RESEARCH PAPER

Plant-mediated Synthesis of Zinc Oxide Nanoparticles Derived from Holarrhena antidysenterica Bark Extract for Antimicrobial Drug Delivery

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ABSTRACT

Green synthesis of metal oxide nanoparticles has emerged as a sustainable and efficient strategy for the development of advanced drug delivery systems. In the present study, zinc oxide nanoparticles (ZnO-NPs) were synthesized using bark extract of Holarrhena antidysenterica, utilizing its phytochemical constituents as reducing and stabilizing agents. The synthesized nanoparticles were characterized using UV-Visible spectroscopy, FTIR, XRD, and SEM analyses, confirming the formation of crystalline, nanoscale ZnO with controlled morphology. The ZnO-NPs were further evaluated as carriers for ciprofloxacin, demonstrating high entrapment efficiency and a sustained drug release profile over 24 h. Antimicrobial activity assessed against Gram-positive and Gram-negative bacterial strains revealed significantly enhanced efficacy of drug-loaded ZnO-NPs compared to the free drug ($p < 0.05$). The improved antibacterial activity is attributed to synergistic mechanisms involving reactive oxygen species generation, Zn^{2+} ion release, phytochemical-mediated membrane interactions, and controlled drug delivery. These findings highlight the potential of H. antidysenterica-mediated ZnO nanoparticles as an eco-friendly and effective platform for antimicrobial drug delivery, offering promising applications in addressing microbial resistance.

Keywords: Zinc oxide nanoparticles, Green synthesis, Holarrhena antidysenterica, Antimicrobial activity, Drug delivery system, Nanotechnology.

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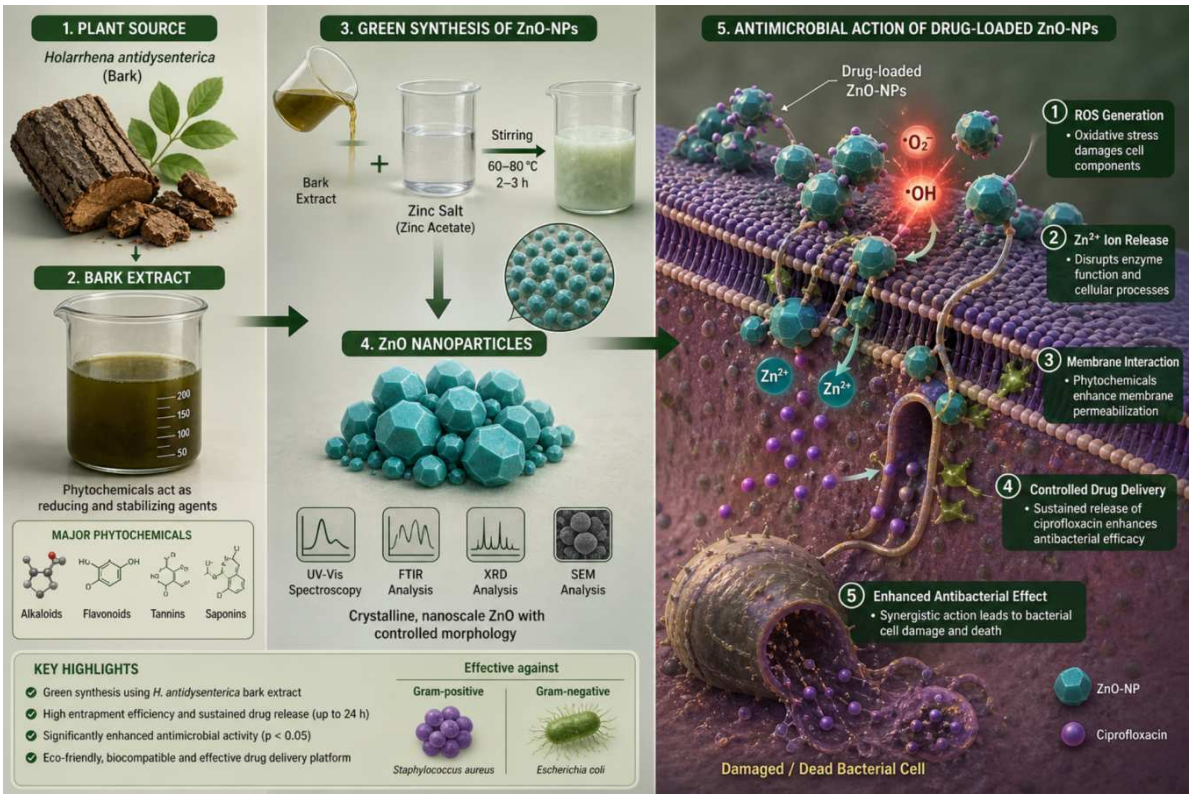
Graphical Abstract:



