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ORIGINAL ARTICLE

Green Synthesis and Characterization of Iron Oxide Nanoparticles from A Novel Strain of *Bacillus Licheniformis* and Evaluating Its Antimicrobial Activity

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Key words: ***Nanotechnology, Antibiotic resistance, Iron nano particles, Metal nano particles***

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ABSTRACT

Background: *Metal reduction through particular metabolic pathways of different organisms has been determined as a possible source of nanoparticles production.* ***Objectives:*** *This study focuses on the synthesis and characterization of iron oxide nanoparticles (IONPs) using a novel strain of Bacillus licheniformis as a source of the reducing and stabilizing agent.* ***Methodology****: Synthesis of iron oxide nanoparticles was examined by ultraviolet–visible spectroscopy, transmission electron microscopy (TEM), and dynamic light scattering (DLS). The antimicrobial activity of NPs against Staphylococcus aureus, E.coli and Candida albicans was assessed. Additionally, this study investigated the combined effects of the IONPs with some commonly used antibiotics and antifungals* ***Results:*** *Synthesis of iron oxide nanoparticles was successful with three molar ratios. TEM showed spherical morphology and 48±9 nm average size distribution, particles with a size range from 34.74-52.44 nm. Iron oxide nanoparticles exhibit potent antibacterial activity and can enhance the efficacy of certain antibiotics..****Conclusion****: This study suggested the potential of IONPs as a promising approach to overcome antimicrobial resistance and improve the treatment of infectious diseases*

INTRODUCTION

Nano science and nanotechnology are fields that deal with the manipulation of matters at the nanoscale, typically between 1-100 nm, at which materials exhibit unique properties such as high surface area that can be applied to create novel materials and devices[1]. Nanotechnology has gained prominence since the innovation of the scanning tunnelling microscope in 1981 and has the potential to revolutionize various fields, diverse applications, and ongoing research challenges[2]. Nanoparticles (NPs) can be used to create scaffolds for tissue regeneration, deliver drugs specifically to infected cells minimizing side effects, used as contrast agents in MRI and CT scan imaging, create surfaces that repel water, oil, and other contaminants, and create stronger, lighter, and highly conductive materials[3]. Traditionally, nanoparticles were synthesized using methods that often involve the use of toxic chemicals, environmental hazards, and often higher temperatures[4]. But the growing awareness of environmental sustainability necessitates interest in green synthesis methods which utilize biological materials such as plants or microorganisms as reducing agents to produce nanoparticles with unique properties[5]. These biological systems are eco- friendly, readily available, biocompatible, can be genetically engineered with specific shapes and sizes, and are more cost effective than traditional chemical methods[6]. Biological techniques for green nanosynthetic methods are generally economical, environmentally safe, and time-efficient, so they yield notable outcomes for nanotechnology. Metal reduction through particular metabolic pathways of different organisms has been determined as a possible source of nanoparticles production[7]. Various bacterial species have been shown to effectively produce iron oxide nanoparticles with antimicrobial activity against pathogens like *Staphylococcus aureus* and *E. coli*[8]. The synthesis occurs via intracellular or extracellular pathways where bacteria convert metal ions into NPs through enzymatic reduction[9]. The use of novel bacterial strains for nanoparticles synthesis can create unique NPs with distinct properties. Specific strains of bacteria have demonstrated the ability to produce NPs with varying sizes and shapes exhibiting antimicrobial activity[10].

Unlike conventional antibiotics, biogenic synthesized nanoparticles can exhibit multiple mechanisms of action to combat multi drug resistant pathogens such as oxidative stress, physical interactions,

or catalysis, reducing the likelihood of resistant development[11]. Nanoparticles provide the hope in solving the problem of antibiotic resistance[12]. Nanoparticle's biocompatibility and the ability to enhance the efficacy of existing traditional antimicrobial agents through synergism make them promising alternative strategies in treating infections and addressing growing challenges of antibiotic resistance[13].

METHODOLOGY

Bacterial strain collection and identification

The novel strain of *Bacillus licheniformis* was previously isolated from soil and identified microscopically and genetically by 16S ribosomal RNA[14]. The pathogenic microorganisms (*Staphylococcus aureus* (MRSA), *E. coli*, and *Candida albicans*) were taken from the Microbiology Laboratory/Department of Clinical and Laboratory Sciences/College of Pharmacy/University of Basrah in which were previously isolated from diabetic foot infections and vaginal swabs.

