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Evaluation of therapeutic activities of synthesized iron oxide nanoparticles

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ABSTRACT:

This study aims to synthesize and characterize iron oxide nanoparticlesand evaluate them for anti-microbial and anti-oxidant activity. Iron oxide nanoparticles were also modified by precipitation with calcium carbonate particles and their therapeutic activity was assessed. Iron oxide nanoparticles were prepared andanalyzed using TEM, XRD, and Zeta Potential measurements. The synthesized iron oxide nanoparticles were analyzed by TEM images, andthe particles werefound to be in ultra-small size of 5-10 nm. XRD and IR Spectroscopy confirmed the formation of the nanoparticle and the zeta potential of -32.6 indicated that the formed particles were stable. Furthermore, the evaluation of antimicrobial and antioxidant activity was done using the agar diffusion method and the DPPH method respectively. The results indicate that the iron oxide nanoparticles possess good antibacterial activity and antioxidant activity.The iron oxide nanoparticles and modified iron oxide nanoparticles showed moderate inhibitory activity against Staphylococcus aureus (MTCC 87) at1000 µg.According to theantioxidant studies, the modified iron oxide had the highest scavenging activity for DPPH when compared to iron oxide nanoparticles (IC5078.2 µg/ml and (IC5087.5 µg/ml respectively).Thus, Iron oxide nanoparticles and modified particles were synthesized, characterized, and found to be promising anti-oxidant and anti-antimicrobial activity, paving the way to fight antibiotic resistance and also deadlier diseases like cancer.

Keywords:Iron oxide nanoparticles, Transmission electron microscopy, X-ray diffraction, antioxidant activity, antibacterial efficacy.

1. Introduction

Iron oxide nanoparticles (NPs), particularly Fe_3O_4 NPs, have captured the spotlight in cancer research for their potential as therapeutic heroes. These tiny particles boast a host of remarkable qualities, from their magnetic targeting prowess to their friendly compatibility with the body, making them stand out in the quest against cancer. With their ability to seamlessly integrate into treatments like magnetic hyperthermia, photodynamic therapy, and photo thermal therapy,Fe_3O_4Nanoparticles have become the go-to warriors in the fight against cancer. Scientists have a variety of methods at their disposal to craft these magnetic marvels, allowing them to tailor the nanoparticles with biocompatible coatings or targeting molecules for even greater precision and effectiveness. Iron oxide nanoparticles have demonstrated remarkable potential for delivering therapeutic agents to specific target sites within the body, guided by external magnetic fields. To optimize the behavior of Fe_3O_4 nanoparticles in biological and environmental contexts, it is imperative to synthesize them with precisely controlled size, shape, and surface properties.(Nguyen et al. 2021; Castellanos-Rubio et al. 2021; Liu et al. 2023)
The 20th century was considered the "golden age" of antibiotic research due to the discovery of antibiotics, which began with the syphilis treatment and was further boosted by the unintentional discovery of penicillin.(Sharland et al. 2018)However, the number of organizations searching for new antibiotics has decreased in the current landscape due to the rising prevalence of antibiotic resistance.(Fernandes and Martens 2017)As metal-based nanoparticles (NPs) can target different bimolecular aspects of resistant strains of bacteria, they have become a popular research topic in the fight against antibiotic resistance. Reactive oxygen species (ROS) production, cation release, ATP depletion, and interaction with cell membranes are some important mechanisms of action. It is recognized that oxidants belong to a class of factors that are linked to various disorders and aging. (Shen et al. 2022; Sies and Jones 2020)

It is also suggested that the emergence of antibacterial NPs holds promise in restraining the evolution of more resistant strains. Commonly utilized metals for fabricating antibacterial NPs include aluminum (Al_2O_3), iron (Fe_2O_3), magnesium (MgO), zinc (ZnO), bismuth, and cerium.(Sánchez-López et al. 2020)Researches have shown that metal nanoparticles are effective in enhancing antimicrobial activity.For instance, studies have highlighted the impact of iron nanoparticle NPs against two pathogens often acquired in hospitals: Staphylococcus aureus and E.coli.(Ma et al. 2015; Romdoni et al. 2023)

In this study, we focus on the synthesis, and characterization of iron oxide particles and hybrid particles and also thereby the evaluating their therapeutic activity to ensure that these metal particles are efficient for antimicrobial resistance against hospital-acquired infections.

