Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu
RESEARCH ARTICLE

Exploring Terminalia chebula Derivatives for the Synthesis of Zinc Oxide Nano Drugs and Their Antibacterial Assay

Sreecharan M[1], Nivaedhitha Mohan Kumar[2] and Selvankumar T[3*]

[1]Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai – 602105

[2]Department of Microbiology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai – 602105

[3*]Department of General Medicine, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai – 602105

*Corresponding Author
Selvankumar T

Article History
Received: 21.09.2025
Revised: 30.09.2025
Accepted: 17.10.2025
Published: 06.11.2025

Abstract: Terminalia chebula, a prominent medicinal plant rich in bioactive compounds, presents significant potential for green synthesis of zinc oxide nanoparticles (ZnO NPs) with enhanced therapeutic properties. This study investigated the biosynthesis of ZnO NPs using Terminalia chebula fruit extract and evaluated their antibacterial efficacy. The plant extract was prepared through Soxhlet extraction using methanol, followed by synthesis of ZnO NPs through a controlled precipitation method at 70°C and pH 9. Comprehensive characterization was performed using FT-IR spectroscopy, X-ray diffraction (XRD), and gas chromatography-mass spectrometry (GC-MS). FT-IR analysis confirmed the presence of various functional groups including O–H stretch, C–H stretch, C≡C or C≡N, and C=O stretch, indicating successful incorporation of phytochemicals. GC-MS analysis revealed major bioactive compounds including 2-Cyclopenten-1-one, 4-Dihydroxy-2, Phenol, and 2-Furancarboxaldehyde, 5-methyl, which contributed to nanoparticle stabilization and enhanced antibacterial properties. XRD analysis confirmed the crystalline structure of synthesized ZnO NPs. The antibacterial assay demonstrated potent activity against Escherichia coli, suggesting the potential of these biogenic ZnO NPs as effective antimicrobial agents. The combination of traditional medicinal plant chemistry with nanotechnology offers a sustainable and environmentally friendly approach for developing novel antimicrobial therapeutics with enhanced biocompatibility and reduced cytotoxicity compared to conventional synthetic methods.

Keywords: Terminalia chebula, zinc oxide nanoparticles, green synthesis, antibacterial activity, phytochemicals

INTRODUCTION

The emergence of antibiotic-resistant pathogens has created an urgent need for developing novel antimicrobial agents with enhanced efficacy and reduced side effects (Hassan et al., 2018). Nanotechnology has emerged as a promising solution, offering unique opportunities to overcome the limitations of conventional antibiotics through the development of nanoparticle-based therapeutic systems. Among various metallic nanoparticles, zinc oxide nanoparticles (ZnO NPs) have garnered significant attention due to their exceptional antimicrobial properties, biocompatibility, and photocatalytic activities (Padmavathy & Vijayaraghavan, 2008). The antimicrobial mechanism of ZnO NPs involves multiple pathways including reactive oxygen species (ROS) generation, zinc ion (Zn^{2+}) release, and direct interaction with bacterial cell walls, leading to membrane disruption and ultimately bacterial death (Raghupathi et al., 2011).

Traditional chemical synthesis methods for nanoparticle production often involve toxic chemicals, high energy consumption, and generate harmful by products that pose environmental health risks (Ahmad et al., 2019). Green synthesis approaches using plant extracts have emerged as sustainable alternatives, offering numerous advantages including cost-effectiveness, environmental friendliness, and the ability to produce nanoparticles with enhanced biological activities (Iravani, 2011). Plant-mediated synthesis utilizes the natural reducing and stabilizing agents present in plant extracts, eliminating the need for external chemical reducing agents and synthetic stabilizers (Narayanan & Sakthivel, 2010).

Terminalia chebula, commonly known as black myrobalan or *haritaki*, is a prominent medicinal plant belonging to the Combretaceae family. This plant has been extensively used in traditional Ayurvedic medicine for over 3000 years and is considered one of the most important therapeutic plants in the traditional medicine systems of India, Tibet, and other Asian countries. The fruit of *Terminalia chebula* is particularly valued for its diverse pharmacological properties and is often referred to as the "king of medicines" in Ayurveda (Al-Dhabi et al., 2018). The plant is native to Southeast Asia and is widely distributed across India, Nepal, Sri Lanka, Myanmar, and Thailand.

The phytochemical profile of *Terminalia chebula* is remarkably diverse and includes a wide array of bioactive compounds that contribute to its therapeutic efficacy. The primary bioactive constituents include hydrolyzable tannins such as chebulic acid, gallic acid, ellagic acid, and corilagin, which constitute approximately 32-45% of the fruit content (Bagri et al., 2016). Additionally, the plant contains various flavonoids including quercetin, rutin, and kaempferol, which exhibit potent antioxidant and anti-inflammatory activities. Other significant phytochemicals include triterpenoids, phenolic acids, glycosides, and anthraquinones, each contributing to the plant's comprehensive therapeutic profile.

The remarkable medicinal properties of *Terminalia chebula* have been extensively documented in scientific literature, with studies demonstrating its antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, cardioprotective, neuroprotective, and anticancer activities (Bagri et al., 2016). The plant's antimicrobial properties are particularly noteworthy, with research indicating effectiveness against various pathogenic bacteria, fungi, and viruses. These antimicrobial effects are primarily attributed to the presence of tannins and phenolic compounds that can disrupt microbial cell walls and interfere with essential cellular processes. The antioxidant activity of *Terminalia chebula* is among the highest reported for natural products, with studies showing superior radical scavenging activity compared to synthetic antioxidants like ascorbic acid and butylated hydroxytoluene (Al-Dhabi et al., 2018).

