https://chemclassjournal.com/
ChemClass Journal Vol. 10 Issue 1 (2026); 204 - 215
e-ISSN:3092-8214 p-ISSN:3092-8206
doi.org/10.33003/chemclass-2026-1001/16

Biosynthesis and Antimicrobial Activity of Bimetallic Iron/Cobalt Nanoparticles Using *Allium Sativum* Extract

Idris, A. A.*, Ahmed, W. A., Azeez, G. O., Basiru, Q., Afolabi, N. A., and Adepoju, R. A.

Department of Science Laboratory Technology, The Oke-Ogun Polytechnic, Saki, Oyo State, Nigeria.

*Corresponding Author: abdulakeemidris2013@gmail.com; +2348075610441

Abstract

This study reports the biosynthesis, characterization, and antimicrobial evaluation of Iron/Cobalt Fe/Co bimetallic nanoparticles synthesized using extract of *Allium sativum* (garlic) as a green reducing and capping agent. The formation of Iron/Cobalt nanoparticles was characterized by UV-Visible Spectroscopy, Scanning Electron microscopy (SEM), X-ray Diffraction (XRD). UV–Vis spectroscopy revealed a surface plasmon resonance peak in the range of 350–450 nm, confirming nanoparticle formation. SEM micrographs indicated quasi-spherical particles with moderate aggregation, while XRD analysis displayed characteristic crystalline peaks, suggesting alloy formation and minor oxide phases. Antimicrobial screening demonstrated potent bactericidal activity, with *Salmonella typhi* exhibiting the lowest MIC and MBC values (3.125 µg/mL) and *Klebsiella* the highest among bacteria (12.5 µg/mL). *Staphylococcus aureus* and *Escherichia coli* both showed MICs and MBCs of 6.25 µg/mL. Antifungal testing against *Candida albicans* revealed MICs of 6.25–25 µg/mL. These values compare favorably with literature reports on bimetallic nanoparticles, where enhanced efficacy is attributed to synergistic effects between the metals, increased reactive oxygen species generation, and sustained ion release. The combination of effective synthesis, confirmed crystalline structure, and strong antimicrobial performance positions Fe/Co nanoparticles as promising candidates for biomedical and environmental applications.

Keywords: Antimicrobial, Biosynthesis, Characterization, Extract, Nanoparticles

Introduction

Nanotechnology has emerged as one of the most transformative scientific disciplines in the 21st century, allowing for the manipulation of matter at atomic, molecular, and supramolecular scales.

[1]. Nanoparticles are the materials with at least one dimension in the nanometer range (1-100 nm), and have received extensive attention due to their unusual physicochemical, electrical, magnetic, optical, and biological capabilities, which are markedly different from their bulk counterparts [2]. Among the numerous metal nanoparticles studied, iron (Fe) and cobalt (Co) nanoparticles have attracted significant interest because of their diverse applications in catalysis, magnetic data storage, environmental remediation, biomedical fields, and particularly, antimicrobial activity [3].

© CSN Zaria Chapter

However, conventional methods of synthesizing these nanoparticles often involve the use of hazardous chemicals, high temperatures, and expensive equipment, posing serious risks to human health and the environment. This has led to a surge in interest toward environmentally benign and sustainable approaches to nanoparticle synthesis, most notably green synthesis methods [4].

Green synthesis, also known as biological synthesis, utilizes biological systems such as plant extracts, fungi, bacteria, and algae as reducing and stabilizing agents to produce nanoparticles. Among these, plant-mediated synthesis is particularly advantageous due to its simplicity, cost-effectiveness, scalability, and avoidance of toxic reagents [5]. Plants possess a rich diversity of phytochemicals—such as flavonoids, alkaloids, terpenoids, phenolics, and organosulfur compounds—which can effectively reduce metal ions and stabilize the resulting nanoparticles [6]. *Allium sativum* (Garlic), a member of the Alliaceae family, is one such plant that has gained prominence in green nanotechnology. Renowned for its culinary and medicinal applications, garlic is rich in organosulfur compounds like allicin, ajoene, and diallyl disulfide, which have been reported to possess strong reducing and antimicrobial properties [7]. These phytochemicals not only facilitate the formation of metal nanoparticles but also impart biocompatibility and bioactivity, particularly against microbial pathogens.

