

Sustainable Fabrication and Characterization of Zinc Nanoparticles Utilizing the Medicinal Herb *Stachytarpheta indica*

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Abstract: This study explores the green synthesis and characterization of zinc nanoparticles (ZnNPs) prepared using an aqueous leaf extract of *Stachytarpheta indica*. UV–Vis spectrophotometry showed a distinct absorption peak at 380 nm, confirming the formation of ZnNPs within the typical surface plasmon resonance (SPR) range. FTIR analysis revealed the presence of hydroxyl, amine, carbonyl, and polysaccharide functional groups, indicating that phenolics, flavonoids, and proteins likely played key roles in reducing and stabilizing the nanoparticles. SEM imaging showed polydisperse ZnNPs with irregular shapes and porous surface structures, reflecting a broad micro- to nanoscale size distribution and potential functional versatility. The physicochemical characteristics of the synthesized ZnNPs align well with those reported for other plant-based nanoparticle fabrication methods. Overall, the findings demonstrate that *S. indica* leaf extract is an effective and sustainable agent for producing stable ZnNPs with potential applications in antimicrobial and environmental fields.

Keywords: Zinc nanoparticles, *Stachytarpheta indica*, green synthesis, UV–Vis spectroscopy, FTIR analysis, SEM imaging, phytochemicals, nanoparticle stabilization, biosynthesis, antimicrobial potential.

1. Introduction

The rapid growth of nanotechnology has enabled the development of nanoparticles with customizable characteristics for diverse biomedical and industrial uses. Among these, zinc oxide nanoparticles (ZnO-NPs) have emerged as notable candidates due to their strong antibacterial properties. According to Sirelkhatim et al. (2015), global interest in ZnO-NPs has risen significantly, primarily because their nanoscale dimensions enhance surface reactivity and promote effective interaction with microbial cells. Since most microorganisms range from a few hundred nanometers to several micrometers in size, ZnO-NPs possess an ideal size range for adhering to bacterial surfaces or entering cells, thereby amplifying their antimicrobial potential.

Zinc oxide is well known for its biocompatibility and exhibits powerful photo-oxidizing and photocatalytic abilities that contribute to its antimicrobial effects. The combination of a high surface area-to-volume ratio and distinctive surface chemistry supports its interaction with biological systems. Research has demonstrated that ZnO-NPs have low cytotoxicity toward human cells while effectively targeting microbial pathogens (Colon et al., 2006; Padmavathy & Vijayaraghavan, 2008). This selective toxicity underscores their suitability for safe and effective antimicrobial applications across healthcare and industrial sectors.

Several mechanisms have been suggested to account for the antibacterial activity of ZnO nanoparticles (ZnO-NPs). One major pathway is the generation of reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), hydroxyl radicals (OH[•]), and superoxide ions (O₂²⁻). These ROS damage bacterial cells by disrupting membrane integrity, increasing permeability, and inducing oxidative stress within cellular components (Seil & Webster, 2012). As noted by Sirelkhatim et al. (2015), ROS-mediated stress leads to membrane destabilization, disruption of the proton motive force, leakage of intracellular contents, and activation of stress-response genes, ultimately resulting in bacterial cell death. Furthermore, structural imperfections on the surface of ZnO-NPs provide abrasive sites that intensify mechanical damage to bacterial membranes.

Another widely discussed mechanism is the release of Zn²⁺ ions from ZnO-NPs into the surrounding environment. These

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ions hinder bacterial metabolic processes by inhibiting essential enzymes and active transport systems (Premanathan et al., 2011; Li et al., 2011). The toxicity associated with ZnO-NPs is strongly influenced by the dissolution of Zn^{2+} , which depends on nanoparticle size, shape, and surface properties (Yang et al., 2009; Xia et al., 2008). For instance, Kasemets et al. (2009) reported that Zn^{2+} release significantly contributed to ZnO toxicity in *Saccharomyces cerevisiae*, a yeast commonly used in food fermentation. Nevertheless, the degree to which Zn^{2+} ions contribute to antibacterial activity remains contested. Some studies assert that Zn^{2+} levels are sufficiently high to produce bactericidal effects (Reddy et al., 2007), whereas others argue that the low solubility of ZnO in aqueous conditions limits the impact of ion release (Sawai, 2003; Jiang et al., 2009).

