

Effect of Ferric Oxide Nanoparticles on *Staphylococcus aureus*

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Abstract

Background: Ferric oxide nanoparticles (FeO NPs) have gained attention for their potential applications in various fields, including medicine and biotechnology. When it comes to *Staphylococcus aureus*, a bacterium known for causing various infections, researchers have explored the use of FeO NPs due to their antimicrobial properties. **Objectives:** These nanoparticles have shown the ability to inhibit the growth and viability of *S. aureus*, making them a promising tool in combating this bacterium, this research can contribute to the development of effective antimicrobial treatments and infection control methods. **Materials and Methods:** Forty-eight samples of *S. aureus* were collected from burns, wounds, and urine, but only 19 were identified as *S. aureus* based on cultural and morphological characteristics and by using polymerase chain reaction technique. To study the effect of FeO NPs on *S. aureus*, they were biosynthesized by pyocyanin pigment produced from *Pseudomonas aeruginosa*. FeO NPs were characterized using atomic force microscopy, Fourier-transform infrared spectroscopy, field emission scanning electron microscope, energy dispersive X-ray spectroscopy, and UV-vis spectroscopy. *S. aureus* was cultured on MHA for determining the antimicrobial effect, and the agar well diffusion method was used. **Result:** The findings demonstrate a clear relationship between the substance's concentration and the inhibition zone's diameter. Specifically, when the concentration was 0.1 µg/mL, the diameter of the inhibition zone was 30 mm, but at a concentration of 0.0125 µg/mL, the diameter was 14 mm. **Conclusion:** The use of FeO NPs has demonstrated significant antimicrobial properties against *S. aureus*, suggesting their potential as a novel therapeutic approach.

Keywords: Biosynthesis, ferric oxide, nanoparticles, *Pseudomonas aeruginosa*, pyocyanin, *Staphylococcus aureus*

INTRODUCTION

Nanotechnology encompasses the scientific, technical, and technological activities that occur at the nanoscale. A wide range of scientific fields, from chemistry and biology to physics and materials science to engineering, can benefit from it. Nanoparticles (NPs) are substances characterized by having two or more dimensions, often with a diameter falling within the range of 1–100 nm.^[1] *Ex vivo* (out of the living) synthesis of NPs is becoming increasingly popular for various uses, including medical treatments, industrial production, and incorporation into materials such as cosmetics or clothing. Increased reactivity and possible biochemical activities are a result of the high surface-to-volume ratio of NPs. However, the underlying chemical mechanisms of how NPs interact with biological systems are mainly undiscovered.^[2]

Staphylococcus aureus is a widespread bacterium that lives as part of the regular flora of the skin, hair, and throat of humans and animals alike. As normal flora, they pose no

threat to the host unless they gain access to the bloodstream through a cut or scratch (as can happen when food handlers and hospital workers scrub their hands too roughly) or when they can bypass the body's other defenses and cause infections.^[3] Boils, impetigo, acne, cellulitis, carbuncles, and abscesses are all skin infections. Staphylococcal scalded skin syndrome is the result of untreated, widespread acne infections. These infections might linger for a while but are not as dangerous as sepsis or bacteremia, which occur when the pathogen enters the body. In the absence of antibiotics, *S. aureus* is known to cause various illnesses, including the potentially fatal toxic shock syndrome and endocarditis.^[4,5] It is a common source of post-op

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infections. One of the hospital's newest issues is the spread of methicillin-resistant *S. aureus* nosocomially, which can be fatal for immunocompromised patients.^[6] Common foods that cause staphylococcal food poisoning include eggs, beef, milk, and other dairy products. In the food industry, preventing staphylococcal food poisoning, which can cause gastroenteritis, is essential during operations where temperatures are slightly above refrigeration. Food poisoning is caused by *S. aureus* enterotoxins.^[7]

MATERIALS AND METHODS

Ferric oxide NP (FeO NP) synthesis

The first step for NP preparation is preparing a flask that contains 100 mL of chloroform containing pyocyanin and 10 g of ferric chloride. The flasks were placed intermittently in a sonicator for 15 min, then placed in a shaker for 48 h. After 48 h. of shaking, both of the solutions in each flask were poured into a plain tube, about 5 mL in each tube. The tubes were placed in a centrifuge at 5000 rpm for 10 min, washed with deionized distill water, and placed again in a centrifuge at 5000 rpm for 10 min, then washed and centrifuged again. The solution was placed in a petri dish and left to dry in the incubator.^[8]

Characterization of iron oxide

The characteristics of NPs were confirmed by Fourier-transform infrared (FTIR) spectroscopy (SHIMADZU, Kyoto, Japan), Energy Dispersive X-ray (EDX) diffraction (Panalytical, Almelo, Netherlands), atomic force microscopy (AFM; Angstrom Advanced, Stoughton, MA, USA), field emission scanning electron microscopy and UV-vis spectroscopy (SHIMADZU).

