



Iron Oxide Nanoparticles Synthesis From Vermicomposting Leachate and its Antioxidant Activities

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Currently, nanotechnology and nanoparticles have been quickly emerged and have gained the attention of scientists due to their massive applications in environmental sectors. Nanotechnology also encompasses the ability to design, characterize, manufacture, and implement nano-sized structures. Today, metal oxide nanoparticles stand out in industrial applications in various fields of applied nanotechnology. Among metal oxide nanoparticles, iron oxide nanoparticles (FeO-NPs) are one of the widely used NPs. Green chemistry-based nanoparticles production is one of the most interesting topics in recent years. In the present study, we used vermicomposting leachate to synthesize FeO-NPs. First, vermicomposting leachate (VCL) was produced and then FeO-NPs was obtained from ferric chloride salt. FeO-NPs was characterized by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) and X-ray diffraction (XRD). Additionally, the antioxidant activities of FeO-NPs synthesized from vermicomposting leachate (VCL-FeO-NPs) were evaluated by DPPH scavenging activity. The highest DPPH activities of VCL-FeO-NPs at 200 mg/L concentration were 93.54%. In addition, the nanoparticles showed significant DNA nuclease activity. The antimicrobial activities of VCL-FeO-NPs were studied in micro dilution methods and it exhibited moderate antimicrobial activity through Gr +ve, Gr -ve, and fungi. The nanoparticles showed more effective microbial cell inhibition activity against *E. coli*. Also, biofilm inhibition results were detected against *S. aureus* and *P. aeruginosa* were 66.05% and 67.29%, respectively.

Keywords: antimicrobial activity, antioxidant activity, biofilm inhibition, iron oxide nanoparticles, microbial cell viability, vermicomposting leachate

INTRODUCTION

Nanotechnology (NT) and nanoscience is one of the remarkable areas of science dealing with using the structure of nanoscale (Kakakhel et al., 2021). NT is used in the medical field, pharmacology, optics, electronics, food industry, textiles, wastewater treatment, agriculture, (Azarang et al., 2014; Fernández et al., 2016). In addition, NT is used extensively in antimicrobial, catalysis and toxicity science (Potbhare et al., 2019; Umekar et al., 2021; Yabalak et al., 2022). The nanoparticles (NPs) are found in different types based on by their chemical structure. Nevertheless, some processes of

nanoparticle's synthesis do not accord economically with their biological aim. Such as, till now, many methods have been used for the synthesis of FeO-NPs. Mostly microorganisms and chemical methods have been used to synthesize FeO-NPs; however, the chemical methods have been considered as ecotoxic (Arslan et al., 2022). Therefore, the present study was aimed to choose biological methods using plant for the fabrication of FeO-NPs. This green process for NPs synthesis is non-toxic, eco-friendly, non-harmful for human health (Ghotekar et al., 2021; Priya et al., 2021). So far, this biotechnology became an important branch followed by nanotechnology (Mohanpuria et al., 2008). Biological synthesis is pretty cheap, an environmentally friendly and safe method (Latha et al., 2018). In biological synthesis, bacteria, fungi, algae, and different sources of plants can be used (Nanda, et al., 2018). For example, Potbhare et al. (2020a) fabricated silver nanoparticles (Ag NPs) on the surface of graphene oxide (GO) nanosheets as a multifunctional antibacterial material by using *Pseudomonas aeruginosa* biomass extract. Antibacterial action of the synthesized Ag-GO NPs was tested against bacterial species *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The results revealed that Ag-GO NPs showed effective antibacterial performance against *E. coli*. In another study, Ag-NPs was synthesized by *Rhizoctonia solani* (R. solani) fungi with particle size of 10–20 nm (Potbhare et al., 2020b). AgNPs were found as an effective antibacterial agent against *S. aureus*. Chaudhary et al. (2021) demonstrated the fabrication of uniformly decorated zinc oxide (ZnO) on reduced graphene oxide (ZnO-rGO) nanocomposites using *Sesbania bispinosa*. The nanocomposites were used as photocatalyst for methylene blue (MB) dye-degradation and they supplied 85% dye degradation efficiency towards MB dye under UV-Visible light irradiation within 70 min.

In recent years, researchers have used various plant extracts to produce metal and metal oxide nanoparticles such as manganese dioxide (MnO₂) (Hashem et al., 2018), zinc oxide (ZnO) (Suresh et al., 2015), iron oxides (FeO, Fe₂O₃, Fe₃O₄, FeOOH, Fe⁰) (Karunakaran et al., 2018), tin oxide (SnO₂), lead oxide (PbO₂), silver (Tavaf et al., 2015), gold, copper oxide (CuO), palladium (Kalaiselvi et al., 2015), cerium (IV) oxide, cerium dioxide, titanium oxide, ZnCuFe nanoparticle, cobalt ferrite (CoFe₂O₄), aluminum oxide (Al₂O₃), magnesium (Mg), and sulfur nanoparticles (Salem et al., 2016). Iron oxide has been one of the extensively investigated transition metal oxides due to its important variable oxidation states, low cost, environmental friendly nature, crystal structures, and magnetic properties (Devi et al., 2019).

Vermicomposting leachate can be also used for synthesis of nanoparticles. The digestion of organic material by earthworms results in the production of vermicomposting (Chaoui et al., 2003). Water is constantly sprayed during vermicomposting process on account of obtain the required moisture level in the reactor. Vermicomposting process also generates leachate due to the delivery of water by microorganisms throughout the separation of organic material (Gutiérrez-Miceli et al., 2008). The surplus water that leaches out is usually familiar as vermicomposting leachate or worm bed leachate.

Vermicomposting leachate is very valuable and can be exploited liquid fertilizer due to its loud concentration of plant nutrition's and the existence of fulvic and humic acids (Gutiérrez-Miceli et al., 2008). However, if the resulting leachate is discharged to the environment, it may reason pollution, exclusively if the site is close to the groundwater source (Katheem Kiyasudeen et al., 2016). Therefore, various methods should be developed for their evaluation.