Materials

The chemicals used involve hydrated ferrous chloride ($FeCl_2.4H_2O$, 98%) which is used as a precursor for metal iron (Loba Chemie Pvt Ltd, India). Nutrient agar and nutrient broth were used as culture media (CDH, India). Antibiotics used in the study were from (HI Media, India).

Preparation of McFarland solution

McFarland standard (0.5) was prepared following established protocols[15]. This standard served as a visual reference to adjust the turbidity of bacterial suspensions to ensure a consistent bacterial cell density. This standardization enhances the reproducibility of microbiological assays[16].

Susceptibility test for selected antimicrobials

The Kirby-Bauer disk diffusion method, as described by Bauer *et al*[17] was used to measure antibiotic and antifungal susceptibility test. Eight antibiotics of different classes and five antifungals were assessed against *S. aureus, E. coli,* and *Candida albicans*. First, bacterial pathogens were cultured in nutrient agar for 24 hours, then the colonies were taken using sterile loops and suspended in 5ml of nutrient broth, adjusting its turbidity compared to a 0.5 McFarland standard[16]. The bacterial suspension was inoculated onto nutrient agar using a sterile cotton swab. Then antibiotic and antifungal disks were placed on the inoculated plates and incubated for 24 hours at 37 °C[17]. The zone of growth inhibition surrounding each disk was measured and classified as sensitive (S), resistant (R), or intermediate (I) as compared to reference tables from the Clinical and Laboratory Standards Institute CLSI[18].

Fabrication and characterization of iron nanoparticles

For the preparation of bacterial supernatant, *B. licheniformis* was first cultivated on nutrient agar for one day, then inoculated in nutrient broth for 24 hours at 30 °C in a 150 rpm shaker incubator[19]. Hydrated ferrous chloride solution was prepared in 10 mM using deionised water. For iron nanoparticle production the iron salt was added to the bacterial extract drop by drop in three molar ratios, 1:1, 1:0.5, and 0.5:1 to evaluate the effect of this variable on the properties and biological effects of the produced nanoparticles. The admixture suspension was then incubated with shaking for two days (about 24 hours) and observed for any change in the reaction colour. After incubation, the result suspension was centrifuged at 6000 rpm for about 20 minutes to remove any remnant bacterial fragments and kept in the refrigerator for subsequent characterization processes[20].

Nanoparticle's size and configuration were investigated using the transmission electron microscope (TEM)[21] (novaNano, USA). The elemental content and impurities of the nanoparticle sample were determined by energy Dispersive X Ray Spectroscopy (EDX)[22] (novaNano, USA) and the size distribution, surface potentials, and nanoparticles stability were analyzed using dynamic light scattering (DLS) zeta sizer and zeta potential (malvern, UK)[23]. While Fourier transform infrared spectroscopy (FTIR) (Bruker, USA) was used to examine the molecular moieties and chemical groups that may have a role in the synthesis reaction[24].

Antimicrobial activity of iron oxide Nanoparticles (invitro)

Microbes like Gram-positive *Staphylococcus aureus*, Gram-negative *E. coli*, and *Candida albicans* were used to evaluate the antimicrobial effects of biosynthesized iron oxide nanoparticles by employing the well diffusion assay[25]. Culture media preparation was done according to the manufacturer's instructions. Preparation of the microbial suspension was done as compared to 0.5 McFarland turbidity standard[26]. Microorganisms are first sub-cultured into nutrient broth overnight then inoculated onto nutrient agar surface. Wells then created into the agar using a sterile cork borer and each well received about 50 µL of each molar ratio 1:1,1:0.5, and 0.5:1 of IONPs[25]. The extract of *B. licheniformis* and the salt of hydrated ferrous chloride ($FeCl_2.4H_2O$, 98%) were loaded into wells as controls. Then the plates were incubated for 24 hours at 37 °C and the zone of bacterial growth inhibition was measured in millimetres[27].

Determination of Minimum Inhibitory concentration (MIC)

A stock suspension of high concentration iron oxide nanoparticle was prepared for the three molar ratios using gravimetric method and diluted using deionized water to obtain a range of nanoparticle concentrations

(0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/ml)[28]. Vancomycin antibiotic and deionized water were used as positive and negative controls, agar well technique was utilized[29]. *S. aureus* was cultivated on nutrient agar plate and wells (6 mm) were punched on the surface of the agar. Each well was filled with iron nanoparticles suspension of a specific concentration and the plates were then incubated at 37 °C. After 18-24 hours of incubation, the distance across each well was measured and evaluated[27]. The lowest concentration at which bacterial growth is inhibited is considered the minimum inhibitory concentration MIC[29].