Staphylococcus aureus collection

Sample collection was done Between October and January suspected samples from wounds, burns, and urine were collected and cultured in the Al-Kindy hospital laboratory using a transport media to be transported to the laboratory, then using mannitol salt agar (MSA) as a selective medium after that incubated at 37°C for 24 h. Lactose fermenting colonies were identified morphologically polymerase chain reaction (PCR) was used to confirm the identification also VITEK-2 system was used for antimicrobial sensitivity test.

Ethical approval

This work was approved by Ethical committee of the Department of Biology/College of Science/ Baghdad University according to the Ref number (CSEC/0992/0085). The samples were collected after the approval of the patients.

Antimicrobial activity of prepared NPs

Antimicrobial efficacy of FeO NPs was examined using the agar well diffusion method to determine their efficiency

against *S. aureus*.^[9] Muller–Hinton agar medium (25 mL) was sterilized and cooled. Subsequently, the liquid was transferred into sterilized Petri plates and left undisturbed to harden at room temperature. Sterile cotton swabs were used to transfer and spread the overnight growth of test microorganisms onto the agar medium. Wells were made in agar by using a cork borer and filled with various concentrations test (0.1, 0.05, 0.025, 0.014, and 0.0125 mg/mL) of FeO NPs, as well as using control of ferric oxide. Placed in incubator for 24 h at 37°C. Then the FeO NPs' efficiency was determined by the diameter of the inhibition zone.

RESULTS

Morphological identification

Staphylococcus aureus was cultured in MSA as it was considered selective and differential, it produced yellow colonies with yellow zones, as shown in Figure 1, while coagulase-negative staphylococci produced small pink or red colonies with no color change to the medium.

Molecular identification of *S. aureus*

Isolates of *S. aureus* were subjected to molecular identification via detecting the *16SrRNA* gene using specific primers. DNA was extracted for screening the presence of *16SrRNA* gene using PCR. The results of the PCR products were confirmed by comparing the amplicon size with DNA ladder bands on the agarose gel. The PCR products have a single amplicon band (149 bp) for *S. aureus*, according to MacroGen.



Figure 1: *Staphylococcus aureus* cultured on MSA

NP characteristics

AFM analysis

AFM was used to determine the size and surface morphology of FeO NPs, testing the contact forces between the tip and surface.^[10] Figure 2 illustrates two- and three-dimensional AFM of FeO NPs which were all the same shape and size. Also, the average size of biosynthesized FeO NPs by pyocyanin according to Table 1 was 39.26 nm.

The result is in correlation with AFM result,^[1] which showed that the average FeO NPs size was in a range from 20 to 40 nm.

FTIR spectroscopy

The bond vibration frequencies can be calculated using FTIR spectrographic investigation. In addition, the

synthesized FeO NPs' infrared-optimized band value was supported by the chemical functional group of FeO NPs. The existence of FeO bonding was verified by FTIR, as shown in Table 2.

Field emission scanning electron microscope (FE-SEM)

The FE-SEM image in Figure 3 reveals that FeO NPs are both uniformly distributed and almost perfectly spherical in shape. The average particle size is about 38.01 nm, and there are some NPs that have a hexagonal form.

The result is in line with the study^[11] which revealed that the biosynthesized FeO NPs had spherical shape and showed less aggregation of particles with diameters of about 20–60 nm.

