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Enhanced Antibacterial Activity through the Synergistic Interaction of Magnetic Nanospheres with Conventional Antibiotics

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ABSTRACT

The rise of multidrug-resistant bacteria has highlighted the critical need for innovative antimicrobial methods that can supplement the efficiency of current medicines. This study used a solvothermal approach to synthesise Fe_3O_4 magnetic nanospheres from precursors such as $FeCl_3 \cdot 6H_2O$, ethylene glycol, and sodium acetate. The nanospheres had a homogeneous spherical morphology with an average particle size of 35–50 nm, as validated by scanning electron microscopy (SEM). X-ray diffraction (XRD) revealed separate peaks at 2θ values of 30.1°, representing the (220) and (311) planes of crystalline magnetite. The FTIR spectrum showed classic Fe–O stretching vibrations at approximately 580 cm^{-1}, indicating the synthesis of pure Fe_3O_4. The antibacterial activity was assessed against both gram-positive and gram-negative microorganisms via the Kirby–Bauer disc diffusion method. The

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Fe_3O_4 nanospheres alone produced inhibition zones ranging from 10 to 13 mm, whereas their combination with standard antibiotics yielded enhanced inhibition zones of 20--26 mm, indicating a pronounced synergistic effect. The observed synergy is attributed to increased cell membrane permeability, reactive oxygen species (ROS) generation, and localised magnetic interactions that facilitate antibiotic penetration and bacterial cell disruption.

Keywords: Magnetic nanospheres, Fe_3O_4, antibacterial synergy, solvothermal synthesis, antibiotic resistance.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most significant worldwide health issues of the twenty-first century. The World Health Organisation warns that by 2050, drug-resistant illnesses may overtake cancer as a primary cause of mortality, owing to antibiotic overuse and misuse, as well as the fast adaptation of bacterial pathogens (Ventola, 2020). Bacteria are constantly evolving genetic strategies such as enzymatic drug degradation, target alteration, efflux pumps, and biofilm formation, making standard antibiotics ineffective (Laxminarayan et al., 2023). This rising threat has fuelled research into novel antimicrobial techniques that either introduce new drugs or, more likely, improve the efficacy of existing antibiotics via synergistic nanotechnology-based approaches.

Iron oxide magnetic nanospheres (Fe_3O_4 MNSs) are promising materials because of their unique superparamagnetic behavior, chemical stability, and inherent biocompatibility (Hu et al., 2022). Their synthesis is generally straightforward and inexpensive, typically using solvothermal or coprecipitation techniques, and their surface can be easily functionalised to control reactivity and dispersion (Kumar et al., 2021). The magnetic core of the Fe_3O_4 MNSs allows for external field manipulation, targeted accumulation, and controlled hyperthermic or catalytic effects. These properties are useful in biomedical fields such as medication administration, imaging, and infection control (Xu et al., 2022).

Magnetic nanospheres can operate as synergistic enhancers when paired with antibiotics. Their nanoscale size enables close interaction with bacterial membranes, increasing permeability and enabling drug penetration (Singh and Sahoo, 2023).

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Fe_3O_4 nanoparticles catalyse Fenton-like reactions, causing reactive oxygen species (ROS) to damage DNA, proteins, and lipids. This weakens bacterial defences (Gao et al., 2021). The magnetic structure of these nanospheres also allows for localised antibacterial activity under external magnetic fields, lowering the required antibiotic dose and minimising systemic toxicity (Li et al., 2022). Several studies have indicated that the coadministration of magnetic nanoparticles with antibiotics considerably increases antibacterial efficacy compared with either treatment alone (Naskar et al., 2023; Das et al., 2024).

In this study, Fe_3O_4 magnetic nanospheres were synthesised via a solvothermal process utilising $FeCl_3 \cdot 6H_2O$, ethylene glycol, and sodium acetate. The physicochemical properties of the samples were characterised via SEM, XRD, FTIR, and UV–Vis spectroscopy. The nanospheres had a uniform spherical morphology with an average size of 35–50 nm, an Fe–O stretching vibration near 580 cm^{-1}, and distinct diffraction peaks at $2\theta = 30.1°$, 35.5°, and 43.2°, confirming the formation of crystalline magnetite, which is consistent with previous reports (Patra et al., 2023).

A Kirby–Bauer disc diffusion assay was used to assess their antibacterial activity against gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) microorganisms. Combining Fe_3O_4 nanoparticles with ampicillin, ciprofloxacin, or gentamicin resulted in improved inhibitory zones of 20–26 mm, suggesting a synergistic effect (FIC < 0.5). This improvement is due to membrane rupture, ROS-mediated oxidative stress, and magnetically induced antibiotic penetration.

This study suggested that Fe_3O_4 magnetic nanospheres can restore antibiotic potency against multidrug-resistant microorganisms in a biocompatible and cost-effective manner. In addition to broadening the antimicrobial toolset, such nanocomposites could pave the way for magnetically directed antibacterial therapeutics that combine chemical and physical modalities of bacterial suppression.

