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ENHANCED ANTIBACTERIAL ACTIVITY OF COPPER-DOPED ZINC FERRITE NANOPARTICLES AGAINST PATHOGENIC BACTERIA

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Abstract

:$ZnFe_2O_4$ nanoparticles, both undoped and doped with copper, were synthesized via a cost-effective sol-gel method and characterized using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). XRD results confirmed the formation of a single-phase cubic spinel structure in the Cu-doped $ZnFe_2O_4$, with an average crystallite size of around 37 nm. This was further supported by TEM analysis, which revealed similar particle sizes and confirmed a well-crystallized, spherical morphology, especially in the sample containing 20 wt% Cu. The antibacterial performance was assessed against *E. coli* and *S. aureus* using the Kirby-Bauer disk diffusion technique. The findings demonstrated that higher copper doping levels significantly enhance the antibacterial activity of $ZnFe_2O_4$ nanoparticles.

Keywords: $ZnFe_2O_4$, Copper Doping, XRD, FTIR, TEM, Antibacterial Activity, Gram-positive, Gram-negative

Introduction

Nanomedicine, which refers to the use of nanotechnology in medical applications, has seen remarkable progress in recent years. Due to their small size and high surface area, nanoparticles exhibit unique properties that distinguish them from bulk materials. These characteristics make them highly effective in a range of fields, particularly in biomedical applications such as drug delivery, diagnostics, and antimicrobial treatments [1].

Over the past two decades, spinel-structured nano ferrites have garnered significant attention due to their versatile applications in areas such as pharmaceuticals, medicine, insulation, and drug delivery systems [2,3]. Ferrites, which have the general formula AB_2O_4, consist of metal cations occupying octahedral and tetrahedral sites within their crystal structure. These

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properties, coupled with their small size and unique morphology, make ferrite nanoparticles particularly valuable in biomedical sciences [4,5].

Among various ferrites, zinc ferrite ($ZnFe_2O_4$) nanoparticles have shown exceptional antibacterial properties, especially against pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [6]. In this study, we explore the enhancement of these properties by doping $ZnFe_2O_4$ with copper (Cu) at concentrations of 10% and 20%. Copper is a well-known antimicrobial agent that can effectively combat a wide range of microorganisms, making it an ideal candidate for enhancing the antibacterial activity of ferrites.

Recent studies have demonstrated that copper-doped materials exhibit rapid and almost complete inactivation of *E. coli* and *S. aureus* [7]. Both of these pathogens are major causes of infections in humans, with *E. coli* leading to gastrointestinal issues and *S. aureus* causing skin and soft tissue infections [8]. Importantly, research has shown that nanoparticles can exhibit selective antibacterial toxicity while having minimal effects on human cells [9–12].

The rise of antibiotic-resistant bacteria has become a critical global health challenge. Many bacterial strains have developed resistance mechanisms, making infections harder to treat. This emphasizes the urgent need for new antimicrobial agents. Copper-doped ferrite nanoparticles hold great potential as an alternative to conventional antibiotics, offering an effective solution to combat resistant bacterial strains like *E. coli* and *S. aureus*.

To date, various methods have been used to synthesize zinc ferrite nanoparticles, including sol-gel [13], co-precipitation [14], microemulsion [15], and combustion methods [16]. However, the structural and compositional properties of ferrites can be highly sensitive to the synthesis technique, which affects their performance in biomedical applications [17].

In this study, we synthesize undoped and copper-doped $ZnFe_2O_4$ nanoparticles using the sol-gel method and characterize them through X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). We then evaluate their antibacterial activity against *S. aureus* and *E. coli*, examining how varying copper concentrations (10% and 20%) influence their antibacterial performance. The aim is to explore the potential of copper-doped $ZnFe_2O_4$ nanoparticles as effective antimicrobial agents.

Materials And Methods

Materials

Zinc nitrate tetrahydrate (Zn(NO3)2·4H2O)(Zn(NO_3)_2·4H_2O)(Zn(NO3)2·4H2O), iron nitrate nonahydrate (Fe(NO3)2·9H2O)(Fe(NO_3)_2·9H_2O)(Fe(NO3)2·9H2O), and citric acid of analytical grade were used in this study. Ethanol served as the solvent in all experimental procedures. Copper-doped zinc ferrite (Cu-ZnFe2O4)(Cu\text{-}ZnFe_2O_4)(Cu-ZnFe2O4) nanoparticles were synthesized using the sol-gel technique.