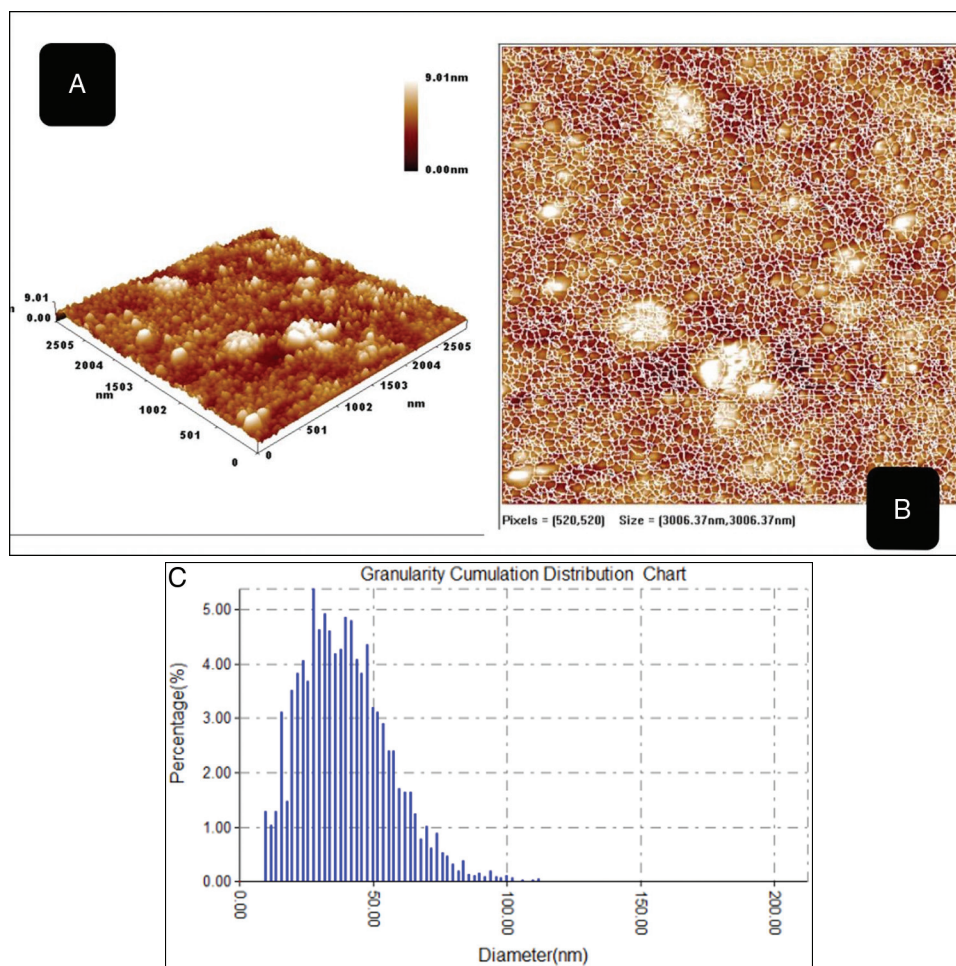


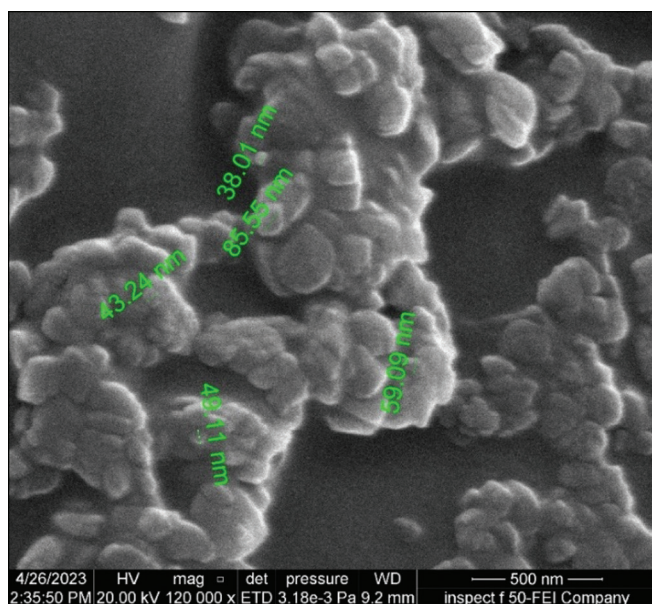
Figure 2: (A) The biosynthesized FeO NPs; (B) 2D and 3D AFM of FeO NPs; (C) chart granularity distribution of FeO NPs

Table 1: The Cumulation size of FeO NPs biosynthesized by pyocyanin by AFM technique

Avg. Diameter:39.26 nm	<=10% Diameter:18.00 nm
<=50% Diameter:36.00 nm	<=90% Diameter:60.00 nm

