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Green Synthesis of Cobalt Oxide Nanoparticles Using *Delonix regia* Leaf

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Abstract

Green synthesis of nanoparticles offers a sustainable and eco-friendly alternative to conventional methods. This study explores the biosynthesis of cobalt oxide nanoparticles (Co_3O_4 NPs) using Delonix regia leaf extract as a reducing and stabilizing agent. The synthesized Co_3O_4 NPs were characterized by UV-visible spectroscopy, Fourier transform infrared (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), confirming their uniform spherical morphology with an average size of 20 nm. The green synthesis method was efficient, cost-effective, and devoid of harmful chemicals, highlighting its potential for large-scale industrial applications. These nanoparticles demonstrated significant antibacterial activity against Escherichia coli, suggesting potential biomedical applications. The advanced technology for synthesizing NPs utilizes natural resources in an environmentally friendly manner. Additionally, green synthesis is preferred to chemical and physical synthesis because it takes less time and effort. The green synthesis of Co_3O_4 NPs has risen due to recently its physicochemical properties. In this study, many functional groups present in Delonix regia leaf extracts are used to stabilize the synthesis of Co_3O_4 NPs. The biosynthesized Co_3O_4 NPs were investigated using UV-visible spectroscopy analysis with highest peak at 436 nm. The XRD analysis showed various peaks ranging from 31.42° to 65.28°, and the highest intensity showed at 36.86°. The particle size ranged from 15 to 35 nm and confirmed that the average particle size is 20 nm. SEM images of green synthesized Delonix regia Co_3O_4 NPs showed agglomerated and uniform spherical particles. The anti-bacterial activity of green synthesized Delonix regia Co_3O_4 NPs was evaluated against Gram-negative Escherichia coli.

Keywords: Green synthesis, cobalt oxide nanoparticles, *Delonix regia*, antibacterial activity, sustainable nanotechnology, biosynthesis

INTRODUCTION

Nanotechnology has revolutionized material science with its applications spanning electronics, catalysis, and medicine. Among these, cobalt oxide nanoparticles (Co_3O_4 NPs) have garnered attention due to their unique optical, magnetic, and catalytic properties. Traditional methods for synthesizing Co_3O_4 NPs often involve hazardous chemicals and energy-intensive processes. To address these challenges, green synthesis using plant extracts has emerged as a sustainable alternative. This study employs *Delonix regia* leaf extract to synthesize Co_3O_4 NPs, leveraging the bioactive compounds in the extract for eco-friendly production [1].

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MATERIALS AND METHODS

Cobalt chloride hexahydrate ($CoCl_26H_2O$), sodium hydroxide pellets (NaOH), and hydrochloric acid (HCl) were purchased from

HiMedia Pvt. Ltd. (India); ethanol (EtOH, 99.5%) was purchase from Scharlau. *Delonix regia* leaves were collected from the local area. Distilled water was used throughout the experiments.

Instruments

- *Laminar air flow*: It is described as airflow in which the direction and velocity of all of the inside a given space are uniform (Figure 1).
- *Magnetic stirrer:* It is a laboratory tool that uses a rotating magnetic field to spin a stir bar (or flea) submerged in a liquid, churning it. A rotating magnet or group of stationary electromagnets positioned beneath the liquid-containing vessel can provide the field (Figure 2).
- *Thermostatic water bath*: It is a specialized liquid heating and cooling device. It can be used in units for chemical, pharmaceutical, environmental protection, scientific research, and other purposes to heat or cool liquids while maintaining a consistent temperature (Figure 3).
- *Centrifuge*: An apparatus that consistently applies centrifugal force a force brought on by rotation is referred to as a centrifuge. In essence, the centrifuge replaces gravity with a force that is equivalent to it but stronger (Figure 4).
- *Vertical autoclave*: An autoclave that uses the moist heat sterilization method is known as a vertical autoclave (Figure 5).

Figure 1. Picture of laminar air flow.

Figure 2. Picture of magnetic stirrer.

Figure 3. Picture of thermostatic water bath.

Figure 4. Picture of centrifuge.

