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Eco-friendly fabrication of iron nanoparticles using *Bauhinia purpurea* and Inhibition of *Staphylococcus aureus* biofilms

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Abstract

Hospital-acquired infections triggered by multidrug-resistant *Staphylococcus aureus* are a major clinical problem because of the bacterium's capacity to build biofilms on medical surfaces. The purpose of this work was to evaluate the antibiofilm activity of green-synthesised iron nanoparticles (FeNPs) produced from *Bauhinia purpurea* leaf extract. The FeNPs were studied by UV-Visible spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM), revealing an absorbance peak at 320–330 nm, a crystalline structure with an average size of 32 nm, and a spherical shape ranging from 100 to 200 nm. The antibiofilm efficiency was investigated using a crystal violet-based microtiter plate assay. The results revealed concentration-dependent inhibition, with a maximum of 92.74% biofilm inhibition at 500 µg/mL and 35.48% inhibition at the lowest dosage of 31.25 µg/mL. SEM imaging revealed the reduced bacterial adhesion and disturbed biofilm architecture on the treated surfaces. This study is unique in that it is the first to demonstrate *B. purpurea*-mediated FeNPs as effective, environmentally friendly antibiofilm agents with prospective implications in avoiding biofilm-associated infections on medical equipment.

Keywords *Bauhinia purpurea*, Biofilm inhibition, Iron nanoparticles, *Staphylococcus aureus*

Introduction

Since the discovery of multidrug-resistant bacterial pathogens in the mid-19th century, a battle has been waged between the scientific community and bacterial pathogens. There we several novel drugs used against fatal microbial infections, and eventually, pathogens overcame all the challenges [1]. Bacteria protect themselves against drugs through several physiological

mechanisms, of which bacterial biofilms play a crucial role [2]. Interestingly, bacterial biofilms were thought to enhance bacterial surface adhesion; however, recent studies suggest that biofilms create a microenvironment for bacteria under which they colonize and initiate infections. In other words, biofilms are the primary physical defense mechanism of bacterial communities. It may be thought that the destruction of bacterial biofilm makes them vulnerable to antibacterial drugs [3].

Bacterial infection caused by bacterial attachment and proliferation is one of the most prevalent and widespread complications associated with implanted medical devices such as stents, heart valves, vascular grafts, and catheters [4]. Upon adhering to implant surfaces, bacteria evade the host defenses and become more resistant to antibiotics by forming biofilms. It has been reported that low

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concentrations of antibiotics can enhance the growth of biofilms, which creates the worst scenario [5]. *S. aureus*, a Gram-positive bacterium, is the leading cause of biofilm-mediated, life-threatening infections, as biofilm formation significantly influences the efficacy of antimicrobial therapy and promotes the dissemination and persistence of infections [6]. It has become clear that the extensive global use of antibiotics in both human and veterinary medicine has led to bacteria developing resistance to a wide range of antibiotics, complicating the treatment of infections [7]. Multidrug resistance (MDR) has become increasingly common, making the treatment of *S. aureus* infections more challenging and posing an escalating risk to the medical community [8].

Chronic infection usually cannot be eradicated unless the implant is removed after several treatments, and this procedure places a heavy burden on patients and medical officials [9]. Developing novel methods to treat biofilm-associated *S. aureus* infections is imperative due to their increasing incidence. The field of nanotechnology provides novel approaches to tackle *S. aureus* biofilm-associated infections. The anti-biofilm activities of nanoparticles are attributed to their enhanced interaction and penetrative capabilities within the biofilm matrix compared to existing free drug molecules [10]. Nanoparticle-based techniques have shown great promise in fighting bacterial biofilms because of their capacity to interfere with microbial structures and disrupt biofilm growth pathways. CdTe-TiO₂ nanocomposites produce reactive oxygen species (ROS) that disrupt bacterial membrane integrity and prevent biofilm formation, especially when activated by visible light [11].