The combined effect of iron oxide nanoparticles with some traditional antibiotics and antifungals

Penicillin G, Amoxicillin/ Clavulanate, Meropenem, Polymyxin B, Streptomycin, Trimethoprim, Gatifloxacin, and Nalidixic acid antibiotics. Fluconazole, Ketoconazole, Itraconazole, Nystatin, and Amphotericin B antifungals were combined with the three molar ratios of IONPs to evaluate their antibacterial and antifungal combined effects against *Staphylococcus aureus, E. coli,* and *Candida albicans* using the Kirby Baur disk diffusion assay[17]. Each antimicrobial disk was impregnated with the iron nanoparticles and strategically placed onto the agar plate which was previously streaked with the tested microorganism and incubated overnight[30]. The diameter of each zone around the disks was measured and data analysis was conducted to investigate whether the combination had synergistic, antagonistic, or additive antibacterial effect[31].

Statistical analysis

Statistical analysis of data was performed using SPSS, IBM Corporation, USA software, version 26. Two-way ANOVA (Analysis of variance) and Least Significant Difference (LSD) post hoc test was performed to assess the significant differences among means. *p*-value of <0.05 is considered statistically significant.

RESULTS

Susceptibility test for selected antibiotics

According to the antimicrobial susceptibility test results as shown in (Table 1), *Staphylococcus aureus* was found to be sensitive to Amoxicillin/ Clavulanate, Meropenem, Streptomycin, Trimethoprim, Gatifloxacin, Nalidixic acid antibiotics, and Polymixin B. *Staphylococcus aureus* showed resistance to Penicillin G. *E. coli* showed sensitivity to Meropenem, Polymyxin B, Amoxicillin/ Clavulanate, and Gatifloxacin antibiotics, and showed resistance to Penicillin G , Trimethoprim and Nalidixic acid. *Candida albicans* was sensitive to Itraconazole antifungal and resistant to Fluconazole, Ketokonazole, Nystatin, and Amphotericin B.

Table 1: The results of antibiotic susceptibility test of *Staphylococcus aureus, E. coli,* and *Candida albicans*

Antibiotics	*Staphylococcus aureus*		*E. coli*		*Candida albicans*		
	ZOI (mm)	Interpretation	ZOI (mm)	Interpretation	Anti-fungal	ZOI (mm)	Interpretation
Penicillin G	0.00	R*	0.00	R	Fluconazole	0.00	R
Amoxicillin/ Clavulanate	22.07±0.31	S**	19.07±0.25	S	Amphotericin B	0.00	R
Meropenem	35.10±0.30	S	35.03±0.15	S	Ketoconazole	0.00	R
Polymyxin B	0.00	R	15.17±0.21	S	Itraconazole	30.09 ±0.41	S
Streptomycin	17.07±0.15	S	17.15±0.2٩	S	Nystatin	0.00	R
Trimethoprim	25.00±0.00	S	0.00	R			
Gatifloxacin	30.20±0.30	S	18.03±0.21	S			
Nalidixic acid	9.00±0.10	S	0.00	R			

*R, Resistant; **S, Susceptible.

Fabrication and characterization of iron nanoparticles

Iron oxide nanoparticles were successfully synthesized using *Bacillus licheniformis* bacterial extract. The formation of nanoparticles was indicated by the reaction colour change from pale brown or yellow to dark brown (Figure 1).

Transmission Electron Microscopy (TEM) analysis of iron oxide nanoparticles showed spherical morphology and 48±9 nm average size distribution (Figure 2). Energy-dispersive-X-ray spectroscopy (EDX) analysis of iron oxide nanoparticles (IONPs) showed the presence of Iron (Fe), Oxygen (O), Chlorine (Cl), and Sodium (Na), Carbon(C), Silicon (Si), and phosphorus (P) (Figure 3).. Dynamic light scattering (DLS) analysis revealed that the synthesized nanoparticles exhibited a mean diameter of 4 nm for a 1:0.5 molar ratio with a relatively broad size distribution, as indicated by a ploydispersity index of 0.490 mV (Figure 4A). Zeta potential measurements performed using electrophoretic light scattering, showed a negative surface charge of -30.9 mV (Figure 4B)..

