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Research Article

MICROBIAL ENZYMATIC REDUCTION OF IRON NANOPARTICLES FOR THE CONTROL OF HUMAN PATHOGENS, *STAPHYLOCOCCUS AUREUS*, AND *SALMONELLA TYPHI*

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ABSTRACT

Objective: The objective of this study was to synthesis, characterize, and evaluation of antimicrobial potential of iron nanoparticles (Fe NPs) using *Serratia marcescens*.

Methods: Fe NPs were fabricated by microbial enzyme using ferric chloride as an agent of reduction and stabilization. Fe NPs formation and their elemental nature were confirmed by ultraviolet (UV)-absorption spectroscopy and energy dispersive X-ray spectroscopy, respectively. The morphology of Fe NPs was characterized by a scanning electron microscope (SEM). Functional groups of biomolecules associated with Fe NPs were inferred from characteristic Fourier transform infrared (FTIR) spectroscopy peaks. The antibacterial activity is determined by the disc diffusion method.

Results: Synthesized Fe NPs exhibited characteristic UV-absorption spectrum peaks at 263nm. FTIR spectroscopy peaks of Fe NPs, 3411.66, 1629.16, 1039.63, and 601.90 cm^{-1} corresponds to carbonyl, disulfides, and ethers groups. SEM study demonstrated that the average size was from 200nm with interparticle distances. The crystalline nature of Fe NPs was confirmed from the X-ray diffraction peaks analysis. The intense diffraction peaks due to Fe NPs at 16.32, 22.56, 35.54, 41.08, 52.36, 61.42, 66.42, 78.1, and 85.08. Corresponding to the 110, 150, 200, 430, 550, and 950 facets of the face-centered cubic crystal structure conformed to the Joint Committee on Powder Diffraction Standards: 89-3722 of iron. Antimicrobial activity of Fe NPs against tested Gram-positive and negative bacterial strains showed significant inhibitory zones.

Conclusion: The inhibitory zones obtained in the present study reveal that the Fe NPs can act as a good antibacterial agent.

Keywords: Microbial synthesis, Nanoparticles characterization, Antibacterial activity, Zone inhibition, *Staphylococcus aureus*, *Salmonella typhi.*

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INTRODUCTION

These biological methods using micro-organisms have been proposed as an alternative, environmentally friendly method in the synthesis of metallic nanoparticles. Biologically synthesized nanoparticles have special applications in health and medical fields since chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface of nanoparticles [1]. The two modes through which the micro-organisms synthesize iron oxide nanoparticles are biologically induced and controlled mineralization mechanisms [2]. Bacteria possess the ability to reduce metal ions and are momentous candidates in nanoparticle preparation [3]. Attractive iron oxide nanoparticles are drawing in expanded consideration due to their fascinating properties that can be applied in an enormous number of uses, for example, catalysis and biomedicine [4]. *Staphylococcus aureus* is both a commensal bacterium and a human pathogen. Around 30% of the human populace is colonized with *S. aureus* [5]. It is driving reason for bacteremia and infective endocarditis, just as osteoarticular, skin and delicate tissue, pleuropulmonary, and gadget-related contaminations [6]. *Salmonella typhi* causes enteric fever (typhoid and paratyphoid fever) portrayed by high fever, stomach torments, cerebral pain, spewing, and looseness of the bowels [7]. Recently, it has been investigated that the resistance of various human pathogenic bacteria against many synthetic drugs is enhanced day by day [8]. Various studies have been carried out to ameliorate antimicrobial functions due to the growing microbial resistance toward common antiseptic and antibiotics. According to *in vitro* antimicrobial studies, the metallic nanoparticles effectively obstruct several microbial species [9]. The antimicrobial effectiveness of the metallic nanoparticles depends on two important parameters: (a) Material employed for the synthesis of the nanoparticles and (b) their particle size. Nowadays, antimicrobial drugs has been resistant to the pathogens, it becomes gradually increased considered hazardous to public health [10]. The most promising approach for abating or avoiding microbial drug resistance is the utilization of nanoparticles. Due to various mechanisms, metallic nanoparticles can preclude or overwhelm the multidrug-resistance and biofilm formation [11]. Iron nanoparticles (Fe NPs) of corn flakes-like morphology gave excellent antibacterial activity [12]. The present study was *Serratia marcescens* enzymatic reduction of Fe NPs synthesized by reduction of Fe ions. Ferric chloride is a precursor material. Nanoparticles are characterized by X-ray diffraction (XRD), scanning electron microscope (SEM), ultraviolet (UV)-visible absorption, and Fourier-transform infrared (FTIR) spectroscopy. The synthesized nanoparticles were investigated against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*S. typhi*). The results were represented that microbial enzymatic reduction of Fe NPs has displayed extremely higher antibacterial activity against bacterial strains tested here.

