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Research Article

# Eco-Friendly Synthesis and Characterization of Zinc Nanoparticles Using Medicinal Plant *Stachytarpheta indica*

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## Abstract

This study investigates the eco-friendly synthesis and characterization of zinc nanoparticles (ZnNPs) using *Stachytarpheta indica* aqueous leaf extract. UV–Vis spectrophotometry revealed a prominent absorption peak at 380 nm, confirming nanoparticle formation within the characteristic surface plasmon resonance (SPR) range. Fourier-transform infrared (FTIR) analysis identified functional groups such as hydroxyl, amine, carbonyl, and polysaccharide moieties, suggesting the involvement of phenolics, flavonoids, and proteins in nanoparticle reduction and stabilization. The Scanning Electron Microscopy (SEM) revealed polydisperse, irregularly shaped ZnNPs with porous surface morphology, indicating micro-to-nanoscale size distribution and potential for functional activity. The synthesized ZnNPs demonstrated structural and chemical features consistent with previous plant-mediated nanoparticle syntheses. These findings highlight the efficacy of *S. indica* extract in producing stable ZnNPs with promising applications in antimicrobial and environmental technologies.

**Keywords:** Zinc Nanoparticles; *Stachytarpheta indica*; Green Synthesis; UV–Vis Spectroscopy; FTIR Analysis; SEM Imaging, Phytochemicals; Nanoparticle Stabilization; Biosynthesis; Antimicrobial Potential

## Introduction

The rapid advancement of nanotechnology has facilitated the synthesis of nanoparticles with tailored properties for biomedical and industrial applications. Among these, zinc oxide nanoparticles (ZnO-NPs) have gained considerable attention for their potent antibacterial effects. Sirelkhatim., *et al*. [1] highlighted the increasing global interest in ZnO-NPs, largely attributed to their nanoscale size, which enhances surface reactivity and interaction with microbial cells. Given that most microorganisms range in size from hundreds of nanometers to several micrometers, ZnO-NPs are ideally sized to interact with bacterial surfaces or penetrate cells, resulting in improved antimicrobial activity.

ZnO is widely recognized as a biocompatible material, possessing strong photo-oxidizing and photocatalytic properties that contribute to its antimicrobial action. Its high surface area-to-volume ratio, combined with unique surface chemistry, underpins its interaction with biological systems. Studies have shown that ZnO-NPs demonstrate minimal cytotoxicity toward human cells, enabling selective targeting of microbial pathogens [2], [3]. This selective toxicity positions ZnO-NPs as promising candidates for safe antimicrobial applications in health and industry.

Several mechanisms have been proposed to explain the antibacterial efficacy of ZnO-NPs. A primary pathway involves the genera-

tion of reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($OH^-$), and superoxide ions ($O_2^{2-}$). These ROS disrupt bacterial membranes, increase permeability, and cause oxidative damage to intracellular components [2]. According to [1], ROS-induced stress contributes to membrane destabilization, proton motive force disruption, intracellular leakage, and activation of stress response genes, ultimately leading to bacterial cell death. Additionally, surface defects in ZnO-NPs provide abrasive textures that exacerbate physical damage to bacterial membranes.

Another proposed mechanism involves the release of $Zn^{2+}$ ions from ZnO-NPs into the surrounding medium. These ions interfere with bacterial metabolism by inhibiting key enzymatic pathways and active transport systems [4,5]. The toxicity of ZnO-NPs is closely linked to the dissolution of $Zn^{2+}$, a process influenced by nanoparticle size, morphology, and surface characteristics [6,7]. For example, Kasemets., *et al*. [8] demonstrated that $Zn^{2+}$ release was a major contributor to ZnO toxicity in *Saccharomyces cerevisiae*, a yeast species widely used in food production. However, the extent of this contribution remains under debate. While some researchers argue that $Zn^{2+}$ concentrations are sufficient to exert antibacterial effects [9] , others contend that the limited solubility of ZnO in aqueous media minimizes its impact [10,11].