The antibacterial efficacy of ZnO nanoparticles (ZnO-NPs) is largely governed by their physicochemical attributes. Pasquet et al. (2014) highlighted two major factors controlling Zn^{2+} ion release: (i) inherent nanoparticle characteristics—such as size, shape, porosity, and concentration—and (ii) external environmental conditions, including pH, UV irradiation, duration of exposure, and the presence of additional chemical species. Among these, morphology is particularly influential in determining the solubility and reactivity of ZnO-NPs. Peng et al. (2011), for instance, demonstrated that spherical nanoparticles release higher amounts of Zn^{2+} compared to rod-shaped counterparts, likely due to their greater surface curvature and enhanced solubility. Comparable observations by Wang et al. (2009) further support the notion that nanoparticle shape regulates dissolution behavior and thus impacts antibacterial effectiveness. Surface modification is also a key factor governing the antimicrobial performance of ZnO nanoparticles (ZnO-NPs). As reported by Leung et al. (2012), both the generation of reactive oxygen species (ROS) and the release of Zn^{2+} ions are largely driven by surface-mediated processes. Consequently, the surface chemistry of ZnO-NPs plays a crucial role in dictating their interactions with bacterial cell membranes. Advanced characterization techniques—including scanning electron microscopy (SEM), X-ray diffraction (XRD), transmission electron microscopy (TEM), and electron spectroscopy imaging (ESI)—have been instrumental in unraveling these mechanisms. Notably, ESI analyses have demonstrated that ZnO nanorods possess a higher oxygen-to-zinc (O:Zn) ratio compared to other morphologies, which correlates with enhanced ROS production and greater oxidative stress in bacterial cells.

ZnO-NPs have also emerged as promising candidates for food safety applications. When integrated into food packaging materials, they function as effective antimicrobial agents by directly interacting with pathogens present on food surfaces, thereby minimizing contamination and prolonging shelf life. According to Sirelkhatim et al. (2015), ZnO-NPs can suppress a wide spectrum of foodborne pathogens through multiple antibacterial mechanisms. Additionally, recent studies suggest potential antiviral properties, with evidence indicating that Zn^{2+} ions may disrupt viral proteins involved in the pathogenesis of herpes simplex virus (HSV-1) (Dwivedi et al., 2014).

2. Material and Methods

Synthesis of Phyto-Extract Mediated Zinc Nanoparticles

For the present investigation, nanoparticles will be synthesized by treating solutions of silver and zinc salts with phytochemical extracts of the selected plant. The preparation will follow the methods described by Sharma et al. (2018), Stan et al. (2016), Venkatesan et al. (2017), and Happy et al. (2019), with appropriate modifications.

Silver nitrate and zinc sulphate solutions will serve as precursors and will be reacted with the phytochemical extract to obtain phytoextract-mediated ZnNPs. The nanoparticles will be formed through the reduction of the precursor solutions by the plant-derived phytochemicals. The progression of nanoparticle formation will be monitored by observing characteristic colour changes and sediment formation under suitable temperature and pH conditions.

Synthesis of Zinc Nanoparticles with Extract

The synthesis of zinc nanoparticles (ZnNPs) using the phytochemical extract of *Stachytarpheta indica* L. was performed following the procedure described by Ramesh et al. (2014), with necessary modifications tailored to the present study. For ZnNPs preparation, a predetermined volume of leaf extract was reacted with 0.1 M zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) under controlled laboratory conditions to facilitate efficient nanoparticle formation.