2. Materials and Methods

Synthesis and characterization of Iron oxide nanoparticles

Magnetic nanoparticles were prepared as mentioned in the literature with slight modifications.(Serov et al. 2019)Briefly, $FeCl_2$ and $FeCl_3$ were taken in a molar concentration of 0.7 and dissolved in deionized water, and stirred at 500 rpm. 12 ml of liquid ammonia was added upon stirring. The precipitate was collected by using a magnet and was later ultra-sonicated. The resulting precipitate of prepared iron oxide nanoparticles was cooled to room temperature and stored.(Serov et al. 2019)TEM analysis was performed using FEI Tecnai TF30HRTEM. Zeta potential was analyzed using Malvern ZetasizerNanosizer with a 655 nm laser. X-ray analysis was carried out in a RigakuX-ray diffractometer over a range of 10 to 90 Bragg angles.

Anti-oxidant activity by the DPPH method

Anti-oxidant studies were done according to the previous Literature.(Chang et al. 2001)To summarize, the stock solution was prepared in concentrations from 12.5µg/mL to 200µg/mL distilled water was used as a control. The reaction mixture was shaken and incubated at room temperature for 20 minutes. The color change from purple to yellow is due to the hydrogen taken up from an antioxidant. After the incubation time, absorbance was measured at 517nm. The intensity of color change indicates the antioxidant activity.

Antibacterial activity

The agar well diffusion technique was utilized to assess the antibacterial activity of IONP.

The antibacterial activity of IONP was evaluated using the agar well diffusion technique.(Kiehlbauch et al. 2000) Briefly,available Muller Hinton Agar Medium (33.8g) was dissolved in 1000 ml water to form the nutrient medium, and the medium was autoclaved for 15 minutes. It's then pouredintoPetri plates of 100mm. nutrient broth was made by dissolving 13g of commercially available nutrient medium (HI Media) and the medium was dissolved by boiling. it was autoclaved for 15 minutes at 121degree. The antibacterial agent streptomycin was used at a concentration of 10 mg/ml.

3. Results and Discussion

The size shape and morphology of iron oxide nanoparticles were analyzed by TEM.Poly dispersed iron nanoparticles of the ultra-small size of 5 to 10 nm; similar to that which is reported in the literature (Sun et al. 2023)were observed in Figure 1. Furthermore, as the nanoparticles have a very small size, they can act along with biological molecules swiftly.