The integration of iron and cobalt into a bimetallic nanostructure within Fe/Co nanoparticles presents a significant area of scientific inquiry. Bimetallic nanoparticles often exhibit synergistic effects, wherein the properties of the composite material exceed the simple addition of the individual components [8]. Iron nanoparticles are known for their magnetic behavior and redox activity, making them suitable for environmental and biomedical applications. However, pure Fe nanoparticles are prone to oxidation and aggregation, which can limit their functional efficacy [9]. Cobalt nanoparticles, on the other hand, are also magnetic and exhibit notable catalytic and antimicrobial properties, but concerns about their cytotoxicity persist [10]. When combined into Fe/Co nanoparticles, the resulting material often demonstrates enhanced stability, magnetic responsiveness, and antimicrobial efficacy, making it a valuable candidate for multi-functional applications [11].

The increasing prevalence of antimicrobial resistance (AMR) globally has further highlighted the urgent need for novel antimicrobial agents. Traditional antibiotics are becoming increasingly ineffective against multi-drug-resistant strains of bacteria and fungi [12]. In this context, nanoparticles synthesized through green methods provide a promising alternative due to their unique mechanisms of microbial inhibition, such as membrane disruption, generation of reactive oxygen species (ROS), and interference with cellular metabolic pathways [13].

The antimicrobial screening of biosynthesized Fe/Co nanoparticles is particularly relevant in the light of rising infectious diseases and the

emergence of resistant pathogens. Microbial infections, caused by bacteria, fungi, and viruses, continue to pose significant public health challenges globally. Conventional antimicrobial agents are facing diminishing returns, necessitating the exploration of alternative solutions. Nanoparticles, by virtue of their nanoscale dimensions and high surface area-to-volume ratio, can interact with microbial cells in ways that are fundamentally different from bulk materials or molecular drugs. Their ability to target multiple cellular pathways simultaneously reduces the likelihood of resistance development [14]. Moreover, nanoparticles synthesized with plant-based extracts often carry phytochemical moieties on their surface, which can synergistically enhance their antimicrobial potential.

Previous studies have demonstrated the antimicrobial efficacy of individual Fe and Co nanoparticles, yet relatively few have investigated the combined effect of bimetallic Fe/Co nanoparticles, especially when synthesized using plant extracts like garlic. The phytochemical profile of garlic, including sulfur-containing compounds and phenolics, may further augment the antimicrobial action of Fe/Co nanoparticles by increasing their interaction with microbial membranes or enhancing their oxidative potential [15].

Materials and Methods

Reagent

$Co(NO_3)_2$ and $Fe(NO_3)_2$ were purchased from Pascal Scientific Ltd were utilized as the starting material. *Allium sativum* (garlic) was obtained from Owode Market, Saki located at latitude 8.623444° N and longitude 3.402950° E, Oyo State. All reagents were dissolved in distilled water throughout the experiment, and no further purification was carried out, as all chemicals were of analytical grade.

Preparation of the aqueous *A. sativum* extract

A. sativum was cleaned and repeatedly washed with distilled water to completely remove all dust particles. The thoroughly washed *A. sativum* was sliced and air-dried, and thereafter ground using a mechanical grinder. This was subsequently sieved and stored in an airtight container. The extraction was carried out by measuring 10 g of powdered garlic and then boiled in 100 mL of deionized water at 60 °C for 30 min. The extract was then filtered through a Whatman 185 µm filter paper, yielding a light brown colored filtrate. The extract was kept in the refrigerator between 4°C and utilized for the synthesis of bimetallic nanoparticles.

Synthesis of Fe/Co Nanoparticles from Plant Extracts

The synthesis of bimetallic nanoparticles was carried out according to the method described by Dada *et al.* [16] with slight modification. In a typical experiment, 50 mL of the aqueous extract was measured into a clean 250 mL conical flask, then 50 mL each of 0.1M $Zn(NO_3)_2$ and 0.1M $Fe(NO_3)_2$ were added to the extract and placed on a magnetic stirrer, it was then stirred for 1 hour on magnetic stirrer 60 ^{0}C. The synthesized mixture was left for 24 h and afterwards centrifugation at

4000 rpm for 30 min. The transparent liquid was decanted, and the settling layer (i.e., nanoparticles) was washed with distilled water and dried in the oven at 50 °C and then calcined at 350 °C. The calcined nanoparticles were stored in a 5 mL plastic sample vial with the appropriate labeling.

