

Synthesis of CoFe_2O_4 Nanoparticles and Evaluation of Their Antibacterial Activity

Listiyani Purwitasari^{1*}, Poedji Loekitowati Hariani¹, Vivi Sisca²

¹ Master's Program in Chemistry, Faculty of Mathematics and Natural Science, Sriwijaya University, Jln. Raya Palembang Prabumulih KM 32 Indralaya Ogan Ilir, Indonesia, 30662

² Research Center for Energy Conversion and Conservation, National Research and Innovation Agency (BRIN), B.J. Habibie Science and Technology Park, South Tangerang, Indonesia, 15313

*Corresponding Author: l.purwitasari@gmail.com

Abstract

Cobalt ferrite (CoFe_2O_4) is a spinel ferrite-based material known for its excellent magnetic properties and chemical stability, making it as a promising candidate for biomedical applications as well as an antibacterial agent. This study aims to synthesize CoFe_2O_4 nanoparticles by a coprecipitation method and evaluate their antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The synthesized products were characterized by using several instruments includes X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), FTIR spectroscopy and Vibrating Sample Magnetometer (VSM) in order to determine its crystal structure, particle morphology, functional groups, and magnetic properties. Antibacterial activity was assessed by using the disk diffusion method. The results showed that CoFe_2O_4 nanoparticles were successfully synthesized with nanometer-scale crystallite sizes and exhibited strong ferromagnetic properties. Antibacterial tests demonstrated inhibition zones against the growth of *S. aureus* and *E. coli*, indicating that CoFe_2O_4 has potential as an antibacterial agent. The effectiveness of antibacterial activity was influenced by nanoparticle concentration and the type of tested bacteria. This research opens up further opportunities for the development of CoFe_2O_4 applications in health and environmental fields.

Keywords: Synthesis, nanoparticle, CoFe_2O_4 , characterization, antibacterial

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Kobalt ferit (CoFe_2O_4) merupakan salah satu material berbasis spinel ferrite yang memiliki sifat magnetik dan stabilitas kimia yang baik, sehingga berpotensi dalam aplikasi biomedis, termasuk sebagai agen antibakteri. Penelitian ini bertujuan untuk mensintesis nanopartikel CoFe_2O_4 melalui metode kopresipitasi serta mengevaluasi aktivitas antibakterinya terhadap bakteri Gram positif (*Staphylococcus aureus*) dan Gram negatif (*Escherichia coli*). Hasil sintesis dikarakterisasi menggunakan teknik X-Ray Diffraction (XRD), spektroskopi FTIR, Scanning Electron Microscopy (SEM), Vibrating Sample Magnetometer (VSM) untuk menentukan struktur kristal, gugus fungsi, morfologi partikel, dan sifat magnetiknya. Uji aktivitas antibakteri dilakukan menggunakan metode difusi cakram (disk diffusion). Hasil menunjukkan bahwa nanopartikel CoFe_2O_4 berhasil disintesis dengan ukuran kristalit dalam skala nanometer dan menunjukkan sifat feromagnetik yang kuat. Pengujian antibakteri memperlihatkan adanya zona hambat terhadap pertumbuhan *S. aureus* dan *E. coli*, yang menunjukkan bahwa CoFe_2O_4 memiliki potensi sebagai agen antibakteri. Efektivitas antibakteri dipengaruhi oleh konsentrasi nanopartikel dan jenis bakteri uji. Penelitian ini membuka peluang untuk pengembangan lebih lanjut aplikasi CoFe_2O_4 dalam bidang kesehatan dan lingkungan.

