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Sustainable Synthesis of Zinc Oxide Nanoparticles Using *Tinospora cordifolia* Extract and Assessment of Antibacterial Activity

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Abstract

In recent years, zinc oxide (ZnO) nanoparticles have gained considerable attention due to their unique physical, chemical, and biological properties, with applications spanning medicine, electronics, and environmental remediation. However, conventional synthesis methods often involve toxic reagents, high energy input, and harsh conditions, raising sustainability and environmental concerns. To address these issues, this study explores a green synthesis approach for ZnO nanoparticles using *Tinospora cordifolia* extract, a medicinal plant known for its antimicrobial, antioxidant, and anti-inflammatory properties. In this eco-friendly method, *T. cordifolia* extract acts as both a reducing and stabilizing agent, enabling the formation of ZnO nanoparticles from zinc nitrate solution under mild, non-toxic conditions. Characterization of the nanoparticles was performed using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM). FTIR analysis indicated the presence of plant-derived functional groups involved in nanoparticle synthesis, while XRD confirmed the crystalline structure of the ZnO nanoparticles. SEM imaging revealed uniformly distributed particles with consistent morphology. The antimicrobial activity of the synthesized ZnO nanoparticles was assessed using the disc diffusion method against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The results showed significant inhibition zones, demonstrating the nanoparticles' potent antimicrobial properties. This green synthesis method offers a sustainable, low-cost alternative to conventional ZnO nanoparticle production.

Keywords: Green synthesis, *Tinospora cordifolia*, Zinc oxide nanoparticles, Antimicrobial activity, Biogenic synthesis

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Introduction

Nanotechnology is a fast-developing field of science that involves the manipulation and synthesis of materials at the nanoscale, often in the 1 to 100 nanometre range. Properties of materials at this scale have distinct physical, chemical, and biological properties that significantly differ from their larger-scale equivalent. These include increased surface area, increased reactivity, and specific optical, electrical, and magnetic behaviors (Selmani *et al*., 2022). Nanoparticles (NPs) are one of the most widely researched materials in nanotechnology because of their enormous applications in medicine, electronics, energy, and environmental science (Altammar, 2023). To this extent, metal oxide nanoparticles (MONPs) like zinc oxide (ZnO), titanium dioxide (TiO_2), and copper oxide (CuO) have attracted considerable interest because of their outstanding physicochemical properties. Particularly, ZnO nanoparticles have also proven to be a versatile nanomaterial with extensive applications ranging from photocatalysis to drug delivery, bioimaging, and antimicrobial activity (Aldeen *et al*., 2022). ZnO is one of the most versatile materials, having a large bandgap energy of 3.37 eV and outstanding photocatalytic properties. Such features render ZnO NPs useful in applications related to UV protection, biosensors, and medicine (Muthuvel *et al*., 2020). ZnO is effective as an antimicrobial, photodetector, and in energy conversion uses like dye-sensitized solar cells (Liu *et al*., 2021). It is particularly notable for its antibacterial action in that it can inhibit a broad range of microorganisms, like Gram-positive and Gram-negative bacteria, fungi, and viruses (Rajendran *et al*., 2021). But traditional synthesis routes for ZnO NPs—i.e., sol-gel, hydrothermal processes, and chemical vapor deposition—tend to involve toxic chemicals, a great deal of energy input, and high temperatures. Such methods are environmentally and health-wise detrimental (Wang *et al*., 2023). Due to this, demand for more environmentally friendly and sustainable synthesis routes has increased.

Green synthesis, or biosynthesis, is a viable alternative. This green method uses plant extracts, microbes, or biomolecules to lower metal salts to nanoparticles without the use of harmful reagents and cutting down on energy (Aggarwal & Alam, 2020). Out of these, plant-based synthesis is especially useful because it is simple, inexpensive, and scalable. It uses naturally occurring phytochemicals like polyphenols, alkaloids, flavonoids, and terpenoids as both reducing and stabilizing agents (Imade *et al*., 2022). Not only do these biomolecules aid in nanoparticle synthesis, but they also confer functional biological characteristics

like antimicrobial, antioxidant, and anticancer behaviour to the synthesized nanoparticles (Alharthi *et al*., 2020). Specifically, the green chemistry-compliant ZnO nanoparticle synthesis with the use of plant extracts has become popular with proven efficiency in various studies (MuthuKathija *et al*., 2023).