Fourier Transform Infrared Spectroscopy (FTIR) spectrum of IONPs synthesized via a green method showed characteristics peaks at [3165,3047,2965,1628,1551,1451,1402,1345,1299,110, 1047, and 658 cm^{-1}], corresponding to O-H,C-H, C=O, N-H, C-H, Si-O, and Fe-O functional groups (Figure 5).

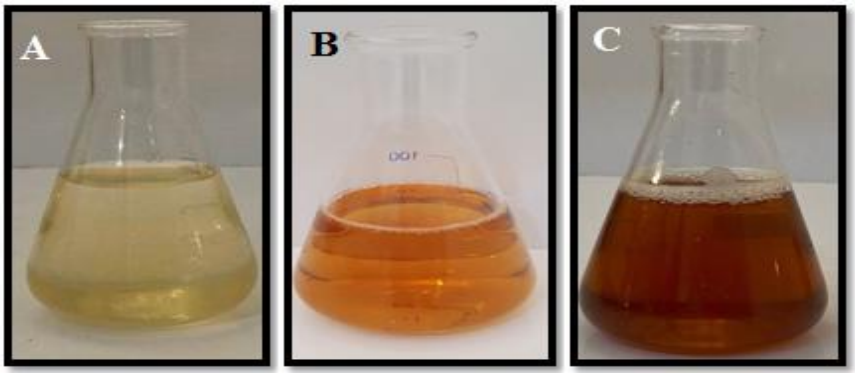

Fig. 1: A: Iron precursor, B: Bacterial extract, C: Colloidal suspension of iron nanoparticles

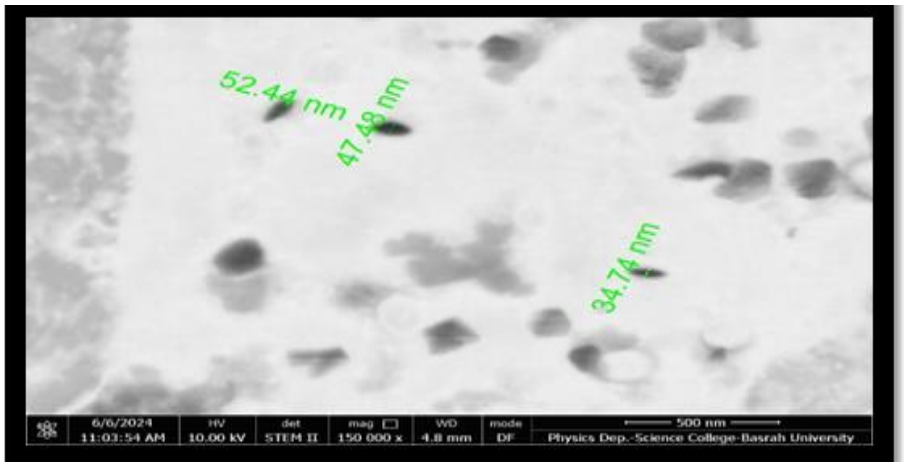

Fig. 2: Transmission Electron Microscopy (TEM) image of 1:0.5 molar ratio iron oxide nanoparticles, showing their spherical morphology and 48±9 nm average size distribution. The image reveals a relatively uniform distribution with minimal agglomeration. The scale bar indicates 5 µm