Kata Kunci: Sintesis, partikel nano, CoFe_2O_4 , karakterisasi, antibakteri

INTRODUCTION

Nanotechnology has brought significant advances in various fields, including medicine, pharmaceutical, and environment. At the nanometer scale, materials often exhibit drastically different physical, chemical, and biological properties compared to their bulk form [1]. One of nanostructured material that has received considerable attention is ferric spinel, especially cobalt ferrite (CoFe_2O_4). CoFe_2O_4 is a transition metal-based ferrite compound that has a cubic spinel crystal structure, high chemical stability, and strong ferromagnetic properties [2]. This combination of characteristics makes CoFe_2O_4 highly attractive for a variety of applications. In electronics, CoFe_2O_4 is widely used as a magnetic storage material, sensors, and energy devices [3]. In catalysis, this material is effective in the degradation of organic pollutants and oxidation-based chemical reactions. Equally important, its magnetic properties allow for easy separation using an external magnetic field, thus supporting the principle of using environmentally friendly and reusable materials. Moreover, CoFe_2O_4 is not only relevant for electronics and catalysis but also have great potential as biomedical ingredients, as well as antibacterial agent.

The increasing resistance toward conventional antibiotic is driving the development of new alternatives for controlling bacterial infections. Metal oxide nanoparticles, including ferrite, exhibit antibacterial activity through various mechanisms such as the generation of reactive oxygen species (ROS), the direct interaction with cell membranes, and the disruption of enzymatic function [4]. In this context, CoFe_2O_4 nanoparticles could be a promising candidate since they not only have antibacterial activity, but also are easily modified and separated using a magnetic field.

Various methods have been used to synthesize CoFe_2O_4 nanoparticles, including sol-gel, coprecipitation, and hydrothermal methods. The choice of synthesis method affects particle size, morphology, and surface properties, which in turn impact antibacterial efficacy [5]. Therefore, it is important to evaluate how a particular synthesis method affects the characteristics of CoFe_2O_4 and its relationship to its biological activity [6].

This study aims to synthesize CoFe_2O_4 nanoparticles using the coprecipitation method, characterize their structure and morphology, and test their antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. It is expected that the results of this study can contribute to the development

of effective and environmentally friendly ferrite-based antibacterial materials [7].

MATERIALS AND METHODS

Materials

The material used include Cobalt Chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) from Merck PA; Sodium Hydroxide (NaOH) from Merck PA; iron (III) chloride (FeCl_3) anhydrate from Merck PA, deionized water, dimethyl Sulfoxide (DMSO) from Merck PA.

Synthesis of CoFe_2O_4

Synthesis of CoFe_2O_4 was conducted by reacting 4.04 g of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and 6.34 g of anhydrous FeCl_3 dissolved in 150 mL of distilled water, then the mixture was stirred for 20 min at room temperature. The pH of the mixture was adjusted to 11 by adding 1 M NaOH dropwise. Stirring was carried out for 2 h until a black precipitate appeared. The precipitate was washed with water several times until the pH became neutral, then dried in an oven at 100 °C for 20 h. Then it was heated in a furnace at 500 °C for 2 h.

Characterization nanoparticle

The nanoparticle CoFe_2O_4 was characterized using the XRD Smartlab Rigaku. The CoFe_2O_4 sample synthesized analyzed using FTIR spectroscopy. Meanwhile, the morphology and elemental composition were determined using a Scanning Electron Microscope equipped with X-ray Energy Dispersion Spectroscopy, and VSM for magnetic test.

Antibacterial activity test

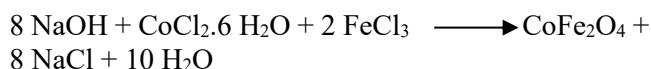
Antibacterial activity testing was conducted using the Kirby-Bauer disc diffusion method, using 5 mm diameter paper discs containing *S. aureus* and *E. coli* as test bacteria. The activity test was repeated three times. The paper discs were soaked in with 20 μL of nanomagnetic dispersion at concentrations of 1000–5000 ppm. The dispersion was prepared by using DMSO as solvent and vortexed for 5 minutes. Treatments of positive control (Amoxicillin) and a negative control (DMSO) were added to the activity test. Using sterile tweezers, the paper discs were placed on the surface of the media. The Petri dishes were incubated for 24 h at 37 °C until a zone of inhibition appeared. Bacterial growth was observed by observing the zone of inhibition formed with a ruler or caliper. The Petri dishes were then incubated at 37 °C for 24 h. Measurements were taken after 24 hours of incubation.