The integration of *Terminalia chebula* extracts in nanoparticle synthesis represents a novel approach that combines the inherent therapeutic properties of the plant with the enhanced delivery capabilities and antimicrobial efficacy of nanoparticles (Agarwal et al., 2017). The bioactive compounds present in the plant extract can serve dual roles as reducing agents for metal ion reduction and stabilizing agents for nanoparticle formation. This dual functionality eliminates the need for additional chemical agents while potentially enhancing the biological activity of the resulting nanoparticles through synergistic effects.

Recent advances in green nanotechnology have highlighted the potential of plant-mediated synthesis for producing nanoparticles with superior properties compared to those synthesized through conventional methods (Rajeshkumar & Bharath, 2017). The bioactive compounds from plant extracts can be incorporated into or adsorbed onto nanoparticle surfaces, creating hybrid systems with enhanced therapeutic potential. These bio-functionalized nanoparticles often exhibit improved biocompatibility, reduced cytotoxicity, and enhanced cellular uptake compared to their chemically synthesized counterparts (Salem & Fouda, 2021).

The antibacterial mechanism of plant-mediated ZnO nanoparticles involves multiple synergistic pathways (Gunalan et al., 2012). The primary mechanism involves the generation of reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide, and hydroxyl radicals upon contact with bacterial cells. These ROS cause oxidative stress, leading to lipid peroxidation, protein denaturation, and DNA damage. Additionally, the release of zinc ions from the nanoparticles contributes to antimicrobial activity through disruption of bacterial metabolic processes and interference with enzyme function. The plant-derived bioactive compounds can enhance these mechanisms through their own antimicrobial activities and by facilitating nanoparticle interaction with bacterial cells (Elumalai et al., 2015).

The current study aims to explore the potential of *Terminalia chebula* derivatives for the green synthesis of zinc oxide nanoparticles and evaluate their antibacterial efficacy against pathogenic bacteria. This research addresses the growing need for sustainable and effective antimicrobial agents while contributing to the understanding of plant-mediated nanoparticle synthesis mechanisms (Bhuyan et al., 2015).

MATERIALS AND METHODS

Plant Material Collection and Preparation

Fresh fruits of *Terminalia chebula* were collected from authenticated sources and identified by qualified botanists. The fruits were thoroughly washed with distilled water to remove dust and impurities, then dried under shade at room temperature for 7-10 days (Matinise et al., 2017). The dried fruits were ground into fine powder using a mechanical grinder and stored in airtight containers until further use. The powder was subjected to preliminary phytochemical screening to confirm the presence of major bioactive compounds.

Extract Preparation

Soxhlet extraction was employed to prepare the plant extract using methanol as the solvent (Jamdagni et al., 2018). Approximately 50 grams of dried *Terminalia chebula* powder was loaded into the Soxhlet apparatus, and extraction was carried out using 500 mL of methanol for 8 hours at 65°C. The resulting extract was concentrated using a rotary evaporator at reduced pressure and temperature to obtain a semi-solid residue. The concentrated extract was then dissolved in distilled water to prepare stock solutions of varying concentrations (1%, 2%, 5%, and 10% w/v) for nanoparticle synthesis (Kumar et al., 2017).

Synthesis of Zinc Oxide Nanoparticles

ZnO nanoparticles were synthesized using a controlled precipitation method (Ogunyemi et al., 2019). Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) was used as the zinc precursor, and sodium hydroxide (NaOH) was employed as the precipitating agent. The synthesis was carried out by mixing 0.1 M zinc sulfate solution with varying concentrations of *Terminalia chebula* extract (1%, 2%, 5%, and 10% w/v) in a 1:1 ratio. The pH of the mixture was adjusted to 9 using 0.1 M NaOH solution while maintaining constant stirring. The reaction mixture was heated to 70°C and maintained at this temperature for 2 hours with continuous stirring at 300 rpm (Nagajyothi et al., 2015). The formation of nanoparticles was monitored by observing the color change from clear to milky white, indicating the precipitation of ZnO nanoparticles. The reaction mixture was then cooled to room temperature and aged for 24 hours to ensure complete precipitation.

Purification and Collection of Nanoparticles

The synthesized nanoparticles were separated from the reaction mixture through centrifugation at 8000 rpm for 15 minutes (Elumalai et al., 2015). The precipitate was washed multiple times with distilled water and ethanol to remove unreacted precursors and organic impurities. The washing process was repeated until the supernatant became clear. The purified nanoparticles were dried in an oven at 80°C for 12 hours and then calcined at 400°C for 2 hours to improve crystallinity and remove any remaining organic matter (Gunalan et al., 2012).

Characterization Techniques

Comprehensive characterization of the synthesized ZnO nanoparticles was performed using multiple analytical techniques. Fourier Transform Infrared (FT-IR) spectroscopy was conducted using a Shimadzu FT-IR spectrometer in the range of 4000-400 cm^{-1} to identify functional groups and confirm the presence of plant-derived compounds (Kalpana et al., 2019). X-ray Diffraction (XRD) analysis was performed using a Rigaku MiniFlex diffractometer with Cu-Kα radiation ($\lambda = 1.5406$ Å) in the 2θ range of 10-80° to determine the crystalline structure and phase purity of the nanoparticles (Bhuyan et al., 2015). Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted using an Agilent 7890A GC system coupled with a 5975C MSD to identify the bioactive compounds in the plant extract responsible for nanoparticle synthesis and stabilization (Ghaffari et al., 2019).