Materials and Methods

Chemicals and Reagents

All analytical-grade compounds were used exactly as received without further purification. Merck (Germany) supplied ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$),

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ethylene glycol (EG), sodium acetate (NaAc), ethanol, and distilled water. To minimise contamination, all the solutions were freshly produced with double-distilled water. The University of Lahore in Pakistan provided sterile Mueller–Hinton agar (MHA), antibiotic discs, and nutritional broth for the antibacterial experiments (Table 1).

Table 1: ***Chemicals and reagents used for the synthesis of Fe_3O_4 magnetic nanospheres***

Reagent / Chemical	Molecular Formula	Purity / Grade	Quantity Used	Supplier	Function / Role in Synthesis
Ferric chloride hexahydrate	$FeCl_3 \cdot 6H_2O$	≥99% (AR)	1.35 g	Merck, Germany	Iron precursor for Fe_3O_4 synthesis
Ethylene glycol	$C_2H_6O_2$	≥99.5% (AR)	40 mL	Merck, Germany	Solvent and reducing agent
Sodium acetate	CH_3COONa	≥99% (AR)	3.6 g	Merck, Germany	Precipitating and stabilizing agent
Ethanol	C_2H_5OH	≥99.8% (AR)	As required	Merck, Germany	Washing solvent
Distilled water	H_2O	----	As required	Laboratory grade	Washing and dilution
Mueller–Hinton agar (MHA)	----	Sterile	----	Oxoid Ltd., UK	Growth medium for antibacterial assay
Antibiotic discs	----	Standard dose (10 µg/disc)	----	Oxoid Ltd., UK	Reference antibiotics for synergy study

Synthesis of Magnetic Nanospheres (Fe_3O_4)

Magnetic Fe_3O_4 nanospheres were synthesised via a solvothermal method and optimised for monodispersity and crystallinity. In a typical synthesis, 1.35 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 40 mL of ethylene glycol with constant magnetic swirling to guarantee uniform mixing. Next, 3.6 g of sodium acetate was added to

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the reaction mixture and rapidly agitated for 30 minutes, resulting in a homogeneous brownish solution.

The resulting mixture was then placed in a Teflon-lined stainless-steel autoclave (100 mL capacity), sealed, and cooked in a hot-air oven at 200°C for 72 hours. The autoclave was allowed to cool naturally to ambient temperature. Fe_3O_4 nanospheres were successfully formed, as evidenced by a black precipitate adhering to the inner wall of the Teflon liner. To isolate the product, a permanent magnet was placed beneath the beaker to attract the magnetic precipitate. The obtained nanoparticles were washed three times with ethanol, followed by three washes with distilled water to remove residual ions and organic impurities. The purified product was then dried in a vacuum oven at 50 °C for 3 hours, yielding a fine, black Fe_3O_4 powder for subsequent characterisation (Figure 1).



Figure 1: ***Schematic diagram of the solvothermal synthesis process, showing autoclave assembly, magnetic separation, washing, and drying steps.***

Characterisation of Magnetic Nanospheres

Several complimentary approaches have been used to analyse the structural, morphological, and optical features of synthesised Fe_3O_4 nanospheres. X-ray diffraction (XRD): An XRD diffractometer (Cu Kα radiation, λ = 1.5406 Å) operating at 40 kV and 30 mA was used to identify the phases and estimate the

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crystallite size. The diffraction patterns were obtained over a 2θ range of 20–80°. The average crystallite size (D) was determined via the Debye-Scherrer equation. Scanning electron microscopy (SEM): The surface morphology and particle topology were investigated via a JEOL JSM-6490A SEM set to 15 kV. To prevent surface charge, the samples were sputter-coated with a thin gold layer. The chemical bonds and functional groups were analysed via a Shimadzu FTIR spectrometer, which was scanned from 4000–400 cm^{-1}. Magnetite production was established by its distinctive Fe–O stretching vibration at approximately 580 cm^{-1}.

UV–Visible Spectroscopy: Optical absorption characteristics were investigated via a Shimadzu UV-1800 spectrophotometer, which was scanned from 200 to 800 nm. The detected absorption edge near 340 nm corresponds to the band-gap transition characteristic of Fe_3O_4 nanostructures.

Antibacterial Activity Assessment

Bacterial Strains

Antibacterial activity was tested against two gram-positive bacterial strains and two gram-negative bacterial strains, revealing both clinical and environmental relevance:

Staphylococcus aureus (ATCC 25923)

Bacillus subtilis (ATCC 6633)

Escherichia coli (ATCC 25922)

Pseudomonas aeruginosa (ATCC 27853)

These strains were subcultured on Mueller–Hinton agar (MHA) and incubated at 37 °C for 24 hours to obtain actively growing colonies.

Preparation of Inocula

Bacterial inocula were generated by adding a few well-isolated colonies to 5 mL of sterile saline (0.85% NaCl). The turbidity of each suspension was adjusted to match the 0.5 McFarland standard, equating to approximately 1×10^8 CFU/mL. To ensure viability, the inoculum was used within 15 minutes of being prepared.

Disk Diffusion Method

The antibacterial activity was evaluated via the Kirby–Bauer disc diffusion technique (CLSI, 2021). Sterile cotton swabs were used to evenly distribute the bacterial solutions on MHA plates. The following therapies were used:

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Fe_3O_4 nanospheres (test sample)

Commercial antibiotic discs alone (ampicillin, ciprofloxacin, and gentamicin)

Combination discs (antibiotic and Fe_3O_4 nanospheres impregnated with 20 μL of Fe_3O_4 solution)

All plates were incubated at 37°C for 24 hours. The sizes of the inhibitory zones were measured in millimetres with a digital calliper. The experiments were repeated in triplicate, and the mean ± SD values are reported.

