

Spectral Investigation of Zn doped CoFe_2O_4 Spinel Ferrites for Antimicrobial Activity

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Abstract: Magnetic Zn-doped cobalt ferrites (CoFe_2O_4) were synthesized using a sol-gel auto-combustion method with urea as the fuel. The synthesized spinel nano-ferrites were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy and UV-visible (UV-Vis) spectroscopy and Antimicrobial activity. The XRD analysis showed that the structures remained the cubic spinel after the substitution of zinc. The crystallite size of the ferrites was found to be in the range of 27.0- 42.0 nm. The SEM images show that the particles are agglomerated and the particle size is in the range of $5\mu\text{m}$ -50 μm . The FT-IR analysis confirmed the formation of metal oxides through the detection of Fe-O stretching vibrations in the range of $400\text{-}750\text{ cm}^{-1}$. The UV-Vis spectroscopy indicated the direct band gap of 4.18 eV to 5 eV suitable for antimicrobial use. A selection of pathogenic microorganisms was evaluated for their antibacterial activity against cobalt ferrites. The best result obtained for Zn-doped cobalt ferrites showed zones of inhibition increased from 16mm to 19mm against *E. Colli* and from 14mm to 21 mm against *B.Subtilis* respectively. Hence, the synthesized metal substituted Cobalt ferrite is considered as a potential candidate material for biomedical applications.

Keywords: Sol-Gel method, Nano ferrite, Microbes, Agar well Diffusion method, UV-Vis spectroscopy.

1. Introduction

Despite extensive research on chemotherapeutic antimicrobial agents, the high incidence of infectious diseases, antibiotic resistance, and associated morbidity and mortality remains a major concern, underscoring the need for new antimicrobial agents. Nano biotechnology has emerged as a promising approach in biomedical applications, leveraging nanoparticles' unique physical and chemical properties such as nanometer scale, high surface area-to-volume ratio, bioavailability, and capability to interact with and cross cell membranes. Inorganic nanoparticles¹, particularly metal oxides like CuO^{+2} , MgO^{+2} , and ZnO^{+2} , demonstrate considerable antibacterial activity and are being extensively explored as antimicrobial agents, exhibiting² a range of properties including metallic, insulating, and semiconductor behavior³.

Metal and metal-oxide nanoparticles, particularly ferrites, are gaining considerable attention for their antimicrobial properties and broad applicability in advanced technologies. Spinel ferrites, such as cobalt ferrite (CF), exhibit desirable optical, magnetic, and electronic characteristics, which are further enhanced when cobalt is partially substituted with metals like zinc, improving their antimicrobial effectiveness⁴. Current studies aim to refine the structural and functional properties of these nanoparticles, especially through sol-gel synthesis, to maximize their potential in biomedical applications, including targeted drug delivery and antibacterial treatments⁵.

Spinel ferrites (MF), with M as a divalent metal (e.g., Ni, Zn, Mg)⁶, have garnered attention for their magnetic, electronic, and optical properties, in addition to their biocompatibility, ease of synthesis, and stability, making them highly attractive for biomedical applications⁶. Interest in doping spinel ferrites with transition metals to enhance antibacterial properties is increasing, although the antibacterial potential of cobalt ferrite nanoparticles, particularly with zinc doping, remains underexplored. The objective of this research is to synthesize and evaluate zinc-doped cobalt ferrite ($\text{Zn}_x \text{Co}_{1-x} \text{Fe}_2 \text{O}_4$) ZCF nanoparticles for their potential applications in biomedical fields⁷.

2. Synthesis and Characterization

The analytical grade chemicals used for the synthesis were cobalt nitrate [AR, ACS, 99%], ferric nitrate [AR, ACS, 98%], zinc nitrate [AR, ACS, 99.5%] and urea (R-ACS) and all were used as received. The Zn Substituted Cobalt ferrite magnetic nanoparticles were synthesized by sol gel auto combustion method. The stoichiometric amount of ferric nitrate ($\text{Fe}(\text{NO}_3)_3$), cobalt nitrate ($\text{Co}(\text{NO}_3)_3$), Zinc nitrate ($\text{Zn}(\text{NO}_3)_3$) and urea were dissolved

in distilled water. The solutions were allowed to heat up to 70°C for 2 Hr to form gel. They were heated in thermodynamic condition with the help of muffle furnace at 800°C temperature followed by grinding and sintering at 800°C for 6 hours. Fine powder of magnetic ZCF nanoparticles is obtained by grinding of sintered samples up to around 1 Hr⁸.