Similarly, hierarchical Au@ZnO nanostructures have increased antibacterial and antibiofilm actions by combining the characteristics of gold and zinc oxide, allowing for deeper biofilm invasion and oxidative damage [12]. Among all types of nanoparticles, iron nanoparticles (FeNPs) are popularised particles as they are nontoxic and readily form oxides [13]. Fe nanoparticles possess a high surface area and excellent dimensional stability. Their physicochemical properties strongly impact the biological functioning of green-synthesized FeNPs. The interaction of nanoparticles with bacterial cells and biofilms is determined by key characteristics such as particle dimension, shape, aggregation state, solubility rate, crystal arrangement, chemical content, surface morphological and surface charge. These characteristics have a direct impact on their capacity to adhere to biofilm matrices, invade microbial populations, produce reactive oxygen species (ROS), and alter bacterial signaling pathways like quorum sensing [14, 15].

Similarly, crystal arrangement and surface morphology impact the production of reactive oxygen species as well as nanoparticle-biomolecule interactions, all of which

are critical for antibacterial effectiveness. The SEM and XRD investigations indicated that the green-synthesized FeNPs had irregular spherical forms and a crystalline structure. These properties most likely led to their potent antibiofilm efficacy against *Staphylococcus aureus*. In addition, surface changes by phytochemicals from *B. purpurea* may have improved FeNP stability and biological activity by capping, reducing accumulation, and regulating interactions at the biological interface. Thus, knowing and managing these nanoparticle properties is crucial for enhancing therapeutic outcomes while avoiding potential toxicity or unexpected consequences [16].

Several fabrication methods have been employed to prepare Fe nanoparticles; however, green synthesis is a simple, low-cost, effective, and eco-friendly method for producing metallic nanoparticles, which utilizes a reducing agent derived from natural product extracts [17]. The primary objective of the present study is to investigate the inhibition of biofilm formation by *S. aureus*, the primary organism in orthopedic implant infections, using Iron nanoparticles synthesized from *B. purpurea*. These nanoparticles are then characterized by UV-vis spectrophotometry, Scanning Electron Microscopy (SEM), and XRD analysis. Furthermore, the surface adherence activity of bio-fabricated nanoparticles on polyvinyl chloride is also analyzed, which can benefit several biomedical applications.

Materials and methods

Collection of samples and extraction

The fresh leaves of *B. purpurea* were collected from and around the Thrissur district in Kerala, India, in December 2023. The leaves were thoroughly washed and dried in the shade at room temperature for 7 days, then ground into a fine powder. The powdered material was stored in tightly closed glass bottles for future research. 10 g of this fine powder was transferred into 250 mL beakers containing 100 mL of distilled water and boiled at 80 °C for 20 min. The mixture was then cooled, centrifuged at 12,000 rpm for 20 min, and filtered to remove debris. The filtrate was used for further analysis. According to the methodology suggested by [10], this method is suitable for preparing an extract to synthesize iron nanoparticles.

Biosynthesis of fenps by the bioactive fraction of *Bauhinia purpurea*

The iron nanoparticles were synthesized using the bioactive fraction of *B. purpurea* extract with slight modifications of the following method [18]. A 10 mL bioactive solution was added to 90 mL of a 2.5 mM aqueous Iron(III) chloride solution. This solution was maintained in a water bath at 60 °C and allowed to run for 4 h for the biosynthesis of Iron nanoparticles. The change in color from pale yellow to black indicated the formation of

nanoparticles. Hence, three sets of centrifugations separated particles at 16,000 rpm for 20 min each. Further, the dried sample was coined as BP-FeNps and proceeded for further characterization. In the present study, we characterized nanoparticles using UV-visible spectrometry, XRD analysis, and scanning electron microscopy, which are considered the standard methods for determining the structural properties of nanoparticles.

Analysis of biofilm inhibition by nanoparticles

The antibiofilm property of the nanoparticles was studied in the ubiquitous bacterial strain *S. aureus* (MTCC No. 902), purchased from MTCC, Chandigarh, India. Nutrient broth, Gentamicin antibiotic solution, Crystal violet solution, 1X PBS, and Sodium acetate were purchased from Himedia, India. Sterile 96-well plates were purchased from Tarsan, India. Conical flasks were from Borosil, India. Spirit lamp, double-distilled water, and test samples. The antibiofilm potential of the produced FeNPs was tested against *Staphylococcus aureus* (MTCC No. 902) utilizing conventional methods. The bacterial strain was grown in nutritional broth at 37 °C and subjected to different doses of FeNP. Nanoparticles affect bacterial attachment, EPS production, and quorum sensing pathways, all of which are necessary for biofilm formation and structural stability.