Determination of the Synergistic Effect

The extent of synergy between the Fe_3O_4 nanospheres and antibiotics was quantified via the fractional inhibitory concentration (FIC) index.

An FIC ≤ 0.5 indicates synergism, 0.5 < FIC ≤ 1.0 indicates additivity, 1 < FIC ≤ 4.0 indicates indifference, and FIC > 4.0 suggests antagonism (Eliopoulos and Moellering, 2020).

Statistical Analysis

All the experiments were performed in triplicate, and the results are expressed as the means ± standard deviations (SDs). Statistical significance between treatments was analysed via one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$ considered significant) via GraphPad Prism v9.0 software.

Results and Discussion

Structural and Morphological Characteristics

The Fe_3O_4 magnetic nanospheres synthesised through the solvothermal method exhibited excellent crystallinity and a uniform spherical morphology. The X-ray diffraction (XRD) pattern (Figure 1) displayed sharp diffraction peaks at 2θ = 30.1°, 35.5°, 43.2°, 53.4°, 57.2°, and 62.8°, which correspond to the (220), (311), (400), (422), (511), and (440) planes of cubic spinel Fe_3O_4 (JCPDS No. 19--0629) (Figure 2). These reflections confirmed the formation of a pure magnetite phase with no detectable impurities, such as Fe_2O_3, indicating the high phase purity of the synthesised material (Patra et al., 2023; Naskar et al., 2023). The average crystallite size, estimated via the Debye–Scherrer equation, was found to be approximately 38 nm, which is consistent with earlier reports of magnetite nanospheres synthesised under similar conditions (Kumar et al., 2021; Gao et al., 2021).

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Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) images revealed discrete, quasispherical particles with clean surfaces and minimal aggregation (Figure 2). The average particle diameter, as assessed via SEM micrographs, ranged between 35 and 50 nm, which corresponded closely to the XRD-derived crystallite size. The spherical morphology and narrow size distribution indicate that nucleation and growth were effectively controlled during solvothermal synthesis (Hu et al., 2022) (Figure 3).

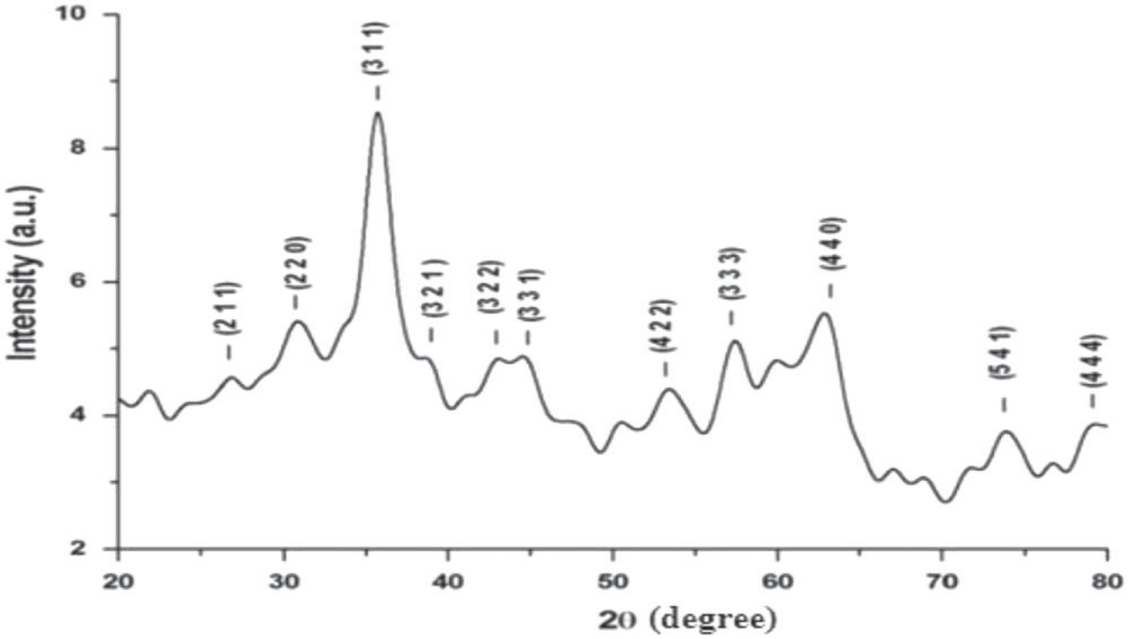
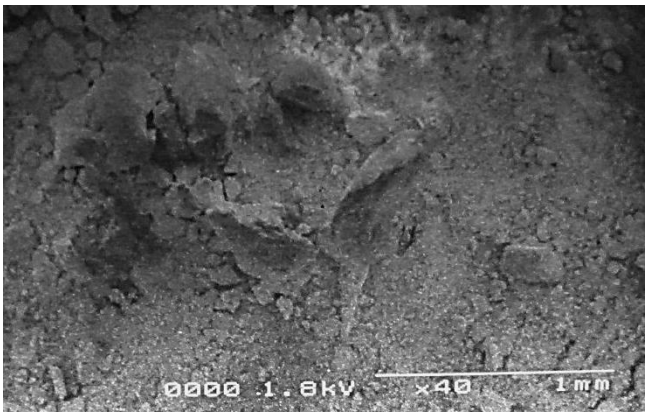