The calcined cobalt ferrite magnetic nanoparticles were characterized using an X-ray diffractometer (XRD), Bruker D8 Advance. Specifications included a $\text{Cu-K}\alpha$ radiation source, with a typical angular range of $2\theta = 10^\circ$ - 80° . This analysis was conducted to study crystal structures, phase identification, and grain sizes in various samples. Scanning electron microscopy (SEM) was performed with either the JEOL JSM-IT500, both capable of high-resolution imaging for analyzing particle size, surface morphology, and material texture. Additional features often included Energy Dispersive X-ray Spectroscopy (EDS) for elemental analysis. Fourier-Transform Infrared (FTIR) Spectroscopy was conducted using a PerkinElmer Spectrum instrument. This technique, Attenuated Total Reflectance (ATR) for solids and liquids, identifies molecular bonds and functional groups, providing information on sample composition and structure. UV-Visible (UV-Vis) Spectroscopy was carried out using a Shimadzu UV-2600 spectrophotometer, covering wavelengths from 200-800 nm. This technique measures absorbance and transmittance to assess optical properties, including band gap and electronic transitions. Using the agar well diffusion method, the antibacterial activity of zinc-substituted cobalt ferrite (ZCF) nanoparticles at 5000 ppm concentration was examined. These ferrites were tested against bacteria *Escherichia coli* and *B. subtilis*. Pathogen growth inhibition was evaluated by measuring the zone of inhibition (ZOI) after 24 hours of incubation for every 30 minutes.

3. Results and discussion

a) Structural Properties

X-ray diffraction (XRD) analysis of cobalt ferrite (CF) nanoparticles in their initial form reveals distinct peaks at 2θ values of 30.12° , 35.54° , 37.12° , 43.13° , 53.41° , 56.89° , and 62.62° , which correspond to planes (220), (311), (222), (400), (442), (511), and (440) respectively. These peaks confirm a cubic spinel structure with the Fd-3m space group, consistent with established data for CF⁹. The average crystallite size D , for each sample was estimated from the most intense peak (311) using Debye Scherrer's formula. The average crystallite size was found to decrease from 43 nm to 27 nm with Zn^{+2} substitution at 800°C ⁹. Differences in lattice parameters and crystallite sizes among the samples are due to variations in the ionic radii of Fe^{3+} and Zn^{2+} .

at tetrahedral and octahedral sites. These structural findings, displayed in tables and figures, illustrate the influence of ionic radius on crystallite size, lattice parameters, cell volume, and density. From Figure 1(b), it is found that As the Zn Conc. increases the peak at 37.48-degree (2 theta) shifts gradually which may be due to the greater atomic geometry of Zn over the Co atom in the spinel structural configuration¹⁰.