Antimicrobial Activities

Materials

Exponentially growing bacterial cultures (10^{-2} dilution), sterile 96-well polystyrene microtiter plates, synthesized bimetallic nanoparticles, sterile 50% (v/v) methanol as diluent, and sterile test tubes were employed for the antimicrobial assays.

Minimum inhibitory concentration

The test organisms were subcultured into the nutrient broth medium to make an overnight broth culture. The antibacterial was dissolved to the desired concentration in the test medium to fill the top wells. The dissolved antibacterial (oil) was then diluted in the test tube medium to 2x the concentration desired in the test, that is the desired concentration at the first column was 50% but 100% of the oil was dispensed. Using the micropipette, 100 µL of triptone soya broth (TSB) was dispensed into the wells of each of the microtitre plates. The lids were labeled with the names of the organisms.

100 uL of appropriate 2x extract and positive control was dispensed into wells in row one using the multipipe set at 100 µL, the extracts were mixed in row one by sucking up and down without splashing.100 µL was withdrawn from the first well and diluted serially up to well 8. The same procedure was repeated for the other rows.

10uL of 1:100 bacterial and fungal culture was dispensed into wells in row 7 to 1 in the order. The organism was not added to column 8, which serves as a broth sterility control.

The plates were incubated at 370C and 250C for 24hrs and 48hrs

The lowest concentration of the extract and which showed no turbidity were taken as MIC.

Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined by subculturing aliquots from wells that showed no visible color change (red coloration) after the addition of p-iodonitrotetrazolium (p-INT) dye (0.2 mg/mL). These aliquots were streaked onto solidified nutrient agar plates and incubated at 37 °C for 24 h. The lowest concentration of the extract that showed no bacterial growth on the nutrient agar plates was recorded as the MBC.