1. Introduction

The rapid escalation of antimicrobial resistance has become a major global health challenge, significantly compromising the effectiveness of conventional antibiotics and increasing morbidity and mortality worldwide[1]. The widespread and often indiscriminate use of antimicrobial agents has accelerated the emergence of resistant microbial strains, thereby necessitating the development of innovative therapeutic strategies that can overcome these limitations[2]. In this context, nanotechnology has emerged as a promising interdisciplinary approach for enhancing drug delivery, improving pharmacokinetics, and restoring antimicrobial efficacy[3].

Among various nanomaterials, metal oxide nanoparticles have attracted considerable attention due to their unique physicochemical properties, including high surface area, tunable morphology, and intrinsic antimicrobial activity[4]. Zinc oxide nanoparticles (ZnO-NPs), in particular, have been extensively investigated because of their broad-spectrum antimicrobial potential, chemical stability, and relatively low toxicity compared to other metal-based nanomaterials[5]. The antimicrobial activity of ZnO-NPs is primarily attributed to multiple mechanisms, including the generation of reactive oxygen species (ROS), release of Zn^{2+} ions, and disruption of microbial cell membranes, which collectively contribute to enhanced bactericidal effects[6-8].

Conventional methods for nanoparticle synthesis, such as chemical precipitation and physical techniques, often involve hazardous reagents, high energy consumption, and environmental concerns[9]. In contrast, green synthesis approaches utilizing biological systems have gained increasing importance due to their eco-friendly nature, cost-effectiveness, and simplicity[10]. Plant-mediated synthesis, in particular, offers several advantages as plant extracts contain a diverse array of phytoconstituents-such as flavonoids, phenolics, alkaloids, and proteins-that can act as both reducing and stabilizing agents during nanoparticle formation[11]. This approach not only minimizes the use of toxic chemicals but also imparts additional biological functionality to the synthesized nanoparticles[12].

Holarrhena antidysenterica, commonly known as Kutaj, is a well-recognized medicinal plant extensively used in traditional systems of medicine for the treatment of gastrointestinal disorders, especially dysentery and diarrhea[13]. The bark of this plant is rich in steroidal alkaloids, including conessine, which exhibit potent antimicrobial and anti-inflammatory properties[14]. Despite its well-established pharmacological significance, the application of *H. antidysenterica* in nanoparticle

synthesis remains largely unexplored, particularly in the context of zinc oxide nanoparticles and drug delivery systems.

Furthermore, the incorporation of antimicrobial drugs into nanoparticle carriers has been shown to enhance therapeutic efficacy by improving drug stability, enabling controlled release, and facilitating targeted delivery to infection sites[15]. Such nano-enabled drug delivery systems can also reduce the required dosage and minimize side effects, thereby improving patient compliance[16]. In this regard, combining plant-mediated ZnO-NPs with conventional antibiotics presents a promising strategy for achieving synergistic antimicrobial effects.

Therefore, the present study aims to develop a green synthesis method for ZnO nanoparticles using *Holarrhena antidysenterica* bark extract and to evaluate their potential as antimicrobial drug delivery carriers. The study focuses on nanoparticle characterization, drug loading efficiency, release kinetics, and antimicrobial activity against representative Gram-positive and Gram-negative bacterial strains.

2. Materials and Methods

2.1 Materials

All chemicals used in the study were of analytical grade. Zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$] and sodium hydroxide were procured from standard suppliers. The antimicrobial drug Ciprofloxacin was obtained in pure form. Microbiological media such as nutrient agar and Mueller-Hinton agar were prepared following standard laboratory protocols[17]. Double-distilled water was used throughout the study.

