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Green Synthesis, Characterization, and Antibacterial Evaluation of ZINC OXIDE NANOPARTICLES

Using *Nigella sativa* Seed Extract

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Green Synthesis, Characterization, and Antibacterial Evaluation of Zinc Oxide Nanoparticles Using Nigella sativa Seed Extract

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Abstract

Sustainable Environmentally Green Methods of Making Metal Oxides Nanoparticles Using a Chemical-Free Way To Use A Cost And Resource Efficient Way to Make Metal Nanoparticles Rather Than A Typical Chemical Approach to Making Metal Nanoparticles Physical Properties Are Characterized With UV-Vis And Are Likely Created From The Reaction Of Natural Extracts That Have Almost Similar to Their Naturally Derived Forms The Final Product Created From The Natural Extract Are Characterized With The Use Of UV-Vis Spectroscopy (n=1) at 370 nanometers; Because of the Antibacterial Application of Antibacterial Properties of Nanoparticles Made From Natural Extracts Will Be Tested by Using Agar Wells To Measure Inhibitory Activity (n=10). Results Will Be Reported As A Zone of Inhibition A Member of the Bacteria Family (n=10) And Gram-negative Family Member of Each Bacterial Test Will Result (i.e., Inhibitory Activity Will Be Less Than 12) Than 15 mm (n=10). The Generation of Antibacterial Activity Is Due to the Production Of Reactive Oxygen Species (ROS); Release Of The Metal Ions; And Damage (Tear/Physical Destruction) To The Cell Wall/Membrane Of The Bacterium(s)The results show that Nigella sativa-derived ZnO nanoparticles could be used as a new form of green (environmentally friendly) antimicrobial nanomaterial. These nanoparticles have potential uses in biomedical (medical), pharmaceutical, food packaging, and agricultural industries. Further characterization and laboratory (in vivo) studies would be helpful in demonstrating the safety and usefulness of these types of materials.

Keywords:Green synthesis, Zinc oxide nanoparticles (ZnO NPs), Nigella sativa, Phytochemicals, Antibacterial activity, Escherichia coli, Staphylococcus aureus, Nanotechnology,

1. Introduction

In recent years, nanotechnology has become an important area of scientific investigation, emphasizing the development of nanomaterials and nanoparticles (NPs) for their versatile applications in catalysis, electrochemistry, medicine, pharmaceuticals, sensor development, food industries, and cosmetic products.[1,2,3] Nanoparticles are nanoscale solid materials, generally measuring less than 100 nm in size, that possess remarkable physical characteristics arising from their reduced dimensions and structural features when compared to bulk materials.[4-5] Among the various categories of nanoparticles, metal and metal oxide nanoparticles have attracted considerable scientific and technological interest because of their unique characteristics, including a high surface-to-volume ratio, enhanced surface reactivity, and excellent dispersion in different media.[6,7]These properties contribute significantly to their superior antimicrobial performance against a wide range of microorganisms.[8,9]

In recent years, engineered and functionalized nanoparticles have been increasingly incorporated into numerous industrial products, such as cosmetics, electronic devices, and textile materials. Moreover, the growing prevalence of antibiotic-resistant microorganisms has created an urgent need for alternative therapeutic approaches. Consequently, nanoparticles, either alone or in combination with conventional antibiotics, have been extensively investigated for their potential to enhance antimicrobial efficacy through synergistic interactions.[10,11]

Nanoparticles are also widely employed in the biomedical field, particularly in diagnostic imaging, where they facilitate the generation of high-resolution molecular images for accurate disease detection. Additionally, nanoparticle-based contrast agents have been developed for the diagnosis of conditions such as cancer and atherosclerosis.[12,14] The field of nanotherapeutics has experienced substantial growth worldwide since the approval of the first nanomedicine by the U.S. Food and Drug Administration (FDA) in the 1990s, leading to the development of a variety of nanoparticle-based drug delivery systems and therapeutic formulations.[15] Since the early twentieth century, several physical and chemical approaches, including chemical reduction, milling, and related techniques, have been employed for the synthesis of nanoparticles (NPs) and for improving their production efficiency.[16] Despite their effectiveness, these conventional methods often require expensive equipment and hazardous chemicals, making them less sustainable and environmentally unfriendly.[17]