Table 2: FTIR of FeO NPs

Type of compound	Frequency of absorption (cm ⁻¹)	Bonds	Compound class of functional groups
Pyocyanin	3421.48–3406.05	O–H stretching	Alcohol
	2954.74–2854.45	O–H stretching (strong broad), O–H stretching (weak broad), N–H stretching, C–H stretching	Carboxylic acid, alcohol, amine salt, alkane
Pyocyanin + ferric sulfate	1110.92–1033.77	C–F stretching	Fluoro compound
	3433.06	O–H stretching	Alcohol
	2925.81–2854.45	O–H stretching	Carboxylic group, alcohol
		N–H stretching	Amine salt
		C–H stretching	Alkane
		O–H stretching	Intramolecular bonded
	1629.74	C=C stretching	cyclic alkane, conjugated alkene
		N–H bending	Amine
			alkane
	1490.87–1433.01	C–H bending	Fluoro compound
Ferric sulfate	1083.92–1029.92	C–F stretching	Amine salt
	536.17–426.24	C–N stretching	Metal oxide
	3390.63–3367.48	C–H bending	Aromatic compound
	1677.95–1649.02	C=N stretching	Imine\oxime
	1091.63	C–O stretching	Aliphatic ether, secondary alcohol
		C–N stretching	Amine
		C–F stretching	Fluoro compound
	671.18–526.53	C–Br stretching	Halo compound
FeO NPs	3541.06–3253.69	O–H stretching	Alcohol
	1650.95–1622.02	*C–H bending,	Aromatic compound, conjugated alkene
	1103.21	C=C stretching	Aliphatic ether, secondary alcohol
	613.32	C–O stretching,	fluoro compound, amine
		C–F stretching,	
		C–N stretching	
		C–Br stretching	*Halo compound

**Figure 3:** Field emission scanning electron microscopy images of FeO NPs**EDX spectroscopy**

The EDX plot of the scanning electron microscope images used to determine the elemental composition of the FeO NPs is depicted in Figure 4. High purity for the synthesized FeO NPs was verified by the EDX spectra, which show that the necessary phases of Fe and O are present in the samples, as shown in Table 3.

UV–vis spectral analysis FeO

UV–visible spectrophotometer (SHIMADZU) scans are used to identify the maximum absorption of biosynthesized FeO. The result showed that biosynthesized FeO NPs exhibited a maximum absorption peak at 250 nm, as shown in Figure 5.

The absorption spectra at 370 nm indicate the formation of FeO NP.^[12] The outcome was similar to a study by Rusianto,^[13] which demonstrated that the FeO NPs show absorbance of 330–450 nm. Additionally, consistent with^[14] investigation, an observation has been made

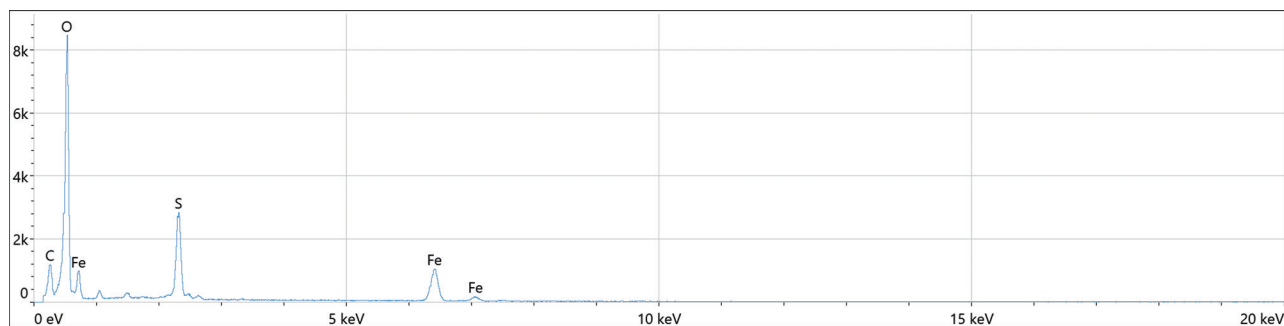


Figure 4: Diagram of elements analysis of FeO NPs

Table 3: Elemental composition of the FeO NPs

Element	Atomic %	Atomic % error	Weight %	Weight % error
C	21.7	0.4	15.7	0.3
O	72.4	0.5	69.7	0.5
S	3.8	0.0	7.3	0.1
Fe	2.2	0.0	7.3	0.1