The antibacterial performance of ZnO-NPs is highly dependent on their physical and chemical characteristics. Pasquet., *et al*. [12] identified two primary categories influencing $Zn^{2+}$ release: (i) intrinsic nanoparticle properties, including porosity, size, concentration, and shape, and (ii) external environmental factors such as pH, UV exposure, duration of contact, and the presence of other chemical species. Morphology has been shown to play a critical role in ZnO-NPs' solubility and reactivity. For instance, Peng., *et al*. [13] found that spherical nanoparticles release more $Zn^{2+}$ ions than rod-shaped ones, likely due to their higher surface curvature and solubility. Similar findings were reported by Wang., *et al*. [14], reinforcing the idea that particle shape governs dissolution behavior and, by extension, antibacterial potency.

Surface modifications have also been shown to significantly influence ZnO-NPs' antibacterial activity. Leung., *et al*. [15] suggested that both ROS generation and $Zn^{2+}$ ion release are surface-driven processes, and thus surface chemistry strongly affects the nanoparticle's interaction with bacterial membranes. Advanced characterization techniques such as scanning electron microscopy (SEM), X-ray diffraction (XRD), transmission electron microscopy (TEM), and electron spectroscopy imaging (ESI) have been instrumental in studying these mechanisms. For example, ESI analysis has revealed that ZnO rods possess higher oxygen-to-zinc (O:Zn) ratios compared to other morphologies, leading to increased ROS production and greater oxidative stress in bacteria.

ZnO-NPs have shown particular promise in food safety applications. When incorporated into food packaging materials, they act as antimicrobial agents by directly interacting with pathogens on food surfaces, thereby preventing contamination and extending shelf life. Sirelkhatim., *et al*. [1] noted the potential of ZnO-NPs to inhibit a broad range of foodborne pathogens through multiple antibacterial pathways. Additionally, emerging research has indicated possible antiviral effects, with studies suggesting that $Zn^{2+}$ ions can interfere with viral proteins, including those involved in herpes simplex virus (HSV-1) pathogenesis [16].

## Materials and Methods

### Synthesis of phyto-extract mediated zinc nanoparticles

For present investigation nanoparticle will be prepared by treating the solution of salt of silver and zinc with phytochemical extracts of plant opted in present study would be prepared according to the method suggested by [17-20], with suitable modifications.

Silver nitrate and Zinc sulphate solution will be used as precursor to treat with phytochemical extract to form phytoextract mediated ZnNPs. The nanoparticles will be prepared by reducing the precursor solutions by phytochemical extract of plant material. The formation of nanoparticle would be monitored by observing the change in colour and formation of sediments at suitable temperature and pH.

### Synthesis of zinc nanoparticles with extract

The synthesis of zinc nanoparticles (ZnNPs) using the phytochemical extract of *Stachytarpheta indica* L. was carried out following the method suggested by Ramesh., *et al*. [21] with suitable modifications for the present study. For ZnNPs synthesis, a known concentration of leaf extract was allowed to react with 0.1 M zinc

acetate dihydrate (Zn $(CH_3COO)_2 \cdot 2H_2O$) solution in a controlled environment to ensure efficient nanoparticle formation. For this purpose, 45 mL of 0.1 M aqueous zinc acetate solution was prepared, and its transparency and clarity were checked. To this solution, 5 mL of *Stachytarpheta indica* L. aqueous extract (prepared from a 100 mg/mL stock solution) was added dropwise under continuous stirring using a magnetic stirrer at 40°C. The gradual addition of the phytochemical extract ensured uniform interaction with the zinc ions, facilitating the reduction and stabilization of the nanoparticles.

A colour change from transparent to light yellow to milky white within an hour indicated the formation of zinc nanoparticles (ZnNPs) [22]. Stirring was maintained for 5 to 6 hours, after which the solution was left to stand overnight for complete reduction. The ZnNPs were then separated by centrifugation at 5000 rpm for 10 minutes at room temperature. The resulting pellet was dried at 45°C for 24 hours, and the obtained ZnNPs powder was re-dispersed in sterile distilled water to obtain the required experimental concentration for further studies.

## Aqueous extraction of crud drug

About 50 grams of fine leaf powder of *Stachytarpheta indica* L. was subjected to defatted in petroleum ether first by keeping the powder dipped in overnight.