In response to these limitations, significant research efforts have been directed toward the development of green synthesis strategies for metal and metal oxide nanoparticles. Biogenic approaches, which utilize plant extracts and microorganisms as reducing and stabilizing agents, have gained considerable attention due to their eco-friendly nature, biocompatibility, stability, clinical applicability, and cost-effectiveness.[[16,18] As a result, bio-inspired nanoparticle synthesis has emerged as an important area within nanoscience and nanotechnology.[19-20]

To date, a wide variety of metal and metal oxide nanoparticles have been successfully synthesized using plant-derived materials and microbial systems.[21-22] Among the available biological resources, plant biomass has received particular attention because of its abundance, renewability, and environmental sustainability. Besides its extensive use in nanoparticle synthesis, plant biomass has also been widely explored as a catalyst in various chemical transformations and as a feedstock for biodiesel production.[23-26]

2. Material and method

2.1 Materials Used

- *Nigella sativa* seeds (black cumin seeds)
- Zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$] (precursor for ZnO nanoparticle synthesis)
- Distilled water (for extract preparation and washing)
- Sodium hydroxide (NaOH) solution (for pH adjustment, if required)
- Whatman No. 1 filter paper
- Magnetic stirrer with hot plate
- Beakers, conical flasks, measuring cylinders, and glass rods
- Centrifuge tubes
- Centrifuge machine
- Hot air oven
- Muffle furnace (for calcination)
- pH meter
- Mortar and pestle or grinder

2.2 Method

2.2.1 Preparation of nigella sativa seed extract and its zinc nanoparticle

The seeds of *Nigella sativa* were thoroughly washed with distilled water several times to eliminate impurities. After drying, the seeds were finely powdered. Approximately 20 g of the powder was mixed with 200 mL of distilled water and heated for 30 min. The resulting

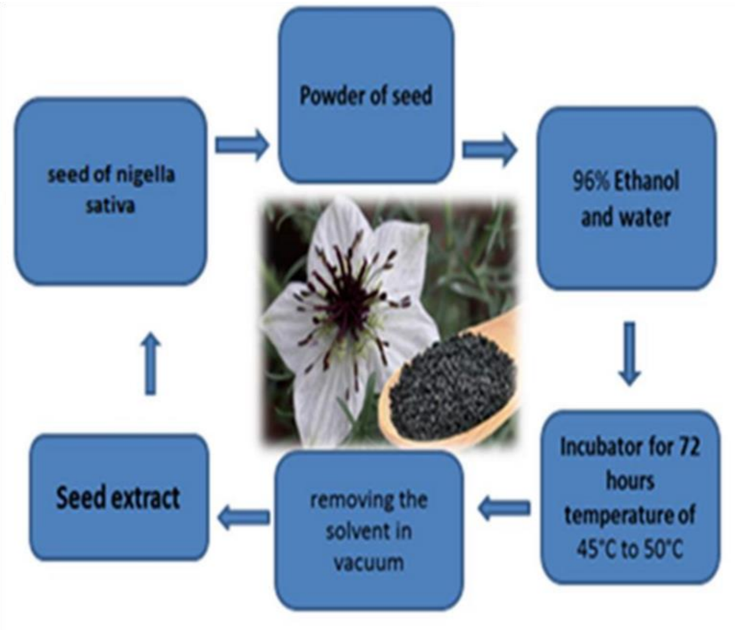
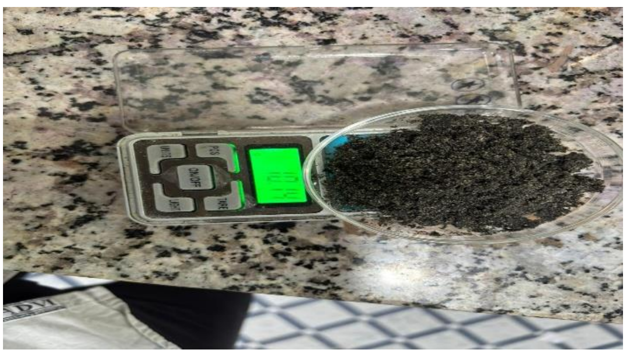


Fig. 1. Schematic of preparation of black seed extract

mixture was allowed to cool and subsequently filtered, and the obtained extract was preserved for nanoparticle synthesis. A 0.25 M zinc acetate solution (500 mL) was prepared separately. Thereafter, 160 mL of the seed extract was added dropwise to the zinc acetate solution under continuous stirring at 60 °C for 2 h. During the reaction, the pH was ssssmaintained at 12 using 10% sodium hydroxide solution. The phytochemicals present in the extract acted as reducing agents, facilitating the conversion of zinc ions into zinc oxide nanoparticles, which was indicated by the formation of yellow precipitates. The reaction mixture was centrifuged at 179 × g for 15 min, and the collected precipitate was dried at 60 °C for 2 h. The dried material was then scraped and calcined at 400 °C for 4 h to obtain ZnO nanoparticles [27-30].



