

## CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF ZINC NANOPARTICLE SYNTHESIZED FROM SOIL BACTERIAL ISOLATES

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### Abstract

Zinc (Zn) nanoparticles have piqued the interest of researchers all over the world due to their numerous potential as antibacterial and antifungal compounds in biomedicine and agriculture. Green nanotechnology has developed as an important approach for the fabrication and production of Zn nanoparticles in recent years. Bacteria have unrivalled capability to convert zinc ions into nanoparticles, which make them the best candidate for the synthesis of Zn nanoparticles because of their rapid growth rates and ease of handling. Therefore, this study was performed to characterize and evaluate the anti-microbial activity of zinc nanoparticles synthesized from soil bacterial isolate. Three different bacteria, J-1, J-2 and J-3 were found in the soil sample. A single bacteria i.e., J-2 with isolated colonies was selected from them and subjected to further processing and later on identified as *Serratia nematodiphila* through biochemical tests and sequencing. The Zn nanoparticles were prepared by mixing zinc sulphate stock solution with the bacterial supernatant culture and incubated in the dark in shaking incubator for 3-5 days. After incubation, the appearance of white color precipitate indicated the successful formation of *Serratia-nematodiphila* mediated Zn nanoparticles. The nanoparticles were further analyzed via UV-Vis, XRD, FT-IR, SEM and TEM analysis. The nanoparticles were pure, spherical, polydispersed and crystalline in nature. The nanoparticles ranged in size from 17 nm to 28 nm with an average size of 24.74 nm. The FTIR values for the synthesized Zn nanoparticles were 510, 620, 880, 1430, 1600, 2400, 3100, 3350, 3600 and 3700 cm<sup>-1</sup>. The highest peaks formed ranged from 3100 to 3700 cm<sup>-1</sup>. Weak peak was formed at 2400 cm<sup>-1</sup>, whereas, medium peak was observed at 1430 and 1600 cm<sup>-1</sup>. The maximum SPR was found at 379 nm. The nanoparticles showed antibacterial activity and antifungal activity against *S. aureus*, *E. coli*, *Sporothrix schenkii* and *Aspergillus fumigatus*, respectively at a concentration of 100 µg/mL and 75 µg/mL, respectively. The zones of inhibitions were reduced with the decrease in nanoparticles concentrations. Hence, according to the findings of the preceding investigations the bacteria-synthesized Zn nanoparticles have a strong antibacterial and antifungal potential.

## INTRODUCTION

Richard Feynman introduced the idea of nanotechnology for the first time in his renowned speech “There’s a plenty of room at the bottom” delivered at the American Institute of Technology. Professor Norio Taniguchi of the Tokyo Science University first coined the term “Nanotechnology” (Guin & Singh, 2023). Nanotechnology is evolving as a “cutting-edge” technology that spans several academic fields, including chemistry, physics, medicine, biology and material science (Saxena, 2023). “Nanos” is a Greek term used as the prefix in the word nanotechnology which means “dwarf” (Rasheed *et al.*, 2023).

There has been a tremendous surge in research interest in the synthesis of metal nanoparticles because of their innovative uses in several industrial areas (Malik *et al.*, 2023). Nanoparticles are solid particles that have at least one dimension ranging from 1 to 100 nm (Sharif *et al.*, 2023). Nanoparticles have opened a number of new avenues for the development of novel materials and the assessment of their properties by modifying particle size, shape and distribution (Sanchis *et al.*, 2023). Metal nanoparticles have received a lot of attention because of their distinctive characteristics, such as catalytic, antibacterial, anticancer, optical and magnetic properties (Roy *et al.*, 2023). The primary characteristic of metal nanoparticles is their large volume and surface ratio, which enhances their molecular interaction (Manoswini *et al.*, 2023). Metal nanoparticles are also used in biochemical sensors, drug delivery, tumor imaging and pharmaceutical treatment procedures (Palanai & Elangovan, 2023).

Metal nanoparticles are generally synthesized and stabilized using either a “bottom up” or “top down” approach (Dhankhar *et al.*, 2023). The “bottom up” approach involves the self-assembly of their atoms into nuclei, which then evolve into nanoscale particles. This type of synthesis involves both chemical and biological approaches (Benkovic *et al.*, 2023). Whereas, the top down approach involves the breakdown of bulk material into small size particles utilizing different chemical and physical approaches (Jagdeo, 2023). The physical approaches include thermal ablation, milling and grinding (Shaheen *et al.*, 2023). However, the chemical approaches includes electrochemistry, photochemical and chemical reduction (Yang *et al.*, 2023). Physical techniques need a large amount of energy, making these processes more capital demanding. Another disadvantage of physical approach is the reduced output of nanoscale materials (Dalmini *et al.*, 2023).

Chemical techniques have been the most preferred strategy for the synthesis of nanoparticles in recent years, due to the demand of less energy in the reduction stage and the synthesis of homogeneous particles with precise shape and size (Sanchis *et al.*, 2023). On the other hand, chemical approaches are ecologically hazardous, due to the use of hydrazine and potassium bitartrate, which induce carcinogenicity, cytotoxicity and genotoxicity (Chatterjee *et al.*, 2022). The chemical approaches for the production of nanoparticles for biomedical applications have been limited because of the instability, toxicity and low-biocompatibility (Hussein *et al.*, 2022). Therefore, the primary focus of nanoparticles synthesis research is currently on developing ecologically friendly technique that successfully modifies the size, shape, stability and features (Zhang *et al.*, 2023).

The use of microorganisms in bio-mediated synthesis has emerged as a possible alternative to existing techniques of nanoparticles production (Srividya *et al.*, 2023). Microbial synthesis is a green strategy that employs biological entities such as bacteria, fungi, algae, actinomycetes, viruses and yeast to produce nanoparticles (Babu & Tirkey, 2023). The microbial mediated synthesis is a safe, cost-effective and reliable method for producing nanoparticles with diversity in shape, size, composition and physio-chemical characteristics (Selvinsimpson & Chen, 2023). This green approach of nanoparticle synthesis is an appealing approach, which enables synthesis with minimum energy and cost requirements and it can be readily scaled-up to larger levels (Singh *et al.*, 2023). Another significant feature of these biological entities is their capacity to act as template for the organization of nanoscale particles into well-defined structures (Kalra *et al.*, 2023).