Initially, 45 mL of 0.1 M aqueous zinc acetate solution was prepared and examined for clarity. Subsequently, 5 mL of *S. indica* aqueous extract (derived from a 100 mg/mL stock solution) was added dropwise to the zinc acetate solution under continuous magnetic stirring at 40°C. The slow addition of the plant extract ensured uniform interaction between the phytochemicals and zinc ions, promoting both reduction and stabilization of the nanoparticles.

A visible color transition from transparent to light yellow and ultimately to milky white within one hour signified the formation of ZnNPs, as reported by Singh et al. (2018). Stirring was continued for 5–6 hours, after which the mixture was left undisturbed overnight to ensure complete reduction. The nanoparticles were then harvested by centrifugation at 5000 rpm for 10 minutes at room temperature. The resulting pellet was dried at 45°C for 24 hours, and the dried ZnNPs were subsequently re-dispersed in sterile distilled water to achieve the desired working concentration for further experimentation.

Aqueous Extraction of Crud Drug

Approximately 50 grams of finely powdered leaves of *Stachytarpheta indica* L. were first defatted using petroleum ether by soaking the powder overnight. After defatting, the leaf powder was spread on a newspaper and air-dried at room temperature for 3–4 hours to ensure complete evaporation of the petroleum ether.

Subsequently, 20 grams of the defatted, dried powder were subjected to Soxhlet extraction using 200 mL of distilled water as the solvent. The Soxhlet extraction was carried out at 70 °C for 24 hours, until complete exhaustion of the marc (Handa, 2008).

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

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

Under continuous stirring, the gradual change in solution color—from transparent to yellow and finally to reddish-brown within an hour—indicates the formation of silver nanoparticles from the plant extract (Valli et al., 2012). Stirring was continued for 5–6 hours, after which the mixture was left undisturbed overnight. The ZnNPs were then separated by centrifugation at 5000 rpm for 10 minutes at room temperature. The resulting pellets were dried at 45 °C for 24 hours, and the obtained powder was re-suspended in sterile distilled water to prepare the desired experimental concentrations.

Characterization Techniques

UV–Vis Spectrophotometry: The optical characteristics of the ZnNPs were evaluated using a UV–Vis spectrophotometer, recording absorbance within the 300–800 nm range.

Fourier-Transform Infrared (FTIR) Spectroscopy: FTIR analysis was performed to identify the functional groups involved in the reduction and stabilization of the nanoparticles.

Scanning Electron Microscopy (SEM): SEM imaging was utilized to examine the morphology, size distribution, and surface features of the synthesized ZnNPs.

Analysis of Synthesized Silver and Zinc Nanoparticles

The synthesized zinc nanoparticles (ZnNPs) were characterized using a range of analytical techniques to verify their formation, stability, and surface morphology. The nanoparticle suspensions were analyzed through UV–Visible (UV–Vis) spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM) to confirm successful synthesis and evaluate their physicochemical attributes.

A. UV–Vis Spectrophotometry

UV–Vis analysis served as an initial confirmation tool by recording the absorption spectra of the nanoparticles within the 200–800 nm wavelength range. The presence of characteristic Surface Plasmon Resonance (SPR) peaks indicated nanoparticle formation. Typically, silver nanoparticles show SPR peaks in the 400–450 nm region, while zinc nanoparticles exhibit peaks around 300–380 nm (Jain et al., 2020).

B. FTIR Analysis

FTIR spectroscopy was used to identify functional groups involved in the reduction and stabilization of the nanoparticles. Dried samples were scanned in the 4000–400 cm^{−1} range to detect biomolecules—such as phenolics, flavonoids, and proteins—that participate in nanoparticle synthesis and capping processes (Mittal et al., 2021).

C. SEM Imaging

SEM imaging was employed to study the size, shape, and surface morphology of the nanoparticles. A drop of the nanoparticle suspension was placed on carbon tape, air-dried, and examined under the microscope at varying magnifications. The resulting micrographs provided detailed information on nanoparticle distribution, structural integrity, and possible aggregation (Sharma et al., 2019).