Recent research has shown that nanoparticles such as FeNPs function by causing oxidative stress, changing membrane integrity, and permeating the biofilm matrix, resulting in a strong antibiofilm effect [19]. In our investigation, FeNPs produced with *B. purpurea* leaf extract inhibited biofilm development in a concentration-dependent manner, corresponding with the previously reported antibacterial characteristics of biosynthesized nanoparticles. The maximum inhibition (92.74%) was seen at 500 µg/mL, demonstrating a substantial interaction between the FeNPs and biofilm components, possibly assisted by phytochemical capping agents. These findings are consistent with the better antibiofilm effectiveness shown in biogenic nanoparticles due to their increased surface reactivity and capacity to interfere with bacterial colonization at early phases.

Microtitre plate assay (MTP) for biofilm inhibition

The microtiter plate assay method investigated the efficacy of inhibiting biofilm using 96-well flat-bottom polystyrene titer plates as a surface for bacterial adhesion [3]. Each well was supplied with 180 µL of BHI broth and inoculated with 10 µL of an overnight culture of *S. aureus*. A 10 µL nanoparticle solution was added from the prepared stock solution, which ranged from 250, 125, 62.5, and 31.25 µg/mL, along with a control (without the test sample). The setup was incubated at 37 °C for 24 h. After the incubation period, the content in the wells was

removed and washed with 0.2 mL of phosphate-buffered saline (PBS) at pH 7.2 to remove free-floating bacteria. The adherence of bacteria was fixed with sodium acetate (2%) and stained with crystal violet (0.1%, w/v). Excessive stain was removed by deionized water wash and kept for drying.

Furthermore, the dried plates were washed with 95% ethanol, and the optical density was determined using a microplate reader (Thermo) at 600 nm. The percentage of biofilm inhibition was determined by comparing the absorbance values of treated and untreated wells. This commonly employed test for measuring nanoparticle-mediated antibiofilm action offers a straightforward and reproducible method of quantifying biofilm suppression [20]. The percentage of biofilm inhibition was calculated using the formula below, and the IC50 value was also determined from the graph.

$$\% \text{ Biofilm inhibition} = (\text{Control OD} - \text{Test OD}) / \text{Control OD} \times 100$$

Surface morphology analysis of biofilm Inhibition by electron microscopy

The surface morphology of biofilm inhibition was analyzed using linear low-density polyethylene (LLDPE) as a surface, modifying the method used by Dey et al. (2020). Freshly cultured *S. aureus* cells were incubated with sterile plastic films of approximately 2 cm² in the nutrient broth medium for 24 h at 37 °C in a 150 RPM shaking condition. The addition of nanoparticles enhanced the test setup. The control was made by adding the same amount of distilled water. After the incubation period, the samples were subjected to scanning electron microscopy to determine the surface morphology of biofilm inhibition [21].

Results

Synthesis and characterization of BP-FeNPs

The bio-fabrication of iron nanoparticles was characterized by a color change from yellowish-brown to greenish-black, resulting from the reduction of FeCl₃ solution to Fe³⁺ ions, which facilitated the formation of iron nanoparticles. The UV-spectrophotometric analysis revealed a sharp peak in the range of 300 to 350 nm, attributed to light scattering, which indicates the formation of nanoparticles in the solution (Fig. 1). The XRD pattern, shown in Fig. 2, indicates the formation of iron nanoparticles. The experimentally obtained diffraction angle was 34.6 (2θ in degrees), whereas the standard diffraction angle was 35.2 (2θ in degrees). The biosynthesis process consists of four stages: collecting and extracting *B. purpurea* leaves, preparing an iron precursor solution (FeCl₃), mixing the plant extract with the FeCl₃ solution at 60 °C for nanoparticle formation (a color transition), and purifying the FeNPs through centrifugation and

