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Original Research Article

Dual Inhibitory and Antibiofilm Activities of Cobalt Oxide Nanoparticles Against Escherichia coli: MIC-Guided Nanostructure–Biofilm Interactions

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Abstract:
Biofilm-associated infections caused by Escherichia coli remain a significant clinical challenge due to their inherent resistance to conventional antibiotics. The emergence of biofilm-forming Escherichia coli strains poses a critical barrier to effective infection control, necessitating the exploration of nanomaterials as alternative antibiofilm agents. Nanomaterials with high surface activity have recently emerged as promising alternatives for combating biofilm formation. In this study, cobalt oxide nanoparticles (Co_3O_4 NPs) were synthesized via a green chemistry route and evaluated for their dual role inhibitory and antibiofilm efficacy against E. coli. Physicochemical characterization using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and Transmission electron microscopy (TEM) confirmed the crystalline cubic spinel structure, surface functional groups, and nanoscale morphology of the particles. Antibiofilm activity was assessed using the crystal violet microtiter plate assay, while minimum inhibitory concentration (MIC) was determined by broth microdilution. The Co_3O_4 NPs exhibited a MIC value of 0.25 mg/mL, effectively inhibiting planktonic growth. Biofilm inhibition assays demonstrated a concentration-dependent reduction in biofilm biomass, with a maximum inhibition of 66.9 % at MIC levels. The antibiofilm efficacy is attributed to the high surface adsorption capacity of cobalt oxide nanoparticles, which facilitates nanoparticle–bacteria interactions. These results highlight the potential of cobalt oxide nanoparticles as multifunctional adsorbents with significant antibiofilm activity, opening avenues for their application in antimicrobial coatings, water treatment, and nano biomaterials.

Keywords: Cobalt oxide nanoparticles, Escherichia coli, Antibiofilm activity, Minimum Inhibitory Concentration (MIC), Nanostructure–biofilm interaction, Biofilm disruption

INTRODUCTION

The emergence of multidrug-resistant (MDR) bacterial strains has intensified the global search for alternative antimicrobial strategies. Among these, biofilm-forming pathogens such as *Escherichia coli* pose a significant challenge due to their ability to resist conventional antibiotics and persist in hostile environments [3]. Biofilms are structured microbial communities encased in extracellular polymeric substances (EPS), which protect bacteria from immune responses and antimicrobial agents, contributing to chronic infections and contamination in medical and industrial settings [4]. Biofilm formation by pathogenic bacteria such as *Escherichia coli* represents a major global challenge, particularly in healthcare and environmental systems. Biofilms confer enhanced resistance to antibiotics and conventional antimicrobial treatments, making infections persistent and difficult to eradicate. *Escherichia coli*, a common Gram-negative bacterium,

is known for its ability to form robust biofilms, contributing to persistent infections and contamination in water systems and medical devices. The extracellular polymeric substances (EPS) within biofilms act as a protective barrier, limiting the penetration of antimicrobial agents and facilitating bacterial survival under hostile conditions [5]. *E. coli*, a Gram-negative facultative anaerobe, is widely recognized for its role in urinary tract infections, gastrointestinal disorders, and nosocomial infections. Its capacity to form biofilms on various surfaces—including medical implants, catheters, and water pipelines—makes it a critical target for antibiofilm research. Traditional antibiotics often fail to penetrate the EPS matrix, necessitating the development of novel materials that can both inhibit bacterial growth and disrupt biofilm architecture. Biofilms are highly structured microbial communities encased in a self-produced extracellular matrix that confer protection against antibiotics and host defences. Among pathogenic bacteria, *Escherichia coli* is a prominent biofilm-former and is responsible for a wide range of infections, particularly urinary tract and device-associated infections, where conventional antibiotics often fail to eradicate the pathogen [1,2]. This has necessitated the search for alternative strategies capable of disrupting biofilms and overcoming microbial resistance [8]. The persistence of *E. coli* biofilms complicates treatment, increases hospitalization costs, and contributes to antimicrobial resistance, underscoring the urgent need for novel biofilm control strategies.