Microorganisms can be used as a potential Nano factory for environmental friendly and low-cost production of various metallic nanoparticles such as copper, silver, zinc, gold and palladium nanoparticles (Agarwal & Darbar, 2023). These tiny structures might have various shapes and forms including nanorods, nanotubes and nanowires (Kumari *et al.*, 2023). These tiny structures have been used as anticancer and antibacterial compounds in biomedical applications (Roy *et al.*, 2023).

Bacteria have unrivalled capacity to convert metallic ions into nanoparticles, making them one of the best candidate for the synthesis of metallic nanoparticles because of their rapid growth rates and ease of handling (Tripathy *et al.*, 2023). In contrast to other microorganisms, bacteria can be readily shaped and

genetically modified for metal ion bio mineralization (Luo *et al.*, 2023). Bacteria are constantly exposed to toxic and harsh environmental conditions resulting from elevated concentrations of metal ions in the environment. However, to deal with these stress conditions, bacteria have evolved a number of defence mechanisms including efflux pump, precipitation, sequestrations and variations in the concentration of ions. Bacteria can effectively use these defense mechanisms to synthesize nanoparticles for a variety of uses (Pal *et al.*, 2022; Selvinsimpson & Chen, 2023).

Over the past ten years, there have been significant developments in the field of bacterial-assisted nanoparticles (Selvinsimpson & Chen, 2023). The bacterial assisted synthesis of nanoparticles is ecofriendly and cost-effective (Alsaiani *et al.*, 2023). They are free from hazardous chemical contaminant, which is a desired characteristic for biomedical applications (Tauseef *et al.*, 2022). Another advantage of the bacterial mediated synthesis of nanoparticles is that it doesn't require an extra step of attachment or capping of bioactive molecules to the surface of nanoparticles (Gahlawat and Choudhury, 2019; Tauseef *et al.*, 2022). The synthesis of bacterial-mediated nanoparticles takes far less time than the physiochemical approaches (Tauseef *et al.*, 2022). For example, Sonbol *et al.* (2021) and Aygun *et al.* (2023) prepared palladium nanoparticles in one step. Other researchers have found diverse bacterial isolates to be useful in quick biosynthesis of several metallic nanoparticles (Yusof *et al.*, 2019; Alao *et al.*, 2022; Morowvat *et al.*, 2023). Rizki and Klaypradit, (2023) also synthesized silver and gold nanoparticles in a single step using marine bacteria, thus demonstrating the significance of nanoparticles synthesis using bacterial isolates. Among other nanoparticles, bacterial-mediated zinc nanoparticles have piqued the curiosity of researchers (Kumar *et al.*, 2023).

Zinc is typically a white color powder, which is insoluble in water (Paramadini *et al.*, 2023). This powder is used as an additive in numerous products including paints, pigments, batteries, ointments, lubricants, adhesives, fire retardants, cement, ceramics, ferrites, glass and as a zinc source in food (Tokeser, 2023). Zinc occurs as mineral zincate naturally in the earth crust; however, the majority of Zn utilized commercially is synthetic. Zinc is a compatible and non-toxic to human skin making it useful additive for fabrics and materials which come in contact with the human skin (Joseph *et al.*, 2023). The effectiveness of the material's function is enhanced by the increased by

the huge surface area of the nanoscale Zn in comparison to bulk Zn (Sun *et al.*, 2023).

The crystal structure of the Zn is wurtzite hexagonal. This crystal structure can only be seen when observed under Scanning Electron Microscope (Nagamalleswari & Modem, 2023). The process of crystal formation determines its exact shape. Crystal of zinc in standard size can be either acicular needles or plate-shaped (Ulusoy, 2023). The induction of the zinc might form several crystalline structures via specific deposition processes, which is a particularly active field research now-a-days (Kolodziejczak & Jesionowski, 2022). Zinc exists in three different crystalline forms, including cubic zinc blende, hexagonal wurtzite and cubic rock salt (Alhoqail *et al.*, 2023). Among them, wurtzite is the most prevalent and stable structure under ambient conditions (M'hid *et al.*, 2023).

Zinc nanoparticles are used in rubber manufacturing, paints, plastics, cosmetics, electronics, sunscreens and several pharmaceutical products (Ahmed *et al.*, 2023). It is used to treat carcinoma cells and leukemia (Thabet *et al.*, 2023). Zn nanoparticles are used as a drug carrier (Sana *et al.*, 2023). It is also used in packaging of different kinds of food, medical care, decoration, construction and textile industries (Zanchettin *et al.*, 2023).

Human life is seriously threatened by rising antibiotic resistance and the global emergence of bacterial diseases (Tariq, 2023). Zinc nanoparticles are now being researched owing to their distinct antibacterial and antifungal properties (Kiani *et al.*, 2023). Zinc is known to have photo oxidizing and photo-catalytic effects (Ahmed *et al.*, 2022). It has been discovered that Zn particles are effective against bacteria with sizes between nanometer to micrometer (Bhosale *et al.*, 2023). Zinc nanoparticles interact with the bacterial cells more easily by penetrating them because of its nanoscale size (Ramzan *et al.*, 2023). The huge ratio of surface to volume and distinct physiochemical characteristics of Zn are primarily responsible for their antibacterial property (El-Bendary *et al.*, 2023).