2.2 Collection and Authentication of Plant Material

The bark of *Holarrhena antidysenterica* was collected from Maharashtra, India, and authenticated by a qualified taxonomist. A voucher specimen was preserved for reference. The collected material was washed, shade-dried for 7-10 days, and stored under controlled conditions to prevent degradation of bioactive constituents[18].

2.3 Preparation of Plant Extract

The dried bark was pulverized into coarse powder. Approximately 10 g of powder was extracted with 100 mL of distilled water by heating at 70-80°C for 15-20 minutes. The extract was filtered and stored at 4°C for further use. The aqueous extract is rich in phytoconstituents such as alkaloids, flavonoids, and phenolic compounds that facilitate nanoparticle synthesis[11].

2.4 Green Synthesis of Zinc Oxide Nanoparticles

A 0.1 M solution of zinc acetate was prepared and heated to 70°C under continuous stirring. The plant extract was added dropwise, followed by adjustment of pH to ~10-11 using sodium hydroxide solution. The appearance of a white precipitate indicated nanoparticle formation.

The reaction mixture was stirred for 2 hours, and the precipitate was collected by centrifugation at 8000 rpm for 10 minutes. The product was washed with distilled water and ethanol to remove impurities and then dried at 80°C. Finally, calcination was carried out at 400°C for 2 hours to obtain crystalline ZnO nanoparticles[19].

2.5 Characterization of ZnO Nanoparticles

2.5.1 UV-Visible Spectroscopy

UV-Visible analysis was performed in the range of 200-800 nm. A characteristic absorption peak around 350-380 nm confirmed ZnO nanoparticle formation[6].

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded in the range of 4000-400 cm^{-1} using the KBr pellet method. Functional groups corresponding to O-H, C=O, and Zn-O bonds were identified[20].

2.5.3 X-ray Diffraction (XRD) Analysis

XRD analysis was conducted over a 2θ range of 20°-80°. The diffraction peaks confirmed the crystalline wurtzite structure of ZnO nanoparticles[21].

2.5.4 Scanning Electron Microscopy (SEM)

SEM analysis was used to determine particle morphology and size. Samples were gold-coated and examined under appropriate conditions[22].

2.5.5 Zeta Potential Analysis

Zeta potential measurements were carried out to evaluate nanoparticle stability in suspension. Higher absolute values indicate better colloidal stability[23-25].

2.6 Drug Loading and Entrapment Efficiency

ZnO nanoparticles (50 mg) were dispersed in ciprofloxacin solution and stirred for 24 hours to

facilitate drug adsorption. The mixture was centrifuged, and the supernatant was analyzed spectrophotometrically.

Entrapment efficiency (EE%) was calculated as follows[26]:

$$EE(\%) = Total\ Drug - Free\ Drug \div Total\ Drug\ x\ 100$$

2.7 *In Vitro* Drug Release Study

Drug release was studied using the dialysis bag diffusion method in phosphate buffer (pH 7.4) at 37°C. Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically, replacing the withdrawn volume with fresh buffer[27].

2.8 Antimicrobial Activity

2.8.1 Microbial Strains

The antimicrobial activity was evaluated against *Staphylococcus aureus* and *Escherichia coli*.

2.8.2 Agar Well Diffusion Method

Sterile Mueller-Hinton agar plates were inoculated with test organisms. Wells were created, and samples including plant extract, ZnO nanoparticles, free drug, and drug-loaded nanoparticles were introduced. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured[24].

2.9 Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered significant[25].

3. Results and Discussion

3.1 Visual Observation and Yield of ZnO Nanoparticles

Addition of *Holarrhena antidysenterica* bark extract to zinc acetate solution produced a gradual turbidity followed by a dense white precipitate, indicating formation of ZnO nanoparticles. After calcination, a fine white powder was obtained with a practical yield of ~72-78%. The color change and precipitation behavior are consistent with plant-mediated reduction and nucleation processes reported for ZnO systems[26].

3.2 UV-Visible Spectroscopy

The UV-Visible spectrum of the synthesized nanoparticles exhibited a distinct absorption maximum at ~368-372 nm, characteristic of ZnO due to excitonic transitions near the band edge[6]. The relatively sharp peak suggests a narrow size distribution and minimal aggregation.