After defatting the leaf powered was allowed to further drying at room temperature by spreading it on newspaper leaving for 3-4 hours leads to complete evaporation of petroleum ether.

Now 20 grams of defatted dried powder was subjected to Soxhlet extraction with 200 ml of distilled water as a solvent. Soxhlation process was allowed to carry out for 24 hours at 70°C temperature till the exhaustion of the merc [23].

The extract so obtained is subjected to evaporation of solvent in boiling water bath

$$\text{Percentage Yield} = \frac{\text{Weight of Extract Drug}}{\text{Weight of Crude Drug}} \times 100$$

At continuous stirring condition change in colour from transparent to yellow to redish brown within hour indicates the formation of Silver Nano Particles of plant extract [24]. The stirring was maintained for 5 to 6 hours, after which the solution was allowed to stand for overnight. This was followed by separation of ZnNPs by centrifugation at 5000 rpm at room temperature for 10 min they drying of pellets was done at 45°C for 24 h and the powder so obtained was dispensed in sterile, distilled water to obtain the required experimental concentration for the experiments.

## Characterization techniques

UV-Vis Spectrophotometry: The optical properties of ZnNPs were analyzed using a UV-Vis spectrophotometer, with absorbance recorded between 300–800 nm.

Fourier-Transform Infrared (FTIR) Spectroscopy: FTIR analysis identified functional groups responsible for nanoparticle reduction and stabilization.

Scanning Electron Microscopy (SEM): SEM imaging provided insights into the morphology, size distribution, and surface characteristics of the synthesized ZnNPs.

## Analysis of synthesized silver and zinc nanoparticles

The characterization of synthesized zinc (ZnNPs) nanoparticles was performed using various analytical techniques to confirm their formation, stability, and surface morphology. The suspected nanoparticle suspensions were subjected to UV-Vis spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM) to validate their synthesis and assess their physicochemical properties.

## UV-Vis spectrophotometry

It was used as a preliminary confirmation method by recording the absorption spectra of the synthesized nanoparticles in the wavelength range of 200–800 nm. The characteristic Surface Plasmon Resonance (SPR) peaks were analyzed to confirm nanoparticle formation, with silver nanoparticles typically exhibiting peaks between 400–450 nm and zinc nanoparticles around 300–380 nm [25].

### FTIR analysis

It was performed to identify the functional groups responsible for the reduction and stabilization of the nanoparticles. The samples were dried and subjected to Fourier Transform Infrared Spectroscopy in the range of 4000–400 $cm^{-1}$, and spectra were analyzed to detect the presence of biomolecules such as phenols, flavonoids, and proteins involved in nanoparticle synthesis [26].

### SEM imaging

It was conducted to determine the size, shape, and surface morphology of the nanoparticles. A drop of the nanoparticle suspension was coated onto a carbon tape, air-dried, and analyzed using Scanning Electron Microscopy at different magnifications. The obtained micrographs provided insights into nanoparticle distribution, structural integrity, and aggregation tendencies [17].

These characterization techniques collectively ensured the successful synthesis and stability of silver and zinc nanoparticles, verifying their suitability for further applications.

## Results and Discussion

### Analysis of zinc nanoparticles

#### A. UV-vis spectrophotometry

Likewise, the UV-Vis spectrophotometric analysis of zinc nanoparticles (ZnNPs) synthesized using *Stachytarpheta indica* aqueous extract exhibited a prominent absorption peak at 380 nm, indicating the successful formation of ZnNPs. UV-Vis absorbance spectrum of *Stachytarpheta indica* aqueous extract and their respective zinc nanoparticles is depicted in figure 1 which was generated on UV-Vis spectrophotometer LabIndia3000+. This peak falls within the characteristic surface plasmon resonance (SPR) range for ZnNPs, which typically appears between 350-400 nm depending on particle size and synthesis conditions.