Figure 5. Picture of vertical autoclave.

Figure 6. Picture of sonicator/ultrasonic bath.

Figure 7. Picture of UV-visible spectrophotometer.

- *Sonicator/ultrasonic bath*: A sonicator is an electronic device that performs sonication, or applying sound energy to the target sample. The apparatus converts electrical energy into ultrasonic sound energy in the form of an ultrasonic bath or an ultrasonic probe (or probe sonicator) (Figure 6).
- *UV-visible spectrophotometer*: A spectrophotometer is an instrument that scientists use to determine information about an object or substance through the analysis of its light properties (Figure 7).

Methods

Preparation of Delonix regia Leaf Extract

The fresh *Delonix regia* (*gulmohar*) leaves were washed thoroughly with tap water and subsequently using distilled water. Then, we allowed it to dry at room temperature. After drying at room temperature, 120 g of *Delonix regia* leaves were weighed and added to 400 mL of de-ionized water. Then these leaves were chopped properly in a mechanical blender [2–4].

Then the mixture was heated on magnetic stirrer for 30 minutes at 60°C after which the mixture was allowed to cool down at room temperature and then centrifuged at 8000 rpm for 15 minutes at room temperature. Then we filtered the mixture through Whatman filter paper to obtain final yellowish color leaf extract which was stored at 4°C for further use (Figure 8(a)–(g)).

Biosynthesis of Co_3O_4 NPs

Prepare 0.1 M cobalt chloride hexahydrate salt solution, that is, 4.72 g of cobalt chloride hexahydrate was added in 200 mL of distilled water. Then allow heating of cobalt salt solution on hot water bath; meanwhile add 20 mL of *Delonix regia* leaf extract drop by drop in cobalt salt solution then heat the final solution for 15 min at 60°C. Upon addition, the solution mixture turned to dark brown color.