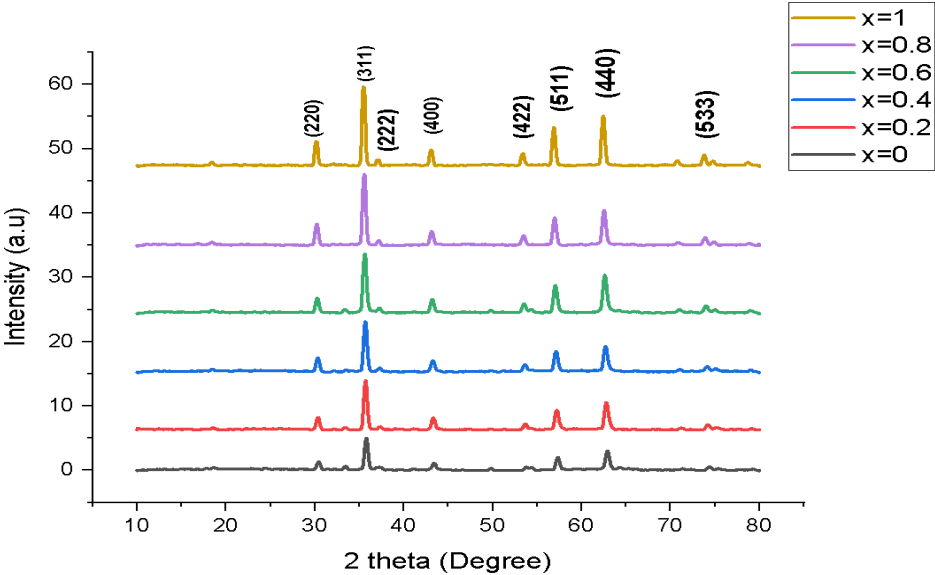


Figure 1(a): XRD patterns of $\text{Zn}_x\text{Co}_{1-x}\text{Fe}_2\text{O}_4$

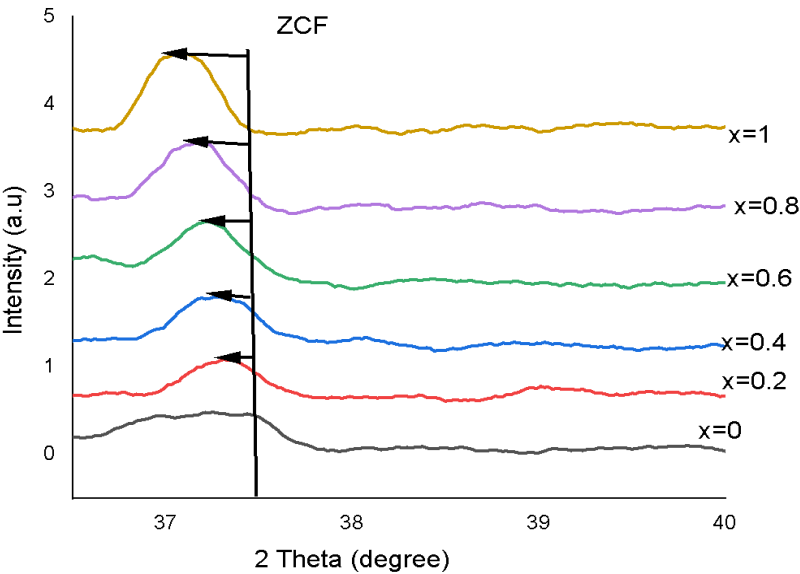


Figure 1(b): XRD Pattern of $\text{Zn}_x\text{Co}_{1-x}\text{Fe}_2\text{O}_4$

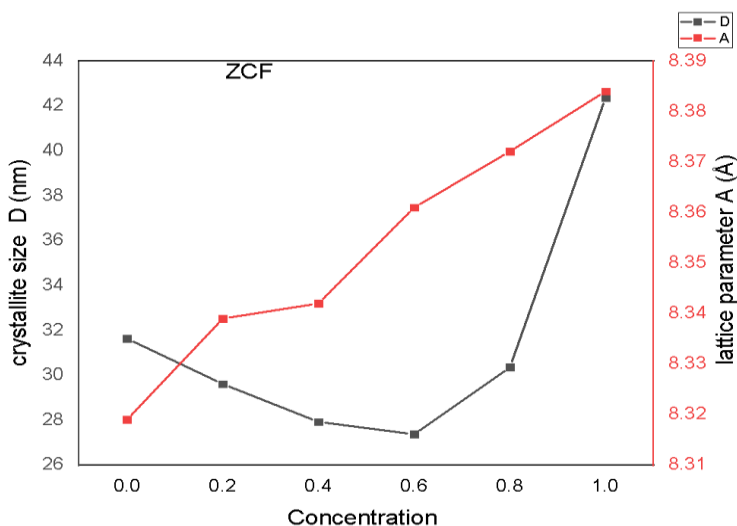


Figure 1(C): XRD Pattern of $\text{Zn}_x\text{Co}_{1-x}\text{Fe}_2\text{O}_4$

The behaviour of crystalline size and lattice parameter against Zn doping is depicted in Figure 1(c). It shows that crystalline size decreases up to 28 nm and then increases gradually. Whereas the continuous increment in the lattice parameter is observed which may be due to the structural adjustment and fitting of foreign Zn atom in spinel ferrite cubic structure and optimised antimicrobial activity is supported by the minimum crystalline size of ZCF¹⁰.

b) Morphological Properties

SEM analysis was used to examine the microstructures of ZCF. The SEM images in Figures 2(a) and 2(c) depict a highly agglomerated, irregularly shaped, nano-grained structure. The study further revealed that the morphology of ZCF particles is heterogeneous and non-uniform. EDX measurements confirmed that the synthesized ZCF materials are stoichiometric, homogeneous, and exhibit a uniform elemental distribution.