METHODS

Fresh bacteria *S. marcescens* was collected from the Department of Microbiology, Periyar University, Salem. All other chemicals used in the experiment were of AR grade and obtained from standard chemical sources.

Synthesis of Fe NPs

Fe NPs were synthesized using the procedure of uses an aqueous ferric chloride solution for the reduction of Fe ions. We used precursor was Ferric chloride. The Fe NPs were prepared by 200 mL of supernatant was added to 200 ml of 0.6 M aqueous ferric chloride solution for the reduction of Fe ions. The effects of temperature on the synthesis rate

and particle size and shape of the prepared Fe NPs were studied by carrying out of reaction. The study was carried out with 0.6 M Fe^{2}+. The Fe NP solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min, followed by redispersion of the pellet in deionized water.

Characterization techniques

The characterization of Fe NPs was carried out using UV-visible spectroscopy (SHIMADZU UV-1800) UV-visible spectrophotometer (Japan) at a range of 250–350 nm, FTIR spectra were done using a PerkinElmer spectrum 2000, and SEM analysis was done using 10 kV ultra-high-resolution SEM (FEI QUANTA-200 SEM).

Antibacterial study

The antibacterial activity of Fe NPs nanoparticles against *S. aureus* and *S. typhi.* was tested by the diameter of inhibition zone (DIZ). The zone of inhibition (ZOI) was measured by agar well-diffusion method. Nutrient broth/agar (peptic digest of animal tissue 5 g, sodium chloride 5 g, beef extract 1.5 g, yeast extract 1.5 g, and agar 15 g dissolved in 1 l of double distilled water) was used to cultivate bacteria. The media was autoclaved and cooled. Fresh nutrient broths were prepared, and each experiment was then performed. Antibacterial activity was measured using methods of disc diffusion plates on agar (Ronald, 1990). One hundred microliters of 24 h mature bacterial broth culture were inoculated and spread using sterile L-shaped glass rod into the nutrient agar (NA) plates. The plates were allowed to stand for 10–15 min to allow the culture to get absorbed in the medium. On each plate, a reference Fe NPs was also applied. The discs were made by cutting discs (10 mm) from a filter paper. Each disc was impregnated with the anticipated antimicrobial Fe NPs at different concentrations such as 400 µl, 450 µl, 500 µl, and 550 µl for using a micropipette. We used positive control as nanoparticle and negative control as distilled water. This was then placed on a plate of sensitivity testing NA; these were left to dry. These plates are kept at room temperature (28–30°C) for 24 h to allow maximum diffusion of the test materials to the surrounding media. The test materials having antibacterial properties inhibit bacterial growth in the media surrounding the disc and thereby yield a clear, distinct area defined as ZOI. The antibacterial activity of the test agent is then determined by measuring the diameters. The DIZ was measured and expressed in millimeters.