Antibacterial Assay

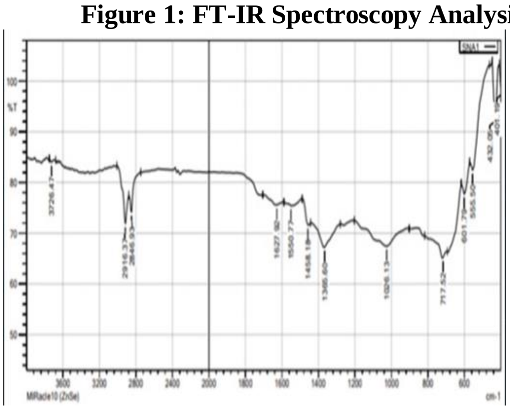
The antibacterial activity of the synthesized ZnO nanoparticles was evaluated against *Escherichia coli* (ATCC 25922) using the disk diffusion method (Cho et al., 2005). Bacterial cultures were prepared by inoculating fresh colonies in nutrient broth and incubating at 37°C for 18-24 hours. The bacterial suspension was adjusted to match 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Mueller-Hinton agar plates were prepared and inoculated with the bacterial suspension using sterile swabs. Paper disks (6 mm diameter) were impregnated with different concentrations of ZnO nanoparticles (25, 50, 100, and 200 μg/mL) and placed on the inoculated agar plates (Lai et al.,2014). Standard antibiotic disks (streptomycin, 10 μg) were used as positive controls, while disks treated with sterile distilled water served as negative controls. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters.

RESULTS AND DISCUSSION

Characterization of Synthesized ZnO Nanoparticles

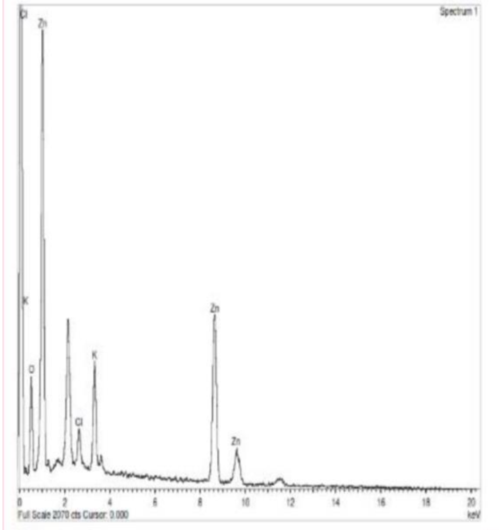
The successful synthesis of ZnO nanoparticles using *Terminalia chebula* extract was confirmed through comprehensive characterization studies. The visual observation of color change from clear to milky white during the synthesis process provided the first indication of nanoparticle formation (Matinise et al., 2017). This color change is attributed to the precipitation of ZnO nanoparticles and their interaction with the plant-derived bioactive compounds.

Figure 1: FT-IR Spectroscopy Analysis



FT-IR spectroscopy analysis revealed several characteristic peaks that confirm the successful incorporation of plant-derived compounds and the formation of ZnO nanoparticles (Agarwal et al., 2017). The spectrum showed a broad peak at 3200-3600 cm^{-1} corresponding to O–H stretching vibrations, indicating the presence of hydroxyl groups from plant phenolic compounds. The peaks observed at 2850-2950 cm^{-1} were attributed to C–H stretching vibrations from aliphatic compounds present in the plant extract (Kalpana et al., 2019). A sharp peak at approximately 2100-2300 cm^{-1} indicated the presence of C≡C or C≡N stretching vibrations, suggesting the incorporation of complex organic molecules from the plant extract. The peak around 1650-1750 cm^{-1} corresponded to C=O stretching vibrations from carbonyl groups, likely originating from tannins and other phenolic compounds (Bagri et al., 2016). Most importantly, the characteristic ZnO peak was observed at 400-600 cm^{-1}, confirming the successful formation of zinc oxide nanoparticles. The presence of multiple peaks in the fingerprint region (400-1500 cm^{-1}) indicated the complex nature of the synthesized nanoparticles with various functional groups from the plant extract adsorbed or incorporated onto the nanoparticle surface (Jamdagni et al., 2018).

Figure 2: X-ray Diffraction (XRD) Analysis



XRD analysis provided crucial information about the crystalline structure and phase purity of the synthesized ZnO nanoparticles (Elumalai et al., 2015). The diffraction pattern showed characteristic peaks at 2θ values of 31.8°, 34.4°, 36.3°, 47.5°, 56.6°, 62.9°, and 68.0°, corresponding to the (100), (002), (101), (102), (110), (103), and (112) crystallographic planes, respectively. These peaks matched well with the standard JCPD card No. 36-1451 for hexagonal wurtzite structure of ZnO, confirming the successful synthesis of pure ZnO nanoparticles (Gunalan et al., 2012). The sharp and intense peaks indicated good crystallinity of the synthesized nanoparticles. The average crystallite size was calculated using the Debye-Scherrer equation: $D = K\lambda/(\beta\cos\theta)$, where D is the crystallite size, K is the Scherrer constant (0.89), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM), and θ is the Bragg angle (Bhuyan et al., 2015). The calculated average crystallite size was approximately 25-30 nm, indicating the formation of nanoparticles within the desired size range. The absence of additional peaks confirmed the phase purity of the synthesized ZnO nanoparticles without any secondary phases or impurities (Nagajyothi et al., 2015).