Researchers are currently thoroughly examining Zn nanoparticles, since multiple studies have shown that they are biocompatible with human cells (Gupta *et al.*, 2023). Pathogenic bacteria have cell surface proteins that aid in adhesion and colony formation. The cell wall also contains teichoic acid and polysaccharides, which protects bacteria in stress conditions from host defense mechanisms (Rastogi & Ghosh, 2023; Zheng *et al.*, 2023). Since all the above macromolecules are charged molecules, the modified nanoparticles are used to elicit particular interactions to disrupt the integrity

of cell wall (Mir *et al.*, 2023). The direct interaction of the Zn nanoparticles with the cell wall results in the disruption of the integrity (Metryka *et al.*, 2023). Zinc nanoparticles are an effective bactericidal agent against both gram negative and gram positive bacteria (Awan *et al.*, 2023). It was recently found that Zn nanoparticles showed enhanced bactericidal activity against *S. aureus* as compared to other nanoparticles (Jabir *et al.*, 2022; Heidari *et al.*, 2022).

Zinc nanoparticles absorb UV-light. This feature of Zn is linked with the conductivity, which increases the contact of Zn nanoparticles with the bacteria (Lallo *et al.*, 2019). These oxygen species desorb from the surface as a result of UV light interaction. Thus a decrease in the surface electron depletion zone leads to improved conductivity (Singh *et al.*, 2020). These species aid nanoparticles in penetrating and destroying bacteria (Abdelghafar *et al.*, 2022). The antibacterial ability of Zn nanoparticles is greatly influenced by the shape of the nanoparticles (Alshameri & Owais, 2022). Controlling the synthesis factors such as type of precursor, solvent, pH and temperature helps in attaining the desired antibacterial characteristics in Zn nanoparticles (Smaoui *et al.*, 2023).

Green nanotechnology has developed as an important approach for the fabrication and production of Zn nanoparticles in recent years. Bacteria have capability to convert zinc ions into nanoparticles. In this study *Serratia nematodiphila*, a bacteria isolated from soil showed the synthesis of Zn nanoparticles which had an excellent antibacterial and antifungal activity.

## METHODOLOGY

### Study Area

The current research on nanoparticles synthesis was conducted in the Microbiology Laboratory of Sarhad Institute of Allied Health Sciences, Sarhad University of Science and IT, Peshawar.

### Sample Collection

A total of 6 sample of soil from a depth of 5 cm was collected in sterile polythene bag from 2 different localities (back of Sarhad University and my village soil sample) Peshawar.

### Bacterial Isolation

Soil 1 gram was suspended in water for time to make a suspension. Then, 1 mL of this suspension was diluted to 10ml. All of the plates were examined for bacterial growth following incubation. The bacterial colonies were picked and again cultured on nutrient agar plates to get pure and isolated bacterial colonies (Raag *et al.*, 2022).

### Bacterial Identification

The isolated bacteria were identified based on morphology, Gram staining and biochemical tests. (Catalase, citrate, blood hemolysis, starch hydrolysis, NAACL tolerance Bergey's manual 1994)

### Phenotypic Identification

The isolated bacterial colonies were examined on nutrient agar plates for their morphological traits including color, margins and shape.

### Gram Staining

Gram staining analysis was done to further identify the bacterial isolate. For accurate results, a 24 hours fresh bacterial colony was picked using a sterilized loop and a smear was made on a glass slide. Smear was fixed with a low heat. The crystal violet was added to the smear and left for almost a minute. Then crystal violet was added to it and again left for a minute. After this, gram iodine was added and washed with tap water after 2 seconds and in the last step, safranin was added to the smear. The smear was then observed under the microscope (Das *et al.*, 2020).

### Biochemical identification

Biochemical tests including catalase, citrate and blood hemolysis analysis was performed to identify the isolated bacteria.

### Catalase Test

Catalase analysis was performed in order to identify bacterial isolates that produce catalase enzyme. It degrades hydrogen peroxide to make oxygen and water. A few drops of H<sub>2</sub>O<sub>2</sub> were dropped onto the slide with a dropper. A clear bacterial colony was taken and mixed with hydrogen peroxide on slide. The rapid production of bubbles indicated positive outcome (Ademola, 2022).

### Citrate Test

The citrate test was carried out to test the citrate consumption ability of the isolated bacteria. The citrate media was prepared and the slants were made. The slants were left for some time to solidify and the isolated bacteria were inoculated in it. The inoculated slants were left in incubator for 24 hours at 37 °C. The following day, the slants were checked for the color change. The color change showed positive outcome (Foreman *et al.*, 2021).

### Blood hemolysis test

This test determines which hemolytic enzymes a bacterium carries. For this test blood agar media was prepared and autoclaved. The autoclaved media was poured into the plates and left to solidify. After solidification, the isolated bacteria was cultured on the plates and incubated. After growth, if the plates become darker, the organism has shown alpha

hemolysis. No noticeable change in the color of the medium indicates gamma hemolysis (Foreman *et al.*, 2021).

#### Starch hydrolysis

This test determines the ability of the isolated bacterium to degrade starch. Pure colony was cultured on the media plate. The inoculated plates were incubated for 48 hours at 37 °C. After this, iodine was added to the plate with the help of a dropper and left for 30 seconds. The excess iodine was removed from the plate. Examine the clear zone surrounding the bacterial growth line (Haile *et al.*, 2022).

#### Sodium chloride tolerance

This test determines the ability of the isolated bacterium to tolerate various concentrations of sodium chloride. Salts disrupt the osmotic equilibrium and membrane permeability. High salt concentrations thereby prevent a number of bacteria from growing while only allowing the growth of salt-tolerant bacteria. Broth media containing 6.5 % NaCl was prepared. The isolated colonies from fresh culture were inoculated

**Table. 1. Primers with sequences**

Primer	Sequences
Forward: 27F	5'-AGA GTT TGA TCM TGG CTC AG- 3'
Reverse: 1492R	5'-GGT TAC CTT GTT ACG ACT T- 3'

#### Trimming and submission of the sequences

The amplified sequences were then trimmed with finchtv software to get clean reads of these sequences to construct a phylogenetic tree. Then for 16s rRNA-based identification, these cleaned sequences were blasted in NCBI's BLAST and compared to other neighboring sequences in the database (Idris *et al.*, 2020).

#### Similar sequences downloading

Sequences showing a high degree of identity to those in the database were obtained and saved in FASTA format after NCBI blast (Martinez *et al.*, 2018).