One such plant is *Tinospora cordifolia*, or *Guduchi,* a climbing shrub of the family Menispermaceae indigenous to the tropical areas of India and Southeast Asia. It is a foundation of Ayurvedic medicine, valued for its immunomodulatory, anti-inflammatory, antioxidant, and antimicrobial activities (Nath *et al*., 2023). Because of its rejuvenating characteristics, it is even called "Amrita" or "nectar of immortality" in classical Indian literature. *T. cordifolia* has a variety of bioactive compounds, including alkaloids, glycosides, flavonoids, and terpenoids responsible for giving it its medicinal potential (Kulkarni *et al*., 2023; Savva *et al*., 2023; Li *et al*., 2024). These compounds can also be used in the green synthesis of nanoparticles as both reducing and capping agents (Saini & Kumar, 2023). Various studies have established its use in synthesizing silver, gold, and zinc oxide nanoparticles with good stability and bioactivity (Nath *et al*., 2023). In addition, the innate antimicrobial capability of *T. cordifolia* enhances the biological activity of ZnO nanoparticles to make them more effective for biomedical applications (Libin *et al*., 2023).

The antimicrobial activity of ZnO nanoparticles largely rests on their capability to produce reactive oxygen species (ROS) like hydroxyl radicals and hydrogen peroxide on their surface. These ROS can lyse microbial cell membranes, damage intracellular proteins, and degrade DNA, ultimately causing microbial cell death (Abebe *et al*., 2020). Due to its small size and high surface area, ZnO NPs increase interactions with microbial cells and provide better antimicrobial activity (Mendes *et al*., 2022). ZnO NPs have been found effective against a wide variety of bacteria, fungi, and viruses, including multidrug-resistant bacteria—a significant problem in global health (Han *et al*., 2021). ZnO NPs offer potential in areas such as medical device coatings, wound dressings, food package coatings, and water treatment systems. Notably, the size, shape, surface charge, and concentration of ZnO nanoparticles have significant impacts on their antimicrobial activity. The synthesis process—such as the application of phytochemical-rich plant extracts such as *T. cordifolia* can be tailored to maximize these parameters for optimal biological activity and low toxicity (Libin *et al*., 2023). Plant-based routes also minimize the possibility of toxic by-products, ensuring biocompatibility and environmental safety (Tran *et al*., 2022).

Materials and Methods

Reagents and Chemicals

T. cordifolia leaf extract, Zinc Nitrate, Sodium Hydroxide, Distilled water, Petri plates, Muller Hinton medium, agar, sterile discs.

Preparation of Leaf Extract

For preparing the *T. cordifolia* leaf extract for green synthesis of ZnO nanoparticles, *T. cordifolia* leaves were freshly harvested, washed well with distilled water to eliminate any impurities or dust, and then air-dried under shade for a few days to retain their bioactive components. After being dried completely, the leaves were ground into fine powder using a sterile mortar and pestle. A calculated amount of powdered leaf sample (10 g) was then introduced into distilled water (100ml) in a conical flask using a plant-to-solvent ratio optimal for efficient extraction. The resulting mixture was heated at 70°C for 1 hour in a water bath with constant heating to aid in the leaching of bioactive phytochemicals. Upon heating, the extract was left at room temperature and then filtered with the help of Whatman No. 1 filter paper to get rid of solid residues. The clear filtrate thus obtained was gathered and kept at 4°C for later use in the synthesis of ZnO nanoparticles (Mustafa *et al*., 2023; Avramova & Vasileva, 2024).

Synthesis of Nanoparticles

The green synthesis of ZnO nanoparticles, 9 g of zinc nitrate($Zn(NO_3)_2$) and 7 g of sodium hydroxide (NaOH) pellets were employed as precursor materials. First, 7 g of NaOH was dissolved in a small amount of distilled water under constant stirring to achieve proper dissolution. Independently, 9 g of $Zn(NO_3)_2$ was dissolved in distilled water, and it was added slowly under constant stirring to the solution of NaOH. The addition caused the precipitation of a white precipitate, which was the initial formation of zinc hydroxide (García & Jaramillo, 2023).