Nanotechnology has emerged as a promising strategy to combat biofilm-related issues, offering Metal oxide nanoparticles with enhanced surface reactivity and tunable physicochemical properties, including high surface area, morphology, and reactive surface chemistry. These features enable nanoparticles to interact directly with bacterial membranes and biofilm matrices, leading to enhanced antimicrobial efficacy [11]. Among various metal oxide nanoparticles, cobalt oxide (Co_3O_4) has gained attention for its unique redox activity, stability and ability to generate reactive oxygen species (ROS) under physiological conditions. However, its role in disrupting biofilm architecture and inhibiting bacterial growth remains underexplored. Nanoparticle-based approaches have gained attention due to their high surface-to-volume ratio, adsorption capability, and ability to generate reactive oxygen species (ROS) that disrupt microbial physiology. Cobalt oxide nanoparticles (Co_3O_4 NPs) are transition metal oxides with unique structural, electrical, and surface properties [12]. Their potential as antimicrobial and antibiofilm agents has been underexplored compared to other nanomaterials such as silver and zinc oxide. In particular, their adsorptive surface interactions with bacterial cells may provide a promising mechanism for biofilm inhibition. Importantly, green synthesis routes using plant extracts provide an eco-friendly, cost-effective, and sustainable alternative to conventional chemical methods, eliminating toxic by-products and enhancing biocompatibility. Green synthesis employs biological resources such as plant extracts, microorganisms, or biopolymers as reducing and stabilizing agents. Plant-based synthesis, in particular, offers several advantages, including rapid synthesis, scalability, biocompatibility, and incorporation of bioactive phytochemicals that may synergistically enhance biological activity [11]. The present study demonstrates the green synthesis of cobalt oxide (Co_3O_4) nanoparticles using ginger extract, representing a clean, cost-effective, and sustainable alternative to conventional chemical and physical synthesis routes. Unlike previous studies that relied on toxic precursors, high energy consumption, or synthetic surfactants, the use of *Zingiber officinale* provides biogenic phytochemicals (gingerol, shogaol, and zingerone) that serve simultaneously as reducing and capping agents. This eliminates hazardous waste generation and aligns with green chemistry principles. Ginger (*Zingiber officinale*), a widely used medicinal plant, has been extensively studied for its therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant effects [12]. The rhizome of ginger contains a diverse array of phytochemicals, such as gingerols, shogaols, paradols, flavonoids, phenolics, and terpenoids [13]. These compounds act as natural reducing agents that facilitate the conversion of cobalt salts into cobalt oxide nanoparticles while simultaneously stabilizing the nanoparticles to prevent aggregation [14]. Moreover, the inherent antimicrobial properties of ginger-derived phytochemicals may impart additional synergistic effects to the nanoparticles, enhancing their antibiofilm efficacy [15]. Recent reports have demonstrated the potential of phytogenic cobalt oxide nanoparticles against a range of bacterial pathogens. For instance, green-synthesized Co_3O_4 NPs have shown significant antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, suggesting broad-spectrum antimicrobial potential [16]. However, systematic investigations into their activity against *E. coli* biofilms, particularly using ginger extract as the reducing agent, remain limited. While ginger-mediated synthesis of silver, zinc oxide, and copper oxide nanoparticles has been widely explored [17–19], cobalt oxide remains relatively underexplored despite its promising redox properties and biofilm inhibition capabilities.

Conventional antibiotic therapy often fails to eliminate biofilms effectively, as bacterial cells within biofilms may be up to 1000 times more resistant to antimicrobial agents compared to their planktonic counterparts [5]. This has fuelled intensive research into alternative approaches, including the use of nanomaterials, which offer unique properties that can overcome the limitations of traditional antimicrobials.

Metal and metal oxide nanoparticles have demonstrated promising antimicrobial and antibiofilm activities, attributed to their nanoscale size, high surface area-to-volume ratio, and ability to interact with microbial membranes and intracellular targets [6]. Among various nanomaterials, cobalt oxide nanoparticles (Co_3O_4 NPs) have emerged as particularly attractive due to their versatile properties. Co_3O_4 is a p-type semiconductor with a spinel structure, known for its redox activity, oxygen storage capability, and catalytic behaviour [7]. These characteristics make it suitable not only for energy and environmental applications but also for biomedical purposes, including antimicrobial therapy. The antimicrobial mechanism of cobalt oxide nanoparticles is thought to involve multiple pathways: (i) generation of reactive oxygen species (ROS) that induce oxidative stress and damage bacterial proteins, lipids, and nucleic acids; (ii) direct interaction with bacterial cell walls and membranes, leading to structural disruption; and (iii) interference with quorum sensing and biofilm formation processes [8,9]. Such multifaceted mechanisms reduce the likelihood of bacterial resistance development, positioning Co_3O_4 NPs as a valuable candidate for biofilm inhibition. Recent reports have demonstrated the potential of phytogenic cobalt oxide nanoparticles against a range of bacterial pathogens. For instance, green-synthesized Co_3O_4 NPs have shown significant antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, suggesting broad-spectrum antimicrobial potential [16]. However, systematic investigations into their activity against *E. coli* biofilms, particularly using ginger extract as the reducing agent, remain limited. While ginger-mediated synthesis of silver, zinc oxide, and copper oxide nanoparticles has been widely explored [17–19], cobalt oxide remains relatively underexplored despite its promising redox properties and biofilm inhibition capabilities. Given the increasing prevalence of biofilm-associated *E. coli* infections and the limitations of existing therapeutic options, there is a pressing need to explore novel, sustainable, and effective nanomaterials. The present study focuses on the green synthesis of cobalt oxide nanoparticles using ginger extract and evaluates their inhibitory and antibiofilm activities against *Escherichia coli*. The work aims to (i) synthesize and characterize Co_3O_4 NPs via a phytogenic route, (ii) determine the minimum inhibitory concentration (MIC) against *E. coli*, and (iii) assess their efficacy in disrupting biofilm formation using Light microscopy. By integrating the dual benefits of nanotechnology and phytochemistry, this research provides valuable insights into the development of eco-friendly antimicrobial nanomaterials with potential applications in combating biofilm-associated infections, in antimicrobial coatings, water purification, and nano biomedical platforms.