Compared to the aqueous extract, which showed a broad absorbance pattern, the ZnNPs exhibited a distinct peak, confirming the reduction and stabilization of zinc ions by phytoconstituents present in the extract. A gradual decline in absorbance beyond 400 nm further supports the stability and nanoscale nature of the synthesized ZnNPs. The reduced absorbance values at higher wavelengths (600-800 nm) indicate minimal aggregation and uniform particle dispersion, essential for potential antimicrobial applications.

These results corroborate previous findings where plant-mediated ZnNPs displayed similar UV-Vis absorbance peaks, confirming the efficiency of *S. indica* extract in nanoparticle synthesis.

The UV-Vis spectrophotometric analysis of zinc nanoparticles (ZnNPs) synthesized using *Stachytarpheta indica* aqueous extract revealed a prominent absorption peak at 380 nm, indicating suc-

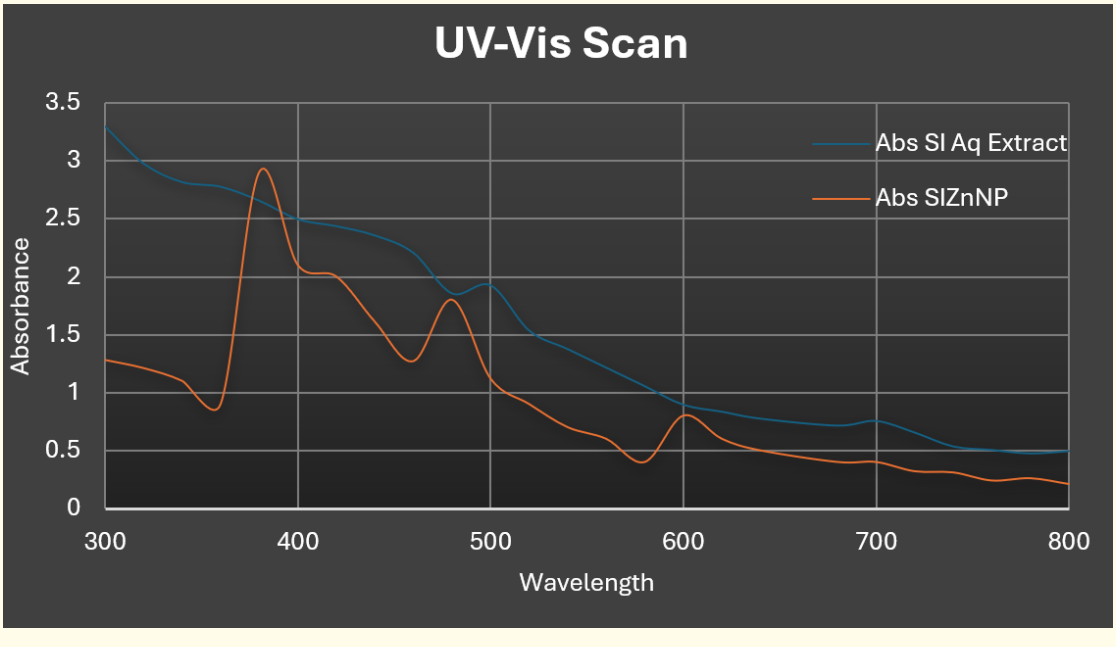


**Figure 1:** UV-Vis Absorbance Spectrum of *Stachytarpheta indica* Aqueous Extract and Zinc Nanoparticles generated on UV-Vis spectrophotometer LabIndia3000+.

cessful nanoparticle formation. This finding aligns with previous studies employing plant extracts for ZnNP synthesis. For instance, ZnNPs synthesized using *Papaver somniferum* extract exhibited a strong surface plasmon resonance (SPR) peak at 360 nm, confirming nanoparticle formation [27].

Similarly, ZnNPs produced with *Deverra tortuosa* extract showed an absorption edge indicative of nanoparticle formation, consistent with the characteristic SPR range for ZnNPs [28]. These consistent SPR peaks across different plant-mediated syntheses underscore the efficacy of phytochemicals in reducing and stabilizing zinc ions into nanoparticles. The uniformity in SPR peaks suggests that plant extracts can effectively control nanoparticle size and dispersion, crucial for applications in antimicrobial therapies.