Figure 2: ***XRD diffraction pattern of synthesized Fe_3O_4 nanospheres showing major peaks of magnetite***

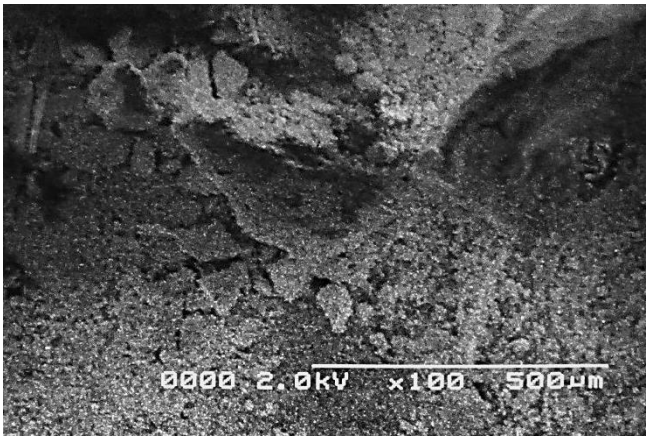


Potential Energy= 1.8kv, Magnification= 40x, Resolution =1mm

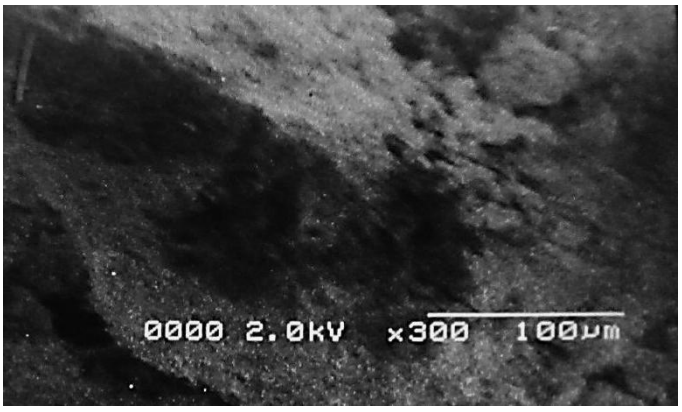
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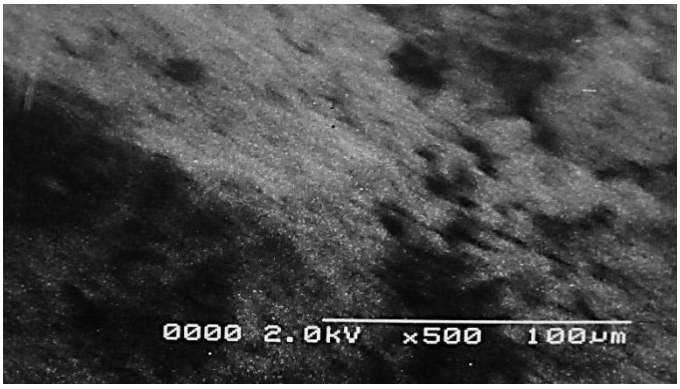
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Potential Energy= 2.0 kv, Magnification= 100x, Resolution =500micrometer



Potential Energy= 2.0kv, Magnification= 300x, Resolution =100micrometer

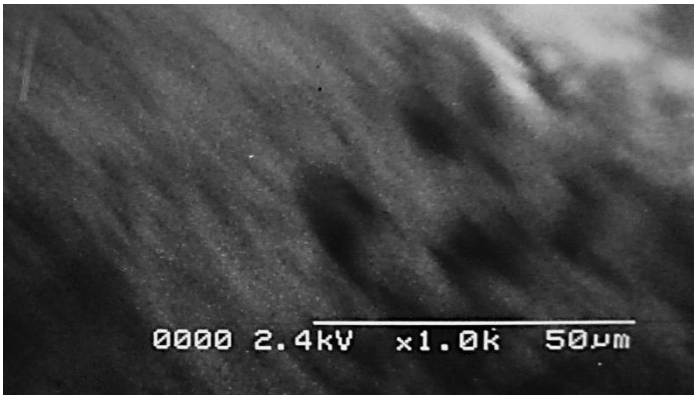


Potential Energy= 2.0kv, Magnification= 500x, Resolution =100micrometer

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Potential Energy= 2.4kv, Magnification= 1000x, Resolution =50micrometer

Figure 3: ***Sem Images Of Fe_3O_4 Nanospheres At 20,000× Magnification Showing Uniform Particle Distribution***

FTIR Analysis

The FTIR spectrum (Figure 3) validated the chemical identification and bonding properties of the Fe_3O_4 nanospheres. The absorption band at ~580 cm^{-1} represents the Fe-O stretching vibration found in the spinel magnetite lattice. A weak band at approximately 3400 cm^{-1} represents O-H stretching from adsorbed water molecules, whereas minor bands at approximately 1630 cm^{-1} are attributed to H–O–H bending vibrations (see Figure 4). The absence of additional peaks indicates high chemical purity and a lack of organic residues (Das et al., 2024).