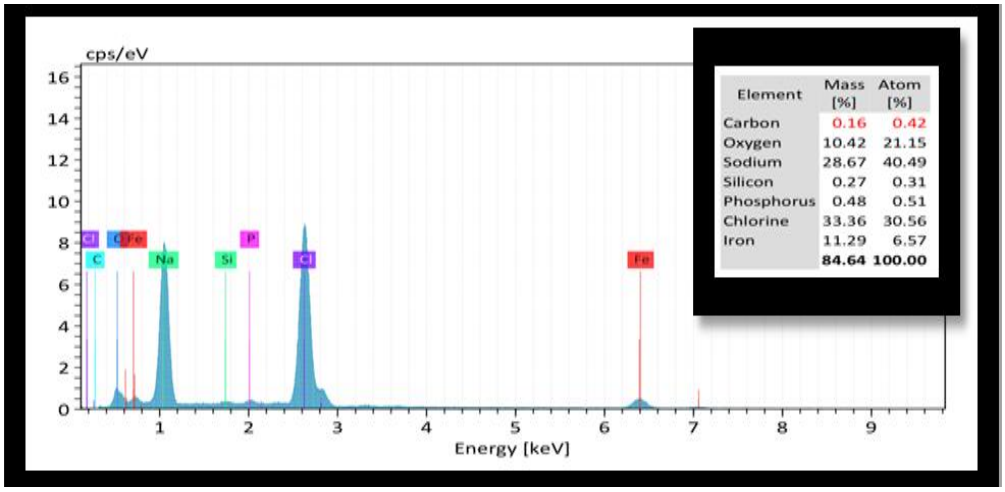

Fig. 3: Energy-dispersive-X-ray spectroscopy (EDX) analysis of iron oxide nanoparticles (IONPs) showed the presence of Iron (Fe), Oxygen (O), Chlorine (Cl), and Sodium (Na), Carbon(C), Silicon (Si), and phosphorus (P). The spectrum confirms the presence of Fe and O as the primary elements, and may indicate trace amounts of C, Si, and P. The peaks correspond to the characteristic X-ray emissions of the respective elements

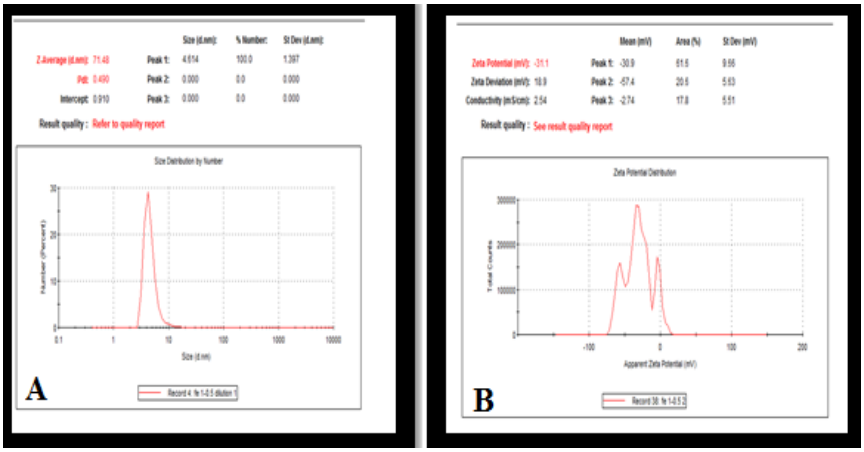

Fig. 4: Dynamic Light Scattering (DLS) data for iron oxide nanoparticles (IONPS) synthesized via green method, highlighting A: the zeta size distribution, B: zeta potential

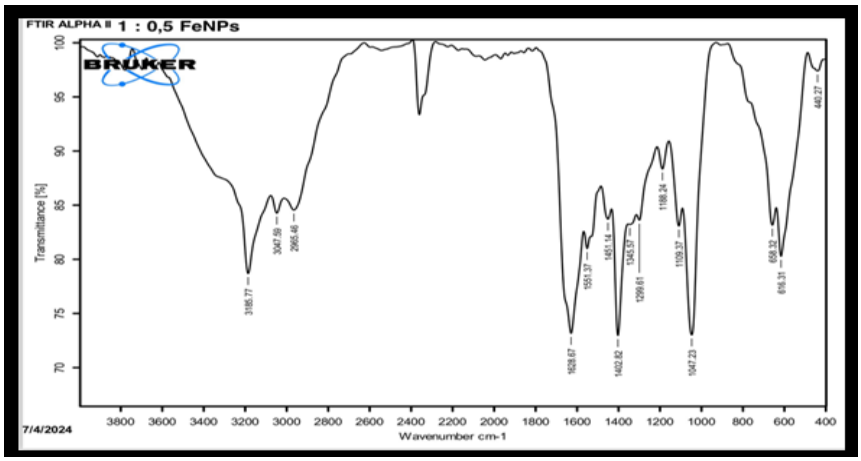

Fig. 5: Fourier Transform Infrared Spectroscopy (FTIR) spectrum of IONPs synthesized via a green method showing characteristics peaks at [3165,3047,2965,1628,1551,1451,1402,1345,1299,1109,1047, and 658]cm^{-1}, corresponding to O-H,C-H, C=O, N-H, C-H, Si-O, and Fe-O functional groups

Antimicrobial effect of iron Nanoparticles (in vitro)

The results presented in (Table 2 and Figure 6) demonstrated that the iron oxide nanoparticles produced using *Bacillus licheniformis* exhibit antibacterial activity against *Staphylococcus aureus* (MRSA) at all tested molar ratios. No significant antibacterial effect was observed against *E. coli* and no anti-fungal effect against *Candida albicans* under the same conditions.