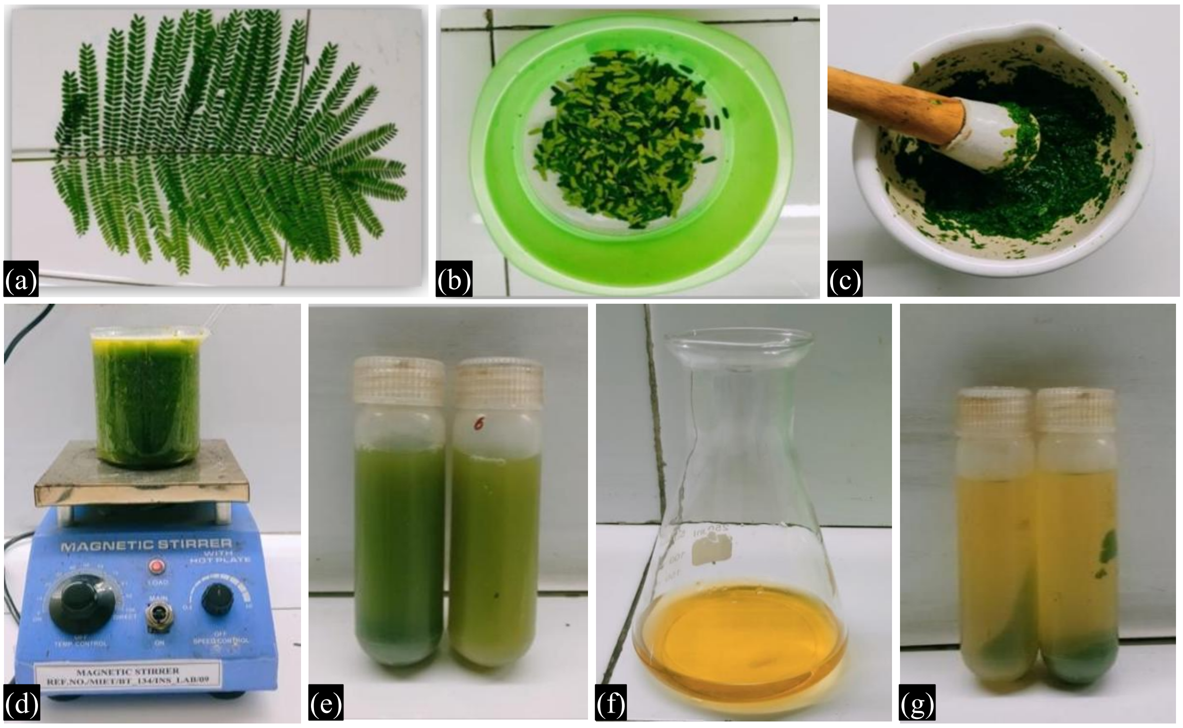

Figure 8. (a) *Delonix regia* plant leaves, (b) washed leaves of *Delonix regia*, (c) crushed leaves of *Delonix regia*, (d) heating of leaves on a magnetic stirrer, (e) leaf extract before centrifugation, (f) leaf extract after centrifugation, and (g) final leaf extract in yellowish color.

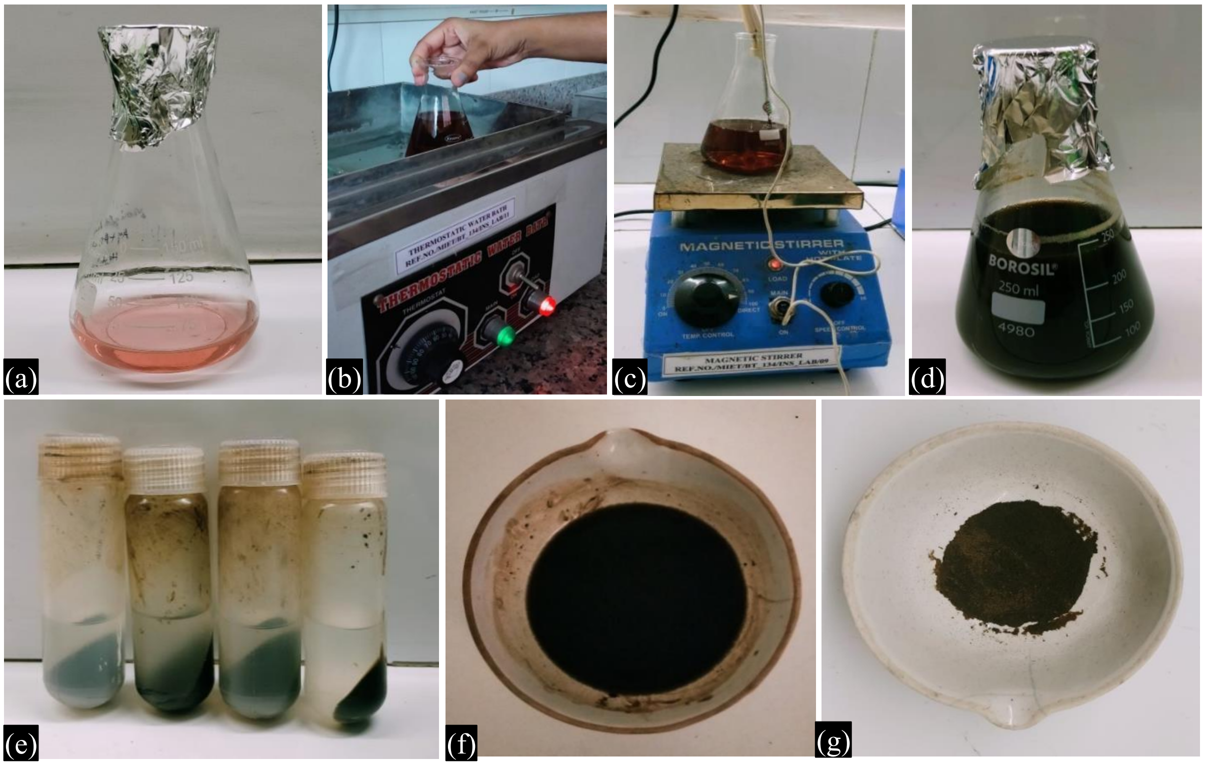

Figure 9. (a) Cobalt chloride hexahydrate solution, (b) cobalt salt solution with 20 mL of *Delonix regia* leaf extract heating on hot water bath, (c) cobalt salt solution with 20 mL of *Delonix regia* leaf extract heating on a magnetic stirrer, (d) final solution which may contains cobalt oxide nanoparticles, (e) after centrifugation we obtained pellets which was collected in a crucible, (f) obtained pellet of cobalt salt solution which contains *Delonix regia* leaf extract, (g) final cobalt oxide nanoparticles.