3. Result

Analysis of Zinc Nanoparticles

A. UV-Vis Spectrophotometry

Similarly, the UV–Vis spectrophotometric analysis of zinc nanoparticles (ZnNPs) synthesized using the aqueous extract of *Stachytarpheta indica* showed a distinct absorption peak at 380 nm, confirming the successful formation of ZnNPs. The UV–Vis absorbance profiles of the plant extract and the corresponding ZnNPs are presented in Figure 1, recorded using a LabIndia 3000+ UV–Vis spectrophotometer. This absorption peak lies within the characteristic surface plasmon resonance (SPR) region for ZnNPs, typically ranging between 350–400 nm depending on nanoparticle size and synthesis parameters.

In contrast to the extract alone, which exhibited a broad and less defined absorbance pattern, the ZnNPs demonstrated a sharp and well-defined peak, indicating effective reduction and stabilization of zinc ions by the phytochemicals present in the plant extract. The gradual decrease in absorbance beyond 400 nm further supports the stability and nanoscale dimensions of the synthesized ZnNPs. Additionally, the low absorbance values observed at higher wavelengths (600–800 nm) suggest minimal nanoparticle aggregation and uniform dispersion, which are critical for their potential antimicrobial applications.

These observations align with earlier reports of plant-mediated ZnNP synthesis, where similar UV–Vis spectral characteristics were noted, thereby reaffirming the efficacy of *S. indica* extract in facilitating nanoparticle formation.

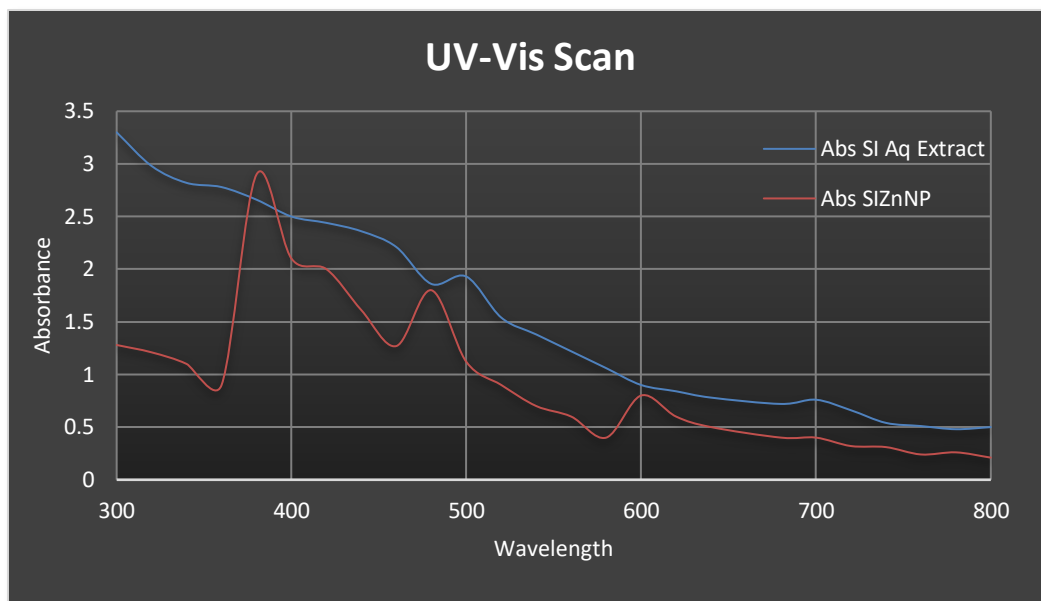


Figure 1: UV-Vis Absorbance Spectrum of *Stachytarpheta indica* Aqueous Extract and Zinc Nanoparticles generated on UV-Vis spectrophotometer LabIndia3000+.