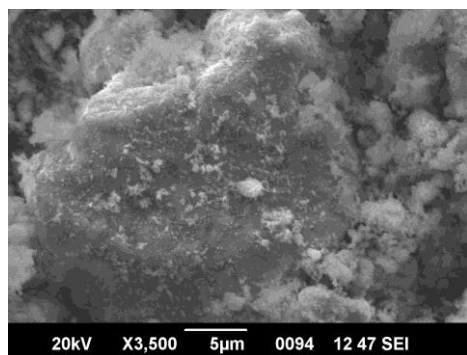


Figure 2(a) SEM of CF

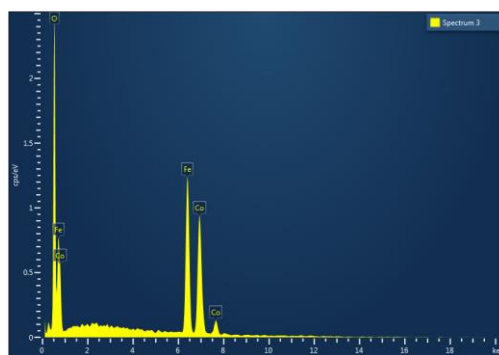


Figure 2 (b) EDAX of CF

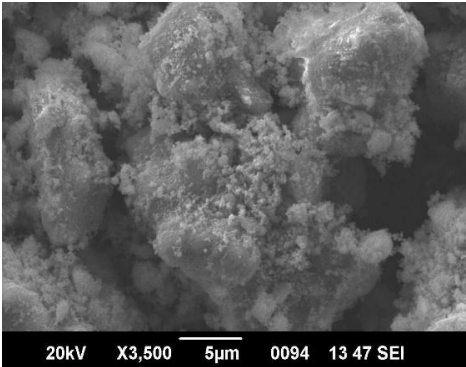


Figure 2 (c) SEM of ZCF-0.4

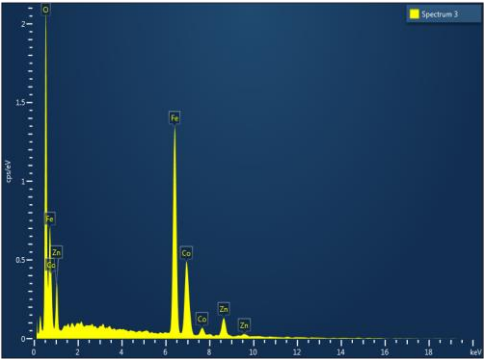


Figure 2. (d) EDAX of ZCF-0.4

The elemental composition of each variant is clearly depicted in Figures 2(b) and 2(d), which present the EDX spectra used to confirm the presence of elements. The spectra revealed characteristic peaks corresponding to Zn, Co, and Fe atoms, confirming the presence and even distribution of each element within the samples¹⁰.

c) SEM-EDAX Analysis

The Figure 3(a), (b), (c), (d), (e) and (f) for (x = 0, 0.2, 0.4, 0.6, 0.8 and 1) represents the optical properties of Zn-doped Co ferrite (ZCF) nanoparticles with varying Zn²⁺ concentrations were investigated using UV-Vis spectroscopy. The bandgap (E_g) values were determined through Tauc plot analysis, revealing a range of 2.87¹⁴ - 5 eV across the samples¹¹.

Table 1

Sample	CF	ZCF-0.2	ZCF-0.4	ZCF-0.6	ZCF-0.8	ZCF-1
Energy Gap	2.87	4.198	4.22	4.790	4.98	5

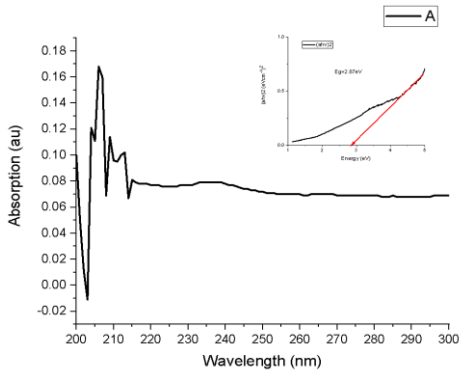


Figure 3 (a)