The formation of a clear zone around the well indicates antibacterial activity. This clear zone was identified as the zone of inhibition, and its diameter was then measured using a caliper and included in the calculation.

Inhibition zone = well diameter – inhibition zone diameter.

RESULTS AND DISCUSSION

The synthesis of CoFe_2O_4 was successfully carried out through the coprecipitation method using $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ precursors as a source of Co^{2+} ions, anhydrous FeCl_3 as a source of Fe^{3+} ions, and NaOH as a precipitating agent. The reaction is following this equation:



In this study, 3.5463 grams CoFe_2O_4 was obtained. The yield of the reaction 88,65%. The coprecipitation process at alkaline pH resulted in metal hydroxide precipitates which were then dried and calcined to form the spinel phase of CoFe_2O_4 . The success of the synthesis was confirmed through characterization results, where the X-ray diffraction (XRD) pattern showed typical peaks of the spinel structure, while FTIR analysis showed absorption bands in the $400\text{--}600 \text{ cm}^{-1}$ region related to the vibrations of metal–oxygen bonds (Fe-O and Co-O). In addition, the SEM-EDX results showed a particle morphology that tended to be agglomerative with the distribution of Co, Fe, and O elements in accordance with the theoretical composition. This confirms that this simple coprecipitation method is effective in producing cobalt ferrite (CoFe_2O_4) with good phase purity [5].

Characterization results

XRD characterization was performed to determine the 2θ angle position, peak intensity, and crystal phase, while also evaluating the success of the nanoparticle synthesis process. The diffractogram of the nanoparticle synthesis results is shown in **Figure 1**.

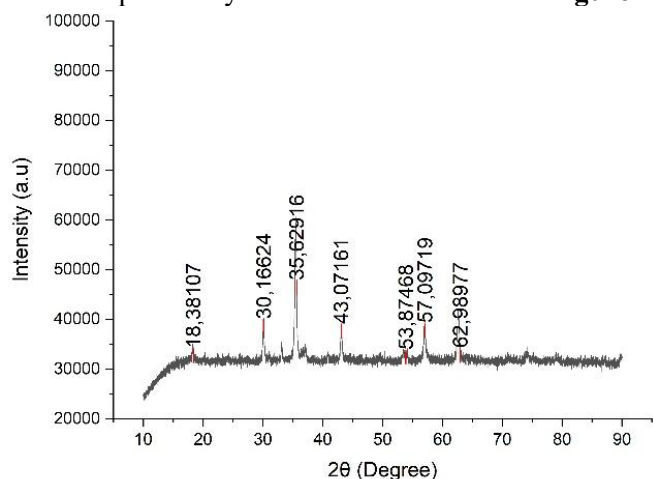


Figure 1. Diffractogram of CoFe_2O_4 nanoparticle

The X-ray diffraction (XRD) pattern in the figure above shows several characteristic peaks located at 2θ angles of approximately 18.38 ; 30.16 ; 35.29 ; 43.07 ; 53.87 ; 57.10 ; and 62.98° . These peaks correspond to the (111), (220), (311), (400), (422), (511), and (440) crystal planes, which are characteristic of the cubic spinel structure of CoFe_2O_4 as stated in JCPDS standard card No. 22-1086 or JCPDS No. 78-1744 [5].

The sharp and well-defined peak intensities indicate that the synthesized material has a high degree of crystallinity. The presence of all major planes without additional peaks confirms that the formed phase is a single-phase CoFe_2O_4 without any impurities such as Fe_2O_3 or CoO [8,9].

The width of the diffraction peaks also provides information about the crystallite size. Using the Scherrer equation, the crystallite size of CoFe_2O_4 is 17.34 nm , which is consistent with the results of the coprecipitation. The synthesized material exhibits a nanometer scale size, which may enhance its antibacterial activity [10,11,12].

FTIR characterization was used to identify functional groups in CoFe_2O_4 . The FTIR spectrum of CoFe_2O_4 is shown in **Figure 2**.