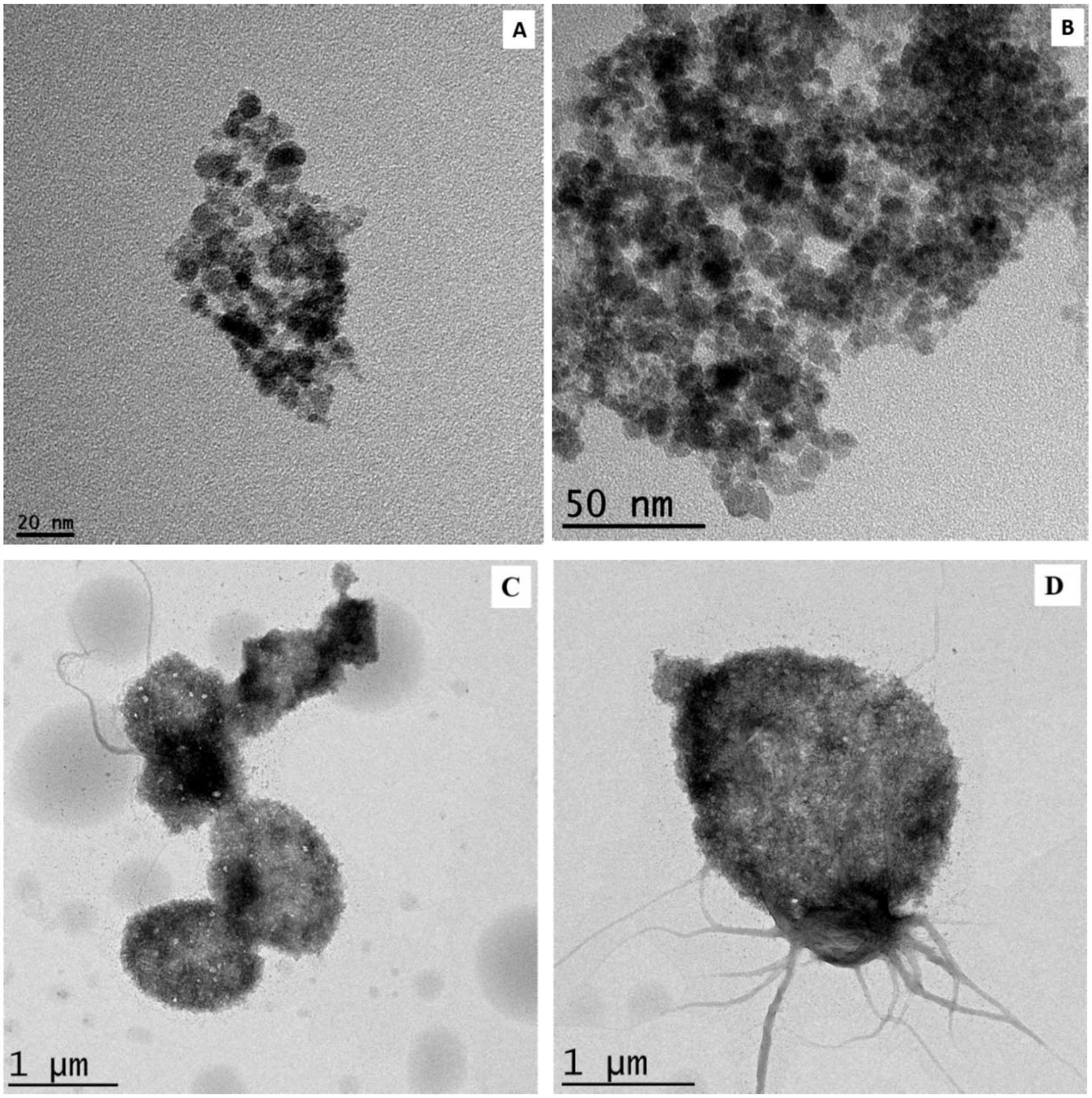

Fig. 1. TEM image of IONPs (A, B) and modified iron oxide particles (C, D)

XRD measurement was done to determine the crystalline structure of the product.(Figure 2) The phase structure of the samples, as shown by the XRD diffraction peaks, is in complies with the standard, which is represented by the ICDD (98-015-8743) with peaks at 2θ of 30.21°, 35°, 43.34°, 53.35°, 57.37°, and 63.07° were attributed to the Fe3O4 reflections at (220),(311), (400), (511), and (440).(Ba-Abbad et al. 2022; Alterary and AlKhamees 2021)In XRD the diffraction peaks at 2θ of (110),(112),(114),(224)confirmed the presence of calcium carbonate particles.(Zhou et al. 2004) and thereby formation of hybrid particles.