The aqueous extract of *Stachytarpheta indica* showed a distinct absorption peak at 380 nm, confirming successful nanoparticle formation. This observation is consistent with earlier reports on plant-mediated ZnNP synthesis. For example, ZnNPs produced using *Papaver somniferum* extract exhibited a pronounced surface plasmon resonance (SPR) peak at 360 nm, validating nanoparticle formation (Muhammad et al., 2019).

Likewise, ZnNPs synthesized with *Deverra tortuosa* extract demonstrated an absorption edge characteristic of ZnNPs, aligning with the typical SPR range (Al-Sharqi et al., 2020). The consistency of these SPR peaks across various plant-based syntheses highlights the effectiveness of phytochemicals in reducing and stabilizing zinc ions into nanoparticles. Such uniformity further suggests that plant extracts can regulate nanoparticle size and dispersion, which is crucial for their potential applications in antimicrobial treatments.

B. FTIR Analysis

The Fourier-transform infrared (FTIR) spectrum of *Stachytarpheta indica* extract-mediated zinc nanoparticles (ZnNPs), presented in Figure 2 and obtained using the Alpha Bruker FTIR instrument, provides valuable insights into the biomolecules involved in the reduction, stabilization, and capping of the nanoparticles.

The broad absorption bands 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 derived from phenolic compounds and proteins, which act as stabilizing agents.

The peak at 3203.56 cm^{-1} is associated with C–H stretching, typically linked to aliphatic compounds.

Peaks observed at 2924.00 cm^{-1} and 2843.65 cm^{-1} represent C–H stretching vibrations of alkane groups, likely originating from plant-derived terpenoids or flavonoids.

The band at 2024.00 cm^{-1} may indicate C≡C stretching, suggesting the presence of alkyne groups.

A prominent peak at 1634.69 cm^{-1} corresponds to C=O stretching of amide or carboxyl groups, implying protein involvement in ZnNP formation.

The peak at 1400.32 cm^{-1} is attributed to C–H bending, confirming the presence of alkanes or aromatic compounds.

Absorption bands at 1029.86 cm^{-1} and 1023.09 cm^{-1} correspond to C–O stretching vibrations of polysaccharides, which contribute to the stabilization of the nanoparticles.

The peak at 871.82 cm^{-1} indicates out-of-plane bending of aromatic C–H bonds.

These functional groups collectively confirm the significant role of phytochemicals in the biosynthesis and stabilization of ZnNPs, consistent with earlier reports highlighting the involvement of flavonoids, proteins, and phenolic compounds in nanoparticle formation.

FTIR spectroscopy thus serves as a crucial tool for identifying the functional groups participating in ZnNP biosynthesis using *Stachytarpheta indica* extract. The broad band around 3446.79 cm^{-1} , representing O–H and N–H stretching, further aligns with findings by Suresh et al. (2015), who demonstrated the importance of phenolic groups in *Cassia fistula* extract during ZnNP synthesis.

The absorption peaks at 2924.00 cm^{-1} and 2843.65 cm^{-1} correspond to C–H stretching vibrations of alkane groups, likely originating from plant-based terpenoids or flavonoids. Such biomolecules have been reported to assist in nanoparticle stabilization, as demonstrated in *Aquilegia pubiflora* extract-mediated ZnNP synthesis where flavonoids acted as effective capping agents (Jan et al., 2020).

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A distinct peak at 1634.69 cm^{-1} is attributed to C=O stretching vibrations of amide or carboxyl groups, indicating possible protein interactions with ZnNPs. This is consistent with the observations of Stan et al. (2016), who reported protein-mediated stabilization of ZnNPs synthesized using *Allium sativum* extract. The band at 1400.32 cm^{-1} , associated with C–H bending vibrations, suggests the presence of alkanes or aromatic compounds. Furthermore, the peaks near 1029.86 cm^{-1} and 1023.09 cm^{-1} are linked to C–O stretching vibrations of polysaccharides, which may also contribute to nanoparticle stability. Similar findings have been reported in earlier studies where plant-derived polysaccharides played a key role in ZnNP stabilization (Sundrarajan et al., 2015).