The implementation of vermicomposting in the recycling of organic and animal wastes has acquired admission in the last two decades (Cambardella et al., 2003; Reynolds 2004). Studies on utilizing vermicomposting leachate focused on their fertilizer properties (Arancon et al., 2005; Gutiérrez-Miceli et al., 2011; Tharmaraj et al., 2011; Vasantharaj et al., 2017; García-Gómez et al., 2008). So far, different types of wastes such as cow manure, cow dung and green forages, vegetable waste have been utilized in studies of vermicomposting leachate.

In this study, we focused on the synthesis of FeO-NPs using the green approach and examined their DPPH activity, DNA cleavage ability, antimicrobial activity, cell viability inhibition, antimicrobial photodynamic therapy, and biofilm inhibition properties. As far as our knowledge, vermicomposting leachate has been used for the first time to synthesize FeO-NPs. The present work has highlighted the easy to scale up method for the fabrication of FeO-NPs and also contributed to the development of less expensive FeO-NPs in the field of antimicrobial activity. Moreover, the prepared FeO-NPs from vermicomposting leachate were characterized using SEM, EDX, and XRD.

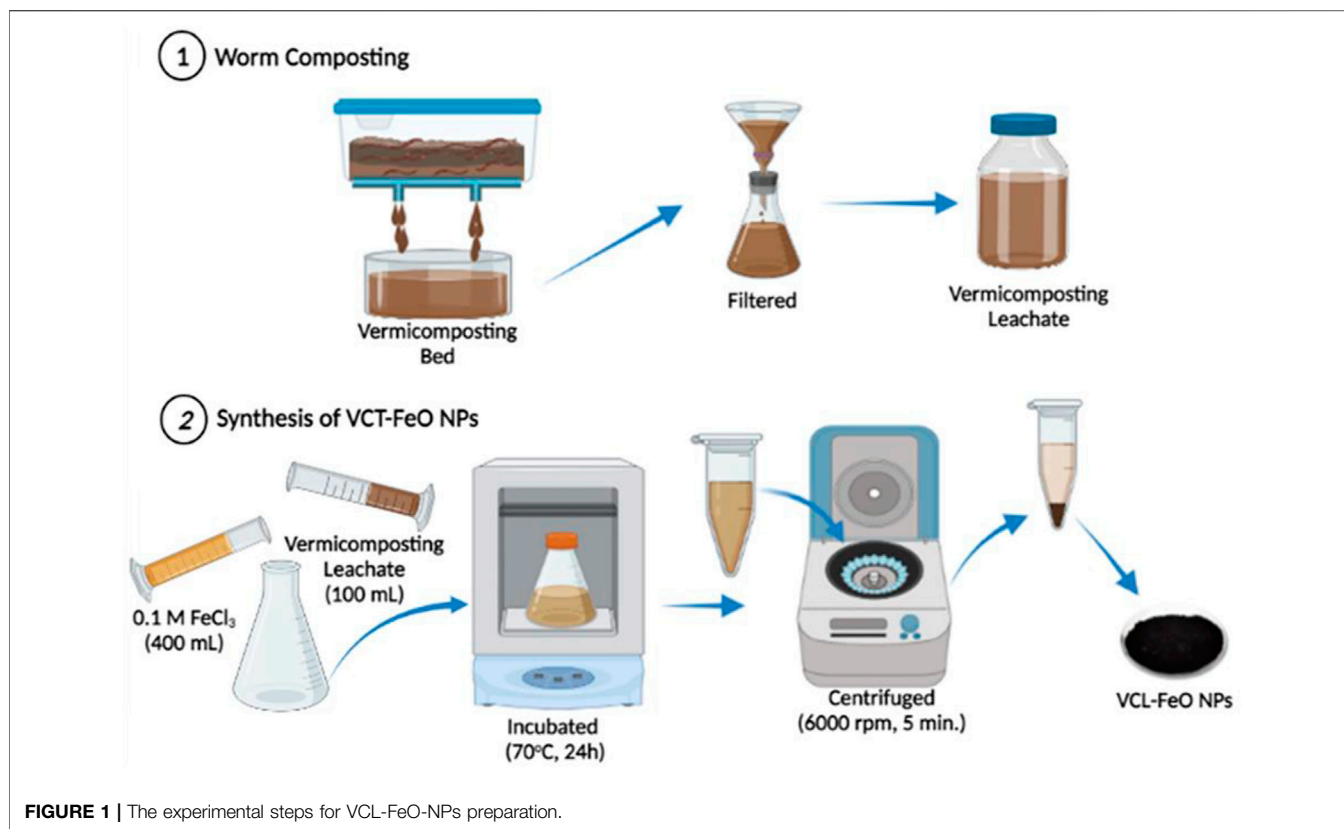
MATERIALS AND METHODS

Materials and Chemicals

Hydrochloric acid (HCl, ACS reagent, 37%), iron (III) chloride (FeCl₃, reagent grade, 97%) and sodium hydroxide [NaOH, reagent grade, ≥98%, pellets (anhydrous)] were purchased from Sigma Aldrich. FeCl₃ salt was utilized as the iron source. The utilized chemicals and reagents of this research were analytical grade. Distilled water used in all experiments was obtained from a two-stage Millipore Direct-Q3UV purification system.

Vermicomposting Leachate Production

Four test beds (40 cm height × 40 cm length × 25 cm depth) were used to obtain vermicomposting leachate. In order to protect the vermicomposting against the sun and rain, the experimental beds were carried out in an indoor environment with an average temperature of 20–25°C, which does not receive direct sunlight. Cow manure and fruit pulp were composted in a thermophilic environment for 2 months, with mechanically turned over every 15 days (Juarez et al., 2015). Cow dung and fruit pulp with 80% moisture content, after a 2-month composting period, worms (*Eisenia fetida*) were left in four test beds in the composted cow manure and fruit pulp. The composite leaks were obtained from the leachate collection tanks during the 2-month vermicomposting process.



Iron Oxide Nanoparticles Synthesis

The vermicomposting leachate collected from four beds was used by filtering through a funnel with a filter paper to remove the solids. The experimental steps for preparation of VCL-FeO-NPs is shown in **Figure 1**. 0.1 M FeCl₃ (400 ml) was prepared and mixed with 100 ml vermicomposting leachate. The mixture was incubated (Microtest MCI 120 incubator) at 70°C for 24 h and the resulting precipitate was separated by centrifugation (Hettich Mikro 22R) at 6,000 rpm for 5 min. NPs was washed with double distilled water and ethanol for five times followed by drying in oven at 80°C.