Then, 30 mL of *T. cordifolia* leaf extract, which was pre-prepared, was added to the reaction mixture gradually under constant stirring. Distilled water was added to bring the volume up to 200mL. Vigorous stirring with a magnetic stirrer at room temperature was conducted for 1 hour in order to permit the plant extract to serve as a reducing and stabilizing agent in the process of synthesis.

The ZnO nanoparticles synthesized were harvested by centrifuging the reaction mixture at 10,000 rpm for 1 hour to ensure proper separation of the nanoparticles from the liquid phase. This was repeated 2–3 times, with the supernatant being removed and the pellet being kept every time. The pellet obtained was washed thoroughly with absolute ethanol several times to ensure the removal of any remaining impurities and unreacted plant extract. The cleaned nanoparticles were then dehydrated using a hot-air oven to remove all the moisture. After dehydration, the solid nanoparticles were subjected to subsequent analysis, using FTIR, SEM, XRD, and EDX.

Antibacterial Activity

In order to determine the antibacterial activities of ZnO nanoparticles, the medium was prepared by adding 2.1 g of Muller Hinton medium and 2 g of agar to 100 mL of distilled water. The medium was sterilized and set into sterile petri dishes, where it was allowed to solidify into a gel under aseptic conditions. After this, 1 gram of the ZnO powder was suspended in 1 mL of sterile distilled water, thus preparing a 1 g/mL suspension of ZnO nanoparticles. Impregnating sterile filter paper discs with this suspension, at approximately 10–20 µL per disc, gave rise to the impregnated discs, which were dried aseptically. Once the agar plates solidified, inoculated overnight cultures of Escherichia coli and Staphylococcus aureus were cross-streaked on separate plates to generate uniform lawns of bacterial growth. The impregnated

discs were subsequently placed on the inoculated plates. The plates were kept undisturbed at a temperature of 27°C to 34°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of clear zones (zones of inhibition) that resulted around each of the discs after the stated incubation period. These zones indicate the level of susceptibility of the bacteria to the ZnO nanoparticles.

Characterization Techniques

Fourier Transform Infrared Spectroscopy (FTIR)

ZnO nanoparticles are characterized by identifying functional groups on their surface using Fourier transform infrared spectroscopy (FTIR). This technique is especially useful to detect surface capping materials, since nanoparticles have a large surface area to volume ratio and surface modifications are important for their stability and functionality. FTIR analysis was used to determine the bioactive molecules that act as stabilizers or capping agents in the synthesis of ZnO nanoparticles. The method is based on the fact that biological molecules absorb infrared light at specific wavelengths due to molecular bond vibrations, and thus gives information about the surface chemistry of ZnO nanoparticles.

Scanning Electron Microscopy (SEM)

High-resolution images of ZnO nanoparticles are scanned using SEM, which gives the morphological, size, and surface structure of these nanoparticles. The atoms in the sample interact with electrons, and the resulting images are formed. The major bulk sample and topographical, compositional, and shape analysis tool is the SEM. SEM is used to characterize ZnO nanoparticles and determine their uniformity and structural properties in a magnification range of 20X to roughly 30,000X, making it a good tool.

X-Ray Diffraction (XRD)

XRD is an essential technique to determine the crystalline structure and the phase-pure ZnO nanoparticles. The hexagonal wurtzite structure of ZnO is confirmed by the diffraction pattern. The peak broadening effects can be used to estimate the crystallite size using the Scherrer equation. XRD analysis is carried out to verify that the synthesized ZnO nanoparticles exhibit the corresponding crystalline phase and structural integrity.

Energy Dispersive X-ray (EDX) of ZnO Nanoparticles

Spectroscopy is used to confirm the elemental composition and purity of ZnO nanoparticles. Zinc (Zn) and oxygen (O) are present and at the correct stoichiometry as confirmed by spectra produced by the EDX detector with peaks corresponding to ZnO. This technique allows for quantitative elemental ratios that can be used to synthesize the correct ZnO nanoparticles as well as detect any impurities.