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Meanwhile prepare 0.1 M NaOH solutions, that is, 0.4 g of NaOH in 50 mL of distilled water. Finally, 0.1 M NaOH solution was added drop by drop as a catalyst till the pH of the mixture reached 12. As the pH of the solution turned to pH 12, color change was observed from brownish to blackish and precipitate was formed and allowed to settle down. Finally, the obtained precipitate was allowed to centrifuge (three times with water and one time with ethanol) at 10 000 rpm for 15 minutes at room temperature. Then the obtained residue was air dried for 1 day at 80°C. The residue was further crushed and calcinated at 500°C for 2 h in a muffle furnace. Finally, a black colored powder was obtained, and further characterization was carried out (Figure 9(a)–(g)) [5–7].

RESULTS AND DISCUSSION

UV-Visible Spectra of Co_3O_4 NPs

The technique employed to characterize these synthesized nanoparticles was UV-visible spectroscopy. The UV-visible absorption spectrum of the created Co_3O_4 NPs is shown in Figure 10. The greatest absorption peaks for Co_3O_4 NPs are at 230 and 436 nm. Due to quantum confinement and tiny molecules, the energy band gaps of the extracts of Co_3O_4 NPs are 3.6 eV and 2.85 eV. The position and shape of plasmon absorption of cobalt nanoclusters were strongly dependent on the particle size, dielectric medium and surface-adsorbed species (Figure 10) [8].

Fourier-Transform Infrared (FTIT) Spectroscopy

FT-IR analysis was performed to find out the different functional groups that are present in leaf extract of *Delonix regia* and are helpful for the synthesized nanoparticles and act as capping and stabilizing agents. The IR spectrum of synthesized NPs is shown in Figure 11.

X-Ray Diffraction (XRD) Analysis

By using XRD analysis, the structural characteristics of Co_3O_4 NPs were identified. By using XRD analysis, the crystal structure of Co_3O_4 NPs was investigated. X-ray diagram of CoNPs is depicted in Figure 12. Additionally, the face-centered cubic (FCC) crystal planes (220), (311), (400), (511) and (440) were represented by the diffraction peaks at 31.42°, 36.86°,45.02°, 59.46° and 65.28°, respectively. The Co_3O_4 NPs diffraction 2θ values correspond to the values from the JCPDS No. 073-1701 standard database. Using Scherer's equation, the crystalline size of Co_3O_4 NPs was determined from the peak with the maximum intensity as shown below:

$$D = 0.9\lambda / \beta \cos\theta$$

where D is the size of the Co_3O_4 NPs crystallites, λ is the wavelength of the X-ray source used in XRD (0.1541 nm), and β is the entire width at half maximum of the diffraction peak. The average crystallite size for as-synthesized Co_3O_4 NPs was found to be 20 nm (Figure 12).

Scanning Electron Microscopy (SEM)

The SEM images of Co_3O_4 NPs at different magnifications clearly exhibit the nanoparticles like morphology indicating well uniform particles with narrow size distribution lies in the range of 25 to 35 nm. The surface of as synthesized nanoparticles is very smooth, which facilitates the better contact with the bacterial cell wall and hence increases bacterial killing ability of NPs. Such behavior of smooth surfaced NPs has already been established in the literature (Figure 13).

Transmission Electron Microscopy (TEM)

TEM was performed to know about the structural features of synthesized Co_3O_4 NPs. TEM showed that the synthesized product possesses single morphology. In the large area TEM image, it can be seen that few NPs are aggregated together in the form of groups. It is believed that these NPs are well dispersed in solution and are aggregated during analyte preparation. In order to know more about morphology of Co_3O_4 NPs, TEM images at higher magnifications were captured. It can be seen that most of the NPOs are spherical shaped and size estimated from the TEM images lies in the range of about 15 to 35 nm (Figure 14).