Iron Oxide Nanoparticles Characterization

The SEM-EDX technique was used to characterize the synthesized FeO-NPs. The surface morphology of NPs was studied using scanning electron microscopy (SEM). (Germany's Zeiss Supra 55). The composition of the elements presents on FeO-NPs surfaces was quantified using energy dispersive X-ray spectroscopy (EDX). The XRD measurements were carried out using *via* X-ray diffraction device (XRD, Bruker, D8 Venture) with 2θ scan range from 20 to 90° at a scan rate of 2°/min.

DPPH Activity

The DPPH activity was utilized to discover the antioxidant activity of VCL-FeO-NPs and the method was applied as previously reported by Salih Ağırtaş et al. (2015). Firstly, 250 μl of VCL-FeO-NPs prepared in five different

concentrations (range from 12.5 to 200 mg/ml) and ascorbic acid and trolox used as standard were taken and put into test tubes. The 1,000 μl of DPPH methanol solution was then added to the test tubes, vortexed to form a homogeneous mixture, and then incubated in the dark for 30 min. Later, the absorbance was detected using spectrophotometer at 517 nm and the percent activity was calculated according to the formula below (1).

$$\text{Capacity (\%)} = \left(\frac{\text{Abs}(\text{control}) - \text{Abs}(\text{sample})}{\text{Abs}(\text{control})} \right) \times 100 \quad (1)$$

Abs_{control}: absorbance value of Ascorbic acid and Trolox,
Abs_{sample}: the absorbance value of the DPPH and test compounds.

DNA Cleavage Ability

Plasmid DNA was used to investigate the impact of newly synthesized VCL-FeO-NPs on DNA. Evaluation of the method was carried out by electrophoresis. The method is principally based on treating different concentrations of VCL-FeO-NPs with *E. coli* plasmid DNA. Briefly, prepared in three different concentrations of VCL-FeO-NPs (125, 250 and 500 mg/L) and 0.1 μg/μl supercoiled plasmid DNA were mixed and incubated at 37°C for 60 min. At the end of the time, loading dye was joined to the mixture and thoroughly blended and then the mixture was loaded onto agarose gel. Moreover, electrophoresis process was carried out at 120 V for 1 h. Later, the gel was lightened with UV lamp and photographed.

Antimicrobial Activity

The antimicrobial activity of the prepared VCL-FeO-NPs was verified using the standard microdilution method. To investigate the antibacterial activity of VCL-FeO-NPs, several types of Gram -ve (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Legionella pneumophila* subsp. *pneumophila*), Gram +ve (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus hirae*) bacteria and yeast (*Candida tropicalis*, *Candida parapsilosis*) were utilized. The strains were grown overnight before microdilution experiment. Two-fold serial dilutions of VCL-FeO-NPs were done for the study. Later, the strains were added to the microplate-wells. Next plates left for 24 h incubation at 37 ± 2 C. MIC was described as the lowest concentration of VCL-FeO-NPs capable of inhibiting microbial growth up to 99%.

Bacterial Viability Inhibition Test and Antimicrobial Photodynamic Therapy

Cell viability inhibition and antimicrobial photodynamic therapy study was also performed to evaluate the inhibition activity of cellular viability of newly synthesized VCL-FeO-NPs. The strain used for this was a Gr -ve *E. coli* (ATCC 10536) strain. The preliminary preparation stage for the test procedure of *E. coli* was explained in detail in our previous study (Gonca et al., 2021). The prepared *E. coli* to be used in both bacterial viability inhibition and antibacterial photodynamic therapy studies. The prepared *E. coli* was treated with VCL-FeO-NPs. Then it was allowed to incubate for 60 min and when the incubation was finished, the dilution step and the cultivation step were performed and incubated at 37°C for 24 h. Finally, the colonies were counted. Finally, Inhibition percentages were calculated according to the Eq. 2 below by counting the colonies. In addition, in the antimicrobial photodynamic therapy study, the method in the microbial cell viability study was applied after the compounds were exposed to LED light for 20 min. Ultimately, the colonies were counted and the inhibition activity computed utilizing with Eq. 2.

$$\% \text{Cell viability inhibition} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100 \quad (2)$$

Inhibition Activity of Biofilm Formation

Biofilm inhibition study was performed as another parameter to evaluate the antimicrobial activity of newly synthesized VCL-FeO-NPs. *S. aureus* (Gr +ve) and *P. aeruginosa* (Gr -ve) were used in the biofilm inhibition study. The details of the study are given in our previous study (Gonca et al., 2021). In summary, VCL-FeO-NPs was prepared at three different concentrations into a 24 well plate and then, the microorganisms were inoculated into the respective wells and incubated. The wells without VCL-FeO-NPs were used as a positive control wells. When incubation time was expired the wells were drained and washed distilled water. Afterwards, the plate was kept in an oven until dry. After the wells dried,

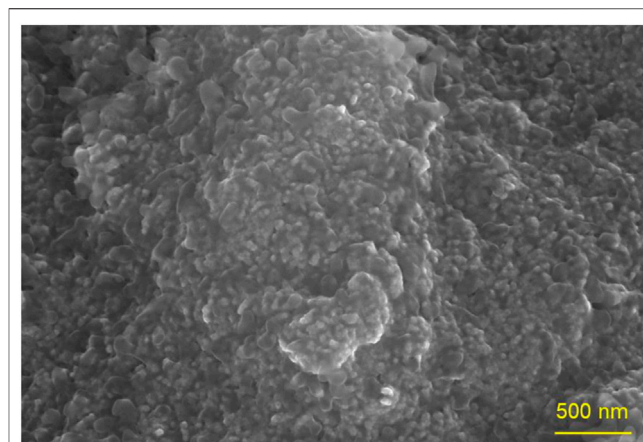


FIGURE 2 | SEM images of synthesized FeO-NPs.