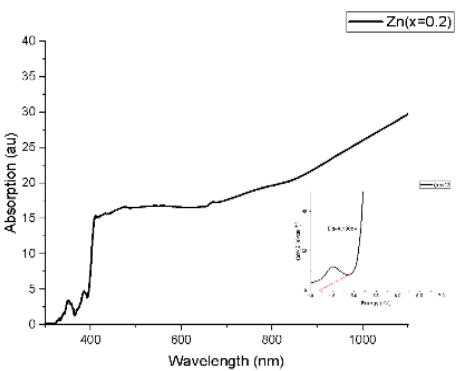


Figure 3 (b)

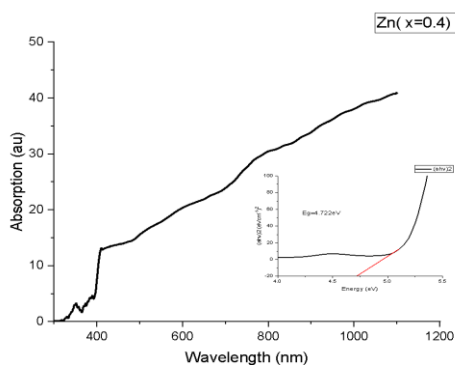


Figure 3 (c)

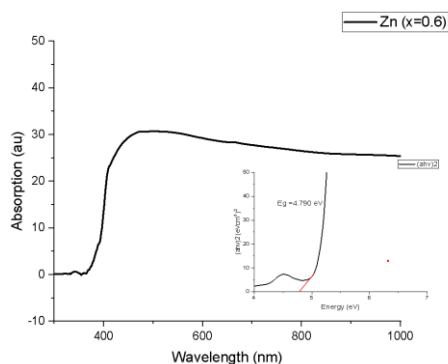


Figure 3 (d)

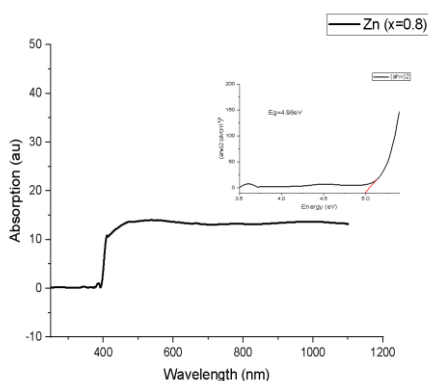


Figure 3 (e)

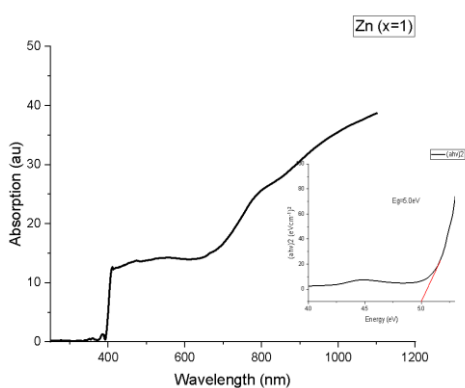


Figure 3 (f)

d) FTIR Spectra

Figure 4 shows the FTIR spectra of Zn doped CoFe_2O_4 prepared by sol gel auto-combustion method using urea as fuel and sintered at 700°C recorded between 4000 cm^{-1} and 400 cm^{-1} ¹⁸. The O–H stretching vibrations interacting through H bonds are observed at 3393 cm^{-1} , 2927 cm^{-1} and the absorption band present at about 1647 cm^{-1} is due to the bending of the absorbed water molecules. The absorption band at 1120 cm^{-1} is characteristic of the cobalt ferrite system and this may be due to the residual FeO-OH . The absorption bands present at about 635 cm^{-1} is due to the stretching vibrations of metal oxide in octahedral group complex Co (II)-O_2 of the cobalt ferrite phase which proves the existence of spinel ferrite. Fading and diminishing of absorption band at 1647 cm^{-1} may be due to the desorption of water molecules after 0.06% Conc. Also, the characteristic 1120 cm^{-1} peak reappears after 0.08% Conc. Is due to the restructuring and affixing the dopant % in the spinel structure with decreased strain¹⁰. Fourier transform infrared (FTIR) absorption bands (ν_1 , ν_2) for ZCF ferrite nanoparticles with $x = 0, 0.2, 0.4, 0.6, 0.8$, and 1 are shown in Figure 4.