MATERIALS

Analytical-grade cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$), nutrient broth, crystal violet, ethanol, and all other chemicals were procured from HiMedia Laboratories (Mumbai, India). Fresh ginger (*Zingiber officinale*) rhizomes were purchased from a local market, thoroughly washed, and air-dried before use. All glassware was cleaned with distilled water and dried prior to synthesis. *Escherichia coli* (MTCC 443) was used as the test strain for antibacterial and antibiofilm studies.

Methodology

Preparation of Ginger Extract

Fresh ginger (*Zingiber officinale*) rhizomes were thoroughly washed with distilled water to remove adhering impurities and surface contaminants. The cleaned rhizomes were then chopped into small pieces and crushed using a mortar and pestle without applying heat to preserve the natural phytochemicals. The resulting pulp was filtered through Whatman No. 1 filter paper, and the obtained filtrate was collected as a concentrated ginger extract. The extract was stored at 4 °C and can used freshly as a reducing and stabilizing agent for the green synthesis of cobalt oxide nanoparticles.

Green synthesis of Cobalt Oxide Nanoparticles

Cobalt oxide nanoparticles (Co_3O_4 NPs) were synthesized via a green route using the prepared ginger extract as a bio reductant and capping agent. A 0.1 M aqueous solution of cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$) was prepared in 50 mL of distilled water and stirred magnetically at room temperature. To this solution, 10 mL of freshly prepared ginger extract was added dropwise under constant stirring. The mixture was stirred for 2 h at ambient temperature, during which the colour gradually changed from pinkish to dark brown, confirming the initiation of nanoparticle formation. The reaction mixture was allowed to stand undisturbed for 24 h to complete the reduction process. The resulting precipitate was centrifuged at 8000 rpm for 15 min, and the collected pellet was washed repeatedly with distilled water and ethanol to remove unreacted species and organic residues. The purified nanoparticles were oven-dried at 80 °C for 6 h and subsequently calcined at 400 °C for 3 h in a muffle furnace to obtain phase-pure crystalline Co_3O_4 NPs suitable for characterization and biological evaluation.

Characterization of Cobalt Oxide Nanoparticles

The synthesized cobalt oxide nanoparticles (Co_3O_4 NPs) were characterized using various analytical techniques. Fourier transform infrared (FTIR) spectroscopy (4000–400 cm^{-1}) identified functional groups from the ginger extract involved in reduction and stabilization, along with characteristic Co–O

stretching vibrations. The crystalline structure and average crystallite size were determined by X-ray diffraction (XRD) using Cu Kα radiation (λ = 1.5406 Å). Transmission electron microscopy (TEM) provided information on particle morphology, size, and dispersion.

Minimum Inhibitory Concentration Assay

The antibacterial activity of the synthesized Co_3O_4 nanoparticles was evaluated against *Escherichia coli* using the broth microdilution method to determine the minimum inhibitory concentration (MIC). Briefly, bacterial cultures were grown overnight in nutrient broth at 37 °C and adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1×10^8 CFU/mL). Two-fold serial dilutions of Co_3O_4 NPs (ranging from 10 to 200 µg/mL) were prepared in sterile 96-well microplates containing nutrient broth. Each well was inoculated with 100 µL of bacterial suspension and incubated at 37 °C for 24 h. Wells without nanoparticles served as positive controls, while sterile broth acted as the negative control. After incubation, bacterial growth was assessed visually and spectrophotometrically at 600 nm. The MIC was recorded as the lowest nanoparticle concentration that completely inhibited visible bacterial growth compared to the control.