crystal violet (CV) dye was added and left for 60 min. The CV was then removed and the plates were gently washed. Afterwards, ethanol was added to the wells and absorbance values were measured. Biofilm inhibition activity was computed in accordance with the Eq. 3 shown below

$$\text{Biofilm Inhibition}(\%) = \left(\frac{\text{Abs}(\text{control}) - \text{Abs}(\text{sample})}{\text{Abs}(\text{control})} \right) \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Characterization of Iron Oxide Nanoparticles

Morphological properties of FeO-NPs obtained from vermicomposting leachate are examined using SEM microscopy. It was observed that FeO-NPs induced are highly agglomerated with distinct particle sizes. The results also showed that FeO-NPs were an irregular and nonuniform semi-spherical shape (Figure 2). The rougher surface was observed, as well as an apparent sticking behavior due to the capping agent on the surface. SEM analysis depicted that FeO-NPs were smaller than 100 nm. It can be clearly seen that the peak of Fe was prominent among another element which indicates the presence of Fe in the NPs (Figure 3). Other elements like oxygen, carbon, and chlorine were also observed which indicated the effective role of organic materials as a capping agent. Similar results were reported by Aisida et al. (2021).

XRD technique was also exploited to obtain more detailed information about FeO-NPs crystallography. A series of predominant peaks observed in XRD were indexing the purity and crystalline nature of the synthesized FeO-NPs (Figure 4). The reflections in the given XRD pattern were identified as iron oxide. The XRD patterns matched with that of Fe-NPs synthesized using avocado fruit rind aqueous extract (Kamaraj et al., 2019).

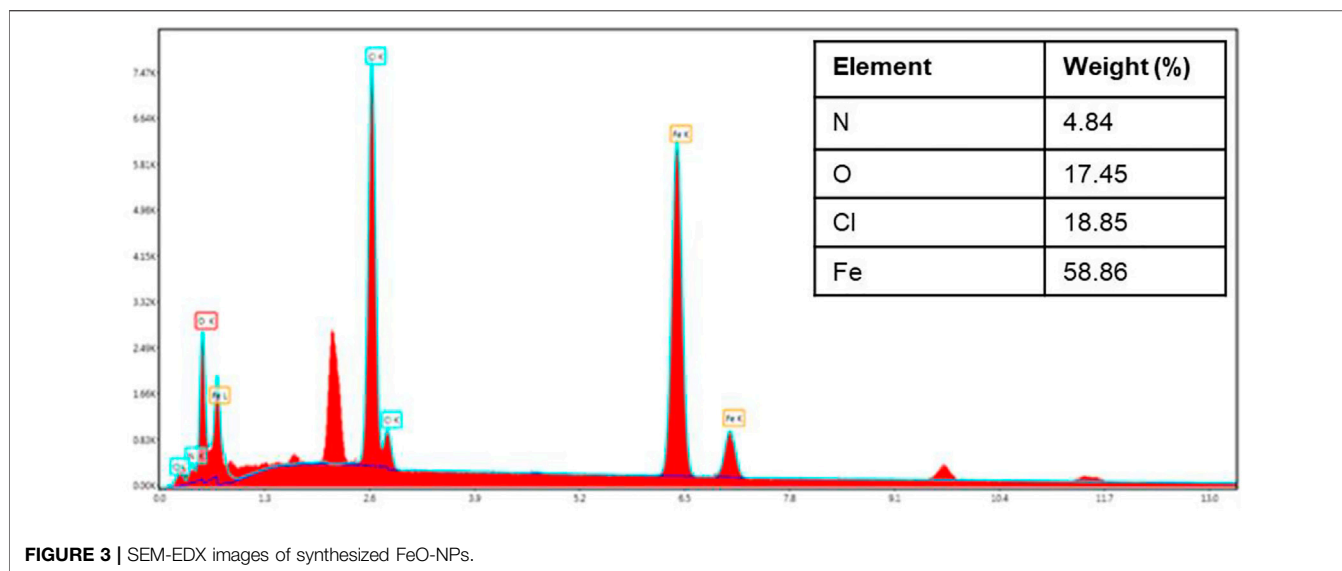


FIGURE 3 | SEM-EDX images of synthesized FeO-NPs.