Table 2: The antimicrobial effect results of iron oxide nanoparticles by the Kirby- Bauer method

IONPs + Controls	Zone of inhibition ZOI (mm)		
	S. aureus	***E. coli***	***C.albicans***
$FeCl_2$ (10mM)	*-	-	-
Bacterial supernatant	-	-	-
1: 0.5 NPs	10.43±1.02mm	-	-
1:1 NPs	14.1±1.55 mm	-	-
0.5:1 NPs	15.26±0.73mm	-	-

* No Bacterial inhibition

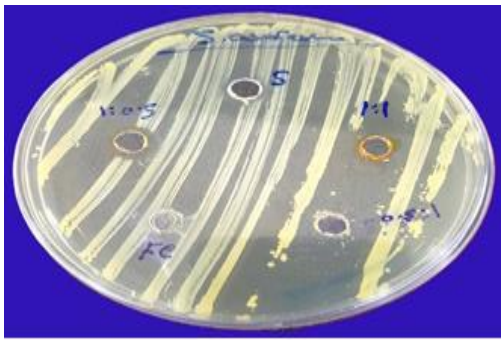

Fig. 6: The antibacterial effect of iron oxide nanoparticles against *S .aureus*

Determination of Minimum Inhibitory concentration (MIC)

The observed MIC values in (Figure 7), the ratios of 1:1 and 1:0.5 recorded the highest MIC value of 8 mg/ml, whereas the ratio of 0.5:1 recorded the lowest MIC value of 2 mg/ml.

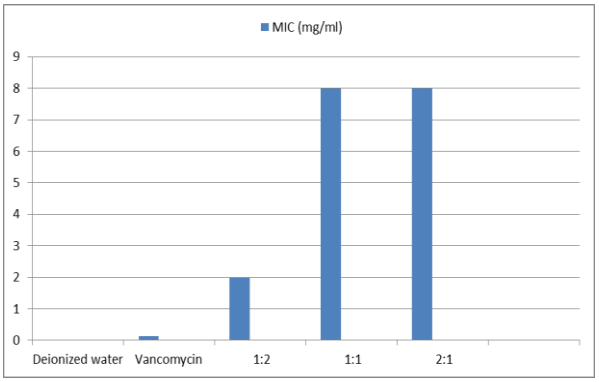

Fig. 7: The minimum inhibitory concentration (MIC) of iron oxide nanoparticles against *Staphylococcus aureus*

The combined effect of iron oxide nanoparticles with some traditional antibiotics and antifungals

The results presented in (Table 3, 4 and 5) demonstrated that the combined effect of the three molar ratios of iron oxide nanoparticles with antimicrobials against tested pathogens, *Staphylococcus aureus*, *E coli*, and *Candida albicans* yielded varied outcomes. The nano particles synthesis variables (bacterial extract: iron salt solution molar ratios of addition) found to be a statistically non-significant (*p*-value>0.05) when combined with all of the tested antibiotics and antifungals indicating a broad range of effective ratios.

Penicillin G, Amoxicillin/ Clavulanate, Polymyxin B, Trimethoprim and Nalidixic acid combined with IONPs against *Staphylococcus aureus*, Amoxicillin/ Clavulanate, Gatifloxacin and Nalidixic acid combined with IONPs against *E coli* resulted in a statistically significant enhancement of antibacterial effect. Meropenem, Streptomycin and Gatifloxacin against *Staphylococcus aureus* and Penicillin G, Meropenem, Polymyxin B, Streptomycin and Trimethoprim against *E coli* led to a statistically non- significant enhancement.

Combining Fluconazole, Ketoconazole, Itraconazole, Nystatin, and Amphotericin B with IONPs in three different molar ratios showed statistically non-significant change or enhancement in the anti- fungal activity against *Candida albicans* (Table 5).