Antibiofilm Assay

The antibiofilm activity of the synthesized Co_3O_4 nanoparticles was evaluated against *Escherichia coli* using the microtiter plate crystal violet assay. Briefly, overnight bacterial cultures were adjusted to 0.5 McFarland standard and diluted 1:100 in fresh nutrient broth supplemented with different concentrations of Co_3O_4 NPs (25–200 µg/mL). Aliquots (200 µL) were dispensed into sterile 96-well microplates and incubated at 37 °C for 24 h under static conditions to allow biofilm formation. After incubation, wells were gently washed three times with phosphate-buffered saline (PBS, pH 7.4) to remove non-adherent cells, air-dried, and stained with 0.1% (w/v) crystal violet for 15 min. The excess stain was removed, and the wells were rinsed thoroughly with distilled water. The bound dye was solubilized using 95% ethanol, and absorbance was measured at 570 nm. The percentage inhibition of biofilm formation was calculated relative to the untreated control. The inhibition by Co_3O_4 nanoparticles was calculated using the formula:

$$\% \text{ Biofilm inhibition} = \frac{[\text{Control recorded at } OD_{570}\text{nm} - \text{Test recorded at } OD_{570}\text{nm}]}{\text{Control recorded at } OD_{570}\text{nm}} \times 100$$

RESULTS AND DISCUSSION

Functional Group Analysis (FTIR)

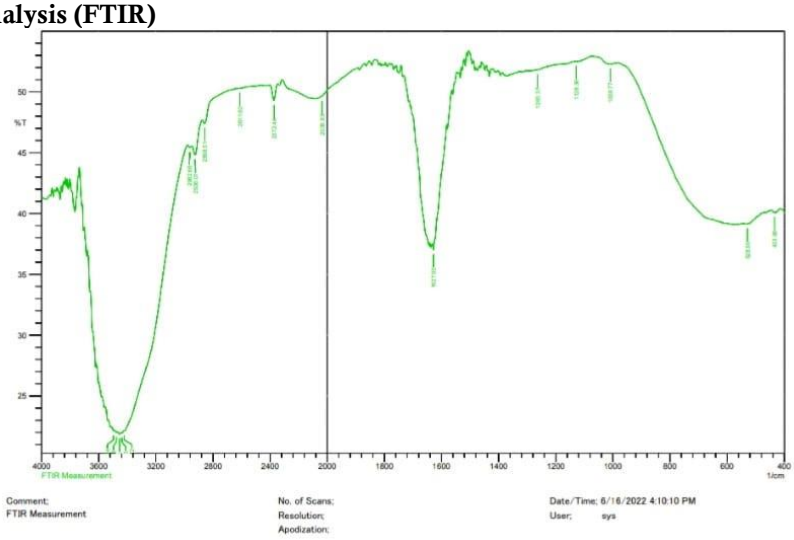

Fig. 1 FTIR Spectrum of cobalt oxide nanoparticles using ginger extract

FTIR spectroscopy revealed the presence of O–H, C=O, and C–O functional groups originating from the ginger extract, confirming the role of phytochemicals in the reduction and stabilization of the nanoparticles. Additionally, characteristic Co–O stretching bands were observed, further validating the successful formation of cobalt oxide. The capping by phytochemicals likely contributed to the enhanced stability and dispersibility of the nanoparticles. A broad band at ~3426 cm^{-1} corresponds to O–H stretching vibrations, indicating hydroxyl groups from water or phenolic compounds that likely contributed to nanoparticle reduction and stabilization. Peaks at ~2911 cm^{-1} and ~1638 cm^{-1} are attributed to C–H and C=O stretching vibrations, suggesting aldehyde or ketone groups in the extract. The absorption at ~1422 cm^{-1} corresponds to C–C stretching of aromatic compounds, while the peak at ~1056 cm^{-1} indicates C–O stretching of primary alcohols. Additionally, bands at ~504 cm^{-1} and ~664 cm^{-1} are characteristic of Co–O stretching vibrations, confirming the formation of spinel-structured Co_3O_4 nanoparticles. These observations

collectively demonstrate that phytochemicals in the ginger extract acted as both reducing and stabilizing agents during the green synthesis process.