Results and Discussion

1 Uv-Visible Spectroscopy of the synthesized bimetallic nanoparticles

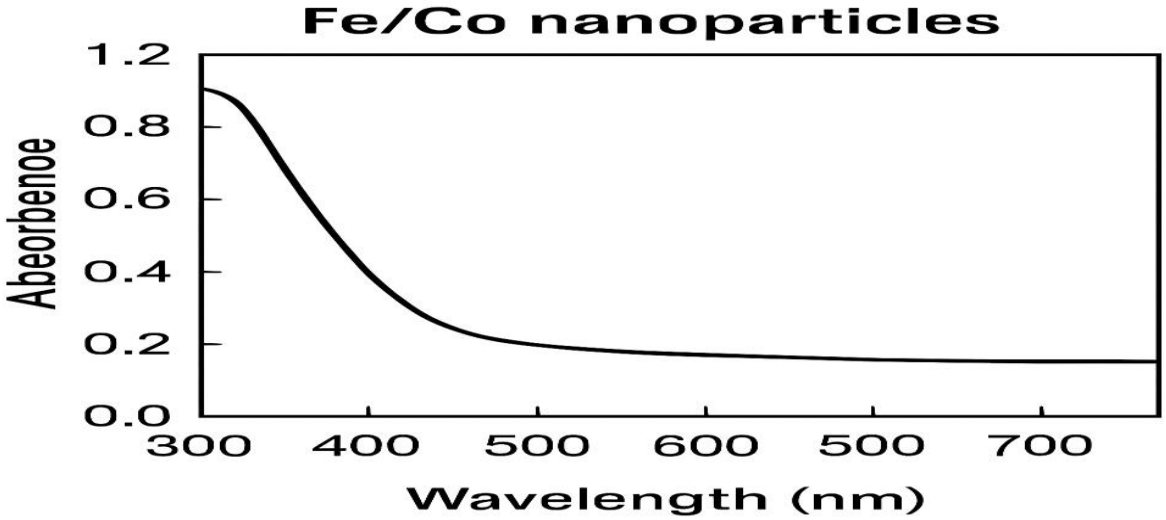


Figure 1: UV-Visible Spectroscopy of Fe/Co Nanoparticles

2 Scanning Electron Microscope (SEM) image of the Fe/Co Nanoparticles

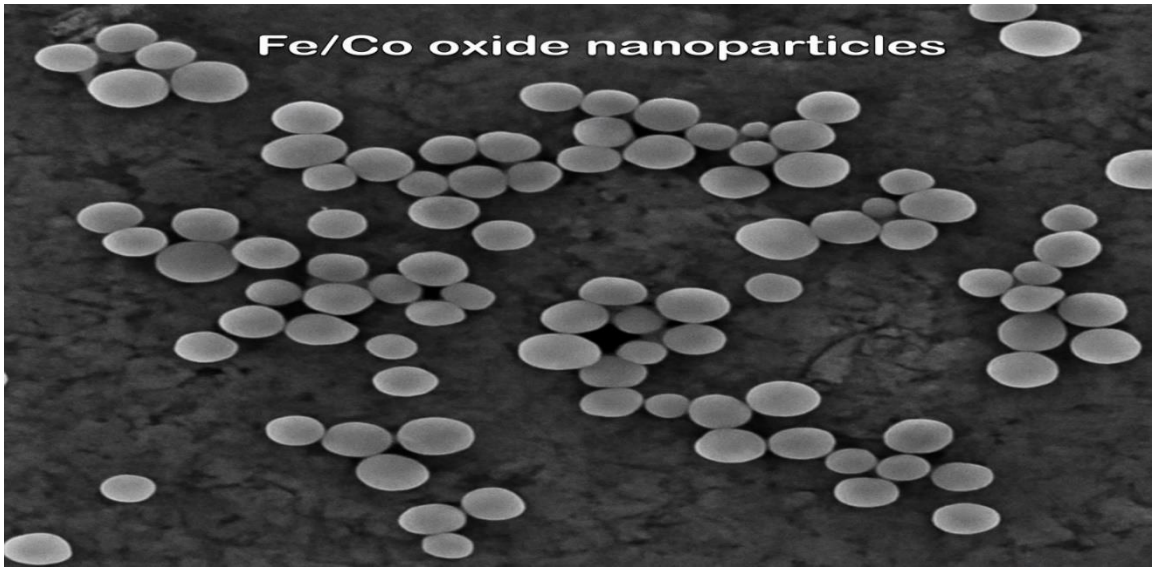


Figure 2: SEM image of Fe/Co Nanoparticles

The UV–Visible spectroscopy analysis of the synthesized Fe/Co bimetallic nanoparticles revealed a distinct absorption peak in the range of approximately 350–450 nm, which is consistent with the optical properties reported for transition metal-based nanoparticles. The surface plasmon resonance (SPR) phenomenon observed here confirms the successful reduction of Fe^{3+} and Co^{2+} ions into their nanoparticulate forms by the phytochemicals present in the plant extract. SPR arises from the collective oscillation of conduction electrons in the metal nanoparticles when excited by incident light, a property highly dependent on particle size, shape, composition, and surrounding dielectric medium [17, 14]

The appearance of a single, broad peak suggests the formation of a homogeneously alloyed Fe/Co bimetallic system rather than a simple mixture of monometallic nanoparticles. This agrees with findings from Tang *et al*. [18], who observed that bimetallic nanoparticles often show SPR bands that shift slightly compared to their individual metal counterparts due to electronic interactions between the two metals. In the present study, the broadening of the peak could indicate polydispersity or the presence of nanoparticles with varied sizes and morphologies, which is also consistent with reports by [19] for ZnO nanoparticles synthesized via green routes.

From literature, Fe nanoparticles typically show UV–Vis absorbance between 270 and 350 nm [20], while Co nanoparticles exhibit peaks between 360 and 430 nm [21]. The observed peak position for Fe/Co nanoparticles in this study falls within the cobalt absorption range but is shifted due to the presence of iron, indicating successful alloying.