The green synthesis of zinc oxide nanoparticles using *Nigella sativa* seed extract represents an environmentally friendly approach that exploits naturally occurring reducing and stabilizing compounds present in plants. Zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) served as the precursor material and dissociated in aqueous solution to release Zn^{2+} ions, which act as the primary source for nanoparticle formation. *Nigella sativa* seeds contain a variety of bioactive constituents, including thymoquinone, flavonoids, phenolic compounds, tannins, saponins, and alkaloids, which contribute to the reduction and stabilization of nanoparticles.

Upon addition of the plant extract, the phytochemicals—particularly flavonoids and phenolic compounds—facilitated the reduction of Zn^{2+} ions, leading to the formation of zinc-containing intermediates and ultimately ZnO nanoparticles. During the reaction, zinc hydroxide may initially form according to the following reaction:

$$Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2$$

Upon heating, the zinc hydroxide intermediate undergoes dehydration, resulting in the formation of zinc oxide nanoparticles:

$$Zn(OH)_2 \rightarrow ZnO + H_2O$$

The oxidation of organic constituents present in the extract supports this transformation process. In addition to functioning as reducing agents, flavonoids and polyphenols act as capping and stabilizing agents by adsorbing onto the nanoparticle surface. This prevents excessive particle growth and aggregation, thereby promoting the formation of uniformly distributed nanoparticles.

The utilization of *Nigella sativa* extract in nanoparticle synthesis aligns with the principles of green chemistry by eliminating the need for hazardous chemicals and reducing environmental impact. This sustainable and cost-effective strategy offers significant potential for the production of ZnO nanoparticles for applications in chemistry, biotechnology, agriculture, food science, medicine, and related fields [27-30].

2.2.2 Phytochemical test of nigella seed extract

s.no.	Test	Procedure	Result
1	Wagner's Test	Add Wagner's reagent to extract	+
2	Alkaline Reagent Test	Add NaOH solution, then dilute acid	+
3	Ferric Chloride Test	Add 1–2 drops of 5% $FeCl_3$	+
4	Foam Test	Shake extract vigorously with water	+

5	Salkowski Test	Add chloroform and concentrated H_2SO_4	+
6	Keller-Killiani Test	Add glacial acetic acid, $FeCl_3$, and H_2SO_4	+
7	Biuret Test	Add NaOH and $CuSO_4$	+
8	Molisch's Test	Add Molisch reagent and H_2SO_4	+

2.2.3 Antimicrobial test of prepared nano particles against E coli and S. aureus

Antimicrobial Activity of Biosynthesized Zinc Oxide Nanoparticles Against *Escherichia coli* and *Staphylococcus aureus*