Synthesis of Cu-doped $ZnFe_2O_4$ nanoparticles

The nanoparticles were prepared via the sol-gel method. The required amounts of precursors were dissolved in ethanol in a beaker, followed by continuous stirring on a magnetic stirrer for

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approximately 2 hours. A homogeneous gel was obtained, which was subsequently dried and calcined at different temperatures to yield Cu-doped $ZnFe_2O_4$ nanoparticles.

Bacterial culture and antibacterial activity evaluation

Collection of microorganisms
For antibacterial assessment, both Gram-negative and Gram-positive bacterial strains were employed: *Escherichia coli* (NCIM 2256) and *Staphylococcus aureus* (NCIM 2901).

Preparation of inocula
Each bacterial strain was maintained by sub-culturing on fresh nutrient agar. Nutrient broth was sterilized at 15 lbs pressure and 121 °C for 15 minutes. A loopful of each bacterial colony was transferred into 5 mL of sterile nutrient broth and incubated overnight at 37 °C. These overnight-grown cultures were used as inocula for all experimental assays. *Growth profiling of bacteria* Fresh bacterial colonies were inoculated into nutrient broth to study growth kinetics. The turbidity of the broth cultures was monitored, and the optical density (OD) at 600 nm was adjusted between 0.08–0.1, following NCCL guidelines, to standardize the inoculum density.

Materials And Methods

Materials
Analytical grade zinc nitrate tetrahydrate (Zn(NO3)2·4H2O)(Zn(NO_3)_2·4H_2O)(Zn(NO3)2·4H2O), iron nitrate nonahydrate (Fe(NO3)2·9H2O)(Fe(NO_3)_2·9H_2O)(Fe(NO3)2·9H2O), and citric acid were used as precursors. Ethanol was employed as the solvent in all experimental steps. Copper-doped zinc ferrite (Cu-ZnFe2O4)(Cu\text{-}ZnFe_2O_4)(Cu-ZnFe2O4) nanoparticles were synthesized using the sol–gel method.

Synthesis of Cu-doped $ZnFe_2O_4$ nanoparticles
The nanoparticles were obtained via a sol–gel process. The required chemicals were dissolved in ethanol and stirred continuously on a magnetic stirrer for about 2 hours until a uniform gel was formed. The resulting gel was then dried and calcined at different temperatures to produce Cu-doped $ZnFe_2O_4$ nanoparticles.

Bacterial culture and antibacterial assay

Collectionm of microorganisms
To evaluate antibacterial activity, both Gram-negative and Gram-positive bacteria were selected as test organisms: *Escherichia coli* (NCIM 2256) and *Staphylococcus aureus* (NCIM 2901). ***Preparation of inocula***
Each bacterial strain was maintained by sub-culturing on fresh nutrient agar. Nutrient broth was sterilized at 121 °C for 15 minutes under 15 lbs pressure. A loopful of bacterial colony was transferred to 5 mL of sterile nutrient broth and incubated overnight at 37 °C. These actively growing cultures were used as inocula for subsequent experiments.

Result And Disscusion

X- RAY DIFFRACTION (XRD)

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igure 1 depicts the X-ray diffraction (XRD) spectra of undoped $ZnFe_2O_4$ along with Cu-incorporated $ZnFe_2O_4$ ferrites. The recorded diffraction peaks were indexed successfully and found to be in excellent agreement with the standard reference pattern provided in JCPDS card No. 22-1012. The Cu-doped $ZnFe_2O_4$ sample shows sharp and well-resolved reflections, which clearly demonstrate the formation of a single-phase material with a cubic spinel lattice. The major diffraction planes corresponding to (311), (420), (531), (422), and (620) were identified, further supporting the cubic spinel phase formation. The crystallite size of the particles was determined from the broadening of diffraction peaks using the Debye–Scherrer equation [19], where D is the average crystallite size, β denotes the full width at half maximum, λ is the X-ray wavelength, and θ is the Bragg angle. The estimated average crystallite size of Cu-doped $ZnFe_2O_4$ nanoparticles was approximately 37 nm.