Results and Discussion

Edx Spectrum of Zno Nanoparticles

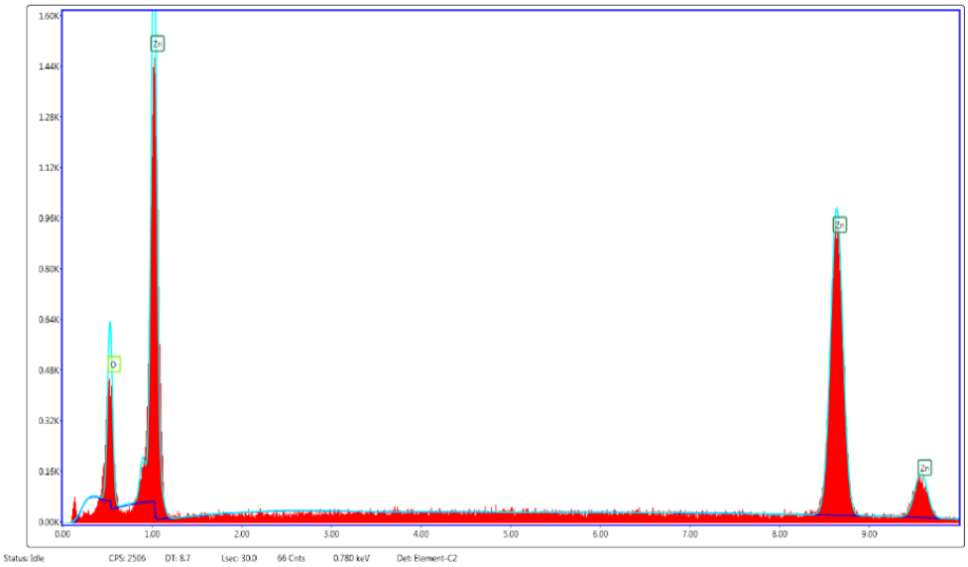
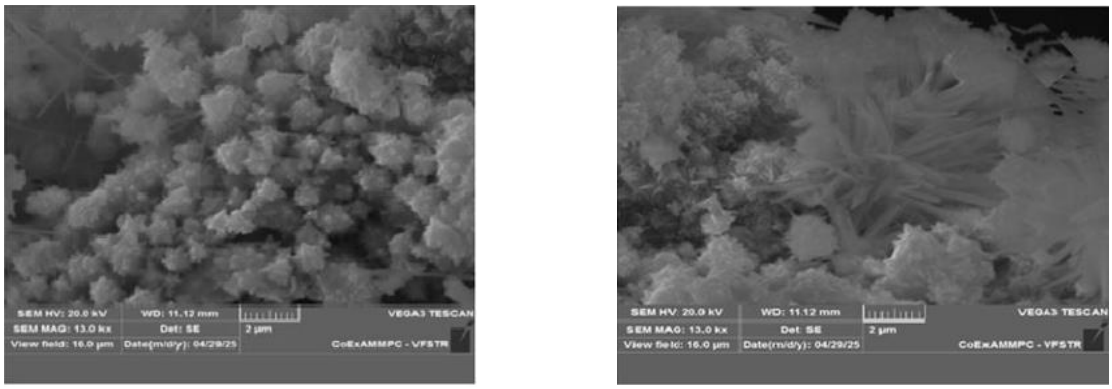


Figure 1. Energy Dispersive X-ray (EDX) spectrum of synthesized ZnO nanoparticles. The spectrum confirms the elemental composition with prominent peaks corresponding to zinc (Zn) and oxygen (O), validating the formation of ZnO nanoparticles.

The EDX spectrum shown in **Figure 1** applies to ZnO nanoparticles and shows the sample's elemental composition as peaks along the energy axis. The largest peaks are observed at approximately 1 keV, 8.6 keV, and 9.6 keV, which correspond to the Zn Lα, Kα, and Kβ emission lines. These strong and sharp peaks suggest that zinc is a major constituent in the sample. There is also a small peak, noticeable at roughly 0.5 keV, which represents the oxygen Kα line, signalling the presence of oxygen. The zinc and oxygen together confirm the synthesis of ZnO nanoparticles instead of metallic zinc or some other zinc compound. Other elements not showing significant peaks suggest the sample is clean and free from contaminants. In addition, the

sharpness and clarity of the peaks suggest the nanoparticles are crystalline, as amorphous materials show broader peaks. The spectrum supports the metallic composition of the sample as consistent with ZnO nanoparticles and confirms high purity of the sample, which can then be used in antimicrobial coatings, photo catalysts, and biomedical devices.