Table 1: Zone of inhibition of iron nanoparticles against *Staphylococcus aureus*

S. no	Organism	Zone of inhibition (mm)				
	Concentration (mg/ml)	Control	400 µl	450 µl	500 µl	550 µl
1.	*Staphylococcus aureus*	0 mm	3 mm	6 mm	8 mm	10 mm

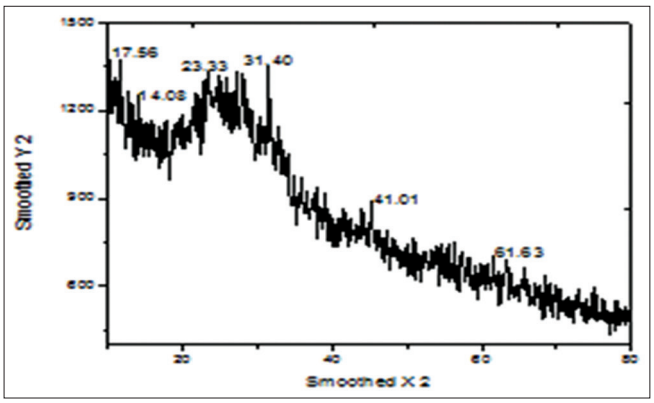

Fig. 1: X-ray diffraction pattern of iron nanoparticles

RESULTS AND DISCUSSION

The synthesized Fe NPs were confirmed by XRD data using Scherrer's equation. FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of ferric chloride to Fe NPs. SEM images provide morphology and size of the biosynthesized Fe NPs, and UV-visible absorption spectroscopy was confirmed the formation of nanoparticles. Fig. 1 shows the XRD pattern of the Fe NPs. The XRD pattern revealed that the synthesized Fe NPs, the intense diffraction peaks at 16.32, 22.56, 35.54, 41.08, 52.36, 61.42, 66.42, 78.1, and 85.08. Corresponding to the 110, 150, 200, 430, 550, and 950 facets of the face-centered cubic crystal structure. Fig. 2 shows FTIR spectrum exhibited a number of major peaks positioned at 3411.66, 1629.16, 1039.63, and 601.90 cm^{-1}. The peak at 3411.66 cm^{-1} developed for O-H stretching vibrations, at 1629.16 cm^{-1} corresponding to the vibration peak of carbonyl (C=O) observed, at 1039.63 cm^{-1} is corresponding to the C–O–C stretching vibration, at 601.90 cm^{-1} developed for disulfides stretch. These peaks confirmed the presence of Fe NPs. Fig. 3 shows that SEM study demonstrated that the average size was from 200 nm with interparticle distances. The observed Fe NPs were irregular in shape.