$$D = \frac{0.89\lambda}{\beta \cos\theta}$$

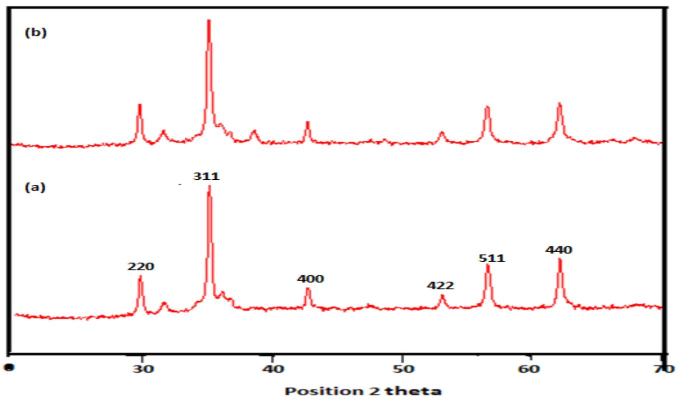

Figure. 1 XRD

Analysis of (a) $ZnFe_2O_4$ and (b)20 wt %Cu doped $ZnFe_2O_4$ nanoparticle

FOURIER TRANSFORM INFRA-RED (FTIR) SPECTROSCOPY

Figure 2 illustrates the FTIR spectrum of $ZnFe_2O_4$ doped with 20 wt% Cu, recorded over the wavenumber region of 400–4000 cm^{-1}. The spectrum reveals several characteristic absorption bands: a broad peak at 3432.45 cm^{-1} corresponding to O–H stretching vibrations, a band at 2926.53 cm^{-1} attributed to sp^3 C–H stretching, and another at 1632.53 cm^{-1} assigned to N=O–O stretching. A distinct peak at 1458 cm^{-1} is also observed, while the strong absorption at 554.28 cm^{-1} corresponds to Fe–O stretching and the band at 464 cm^{-1} is associated with Zn–O stretching vibrations. These latter two bands represent the fundamental metal–oxygen stretching modes, which are characteristic features of spinel ferrite structures. The observed variations in band positions are attributed to differences in bond lengths between cations situated at tetrahedral and octahedral sites within the lattice [20]. characteristics peaks of spinel structure 20wt % Cu doped ZnFe2O4.

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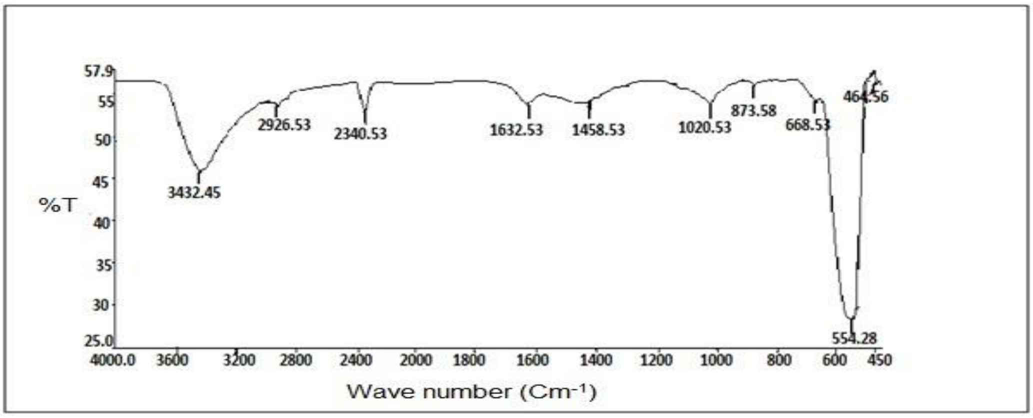

(b

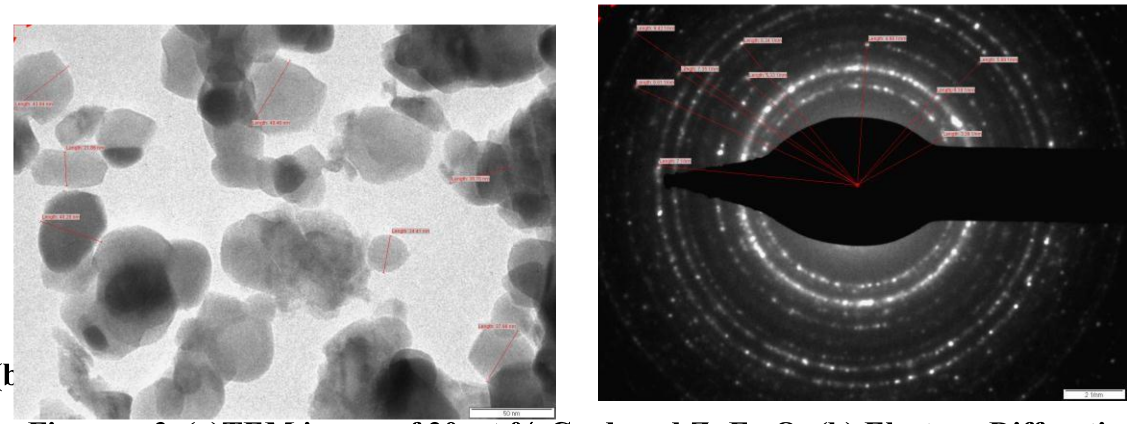

Figure. - 3. (a)TEM image of 20 wt % Cu doped $ZnFe_2O_4$ (b) Electron Diffraction

