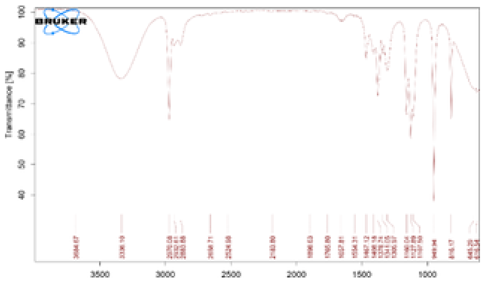

Figure 5. Biosynthesis of zinc oxide nanoparticles Streptomyces SN 1.

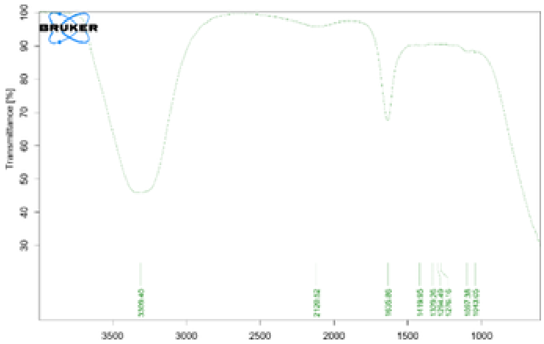

Figure 6. Biosynthesis of zinc oxide nanoparticles Streptomyces SN 2.

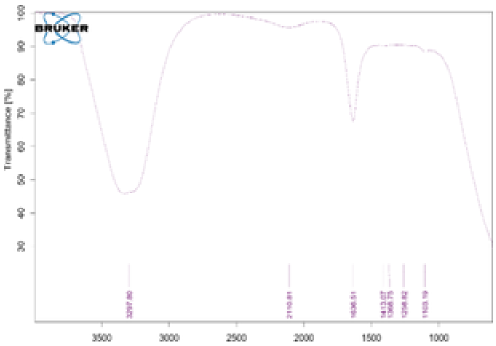

Figure 7. Biosynthesis of zinc oxide nanoparticles Streptomyces SN 3.

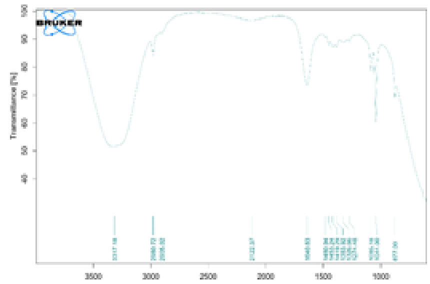

Figure 8. Chemical synthesis of zinc oxide nanoparticles.

UV visible spectroscopy

The UV-Vis spectroscopy (Module: UH5300) analysis revealed the presence of zinc oxide metal ions in the prepared nanoparticle samples, with absorption peaks occurring between 200-300 nm, specifically at 206.5 nm for biologically synthesized zinc oxide nanoparticles (using Actinomycetes) and256.5 nm for chemically synthesised zinc oxide nanoparticles in Figure 9. Similarly, Mohanta et al., reported that UV-Vis absorption spectrum of ZnO nanoparticles showed a characteristic band 420 nm confirming the successful synthesis and optical properties of ZnO nanoparticles [43].

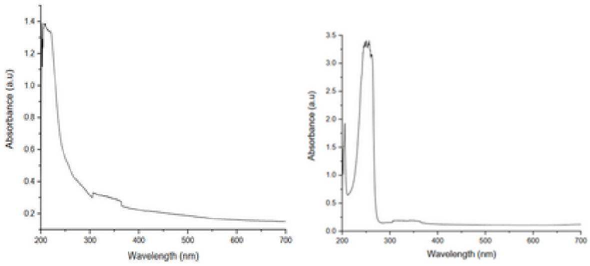

Figure 9. UV Analysis of zinc oxide nanoparticles A. Chemical synthesis B. Streptomyces SN 1.