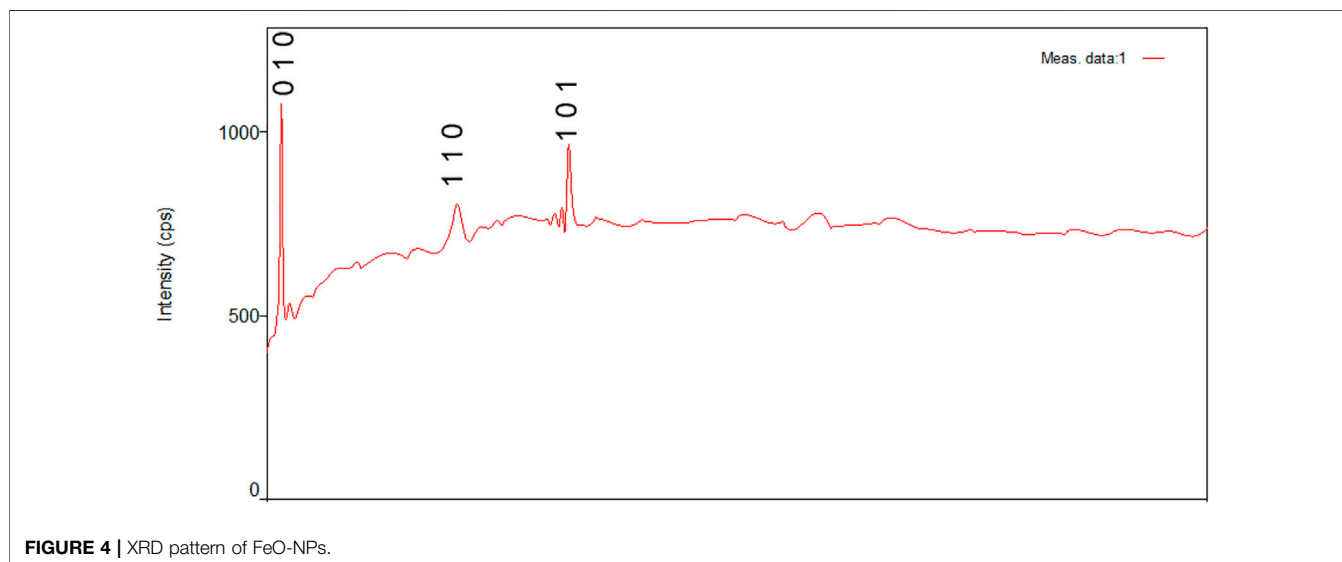
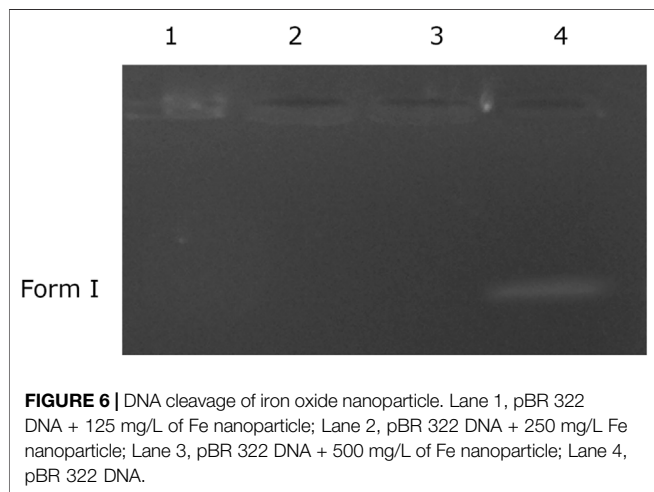
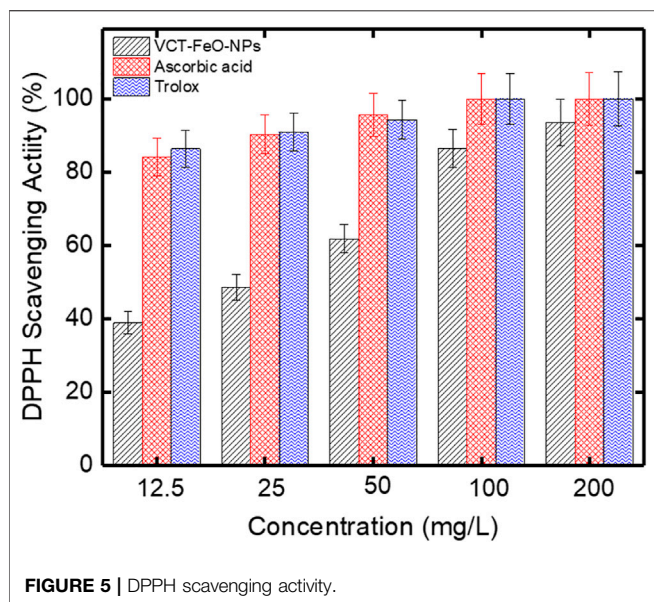


FIGURE 4 | XRD pattern of FeO-NPs.

DPPH Radical Scavenging Activity

As it is known, free radicals are necessary to kill microorganisms, activate enzymes and hormones, and produce energy. However, overproduction of free radicals causes many diseases related to oxidative stress such as oxidative stress-related neurological disorder, cardiac arrest, and cancer cell growth in humans. Antioxidants, which are called radical scavengers, normally regulate free radicals in the human body (Stephen Inbaraj and Chen, 2020). In this study, the DPPH method was used to investigate the free radical scavenging activity of FeO-NPs produced using compost leachate. The DPPH method is a commonly used technique for determining antioxidant activity. The color of the DPPH solution, which has a purple color, changes towards yellow in the presence of antioxidant substances in the environment. The DPPH activity results of VCL-FeO-NPs and also of ascorbic acid and trolox used as

standard are shown in the Figure 5. DPPH activity results of newly synthesized VCL-FeO-NPs were 38.88%, 48.49%, 61.83%, 86.51%, and 93.54% at 12.5, 25, 50, 100, and 200 mg/L concentrations, respectively. DPPH activity results of Ascorbic acid and Trolox at the same concentrations were in the range from 84.12% to 100.00% and 86.39%–100.00%, respectively. Also, as can be clearly seen in the Figure 5, the free radical scavenging activity increased in parallel with the concentration. The free radical scavenging activity of the surface reaction between DPPH radical and VCL-FeO-NPs might be due to an electron shift in oxygen at the radical's surface (Mirza et al., 2018). Antioxidant activities of FeO-NPs were also investigated in studies conducted by different researchers. Some of these studies are as follows. Chavan et al. (2020) synthesized FeO-NPs using *Blumea eriantha* DC extract (BEDC). They studied that of BEDC-FeO-NPs DPPH activity and reported that it was showed scavenging activity as $17.25 \pm 1.19\%$ at



50 $\mu\text{g ml}^{-1}$ concentration. Dowlath et al. (2021) synthesized FeO-NPs in two ways including GS (green synthesis) and CS (chemical synthesis) and investigated the antioxidant activity of both FeO-NPs by DPPH method. They found that GS FeO-NPs and CS FeO-NPs showed $79.99 \pm 0.92\%$ and $15.11 \pm 0.65\%$ DPPH inhibition activity at 100 $\mu\text{g/ml}$ concentration, respectively. Singh et al. (2020) used *Coriandrum sativum* leaf extract to synthesize iron oxide nanoparticles and reported that it showed DPPH activity with ranged from 32.54 to 84.28% at different concentrations (50–250 μg). Kumar et al. (2020) synthesized *Citrus paradisi* peel extract-mediated FeO-NPs and reported that scavenging activity of the CPPE-FeO-NPs was 26.52% at 100 μg the quantity of FeO-NPs. DPPH activity results obtained in the presented study were found to have better results compared to the above-mentioned studies. Therefore, FeO-NPs synthesized using compost leachate seem to be the good candidate for be antioxidant agent because of its good antioxidant potential.

TABLE 1 | The minimum inhibition concentration (MIC) of test microorganisms.