Morphology and Size (TEM)

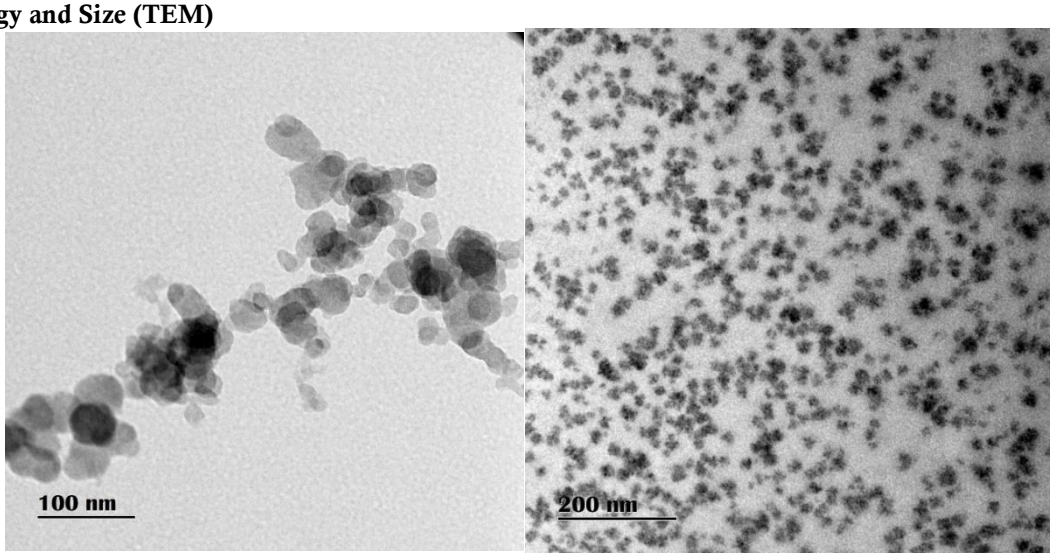

Fig. 2 TEM image of Cobalt oxide nanoparticles using ginger extract

TEM analysis (Fig. 3) demonstrated that the Co_3O_4 nanoparticles were predominantly spherical, with a size range of approximately 15–25 nm. Minimal aggregation was observed, indicating effective stabilization by ginger phytochemicals. The nanoscale size and uniform morphology enhance surface interactions with bacterial cells, supporting their antibacterial and antibiofilm efficacy. Compared to previously reported Co_3O_4 nanoparticles synthesized using other plant extracts or chemical routes, the ginger-mediated approach achieved enhanced bioactivity due to synergistic interaction between bioactive phytochemicals and metal oxide surfaces, promoting ROS generation and EPS degradation. Additionally, the particles displayed stable, uniform morphology and biocompatible surface chemistry, minimizing potential ecotoxic effects often associated with chemically synthesized nanomaterials.

Crystallinity (XRD)

XRD patterns (Fig. 4) confirmed the crystalline spinel structure of Co_3O_4 nanoparticles. Sharp and well-defined diffraction peaks indicated high crystallinity. The average crystallite size, calculated using the Debye–Scherrer equation, was consistent with TEM observations, further confirming the nanoscale nature of the particles. The diffraction pattern displayed prominent peaks at 31.72°, 36.90°, 44.44°, 59.47°, and 65.29°, corresponding to the (220), (311), (222), (400), and (440) planes, respectively. These reflections are characteristic of the face-centered cubic (FCC) spinel structure of Co_3O_4. The average crystallite size, calculated using the Debye–Scherrer equation, ranged from 15 to 25 nm, with an estimated mean size of approximately 23.82 nm, in agreement with the Joint Committee on Powder Diffraction Standards (JCPDS card No. 42-1467). These results confirm the formation of a pure, well-crystalline Co_3O_4 phase.