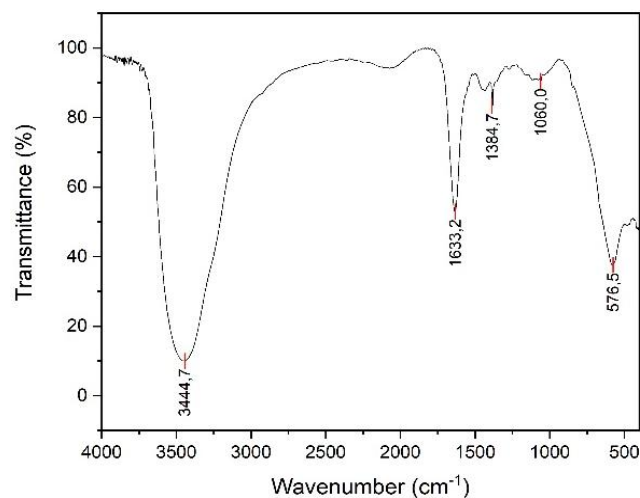


Figure 2. Spectrum of FTIR CoFe_2O_4 nanoparticle

The spectrum shows a broad band at 3444.7 cm^{-1} , which is related to the stretching vibration of water molecules both in the free and adsorbed form. The peak at 1633.2 cm^{-1} is associated with the H-O-H bending vibrations of water molecules [11-13]. Its presence further strengthens the indication of the presence of residual bound or adsorbed water on the surface of the CoFe_2O_4 material. The Absorption band at 1384.7 cm^{-1} is usually associated with C–O vibrations of residual carbonate ions (CO_3^{2-}) which may originate from reagents or synthesis processes (e.g. the use of a strong base such as NaOH which can react with CO_2 from the air) [5].

The absorption bands appearing at low wavenumbers are characteristic of the stretching vibrations of metal–oxygen bonds (Fe–O and Co–O) in the spinel structure [14-15]. The appearance of these bands confirms the formation of the CoFe_2O_4 spinel phase, as metal–oxygen vibrations generally occur in the range of $400\text{--}600\text{ cm}^{-1}$. Accordingly [16-17], in the

CoFe_2O_4 spectrum, a sharp peak is seen at 576 cm^{-1} which is associated with the vibration of the Fe–O bond.

Meanwhile, material characterization was also carried out using the SEM-EDX instrument and the results are shown in **Figure 3**.