SEM (Scanning Electron Microscope)

The Scanning Electron Microscopy (SEM) analysis revealed that the synthesized zinc oxide nanoparticles in Figure 10. The sample was mounted on the aluminium holder stubs using 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. Then the size of zinc oxide nanoparticles ranges from approximately 70-90nm with spindle or rod-shaped structures are produced. Similarly, Shanmugasundaram & Balagurunathan worked on the same area where they synthesised the zinc oxide nanoparticles with the size of 15-30nm [44].

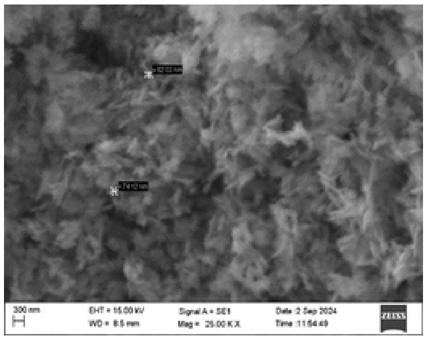

Figure 10. SEM analysis of zinc oxide nanoparticles.

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Antimicrobial activity

The antimicrobial efficacy of zinc oxide nanoparticles synthesized from Actinomycetes was compared to chemically synthesized nanoparticles against pathogens (E. coli, Klebsiella sp., Candida sp., Pseudomonas sp., and Staphylococcus aureus), revealing an enhanced inhibitory zone (1-8 mm) and superior antimicrobial activity of biosynthesized zinc oxide nanoparticles [Figure 11].

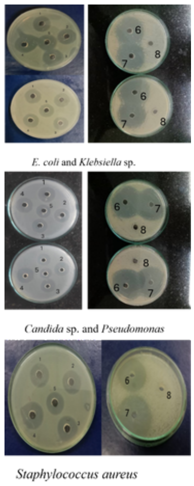

Figure 11. Antimicrobial activity by agar well diffusion method;1-Culture filtrate 1, 2-Chemical synthesis sample, 3- zinc oxide nanoparticles 1, 4- zinc oxide nanoparticles 2, 5- zinc oxide nanoparticles 3, 6-Culture filtrate 2, 7-Culture filtrate 3, 7-Culture filtrate 3, 8-Negative Control.

Table 3: Zone of inhibition of zinc oxide nanoparticles against test organisms.

DIAMETER OF CLEAR ZONE IN mm							
Organisms	**ZnO NPs1**	**ZnO NPs2**	**ZnO NPs3**	**Chemical synthesis**	**Culture filtrate1**	**Culture filtrate2**	**Culture filtrate3**
E. coli	26	28	23	23	21	23	21
Klebsiella sp.	25	23	20	19	22	19	23
Candida sp.	23	25	23	19	21	19	21
Pseudomonas sp.	18	21	20	16	18	19	17
Staphylococcus aureus	22	24	30	19	24	20	19

Conclusions

Biosynthesis of zinc oxide nanoparticles (ZnONPs) by actinomycetes, specifically Streptomyces strains, presents a novel, eco-friendly alternative to traditional nanoparticle production methods. This soil-based approach leverages the microorganisms' ability to reduce zinc ions, offering a cost-effective and scalable solution. The resulting zinc oxide nanoparticles exhibit a broad range of therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities, making them suitable for various applications such as wound healing, cancer treatment, infectious disease management, and neurodegenerative disease management. These nanoparticles enhance collagen synthesis, induce apoptosis in cancer cells, and demonstrate antibacterial, antiviral, and antifungal properties while mitigating oxidative stress and inflammation. Future research will focus on optimizing biosynthesis, evaluating toxicity and biocompatibility, developing targeted delivery systems, and exploring combination therapies. This eco-friendly production method, combined with zinc oxide nanoparticles unique properties, holds significant promise for medical and environmental applications, offering a sustainable solution for nanoparticle production.

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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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