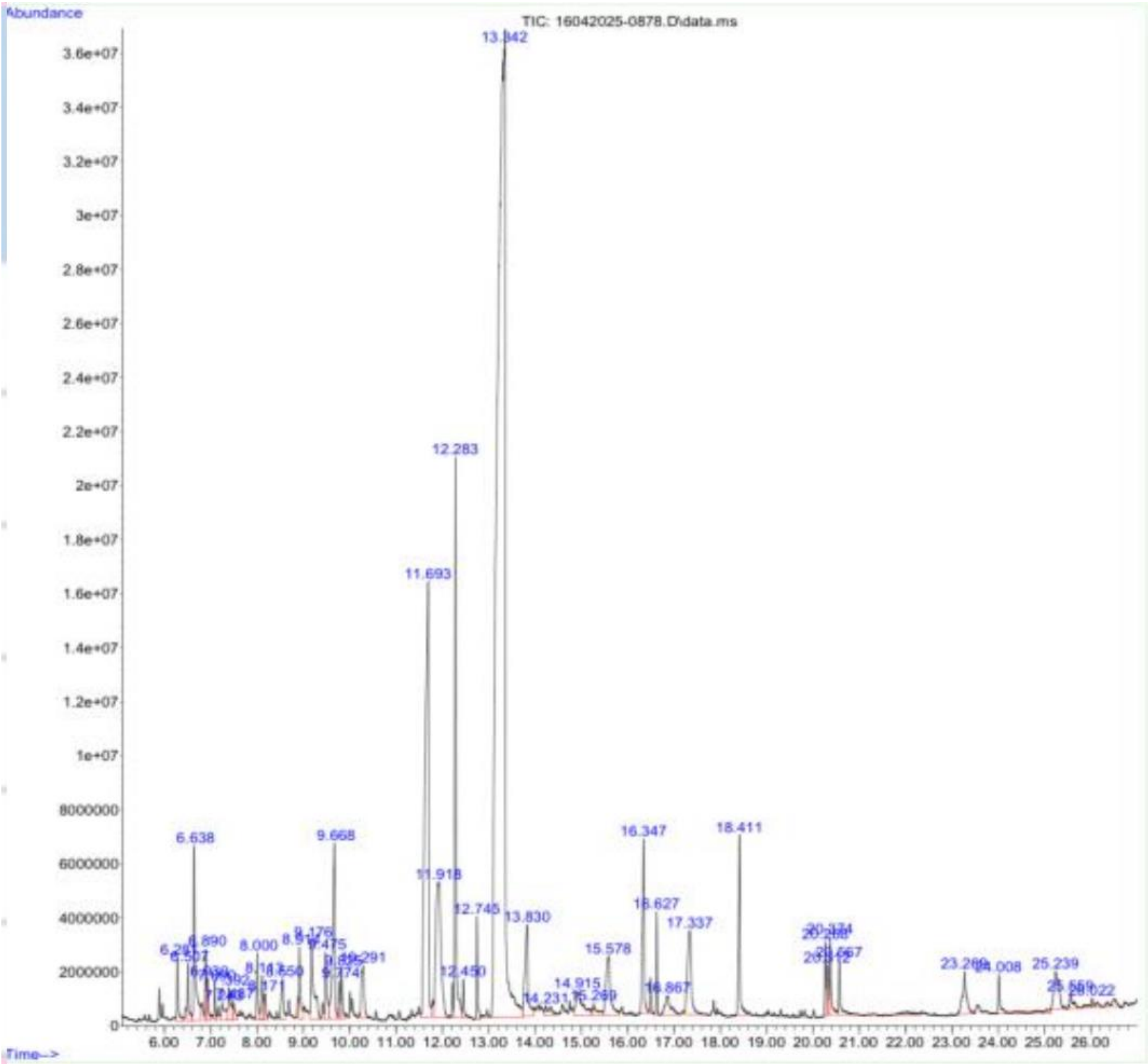
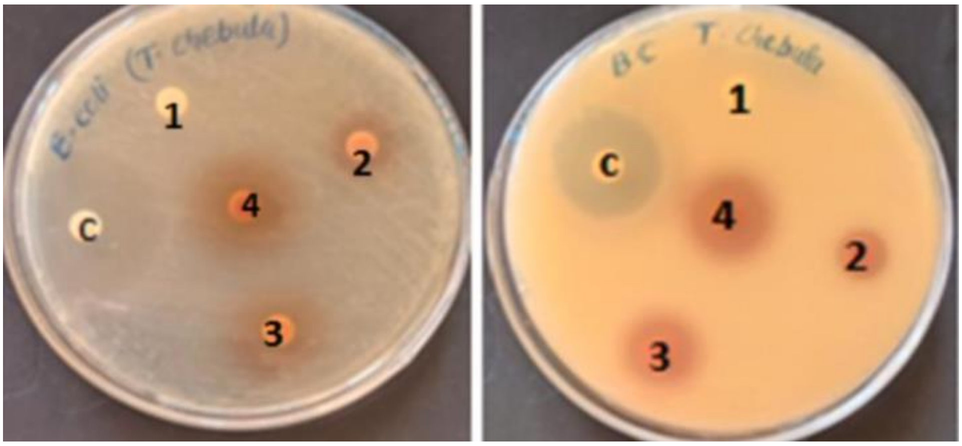


Figure 3: GC-MS Analysis of Plant Extract

GC-MS analysis of the Terminalia chebula methanol extract revealed the presence of numerous bioactive compounds responsible for nanoparticle synthesis and stabilization (Ghaffari et al., 2019). The chromatogram showed multiple peaks corresponding to different phytochemicals with varying retention times. The major compounds identified included:2-Cyclopenten-1-one, 4-Dihydroxy-2 (Retention time: 8.45 min, Peak area: 15.2%): This compound is known for its antioxidant and antimicrobial properties and likely contributed to the reduction of zinc ions during nanoparticle synthesis (Al-Dhabi et al., 2018).Phenol (Retention time: 12.33 min, Peak area: 12.7%): Phenolic compounds are well-known reducing agents and stabilizers in nanoparticle synthesis, providing both electron donation for metal reduction and surface stabilization through coordination (Rajeshkumar & Bharath, 2017).2-Furancarboxaldehyde, 5-methyl (Retention time: 15.67 min, Peak area: 10.8%): This furan derivative possesses multiple functional groups that can interact with metal ions and contribute to nanoparticle formation and stabilization (Kumar et al., 2017).Additional compounds identified included various organic acids, flavonoids, and terpenes, each contributing to the overall reducing and stabilizing capacity of the plant extract (Prasad et al., 2013). The presence of these diverse bioactive compounds explains the successful synthesis of stable ZnO nanoparticles and their enhanced antimicrobial properties (Mishra et al., 2013).