Microorganisms	VCL-FeNPs*	Ampicillin	Fluconazole
<i>E. coli</i>	256	0.5	—
<i>P. aeruginosa</i>	256	0.5	—
<i>L. pneumophila pneumophila</i>	256	0.5	—
<i>E. hirae</i>	128	0.5	—
<i>E. fecalis</i>	256	1	—
<i>S. aureus</i>	128	1	—
<i>C. parapsilosis</i>	256	—	0.5
<i>C. tropicalis</i>	256	—	0.5

* $\mu\text{g/ml}$.

DNA Cleavage Ability

The bio-efficiency of most antimicrobial and anticancer agents is frequently connected to its capability to interact with DNA. Such compounds can cause damage to the structure of DNA in cancer and microbial cells as well as induce cellular death by apoptosis/necrosis (El-ghamry et al., 2021) and these DNA damages may have been caused by single oxygen radicals (Chouke et al., 2022). One of the studies carried out in the presented study was the DNA cleavage study. For this, the effect of VCL-FeO-NPs on *E. coli* pBR 322 plasmid DNA was investigated by agarose gel electrophoresis method. To investigate the effect of VCL-FeO-NPs on plasmid DNA, DNA was treated with 3 different (125, 250, and 500 mg/L) concentrations of NPs. The results of the DNA cleavage study are given in the **Figure 6**. Possible results in DNA cleavage study are as follows; 1) Single strand break in plasmid DNA and transition from Form I to Form II. 2) Occurrence of double strand break in plasmid DNA and transition from Form I to Form III. 3) Another expected situation is the complete degradation of DNA by exposure to the substances (VCL-FeO-NPs for this study). When faced with the situation in the 3rd option, no band is observed in the gel because the DNA is divided into small pieces. In the presented study, there is no band in the **Figure 6** because the DNA was completely fragmented. That is, the synthesized nanoparticle is highly effective on DNA and its effect on DNA is a very valuable result when considered from a pharmacological point of view. It has also been shown by studies that nanoparticles cause damage to DNA. El-ghamry et al. (2021) studied on nanoparticle-size metal complexes and showed its DNA cleavage ability. Gur et al. (2022) synthesized that biogenic zinc oxide nanoparticles and they showed that its DNA damage increases depending on the concentration. As a result, newly synthesized VCL-FeO-NPs can be used as a chemical nuclease agent after further studies.

Antimicrobial Activity

In the present study, the antimicrobial capability of the synthesized VCL-FeO-NPs were investigated through microdilution processes. The antimicrobial activity results are given in **Table 1**. Ampicillin and Fluconazole used as controls for bacterial and fungal strains, respectively. VCL-FeO-NPs demonstrated lower antimicrobial activity than controls. Accordingly, the MIC values of the nanoparticle are 128 $\mu\text{g/ml}$ against *E. hirae* and *S. aureus* and 256 $\mu\text{g/ml}$ against *E. coli*, *P.*

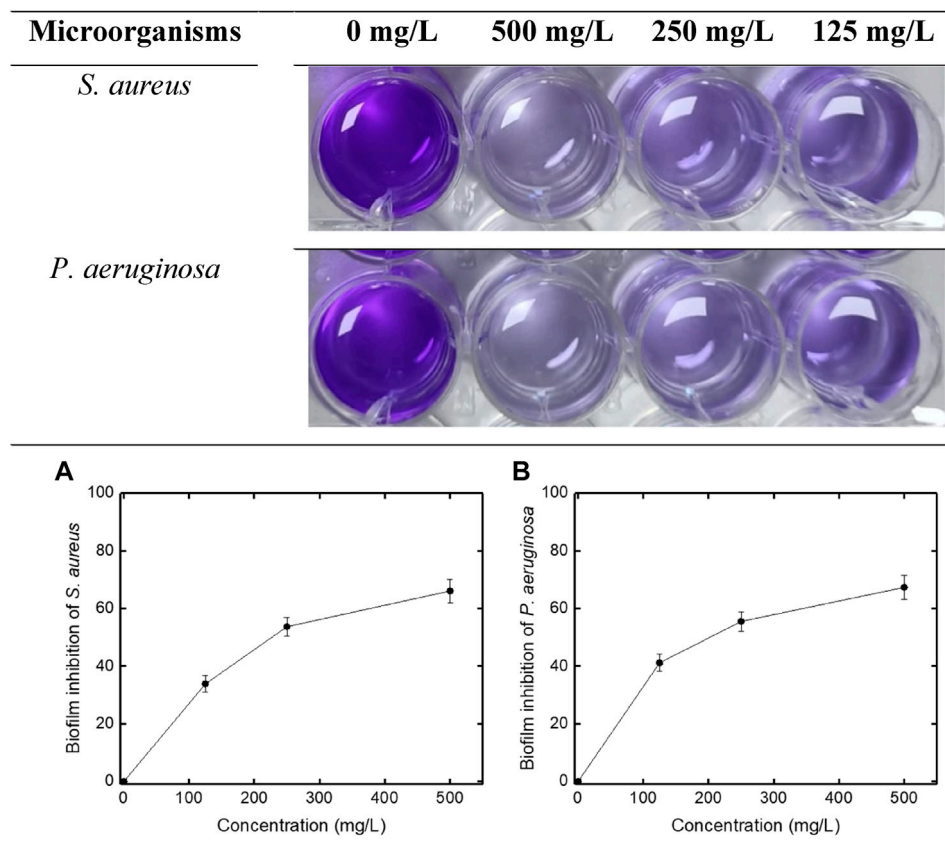


FIGURE 7 | Biofilm inhibition.