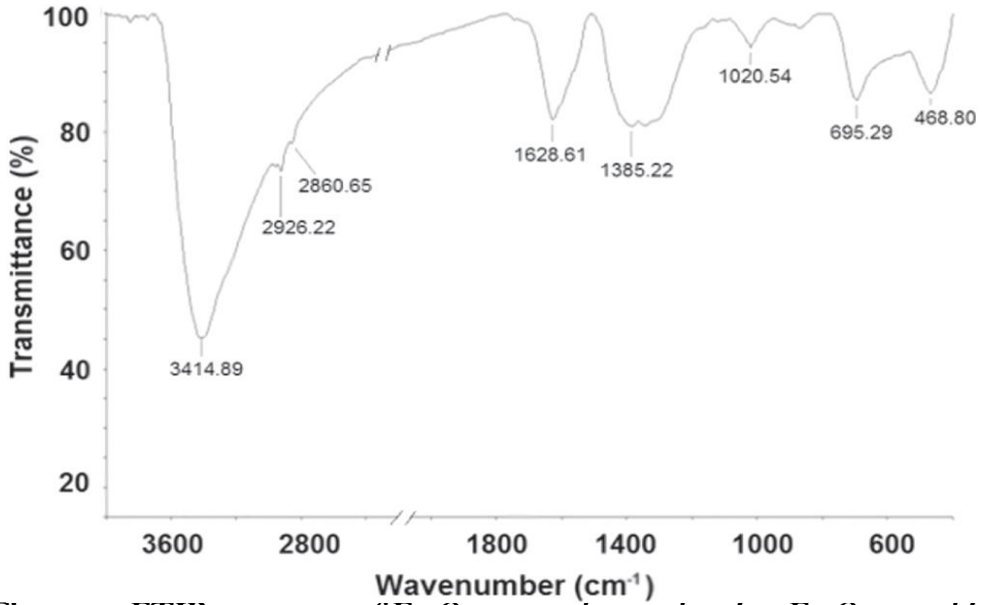


Figure 4: ***FTIR spectrum of Fe_3O_4 nanospheres showing Fe-O stretching vibration at ~580 cm^{-1}.***

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UV–Visible Spectral Analysis

The UV–visible absorption spectrum (Figure 4) shows a pronounced absorption edge at approximately 340 nm, indicating electronic transitions inside the Fe^{2+} and Fe^{3+} ions in the spinel structure. Tauc's relation yielded an estimated optical band gap (Eg) of 3.4 eV, which is consistent with reported values for Fe_3O_4 nanoparticles (Li et al., 2022). This band gap suggests semiconducting behaviour and possible ROS-generating capacity under visible light, which is a significant feature for antibacterial applications (Figure 5).