#### Multiple sequences alignment

The MEGA-7 software was used to align specific sequences from various species with the query sequences commonly called as contigs in the FASTA format, in order to detect the evolutionary conserved regions (Mahmoodi *et al.*, 2018).

#### Construction of phylogenetic tree

Phylogenetic tree was constructed based on nucleotide sequences using the molecular genetic analysis MEGA-7 software (Mahmoodi *et al.*, 2018).

#### Preparation of bacterial culture

The isolated colonies of the identified bacterial species were picked through loop and inoculated in nutrient broth. The inoculated culture was placed in shaking

using loop in broth. This inoculated tube was left in the ambient air for 2 days. After 2 days, the color change and turbidity of the tubes was observed (An *et al.*, 2021).

#### Deoxyribose Nucleic Acid Extraction

Isolated bacterial colony was picked with a sterilized toothpick. The isolated colony was mixed with 50 µL of sterile distilled water. This bacterial suspension was heated in a water bath for 10 minutes at 97 °C. After heating, the culture was centrifuged for 10 minutes at 10000 rpm and the bacterial DNA-containing supernatant was collected. The concentration of DNA in the supernatant was determined by measuring its absorbance at 260 nm with a UV spectrophotometer.

#### Molecular identification based on sequencing

The extracted DNA was sent to Macrogen, Korea. PCR was performed by utilizing universal primers to amplify 16s rRNA fragment. The results of the sequencing of the 16s rRNA products were provided by the Macrogen. Universal primers used for sequencing are listed in table 3.1 (Idris *et al.*, 2020; Martinez *et al.*, 2018).

incubator for 24 hours. After this, the culture was centrifuged for 20 minutes at 7000 rpm and the supernatant was collected (De Silve *et al.*, 2020).

#### Preparation of 1mM stock solution

To prepare 1mM zinc sulphate stock solution, 0.17 gram of zinc sulphate was mixed with 1000 mL distilled water (Keshavarz & Torkian, 2022).

#### Biosynthesis of Zn nanoparticles

90 mL of stock solution was mixed with the *Serratia nematodiphila* supernatant. This bacterial and stock solution culture was then kept in the dark in shaking incubator for 3- 5 days. After incubation, the mixture was observed and the color change was noted (De Silve *et al.*, 2020).

#### Purification of Zn nanoparticles

The synthesized Zn nanoparticles were purified for 20 minutes at 12000 rpm in a centrifuge. The precipitate was then dissolved in distilled water in order to remove the residues. The precipitate was then dried to 90 °C in oven to ensure complete drying (Alao *et al.*, 2022).

#### Characterization of Zn nanoparticles

The following spectroscopic analysis was performed in CRL lab Peshawar University to characterize the synthesized Zn nanoparticles.

#### UV/Vis Spectroscopy

A UV/Vis spectrophotometer (Hitachi U-2201) was used to analyze the UV/Vis spectrum at resolution of 1 nm ranging from 200-600 nm. This spectroscopy was performed to identify the highest peak of the synthesized Zn nanoparticles on the basis of surface plasmon excitation (Abdo *et al.*, 2021).

#### FTIR spectroscopy

By using FTIR (Perkin Elmer, spectrum-RS1), the functional groups of Zn nanoparticles falling in the range of 500 nm-4000 nm was observed. This spectroscopy was conducted to explore the involvement of bacterial metabolites stabilization, capping and reduction of Zn nanoparticles (Amin *et al.*, 2021).

#### XRD spectroscopy

Powder X-Ray Diffractometer (BRUKER, Germany) was used to determine the purity and crystalline structure of the Zn nanoparticles. The operating temperature, angle and voltage of XRD was 0°-80 °C, 0°-80° and 40 KV, respectively. The size of Zn nanoparticles was calculated on the basis of XRD analysis by using the Debye Scherer equation as follows;

Average size of Zn nanoparticle (D) =  $\frac{0.9\lambda}{B \cos \theta}$  (Soliman *et al.*, 2021).

$B \cos \theta$

#### TEM spectroscopy

TEM spectroscopy was conducted to explore the morphological properties of Zn nanoparticles produced by bacteria (TEM-JEO, Tokyo, Japan). The carbon grid of TEM was loaded with a few drops of Zn nanoparticle. A blotting paper was used to remove the excess solution of nanoparticles. The grid was allowed to dry and analyzed after drying (Nilavukkarasi *et al.*, 2020).

#### SEM spectroscopy

SEM spectroscopy was conducted to explore the surface topology and stability of Zn nanoparticle at various magnifications (Moghazy, 2021).

#### Antibacterial activity of Zn nanoparticles

Zinc nanoparticles synthesized by bacteria were evaluated for antibacterial activity against pathogenic drug resistant *S. aureus* and *E. coli*. For this purpose, Muller Hinton Agar media plates were prepared. The inoculum of the tested bacterial isolates were adjusted at O.D 1 and spread onto the plates. A Sterilized well borer was used to make 0.8 mm wells on the plates. A total of 100 µL of prepared nanoparticles were added to the wells. The activity of various concentrations of Zn nanoparticles *ie.*, 100, 75, 50 and 25 µg/mL was investigated to determine the MIC. To ensure the successful dispersion of the nanoparticles the plates

were kept for 2 hours in the refrigerator. After 2 hours, the plates were transferred to the incubator. After incubation at 36 °C, the zones formed around the wells were measured in mm and noted. This procedure was conducted in triplicate (Abdo *et al.*, 2021).

The percent inhibition of the bacterial isolates was calculated using the following formula.