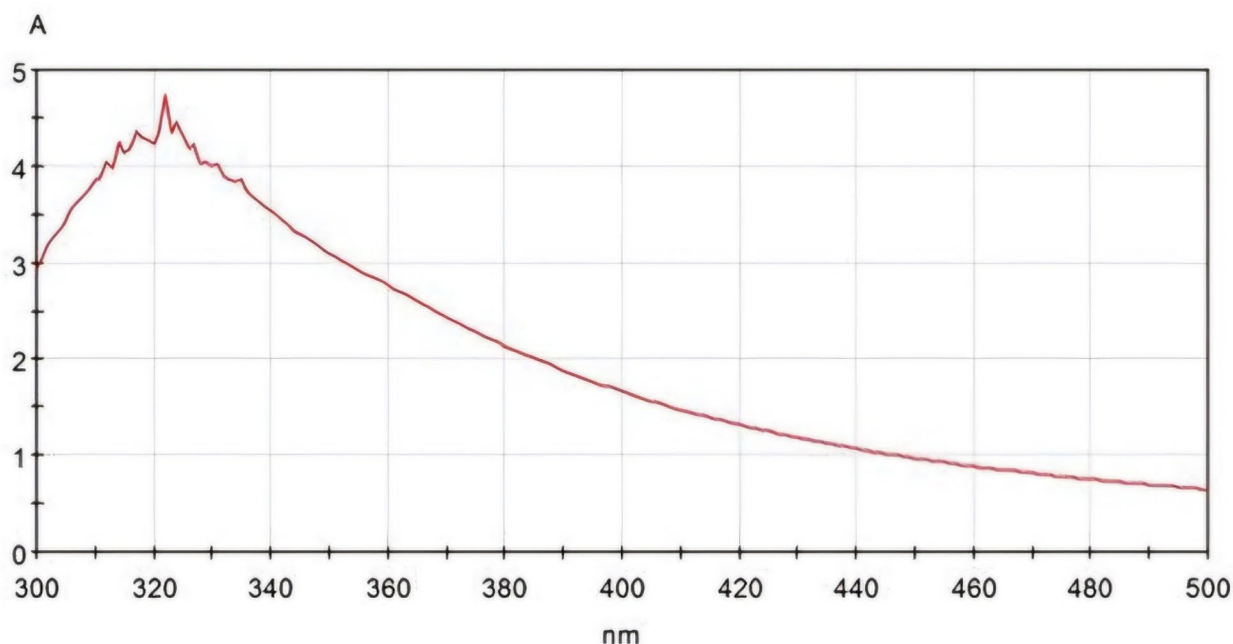


Fig. 1 Spectrophotometric peak showing the synthesis of FeNPs

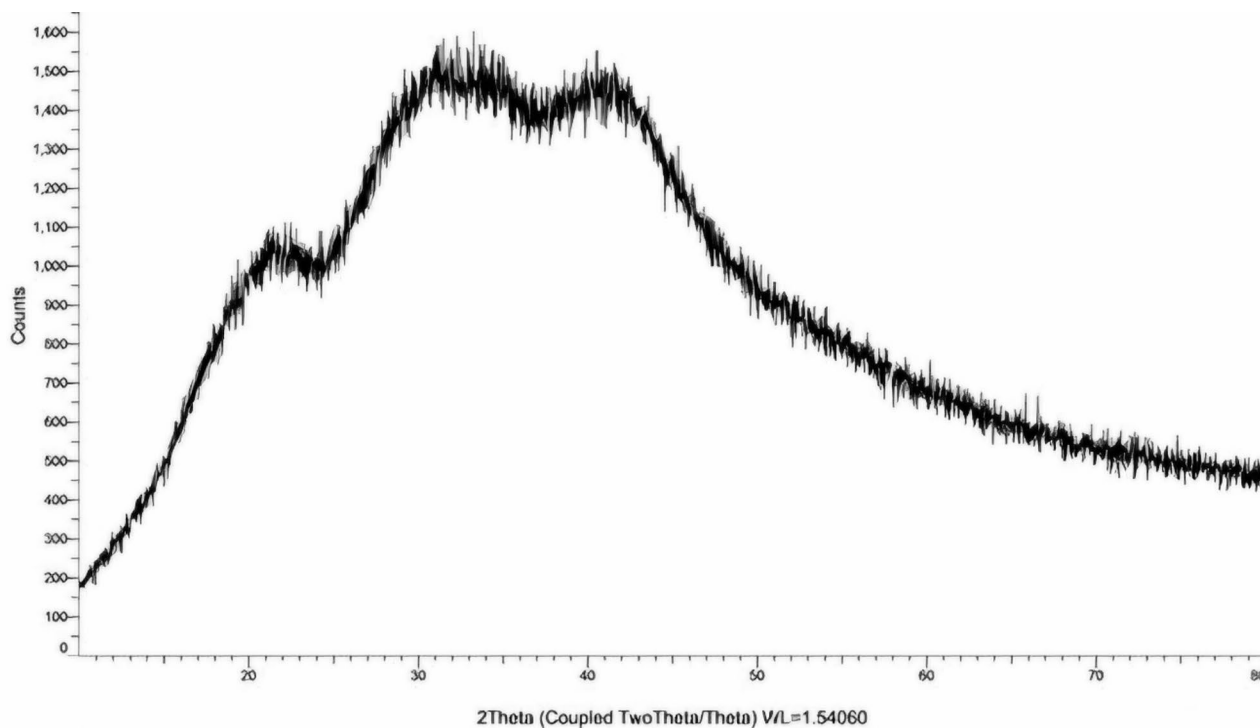


Fig. 2 XRD pattern of BP-FeNPs

drying for further characterizing. This approach is environmentally safe, avoids hazardous chemicals, and uses bioactive plant-based constituents for both reduction and encapsulation of nanoparticles [22].

The synthesis of iron nanoparticles (FeNPs) was confirmed using UV-visible spectroscopy. FeNPs exhibit a significant absorbance peak at around 320–330 nm, which corresponds to their surface plasmon resonance (SPR). The peak shows that the *B. purpurea* extract's

phytochemicals effectively reduce Fe^{3+} ions, resulting in stable FeNPs. The broad nature of the peak indicates minor polydispersity and the existence of bioorganic chemicals on the nanoparticle surface, which is consistent with phytochemical encapsulation and stabilization. These findings are consistent with previous studies on green-synthesized FeNPs.

The observed peaks were compared with the standard powder diffraction card, and the results agreed with the XRD standard patterns of FeNPs. The average crystalline size was calculated using the Debye-Scherrer equation, which yields a value of 32 nm for the peak. SEM images were obtained for the iron nanoparticles, as shown in Fig. 3. The SEM micrograph revealed that the synthesized iron nanoparticles were aggregated into irregular spherical shapes, ranging from 100 to 200 nm, with an inter-particle distance. The larger size of the particle is due to the capping agent binding the FeNPs.

The substantially larger particle size (100–200 nm) observed in SEM is attributed to the presence of bioactive phytochemicals in *B. purpurea*, which serve as encapsulating and stabilizing agents.

These phytochemicals, such as flavonoids, tannins, and phenolics, adhere to the nanoparticle surface, limiting aggregation while simultaneously facilitating particle size expansion caused by surface passivation. This bio-capping effect is consistent with recent observations on green-synthesized FeNPs, where the organic layer produced by plant-derived substances leads to a greater hydrodynamic diameter than their uncapped or chemically fabricated counterparts.

Spectrophotometric analysis of biofilm Inhibition

The capability of BP-FENPs in inhibiting bacterial biofilm formation was studied calorimetrically. The results suggested a decrease in biofilm formation when the concentration of nanoparticles was increased in the solution (Fig. 3). The maximum inhibition was observed at the highest tested concentration of 500 $\mu\text{g/mL}$, with 92.74% inhibition. Even the minimum tested concentration of 31.25 $\mu\text{g/mL}$ demonstrated adequate biofilm inhibition,

reducing it by 35.48% compared to the untreated control sample. It was also apparent that the concentration of nanoparticles remained in direct proportion to biofilm inhibition.