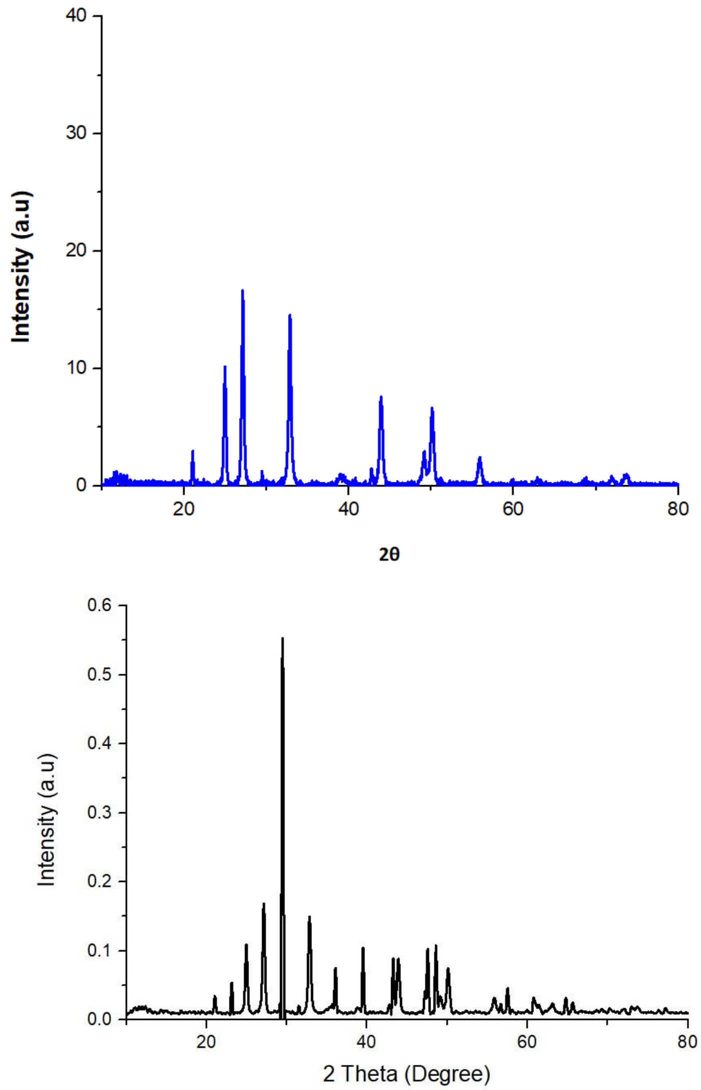

Figure 2.XRD diffraction patterns of Fe_3O_4nanoparticles and modified iron oxide nanoparticles.

Zeta potential shows the stability of the colloidal dispersion, and the results show that the synthesized particles have good stability as the zeta potential value was -32.6 mv. It shows that particles have a lesser tendency to agglomerate.

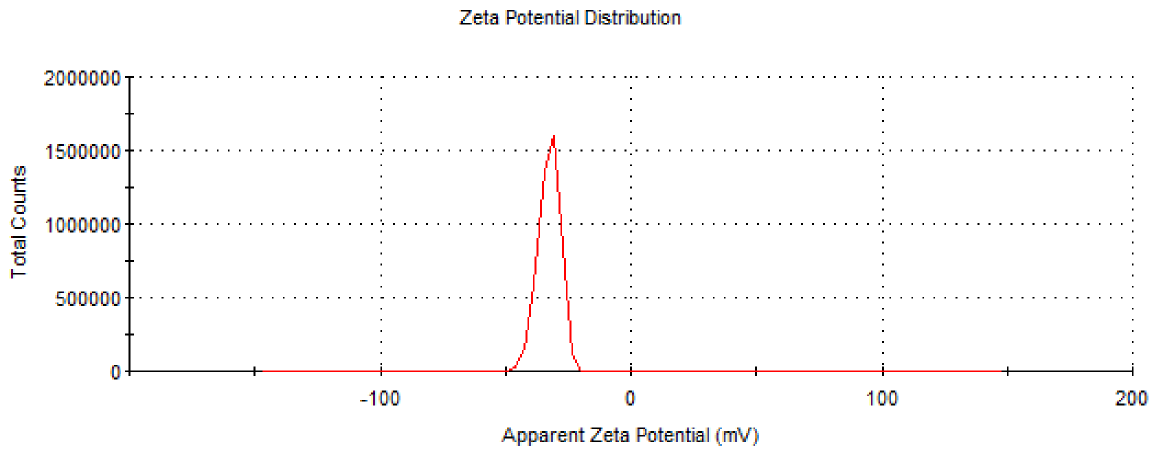

Fig. 3. Zeta potential analysis of IONPs.

Antioxidants protect against oxidative damage of reactive oxygen species. The assay called DPPH (2,2-Diphenyl-1-picrylhydrazyl) is done to evaluate the antioxidant capacity of inoxidin-3-one.Antioxidants protect us from oxidative damage caused by things like stress and pollution. They do this by using different methods, such as deactivating harmful enzymes and grabbing onto rogue molecules called free radicals. In a study(Polumbryk, Ivanov, and Polumbryk 2013)they tested how well different extracts could do this using something called the DPPH assay. IC_{50} is used to determine the antioxidant activity. A lower IC_{50}value indicates higher antioxidant activity.IC_{50}value indicates the amount of antioxidants required to reduce the free radical by 50 percent. (Ge, Cao, and Chu 2022).