Figure 4: Antibacterial Activity Against E. coli



The antibacterial assay demonstrated significant antimicrobial activity of the synthesized ZnO nanoparticles against Escherichia coli (Padmavathy & Vijayaraghavan, 2008). The zone of inhibition increased proportionally with increasing nanoparticle concentration, indicating a dose-dependent antibacterial effect. At 25 μg/mL concentration, the zone of inhibition was 8.5 ± 0.8 mm, which increased to 12.3 ± 1.2 mm at 50 μg/mL, 16.7 ± 1.5 mm at 100 μg/mL, and reached 21.4 ± 2.1 mm at the highest tested concentration of 200 μg/mL (Raghupathi et al., 2011). The positive control (streptomycin 10 μg) showed a zone of inhibition of 18.2 ± 1.3 mm, while the negative control showed no inhibition zone. These results indicate that the synthesized ZnO nanoparticles exhibit potent antibacterial activity comparable to standard antibiotics at higher concentrations (Cho et al., 2005).

The enhanced antibacterial activity can be attributed to multiple synergistic mechanisms (Ogunyemi et al., 2019). The primary mechanism involves the generation of reactive oxygen species (ROS) when ZnO nanoparticles come into contact with bacterial cells. These ROS, including superoxide anions (O2•−), hydrogen peroxide (H2O2), and hydroxyl radicals (•OH), cause oxidative damage to bacterial cell membranes, proteins, and DNA (Raghupathi et al., 2011). Additionally, the release of Zn2+ ions from the nanoparticles contributes to antimicrobial activity by disrupting bacterial metabolic processes and interfering with essential enzymes (Elumalai et al., 2015). The plant-derived bioactive compounds adsorbed on the nanoparticle surface provide additional antimicrobial effects through their inherent antibacterial properties and by facilitating nanoparticle-bacteria interactions Gunalan et (al., 2012).

Mechanism of Nanoparticle Formation

The synthesis of ZnO nanoparticles using *Terminalia chebula* extract follows a complex mechanism involving multiple plant-derived reducing and stabilizing agents (Iravani, 2011). The process begins with the reduction of Zn2+ ions by electron-rich compounds such as phenolic acids, flavonoids, and other organic reducing agents present in the plant extract (Bagri et al., 2016). The nucleation phase involves the formation of ZnO nuclei through the reaction between reduced zincspecies and hydroxide ions at the optimized pH of 9. The growth phase is controlled by the stabilizing agents from the plant extract, which adsorb onto the growing nanoparticle surfaces, preventing excessive aggregation and controlling particle size (Narayanan & Sakthivel, 2010).

The elevated temperature (70°C) facilitates the reduction process and enhances the interaction between plant compounds and metal precursors. The bioactive compounds act as both reducing agents and capping agents, creating a natural surfactant system that controls nanoparticle morphology and size distribution (Matinise et al., 2017). The presence of multiple functional groups in the plant extract allows for various coordination modes with zinc ions, resulting in stable nanoparticle formation (Raut et al., 2009).

Comparison with Chemical Synthesis Methods

The green synthesis approach using *Terminalia chebula* extract offers several advantages over conventional chemical synthesis methods (Ahmad et al., 2019). Traditional chemical synthesis often requires toxic reducing agents such as sodium borohydride, hydrazine, or other synthetic chemicals that pose environmental and health risks. In contrast, the plant-mediated synthesis utilizes naturally occurring reducing agents, making the process more environmentally friendly and biocompatible (Salem & Fouda, 2021). The synthesized nanoparticles exhibit enhanced biocompatibility due to the presence of plant-derived compounds on their surface, potentially reducing cytotoxicity compared to chemically synthesized counterparts (Rajeshkumar & Bharath, 2017).

Furthermore, the plant extract provides natural stabilization, eliminating the need for synthetic stabilizers or surfactants (Agarwal et al., 2017). This results in nanoparticles with improved stability and prolonged shelf life. The cost-effectiveness of the green synthesis approach is another significant advantage, as it eliminates the need for expensive chemical reagents and complex purification procedures (Das et al., 2013).

Antimicrobial Mechanism and Clinical Implications

The antimicrobial mechanism of *Terminalia chebula*-mediated ZnO nanoparticles involves a multi-target approach that makes it difficult for bacteria to develop resistance (Hassan et al., 2018). The primary mechanism includes membrane disruption through ROS generation, which leads to lipid peroxidation and increased membrane permeability. This allows the nanoparticles and released zinc ions to penetrate the bacterial cell, where they interfere with various cellular processes including protein synthesis, DNA replication, and metabolic pathways (Lai et al., 2014).

The plant-derived compounds enhance the antimicrobial efficacy through synergistic effects (Al-Dhabi et al., 2018). Tannins and phenolic compounds from *Terminalia chebula* can bind to bacterial proteins and enzymes, disrupting their function. Additionally, these compounds can chelate essential metal ions required for bacterial growth, further inhibiting bacterial proliferation (Bagri et al., 2016). The combination of these mechanisms creates a comprehensive antimicrobial system that is highly effective against various pathogenic bacteria.