Scanning Electron Microscopy (SEM) of ZnO Nanoparticles



a) b)

Figure 2. SEM micrographs of green synthesized zinc oxide particles using *T. cordifolia* extract. a) Densely aggregated, quasi-spherical particles with rough surface morphology, b) Mixture of spherical aggregates and needle-like or rod-shaped nanostructures

The sequence of Scanning Electron Microscopy (SEM) images points irreversibly toward the involvement and essential role of nanoparticles in the generation of the microstructures documented. Taken at high magnifications from 13,000x to 65,000x, the images indicate nanometer and micrometer-scale features, which perforce imply participation of building blocks at the nanoscale. Image of **Figure 2a** displays closely aggregated, plate-like particles whose fine texture and size in the range of 500 nm suggest they are either nanoparticle aggregates or extremely thin nanoplates. Image of **Figure 2b** displays a more complex, hierarchical morphology, with larger, less ordered plate-like particles accompanied by radiating needle- or rod-like features. These latter structures are especially characteristic of nanoparticle assembly, with nanometer-sized diameters and oriented growth indicating self-assembly through nanoparticle interactions. The roughness of the surface texture uniformly seen for both the plate-like and needle-like/rod-like components further indicates their assembly from smaller units of nanoparticles. Consistent with these observations, the probable shapes of the constituent nanoparticles are both plate-like and rod-like or needle-like morphologies. The SEM images taken together indicate a material with a complex microstructure composed of nanoscale building blocks of different shapes. Although these images provide strong morphological evidence, conclusive proof, and determination of individual nanoparticle size, shape, and distribution.

FTIR Analysis of ZnO Nanoparticles

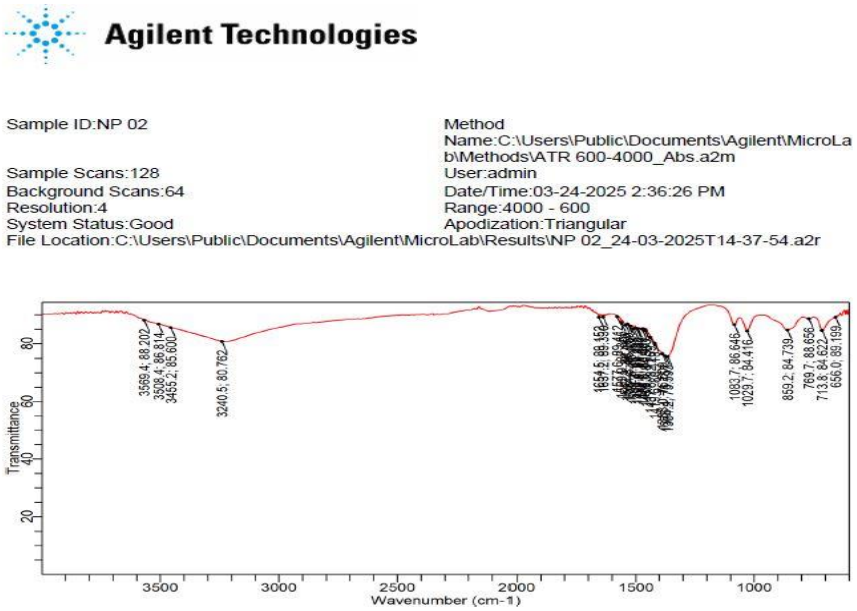


Figure 3. FTIR spectrum of ZnO nanoparticles synthesized using *T. cordifolia* extract, showing characteristic Zn–O stretching vibrations and functional groups from plant phytochemicals.

Table 1. FTIR peak analysis of ZnO nanoparticles synthesized using *T. cordifolia* extract

Peak Number	Wavenumber (cm^{-1})	Intensity
1	656.01146	89.19905
2	713.78520	84.62243
3	769.69526	88.65599
4	859.15137	84.73884
5	1029.67708	84.41569
6	1083.72348	86.64612
7	1364.20565	75.59285
8	1375.38766	75.75651
9	1387.96743	76.50892
10	1419.64980	80.11758
11	1437.82057	82.26929
12	1458.32093	84.56322
13	1465.77560	85.16108
14	1474.62803	85.09563
15	1490.93513	85.19981
16	1498.38981	85.38088
17	1508.17407	85.21707
18	1522.61751	86.13075
19	1534.26544	86.75883
20	1542.18603	86.48930
21	1559.89088	87.35480
22	1577.59574	89.41169
23	1637.23314	89.32954
24	1654.47208	89.15218
25	3240.45433	80.76211
26	3455.24218	85.59998
27	3508.35674	86.81430
28	3569.39190	88.20236