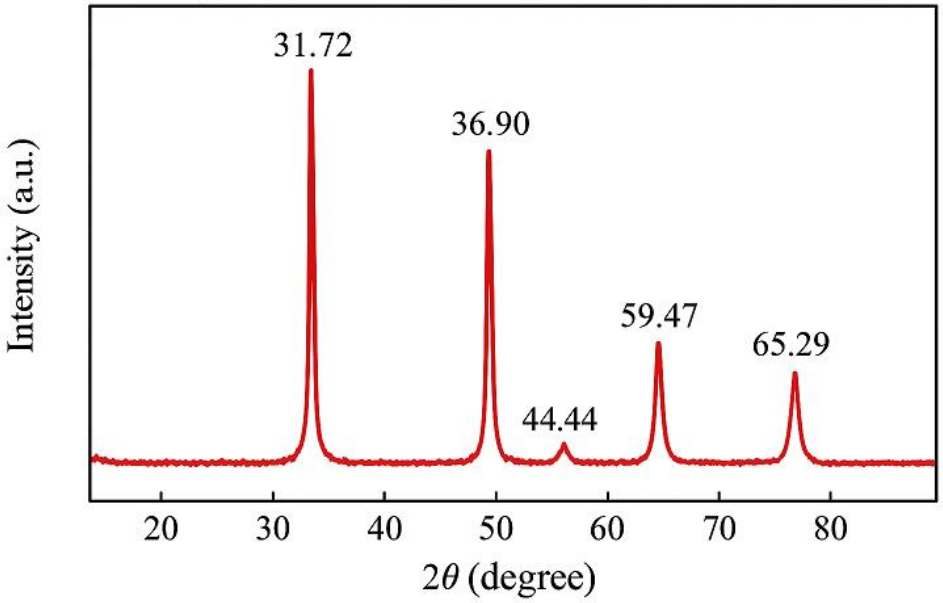

Fig. 3 XRD analysis of Cobalt oxide nanoparticles using ginger extract

Minimum Inhibitory Concentration (Antibacterial Assay) of Co3O4-Nps

The antibacterial efficacy of green-synthesized Co_3O_4 nanoparticles (NPs) was evaluated against *Escherichia coli* using the broth dilution method, and the results are summarized in Table 1. A concentration-dependent inhibitory effect was observed, indicating that bacterial growth decreased progressively with increasing nanoparticle concentration. At the lowest tested concentration (0.007 mg/mL), the bacterial growth inhibition was minimal (OD = 0.457), whereas at 1 mg/mL, the optical density significantly reduced to 0.001, confirming strong antibacterial activity. The minimum inhibitory concentration (MIC) of Co_3O_4 NPs against *E. coli* was determined to be 0.25 mg/mL, where a substantial reduction in bacterial growth (OD = 0.086) was observed compared with the untreated bacterial control (BC = 1.438). Beyond this concentration, a further increase in inhibition was evident, suggesting a dose-responsive antibacterial effect. The positive control (PC) exhibited complete inhibition, validating the test accuracy. The strong antibacterial activity of Co_3O_4 nanoparticles can be attributed to multiple mechanisms. The nanoscale size and high surface reactivity facilitate strong interaction with bacterial cell walls, leading to membrane disruption and leakage of intracellular contents. Additionally, Co_3O_4 NPs are known to generate reactive oxygen species (ROS) such as superoxide and hydroxyl radicals, which cause oxidative stress, protein denaturation, and DNA damage in bacterial cells. The release of Co^{2+} ions may further interfere with essential enzymatic processes and metabolic pathways, contributing to bacterial cell death.

The percentage inhibition of *E. coli* growth at different concentrations of Co_3O_4 nanoparticles is presented in Figure 5. The inhibition increased sharply with concentration, reaching ~99.9% at 1 mg/mL, indicating almost complete bacterial suppression. At the MIC value (0.25 mg/mL), ~94% inhibition was observed, confirming potent antibacterial efficacy even at low doses. Below 0.015 mg/mL, inhibition dropped below 75%, reflecting a concentration-dependent response. The logarithmic trend of inhibition suggests that the antibacterial efficiency is dominated by nanoparticle–cell interactions that intensify with increasing surface availability of Co_3O_4 NPs. The sigmoidal pattern also indicates that a threshold nanoparticle concentration is required to disrupt bacterial membranes effectively and induce oxidative stress through reactive oxygen species (ROS) generation. Overall, these results demonstrate that biosynthesized Co_3O_4 nanoparticles exhibit strong and dose-dependent antibacterial activity against *E. coli*, with a minimum inhibitory concentration (MIC) of 0.25 mg/mL**.**

Concentration of Co_3O_4 NPs (mg/ml)	**Test with *Escherichia coli***
1	0.001
0.5	0.027
0.25	0.086
0.12	0.093
0.062	0.152
0.031	0.235
0.015	0.372
0.007	0.457

BC	1.438
PC	-

Table. 1 Minimum Inhibitory Concentration of Cobalt oxide nanoparticles against *Escherichia coli*

These findings are consistent with previous reports indicating that metal oxide nanoparticles, particularly cobalt- and nickel-based nanomaterials, exert potent antibacterial effects through physical disruption and oxidative mechanisms. The results confirm that the biosynthesized Co_3O_4 nanoparticles possess significant antibacterial activity against *E. coli*, highlighting their potential application in wastewater disinfection, biomedical coatings, and antimicrobial packaging materials.