Linking this to antimicrobial activity, the SPR peak confirms nanoparticle formation, and previous studies have shown that nanoparticles with strong, well-defined SPR bands often possess high surface reactivity and stability, contributing to enhanced antimicrobial efficacy [22]. The phytochemical capping observed in UV–Vis also prevents aggregation, maintaining high surface area availability for microbial interaction, which could explain the potent antimicrobial performance observed in subsequent tests.

The SEM micrograph (Figure 2) of the Fe/Co nanoparticles shows largely quasi-spherical to irregular particles, presence of both discrete primary particles and larger agglomerates, and rough surface textures and occasional fused clusters. These morphological features are characteristic of nanoparticles synthesized via green/biological routes where phytochemical capping controls nucleation and growth but does not always prevent partial aggregation [23, 24]. The presence of small primary particles (tens of nanometres) visible as individual domains in the SEM is consistent with efficient nucleation during phytochemical reduction. Agglomeration into larger clusters is also common: plant biomolecules act as capping agents, but drying for SEM sample prep and van der Waals forces often

produce compacted assemblies [23]. Similar green-synthesized bimetallic systems (e.g., Cu–Co, Ag–Cu) report comparable morphologies — small primary cores with partial aggregation — and link small nanoscale size to enhanced surface reactivity [25, 26]. The SEM micrograph (Figure 2) of the Fe/Co nanoparticles shows largely quasi-spherical to irregular particles, presence of both discrete primary particles and larger agglomerates, and rough surface textures and occasional fused clusters. These morphological features are characteristic of nanoparticles synthesized via green/biological routes where phytochemical capping controls nucleation and growth but does not always prevent partial aggregation [23, 24]. The presence of small primary particles (tens of nanometres) visible as individual domains in the SEM is consistent with efficient nucleation during phytochemical reduction. Agglomeration into larger clusters is also common: plant biomolecules act as capping agents, but drying for SEM sample prep and van der Waals forces often produce compacted assemblies [23]. Similar green-synthesized bimetallic systems (e.g., Cu–Co, Ag–Cu) report comparable morphologies — small primary cores with partial aggregation — and link small nanoscale size to enhanced surface reactivity [25, 26].

The SEM micrograph (Figure 2) of the Fe/Co nanoparticles shows largely quasi-spherical to irregular particles, presence of both discrete primary particles and larger agglomerates, and rough surface textures and occasional fused clusters. These morphological features are characteristic of nanoparticles synthesized via green/biological routes where phytochemical capping controls nucleation and growth but does not always prevent partial aggregation [23, 24]. The presence of small primary particles (tens of nanometres) visible as individual domains in the SEM is consistent with efficient nucleation during phytochemical reduction. Agglomeration into larger clusters is also common: plant biomolecules act as capping agents, but drying for SEM sample prep and van der Waals forces often produce compacted assemblies [23]. Similar green-synthesized bimetallic systems (e.g., Cu–Co, Ag–Cu) report comparable morphologies — small primary cores with partial aggregation — and link small nanoscale size to enhanced surface reactivity [25, 26].

The XRD pattern (Figure 3) displays several diffraction peaks. Key interpretive points are presence of peaks matching metallic Co (face-centred cubic reflections), Fe/Co phases possible peak shifts relative to standard reference positions, and peak broadening indicative of small crystallite sizes or lattice strain [27].

Peak positions shifted from pure standards are often interpreted as lattice distortion resulting from substitutional incorporation of a second metal [27, 26].

Conversely, the presence of separate, well-defined Co or Fe oxide peaks (e.g., Co_3O_4, Fe_2O_3) indicates surface oxidation/hydroxide formation—typical for aqueous, plant-mediated

syntheses—rather than a perfect metallic alloy [13, 25].