Percent inhibition =  $\frac{\text{Zones of inhibition of nanoparticles}}{\text{Zones of inhibition of control}} \times 100$

#### Antifungal activity of Zn nanoparticles

Zinc nanoparticles synthesized by bacteria were evaluated for antifungal activity against pathogenic fungi *Sporothrix schenckii* and *Aspergillus fumigatus*, respectively. For this purpose, tube dilution assay was performed. Stock solution of Zn nanoparticles (24 mg/mL) was prepared. Potato Dextrose Agar (PDA) (Oxoid) slants were prepared. A total of 66.6 µL of the stock solution was added to the test tubes and solidified. The inoculum of the tested bacterial isolates were adjusted at O.D 1 and streaked in the test tubes. The activity of various concentrations of Zn nanoparticles *ie.*, 100, 75, 50 and 25 µg/mL was investigated. The plates were transferred to the fungal incubator. After incubation, the linear growth inhibition was measured using the following formula (Abdo *et al.*, 2021).

Percent growth inhibition =  $\frac{\text{linear growth in the tested sample}}{\text{linear growth in control}} \times 100$

## RESULTS

### Processing of soil sample

Two different bacteria, J-1, and J-2 were isolated from the soil sample collected from Sarhad University of Science and Information Technology, Peshawar. A single bacteria *ie.*, J-2 with isolated colonies was selected from them and subjected to further processing. Because J1 was identified as *Bacillus subtilis* it is commonly occur in soil. The soil temperature and pH recorded at the time of sampling was 42 °C and 8.0, respectively.

### Bacterial identification

#### Phenotypic identification

The J-2 bacterial isolate on nutrient agar formed red color, smooth and circular colonies with entire margins.

#### Gram staining

The bacterial isolate formed pink color, rod shaped tiny colonies, which occurs individually when observed at 100x magnification under the microscope.

#### Biochemical identification

The isolate tested positive for catalase, citrate and blood hemolysis. Starch hydrolysis and 6.5 NaCl utilization were both negative for the tested isolates in table No 4.1.

**Table 2. Biochemical identification of J-2 isolate**

S. No.	Biochemical test	Result
1.	Catalase	Positive
2.	Citrate	Positive
3.	Blood hemolysis	Positive
4.	Starch hydrolysis	Negative
5.	NaCl	Negative
6.	Similarity of bacteria	<i>Serratia</i> spp.

**Table 2. Sequences of 16s rRNA gene of J-2 isolate**

**Sequences of 16s rRNA gene of J-2 isolate**

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AGTCGGCGTCTCCAGGCGGTTCGATTTACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCA
CAACCTCCAAATCGACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTC
CCCACGCTTTTCGACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATT
CCTCCAGATCTCTACGCATTTACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTC
TAGCTTGCCAGTTTCAAATGCAGTCCCAGGTTGAGCCCGGGGATTCACATCTGACTTAA
CAAACCGCCTGCGTGCGCTTTACGCCAGTAATTCCGATTAACGCTTGACCCTCCGTATT
ACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGAGTAACGTCAATTGATGA
GCGTATTAAGCTCACCACCTTCTCCTCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTT
CACACACGCGGCATGGCTGCATCAGGCTTGCGCCATTGTGCAATATTCCTCACTGCTGCC
TCCCGTAGGAGTCTGGACCGTGTCTCAGTTCAGTGTGGCTGGTCATCCTCTCAGACCAGC
TAGGGATCGTCGCCTAGGTGAGCCATTACCCACCTACTAGCTAATCCCATCTGGGCACAT
CTGATGGCAAGAGGCCCGAAGGTCCCCCTTTGGTCTTGCGACGTTATGCGGTATTAGCT
ACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCAGACATACTCACCCGTCGG
CCGCTCGTCACCCAGGGAGCAAGCTCCCCTGTGCTACCGCTCGACTTGCATGTGTTAAGCC

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**Fig 1. Phylogenetic tree with query sequence identified as *Serratia nematodiphila***

**Biosynthesis of Zn nanoparticles**

The appearance of white color precipitate, a sign of the reaction mixture of the *Serratia nematodiphila* and the

precursor salt *i.e.*, zinc sulphate indicated the successful formation of *Serratia-nematodiphila* mediated Zn

nanoparticles. After the 1 mM Zinc sulphate solution was added to the *Serratia nematodiphila* culture drop by drop, white precipitate developed and the reaction mixture changed from colorless to white due to zinc ion reduction, indicating the formation of Zn nanoparticles. (De silve *et al.*, 2020)

Characterization of Zn nanoparticles

#### UV/Vis spectroscopic analysis

The color shift of the precipitate from colorless to white demonstrated the effective bacterial production of Zn nanoparticles. UV/Vis spectroscopic analysis at a wavelength of 300 to 600 nm was performed to confirm this color change and the highest Surface Plasmon Resonance (SPR). Because complex Zinc nanoparticle peak occur in 300 to 600nm range. The zinc sulphate was successfully transformed into the end product (Zn nanoparticles), as evidenced by the maximum SPR being found at 379 nm.

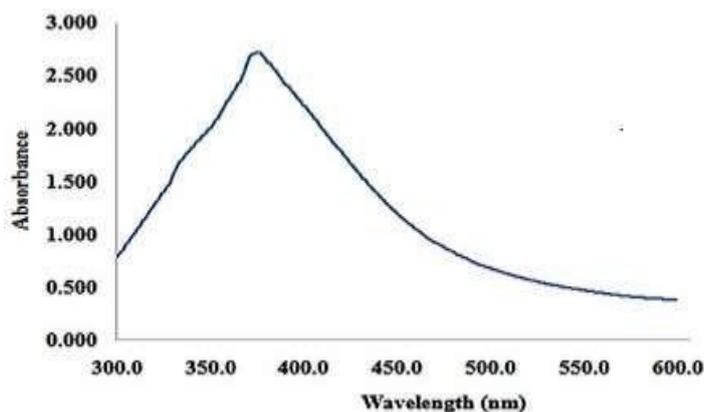
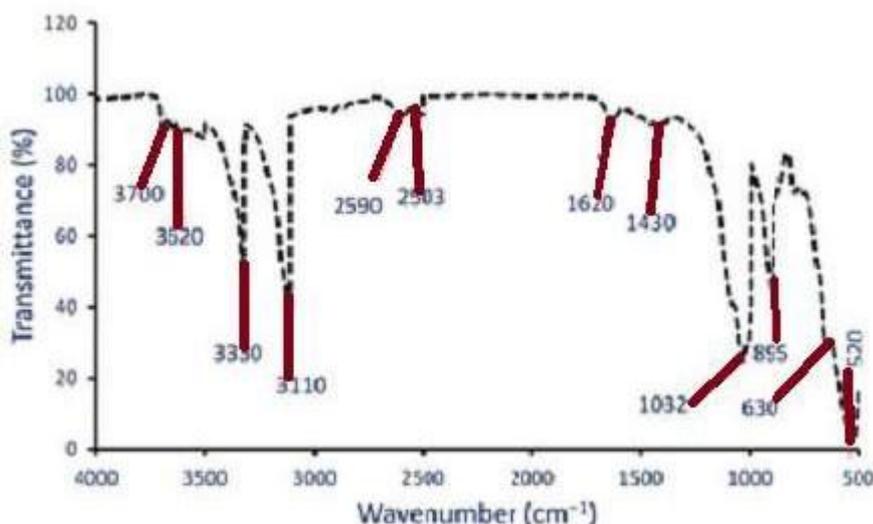