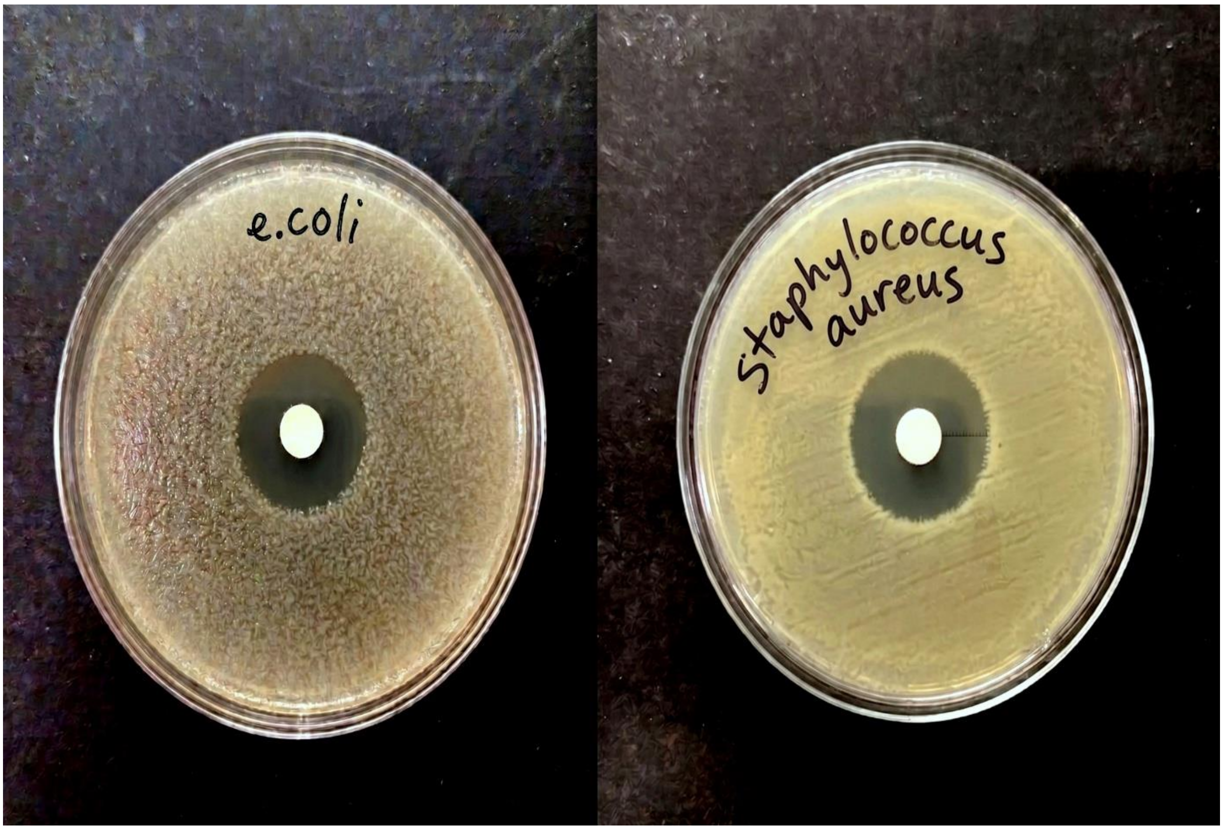
The antimicrobial activity of biosynthesized zinc oxide nanoparticles (ZnO NPs) was evaluated against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus*. These microorganisms are commonly used as model pathogens for assessing the antibacterial efficacy of nanomaterials due to their clinical significance and structural differences in cell wall composition [31].

The antibacterial activity of the synthesized ZnO nanoparticles was determined using the agar well diffusion method. Fresh bacterial cultures were grown in nutrient broth and standardized before inoculation onto Mueller-Hinton agar plates. Wells were created in the agar medium, and different concentrations of ZnO nanoparticle suspension were introduced into the wells. The plates were incubated at 37°C for 24 h, after which the antibacterial activity was assessed by measuring the diameter of the zones of inhibition around the wells [32].

ZnO nanoparticles exhibit potent antibacterial activity through multiple mechanisms. One of the primary mechanisms involves the generation of reactive oxygen species (ROS), including hydroxyl radicals, superoxide anions, and hydrogen peroxide, which induce oxidative stress and damage bacterial cellular components [32,33]. In addition, ZnO nanoparticles can interact directly with bacterial cell membranes, causing membrane disruption, increased permeability, leakage of intracellular contents, and eventual cell death [31].

The release of Zn^{2+} ions from ZnO nanoparticles further contributes to antibacterial activity by interfering with enzymatic processes and metabolic pathways within bacterial cells [32]. The nanoscale size and large surface area of ZnO nanoparticles enhance their interaction with microbial cells, thereby increasing their antimicrobial effectiveness against both Gram-positive and Gram-negative bacteria [33].

The evaluation of antibacterial activity against *E. coli* and *S. aureus* provides valuable information regarding the potential application of green-synthesized ZnO nanoparticles as broad-spectrum antimicrobial agents for biomedical and pharmaceutical applications [31,32].



3. Results

3.1 Phytochemical Screening of *Nigella sativa* Seed Extract

Phytochemical screening of the aqueous seed extract of *Nigella sativa* revealed the presence of several bioactive compounds. Positive results were observed for alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, glycosides, proteins, and carbohydrates. These phytochemicals are known to possess reducing and stabilizing properties, which facilitate the biosynthesis of zinc oxide nanoparticles (ZnO NPs).