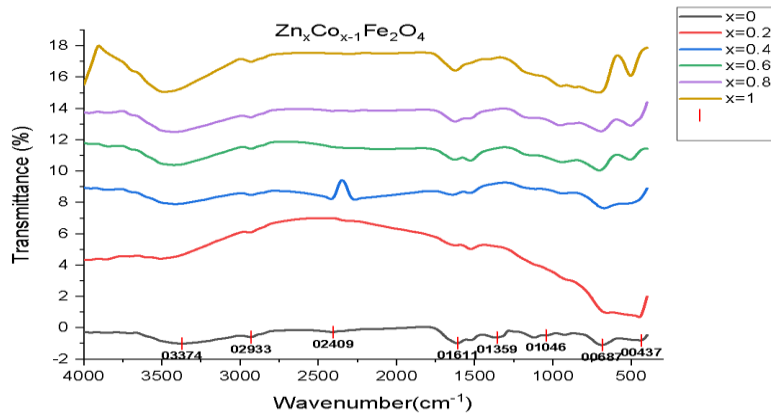


Figure 4: Fourier transform infrared spectra of $\text{Zn}_x\text{Co}_{1-x}\text{Fe}_2\text{O}_4$

It is well known that spinel ferrites exhibit two FTIR-active bands, designated as ν_1 and ν_2 . The higher-frequency band, ν_1 , is generally observed in the range of $500\text{--}700\text{ cm}^{-1}$, while the lower-frequency band, ν_2 , typically appears in the range of $350\text{--}450\text{ cm}^{-1}$. These bands correspond to the stretching vibrations of metal-oxygen bonds in the tetrahedral and octahedral sites, respectively. Both bands gradually decrease as the zinc concentration increases in cobalt ferrite¹².

Table 2: absorption bands (ν_1, ν_2) for ZCF ferrite

Ferrite	ν_1	ν_2
CF	687	437
ZCF-0.2	507	410
ZCO-0.4	512	405
ZCF-0.6	502	406
ZCF-0.8	501	401
ZCF-1	500	396

e) Antimicrobial Activity

Antibacterial activity of ZCF is evaluated against *E. coli* and *B. subtilis* by agar well diffusion assay is shown in Figure respectively. Also the antibacterial activity measurements in terms of zone of inhibition are summarized in Table 3. All samples showed inhibitory effect on both *E. coli* and *B. subtilis* in agar well diffusion method, and their inhibitory effects increased in concentration dependent manner. The ZCF-0.8 is more inhabitant against *E coli* whereas ZCF-0.4 is found to be more sensitive against *B. subtilis* which could be due to the structural differences in their cell wall. The table 3 shows the range of inhibition zone against *E coli* and *B. subtilis* for different concentrations.