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was employed to investigate the morphology and particle size of the synthesized Cu-doped $ZnFe_2O_4$ nanoparticles. Representative TEM micrographs of the 20 wt% Cu-doped $ZnFe_2O_4$ sample are shown in Figure 3.The TEM analysis revealed that the nanoparticles possessed a predominantly spherical morphology with slight agglomeration, which is commonly observed in ferrite nanoparticles due to their magnetic interactions. The average particle size was found to be ~37 nm, which was in close agreement with the crystallite size estimated from XRD data using the Debye–Scherrer equation. This correlation between XRD and TEM confirms the reliability of the structural characterization.In addition to particle morphology, high-resolution TEM (HRTEM) images displayed well-defined lattice fringes, confirming the crystalline nature of the nanoparticles. The measured interplanar spacing values were consistent with the characteristic planes of the cubic spinel structure of $ZnFe_2O_4$, further supporting the XRD findings.Furthermore, selected area electron diffraction (SAED) patterns exhibited concentric diffraction rings corresponding to the (311), (420), (511), and (440) planes of spinel ferrites, thereby validating the polycrystalline nature of the synthesized nanoparticles.Overall, TEM analysis corroborated the XRD results, demonstrating that the synthesized 20 wt% Cu-doped $ZnFe_2O_4$ nanoparticles were spherical, crystalline, and of nanoscale dimensions (~37 nm).inhibition zone measurements with increase

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the doping of copper. From the results clear that 20 wt % Cu doped $ZnFe_2O_4$ is best antibacterial agent than 10 wt % Cu doped $ZnFe_2O_4$ and pure $ZnFe_2O_4$.

Antibacterial Study

The antibacterial performance of pure $ZnFe_2O_4$ and Cu-doped $ZnFe_2O_4$ nanoparticles was investigated using the Kirby–Bauer disc diffusion method. The inhibitory activity was assessed against two pathogenic bacterial strains, *Staphylococcus aureus* and *Escherichia coli*. The outcomes are summarized in Table 1 and presented in Figure 4 (a–f). Tetracycline served as the positive control, while blank solvent acted as the negative control.

The results revealed that the antibacterial efficacy improved markedly with Cu doping. For 10 wt% Cu-doped $ZnFe_2O_4$, inhibition zones of 17 mm against *S. aureus* and 15 mm against *E. coli* were recorded. At 20 wt% Cu doping, the inhibition zones increased to 20 mm and 18 mm, respectively. These findings clearly demonstrate that Cu incorporation enhanced the antibacterial properties of $ZnFe_2O_4$ compared to the undoped material.

Interestingly, *S. aureus* exhibited greater susceptibility than *E. coli*. This agrees with the earlier findings of Sonohara et al. [21], who reported that the *S. aureus* membrane carries a lower negative surface charge compared to *E. coli*. This lower charge density allows greater penetration of negatively charged free radicals, leading to oxidative stress, membrane disruption, and eventual cell death in *S. aureus* at relatively lower nanoparticle concentrations [22].

The improved antibacterial activity can be directly correlated with the structural and morphological features characterized by XRD and TEM analyses. XRD results confirmed the formation of a crystalline cubic spinel structure with an average crystallite size of ~37 nm, while TEM micrographs verified the spherical morphology and nanoscale dimensions of the particles. The small particle size and high surface-to-volume ratio of the Cu-doped nanoparticles provide a larger contact area for bacterial interaction, facilitating enhanced membrane penetration and reactive oxygen species (ROS) generation. Moreover, the incorporation of Cu ions further contributes to oxidative stress, thereby improving bactericidal efficiency.