The antibiofilm activity of BP-FeNPs was tested using a microtiter plate assay with crystal violet staining. A concentration-dependent inhibitory pattern was identified, with rising nanoparticle concentrations causing a significant decrease in biofilm biomass. At the highest level tested (500 $\mu\text{g/mL}$), BP-FeNPs obtained 92.74% inhibition, while the lowest dosage (31.25 $\mu\text{g/mL}$) only demonstrated 35.48% inhibition.

These findings indicate that BP-FeNPs effectively inhibit the development and maturation of biofilms by *S. aureus*, most likely by interfering with surface adherence and extracellular polymeric substance (EPS).

Surface morphology analysis of biofilm Inhibition

Scanning electron micrography revealed the surface morphology of bacterial biofilm inhibition (Fig. 4). It was observed that the surface of the untreated sample consisted of invaginations and grooves due to bacterial biofilm and degradation, whereas the treated sample was smooth, indicating the absence of bacterial-mediated activities. Therefore, it was evident that in the presence of the extract, bacteria cannot attach themselves to a surface, whereas it is the contrary observation in the absence of an antagonist (Fig. 5).

Discussion

Bacterial biofilm may be considered the biostructure by which bacteria attach to a host surface and initiate colonization. Biofilm formation typically begins with a reversible attachment, followed by a permanent attachment supported by adhesive structures, primarily exopolysaccharides (EPS) [23]. The planktonic bacterial cells undergo a series of attachment and detachment events to a new surface, resulting in increased cAMP levels within the cells through the surface sensing system, which in turn triggers permanent attachment [24]. The study suggested that there is a considerable decrease in