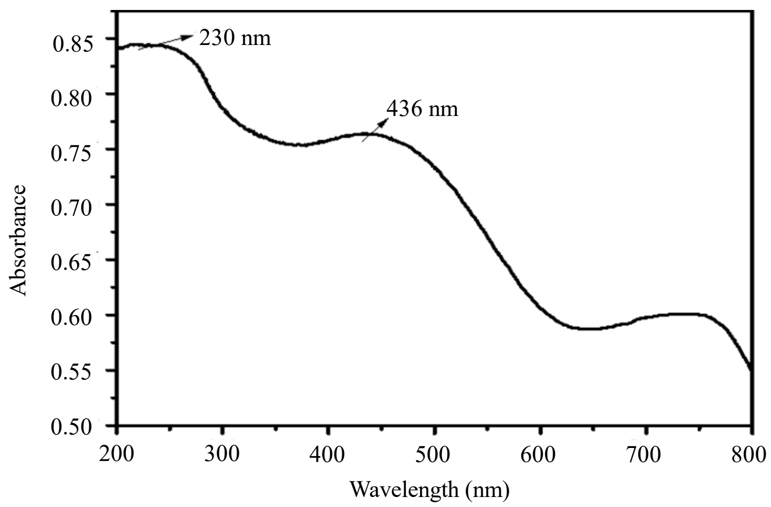

Figure 10. UV–vis spectra of bio-synthesized Co_3O_4 nanoparticles (NPs).

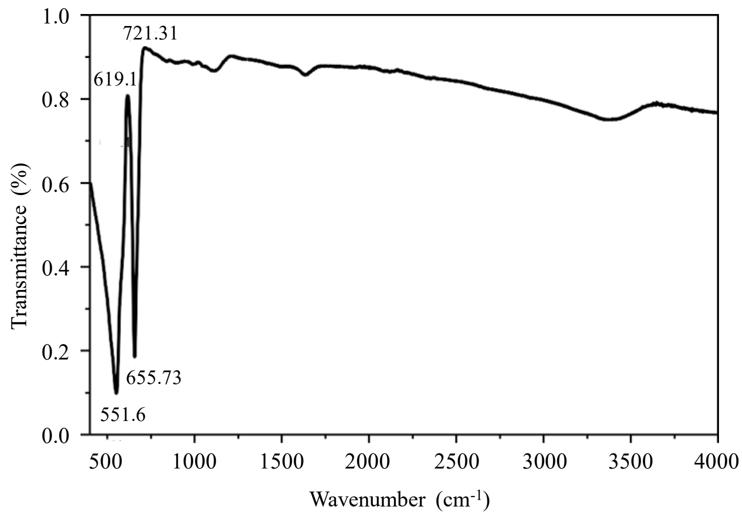

Figure 11. Fourier transform infrared (FTIR) spectrum of bio-synthesized Co_3O_4 nanoparticles (NPs).

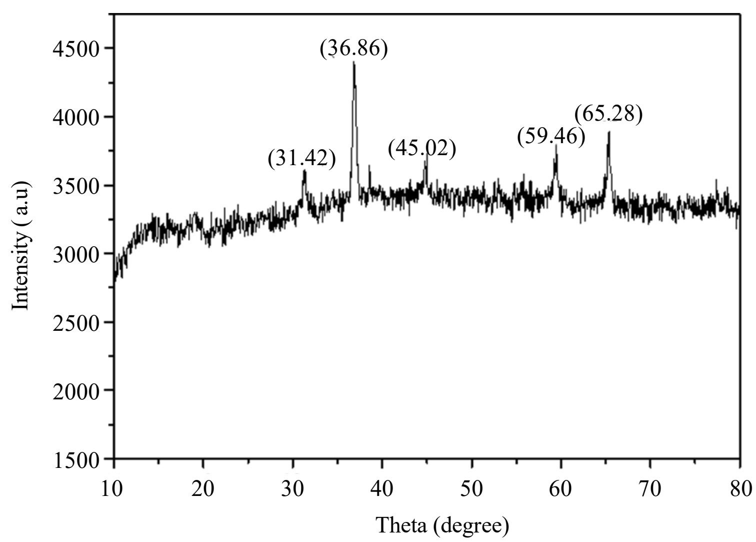

Figure 12. X-ray diffraction pattern of synthesized Co_3O_4 nanoparticles (NPs).