## FTIR analysis

The Fourier-transform infrared (FTIR) spectrum of *Stachytarpheta indica* extract-mediated zinc nanoparticles (ZnNPs) as depicted in figure 2 was generated from FTIR instrument Alpha Bruker provides insight into the biomolecules responsible for reduction, stabilization, and capping.

- Broad peaks at 3446.79 $cm^{-1}$ and 3421.72 $cm^{-1}$ correspond to O-H and N-H stretching vibrations, indicating the presence of hydroxyl and amine groups from phenolic compounds and proteins, which act as stabilizing agents.
- Peak at 3203.56 $cm^{-1}$ suggests C-H stretching, which is commonly associated with aliphatic compounds.
- The peaks at 2924.00 $cm^{-1}$ and 2843.65 $cm^{-1}$ represent C-H stretching vibrations of alkane functional groups, possibly from plant-derived terpenoids or flavonoids.
- Peak at 2024.00 $cm^{-1}$ could indicate C≡C stretching, possibly from alkynes.
- Significant peak at 1634.69 $cm^{-1}$ corresponds to C=O stretching of amides or carboxyl groups, suggesting protein interaction with ZnNPs.
- Peak at 1400.32 $cm^{-1}$ is associated with C-H bending, indicating the presence of alkanes or aromatic compounds.
- The region around 1029.86 $cm^{-1}$ and 1023.09 $cm^{-1}$ is attributed to C-O stretching of polysaccharides, which contribute to nanoparticle stability.
- A peak at 871.82 $cm^{-1}$ suggests out-of-plane bending of aromatic C-H bonds.

These functional groups confirm the role of phytochemicals in nanoparticle synthesis, aligning with previous studies that report the involvement of flavonoids, proteins, and phenolics in ZnNP formation.

Fourier-transform infrared (FTIR) spectroscopy is instrumental in identifying functional groups involved in the biosynthesis and stabilization of zinc nanoparticles (ZnNPs) mediated by *Stachytarpheta indica* extract. The FTIR spectrum of these ZnNPs reveals several key absorption bands indicative of various biomolecular interactions.

The broad absorption peak around 3446.79 $cm^{-1}$ corresponds to O-H and N-H stretching vibrations, signifying the presence of hydroxyl and amine groups from phenolic compounds and proteins. These functional groups are known to act as reducing and stabilizing agents in nanoparticle synthesis. Similar observations were reported by Suresh., *et al*. [29] where phenolic groups in *Cassia fistula* extract played a crucial role in the formation of ZnNPs.

The peaks at 2924.00 $cm^{-1}$ and 2843.65 $cm^{-1}$ are attributed to C-H stretching vibrations of alkane groups, possibly originating from plant-derived terpenoids or flavonoids. These biomolecules have been implicated in nanoparticle stabilization, as evidenced in studies involving *Aquilegia pubiflora* extract-mediated ZnNPs, where flavonoids served as capping agents [30].

A prominent peak at 1634.69 $cm^{-1}$ corresponds to C=O stretching vibrations of amide or carboxyl groups, suggesting protein interactions with ZnNPs. This aligns with findings by Stan., *et al*. [18] who noted protein involvement in stabilizing ZnNPs synthesized using *Allium sativum* extract. The absorption band at 1400.32 $cm^{-1}$ is associated with C-H bending vibrations, indicative of alkanes or aromatic compounds. Additionally, peaks around 1029.86 $cm^{-1}$ and 1023.09 $cm^{-1}$ are attributed to C-O stretching vibrations of polysaccharides, contributing to nanoparticle stability. These observations are consistent with previous studies where polysaccharides from plant extracts facilitated ZnNP stabilization [31].