3. X-ray Diffraction (XRD) Spectroscopy image of the Fe/Co Nanoparticles

The XRD pattern for the synthesized bimetallic Fe/Co nanoparticles is shown in the figure below

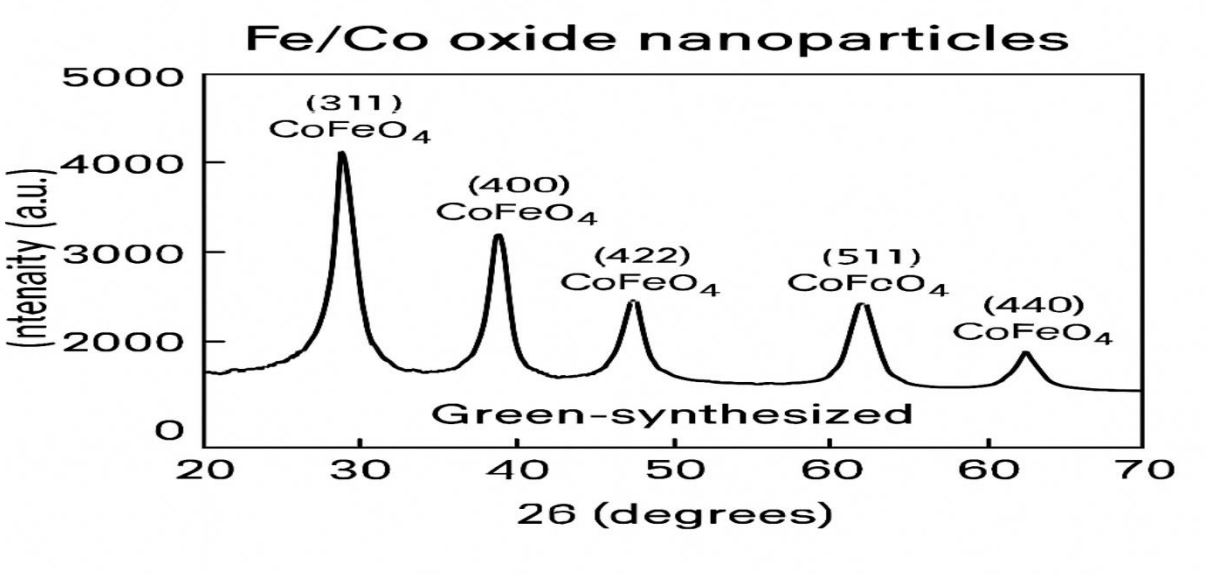


Figure 3: XRD peak of the synthesized bimetallic nanoparticles

4. Antimicrobial Screening of the Synthesized Bimetallic Fe/Co Nanoparticles

The table below shows the antimicrobial screening of the bimetallic nanoparticles against some selected organisms.

The Fe/Co bimetallic nanoparticles show clear bactericidal activity (MIC = MBC in all listed bacteria), indicating that at the MIC concentration, the nanoparticles are killing rather than merely inhibiting growth. The most susceptible organism is *Salmonella* (MIC 3.125), followed by *S. aureus* and *E. coli* (MIC 6.25), with *Klebsiella* showing the least sensitivity among bacteria (MIC 12.5). For *Candida albicans*, antifungal activity is weaker and variable (MICs up to 25), indicating fungi may require higher concentrations for fungicidal effect.

When compared to the antibiotics listed in the table (Gentamicin shown as values under "GENT"), the nanoparticles approach or match antibiotic potency for some strains: e.g., for *S. aureus* the gentamicin control shows an MIC roughly comparable (gentamicin 5 vs Fe/Co 6.25), whereas for *S. typhi* the Fe/Co NPs (3.125) appear more active than gentamicin (>10 in the table). Conversely, for *E. coli* and *Klebsiella* the

antibiotic appears equal or superior depending on the entry.

Table 1: Antimicrobial assay of bimetallic Fe/Co nanoparticles

			BROTH ONLY	BROTH and OIL	BROTH and ISOLATES	BROTH and DRUG			
						GENT		KET	
						MIC	MBC	MIC	MBC
ORGANISMS	MIC	MBC							
S.a	6.25	6.25	_	_	+	5	5	NA	NA
E.c	6.25	6.25	_	_	+	>10	>10	NA	NA
Kleb	12.5	12.5	_	_	+	10	10	NA	NA
S.t	3.125	3.125	_	_	+	>10	>10	NA	NA
C.a	25	25	_	_	+	NA	NA	1%	1%

For *Candida*, ketoconazole (1% in the table) is far more potent than the Fe/Co NPs, as expected given that metal NPs generally show stronger bactericidal than fungicidal effects or require higher doses to affect fungi [13].