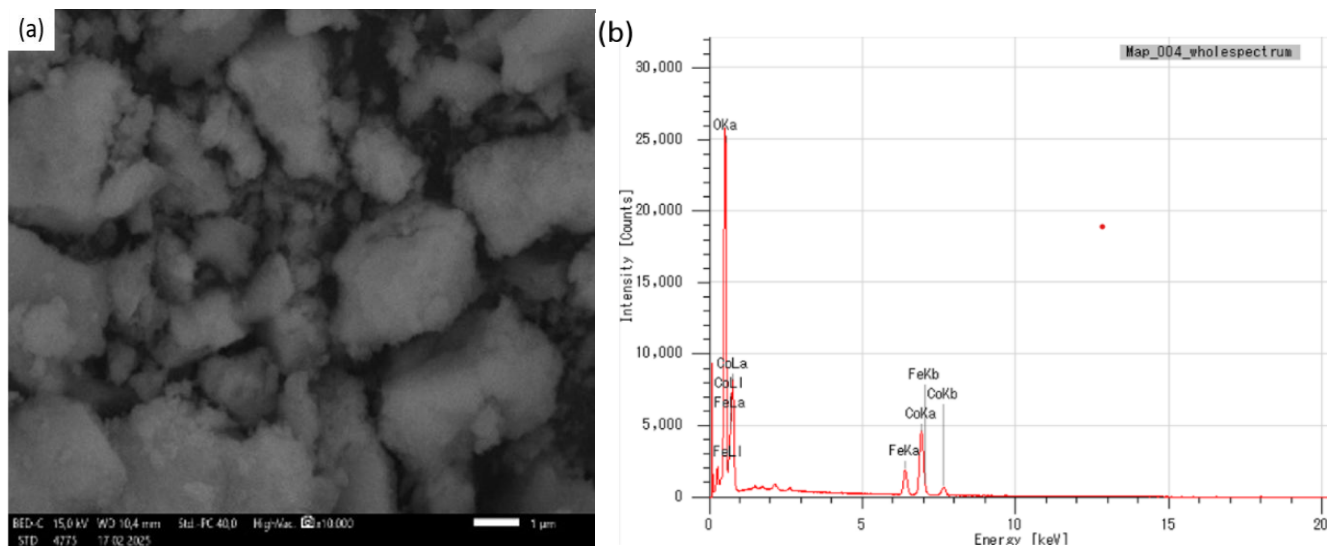


Figure 3. (a) Morphological CoFe_2O_4 nanoparticle and (b) element analysis

SEM images at 10,000x magnification in **Figure 3(a)** show that CoFe_2O_4 particles have a morphology that tends to be aggregative with irregular particle shapes. It can be seen that small particles combine to form clumps (agglomerates) [13-19], which generally occurs due to the presence of interparticle magnetic (dipole–dipole interaction) forces and high surface energy in nanoparticles [20,21]. The observed particle sizes are on the submicron to nanometer scale, in accordance with the characteristics of ferrite spinel-based materials. Each nanoparticle possesses a strong magnetic moment, leading to magnetic attraction and cluster formation. In addition, van der Waals forces further enhance interparticle adhesion. Because of the high surface-to-volume ratio, CoFe_2O_4 nanoparticles exhibit high surface energy, and to minimize this energy, particles tend to attach to one another [18].

The absence of stabilizing agents or surfactants during synthesis exacerbates this behavior. Without steric or electrostatic barriers, particles interact directly and form aggregates [17]. Furthermore, calcination at high temperatures can induce sintering, resulting in the fusion of smaller grains into larger ones. During SEM sample preparation, rapid solvent evaporation may also cause particle accumulation in localized regions due to capillary drying effects [22,23].

Synthesis conditions such as pH, precursor concentration, and reaction rate also influence

agglomeration. Uncontrolled nucleation and rapid crystal growth lead to particle clustering [17]. Overall, agglomeration in CoFe_2O_4 is attributed to the combined effects of magnetic attraction, van der Waals forces, surface energy, and synthesis parameters. To reduce agglomeration, surfactants (such as PVP, citrate, or oleic acid) can be used, along with optimized calcination conditions and controlled reaction parameters [24].

The EDX analysis results in **Figure 3(b)** show the characteristic peaks of the material's main constituent elements: cobalt (Co), iron (Fe), and oxygen (O). The presence of Co and Fe peaks in the energy range of approximately 0.7–7 keV confirms that the synthesized material contains cobalt and iron ions, consistent with the theoretical composition of CoFe_2O_4 . Meanwhile, the presence of oxygen (O_4) peaks confirms that metal elements have bonded with oxygen to form oxide compounds in the spinel structure.

The relatively high intensity of the Co and Fe peaks compared to oxygen indicates the dominance of metal elements on the sample surface, which is common in ferrite-based materials. The absence of other element peaks indicates that the synthesis process produced a material of good purity without significant contaminants from precursors or other additives.

Thus, these EDX results support the previous XRD and FTIR analyses and confirm that the synthesis

successfully produced CoFe_2O_4 nanoparticles with a composition consistent with the spinel structure. In addition, magnetic strength tests were carried out using a VSM instrument, the results are shown in **Figure 4**.