Prior studies have demonstrated that IONPs have superior antioxidant capabilities.(Majeed et al. 2021; Ge, Cao, and Chu 2022)They give away electrons or hydrogen atoms to those free radicals, stopping them from causing harm to important parts of our cells like lipids and proteins.

Thereby it can stop the oxidation process of nucleic acid, lipids, and proteins.(Gulcin and Alwasel 2023)As a result, IONPs' possible antioxidant activity may one day be employed as an anticancer and other therapeutic agent.Figure 4 displays the antioxidant activity of several IONP concentrations (12.5–200 µg/ml). At the highest concentration (200 µg/ml), IONPs demonstrated 63% DPPH radical scavenging, while standard ascorbic acid demonstrated 94% antioxidant activity. From our study, it was found that the DPPH scavenging capacity of biosynthesized modified IONP, IONPs, and standard ascorbic acid had IC_{50} values of 78.2, 87.5 µg/mL and 19.9 µg/mL, respectively.

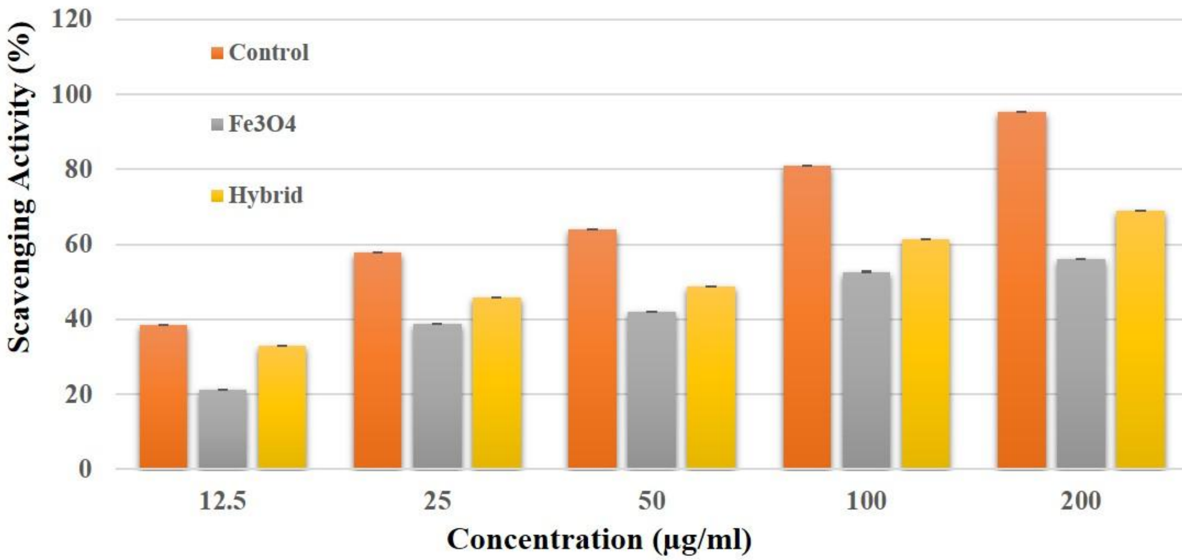

Figure 4. Antioxidant activity of IONP by DPPH Assay

Recently iron oxide nanoparticles and their modified particles are widely researched as an alternative to antibiotics as an alternative solution to multidrug-resistant problems. In antimicrobial study, the test organisms are seeded on the plate and the antimicrobials in the samples are allowed to interact and diffuse out forming uniform inhibition zones due to their confluent growth. The zone of inhibition is measured in millimeters. The antibacterial properties of samples were analysed in various concentrations ranging from 250 and 1000 µg using agar well diffusion method. The bacteria used in this study were *Staphylococcus aureus* (ATCC 25923) and *E. coli* (ATCC 25922).