aeruginosa, *L. pneumophila* subsp. *pneumophila*, *E. fecalis*, *C. parapapillosis*, and *C. tropicalis*. According to our antimicrobial activity results, Gram +ve bacteria including *E. hirae* and *S. aureus* were found to be more sensitive to FeO-NPs. This situation can be explained as follows; because of that in Gram +ve bacteria formed a thick peptidoglycan layer compared to the Gram -ve bacteria is perhaps an effective degree of contact between nanoparticles and organisms due to its small size (Vitta et al., 2020). Previous reports also showed that higher antimicrobial activity of FeO-NPs on Gram +ve bacteria as compared to Gram -ve ones (Shaker Ardakani et al., 2021). Moreover, antimicrobial activities of iron nanoparticles against different microorganisms have been reported in various studies. Some of these studies are as follows; Salem et al. (2019) biosynthesized Fe₃O₄ NPs using aqueous extracts of seaweed *Colpomenia sinuosa* and *Pterocladia capillacea* and they reported that synthesized Fe₃O₄ NPs exhibited wide spectrum of antibacterial potency against the growth of Gram -ve and Gram +ve. Chavan et al. (2020) synthesized FeO-NPs using alcoholic *Blumea eriantha* DC plant extract, and they found that it showed antibacterial activity against *S. aureus*, *B. subtilis*, *B. cereus* and *E. coli*. Vasantharaj et al. (2019) synthesized FeO-NPs using *Ruellia tuberosa* leaf aqueous extract and studied their antimicrobial activity. They finally reported that while it exhibited better antimicrobial activity against *E. coli* and *Klebsiella*

pneumoniae, it was less active against *S. aureus*. According to our antimicrobial activity results, it is seen that the synthesized NPs has an antimicrobial effect on the strains studied. Therefore, we think that it can be used in various fields as an antimicrobial active substance after further studies.

Biofilm Inhibition Activity

Multidrug resistance of microorganisms results is not only from structure transformation or gene changing of planktonic bacteria, but also from their ability to form biofilms. Combating biofilm formation is one of the important strategies in the development of new antibacterial agents (Srinivasan et al., 2021; Li et al., 2020). In this respect, nanoparticles may emerge as promising new effective substances. In the study, the biofilm inhibition activity of VCL-FeO-NPs was also investigated against *S. aureus* and *P. aeruginosa*. Biofilm inhibition activity results are given in **Figure 7**. Accordingly, as the concentration of VCL-FeO-NPs increased against both bacteria, the biofilm inhibition also increased. Biofilm inhibition activity results were 33.81%, 53.66%, and 66.05% for *S. aureus* and 41.11%, 55.50%, and 67.29% for *P. aeruginosa* at 125 mg/L, 250 mg/L, and 500 mg/L concentrations, respectively. The antimicrobial activity of metal and metal oxide NPs is thought to be mediated by the release of reactive oxygen species (ROS) and effective metal-ions from and

