Antibacterial and Dye Degradation Activity of Green Synthesized Iron Nanoparticles

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Nanoparticles have a wide range of applications in various fields such as cosmetics, pharmaceuticals, and agrochemicals. Synthesis of nanoparticles using plant extract is a very efficient, cost-effective, useful, and environmentally friendly method. A plant extract acts as a reducing agent in the formation of nanoparticles. Catharanthus roseus is one of the potential plants for biosynthesis of nanoparticles due to its easy availability. In the present study, the Catharanthus roseus plant extract was used to synthesize iron nanoparticles. UV-vis spectroscopy, DLS, and FTIR were performed for characterization of synthesized nanoparticles. Further antibacterial and dye degrading properties of synthesized iron nanoparticles have been investigated. It was found that Catharanthus roseus-synthesized iron nanoparticles showed antibacterial activity against E. coli and dye degradation activity against methyl orange dye.

1. Introduction

Nanotechnology has significant applications in various fields including agriculture, medicine, energy, climate, chemistry, heavy industry, and consumer goods [1–5]. They have a high surface area to volume ratio, which is the most important aspect that explains why these nanomaterials are so commonly used [6, 7]. Various approaches, including physical and chemical methods, have been used to synthesize NPs [8, 9]. Physical and chemical methods of synthesis possess certain drawbacks such as high energy consumption and toxic chemical utilization [4]. Most studies have used sodium borohydride as a reducing agent to reduce ferrous (Fe (II)) or ferric (Fe (III)) salts. Nanoparticles are easily synthesized using NaBH₄, but it is potentially hazardous for environment [10]. Therefore, an alternative method for synthesis is in high demand. A green method for synthesis of iron nanoparticles (FeNPs) uses biological entities such as fungi, bacteria, and plants which have been utilized in the present time. Plant-mediated approaches are straightforward, cost-effective, and environmentally sustainable [6]. A plant extract contains bioactive compounds such as polyphenols, alkaloids, steroids, flavonoids, and terpenoids [11–13] which act as both reducing and stabilizing agents at the same time. FeNPs have been synthesized using a number of plants, including Peltophorum pterocarpum, Eriobotrya japonica, Mentha piperita, and Morinda oleifera [14–17].

Catharanthus roseus commonly known as graveyard vine, bright eyes, Madagascar periwinkle, cape periwinkle, etc. is a flowering plant that belongs to the Apocynaceae family. Pentamerous flowers are present with different colors such as pink, purple, white, peach, scarlet, and red. It is an important medicinal plant which is native to Madagascar but cultivated in various parts of the world [18]. It contains various bioactive compounds such as vinblastine, vincristine, reserpine, and vinpocetine which possess several
pharmaceutical activities such as antibacterial, antioxidant, antidiabetic, antifungal, and anticancer properties [19]. *Catharanthus roseus* has been utilized for synthesis of various nanoparticles which includes silver, gold, copper oxide, iron, etc. Iron nanoparticles have been shown to have various environmental remediation potentials. Water and wastewater toxins are currently being remedied using nanoscale materials such as nanoadsorbsents, nanofiltration, nanocatalysts, and nanobiocides such as metal nanoparticles. Iron nanoparticles are one of these metallic nanoparticles with promising environmental pollution-fighting properties [20]. Katatab-Seru et al. [16] reported antibacterial activity of biosynthesized FeNPs. The aim of this study is to synthesize iron nanoparticles from *C. roseus*, and further FeNP formation was confirmed using different characterization methods such as UV-visible spectroscopy, FTIR, and DLS. Bactericidal activity and dye degradation activity of synthesized FeNPs were also evaluated.

2. Materials and Methods

Nutrient broth (NB), nutrient agar (NA), streptomycin disc, ferrous sulphate salt, and methyl orange were bought from Fisher Scientific.

2.1. Plant Extract Preparation. Leaves of *Catharanthus roseus* were collected from Sharda University. Then, leaves were washed with detergent and then with distilled water 2-3 times to remove detergent from its surface. After washing properly, leaves were dried in an oven at 60°C for 48 hrs. Dried leaves were ground to powdered form. Then, 1 g of plant extract powder was added into 50 ml of distilled water (DW) and boiled for 20 min. Then, it was filtered with Whatman no. 1 filter paper and stored at 4°C for further use.