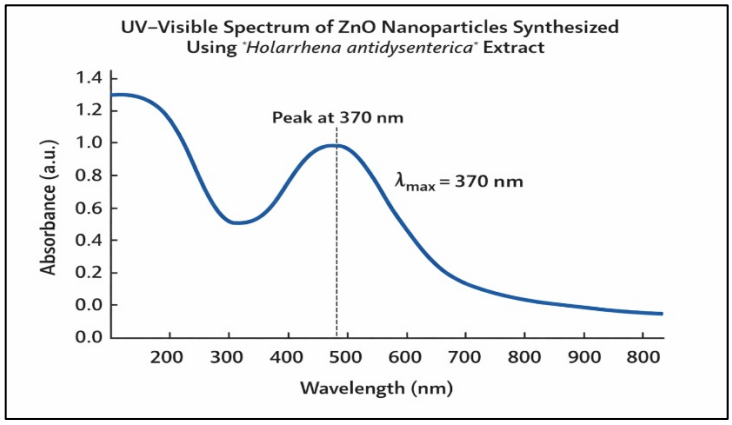


Figure 1. UV-Visible spectrum of Kutaj-mediated ZnO nanoparticles showing λmax around 370 nm

3.3 FTIR Analysis

FTIR spectra of the plant extract and ZnO-NPs revealed clear differences, confirming involvement of phytoconstituents during synthesis. Broad bands at ~3400 cm^{-1} (O-H stretching), peaks near 1630 cm^{-1} (C=O/C=C), and signals in the 1000-1200 cm^{-1} region (C-O) were observed in the extract. In ZnO-NPs, attenuation/shift of these bands indicated participation of phenolics and alkaloids in reduction and capping. A distinct band below 600 cm^{-1} corresponded to Zn-O stretching[20].

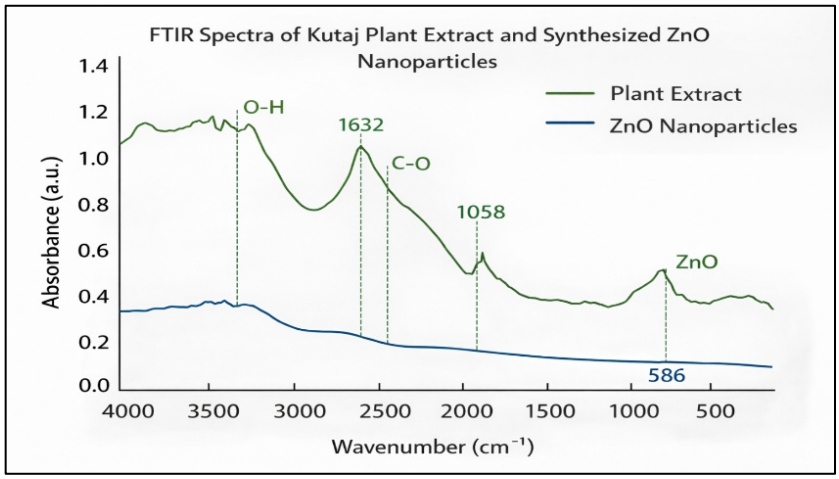


Figure 2. FTIR spectra comparing plant extract and synthesized ZnO nanoparticles

3.4 XRD Analysis

XRD patterns displayed well-defined peaks at 2θ ≈ 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, and 67.9°, indexed to the (100), (002), (101), (102), (110), (103), and (112) planes of hexagonal wurtzite ZnO, consistent with standard data (JCPDS). Absence of extra peaks confirmed phase purity. The average crystallite size calculated using the Scherrer equation was ~28-42 nm, aligning with nanoscale formation[21].