The FTIR spectrum of the ZnO nanoparticles synthesized showed in **Figure 3** and peak analysis showed in **Table 1** proved the successful formation of Zn–O bonds, as suggested by characteristic peaks at 656, 713, and 770 cm^{-1}, which are attributed to Zn–O stretching vibrations commonly seen in metal oxide nanostructures (Perumal *et al*., 2024). Other peaks between 859 and 1083 cm^{-1} can be attributed to C–O or C–O–C stretching, indicating the presence of organic residues or capping agents. Bands in the range of 1364–1577 cm^{-1} suggest C–H bending and potential aromatic C=C stretching, whereas bands at 1637 and 1654 cm^{-1} are generally assigned to adsorbed water and carbonyl groups (Ashour & Abd-Elhalim, 2024). Broad absorption bands between 3240–3569 cm^{1} establish the existence of hydroxyl (–OH) groups, which could be due to surface hydroxylation or atmospheric humidity. These surface functional groups have been reported to increase the antibacterial efficacy of ZnO nanoparticles by mediating the formation of reactive oxygen species (ROS), enhancing membrane disruption, and facilitating zinc ion release. The peak FTIR observed confirms the structural stability and surface reactivity of the nanoparticles, which are likely to account for their efficiency against Escherichia coli and Staphylococcus aureus.

XRD Analysis of ZnO Nanoparticles

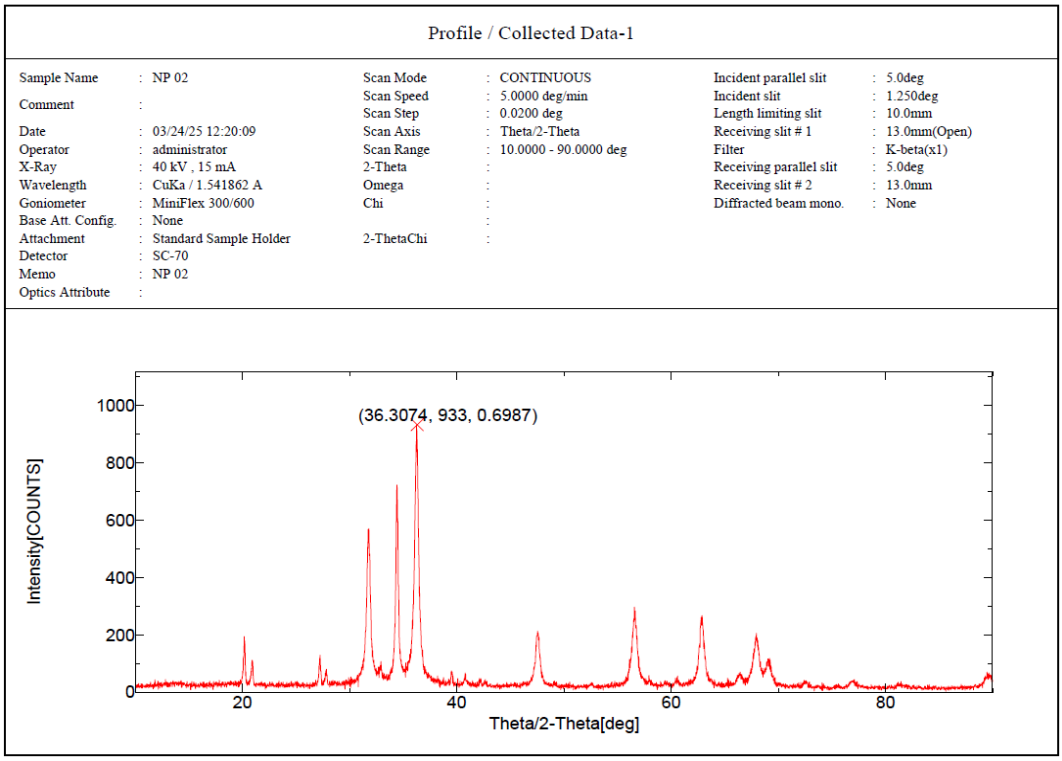


Figure 4. XRD pattern of ZnO nanoparticles synthesized using *T. cordifolia* extract, showing distinct peaks corresponding to the hexagonal wurtzite structure.