Overall, the FTIR profile highlights the significant involvement of phytochemicals—such as phenolics, flavonoids, proteins, and polysaccharides—in the green synthesis and stabilization of ZnNPs using *Stachytarpheta indica* extract. These results align well with existing literature on plant-mediated nanoparticle synthesis, reinforcing the effectiveness of plant extracts in producing stable and functional ZnNPs.

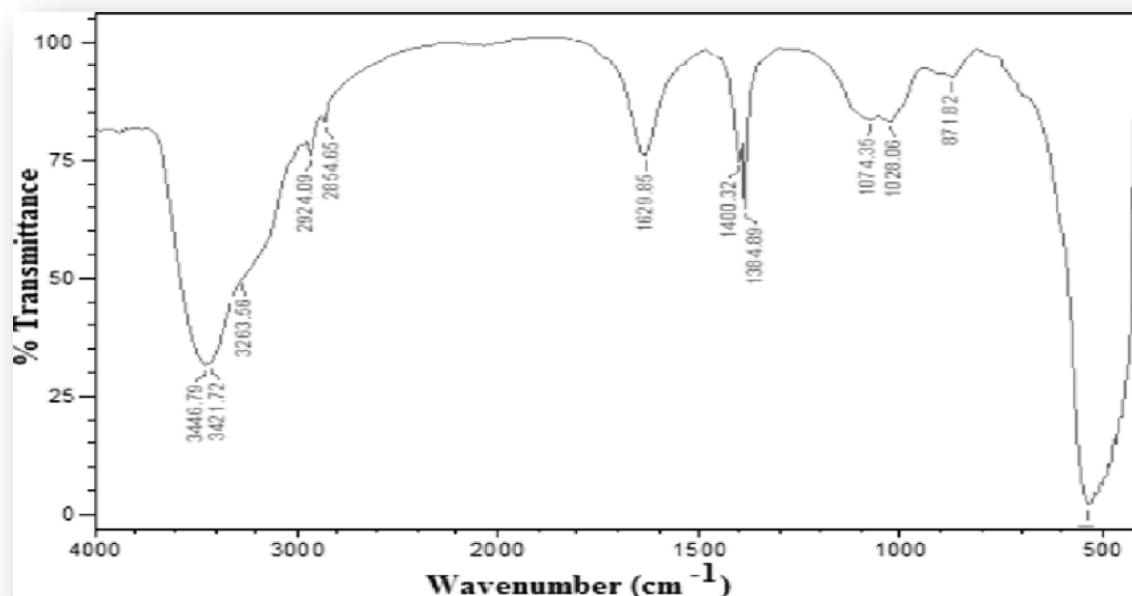


Figure 2: FTIR Spectrum of *Stachytarpheta indica* Extract-Mediated Zinc Nanoparticles Showing Functional Groups Involved in Synthesis and Stabilization.

C. SEM Imaging

The SEM micrograph of *Stachytarpheta indica* extract-mediated zinc nanoparticles (SIZnNPs), shown in Figure 3 and captured at 1400X magnification, provides detailed insights into the morphological features of the synthesized nanoparticles:

1. Particle Distribution and Morphology

The SEM image reveals that SIZnNPs exhibit an irregular and polydisperse morphology. The nanoparticles appear aggregated, displaying a mixture of sharp-edged and flake-like structures. This non-uniformity in shape likely reflects the influence of phytochemicals from *Stachytarpheta indica*, which act as reducing and stabilizing agents. Similar morphologies have been reported in other bio-mediated zinc nanoparticles, where organic compounds such as flavonoids, phenolics, and alkaloids govern particle formation and stabilization.