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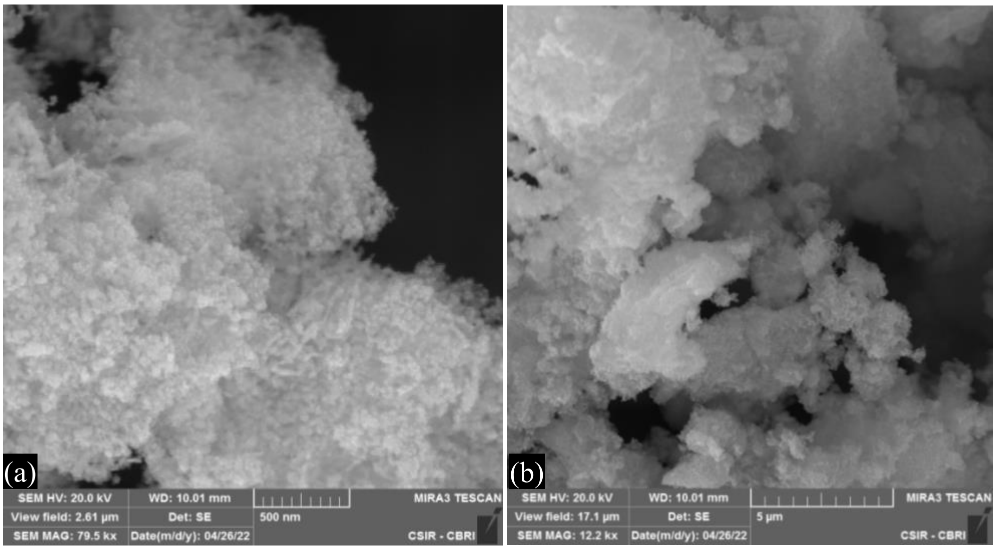

Figure 13. Scanning electron microscopy (SEM) images of the green synthesized Co_3O_4 nanoparticles (NPs).

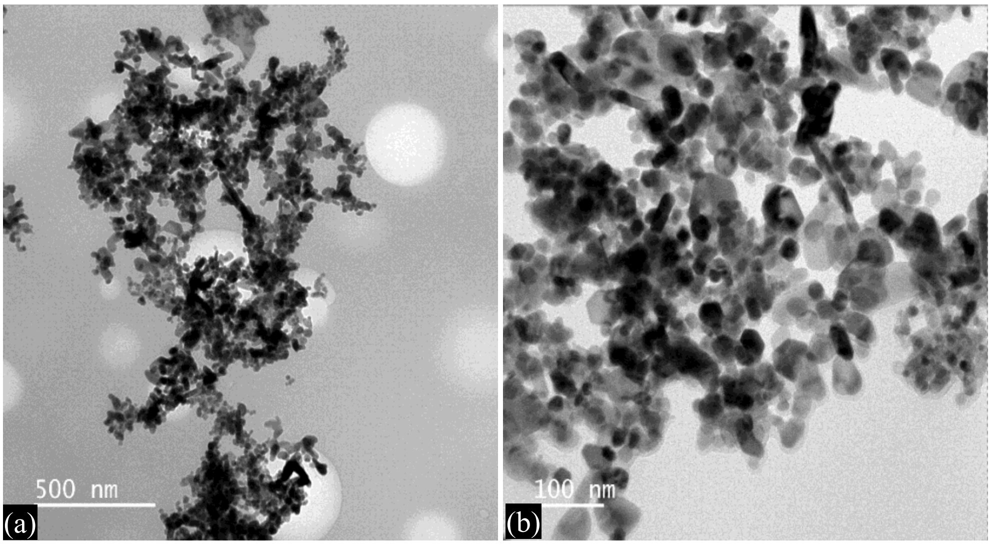

Figure 14. Transmission electron microscopy (TEM) images of the green synthesized Co_3O_4 nanoparticles (NPs).