Table 3: The synergistic antibacterial (AB) activity of iron oxide nanoparticle against *Staphylococcus aureus and E. coli.* using Beta-Lactams and Cell Wall Inhibitors antibiotics

Antibiotics	Molar ratio	*S. aureus*			*E. coli*		
		Zone of inhibition (mm)		Significance	Zone of inhibition (mm)		Significance
		AB alone	NP+AB		AB alone	NP+AB	
Penicillin G	0.5:1	-	10.03±0.21	*S	-	-	**NS
	1:1	-	10.17±0.40		-	20.03±0.31	
	1:0.5	-	-		-	-	
Significance	S				NS		
Amoxicillin/ Clavulanate	0.5:1	22.1 ± 0.30	31.07 ± 0.25	NS	19.07±0.25	25.27±0.45	NS
	1:1	22.1 ± 0.30	25.07 ± 0.31		19.07±0.25	20.10±0.45	
	1:0.5	22.1 ± 0.30	40.07 ± 0.15		19.07±0.25	20.33±0.40	
P value	S				S		
Meropenem	0.5:1	35.23±0.30	35.03 ± 0.35	NS	35.03±0.15	35.33±0.61	NS
	1:1	35.23±0.30	35.13 ± 0.40		35.03±0.15	36.13±0.40	
	1:0.5	35.23±0.30	40.07 ± 0.25		35.03±0.15	35.27± 0.40	
Significance	NS				NS		

*S: Statistically significant (*p*-value of < ٠,٠٥), ** NS: Statistically Non-Significant (*p*-value of ≥ 0.05). (-) is considered (6 mm) for statistical purposes as the disk diameter

Table 4: The synergistic antibacterial (AB) activity of iron oxide nanoparticle against *Staphylococcus aureus and E. coli.* using cell membrane, protein and DNA Synthesis Inhibitors antibiotics

Antibiotics	Molar ratio	*S. aureus*			*E. coli*		
		Zone of inhibition (mm)		Significance	Zone of inhibition (mm)		Significance
		AB alone	NP+AB		AB alone	NP+AB	
Polymyxin B	0.5:1	-	15.03 ± 0.21	**NS	15.13±0.21	19.13±0.45	NS
	1:1	-	20.13 ± 0.21		15.13±0.21	17.07±0.40	
	1:0.5	-	15.00 ± 0.30		15.13±0.21	15.23±0.76	
Significance	*S				NS		
Streptomycin	0.5:1	17.1±0.20	15.07 ± 0.15	NS	17.15±0.29	16.13±0.35	NS
	1:1	17.1±0.20	20.13 ± 0.35		17.15±0.29	17.30±0.57	
	1:0.5	17.1±0.20	17.07 ± 0.21		17.15±0.29	17.00±0.45	
Significance	NS				NS		
Trimethoprim	0.5:1	25.00±0.00	15.00 ± 0.20	NS	-	-	NS
	1:1	25.00±0.00	20.03 ± 0.35		-	16.33±0.40	
	1:0.5	25.00±0.00	15.07 ± 0.21		-	-	
Significance	S				NS		
Gatifloxacin	0.5:1	30.2 ±0.30	30.27 ± 0.21	NS	18.03±0.21	14.10±0.40	NS
	1:1	30.2 ±0.30	30.17 ± 0.25		18.03±0.21	19.20±0.36	
	1:0.5	30.2 ±0.30	30.17 ± 0.40		18.03±0.21	12.10±0.36	
Significance	NS				S		
Nalidixic acid	0.5:1	9.00 ± 0.10	15.07 ± 0.25	NS	-	-	NS
	1:1	9.00 ± 0.10	16.07 ± 0.15		-	10.10±0.25	
	1:0.5	9.00 ± 0.10	20.23 ± 0.30		-	10.03±0.21	
Significance	S				S		

*S: Statistically significant (p-value of < 0.05), ** NS: Statistically Non-Significant (p-value of ≥ 0.05). (-) is considered (6 mm) for statistical purposes as the disk diameter.