Fig. 2. UV/Vis absorption spectra of complex Zn nanoparticles

#### FTIR spectroscopy

The FTIR values for the synthesized Zn nanoparticles were 520, 630, 855, 1032, 1430, 1620, 2503, 2590, 3110, 3330, 3520 and 3700  $\text{cm}^{-1}$ . The highest peaks formed ranged from 3100 to 3700  $\text{cm}^{-1}$ . Weak peak was observed at 2503  $\text{cm}^{-1}$ , whereas, medium peak was formed at 1430 and 1620  $\text{cm}^{-1}$ .

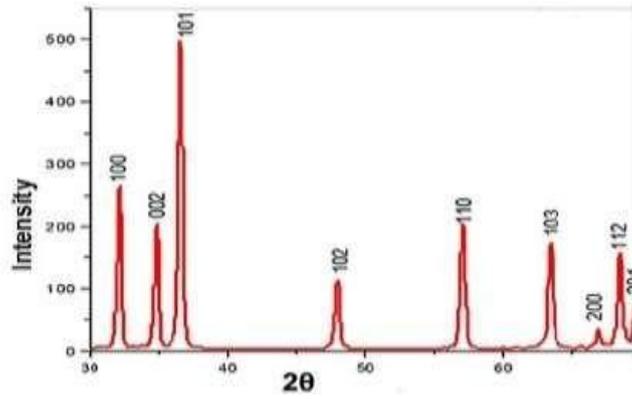
Fig. 3. FTIR of synthesized complex Zn nanoparticles



#### XRD spectroscopy

The composition and structure of Zn nanoparticles was confirmed by XRD crystallographic analysis. The XRD pattern with sharp peaks showed that the Zn

nanoparticles were pure and crystalline in nature. The average size of Zn nanoparticles was 24.74 nm, which was observed at highest peak.

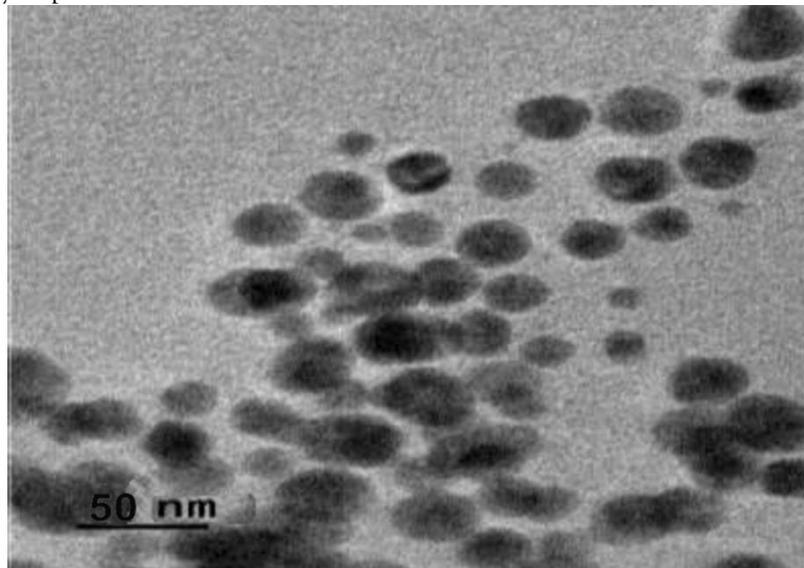


*Fig. 4. XRD pattern of synthesized complex Zn nanoparticles*

TEM spectroscopy

According to TEM micrographs, the nanoparticles were spherical and poly-dispersed.

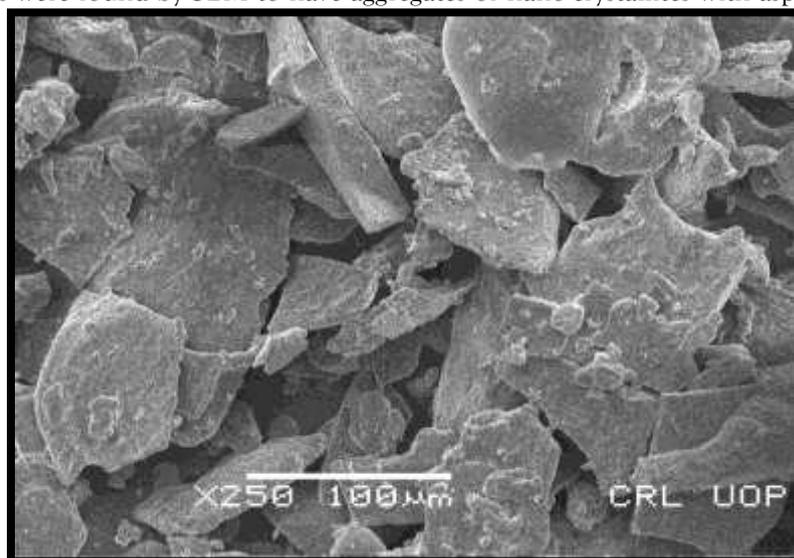
The nanoparticles ranged in size from 17 nm to 28 nm.