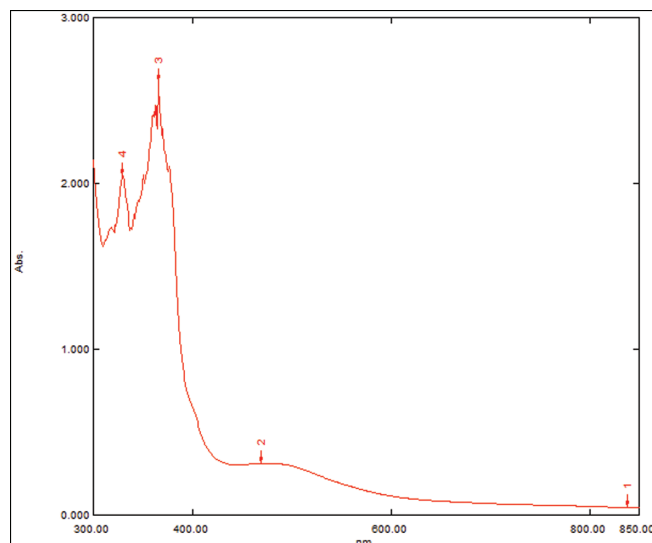


Figure 5: UV-vis spectrophotometry of ferric oxide nanoparticles

indicating the presence of a UV absorption band within the wavelength range of 330–450 nm.

NP synthesis

NPs were effectively synthesized by the biosynthesis technique utilizing chloroform with pyocyanin pigment [Figure 6], hence presenting a promising avenue for further investigation into the potential of NP biosynthesis.

Determination of the highest resistance isolates

The selection of the multidrug-resistant isolate was based on the outcomes obtained from the VITEK-2 system. The findings indicated that a significant proportion of isolates (85%) exhibited multidrug resistance, demonstrating resistance to a minimum of three distinct categories of the antibiotics that were tested.



Figure 6: FeO NPs

DISCUSSION

FeO NPs have exhibited antibacterial properties against *S. aureus*. The impact of various doses (0.1, 0.05, 0.025, 0.014, and 0.0125 µg/mL) of FeO NPs on bacterial cell viability was examined using Muller–Hinton agar. The findings revealed that the strongest inhibition zone was about 30mm at 0.1 µg/mL, while the weakest inhibition zone was 12mm at 0.014 µg/mL [Figure 7]. The results were in excellent agreement with previous studies demonstrating the minimum inhibitory concentration of FeO NPs.^[15] The same effects were mentioned for another green synthesized nanoparticle against several pathogenic bacteria^[16–18].

The utilization of green-synthesized FeO NPs exhibits promising promise as an antibacterial agent for the treatment of bacterial-induced illnesses. The utilization of green synthesis presents some notable benefits in comparison to alternative methodologies.^[19] The approach employed in this study is environmentally sustainable and offers compatibility with biological systems in

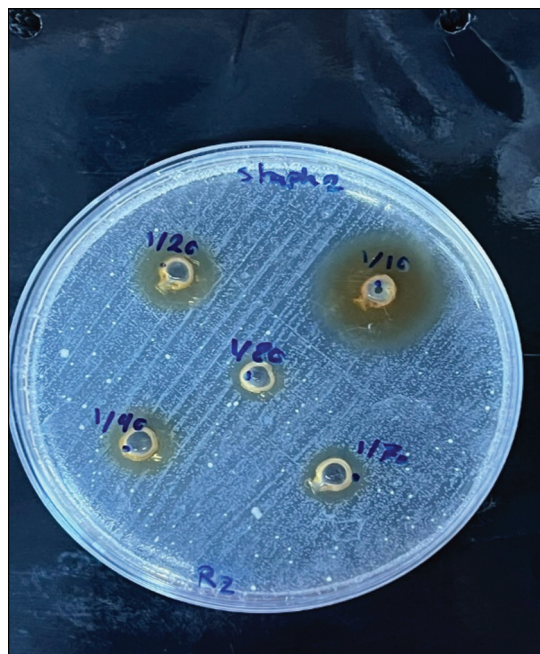


Figure 7: Effect of FeO NPs on *S. aureus*

several fields, such as pharmaceuticals, biomedicine, and cosmetics^[20] as it avoids the use of hazardous chemicals during the synthesis process. The cost-effectiveness of green synthesis has also been demonstrated.

CONCLUSIONS

- The use of FeO NPs has demonstrated significant antimicrobial properties against *S. aureus*, suggesting their potential as a novel therapeutic approach.
- The pyocyanin serves as a valuable and effective tool in producing NPs with potential application in various fields.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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