2. Size and Structural Features

The particle sizes range from $9.72\text{ }\mu\text{m}$ to $16.59\text{ }\mu\text{m}$, indicating micro-to-nanoscale dimensions. Such variation in size suggests multiple nucleation and growth phases during synthesis. The SEM also reveals a rough and porous surface topology, which implies a high surface area and potential for enhanced functional activity, particularly in antimicrobial and catalytic applications.

3. Surface Characteristics and Aggregation

The high-contrast regions observed in the SEM micrograph indicate zinc clusters, suggesting possible agglomeration. This clustering may result from electrostatic interactions between the nanoparticles and phytochemicals, a phenomenon commonly reported in green nanoparticle synthesis. Additionally, factors such as pH, temperature, and extract concentration likely influenced the morphology, consistent with findings in previous studies on biosynthesized ZnNPs.

In summary, the SEM analysis confirms the successful synthesis of zinc nanoparticles with irregular and polydisperse morphology, highlighting the significant role of *Stachytarpheta indica* phytochemicals in reduction, capping, and stabilization. The observed structural characteristics indicate effective bio-mediated synthesis, supporting potential applications in antimicrobial, catalytic, and environmental remediation fields.

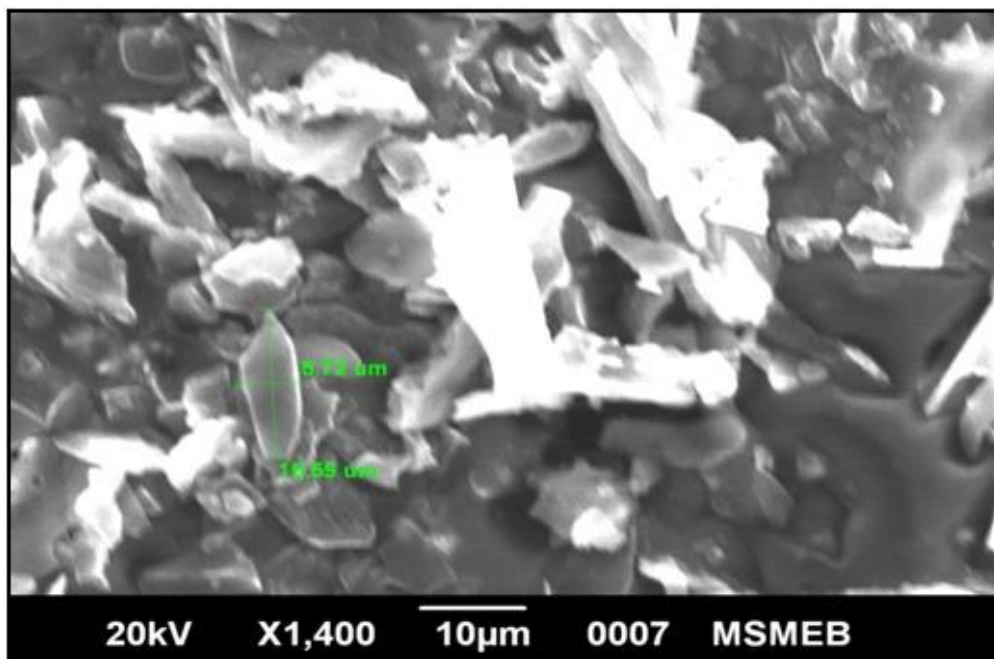


Figure 3: SEM micrograph of sample SZnNP a *Stachytarpheta indica* extract-mediated zinc nanoparticles captured at 1400X magnification.

The biosynthesis of zinc nanoparticles (ZnNPs) using *Stachytarpheta indica* extract, as revealed by SEM micrographs, corroborates previous studies highlighting the crucial role of plant-derived phytochemicals in nanoparticle formation. The observed irregular morphology and polydispersity are in agreement with earlier reports suggesting that biosynthesized ZnNPs adopt diverse shapes due to the influence of biomolecules, which function as both reducing and stabilizing agents (Alavi & Karimi, 2021). Capping agents such as flavonoids and alkaloids further stabilize these nanoparticles, limiting excessive aggregation (Rajiv et al., 2017).