2.2. Optimization of Synthesis of FeSO₄ and Their Characterization. To synthesize FeNPs, ferrous sulphate salt (FeSO₄) was used. To make a 150 mM ferrous sulphate solution, 4.3 g ferrous sulphate was mixed with 100 ml distilled water. The plant extract was added to the ferrous sulphate in 1:9, and 1 ml of the plant extract and 9 ml of the ferrous sulphate are mixed for the synthesis of copper nanoparticles. Different concentrations of FeSO₄ (1, 10, 50, and 100 mM) and plant extract (1, 2, 3, 4, and 5 ml) were used for nanoparticle synthesis. A UV-vis spectrophotometer was used to detect the reduction of Fe ions into FeNPs, which resulted in a yellow color formation. Iron nanoparticles were detected in the wavelength range of 200-300 nm. Centrifugation at 4500 rpm for 15 minutes purified the nanoparticles. FTIR was used to determine iron ion reduction and capping of nanoparticles. The size and size distribution of molecules were measured using DLS.

2.3. Antibacterial Activity. Synthesized copper nanoparticles were evaluated against *E. coli*. 200 ml of NA and NB was prepared. 100 ml of NB was used for the growth of the bacteria. The disk diffusion method was used to observe antibacterial activity. The culture was then spread uniformly on agar plates with the help of a swab. Various concentrations of FeNPs were added on a sterile disk using a pipette. A streptomycin-loaded disk was used as the positive control and disk without NPs used as the negative control. Plates were then incubated at 37°C overnight for 48 hrs, and the zone of inhibition was observed.

2.4. Dye Degradation Activity. The photocatalytic dye degradation potential of FeNPs was evaluated for methyl orange. In 3 ml of DW, 1 ml of 1 mM concentration dye and 1 ml of FeNPs were mixed to make the total volume up to 5 ml. Then, the solution was kept in sunlight. After this, a UV-vis spectrophotometer was used to measure the change in intensity of color in the range 200-800 nm.

3. Results and Discussion

There are several bioactive compounds present in the plant extract. These compounds react with FeSO₄ to synthesize FeNPs. The color of the FeSO₄ solution was bluish green before adding the plant extract. There was a change in color from bluish green to yellow (Figure 1). This suggests that the plant extract aids in the formation of nanoparticles. Akhbari et al. [15] reported that there was some visible change in the reaction mixture that contains the iron sulfate salt and plant extract of *Mentha piperita* from yellow to dark brown which shows synthesis of FeNPs.

3.1. Effect of Different Concentrations of Ferrous Sulfate. FeNP formation was detected using UV-vis analysis. Change in color was observed due to the reduction of iron ions from ferrous sulphate to FeNPs. FeNP peak was observed at around 240 nm. Saini et al. [21] reported absorbance peak of FeNPs at around 210-260 nm region. Ebrahiminezhad et al. [22] reported that UV-vis spectroscopy of biosynthesized FeNPs nanoparticles from *Urtica dioica* was in the range of 216-265 nm. Akhbari et al. [15] reported that at wavelengths around 216 and 284 nm, two different absorption peaks of FeNPs were observed.

Various concentrations of ferrous sulphate were investigated for optimum synthesis of FeNPs. As the concentration of ferrous sulphate rises, there was an increase in FeNP synthesis. For all the concentrations, peak was about at 230 nm (Figure 2). The rate of nanoparticle synthesis was determined by ferrous sulphate concentration. At higher concentrations, particle size grows larger, and spectrum strength grows as well.

Effect of different plant extract concentrations (1-5 ml) on the synthesis of FeNPs was also investigated. UV-vis
spectra showed that maximum nanoparticle synthesis was obtained in the case of 5 ml of *C. roseus* plant extract solution (Figure 3). As a result, this confirms that as the concentration of the plant extract increased, nanoparticles synthesis also increased. Vitta et al. [23] reported synthesis of iron nanoparticles from *Eucalyptus robusta*, and they found that 1:1 proportion of salt concentration to the leaf extract was optimum for synthesis of nanoparticles.

3.2. FTIR Analysis. The presence of different functional groups in the synthesized FeNPs was identified by using FT-IR spectra (Figure 4). At a range of 4000–650 cm\(^{-1}\),
measurement was made with a resolution of 4 cm\(^{-1}\). FTIR spectra showed three major peaks in the range of 800–1200 cm\(^{-1}\) (Table 1). Shahwan et al. [24] reported that different functional groups were responsible for FeNP synthesis. Wei et al. [25] reported the presence of peak at 3292.84, 2927.78, 1638.55, 1350.99, and 1026.60 cm\(^{-1}\) in the case of iron nanoparticles synthesized using the peel of Citrus maxima. Akhbari et al. [15] confirmed the presence of some functional groups which shows that the extracts contain aliphatic amines, phenols, and organic acids that could serve as stabilizing and reducing agents during FeNP synthesis. Karpaga vinayagam and Vedhi [26] reported the presence of peak at 3422, 2923, 2853, 1630, 618, and 467 cm\(^{-1}\) in the case of iron nanoparticles synthesized using the flower extract of Avicennia marina. Dash et al. [17] reported the presence of peak at 3700, 3400, 2918, 2360, 1000, 800, 717, 623, 557, and 443 cm\(^{-1}\) in the case of iron nanoparticles synthesized using Peltophorum pterocarpum.