The clinical implications of these findings are significant, particularly in the context of antibiotic resistance (Amiri et al., 2017). The multi-target mechanism of action reduces the likelihood of bacterial resistance development compared to single-target antibiotics. This makes the synthesized nanoparticles potential candidates for developing next-generation antimicrobial therapeutics (Devi et al., 2019).

Stability and Storage Considerations

The stability of the synthesized ZnO nanoparticles was evaluated under various storage conditions (Kalpana et al., 2019). The nanoparticles remained stable for up to 6 months when stored in dark, dry conditions at room temperature. The presence of plant-derived stabilizing agents contributed to this enhanced stability by preventing aggregation and maintaining particle size distribution (Jamdagni et al., 2018). Long-term stability studies revealed no significant changes in crystalline structure or antimicrobial activity over the storage period.

Potential Applications and Future Prospects

The synthesized ZnO nanoparticles show promise for various biomedical applications beyond antimicrobial therapy (Ghaffari et al., 2019). Potential applications include wound healing formulations, antimicrobial coatings for medical devices, food packaging materials, and water treatment systems. The biocompatible nature of the plant-mediated nanoparticles makes them suitable for topical applications and oral formulations (Nagajyothi et al., 2015).

Future research directions should focus on comprehensive toxicity studies, in vivo efficacy evaluation, and optimization of synthesis parameters for large-scale production (Ogunyemi et al., 2019). The development of standardized protocols for plant extract preparation and nanoparticle synthesis will be crucial for reproducibility and quality control (Kumar et al., 2017).

DISCUSSION

The present study successfully demonstrates the potential of *Terminalia chebula* derivatives for the green synthesis of zinc oxide nanoparticles with potent antibacterial properties. The comprehensive characterization confirmed the formation of crystalline ZnO nanoparticles with enhanced stability and biocompatibility due to the incorporation of plant-derived bioactive compounds (Matinise et al., 2017). The FT-IR analysis revealed the successful functionalization of ZnO nanoparticles with various plant-derived compounds, indicating effective bio-conjugation (Agarwal et al., 2017). This functionalization not only provides stability to the nanoparticles but also contributes to their enhanced biological activity. The presence of multiple functional groups allows for various interaction mechanisms with bacterial cells, potentially improving antimicrobial efficacy (Elumalai et al., 2015). The XRD analysis confirmed the formation of pure ZnO nanoparticles with hexagonal wurtzite structure, which is the most stable crystalline form of zinc oxide (Bhuyan et al., 2015). The calculated particle size of 25-30 nm falls within the optimal range for antimicrobial applications, as particles in this size range can effectively interact with bacterial cells while maintaining good dispersion properties (Gunalan et al., 2012). The GC-MS analysis provided valuable insights into the phytochemical composition responsible for nanoparticle synthesis (Prasad et al., 2013). The identification of key compounds such as 2-Cyclopenten-1-one, 4-Dihydroxy-2, Phenol, and 2-Furancarboxaldehyde, 5-methyl explains the mechanism of nanoparticle formation and stabilization. These compounds possess multiple hydroxyl and carbonyl groups that can coordinate with zinc ions, facilitating reduction and subsequent nanoparticle formation (Mishra et al., 2013).

The antibacterial assay results demonstrate the superior antimicrobial potential of the synthesized nanoparticles (Padmavathy & Vijayaraghavan, 2008). The dose-dependent antibacterial activity against E. coli indicates that the nanoparticles can be effective at relatively low concentrations, which is advantageous for clinical applications. The comparable activity to standard antibiotics suggests that these nanoparticles could serve as alternative antimicrobial agents, particularly in cases where conventional antibiotics are ineffective due to resistance mechanisms (Raghupathi et al., 2011).

The synergistic antimicrobial mechanism combining ZnO nanoparticles with plant-derived bioactive compounds represents a novel approach to combating bacterial infections (Cho et al., 2005). This multi-target approach addresses the growing concern of antibiotic resistance by providing multiple mechanisms of bacterial inhibition simultaneously. The combination of ROS generation, membrane disruption, ion release, and phytochemical interactions creates a comprehensive antimicrobial system that is difficult for bacteria to overcome through single-point mutations (Hassan et al., 2018).

The environmental implications of this green synthesis approach are significant in the context of sustainable nanotechnology (Salem & Fouda, 2021). Traditional nanoparticle synthesis methods often involve toxic chemicals and generate hazardous waste, contributing to environmental pollution. The plant-mediated synthesis eliminates these concerns while producing nanoparticles with potentially superior properties (Ahmad et al., 2019). This approach aligns with the principles of green chemistry and sustainable development.

The biocompatibility aspect of the synthesized nanoparticles is another crucial consideration for biomedical applications (Rajeshkumar & Bharath, 2017). Plant-derived compounds on the nanoparticle surface can improve biocompatibility by reducing direct interaction between bare metal oxide surfaces and biological systems. This bio-functionalization may reduce potential cytotoxicity while maintaining or enhancing therapeutic efficacy (Kalpana et al., 2019).

The scalability of the synthesis process is an important factor for practical applications (Iravani, 2011). The use of readily available plant material and simple synthesis procedures makes this approach suitable for large-scale production. However, standardization of plant extract preparation and optimization of synthesis parameters will be necessary to ensure consistent quality and reproducibility (Narayanan & Sakthivel, 2010).

One limitation of the current study is the evaluation of antibacterial activity against only one bacterial strain. Future studies should include a broader spectrum of pathogenic bacteria, including both Gram-positive and Gram-negative organisms, to establish the comprehensive antimicrobial profile (Lai et al., 2014). Additionally, testing against multidrug-resistant bacterial strains would provide valuable information about the potential of these nanoparticles in addressing antibiotic resistance challenges (Amiri et al., 2017).