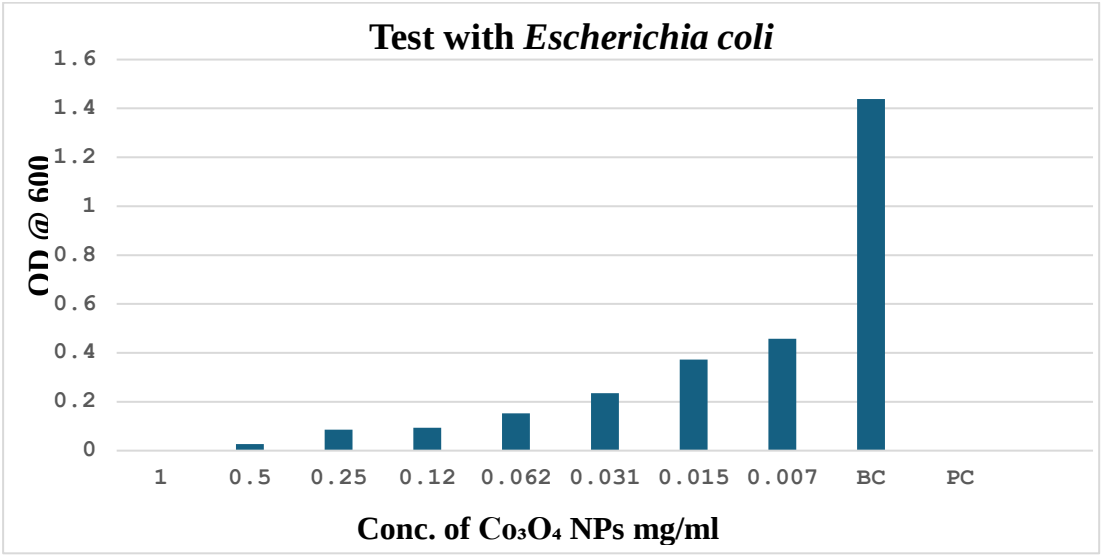

Fig. 4 Concentration-dependent inhibition of *E. coli* growth by Co_3O_4 nanoparticles in antibacterial assay."

Biofilm Inhibition Activity

The antibiofilm activity of green-synthesized cobalt oxide (Co_3O_4) nanoparticles (NPs) using *Zingiber officinale* (ginger) extract was evaluated against *Escherichia coli* using the crystal violet assay. The biofilm biomass was quantified spectrophotometrically at 570 nm, and the corresponding absorbance values are presented in Table 2. The results revealed a clear concentration-dependent inhibition of biofilm formation. The untreated bacterial control (BC) showed a high absorbance value (0.154), indicating dense biofilm formation, while biofilm biomass significantly decreased in NP-treated samples. At the highest tested concentration (1 mg/mL), the absorbance value reduced to 0.051, corresponding to ~66.9 % biofilm inhibition, whereas at the MIC concentration (0.25 mg/mL), inhibition was approximately 58.4 %**.** Even at the lowest concentration (0.007 mg/mL), a moderate inhibition of ~26.0 % was observed.

The results indicate that Co_3O_4 NPs effectively suppressed biofilm development in a dose-dependent manner (Figure 6). The antibiofilm potential of Co_3O_4 NPs can be attributed to multiple interacting mechanisms. The nanoparticles interfere with bacterial adhesion during the initial stages of biofilm formation by altering the surface charge and disrupting cell–substrate interactions. The generation of reactive oxygen species (ROS) such as hydroxyl and superoxide radicals further damage the bacterial cell wall and extracellular polymeric substance (EPS) matrix, leading to biofilm destabilization and reduced structural integrity. Moreover, the small particle size and high surface-to-volume ratio of the biosynthesized nanoparticles enable better penetration into the biofilm matrix, allowing effective disruption of the deeper bacterial layers. The use of *Zingiber officinale* extract as a reducing and stabilizing agent not only promotes the eco-friendly synthesis of Co_3O_4 NPs but also enhances their antibiofilm efficacy. Ginger phytochemicals, including gingerols, shogaols, and polyphenols, are known to contribute to antimicrobial and antioxidant activity, thereby improving the nanoparticles' biofilm inhibition efficiency. Thus, the present study confirms that the ginger-mediated Co_3O_4 nanoparticles possess potent antibiofilm and antibacterial inhibition against *E. coli*, highlighting their potential use in controlling biofilm-associated contamination in water purification systems, biomedical surfaces, and antimicrobial coatings. This dual action highlights the practical applicability of the nanoparticles in controlling microbial contamination in wastewater treatment plants, hospital surfaces, food processing environments, and industrial pipelines where biofilm formation is a major challenge.