The growth inhibition curves presented in Figure 5 (a, b) highlight the dose-dependent nature of antibacterial activity, where higher Cu doping concentrations led to larger inhibition zones. These findings confirm that structural modifications achieved through Cu doping not only altered the physicochemical characteristics of $ZnFe_2O_4$ nanoparticles but also significantly enhanced their biological performance.inhibition zone measurements with increase the doping of copper. From the results clear that 20 wt % Cu doped $ZnFe_2O_4$ is best antibacterial agent than 10 wt % Cu doped $ZnFe_2O_4$ and pure $ZnFe_2O_4$. **Bacterial growth profiling**or growth studies, fresh cultures were inoculated into nutrient broth. The turbidity of the broth was monitored, and the optical density (OD) was adjusted between 0.08–0.1 at 600 nm, in accordance with NCCL guidelines, to standardize the bacterialoncentration.

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E. Coli **S.aureus**

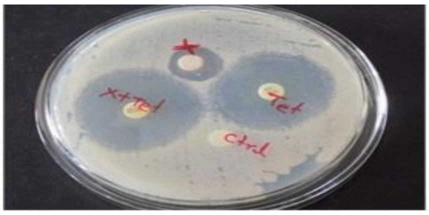

Fig. 4 (a), x= $ZnFe_2O_4$

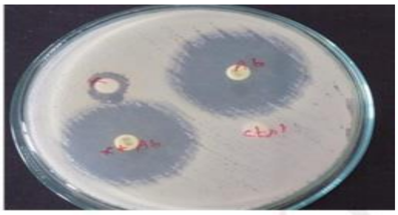

Fig. 4 (b), x= $ZnFe_2O_4$

Fig. 4 (c), Y= Cu 10 wt % $ZnFe_2O_4$

Fig. 4 (d), Y= Cu 10 wt % $ZnFe_2O_4$

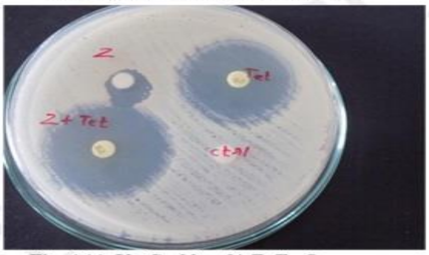

Fig. 4 (e), Y= Cu 20wt % $ZnFe_2O_4$

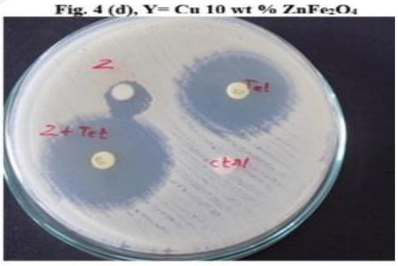

Fig. 4 (f), Y= Cu 20 wt % $ZnFe_2O_4$

Sr. No.	Sample	Test Organism	Zone of Inhibition (mm) – Nanoparticles	Zone of Inhibition (mm) – Standard Antibiotic	Zone of Inhibition (mm) – Antibiotic + Nanoparticles
1	$ZnFe_2O_4$	*E. coli*	13	31	36
2	10 wt% Cu–$ZnFe_2O_4$	*E. coli*	16	33	37
3	20 wt% Cu–$ZnFe_2O_4$	*E. coli*	19	32	37
4	$ZnFe_2O_4$	*S. aureus*	15	33	36
5	10 wt% Cu–$ZnFe_2O_4$	*S. aureus*	18	32	35
6	20 wt% Cu–$ZnFe_2O_4$	*S. aureus*	22	32	37

so

Table 1 Antibacterial activity of $ZnFe_2O_4$ and 20 wt % Cu doped $ZnFe_2O_4$ against E.