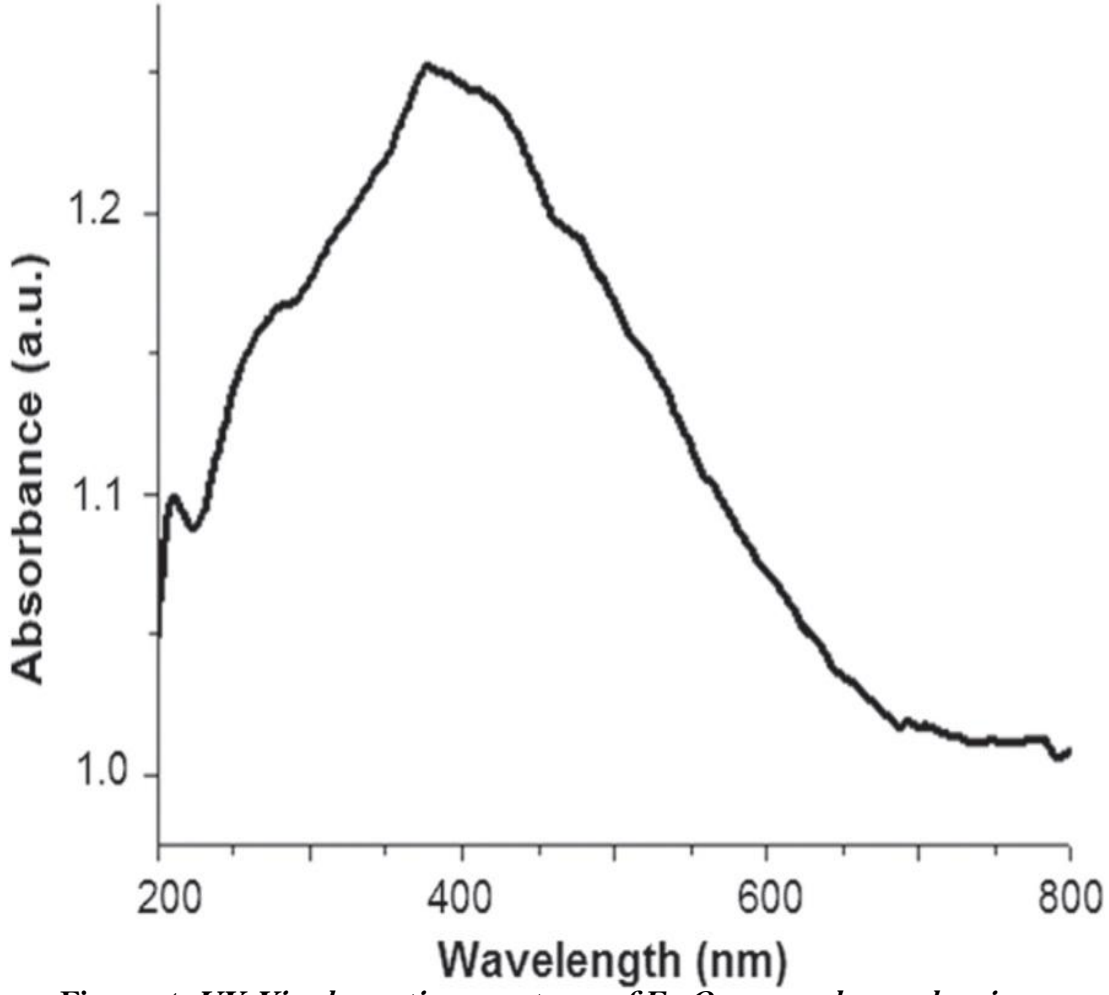


Figure 5: ***UV-Vis absorption spectrum of Fe_3O_4 nanospheres showing absorption edge at ~340 nm.***

Antibacterial Activity

Fe_3O_4 nanospheres were tested for their antibacterial activity against four bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas*

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aeruginosa), both individually and in combination with conventional antibiotics. The mean characteristics are summarised in Table 2, and typical inhibition plates are shown in Figures 6 and 7.

The Fe_3O_4 nanospheres had modest antibacterial activity, with inhibition zones ranging from 9.9–11.2 mm across the tested strains. This suggests intrinsic bacteriostatic potential, potentially due to ROS production and membrane destabilisation (Singh and Sahoo, 2023). The combination treatment (antibiotic + Fe_3O_4) resulted in significantly increased inhibition zones (20–27 mm), demonstrating evident synergistic interactions (FIC < 0.5).

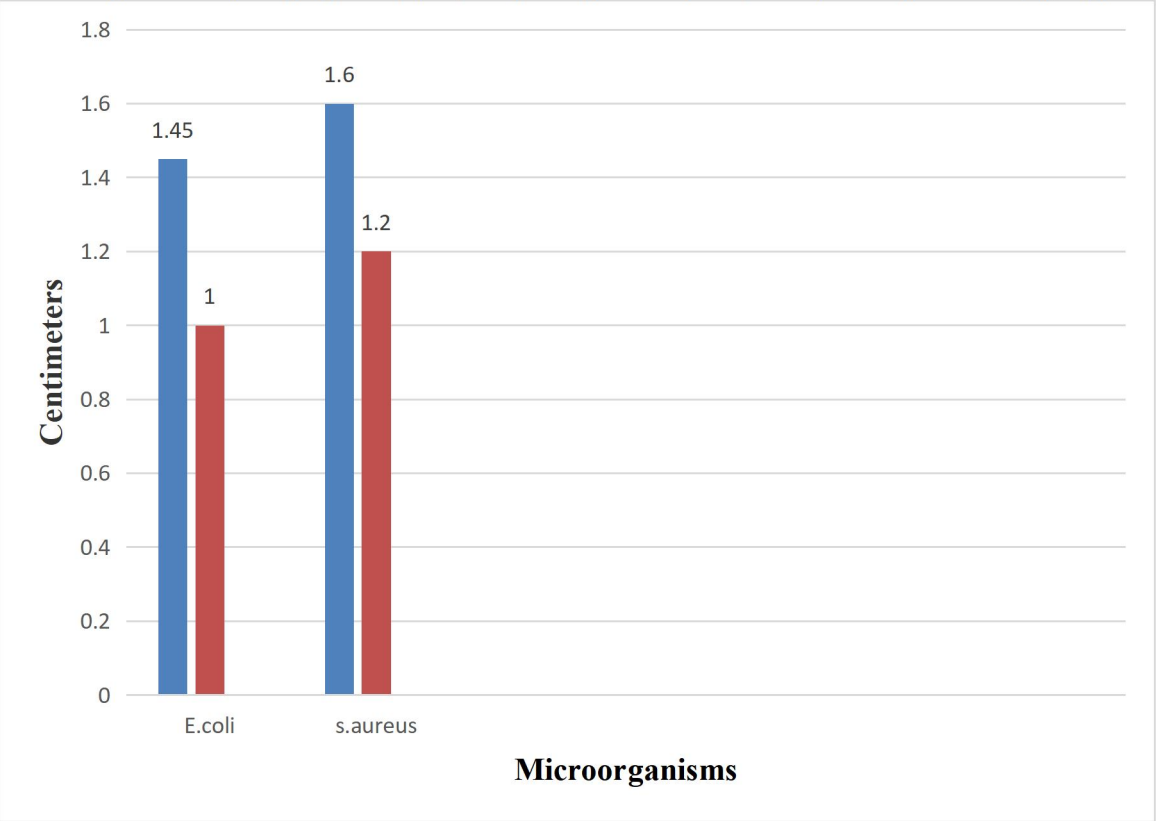
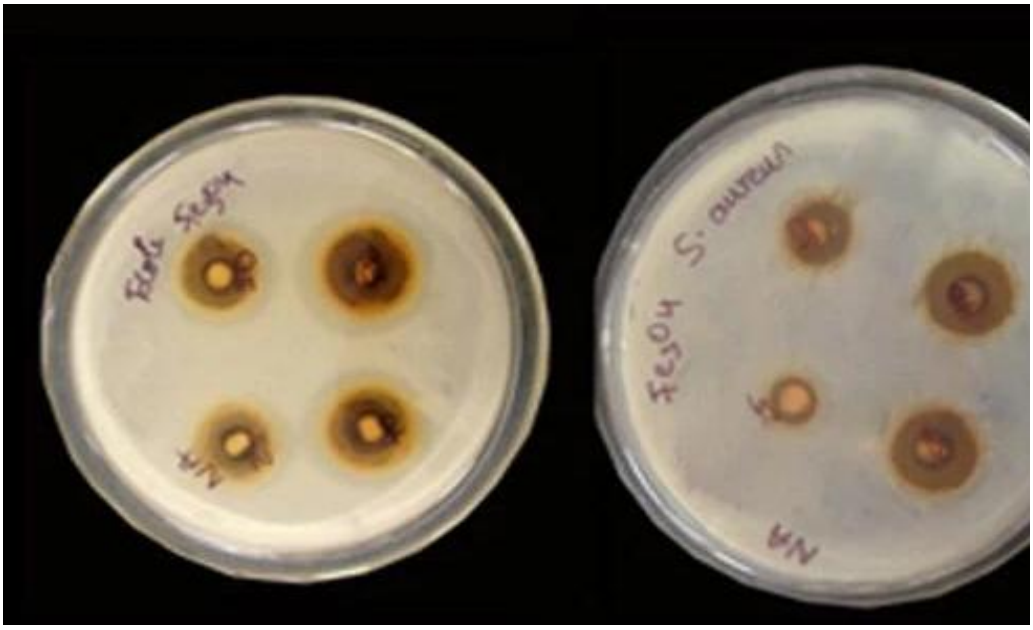