Collectively, the FTIR spectrum underscores the pivotal role of phytochemicals—such as phenolics, flavonoids, proteins, and polysaccharides—in the green synthesis and stabilization of Zn-

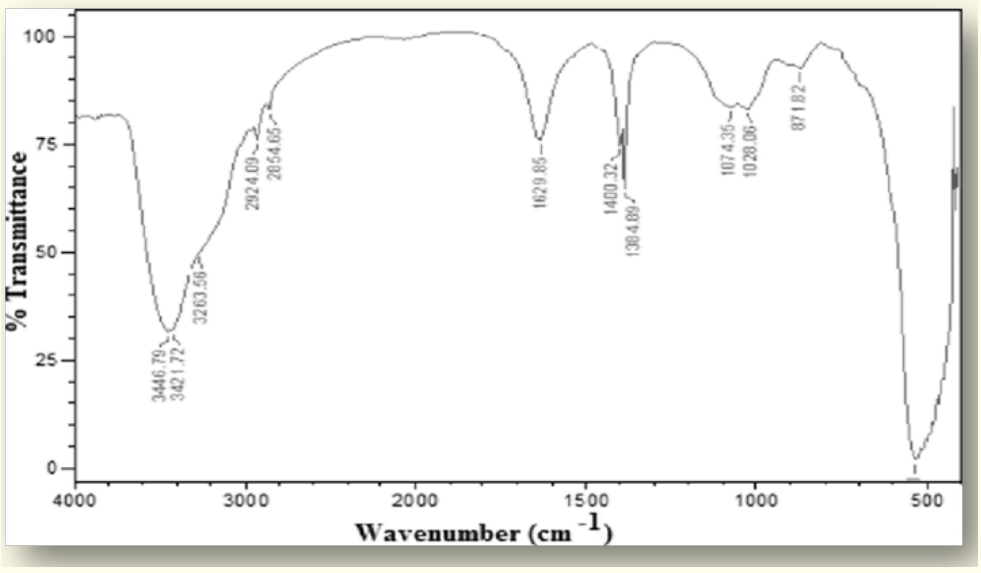


**Figure 2:** FTIR Spectrum of *Stachytarpheta indica* Extract-Mediated Zinc Nanoparticles Showing Functional Groups Involved in Synthesis and Stabilization.

NPs using *Stachytarpheta indica* extract. These findings are in concordance with existing literature on plant-mediated nanoparticle synthesis, highlighting the efficacy of plant extracts in producing stable and functional ZnNPs.

## SEM imaging

The SEM micrograph of *Stachytarpheta indica* extract-mediated zinc nanoparticles (SIZnNP) as depicted in figure 3 captured at 1400X magnification provides key insights into the morphological characteristics of the synthesized nanoparticles which are as follows.

## Particle distribution and morphology

The SEM image of *Stachytarpheta indica* extract-mediated zinc nanoparticles (SIZnNPs) captured at 1400X magnification reveals an irregular and polydisperse morphology. The particles appear aggregated, with a mixture of sharp-edged and flake-like structures. The non-uniformity in shape suggests the influence of phytochemicals in stabilizing and capping the nanoparticles. Similar patterns have been observed in bio-mediated zinc nanoparticles, where organic compounds such as flavonoids, phenolics, and alkaloids regulate nanoparticle formation.

## Size and structural features

The measured particle sizes (ranging from 9.72 µm to 16.59 µm) indicate that the synthesized ZnNPs exhibit micro-to-nanoscale dimensions. The variation in particle size suggests different nucleation and growth phases during synthesis. The SEM image also highlights a rough and porous surface topology, which is indicative of high surface area and potential functional activity, particularly in antimicrobial and catalytic applications.

## Surface characteristics and aggregation

The high contrast regions in the SEM micrograph suggest the presence of zinc clusters with possible agglomeration. The observed clustering may result from electrostatic interactions between the nanoparticles and phytochemicals, a phenomenon frequently reported in green nanoparticle synthesis. Factors such as pH, temperature, and extract concentration could have played a significant role in determining nanoparticle morphology, as seen in previous studies on biosynthesized ZnNPs.

Overall, the SEM micrograph confirms the successful synthesis of zinc nanoparticles with irregular and polydisperse morphology, indicating the potential role of *Stachytarpheta indica* phytochemicals in the reduction, capping, and stabilization of ZnNPs. The observed structural characteristics suggest effective bio-mediated synthesis, with possible applications in antimicrobial, catalytic, and environmental remediation fields.