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Coli and S. aureus

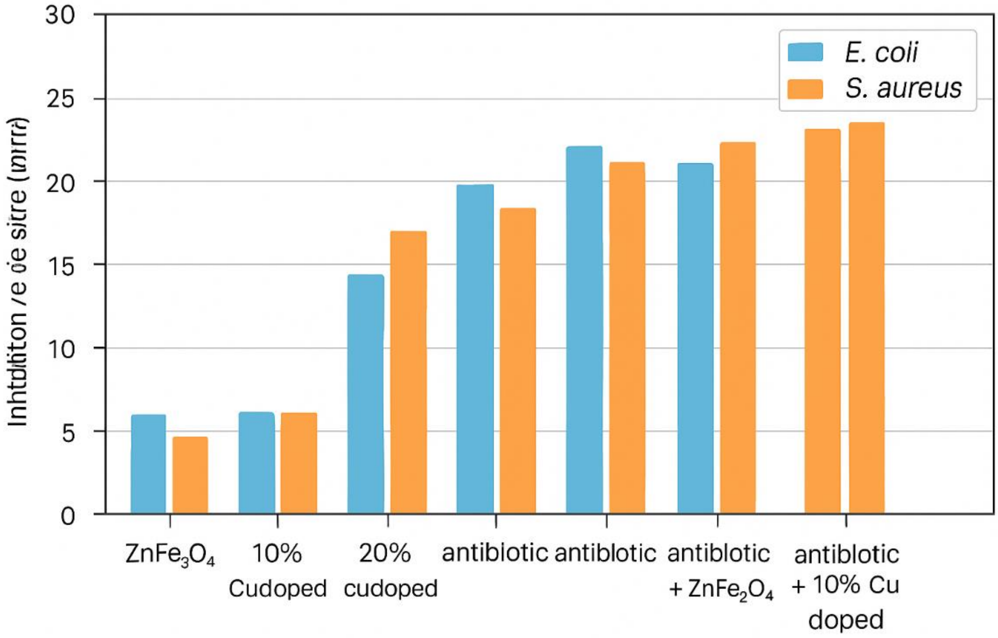

Figure. 5 (a,b)graph showing zone of inhibition of undoped and Cu doped nanonmaterial against E. Coli. S.aureus

Conclusion

In the present study, spinel Cu-doped $ZnFe_2O_4$ nanoparticles with an average size of ~37 nm were successfully synthesized using the sol–gel method. Structural characterization by X-ray diffraction (XRD) confirmed the formation of a cubic spinel phase, while Fourier-transform infrared spectroscopy (FTIR) verified the presence of functional groups. Transmission electron microscopy (TEM) further validated the crystalline nature and spherical morphology of the nanoparticles.

The antibacterial activity of pure $ZnFe_2O_4$ and Cu-doped $ZnFe_2O_4$ (10 wt% and 20 wt%) nanoparticles was assessed using the Kirby–Bauer disc diffusion method. The results demonstrated that doping with copper significantly enhanced antibacterial performance. In particular, 20 wt% Cu-doped $ZnFe_2O_4$ nanoparticles exhibited the largest inhibition zones against *Escherichia coli* and *Staphylococcus aureus*, confirming their strong antibacterial potential. Notably, *S. aureus* was found to be more sensitive than *E. coli*, which can be attributed to differences in bacterial cell wall structure and surface charge.

Overall, the study establishes 20 wt% Cu-doped $ZnFe_2O_4$ nanoparticles as promising candidates for antibacterial applications. In future work, the scope of antibacterial studies can be extended to a wider range of pathogenic strains. Furthermore, the potential of these nanomaterials for advanced biomedical applications such as targeted drug delivery and the removal of microbial contaminants from water systems should be explored.

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Future Scope

The present work highlights the successful synthesis, characterization, and antibacterial evaluation of Cu-doped $ZnFe_2O_4$ nanoparticles. While the findings are promising, several directions can be explored in future research to broaden the scope and practical applications of these nanomaterials:

1. **Extended Antibacterial Studies**
    - Investigate the antibacterial activity of Cu-doped $ZnFe_2O_4$ nanoparticles against a wider range of Gram-positive and Gram-negative human pathogens.
    - Assess their antifungal and antiviral properties to determine broader antimicrobial potential.
2. **Mechanistic Insights**
    - Conduct detailed studies on the mechanism of antibacterial action, focusing on reactive oxygen species (ROS) generation, membrane disruption, and interactions with bacterial DNA and proteins.
    - Use advanced techniques such as flow cytometry, confocal microscopy, and zeta potential analysis to better understand nanoparticle–bacteria interactions.
3. **Biomedical Applications**
    - Explore the potential of Cu-doped $ZnFe_2O_4$ nanoparticles in drug delivery systems, where their magnetic properties could allow targeted delivery under an external magnetic field.
    - Evaluate their cytotoxicity and biocompatibility using mammalian cell lines to assess suitability for therapeutic use.
4. **Environmental Applications**
    - Study the role of these nanoparticles in the purification of water contaminated with microbial pathogens.
    - Assess their efficiency in removing heavy metals and other pollutants due to their high surface reactivity.
5. **Optimization of Synthesis**
    - Investigate the effect of varying synthesis parameters (calcination temperature, Cu concentration, solvent ratios) on particle size, morphology, and antibacterial efficiency.
    - Scale up the synthesis process for potential industrial applications.
6. **Multifunctional Nanomaterials**
    - Combine Cu-doped $ZnFe_2O_4$ with other functional nanomaterials (e.g., silver, graphene oxide, or TiO_2) to create hybrid composites with enhanced antibacterial and catalytic properties.

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