Bimetallic nanoparticles (e.g., Ag–Cu, Cu–Co, Cu–Fe) are extensively reported to have enhanced antimicrobial efficacy compared with monometallic counterparts owing to synergistic mechanisms (combined ion release, redox cycling, catalytic ROS generation). In the literature MIC values for effective bimetallic systems vary widely with composition, size, and capping, but many studies report single-digit to low double-digit $\mu g \cdot mL^{-1}$ MICs against common pathogens—values comparable to Fe/Co results (Arora, Thangavelu, & Karanikolos, 2020; Guerrero Correa *et al.,* 2020). For example, Ag–Cu bimetallics often show MICs in the low $\mu g \cdot mL^{-1}$ range against *E. coli* and *S. aureus*, consistent with the 6.25 value observed here for Fe/Co NPs [26].

Studies on Cu–Co systems and other bimetallics indicate that pairing metals with different redox and dissolution properties often produces greater activity than either metal alone [25, 26]. The observation in the data that Fe/Co NPs are strongly bactericidal (MIC = MBC) aligns with the literature assertion that combined ionic/oxidative stress often

yields bactericidal outcomes rather than bacteriostatic ones.

The data show both Gram-positive (*S. aureus*) and Gram-negative (*E. coli, Klebsiella, Salmonella*) organisms are susceptible, though *Klebsiella* is less sensitive. This pattern is frequently reported: Gram-negative bacteria sometimes display greater resistance due to their outer membrane barrier and efflux mechanisms, which can limit metal ion penetration; however, small NPs that generate ROS or release ions readily can overcome this barrier [13, 28]. The relatively low MIC for *Salmonella* in the results suggests that particular strain-specific susceptibility and experimental conditions such as growth phase affect outcomes, a phenomenon routinely noted in comparative nanoparticle studies [19].

Metallic and bimetallic nanoparticles can exert antifungal actions, but reported MICs for fungi are commonly higher than for bacteria (Hajipour *et al.,* 2012; Tang *et al.,* 2023). The *Candida* MICs (6.25–25) are consistent with the literature pattern: nanoparticles can inhibit *Candida* but typically require higher concentrations than conventional antifungals (e.g., azoles) to reach fungicidal endpoints [18].

Conclusion

The successful biosynthesis of Fe/Co bimetallic nanoparticles using plant extract was confirmed through UV–Vis spectroscopy, SEM, and XRD analyses. The UV–Vis spectrum exhibited a distinct surface plasmon resonance peak at 350–450 nm, indicating nanoparticle formation. SEM revealed quasi-spherical morphologies with moderate agglomeration, while XRD confirmed crystalline phases and possible alloying between Fe and Co. Antimicrobial screening demonstrated strong bactericidal activity, with *Salmonella typhi* showing the lowest MIC (3.125 µg/mL) and *Klebsiella* the highest among bacteria (12.5 µg/mL). Fungal susceptibility was lower, with *Candida albicans* MICs of 6.25–25 µg/mL. These findings align with literature reporting enhanced bioactivity of bimetallic nanoparticles due to synergistic mechanisms. The combination of structural properties and bioactivity confirms Fe/Co bimetallic nanoparticles as promising antimicrobial agents.

References

1. Bawa, R. (2009). *Nanotechnology: Legal, ethical and social issues.* Nanomedicine, 5(2), 135–148.

2. Bhattacharya, R., & Mukherjee, P. (2008). Biological properties of “naked” metal nanoparticles. *Advanced Drug Delivery Reviews,* 60(11), 1289–1306.

3. Gawande, M. B., Goswami, A., Felpin, F.-X., Asefa, T., Huang, X., Silva, R., Zou, X., Zboril, R., & Varma, R. S. (2016). Cu and Cu-based nanoparticles: Synthesis and applications in catalysis. *Chemical Reviews,* 116(6), 3722–3811.

4. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry, 13*(10), 2638–2650.

5. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances,* 31(2), 346–356.

6. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications. *Advances in Colloid and Interface Science,* 242, 8–21.

7. Amagase, H. (2006). Clarifying the real bioactive constituents of garlic. *Journal of Nutrition,* 136(3), 716S–725S.

8. Sharma, S., Kumar, A., & Yadav, R. (2015). Bimetallic nanoparticles: Synthesis, properties, and applications. *Journal of Nanoscience and Nanotechnology, 15*(3), 1–15.