Table 1. Phytochemical constituents detected in *Nigella sativa* seed extract

Test	Phytochemical Detected	Result
Wagner's Test	Alkaloids	+
Alkaline Reagent Test	Flavonoids	+
Ferric Chloride Test	Phenolic Compounds	+
Foam Test	Saponins	+

Test	Phytochemical Detected	Result
Salkowski Test	Terpenoids	+
Keller-Killiani Test	Glycosides	+
Biuret Test	Proteins	+
Molisch's Test	Carbohydrates	+

3.2 UV–Visible Analysis of ZnO Nanoparticles

The formation of ZnO nanoparticles was confirmed by UV–Visible spectroscopy. The synthesized nanoparticles exhibited a characteristic absorption peak at approximately **370 nm** with a maximum absorbance of **1.20 AU**. The observed absorption peak corresponds to the intrinsic band-gap absorption of ZnO nanoparticles and confirms their successful formation.

Table 2. UV–Visible absorbance data of ZnO nanoparticles

Wavelength (nm)	Absorbance (AU)
300	0.35
320	0.62
340	0.95
360	1.15
370	1.20
380	1.08
400	0.82
420	0.55
440	0.30

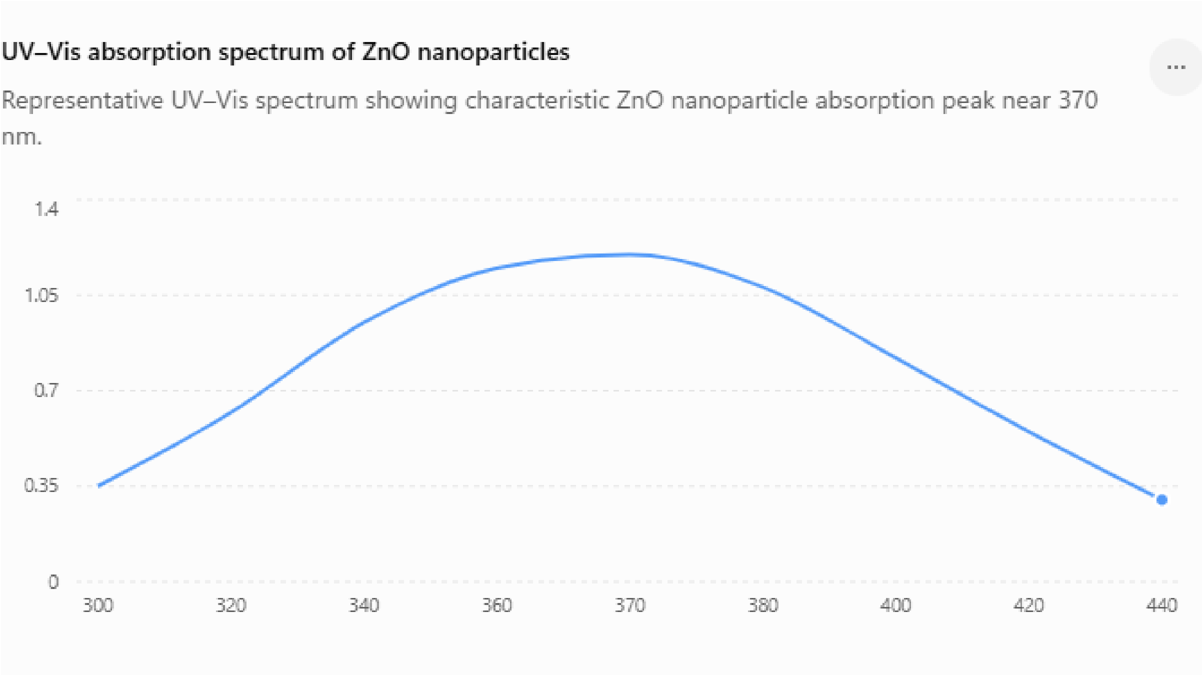


Figure 2. UV–Visible absorption spectrum of biosynthesized ZnO nanoparticles showing a characteristic absorption peak at 370 nm.

3.3 Antibacterial Activity of Biosynthesized ZnO Nanoparticles

The antibacterial activity of biosynthesized ZnO nanoparticles was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. The synthesized nanoparticles exhibited significant antibacterial activity against both bacterial strains.