Table 5: The synergistic antifungal (AF) activity of iron oxide nanoparticle against *Candida albicans*

Antifungals	Molar ratio	Zone of inhibition (mm)		Significance
		AF alone	NP+AF	
Fluconazole	0.5:1	-	*-	**NS
	1:1	-	-	
	1:0.5	-	-	
Significance	NS			
Ketoconazole	0.5:1	-	-	NS
	1:1	-	-	
	1:0.5	-	-	
Significance	NS			
Itraconazole	0.5:1	30.09±0.41	30.08 ± 0.25	NS
	1:1	30.09±0.41	30.27 ± 0.39	
	1:0.5	30.09±0.41	35.14 ± 0.47	
Significance	NS			
Nystatin	0.5:1	-	-	NS
	1:1	-	-	
	1:0.5	-	-	
Significance	NS			
Amphotericin B	0.5:1	-	-	NS
	1:1	-	-	
	1:0.5	-	-	
Significance	NS			

*(-) is considered (6 mm) for statistical purposes as the disk diameter. **NS: Statistically Non-Significant (p-value of ≥ 0.05).

DISCUSSION

The results of this study documented that the colour change during the biosynthesis of iron oxide nanoparticles by the novel strain of *Bacillus licheniform* was also observed by previous researchers[32]. While it has limitations, visual observation of colour change is a simple and valuable tool for monitoring nanoparticles production[33]. Many nanoparticles exhibit distinct colours due to their unique optical properties[34].

The image of Transmission Electron Microscopy (TEM) analysis reveals a relatively uniform distribution with minimal agglomeration (Figure 2).

The results of the spectrum of Energy-dispersive-X-ray spectroscopy (EDX) analysis of iron oxide nanoparticles (IONPs) confirms the presence of Fe and O as the primary elements, and may indicate trace amounts of C, Si, and P (Figure 3). By Dynamic light scattering (DLS) analysis, the high absolute value of zeta potential suggests good colloidal stability, indicating minimal interparticle interactions and reduced likelihood of aggregation[35] (Figure 4). Fourier Transform Infrared Spectroscopy (FTIR) spectrum of IONPs synthesized via a green method indicates nanoparticles surface chemistry and the involvement of organic molecules from the bacterial extract, including proteins, carbohydrates, lipids, and other biomolecules in the synthesis process as reducing and capping agents for the nanoparticles[36](Figure 5).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the most life-threatening pathogens, thus its prevention has become a crucial public health concern[37]. These findings suggest that the antibacterial properties of iron oxide nanoparticles may be specific to certain bacterial strains, warranting further investigation into the underlying mechanisms of action[11] (Table 2 and Figure 6).

The results of Figure 7 suggest that the synthesized iron oxide nanoparticles possess potent antibacterial activity and a relatively narrow window of effective antibacterial concentrations. These relatively low MIC values are promising, as they indicate the potential for effective antimicrobial action at clinically relevant concentrations[37].

The observed variations in the combined effect of IONPs with antibiotics on different microbes offer valuable insights into the potential of combination therapies for combating antimicrobial resistance[38]. The instances of enhanced antimicrobial activity demonstrate the potential for synergistic interactions between nanoparticles and antibiotics, which could lead to a more effective treatment with reduced side effects[39].

CONCLUSION

This study explored the antimicrobial potential of green synthesized iron oxide nanoparticles (IONPs). Characterization techniques, including, TEM, EDX, DLS, and FTIR, revealed the successful synthesis of the IONPs with spherical shape and less than 100 nm size. The IONPs demonstrated antibacterial activity against S. *aureus*, but not against *E. coli*, or *Candida albicans*. The synergistic effects were observed when combined with some antibiotics. These findings have important implications for the development of novel antimicrobial strategies to combat the growing threat of antibiotic resistance. However, further studies are necessary to investigate the potential toxicity of the IONPs, evaluate their long term effects, and translate these findings into effective therapeutic interventions and optimize their delivery methods for in vivo applications. Additionally, exploring the mechanisms of antimicrobial action and the factors influencing synergistic interactions with antibiotics will provide a deeper understanding of their potential as therapeutic agents.

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Conflicts of Interest
The author declared no conflict of interest.

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Ethics Statements
This experiment did not need ethical approval since it did not include animals or humans**.**

Authors Contribution
The authors confirm contribution to the paper as follows: study conception and design: Enas A. Bady, Hawraa R. Dodan, and Ahmed A. A. Alsaad.; data collection: Enas and Hawraa; practical part: all authors; analysis and interpretation of results: Enas A. Bady, Hawraa R. Dodan and Ahmed A. A. Alsaad; draft manuscript preparation: Hawraa R. Dodan. All authors reviewed the results and approved the final version of the manuscript.

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