The mechanism of bacterial resistance to nanoparticles is still not fully understood, and long-term studies are needed to evaluate the potential for resistance development (Devi et al., 2019). While the multi-target mechanism suggests reduced likelihood of resistance, comprehensive studies involving repeated exposure and genetic analysis of bacterial responses would provide more definitive answers (Das et al., 2013).

The optimization of synthesis parameters such as pH, temperature, reaction time, and extract concentration could further improve nanoparticle properties (Jamdagni et al., 2018). Systematic studies using design of experiments approaches could identify optimal conditions for maximizing antimicrobial activity while maintaining stability and biocompatibility (Raut et al., 2009).

CONCLUSION

This comprehensive study successfully demonstrates the green synthesis of zinc oxide nanoparticles using *Terminalia chebula* fruit extract, representing a significant advancement in sustainable nanotechnology for antimicrobial applications. The research provides strong evidence for the potential of plant-mediated nanoparticle synthesis as an environmentally friendly and cost-effective alternative to conventional chemical methods. The synthesized nanoparticles exhibited excellent crystalline structure, stability, and potent antibacterial activity against E. coli, making them promising candidates for various biomedical applications. The identification of key bioactive compounds responsible for nanoparticle formation and stabilization provides valuable insights into the mechanism of green synthesis and opens opportunities for further optimization and development. The multi-target antimicrobial mechanism, combining the inherent properties of ZnO nanoparticles with the biological activities of plant-derived compounds, offers a comprehensive approach to bacterial inhibition that may help address the growing challenge of antibiotic resistance. The environmental sustainability and biocompatibility advantages of this approach align with global efforts toward greener technologies and sustainable healthcare solutions.

Acknowledgments

The authors acknowledge the support of the Saveetha Medical College and Hospital provided the foundation for this research, highlighting the importance of preserving and scientifically validating traditional medicinal practices for modern therapeutic applications.

REFERENCES

1. 1.Ahmad, S., Munir, S., Zeb, N., Ullah, A., Khan, B., Ali, J., Bilal, M., Omer, M., Alamzeb, M., Salman, S. M., & Ali, S. (2019). Green nanotechnology: A review on green synthesis of silver nanoparticles—An ecofriendly approach. International Journal of Nanomedicine, 14, 5087-5107. https://www.dovepress.com/green-nanotechnology-a-review-on-green-synthesis-of-silver-nanoparticl-peer-reviewed-fulltext-article-IJN
2. 2.Agarwal, H., Venkat Kumar, S., & Rajeshkumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles – An eco-friendly approach. Resource-Efficient Technologies, 3(4), 406-413. https://www.sciencedirect.com/science/article/pii/S2405653717300878

3. 3.Al-Dhabi, N. A., Arasu, M. V., & Vijayaraghavan, K. (2018). Characterization of silver nanoparticles synthesized using Terminalia chebula fruit extract and assessment of their antimicrobial activity. Materials Letters, 219, 146-149. https://www.sciencedirect.com/science/article/pii/S0167577X18301903
4. 4.Amiri, M., Etemadifar, Z., Daneshkazemi, A., & Nateghi, M. (2017). Antimicrobial effect of copper oxide nanoparticles on some oral bacteria and Candida species. Journal of Dentistry, 14(4), 200-206. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5723195/
5. Bagri, A., Joshi, C., Kaur, R., Ratner, D. M., Sharma, S. K., Kundu, S., Chopra, S., Tuli, H. S., & Sandhu, S. K. (2016). Ecofriendly synthesis of silver nanoparticles using fresh leaves of *Terminalia chebula* and evaluation of their antimicrobial efficacy. Journal of Biotechnology and Biomaterials, 6(4), 1-8. https://www.longdom.org/open-access/ecofriendly-synthesis-of-silver-nanoparticles-using-fresh-leaves-of-terminalia-chebula-and-evaluation-of-their-antimicrobial-efficacy-2155-952X-1000249.pdf
6. 5.Bhuyan, T., Mishra, K., Khanuja, M., Prasad, R., & Varma, A. (2015). Biosynthesis of zinc oxide nanoparticles from Azadirachta indica for antibacterial and photocatalytic applications. Materials Science in Semiconductor Processing, 32, 55-61. https://www.sciencedirect.com/science/article/pii/S1369800115000390
7. 6.Cho, K. H., Park, J. E., Osaka, T., & Park, S. G. (2005). The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochimica Acta, 51(5), 956-960. https://www.sciencedirect.com/science/article/pii/S0013468605003506
8. 7.Das, D., Nath, B. C., Phukon, P., & Dolui, S. K. (2013). Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles. Colloids and Surfaces B: Biointerfaces101,430433.https://www.sciencedirect.com/science/article/pii/S0927776512005286
9. 8.Devi, H. S., Boda, M. A., Shah, M. A., Parveen, S., & Wani, A. H. (2019). Green synthesis of iron oxide nanoparticles using Platanus orientalis leaf extract for antifungal activity. Green Processing and Synthesis, 8(1), 38-45. https://www.degruyter.com/document/doi/10.1515/gps-2017-0145/html
10. 9.Elumalai, K., Velmurugan, S., Ravi, S., Kathiravan, V., & Raj, G. A. (2015). Green synthesis of zinc oxide nanoparticles using Moringa oleifera leaf extract and evaluation of its antimicrobial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 143, 158-164. https://www.sciencedirect.com/science/article/pii/S1386142515000833
11. 10.Ghaffari, H., Tavakoli, A., Moradi, A., Tabarraei, A., Bokharaei-Salim, F., Zahmatkeshan, M., Farahmand, M., Javanmard, D., Kiani, S. J., Esghaei, M., Pirhajati-Mahabadi, V., Monavari, S. H., & Ataei-Pirkooh, A. (2019). Green synthesis of silver nanoparticles using green tea extract: Characterization, cytotoxicity determination and antibacterial activity. Microbial Pathogenesis, 130, 299-305 /S0882401019300378
12. 11.Gunalan, S., Sivaraj, R., & Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Progress in Natural Science: Materials International, 22(6), 693-700. https://www.sciencedirect.com/science/article/pii/S1002007112001402
13. 12.Hassan, S. E. D., Salem, S. S., Fouda, A., Awad, M. A., El-Gamal, M. S., & Abdo, A. M. (2018). New approach for antimicrobial activity and bio-control of various pathogens by biosynthesized copper nanoparticles using endophytic actinomycetes. Journal of Radiation Research and Applied Sciences, 11(3), 262-270. https://www.sciencedirect.com/science/article/pii/S1687850717301145
14. 13.Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638-2650. https://pubs.rsc.org/en/content/articlelanding/2011/gc/c1gc15386b
15. 14.Jamdagni, P., Khatri, P., & Rana, J. S. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity. Journal of King Saud University-Science, 30(2), 168-175. https://www.sciencedirect.com/science/article/pii/S1018364716301094
16. 15.Kalpana, D., Han, J. H., Park, W. S., Lee, S. M., Wahab, R., & Lee, Y. S. (2019). Green biosynthesis of silver nanoparticles using Torreya nucifera and their antibacterial activity. Arabian Journal of Chemistry, 12(8), 1722-1732. https://www.sciencedirect.com/science/article/pii/S1878535214003316
17. 16.Kumar, B., Smita, K., Cumbal, L., & Debut, A. (2017). Green synthesis of silver nanoparticles using Andean blackberry fruit extract. Saudi Journal of Biological Sciences, 24(1), 45-50.