The X-ray diffraction (XRD) pattern of the synthesized ZnO nanoparticles presented in **Figure 4** (Sample ID: NP 02) showed sharp and well-defined peaks, which is a measure of high crystallinity. The highest intensity peak at $2\theta \approx 36.30°$ is the (101) crystal plane of ZnO. Other peaks of interest that appear at 2θ positions around 31.7° (100), 34.4° (002), 47.5° (102), 56.6° (110), 62.8° (103), and 67.9° (112) correspond to the characteristic diffraction pattern of the hexagonal wurtzite form of ZnO, as cited by the Joint Committee on Powder Diffraction Standards (JCPDS card no. 36-1451) (Al Hassan *et al*., 2021). The lack of any impurity peaks guarantees the phase purity of the sample. High crystallinity and phase purity are crucial in guaranteeing the functional performance of ZnO nanoparticles, particularly in bio applications. ZnO nanoparticles that are crystalline have been shown to improve antibacterial activity based on their high surface energy, efficient charge separation, and the capacity to form reactive oxygen species (ROS) that destroy bacterial membranes and intracellular structures (Shawki *et al*., 2022; El-Khawaga *et al*., 2025). These XRD data support the FTIR information and inhibitory antibacterial zones of inhibition, proving successful synthesis of pure and bioactive ZnO nanoparticles with potent efficacy against Escherichia coli and Staphylococcus aureus.

Antibacterial activity of ZnO Nanoparticles

a) b)

Figure 5. Zone of inhibition of *T. cordifolia* -mediated zinc oxide nanoparticles against a) *Staphylococcus aureus,* b) *Escherichia coli*

From the **Figures 5a and 5b**, it can be seen that the tested material, presumably Zinc Oxide nanoparticles, demonstrates antimicrobial effects against E. coli and Staphylococcus aureus. In each of the Petri dishes, there is a distinct circular zone of inhibition around

the middle disc, showing a region where the growth of bacteria has been inhibited. A comparative visual inspection appears to indicate that the E. coli demonstrates a reportedly greater zone of inhibition than that of the Staphylococcus aureus, suggesting a possibly greater susceptibility of E. coli to the agent in question under these particular experimental conditions.

Calculation of the Zone of Inhibition

Zone of inhibition=diameter of the zone-diameter of the disc

The diameter of the disc that I have placed is about 6mm.

The zone of inhibition obtained using zinc oxide nanoparticles against *E. coli* is 40mm (diameter of the zone) - 6mm (diameter of disc) =34mm.

The zone of inhibition obtained using zinc oxide nanoparticles against Staphylococcus aureus is 32mm (diameter of the zone)-6mm (diameter of disc) =26mm.

The current research is able to effectively show the green synthesis of zinc oxide (ZnO) nanoparticles with *T. cordifolia* extract, which was confirmed using extensive characterization and antibacterial tests. The synthesis method is in consonance with sustainable nanotechnology strategies that focus on environmental friendliness, affordability, and biological compatibility (Faisal *et al*., 2021; Hussain *et al*., 2022).

The biosynthetic pathway used capitalizes on the phytochemical diversity of *T. cordifolia,* consisting of alkaloids, flavonoids, terpenoids, and glycosides. These biomolecules would serve as reducing and capping agents that enable nanoparticle formation and provide stability and surface functionality (Ghazal *et al*., 2021). The bimodal functionality of phytochemicals not only allows for a green, non-toxic synthetic route but also increases the biological activity of the final nanomaterials (Saravanan *et al*., 2022).

EDX analysis verified the elemental nature of the nanoparticles, with intense peaks for zinc and oxygen and no presence of any foreign elements. The sharp Zn Lα, Kα, and Kβ peaks at 1.0, 8.6, and 9.6 keV, respectively, verified high purity and the lack of metallic or impure zinc species. The high level of crystallinity deduced from peak sharpness is of particular importance because crystalline substances tend to have better physicochemical and biological performance than amorphous ones (Al Hassan *et al*., 2021).

SEM imaging showed a diverse morphological topography of clustered plate- and needle-shaped nanostructures. These characteristics are typical of nanoparticle self-assembly, presumably guided by plant-based capping agents (Abel *et al*., 2021). The coarse nanoparticle surfaces also indicate the presence of organic residues, which could facilitate interaction with bacterial membranes. Yet, although SEM was useful in revealing morphological properties, other methods like TEM or DLS are advisable to ensure precise particle size quantitation.