Percentage	***Escherichia coli***

1 mg/ml	0.051
0.5 mg/ml	0.056
0.25 mg/ml	0.064
0.12 mg/ml	0.076
0.06mg/ml	0.083
0.03 mg/ml	0.092
0.015 mg/ml	0.109
0.007 mg/ml	0.114
BC	0.154
PC	_ _ _

Table. 2 Biofilm Inhibition Activity of Comparative assessment of biofilm formation inhibition by Cobalt Oxide Nanoparticles across *Escherichia coli*

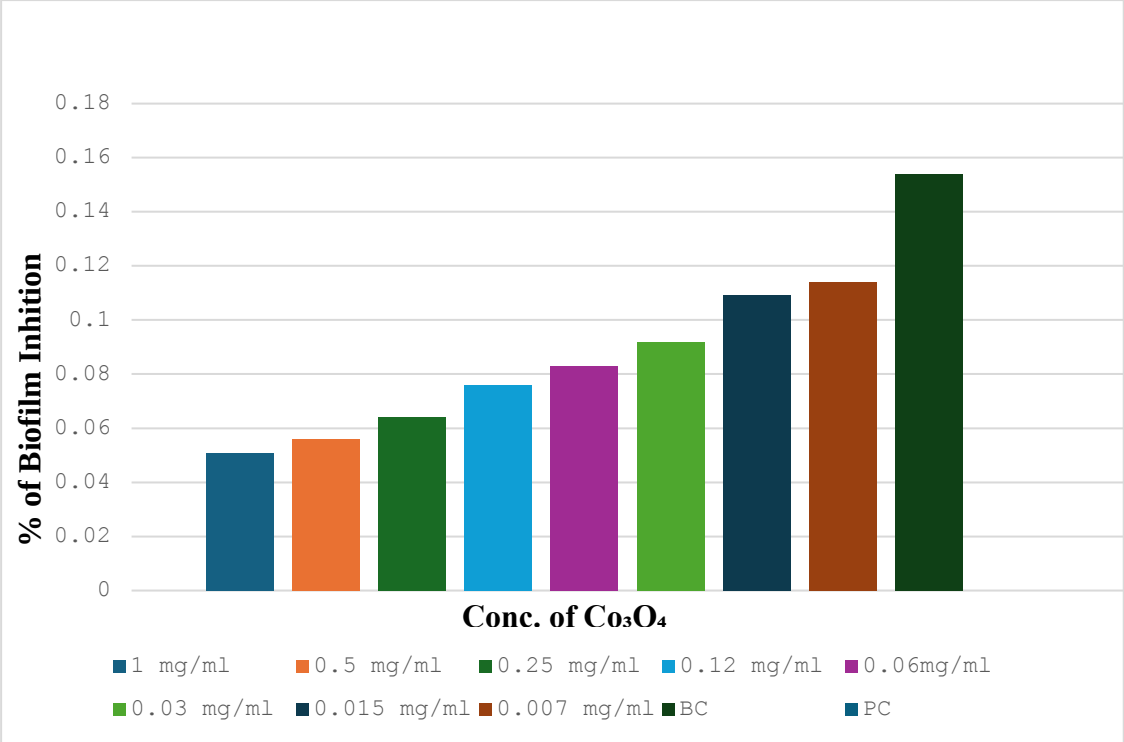

Fig. 5 Biofilm inhibition of *Escherichia coli* by ginger-mediated Co_3O_4 nanoparticles at different concentrations. Statistical significance was determined using Dunnett's test, showing significant differences between the treated groups and the control ($p < 0.05$).

CONCLUSION

This research presents an environmentally sustainable route for synthesizing cobalt oxide (Co_3O_4) nanoparticles using *Zingiber officinale* (ginger) extract as a natural reducing and stabilizing agent. The green synthesis process eliminates the need for toxic chemicals and minimizes hazardous waste generation, contributing to eco-friendly nanomaterial production. The biosynthesized Co_3O_4 nanoparticles exhibited strong antibacterial activity against *Escherichia coli*, with a minimum inhibitory concentration (MIC) of 0.25 mg mL^{-1}, and effectively inhibited biofilm formation by up to 66.9% at 1 mg mL^{-1}. From an environmental perspective, this study provides a green nanotechnological approach for mitigating microbial contamination and biofilm-associated pollution in aquatic environments. The ability of these nanoparticles to inhibit bacterial adhesion and disrupt biofilms suggests their applicability in wastewater treatment systems, biofouling control in industrial pipelines, and antimicrobial coatings for water-handling surfaces. The integration of ginger extract not only promotes sustainable synthesis but also enhances the biological functionality of the nanoparticles through synergistic phytochemical effects. Overall, the research contributes to the development of eco-safe, biodegradable, and efficient nanomaterials for environmental protection and public health applications. Future work may explore the reusability, large-scale synthesis, and toxicity assessment of these nanoparticles to advance their implementation in green environmental remediation technologies.

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Author contributions

Author: C. Ginu Rose: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft.

Co-author: Dr. N. K. Amaliya: Supervision and validation of the manuscript

Availability of data and materials

All data supporting the findings of this study are included within the article and its supplementary information. Additional datasets are available from the corresponding author upon reasonable request.

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