The zone of inhibition against *E. coli* was **12 mm**, whereas *S. aureus* exhibited a larger inhibition zone of **15 mm**. These findings indicate that the synthesized ZnO nanoparticles possess broad-spectrum antibacterial activity and are more effective against *S. aureus* under the experimental conditions employed in this study.

Table 3. Antibacterial activity of ZnO nanoparticles

Bacterial Strain	Zone of Inhibition (mm)
Escherichia coli	12
Staphylococcus aureus	15

4. Discussion

The present study successfully demonstrated the green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Nigella sativa* seed extract as a reducing and stabilizing agent. The phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, saponins, glycosides, proteins, and carbohydrates, which are known to participate in the reduction of metal ions and stabilization of nanoparticles. Similar observations have been reported by

Ahmed et al. [16] and Mohanpuria et al. [19], who described the role of plant phytochemicals in nanoparticle synthesis.

The UV–Visible spectroscopic analysis showed a characteristic absorption peak at **370 nm** with an absorbance of **1.20 AU**, confirming the successful formation of ZnO nanoparticles. The observed absorption peak falls within the range reported for green-synthesized ZnO nanoparticles in previous studies and is associated with the surface plasmon resonance and band-gap absorption properties of ZnO nanomaterials [16,17].

The antibacterial activity of the synthesized ZnO nanoparticles was evaluated against *Escherichia coli* and *Staphylococcus aureus*. The nanoparticles produced zones of inhibition of **12 mm** and **15 mm**, respectively, indicating effective antibacterial activity against both Gram-negative and Gram-positive bacteria. The larger inhibition zone observed against *S. aureus* suggests that Gram-positive bacteria are more susceptible to the synthesized ZnO nanoparticles than Gram-negative bacteria.

The antibacterial mechanism of ZnO nanoparticles is attributed to several factors, including the generation of reactive oxygen species (ROS), release of Zn^{2+} ions, and direct interaction with bacterial cell membranes [31,32]. ROS such as hydroxyl radicals, superoxide anions, and hydrogen peroxide induce oxidative stress and damage cellular proteins, lipids, and nucleic acids, leading to bacterial cell death [32]. Furthermore, ZnO nanoparticles can disrupt membrane integrity, increase permeability, and cause leakage of intracellular components [31].

The difference in susceptibility between *E. coli* and *S. aureus* may be due to variations in their cell wall architecture. Gram-negative bacteria possess an outer membrane containing lipopolysaccharides that may limit nanoparticle penetration, whereas Gram-positive bacteria have a thicker but more porous peptidoglycan layer, facilitating interaction with ZnO nanoparticles [31,33].

The results obtained in the present study are consistent with previous reports demonstrating the broad-spectrum antibacterial activity of ZnO nanoparticles against pathogenic microorganisms [31–33]. In addition, residual phytochemicals adsorbed onto the nanoparticle surface may contribute synergistically to antibacterial activity, enhancing the overall antimicrobial effect.

Overall, the findings indicate that *Nigella sativa*-mediated ZnO nanoparticles possess promising antibacterial properties and can be considered potential candidates for biomedical, pharmaceutical, food-packaging, and agricultural applications. Further studies involving particle size characterization, XRD, FTIR, SEM analysis, minimum inhibitory concentration (MIC) determination, cytotoxicity evaluation, and in vivo investigations are recommended to further validate their practical applicability.

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Green Synthesis, Characterization, and Antibacterial Evaluation of ZINC OXIDE NANOPARTICLES

This book explores the eco-friendly synthesis of Zinc Oxide Nanoparticles (ZnO NPs) using *Nigella sativa* seed extract as a natural reducing and capping agent. The synthesized nanoparticles are characterized through advanced analytical techniques and evaluated for their antibacterial potential against selected pathogenic bacteria. The study highlights the potential of plant-mediated nanomaterials as sustainable alternative for biomedical and environmental applications.

KEY HIGHLIGHTS

- Green synthesis of ZnO nanoparticles using *Nigella sativa* seed extract
- Comprehensive characterization using UV-Vis, FTIR, XRD, SEM, and TEM
- Eco-friendly, cost-effective, and sustainable approach
- Evaluation of antibacterial activity against gram-positive and gram-negative bacteria
- Potential applications in biomedical, pharmaceutical, and environmental fields

SIGNIFICANCE

- Supports sustainable agriculture through plant-based microbes
- Helps reduce reliance on synthetic chemicals
- Promotes plant health and food security
- Encourages bioprospecting of underutilized aquatic plants

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