The curve shows a relatively narrow S-shape, indicating that CoFe_2O_4 has ferrimagnetic properties with the possibility of approaching superparamagnetic behavior at nanoparticle size. The smaller the crystallite size, the weaker the coercive field (H_c) formed. The saturation magnetization value (M_s) is obtained at a field of around ± 1 T, with a value of around 7–8 emu/g. This value is in line with the magnetic properties of CoFe_2O_4 in nanoparticle form, which are generally lower than bulk CoFe_2O_4 (80–90

emu/g), due to the influence of small size, crystal imperfections, and surface effects. [38,22]. From the graph, H_c appears quite small, in the range of hundreds of Oe. This indicates that the material has magnetic properties that are relatively easy to reverse in direction (soft magnetic). This low coercivity is suitable in applications for magnetic data storage, sensor materials, and biomedical applications (drug delivery, hyperthermia therapy). The remanence value (M_r) appears small compared to M_s . This further confirms that the synthesized CoFe_2O_4 has properties approaching superparamagnetic, which is greatly influenced by the size of the nanoparticles and their distribution [16,17,24].

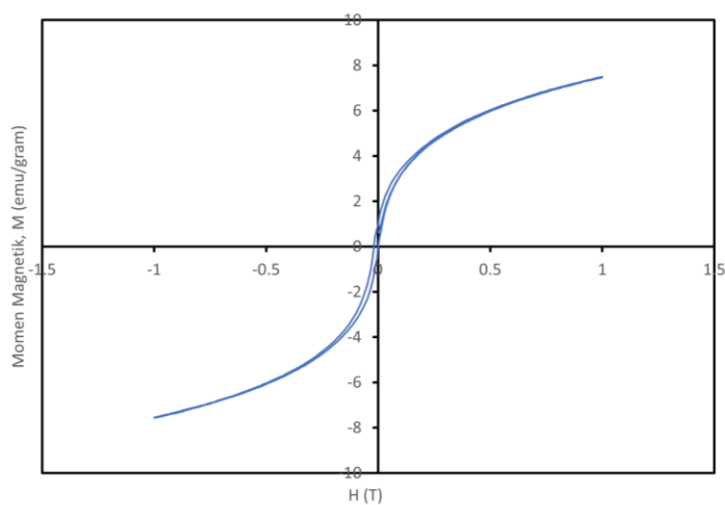


Figure 4. VSM results of CoFe_2O_4

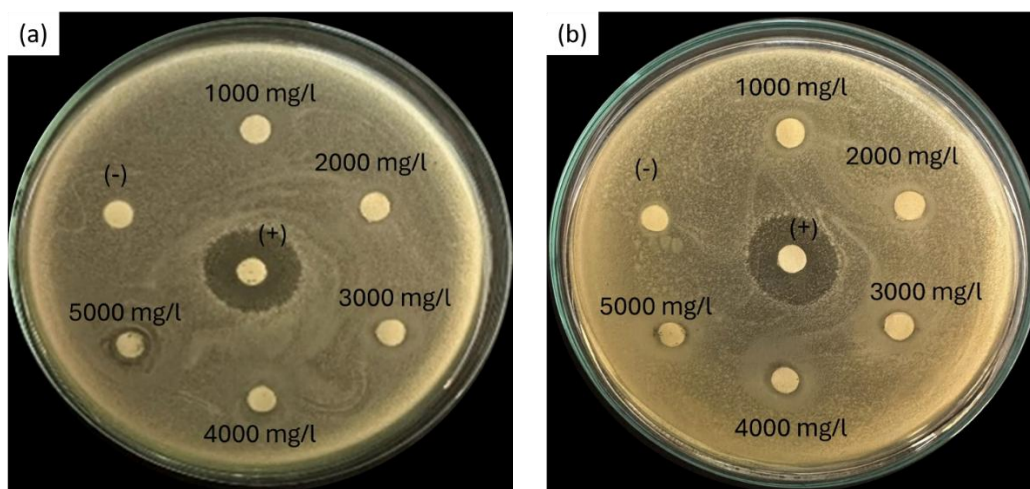


Figure 5. CoFe_2O_4 nanoparticle for antibacterial test of a) *S. aureus* b) *E. Coli*

Antibacterial activity

Finally, an antibacterial activity test was carried out involving *S. aureus* and *E. Coli* bacteria, and the results are presented in **Figure 5**.

Antibacterial activity tests showed that the CoFe_2O_4 nanoparticle was able to inhibit the growth of both bacteria. The inhibition zone is indicated by a clear area around the paper disc. Although it is not

clearly visible, the inhibition zone increasing with increasing concentration [8].