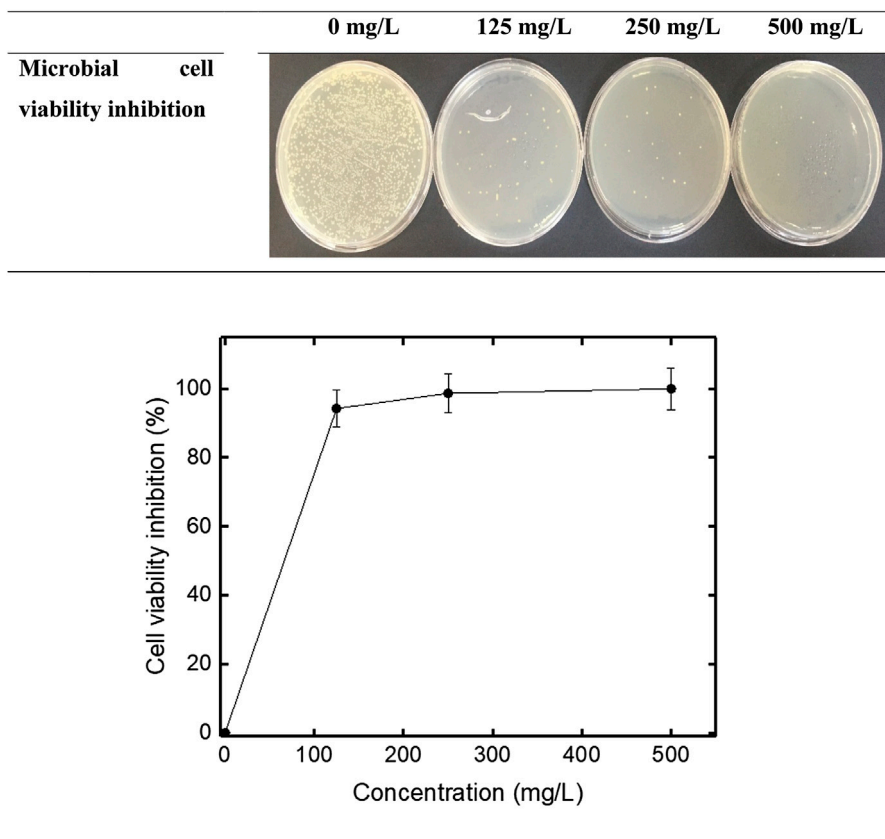


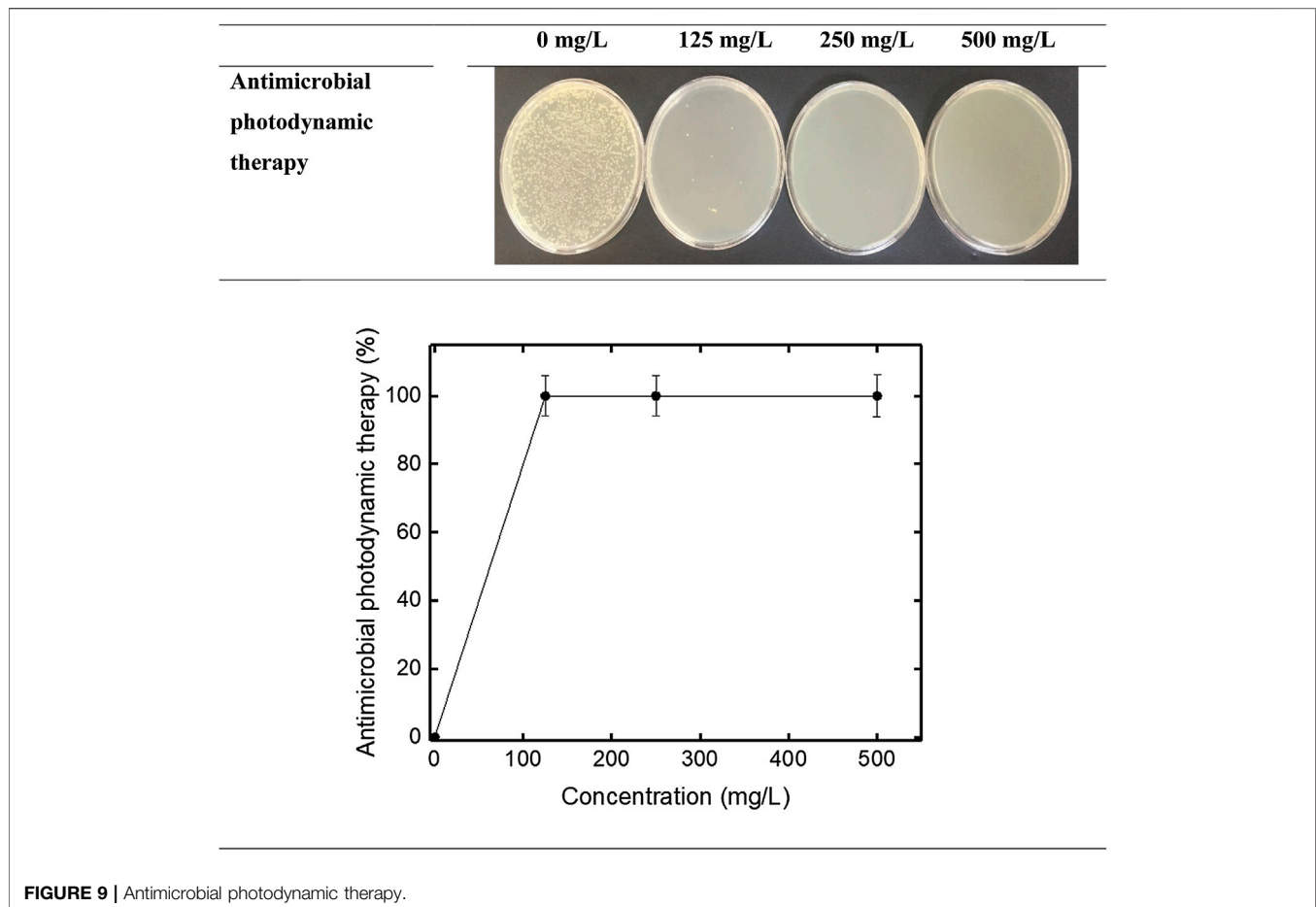
FIGURE 8 | Cell viability inhibition.

its increased surface-to-volume ratios (Khalid et al., 2019). Antibiofilm effect of nanoparticles against various microorganisms has been demonstrated in many studies. Some of these studies are as follows; Salari et al. (2018) reported that Fe_3O_4 -NPs inhibited biofilm formation of *Candida spp.* Kamble and Shinde (2018) reported that synthesized AgNPs using *Curcuma longa* showed potent anti-biofilm activity against *S. aureus* and *S. pneumoniae*. Pham et al. (2019) reported that synthesized and characterized iron NPs inhibited of biofilm formation of *P. aeruginosa* and this inhibition raised as the FeOOH -NP concentration increased. It seems that, these results ensure to anti-biofilm activity of a new synthesized VCL- FeO -NPs, contributing to the research for an efficient material to fight *S. aureus* and *P. aeruginosa* infections resulted from biofilm generation.

Bacterial Viability Effect and Antimicrobial Photodynamic Therapy

In the study, another method used to investigate the antimicrobial activity of CT- FeO -NPs were cell viability inhibition. *E. coli* bacteria were used for this in the method. The results of cell viability inhibition study are shown in the **Figure 8**. As seen **Figure 8**, cell viability inhibition results of VCL-

FeO -NPs were 94.3%, 98.7%, and 99.99% at 125 mg/L, 250 mg/L, and 500 mg/L concentrations, respectively. According to our results, we think that the possible antimicrobial activity is similar to that of Fe_2O_3 NPs. The Fe_2O_3 NPs breaks the bacterial cell membrane and consequently the potential of membrane turn into unsteady and the active transport collapsed. Contemporaneously, Fe_2O_3 NPs enter *via* the impaired membrane and inactivate the enzyme as well as disrupt the DNA, which conduces to death of the bacterial cell. (Ahmad et al., 2020). Also, antimicrobial photodynamic therapy study was performed by exposing the compounds prepared as mentioned above to LED light for 20 min (**Figure 9**). As a result, inhibition rates of antimicrobial photodynamic therapy were determined as 99.99%, 100%, and 100% at 125, 250, and 500 mg/L, respectively. The production of ROS, which damages the protein and DNA of bacteria, is another possible mechanism for the antimicrobial activity of iron NPs, and after exposure to light the antimicrobial effect may have occurred as mentioned (Vasantharaj et al., 2019; Vitta et al., 2020). In addition to these, it is well known that metal oxide nanoparticles can create the formation of ROS in microbial cells. These ROS molecules especially can cause deterioration of the membrane structure (Chaudhary et al., 2019). According to cell viability and antimicrobial photodynamic therapy results, newly



synthesized FeO-NPs seems to be quite effective on *E. coli* bacteria. After further studies, we can say that NPs can be used as antimicrobial agent.

CONCLUSION

Vermicomposting leachate was used to synthesize FeO-NPs. The highest DPPH activities of VCL-FeO-NPs at 200 mg/L concentration were 93.54%. In addition, the nanoparticle showed significant DNA nuclease activity. The antimicrobial activity of VCL-FeO-NPs showed moderate antimicrobial activity against Gram positive, Gram negative, and fungi. However, the nanoparticles showed more effective microbial cell inhibition activity against *E. coli*. Also, biofilm inhibition results were detected against *S. aureus* and *P. aeruginosa* were 66.05% and 67.29%, respectively. It clearly indicated that green synthesized FeO-NPs showed good biological properties, which could be used to produce and formulate new drug and biomedical applications. In addition, we hope that the results of our study will inspire readers to explore new

avenues for the synthesis of FeO-NPs of various morphologies for future antimicrobial applications in nanomedicine. Overall, these results suggest that green synthesis of FeO-NPs has potential as an operative, low-cost, and beneficial process for many scientific and technical applications in future, including environmental applications.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HA, SG, ZI, SÖ, and MY: Concept, Data Curation, Investigation, Writing—Original Draft. ND, BD, GA: Conceptualization, Writing—Original Draft, Formal analysis, Review and; Editing.

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