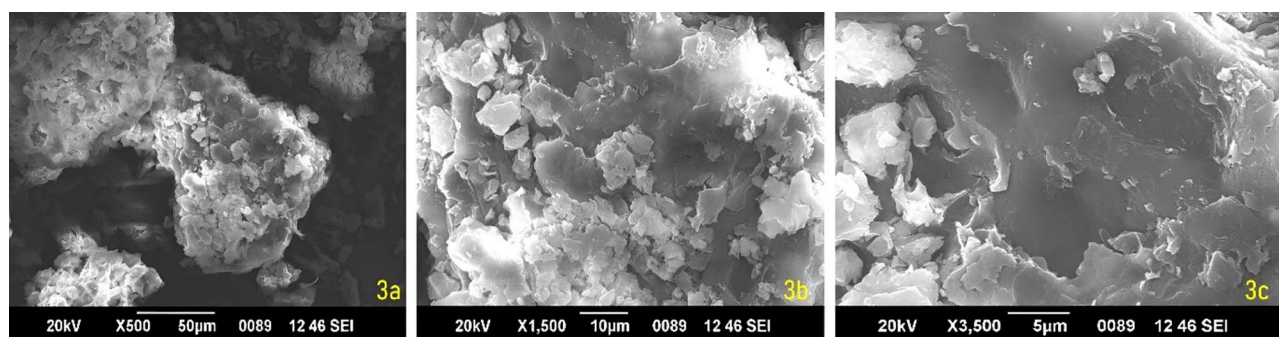


Fig. 3 FeNPs 3a: 500x, 3b 1500 x and 3c 3500 x

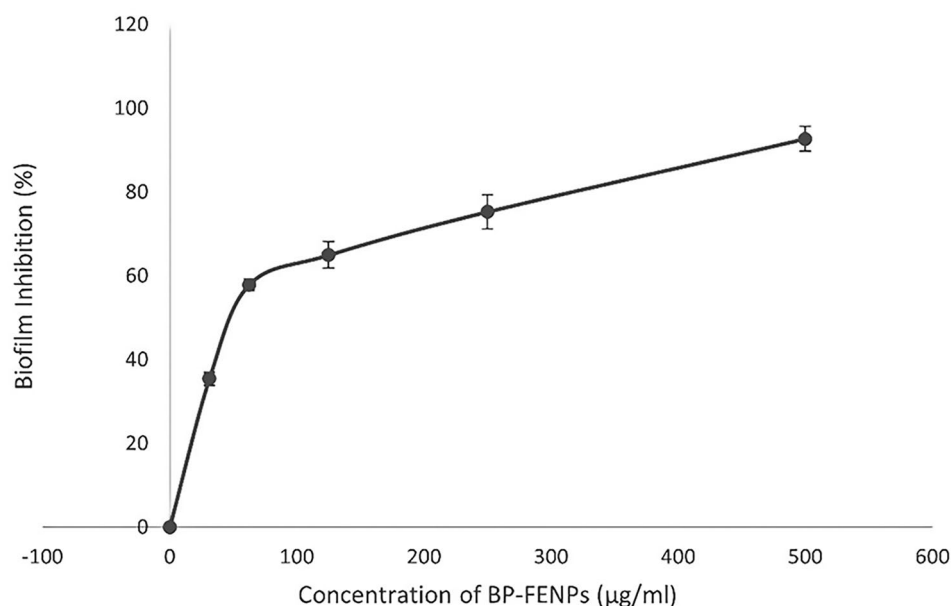


Fig. 4 Effect of nanoparticles on inhibition of biofilm

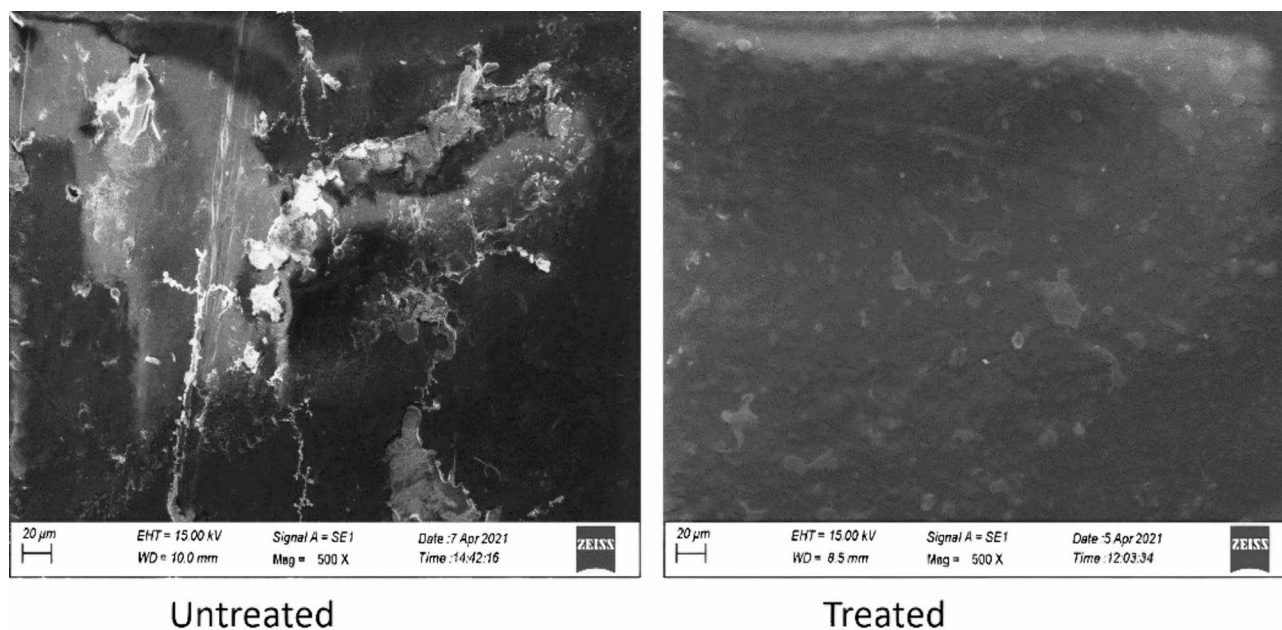


Fig. 5 Surface morphology analysis of biofilm inhibition

the formation of bacterial biofilm when treated with iron nanoparticles. It may be due to several factors, such as interference with the quorum-sensing system, modulation with the second nucleotide messenger signaling, interference with biofilm maturation, or the direct disruption of mature biofilms [25].

The fluorescence assay revealed the presence of loosely arranged biofilms in the treated sample, as compared to the untreated sample, suggesting biofilm formation.