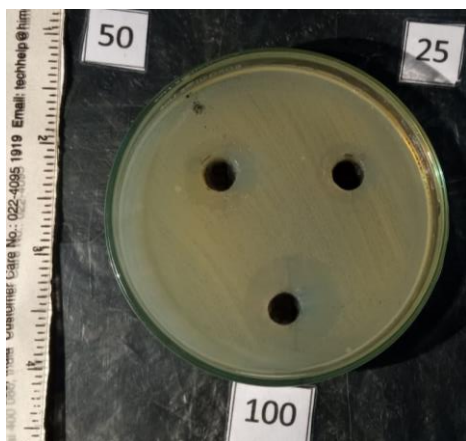
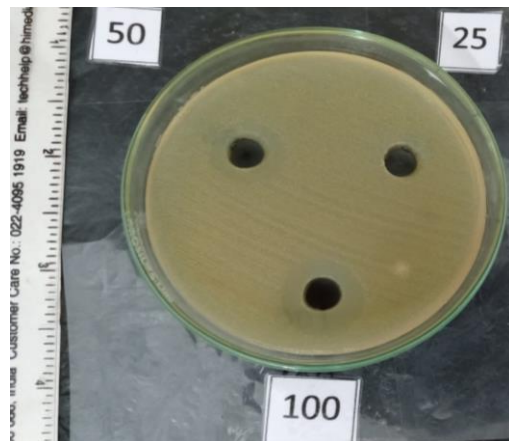
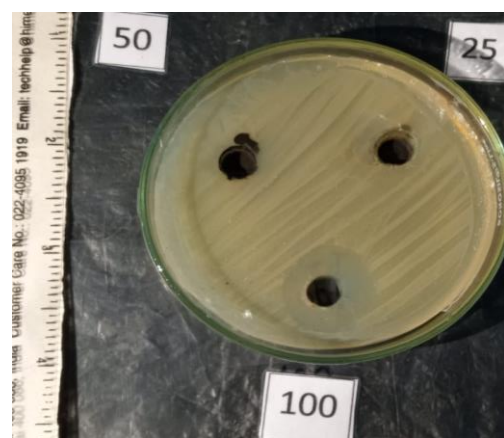
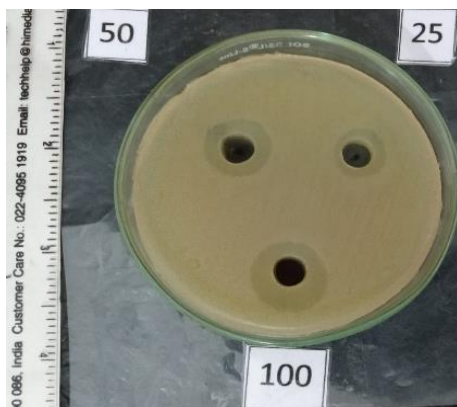
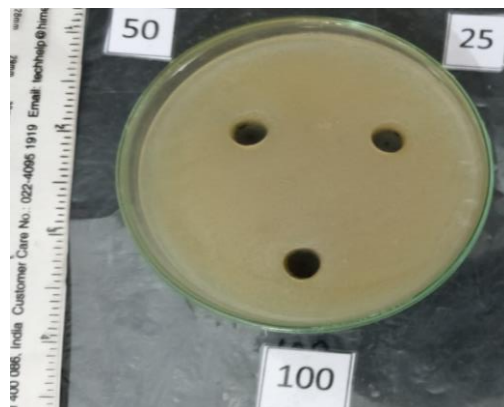
**BS11-CF-0.0****BS11-ZCF-0.4****BS11-ZCF-0.8****EC21-CF-0.0****EC21-ZCF-0.4****EC21-ZCF-0.8**

Table 3: Antibacterial activities of zinc cobalt ferrite (ZCF) nanoparticles against selected pathogenic microbes in terms of zone of inhibition (ZOI) (in mm)

Antibacterial activity of ZCF against <i>Bacillus subtilis</i>			
Zone of Inhibition in mm			
Organisms	25µl	50 µl	100 µl
BS11-CF-0.0	14	15	18
BS11-ZCF-0.4	15	16	21
BS11-ZCF-0.8	NI	15	16
Note:- NI-No Inhibition			

Antibacterial activity of ZCF against <i>Escherichia coli</i>			
Zone of Inhibition in mm			
Organisms	25µl	50 µl	100 µl
EC21-CF-0.0	NI	NI	17
EC21-ZCF-0.2	NI	16	18
EC21-ZCF-0.8	NI	NI	19
Note:- NI-No Inhibition			

4. Conclusion

The integration of metals ion zinc into CF nanoparticles was studied. The sol–gel auto-combustion manufacturing technique used eco-friendly and cost-effective nanoparticle formation. Complete identification and analysis revealed the crystallite size, the surface structural morphology, elemental compositions, band gap, and functional groups of the synthesized nano ferrites. The crystallite size, determined from XRD, ranged from 43 nm for 27 nm ZCF ferrite. It is generally known that the spinel ferrites exhibit two FTIR-active bands, designated as ν_1 and ν_2 . The higher frequency band ν_1 , observed in the range $550\text{--}750\text{ cm}^{-1}$, and lower frequency band ν_2 , in the range $400\text{--}450\text{ cm}^{-1}$, are associated with the stretching of bonds between the metal ions and oxygen in the tetrahedral and octahedral sites, respectively. EDX measurements indicated that ZCF nanoparticles were homogeneous¹³⁻¹⁴ with a uniform elemental distribution. ZCF nanoparticles also has strong bactericidal efficacy against *E. coli* (19.0 mm ZOI) for ($x = 0.8$) and *Bacillus subtilis* (21.0 mm ZOI) for ($x = 0.4$) Zn conc. in Cobalt spinel ferrite and reflects the most potent, possessing strong antimicrobial activity toward selected pathogenic which makes it potential candidate in dermatological and biomedical treatments.

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