Green-synthesized ZnNPs have been reported to possess potent antimicrobial and catalytic activities, rendering them highly applicable in biomedical and environmental contexts (Siddiqi et al., 2018). The structural variations seen in SEM images may result from synthesis parameters, including pH, extract concentration, and reaction temperature, as previously documented (Ovais et al., 2019). Additionally, biosynthesized ZnNPs often demonstrate superior biocompatibility compared to chemically synthesized nanoparticles (Sharma et al., 2020).

Collectively, these findings align with existing literature on bio-mediated ZnNP synthesis, underscoring the role of *S. indica* phytochemicals in nanoparticle stabilization. The observed morphological characteristics suggest promising applications in antimicrobial formulations, drug delivery systems, and wastewater treatment.

4. Discussion

The present study demonstrates the successful green synthesis of zinc nanoparticles (ZnNPs) using an aqueous leaf extract of *Stachytarpheta indica*. The UV–Visible spectroscopic analysis showed a distinct absorption peak at 380 nm, providing evidence for nanoparticle formation. This absorption falls within the characteristic optical region reported for plant-mediated ZnNPs and is comparable with previous observations in which ZnNPs synthesized using *Papaver somniferum* extract exhibited an absorption maximum around 360 nm (Muhammad et al., 2019). The observed spectral characteristics indicate that phytochemicals present in *S. indica* may facilitate the reduction and stabilization of zinc ions during nanoparticle synthesis.

FTIR analysis further supported the involvement of plant-derived biomolecules in nanoparticle formation. The presence of O–H, N–H, C=O, C–H and C–O functional groups suggests the participation of phenolics, proteins, flavonoids and polysaccharides in reduction, capping and stabilization processes. Similar involvement of phenolic compounds and other phytochemicals has been reported in plant-mediated ZnNP synthesis (Suresh et al., 2015; Jan et al., 2020). Protein-associated functional groups may also contribute to nanoparticle stabilization, as reported for ZnNPs synthesized using *Allium sativum* extract (Stan et al., 2016).

SEM analysis revealed irregular, polydisperse and aggregated particles with rough and porous surface features. Such morphological variation may arise from differences in nucleation and growth conditions and from interactions between nanoparticle surfaces and plant-derived capping molecules. Similar morphology-dependent variation has been reported for biosynthesized ZnNPs (Alavi & Karimi, 2021; Rajiv et al., 2017). Moreover, factors such as pH, temperature and extract concentration can substantially influence nanoparticle morphology and aggregation (Ovais et al., 2019).

Overall, the UV–Vis, FTIR and SEM findings collectively support the successful phytogenic synthesis of ZnNPs using *S. indica*. The involvement of naturally occurring phytochemicals makes this approach attractive as a relatively sustainable alternative to conventional nanoparticle synthesis and provides a basis for further investigation of their antimicrobial and environmental applications.

5. Conclusion

This study successfully demonstrates an eco-friendly approach for synthesizing zinc nanoparticles (ZnNPs) using aqueous extract of *Stachytarpheta indica* as both a bioreducing and stabilizing agent. UV–Vis spectrophotometric analysis confirmed the formation of nanoparticles, evidenced by a distinct absorption peak at 380 nm, characteristic of zinc's surface plasmon resonance. FTIR analysis identified functional groups—including hydroxyls, amines, carbonyls, and polysaccharides—highlighting the phytochemicals responsible for reduction and capping. SEM imaging revealed irregular, polydisperse nanoparticles with micro-to-nanoscale dimensions and rough surface morphology, indicating a high surface area and potential for enhanced biological activity. These results underscore the effectiveness of *S. indica* phytoconstituents in enabling a green, cost-effective, and sustainable method for ZnNP synthesis. The synthesized nanoparticles hold promise for applications in antimicrobial, pharmaceutical, and environmental domains. Future research should investigate their bioactivity and toxicity to confirm suitability for practical applications.

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