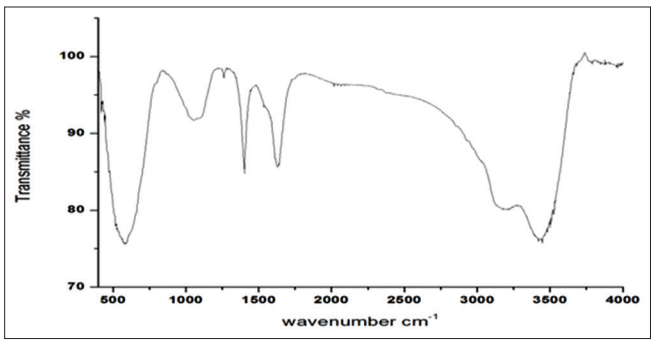

Fig. 2: Fourier-transform infrared studies of iron nanoparticles

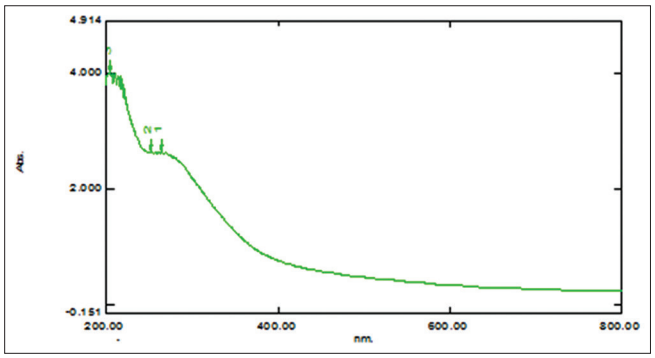

Fig. 3: Ultraviolet-visible spectra recorded of iron nanoparticles

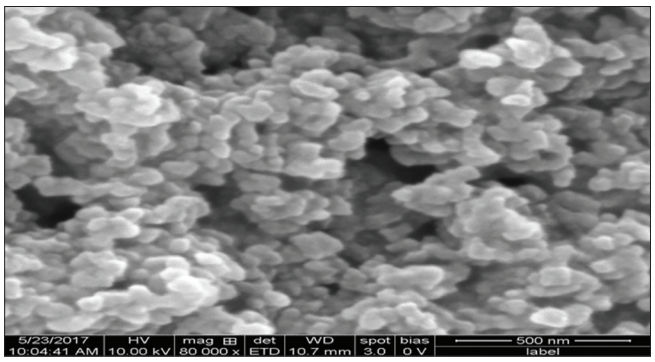

Fig. 4: Scanning electron microscope image of iron nanoparticles (500 nm views)

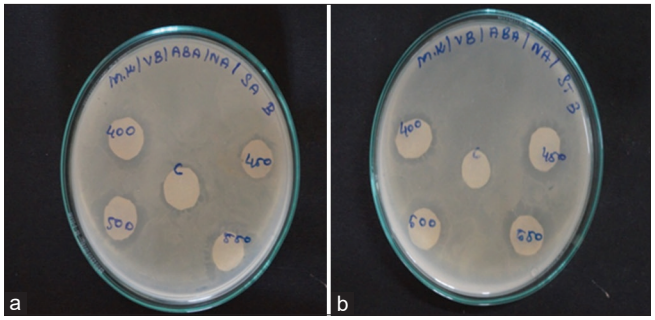

Fig. 5: (a and b) Zone of inhibition of iron nanoparticles against *Staphylococcus aureus* and *Salmonella typhi*

Table 2: Zone of inhibition of iron nanoparticles against *Salmonella typhi*

S. no	Organism	Zone of inhibition (mm)				
	Concentration (mg/ml)	Control	400 µl	450 µl	500 µl	550 µl
1	*Salmonella typi*	0 mm	5 mm	7 mm	9 mm	12 mm

Fig. 4 shows that the UV absorption of Fe NPs recorded from the reaction medium at room temperature using 50% cured enzyme extract with 6 Mm $Fecl_3$ exposure to 2 days reaction time is shown. The synthesized Fe NPs, was investigated against the human pathogens, *Staphylococcus aureus* and *Salmonella typhi* by well diffusion method (Fig. 5). The antibacterial activity of the synthesized Fe NPs, in 400, 450, 500 and 550 µL concentrations were quantitatively measured by the formation of zones of inhibition. The synthesized Fe NPs, was tested against the human pathogen, Staphylococcus aureus developed inhibitory zones measuring 3 mm, 6 mm, 8 mm and 10 mm after 24 h of treatment with 400, 450, 500 and 550 µL concentrations respectively (Table 1). For the Salmonella typhi developed inhibitory zones measuring 5mm, 7mm, 9mm and 12mm after 24 h of treatment with 400, 450, 500 and 550 µL concentrations respectively (Table 2).

CONCLUSION

This study showed that biologically synthesized Fe NPs in quantitatively assessed on the basis of ZOI showed inhibitory zones of 5 mm, 7 mm, 9 mm, and 12 mm for *S. typhi* at 400 µl, 450 µl, 500 µl, and 550 µl concentrations, respectively, and inhibitory zones of 3 mm, 6 mm, 8 mm, and 10 mm for *S. aureus* at 400 µl, 450 µl, 500 µl, and 550 µl, respectively. Fe NPs inversely proportional to their size, with smaller nanoparticles exhibiting more potent antimicrobial activity, were assessed against Gram-positive bacteria *S. aureus* as compared to Gram-negative bacteria *S. typhi* which can act as a good antibacterial agent.

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CONFLICTS OF INTEREST

I assured you do not have any conflicts of interest by authors.

AUTHORS CONTRIBUTION

Project administration, supervision, visualization, investigation, writing – review and editing, writing – original draft preparation, and methodology are done by corresponding and main author – MUNUSAMY C.

Data Curation, Resources, and Software are done by coauthor – CHANDERSAKER K.

Conceptualization and formal analysis are done by coauthor – PREMKUMAR M.

AUTHORS FUNDING

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