*Fig. 5. TEM micrograph of Zn nanoparticles*

SEM spectroscopy

Zn nanoparticles were found by SEM to have aggregates of nano-crystallites with aspherical shape.



*Fig. 6. SEM micrograph of complex Zn nanoparticles*

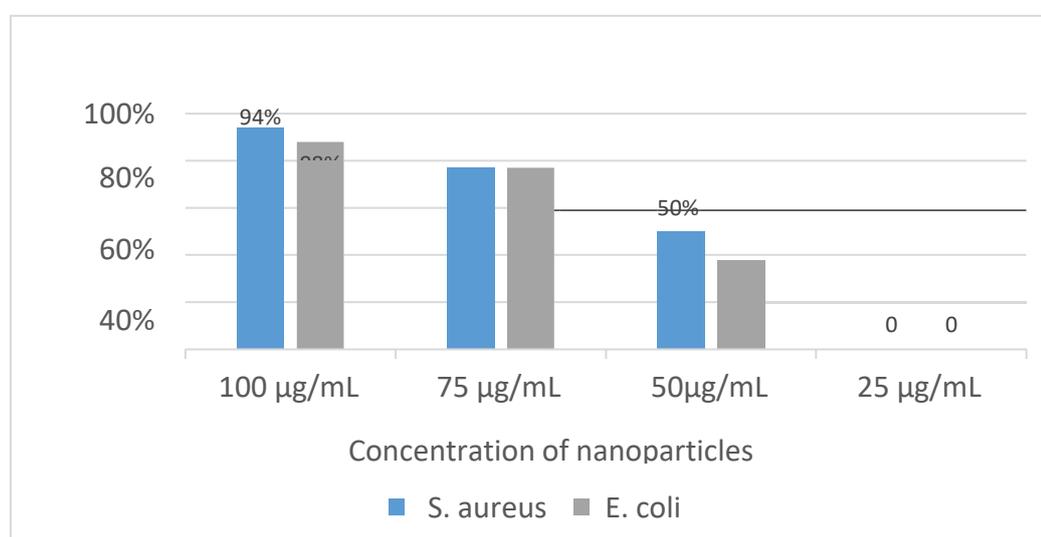
### Antibacterial activity of Zn nanoparticles

Zinc nanoparticles synthesized by bacteria were evaluated for antibacterial efficacy against pathogenic drug resistant *S. aureus* and *E. coli*. The nanoparticles revealed antibacterial activity against *S. aureus* and *E.*

*coli* at a concentration of 100 µg/mL and 75 µg/mL, respectively. The zones of inhibitions were reduced with the decrease in nanoparticles concentrations. The inhibitions zones formed at various concentrations are listed in the table below.

**Table 4. Antibacterial activity of Zn nanoparticles against drug resistant *S. aureus* and *E. coli***

S. No.	Concentration of nanoparticles	Zones of Inhibitions		% Inhibition	
		<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
1.	100 µg/mL	17 mm	16 mm	94 %	88 %
2.	75 µg/mL	14 mm	14 mm	77 %	77 %
3.	50 µg/mL	9 mm	7 mm	50 %	38 %
4.	25 µg/mL	No zone	No zone	---	---



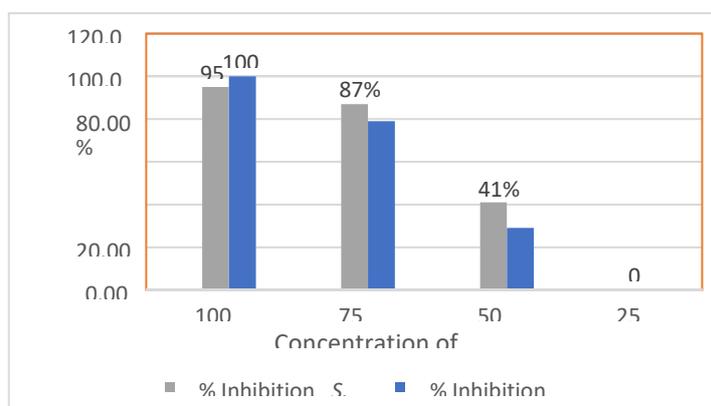
**Fig. 7. Antibacterial activity of Zn nanoparticles against drug resistant *S. aureus* and *E. coli***

#### 4.1. Antifungal activity of Zn nanoparticles

Zinc nanoparticles synthesized by bacteria were evaluated for antifungal activity against pathogenic fungi *Sporothrix schenkii* and *Aspergillus fumigatus*, respectively. The Zn nanoparticles reduced the growth of both the fungal species at a concentration of 100 µg/mL and 75 µg/mL, respectively, whereas, Zn nanoparticles showed reduced activity at a concentration of 50 and 25 µg/mL, respectively in table 4.5 below.

**Table 5. Antifungal activity of Zn nanoparticles**

S. No.	Concentration of Zn nanoparticles	Zones of Inhibition		% Inhibition	
		<i>Sporothrix schenckii</i>	<i>Aspergillus fumigatus</i>	<i>Sporothrix schenckii</i>	<i>Aspergillus fumigatus</i>
1.	100 µg/mL	23 mm	24 mm	95 %	100 %
2.	75 µg/mL	21 mm	19 mm	87 %	79 %
3.	50 µg/mL	10 mm	7 mm	41 %	29 %
4.	25 µg/mL	No zone	No zone	-----	-----



**Fig. 8. Antifungal activity of Zn nanoparticles against drug resistant *S. aureus* and *E. coli***

## DISCUSSION

The biological synthesis of complex nanoparticles is a safe, low-cost, non-toxic and environmental friendly approach (El-Ghwas, 2022). Several bacteria have been identified as producers of metal nanoparticles (Sahin *et al.*, 2023). The bacteria used in the synthesis process have a strong control over the physical properties of the synthesized nanoparticles, including their size, shape and crystallinity (Mousavi *et al.*, 2022). The synthesis of Zn nanoparticles has been found to be mediated by several bacterial isolates, including *P. aeruginosa*, *S. aureus*, *Bacillus* and *Serratia* (Ihsan *et al.*, 2023; Jain *et al.*, 2020).