However, the nanoparticle is degrading as formed, which is considered the fastest and simplest mechanism of biofilm inhibition. From this data, it can be assumed that the bacteria can synthesize a biofilm in the presence of an antagonist; however, the degradation of this biofilm is dose-dependent. This assumption can be supported by the graph obtained (Fig. 4), which suggests that biofilm degradation is directly proportional to the concentration of nanoparticles.

A similar observation was made by Majumdar and Misra [26], who suggested that the small size of nanoparticles enables them to pass through the matrix and cause significant damage to the membrane's integrity, thereby facilitating their attachment. It has also been proven that Iron oxide nanoparticle-mediated hyperthermia stimulates dispersal in bacterial biofilms and enhances the efficacy of antibiotics [27]. The major advantage of nano drugs when compared to conventional drugs is their ability to react against a microbe, which is due to their large cumulative surface area, which provides more contact with microbes than conventional drugs as they penetrate more and attack microbes by protein function inhibition, DNA damage, translation disturbance, and transcription dysregulation [28].

The plant that was used for the synthesis of iron nanoparticles was *B. purpurea*. *B. purpurea* (Leguminosae) is a medium-sized tree native to the Indian sub-continent, and it is used in traditional medicine to treat bacterial infections, fungal infections, rheumatism, convulsions, delirium, septicemia, dropsy, and pain [29]. The plant is also reported for its constituents, including flavone glycosides, foliar flavonoids, 6-butyl-3-hydroxy-flavanone, amino acids, phenyl fatty ester, luteolin, and β -sitosterol [30]. It may be implicit that the reducing agents present in the extract play an integral part in the bio-fabrication of nanoparticles. According to the surface morphology analysis conducted in this study, it is evident that the nanoparticle possesses the ability to inhibit bacterial attachment on Linear Low-Density Polyethylene (LLDPE). The microscopic analysis suggests evidence of bacterial attachment on the surface in the absence of BP-FNPs and an intact surface in its presence. This observation suggests the potential application of these nanoparticles in medical devices to protect them against in vivo bacterial colonization.

S. aureus biofilm development is a multistage process that includes surface adherence, microcolony creation, EPS production, and gene-regulated development. FeNPs derived from *B. purpurea* disrupt this process via numerous methods. First, their nanoscale size and surface activity allow for more penetration into biofilms, causing structural disruption. Second, ROS production and surface charge interactions cause membrane instability and bacterial mortality. Furthermore, FeNPs have been shown to influence gene expression related to biofilm control, including *icaA*, *icaD*, *sarA*, and *agr*, which govern EPS generation and quorum sensing [31]. Our findings are consistent with earlier research suggesting that phytochemical-capped nanoparticles can disrupt quorum sensing systems, diminish polysaccharide intercellular adhesin (PIA) synthesis, and inhibit biofilm-associated genes. This combined action—physical disruption and genetic suppression—increases the antibiofilm capability

of green nanomaterials. These findings lend support to the use of plant-mediated FeNPs in biomedical devices to avoid persistent implant-related infections.

Conclusion

The study reveals that bio-fabricated iron nanoparticles (FeNPs) generated with *B. purpurea* possess significant potential for preventing biofilm formation by *S. aureus*. The study found that increasing the concentration of these nanoparticles led to a greater reduction in biofilm formation, with the highest concentration preventing more than 92% of biofilm formation. UV-visible spectroscopy, XRD, and SEM were used to validate the successful production and shape of the nanoparticles. Nanoparticles damage bacterial biofilm by interference with systems such as quorum sensing, biofilm maturation, and bacterial adhesion to surfaces. This suggests that FeNPs can be utilized in medical settings to prevent biofilm-associated illnesses, particularly for implants and devices that are susceptible to bacterial colonization.

Author contributions

AE: Conceptualization, Investigation, Writing original draft. JJ: Investigation, Supervision, Visualization. KV: Investigation, Project administration, Validation, Supervision.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval

There is no ethical standard related to the present article.

Research involving human participants and/or animals

This study did not involve animal studies.

Informed consent

All authors have read and approved the manuscript, and all are aware of its submission to your esteemed journal. The authors declare that they have no conflicts of interest to disclose. This manuscript has not been published and is not under consideration for publication elsewhere.

Competing interests

The authors declare no competing interests.

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