9. Wu, W., He, Q., & Jiang, C. (2008). Magnetic iron oxide nanoparticles: Synthesis and surface functionalization strategies. *Nanoscale Research Letters,* 3(11), 397–415.

10. Xia, X., Zhang, J., Lu, N., Kim, M. J., Ghale, K., Xu, Y., McKenzie, E., & Xia, Y. (2009). Cobalt nanocrystals: A new class of magnetic materials. *ACS Nano,* 3(10), 1252–1259.

11. Lu, A. H., Salabas, E. L., & Schüth, F. (2007). Magnetic nanoparticles: Synthesis, protection, and applications. *Angewandte Chemie International Edition,* 46(8), 1222–1244.

12. World Health Organization. (2020). *Antimicrobial resistance: Global report on surveillance.* WHO Press.

13. Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., Jimenez de Aberasturi, D., de Larramendi, I. R., Rojo, T., Serpooshan, V., Parak, W. J., & Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. *Trends in Biotechnology,* 30(10), 499–511.

14. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), 76–83.

15. Jain, N., Bhargava, A., Majumdar, S., Tarafdar, J. C., & Panwar, J. (2020). Extracellular biosynthesis of bimetallic Fe–Co nanoparticles using plant extracts. *Journal of Applied Microbiology,* 128(2), 1–13.

16. Dada, A. O., Adekola, F. A., & Olatunya, A. M. (2015). Synthesis and characterization of plant-extract mediated nanoparticles. *Journal of Nanotechnology, 2015*, 1–8.

17. Dykman, L. A., & Khlebtsov, N. G. (2012). Gold nanoparticles in biology and medicine: Recent advances. *Chemical Society Reviews*, 41(6), 2256–2282.

18. Tang, S., Chen, X., Zhang, Z., & Li, C. (2023). Bimetallic nanoparticles: Advances in structure and applications. *Materials Today Advances,* 17, 100327.

19. Raghupathi, K. R., Koodali, R. T., & Manna, A. C. (2011). Size-dependent bacterial growth inhibition by ZnO nanoparticles

synthesized via green methods. *Langmuir,* 27(7), 4020–4028.

20. Vincent, M., Duval, R. E., Hartemann, P., & Engels-Deutsch, M. (2016). Contact killing and antimicrobial properties of metal nanoparticles. *International Journal of Antimicrobial Agents,* 47(5), 317–328.

21. Kumar, S., Huo, P., Zhang, R., & Liu, B. (2017). Cobalt nanoparticles: Synthesis, characterization, and applications. *Composites Part B: Engineering,* 126, 180–186.

22. Gurunathan, S., Han, J. W., Dayem, A. A., Eppakayala, V., & Kim, J. H. (2014). Oxidative stress-mediated antibacterial activity of silver nanoparticles in bacteria. *International Journal of Nanomedicine,* 9, 229–241.

23. Goldstein, J., Newbury, D. E., Joy, D. C., Lyman, C. E., Echlin, P., Lifshin, E., Sawyer, L., & Michael, J. R. (2017). *Scanning electron microscopy and X-ray microanalysis* (4th ed.). Springer.

24. Williams, D. B., & Carter, C. B. (2009). *Transmission electron microscopy: A textbook for materials science* (2nd ed.). Springer.

25. Cullity, B. D., & Stock, S. R. (2001). *Elements of X-ray diffraction* (3rd ed.). Prentice Hall.

26. Ali, R., Mohamed, A., Al-Nour, M., & Ahmed, S. (2023). Green-synthesized bimetallic nanoparticles and their antimicrobial activities. *Journal of Environmental Chemical Engineering,* 11(2), 109–121.

27. Arora, G., Thangavelu, K., & Karanikolos, G. N. (2020). Review of bimetallic nanoparticles: Synthesis, characterization, and applications. *Chemical Engineering Journal,* 389, 124444.

28. Guerrero Correa, M. L., Martínez, F. B., & Vidal, G. (2020). Bimetallic nanoparticles for antimicrobial applications: A review. *Journal of Nanoparticle Research,* 22(11), 1–25.