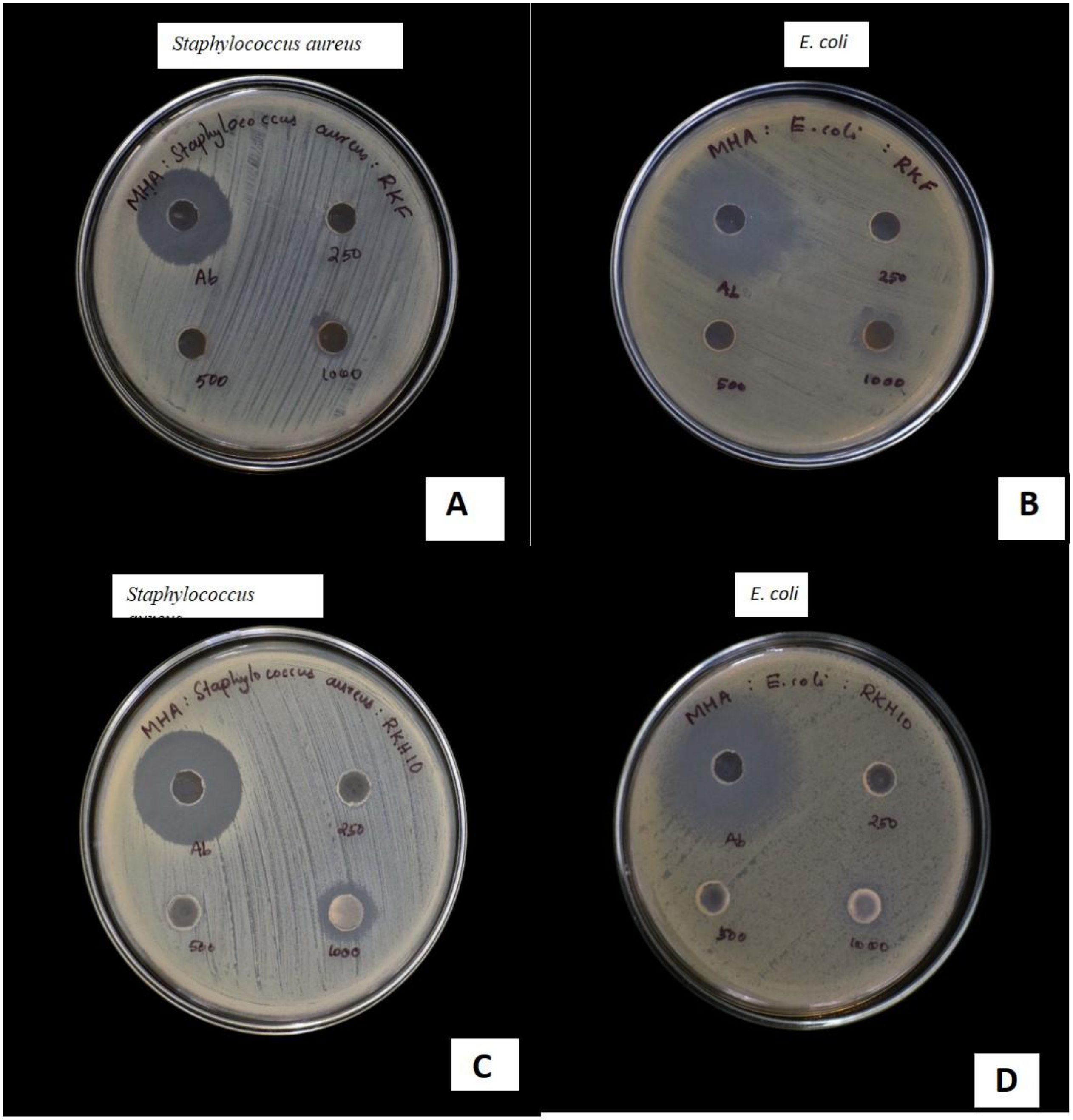

Fig. 5. Antibacterial effect of IONP (A, B) and modified IONP(C, D) against *Staphylococcus aureus* and *E. coli*