The obtained data show that the inhibition zones produced by CoFe_2O_4 nanoparticles were relatively small, ranging between 6 and 7.83 mm. These values are significantly lower than those of the positive control (amoxicillin), which demonstrated inhibition zones of 17.83 mm for *S. aureus* and 19.17 mm for *E. coli*. In contrast, the negative control (DMSO) showed no inhibition zone, confirming that the antibacterial effects were solely due to the nanoparticle. The complete results of the antibacterial tests are presented in Table 1.

Table 1. CoFe_2O_4 nanoparticle for antibacterial test of *S. aureus* and *E. Coli*

Nanoparticle Concentration	<i>S. Aureus</i> (mm)	<i>E. Coli</i> (mm)
Amoxicillin Positive Control (1000 mg/L)	17.83	19.17
DMSO Negative Control (1000 mg/L)	6 ± 0	6 ± 0
Nanoparticle 1000 mg/L	6 ± 0	6 ± 0
Nanoparticle 2000 mg/L	6 ± 0	6 ± 0
Nanoparticle 3000 mg/L	6.5 ± 0.5	6.83 ± 0.4
Nanoparticle 4000 mg/L	7 ± 0	7.5 ± 0.5
Nanoparticle 5000 mg/L	7.67 ± 0.8	7.83 ± 0.9

A gradual increase in inhibition zone diameter was observed as the concentration of CoFe_2O_4 nanoparticles increased from 1000 to 5000 mg/L, indicating a strong concentration-dependent antibacterial effect. However, the overall activity remained weak, suggesting that CoFe_2O_4 possesses limited bactericidal capability under the tested conditions. The weak antibacterial response could be attributed to several physicochemical factors. CoFe_2O_4 nanoparticles are prone to agglomeration, which decreases their effective surface area and reduces direct contact with bacterial cell membranes. This aggregation limits the nanoparticle's ability to disrupt cell walls or induce oxidative stress [28].

Furthermore, CoFe_2O_4 has a highly stable spinel structure that restricts the release of metal ions such as Co^{2+} and Fe^{3+} . The antibacterial activity of many metal oxide nanoparticles depends on ion release and the generation of reactive oxygen species (ROS), which can damage bacterial membranes and intracellular components. Limited ion dissolution and low ROS generation likely contribute to the minimal inhibition observed [23,24].

Comparatively, *E. coli* exhibited slightly larger inhibition zones than *S. aureus*, suggesting that Gram-negative bacteria were marginally more susceptible to

CoFe_2O_4 nanoparticles. This observation may be explained by the thinner peptidoglycan layer in Gram-negative bacteria, which allows easier interaction and penetration of nanoparticles or released ions, whereas the thick peptidoglycan layer of *S. aureus* offers stronger resistance to nanoparticle intrusion.

Overall, the results indicate that CoFe_2O_4 nanoparticles display weak antibacterial performance, primarily due to their chemical stability, particle aggregation, and limited oxidative reactivity. Nevertheless, the increasing trend in inhibition zone with higher nanoparticle concentration suggests that the antibacterial effect is dose-dependent.

CONCLUSION

CoFe_2O_4 nanoparticle were successfully synthesized using the coprecipitation method. Characterization results using XRD and FTIR proved the successful formation and composition of the material. However, CoFe_2O_4 nanoparticles exhibit relatively weak on antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. The low activity is likely associated with particle agglomeration, high chemical stability, and limited release of Co^{2+} and Fe^{3+} ions, which reduce the formation of reactive oxygen species (ROS) responsible for bacterial cell damage. SEM-EDX result may indicate the possibility of particle agglomeration. Nevertheless, the slight increase in inhibition zone with increasing concentration implies a concentration-dependent trend. Future improvements could focus on optimizing synthesis conditions to obtain smaller, well-dispersed nanoparticles or incorporating CoFe_2O_4 with other antibacterial agents to enhance its overall bactericidal performance.

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