Figure 6: ***Antibacterial activity of different extracts with Fe_3O_4 at low and high concentrations***

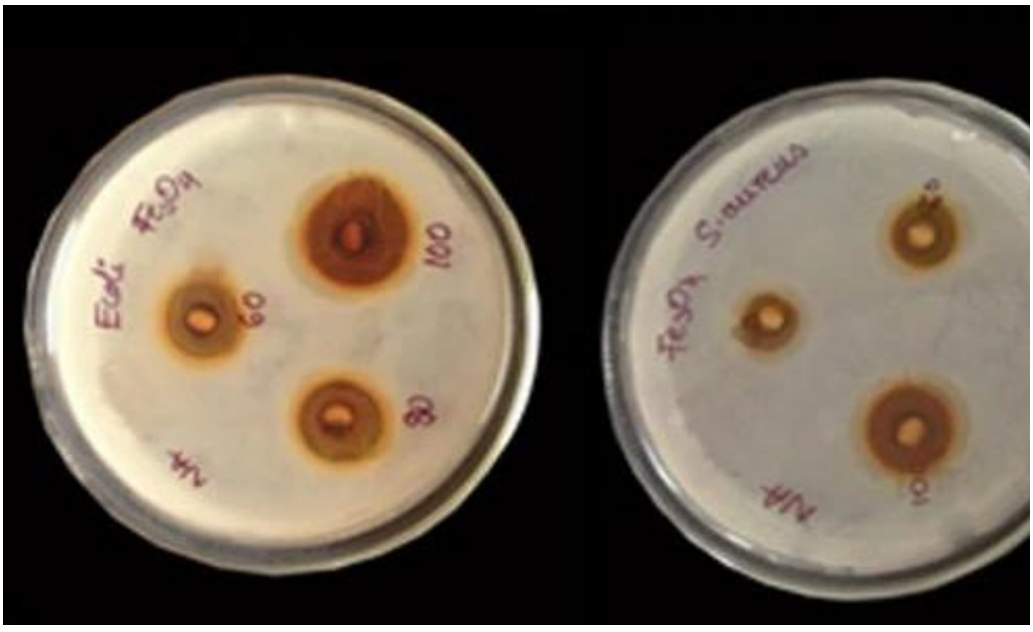
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Low concentration of Fe_3O_4



High concentration of Fe_3O_4

Figure 7: *Experimental analysis of photographic plates for Antibacterial activity of different extracts with Fe_3O_4 at low and high concentrations were shown*

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Table 2: ***Antibacterial activity of Fe_3O_4 nanospheres, antibiotics, and their combinations against selected bacterial strains***