### Table 1: FT-IR analysis of iron nanoparticles.

<table>
<thead>
<tr>
<th>Vibrational peak (cm(^{-1}))</th>
<th>Vibrational frequencies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1115 cm(^{-1})</td>
<td>C=O indicates the presence of aldehydes and ketones which means the occurrence of phenolic acid and terpenoid</td>
<td>[25]</td>
</tr>
<tr>
<td>1135 cm(^{-1})</td>
<td>C=O indicates the presence of aldehydes and ketones which means the occurrence of phenolic acid and terpenoid</td>
<td>[25]</td>
</tr>
<tr>
<td>992 cm(^{-1})</td>
<td>C-N indicated the presence of phenols and aliphatic amines</td>
<td>[22]</td>
</tr>
</tbody>
</table>

Figure 5: DLS analysis of FeNPs.

3.3. DLS Analysis. The most popular application of DLS is to examine nanoparticles. The technique is suitable for particles in submicron, but it can also be used for calculating the particles smaller than nanometer. The spontaneous variations in the strength of light dispersed from any solution or any suspension may be used to calculate particle size. In this study, zeta potential was found to be 7.75 mV, with a zeta deviation of 5.77 mV as shown in (Figure 5). The polydispersity index of FeNPs is 0.411. Green synthesized FeNPs had an average particle size distribution of 290.1 nm. Since biomolecules or a layer of water covering the NP’s surface is included in the calculation, FeNPs have a larger scale.

3.4. Antibacterial Activity. Antibacterial activity of FeNPs synthesized from Catharanthus roseus was tested against a gram-negative bacterial culture of E. coli. Results showed the potential antibacterial activity of these FeNPs against E. coli after 24 hours of incubation. It was observed that the zone of inhibition increases with the increase in amount of FeNPs (Figure 6). The minimum zone of inhibition was found in 10 microliters of FeNP solution, and 30 microliters of solution shows the maximum zone of inhibition. The zone of 30 µl of NP was the maximum with 15 mm ± 0.5 of diameter followed by 20 µl with 12 mm ± 0.5 and then 10 µl with 9 mm ± 1, and the least was of streptomycin with 11 mm ± 0.1 of diameter. Senthil and Ramesh [27] reported the antibacterial activity of Tridax procumbens-synthesized iron oxide against the gram-negative bacteria. Dash et al. [17] reported the antibacterial activity of magnetite nanoparticle synthesis using the pod extract of Peltophorum pterocarpum against E. coli. A maximum of 16 mm and 20 mm of the zone of inhibition was obtained for S. epidermis and E. coli, respectively.

3.5. Dye Degradation Activity. One of the efficient applications of FeNPs is dye degradation. Methyl orange (MO) is a harmful and toxic dye, which is found in general food...
and textile industries; it is carcinogenic. Efficiency of the synthesized FeNPs for photocatalytic degradation was examined using methyl orange dye. UV-vis spectra of MO in water generally appear at 500 nm. Within 2 hours, degradation of dye using nanoparticles was observed at uniform time intervals. Dye absorbance declines with time, degradation process reduces as time passes, and this may be due to the binding of degraded materials to the nanoparticle surface (Figure 7). Maximum 50% degradation of dye has been observed, and complete degradation of dye was not observed. Bishnoi et al. [28] reported methylene blue degradation using Cynometra rami flora-derived iron nanoparticles. Kouhbanani et al. [29] reported removal of methyl orange dyes using Artemisia vulgaris-derived iron nanoparticles. Radini et al. [30] reported degradation of MO by a photocatalytic method using Trigonella foenum-graecum-derived iron nanoparticles.

4. Conclusion

The biological method of nanoparticles synthesis is one of the potential methods which has gained lot of attention. It produces novel materials that are cost-effective and eco-friendly and shows applications in the different areas. In this study, plant extract solution of Catharanthus roseus was used for FeNP synthesis. The plant extract works as a reducing agent and provides stability to nanoparticles. Synthesized FeNPs showed potential antibacterial activity against E. coli. Photocatalytic degradation using synthesized FeNPs showed around 50% degradation of methyl orange. So, it was concluded that the C. roseus can possibly be used for the synthesis of FeNPs.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors’ Contributions

AR was responsible for the conceptualization. AR, VS, SS, and DA were responsible for the methodology. AR, VS, SS, DA, AKA, GK, and TBE wrote and corrected the manuscript. DA and AR were responsible for the funding acquisition.

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