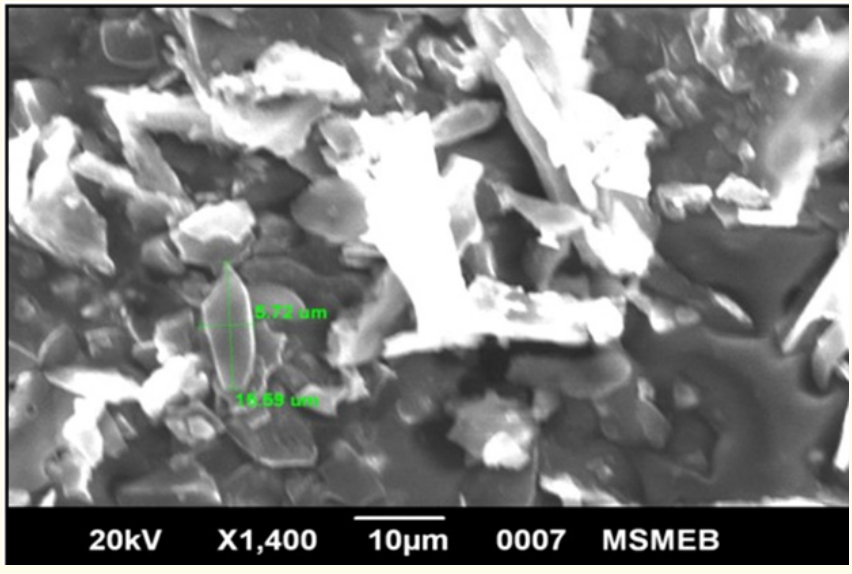


**Figure 3:** SEM micrograph of sample SIZnNP a *Stachytarpheta indica* extract-mediated zinc nanoparticles captured at 1400X magnification.

The biosynthesis of zinc nanoparticles (ZnNPs) using *Stachytarpheta indica* extract, as evidenced by the SEM micrograph, aligns with previous studies that emphasize the role of plant phytochemicals in nanoparticle formation. The irregular morphology and polydisperse nature observed in the micrograph are consistent with prior research indicating that biosynthesized ZnNPs exhibit diverse shapes due to the influence of biomolecules acting as reducing and stabilizing agents [32]. The presence of capping agents, such as flavonoids and alkaloids, contributes to the stabilization of these nanoparticles, preventing excessive aggregation [33].

Studies have shown that green-synthesized ZnNPs exhibit strong antimicrobial and catalytic properties, making them valuable in biomedical and environmental applications [34]. The structural variation observed in the SEM image could be attributed to reaction conditions such as pH, extract concentration, and synthesis temperature, as reported by [25]. Furthermore, biosynthesized ZnNPs have been found to exhibit enhanced biocompatibility compared to chemically synthesized counterparts [29].

Overall, the findings support existing literature on bio-mediated ZnNP synthesis, reinforcing the potential of *S. indica* phytochemicals in nanoparticle stabilization. The observed morphology suggests potential applications in antimicrobial formulations, drug delivery, and wastewater treatment.

## Conclusion

The present study successfully demonstrates the eco-friendly synthesis of zinc nanoparticles (ZnNPs) using *Stachytarpheta indica* aqueous extract as a bioreducing and stabilizing agent. The UV–Vis spectrophotometric analysis confirmed nanoparticle formation through a distinct absorption peak at 380 nm, characteristic of zinc's surface plasmon resonance. FTIR analysis revealed the presence of functional groups—such as hydroxyls, amines, carbonyls,

and polysaccharides—indicative of phytochemicals involved in the reduction and capping processes. SEM imaging further established the formation of irregular, polydisperse nanoparticles with micro-to-nanoscale dimensions and rough surface morphology, suggesting high surface area and potential for biological activity. These findings affirm the role of *S. indica* phytoconstituents in facilitating a green, cost-effective, and sustainable method for ZnNP synthesis. The synthesized nanoparticles show potential for applications in antimicrobial, pharmaceutical, and environmental fields. Future studies may explore the bioactivity and toxicity profile of these ZnNPs to validate their applicability in real-world scenarios.

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