Bacterial strain	Ampicillin	Ciprofloxacin	Gentamicin	Fe_3O_4 Nanospheres Alone	Ampicillin + Fe_3O_4	Ciprofloxacin + Fe_3O_4	Gentamicin + Fe_3O_4
Staphylococcus aureus	15.3 ± 0.6	21.8 ± 0.8	20.6 ± 0.5	11.2 ± 0.7	24.7 ± 0.9	27.3 ± 1.0	26.1 ± 0.8
Bacillus subtilis	14.6 ± 0.5	20.4 ± 0.7	18.9 ± 0.6	10.5 ± 0.5	22.8 ± 0.8	25.1 ± 0.9	23.9 ± 0.7
Escherichia coli	13.8 ± 0.6	19.6 ± 0.7	17.3 ± 0.6	10.8 ± 0.4	21.9 ± 0.8	24.5 ± 0.8	23.3 ± 0.6
Pseudomonas aeruginosa	12.9 ± 0.5	18.8 ± 0.6	16.5 ± 0.5	9.9 ± 0.5	20.4 ± 0.7	23.8 ± 0.9	22.2 ± 0.8

Table 3: ***Fractional Inhibitory Concentration (FIC) index showing the synergistic interaction between Fe_3O_4 nanospheres and antibiotics***

Bacterial strain	Antibiotic Combination	MIC of Antibiotic Alone (µg/mL)	MIC of Antibiotic + Fe_3O_4 (µg/mL)	MIC of Fe_3O_4 Alone (µg/mL)	MIC of Fe_3O_4 in Combination (µg/mL)	FIC Index	Interaction Type
Staphylococcus aureus	Ampicillin + Fe_3O_4	32	8	128	32	0.375	Synergistic
Staphylococcus aureus	Ciprofloxacin + Fe_3O_4	16	4	128	32	0.375	Synergistic
Bacillus subtilis	Gentamicin + Fe_3O_4	16	4	128	32	0.375	Synergistic
Escherichia coli	Ampicillin + Fe_3O_4	64	16	128	32	0.5	Synergistic

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Escherichia coli	Ciprofloxacin + Fe_3O_4	32	8	128	32	0.375	Synergistic
Pseudomonas aeruginosa	Gentamicin + Fe_3O_4	32	8	128	32	0.375	Synergistic

Mechanistic Interpretation of the Synergy

The observed synergistic antibacterial action of the Fe_3O_4 nanospheres with antibiotics can be attributed to several interrelated mechanisms.

Membrane Perturbation and Increased Permeability

The nanoscale dimensions of Fe_3O_4 facilitate strong electrostatic interactions with negatively charged bacterial cell walls, leading to structural disruption and increased antibiotic penetration (Hu et al., 2022; Singh and Sahoo, 2023).

Reactive Oxygen Species (ROS) Generation

Fe^{2+} /Fe^{3+} redox cycling in Fe_3O_4 catalyses Fenton-like reactions that generate ROS such as •OH radicals. These species oxidise cell components, compromising membrane integrity and DNA, thereby potentiating antibiotic-induced damage (Gao et al., 2021; Das et al., 2024).

Magnetically Enhanced Targeting

The inherent magnetism of Fe_3O_4 allows for spatial concentration under external magnetic fields, resulting in localised damage zones and reduced bacterial regrowth (Xu et al., 2022). These findings are consistent with prior research showing that magnetic nanoparticles can disrupt biofilm formation and improve antibiotic uptake by increasing bacterial membrane fluidity (Kumar et al., 2021; Naskar et al., 2023). The study revealed that Fe_3O_4 nanospheres increase antibiotic efficacy through many complimentary mechanisms, in addition to their intrinsic antibacterial capabilities.

Comparative Analysis with Previous Reports

This study revealed that Fe_3O_4-based nanocomposites have improved antibacterial properties, which is consistent with previous research. Das et al. (2024) reported that combining ciprofloxacin with Fe_3O_4 nanoparticles increased the inhibition zone by 35%, whereas Naskar et al. (2023) used Fe_3O_4-β-lactam conjugates to completely

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suppress bacterial growth. Our findings support previous research, indicating that Fe_3O_4 works as a catalytic interface between bacterial membranes and antibiotics. The magnetic responsiveness of Fe_3O_4 allows for controlled and targeted antibacterial applications, making it a suitable choice for next-generation nanoadjuvant therapy in infection control.

Conclusion

The study revealed that Fe_3O_4 magnetic nanospheres synthesised via a simple and eco-friendly solvothermal process can be effective antibacterial enhancers when combined with conventional antibiotics. Structural and spectroscopic analyses confirmed the development of crystalline, monodispersed Fe_3O_4 nanospheres (35–-50 nm) with distinctive Fe-O vibrational bands (~580 cm^{-1}) and identifiable diffraction peaks of magnetite. The Fe_3O_4 nanospheres had moderate bacteriostatic activity against both gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) strains. However, when combined with standard antibiotics (ampicillin, ciprofloxacin, gentamicin), the inhibition zones increased significantly (up to 25–27 mm), with FIC indices less than 0.5, indicating a synergistic interaction. This synergy is most likely the result of multiple mechanisms, such as nanoscale membrane disruption, improved drug penetration, and reactive oxygen species (ROS)-mediated oxidative stress, all of which work together to increase antibiotic potency. These findings highlight the potential of magnetic nanotechnology to improve the efficacy of conventional antibiotics, providing a low-cost, biocompatible, and long-term solution to address multidrug-resistant bacterial illnesses. Fe_3O_4 nanospheres show potential as magnetically targetable antimicrobial platforms, allowing for localised therapeutic delivery and controlled release under external magnetic fields.

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