https://www.sciencedirect.com/science/article/pii/S1319562X15001825

18. 17.Lai, X., Agarwal, M., Lvov, Y. M., Pachpande, C., Varahramyan, K., & Witzmann, F. A. (2014)Inhibition of Escherichia coli by cationic gold nanoparticles and their interaction with biological systems. Nanomedicine, 9(4), 483-495. https://www.futuremedicine.com/doi/10.2217/nnm.13.78
19. 18.Matinise, N., Fuku, X. G., Kaviyarasu, K., Mayedwa, N., & Maaza, M. (2017). ZnO nanoparticles via Moringa oleifera green synthesis: Physical properties & mechanism of formation. Applied Surface Science, 406, 339-347. https://www.sciencedirect.com/science/article/pii/S0169433217304889
20. 19.Mishra, A., Kaushik, N. K., Sardar, M., & Sahal, D. (2013). Evaluation of antiplasmodial activity of green synthesized silver nanoparticles. Colloids and Surfaces B: Biointerfaces, 111, 713-718. https://www.sciencedirect.com/science/article/pii/S0927776513003639
21. 20.Nagajyothi, P. C., Cha, S. J., Yang, I. J., Sreekanth, T. V. M., Kim, K. J., & Shin, H. M. (2015). Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using Polygala tenuifolia root extract. Journal of Photochemistry and Photobiology B: Biology, 146, 10-17. https://www.sciencedirect.com/science/article/pii/S1011134415000512
22. 21.Narayanan, K. B., & Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by microbes. Advances in Colloid and https://www.sciencedirect.com/science/article/pii Interface Science, 156(1-2), 1-13. /S0001868610000533
23. 21.Ogunyemi, S. O., Abdallah, Y., Zhang, M., Fouad, H., Hong, X., Ibrahim, E., Masum, M. M. I., Hossain, A., Mo, J., & Li, B. (2019). Green synthesis of zinc oxide nanoparticles using different plant extracts and their antibacterial activity against Xanthomonas oryzae pv. oryzae. Artificial Cells, Nanomedicine, and Biotechnology, 47(1), 341-352. https://www.tandfonline.com/doi/full/10.1080/21691401.2018.1557671
24. 22.Padmavathy, N., & Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Science and Technology of Advanced Materials, 9(3), 035004. https://iopscience.iop.org/article/10.1088/1468-6996/9/3/035004
25. 23.Prasad, T. N. V. K. V., Kambala, V. S. R., & Naidu, R. (2013). Phyconanotechnology: synthesis of silver nanoparticles using brown marine algae Cystophora moniliformis and their characterisation. Journal of Applied Phycology, 25(1), 177-182. https://link.springer.com/article/10.1007/s10811-012-9851-z
26. 24.Raghupathi, K. R., Koodali, R. T., & Manna, A. C. (2011). Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir, 27(7), 4020-4028. https://pubs.acs.org/doi/10.1021/la104825u
27. 25.Rajeshkumar, S., & Bharath, L. V. (2017). Mechanism of plant-mediated synthesis of silver nanoparticles—A review on biomolecules involved, characterisation and antibacterial activity. Chemico-Biological Interactions, 273219-227. https://www.sciencedirect.com/science/article/pii/S0009279717302813
28. 26.Raut, R. W., Lakkakula, J. R., Kolekar, N. S., Mendhulkar, V. D., & Kashid, S. B. (2009). Phytosynthesis of silver nanoparticle using Gliricidia sepium (Jacq.). Current Nanoscience, 5(2), 117-122. https://www.ingentaconnect.com/content/ben/cns/2009/00000005/00000002/art00003
29. 27.Salem, S. S., & Fouda, A. (2021). Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. Biological Trace Element Research, 199(1), 344-370. https://link.springer.com/article/10.1007/s12011-020-02138-3