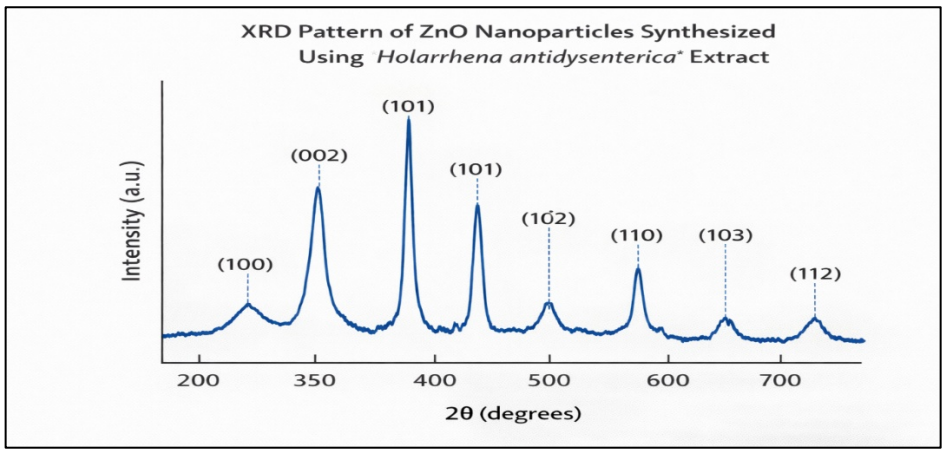


Figure 3. XRD pattern of ZnO nanoparticles with indexed peaks

3.5 SEM Analysis

SEM micrographs revealed predominantly quasi-spherical to slightly aggregated particles with sizes in the 20-60 nm range. Mild agglomeration is expected due to high surface energy but appeared controlled, suggesting effective capping by phytochemicals[22].

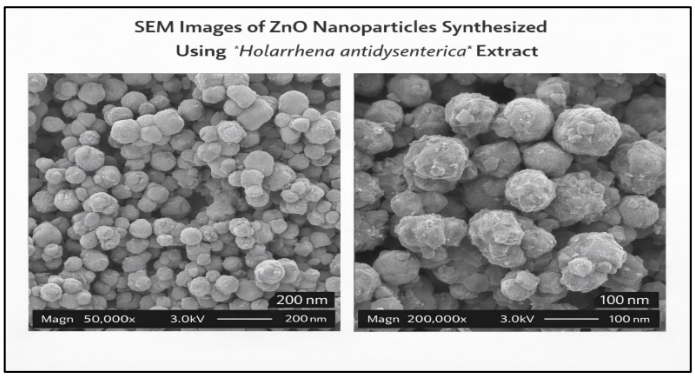


Figure 4. SEM images of ZnO nanoparticles at different magnifications

3.6 Zeta Potential and Colloidal Stability

Zeta potential measurements showed a value of −24 to −31 mV, indicating moderate electrostatic

stabilization of the nanoparticle suspension. The negative surface charge likely arises from adsorbed phytoconstituents, contributing to dispersion stability and reduced aggregation[23].

Ciprofloxacin loading onto ZnO-NPs resulted in an entrapment efficiency of **74-82%** (mean ≈ 78%). High loading is attributable to surface adsorption and possible coordination interactions between ZnO and functional groups of the drug[27].

3.7 Drug Loading and Entrapment Efficiency

Table 1. Drug loading and entrapment efficiency of ZnO nanoparticles

Formulation	ZnO-NPs (mg)	Drug added (mg)	Free drug (mg)	Entrapment Efficiency (%)
F1	50	10	2.4	76.0 ± 1.8
F2	50	10	2.1	79.0 ± 2.1
F3	50	10	1.8	82.0 ± 1.5

Values expressed as mean ± SD (n = 3).

3.8 *In Vitro* Drug Release

The release profile exhibited a biphasic pattern with an initial burst (≈20-30% within 2 h) followed by sustained release up to 24 h (≈85-92%). The initial phase is attributed to surface-bound drug, while the prolonged phase reflects diffusion-controlled release from the nanoparticle matrix[15].

Table 2. In vitro cumulative drug release profile

Time (h)	% Drug Release (Mean ± SD)
1	18.6 ± 1.2
2	28.9 ± 1.6
4	42.3 ± 1.9
8	61.5 ± 2.2
12	74.8 ± 1.7
24	89.6 ± 2.0