The FTIR spectrum also supported nanoparticle formation. Unique Zn–O stretching vibrations at 656, 713, and 770 cm^{-1} attested to the synthesis of ZnO. Additional bands within 859–1083 cm^{-1} (C–O or C–O–C stretching), 1364–1577 cm^{-1} (bending of C–H, aromatic C=C), and 1637–1654 cm^{-1} (bending of carbonyl and water) indicate the occurrence of organic functional groups from *T. cordifolia* (Royani *et al*., 2023). The wide band at 3240–3569 cm^{-1} is assigned to hydroxyl groups, which can be derived from both plant metabolites and ambient moisture. These surface groups are of significant importance for biological interactions, as they can enhance the adhesion of nanoparticles and aid in reactive oxygen species (ROS) formation (Chan *et al*., 2021).

XRD analysis established the hexagonal wurtzite crystalline nature of the obtained ZnO nanoparticles, whose peak positions are consistent with the standard JCPDS card no. 36-1451. The lack of extraneous peaks confirmed the phase purity, whereas the sharp diffraction patterns confirmed a high crystallinity level. These structural features are vital for applications involving reproducible particle behavior, including antimicrobial coatings and biomedical devices. Crystallinity increases ROS production, which is one of the primary mechanisms of antibacterial action of ZnO nanoparticles (Shawki *et al*., 2022).

Antibacterial assays with the agar well diffusion technique showed significant inhibitory action on both Staphylococcus aureus and Escherichia coli, but greater inhibition zones were seen for *E. coli*. This variation in sensitivity could be due to differences in bacterial cell wall composition. The reduced peptidoglycan layer and outer membrane structure of Gram-negative *E. coli* might facilitate nanoparticle entry and oxidative harm more easily, while the greater peptidoglycan thickness in Gram-positive *S. aureus* provides partial protection (Padmavathy & Vijayaraghavan, 2008). The antibacterial action of ZnO nanoparticles is multi-faceted and involves ROS-mediated membrane disruption, Zn^{2+} ion release, interaction with proteins and DNA, and direct membrane destabilization (Aliyu *et al*., 2024). Surface hydroxyl groups, as established through FTIR, may also further increase surface reactivity and allow for these interactions.

This work agrees with earlier accounts that green-synthesized ZnO nanoparticles display strong antimicrobial activities, high crystallinity, and active surface groups to increase biological activity (Chan *et al*., 2021; Shawki *et al*., 2022). The introduction of *T. cordifolia* adds another aspect of biocompatibility because it has been used for a long time in traditional medicine and is known to have therapeutic effects (Royani *et al*., 2023). Overall, the green synthesis of ZnO nanoparticles from mutants of *T. cordifolia* is an effective, eco-friendly, and biologically significant method. The obtained nanoparticles have superior structure and antibacterial activity, and thus, they can be ideal candidates for biomedical, environmental, and pharmaceutical applications.

Conclusion

Green nanotechnology is an eco-friendly and sustainable approach to conventional physical and chemical processes, which are usually toxic and energy-intensive. Here, *T. cordifolia* leaf extract was successfully employed as a reducing agent and stabilizing agent for green synthesis of zinc oxide (ZnO) nanoparticles. Precipitation of white precipitate after adding plant extract to zinc nitrate-sodium hydroxide solution under controlled stirring proved that nanoparticle formation had been achieved. The process of

synthesis using centrifugation, washing with ethanol, and drying was found to be easy, reproducible, and economical, thus being feasible for bulk production.

Characterization methods like FTIR, SEM, XRD, and EDX proved the successful synthesis of ZnO nanoparticles. FTIR analysis confirmed the participation of plant phytochemicals in particle formation, while SEM established surface topography and size distribution. XRD validated the crystallinity of the particles, and EDX confirmed elemental composition and purity. Antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, employing the disc diffusion method, exhibited clear zones of inhibition, demonstrating strong antimicrobial potential. The resulting bioactivity most probably arises from the generation of reactive oxygen species (ROS), membrane disruption, and interaction with microbial proteins and DNA, additionally facilitated by remaining phytochemicals from *T. cordifolia* as synergistic contributors to antibacterial activity.

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Ethics statement: None

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