The nanoparticles were analyzed for antimicrobial properties and the results indicates that both gram-negative and gram-positive bacteria demonstrated good activity for the samples. The antibiotic streptomycin showed a 28–32 mm inhibition zone as a positive control. Iron oxide nanoparticles at 1000 µg showed antibacterial activity against gram-positive and gram-negative bacteria, while smaller concentrations, like 250 µg and 500 µg, showed no antibacterial properties, according to the results. The results of the antibacterial study are shown in Figure 5 and Table 1.At a concentration of 1000 µg, iron oxide nanoparticles demonstrated a zone of inhibition against S. aureus measuring 11 ± 0.6 mm, and *E. coli* measuring 14 ± 1.2 mm. The hybrid particles show a slightly higher activity against *S. aureus* measuring 13 ± 0.9 mm, and E. coli measuring 16 ± 0.5 mm.

Table 1: Antibacterial activity of IONP and modified IONP against various bacterial strains.

Diameter of inhibition zones (mm)				
Bacteria/Conc. IONPs (µg)	**250 µg**	**500 µg**	**1000µg**	**Streptomycin (100µg)**
Escherichia coli	Nil	Nil	14± 1.2 mm	32± 0.9 mm
Staphylococcus aureus	Nil	Nil	11 ± 0.6mm	28± 0.4 mm

Diameter of inhibition zones (mm)				
Bacteria/Conc. Modified IONPs (µg)	**250 µg**	**500 µg**	**1000 µg**	**Streptomycin (100µg)**
Escherichia coli	Nil	Nil	16± 0.5 mm	32± 0.7mm
Staphylococcus aureus	Nil	Nil	13 ± 0.9mm	28± 0.2 mm

Recent studies have shown the antibacterial potential of iron oxide nanoparticles against both gram-positive and gram-negative bacteria.(Zakariya, Majeed, and Jusof 2022; Tabassum et al. 2023; Gabrielyan et al. 2019) The antibacterial effect of these small size nanoparticles is due to the their higher membrane permeability.(Suba et al. 2021)

The mechanism may be explained as the electromagnetic attraction between the positive charge of the nanoparticle and the negative charge of the microorganism. This attraction causes the microbes to die due to oxidation. The thiol group on bacterial surface proteins reacts with the nanomaterials to cause cell lysis.(Rezaei-Zarchi et al. 2010; Prabhu et al. 2015)furthermore, the iron nanoparticles cause oxidative stress through the Fentonprocess and reactive oxygen species altering cell membrane permeability. Hydrogen peroxide is formed by combination of oxygen and Fe^{2+} and this damage the biological macromolecules. (Majeed et al. 2021)Reactive oxygen species including radicals like superoxide, hydroxyl and hydrogen peroxide radcials damage bacterial protiens and DNA. By producing reactive oxygen species, iorn oxide nanoparticles can inhibit most pathogenic bacteria including S. aureus and E. coli.(Kim et al. 2001)Moreover, the ionic transport chain becomes disrupted as a result of nanoparticles adhering to membrane cells. Furthermore, the breakdown of proteins and lipopolysaccharides in the membrane may lead to cell death.(Zakariya, Majeed, and Jusof 2022) IONPs may therefore be considered as an alternate means of treating bacterial multidrug resistance against pathogens.

4. Conclusion

Combining nanotechnology with nanoparticlesis expected to help tackle many biological and healthcare challenges since lots of important processes happen on a super tiny scale. But to make the most of nanoparticles for medical purposes, we need to carefully study how different modifications change their behavior. It's been suggested that nanoparticles might be able to fight off common drug-resistant bacteria and other bugs, which is crucial since we're seeing more and more bacteria becoming resistant to antibiotics because we use them too much. Our research shows that around 1000 µg, the iron oxide and modified iron oxide nanoparticles demonstrated a moderate level of inhibitory activity against Staphylococcus aureus (MTCC 87). The modified iron oxide nanoparticles had the highest DPPH scavenging activity (IC50 78.2 µg/ml and IC50 87.5 µg/ml, respectively), according to antioxidant studies. To combat antibiotic resistance as well as more deadly illnesses like cancer, iron oxide nanoparticles and modified particles were created, characterized, and found to have promising anti-oxidant and anti-antimicrobial activity. Hence, from the study, it is possible to concludethat synthesized nanoparticles are potent antibacterial agents having good antioxidant properties.

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