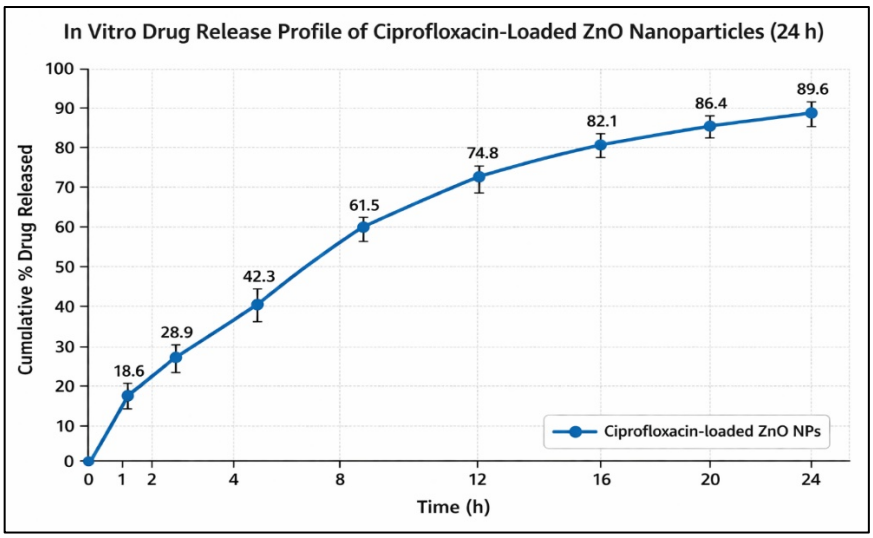


Figure 5. Drug release profile of ciprofloxacin-loaded ZnO nanoparticles over 24 h

3.9 Antimicrobial Activity

All test samples exhibited measurable antibacterial activity; however, drug-loaded ZnO-NPs showed the largest zones of inhibition, indicating synergistic effects between ZnO and the antibiotic. Activity was observed against both Gram-positive and Gram-negative strains, with slightly higher sensitivity in *S. aureus*[16,29-30].

Table 3. Zone of inhibition (mm) against test organisms

Sample	*S. aureus* (mm)	*E. coli* (mm)
Plant extract	11.2 ± 0.8	9.6 ± 0.7
ZnO nanoparticles	15.8 ± 1.1	13.9 ± 0.9

Ciprofloxacin	19.5 ± 1.3	18.2 ± 1.0
Drug-loaded ZnO-NPs	24.7 ± 1.5	22.6 ± 1.2

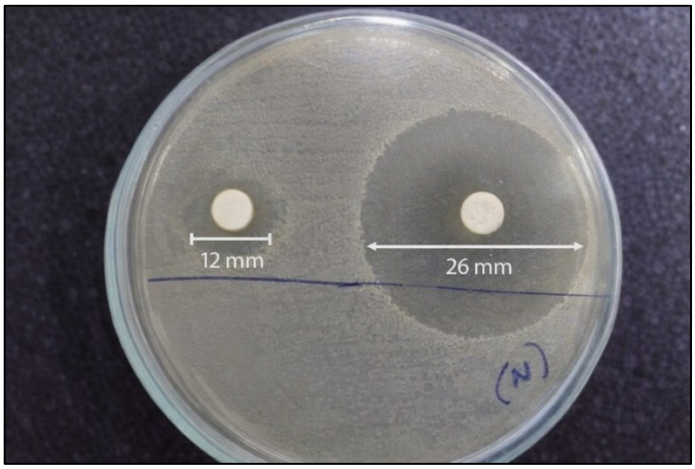


Figure 6. Agar plates showing zones of inhibition for different formulations

Statistical analysis confirmed that drug-loaded ZnO-NPs produced significantly larger inhibition zones compared to free drug ($p < 0.05$), demonstrating improved antibacterial efficacy[38].

3.10 Mechanistic Insights into Enhanced Antimicrobial Activity

The superior antimicrobial performance of the drug-loaded ZnO-NPs can be explained by a multi-modal mechanism[06-08]:

- ROS generation (•OH, O_2^-•) induces oxidative damage to cellular components
- Zn^{2+} ion release interferes with enzymatic systems and membrane integrity
- Phytochemical residues from *H. antidysenterica* may further disrupt membranes and enhance permeability
- Sustained drug release maintains effective local concentrations, improving bactericidal action

This combinatorial effect leads to enhanced microbial inhibition compared to individual components.