Antibacterial Assay

Green synthesized Co_3O_4 NPs were analyzed for antibacterial activity against *Escherichia coli* by agar well diffusion method. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The result indicated the synthesized Co_3O_4 NPs using *Delonix regia* leaf extract have stronger antibacterial activity. The surfaces of the CoNPs might have interacted directly with the bacterial outer membrane, causing the membrane to rupture thereby killing the organism. So, the antibacterial activity exhibited by the Co_3O_4 NPs here is attributed to their small size and high peptidoglycan wall with less permeability. So based upon the structural characteristics of cell walls of both type of bacteria, we assume an enhanced permeability and hence lower activity of Co_3O_4 NPs for Gram-negative bacteria. Such results indicate the potential of these NPs against human pathogens. In this, bacterial activity minimum zone of inhibition (16.0 ± 0.8) was found for *Escherichia coli*. The Gram-negative bacteria have thin peptidoglycan cell wall and non-porous in nature with lower permeability and such a structure of cell wall facilitates the minimum absorption of these NPs. Also, the size of NPs is small which ensures the maximum absorption of NPs by bacterial species and hence resulting into their death [9, 10].

DISCUSSION

The green synthesis of Co_3O_4 NPs involves reducing cobalt chloride with *Delonix regia* leaf extract, leading to uniform NPs with a particle size ranging between 15 and 35 nm. UV-vis spectroscopy revealed characteristic absorption peaks at 436 nm, indicative of Co_3O_4 NPs. FTIR analysis identified functional groups responsible for stabilizing the NPs. XRD confirmed the crystalline structure, while SEM and TEM analyses highlighted their spherical morphology. The antibacterial assay demonstrated significant zones of inhibition against *Escherichia coli*, suggesting enhanced interaction of NPs with bacterial membranes due to their nanoscale size and high surface area.

The green synthesis method aligns with sustainability goals, minimizing environmental hazards and reducing costs. However, challenges such as scalability and precise control over particle size and shape warrant further research. Optimizing reaction parameters and exploring diverse plant extracts could enhance the versatility of this approach [11, 12].

CONCLUSION

The study underscores the potential of *Delonix regia* leaf extract in synthesizing Co_3O_4 NPs, combining efficiency with environmental sustainability. The synthesized NPs exhibit promising antibacterial properties, paving the way for their application in biomedicine and environmental remediation. Further studies should focus on scaling up the process and exploring its efficacy against other pathogens. Co_3O_4 NPs were successfully synthesized using *Delonix regia* leaves, employing a green, eco-friendly, and cost-effective method. Characterization techniques like FTIR, XRD, TEM, and SEM confirmed the synthesis. The NPs exhibited antibacterial activity against *Escherichia coli*, with activity increasing with concentration. The particle size ranged from 21 to 55 nm, suggesting potential for treating infections caused by these bacteria. Though there is significant research on the biosynthesis of other NPs (gold, silver, zinc oxide), cobalt oxide synthesis deserves further attention. The green synthesis method requires refinement to control particle size and shape. More plant species should be explored for the biogenic synthesis of metal oxide NPs, and further research is needed to understand the mechanism and improve applications.

Future Aspects

Nanoparticles are crucial for enhancing catalytic reactions due to their unique properties. Green synthesis methods, as opposed to physical and chemical approaches, are more environmentally friendly. However, toxicity and antibacterial effectiveness need further investigation, including clinical studies on cobalt dressings. Research in green nanotechnology will improve nanoparticle synthesis processes and their applications in various fields. The role of functional groups in nanoparticle synthesis should be explored to optimize the process for industrial use in chemistry, electronics, medicine, and agriculture.

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