In investigation, the bacterial species isolated from soil sample used to synthesize Zn nanoparticles. Similarly, Iqtidar *et al.* (2020) utilized bacterial specie isolated from soil sample to synthesize Zn nanoparticles. In another study by El-Ghwas *et al.* (2022) utilized bacterial specie isolated from farm soil. In our study, the bacterial specie isolated was identified as *Serratia* specie. The *Serratia* specie formed whitish and red, opaque, round colonies with white margins. These findings were further supported by Adan *et al.* (2020). The specie was further confirmed to genus and specie level based on the findings of morphological,

biochemical and physiological tests as *Serratia nematodiphila*. The phylogenetic analysis showed that the isolated bacteria is *Serratia nematodiphila*.

The *Serratia* specie in our study successfully synthesized Zn nanoparticles. Similarly, Verma *et al.* (2021) successfully synthesized Zn nanoparticles utilizing *Aeromonas hydrophila*. This finding was further supported by Mohd Yusof *et al.* (2019). Our results were further in consistence with the findings of Salman *et al.* (2018), who reported the synthesis of Zn nanoparticles from *Lactobacillus* specie.

The synthesis of Zn nanoparticles in study was the appearance of white precipitate at the bottom of the flask. These findings were similar to Rajapriya *et al.* (2020). Similarly, Mustapha *et al.* (2020) also confirmed the formation of Zn nanoparticles by appearance of milky white precipitates. Several other authors, including Archana *et al.* (2022), Pai *et al.* (2019) and Varadavenkatesan *et al.* (2019) also supported our result.

Our characterization results were supported by Adegoke & Gbenga. (2023), who reported crystalline and poly dispersed Zn nanoparticles of size 25 nm. Similar to our study, Kalaba *et al.* (2021) reported poly dispersed,

crystalline Zn nanoparticles with spherical shape. Similarly, Thi Tran *et al.* (2021) also reported Zn nanoparticles with crystalline nature.

The maximum SPR in the current study was observed at 379 nm. This analysis was concurrent with the analysis of Jain *et al.* (2020). Similar to this, Abdo *et al.* (2021) reported the maximum SPR peak at 361 nm. Our analysis was further supported by Kaur *et al.* (2022). Another author, Ibrahim *et al.* (2020) also reported highest resonance peak of Zn nanoparticles at 379 nm. The UV/Vis analysis performed by Anjum *et al.* (2021) was also concurrent with our analysis. Similarly, Chen *et al.* (2022) also reported Zn nanoparticles with highest SPR peak at 379 nm.

The composition and structure of Zn nanoparticles was confirmed by XRD crystallographic analysis. Our XRD and FT-IR analysis was supported by Jayappa *et al.* (2020). Several other authors reported the crystalline nature of the synthesized Zn nanoparticles, which similar to our XRD analysis (Matussin *et al.*, 2021; Zhang *et al.*, 2019; Mohammed *et al.*, 2022). Similarly, Modwi *et al.* (2021) reported Zn nanoparticles with hexagonal shape. All these analysis showed results similar to our study.

In our study, the nanoparticles showed antibacterial activity against *S. aureus* and *E. coli* at a concentration of 100 µg/mL and 75 µg/mL, respectively. Similar to our study, Obeizi *et al.* (2020) Zn nanoparticles with inhibitory activity against *K. pneumoniae* with maximum zone of 19 mm at a concentration of 100 µg/mL. Similarly, Sharma *et al.* (2023) reported Zn nanoparticles with inhibitory activity against *S. aureus*. Certain other authors also reported anti-inhibitory activity of the synthesized Zn nanoparticles at a concentration of 100 µg/mL and 75 µg/mL against pathogenic bacterial isolates (Chunchegowda *et al.*, 2021; Wang *et al.*, 2021; Malathi *et al.*, 2021).

In our study, Zn nanoparticles were evaluated for antifungal activity against pathogenic fungi *Sporothrix schenckii* and *Aspergillus fumigatus*, respectively. The Zn nanoparticles reduced the growth of both the fungal species at a concentration of 100 µg/mL and 75 µg/mL, respectively, whereas, Zn nanoparticles showed reduced activity at a concentration of 50 and 25 µg/mL, respectively. This data was supported by Sanjivkumar *et al.* (2022), who reported the antifungal activity of Zn nanoparticles against *Candida fructus* with an inhibition zone of 20 mm. Similarly, Sebesta *et al.* (2022) reported the inhibitory activity of Zn nanoparticles against *Sporothrix schenckii* at a concentration of 100 µg/mL. Similar to our antifungal analysis, Stevanovic *et al.* (2020) evaluated the anti-fungal activity of Zn

nanoparticles against *Sporothrix schenckii*, which showed promising results. Similarly, Ghosh *et al.* (2022) also reported Zn nanoparticles with inhibitory activity against *Sporothrix schenckii*, while Slavin & Bach. (2022) synthesized Zn nanoparticles with inhibitory activity against *Aspergillus fumigatus* at a concentration of 100 µg/mL.

## CONCLUSION

In the current study, *Serratia nematodiphila* successfully synthesized Zn nanoparticles using green route. The bacterial-mediated synthesis of Zn nanoparticles is simple, environmental friendly and cost-effective approach. According to TEM and SEM micrographs, the nanoparticles were spherical and poly-dispersed with a size in the range of 17 nm to 28 nm. The nanoparticles showed antibacterial activity against *S. aureus* and *E. coli* at a concentration of 100 µg/mL and 75 µg/mL, respectively. On the other hand, the nanoparticles effectively inhibited the growth of pathogenic fungi *Sporothrix schenckii* and *Aspergillus fumigatus* in a dose-dependent manner, which greatly increased the applicability of the synthesized Zn nanoparticles.

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