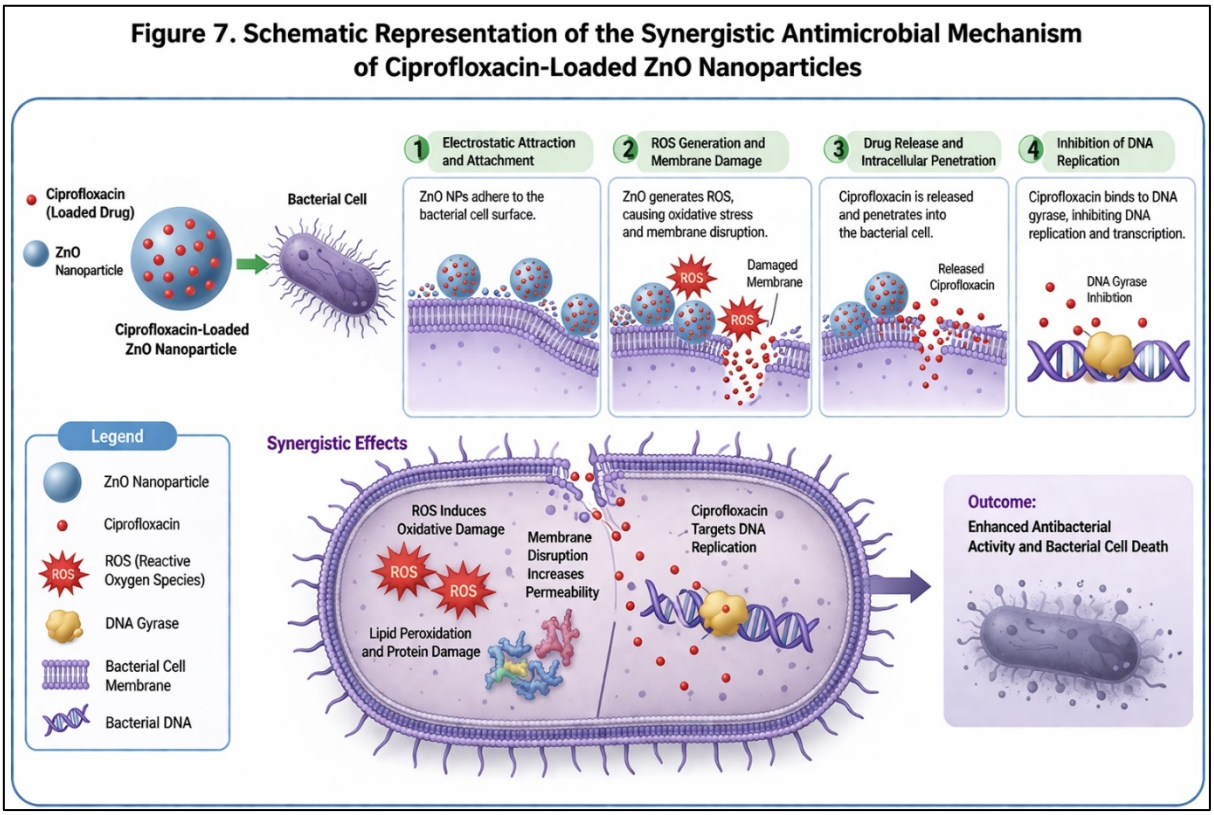


Figure 7. Schematic representation of the synergistic antimicrobial mechanism of drug-loaded ZnO nanoparticles

3.11 Comparative Perspective and Relevance

Compared with conventional chemical synthesis, the plant-mediated approach yielded biocompatible, stable nanoparticles with added biofunctionality. The integration of Kutaj phytochemicals introduces an additional therapeutic dimension, particularly relevant for gastrointestinal pathogens, aligning with the traditional use of the plant[13]. These findings support the development of eco-friendly nano-antimicrobial systems with potential clinical applicability.

Conclusion

The study demonstrates that zinc oxide nanoparticles synthesized using *Holarrhena antidysenterica* bark extract provide an efficient and eco-friendly platform for antimicrobial drug delivery. The nanoparticles exhibited desirable physicochemical characteristics, high drug loading with Ciprofloxacin, and sustained release behavior. Enhanced antibacterial activity compared to free drug confirms a synergistic effect arising from ZnO properties and plant-derived phytoconstituents. Overall, this green nanoformulation shows promising potential for developing effective antimicrobial therapies.

DECLARATIONS

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

This article does not contain any studies involving human participants or animals performed by any of the authors.

Informed Consent

Not applicable.

Author Contributions

All authors contributed equally to the conception, design, literature review, drafting, and revision of the manuscript. All authors have read and approved the final version of the manuscript.

Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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Consent for Publication
All authors have reviewed and approved the manuscript and consent to its publication.

Plagiarism Statement
The authors confirm that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere.

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