



Synthesis of a Novel Supermagnetic Fe₃O₄ Nanoparticles and their Congo Red Dye Removal, Cytotoxic, Antioxidant, and Antimicrobial Activities

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Abstract

Plantago lanceolata is a traditional medicinal plant that has attracted significant interest from researchers due to the use of its physiologically active components, particularly polyphenolics (flavonoids, hydroxycinnamic acids), in various fields. The aim of this study is to synthesize iron oxide (PLE@FeNPs) nanoparticles using a green synthesis approach with *Plantago lanceolata* (*P. lanceolata*) leaf extracts, characterize them, evaluate their in vitro effects, and assess their use in the removal of Congo red (CR) from wastewater. We carried out the physicochemical characterization of the nanoparticles using UV–Vis, FT-IR, and XRD spectroscopies; TEM and SEM microscopy; and Zetasizer particle size analysis. While the distinct peaks in XRD confirm the crystalline structure, TEM has determined an average particle size (8 nm) for PLE@NPs with deformed spherical nanoparticles. The FT-IR spectra showed that bioactive compounds from *P. lanceolata* were involved in the participation of PLE@FeNPs. EDX confirmed the presence of iron in the designed PLE@FeNPs. The antibacterial, antioxidant, and anticancer analyses of the studied PLE@FeNPs revealed significant activities. We investigated the adsorption kinetics of CR on PLE@FeNPs, taking into account initial dye concentration, different pH levels, adsorbent dosages, and temperature. At optimal conditions (concentration, 50 ppm; dosage, 15 mg; pH, 8), the degradation of CR dye in sunlight was found to be 99%. The small size of PLE@NPs (8 nm) and the more negative zeta potential (− 12.2 mV) support this situation. The equilibrium data demonstrated a good fit to the Langmuir isotherm model, outperforming the Freundlich isotherm model. The results showed that the pseudo-second-order kinetic model accurately described the kinetic data. PLE@NPs exhibited significant antioxidant, antimicrobial, and anticancer activities. This situation suggests that the nanocomposition of PLE@NPs obtained through the green route may have improved efficiency due to various synergistic effects. Overall, these results pave the way for further applications in dye removal and biological applications of environmentally friendly PLE@FeNPs.

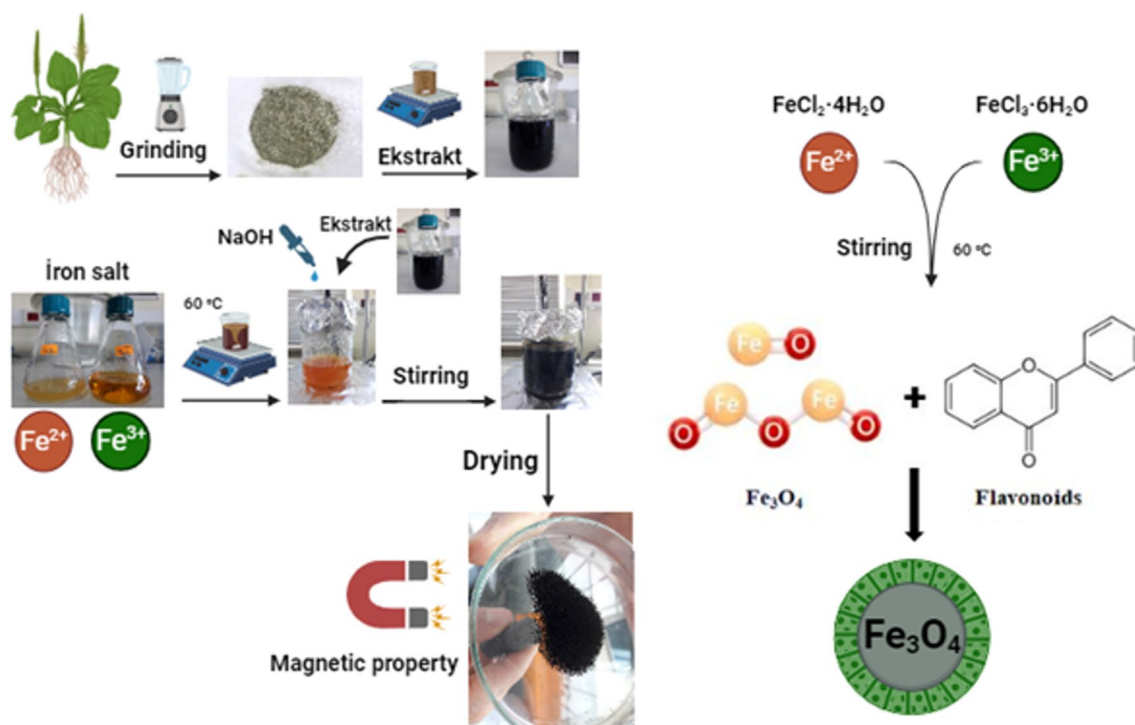
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Graphical Abstract



Keywords Anticancer · Antimicrobial · Congo red · PLE@FeNPs · Langmuir isotherm · *Plantago lanceolata*

Abbreviations

TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
FT-IR	Fourier transform infrared
CR	Congo red
NP	Nanoparticle
PLE@FeNPs	<i>Plantago lanceolata</i> L. extracts/iron nanoparticles
ZP	Zeta potential
A549	Human lung cancer cell line
SH-SY5Y	Human neuroblastoma cell line
MIC	Minimum inhibition concentration
XRD	X-ray diffraction

Introduction

Increasing population, changes and diversity in production processes, and environmental changes that accompany with the industrial development endanger the quality of water and cause increased water contamination [1]. Synthetic dyes are the primary causes of water pollution [2]. Therefore, the removal of these dye components from water has become a major research area.

Scientists have developed several methods for cleaning water to remove the dye wastes. These methods include reverse osmosis, chemical precipitation, ion exchange, and electrocatalysis. However, the application of these methods is hindered by low efficiency, high energy consumption, and toxicity. Extensive research is being carried out to develop alternative methods to overcome these problems [1]. Adsorption is a simple, economical process with a high processing efficiency and reusability of the adsorbents for dye removal from the wastewater. Interest in the field of nano-adsorbents has increased as the conventional adsorbents have limited efficiency, with low surface or active area, lack of selectivity, and adsorption kinetics [3]. The selectivity for surface area or volume in nano-adsorbents is better than traditional adsorbents. There are many ways to make nanoparticle by chemicals methods, but the biological approach, which uses plant extracts as reducing and stabilizing agents, is one of the most appealing because it is cheap and good for the environment [4–9]. Metal oxides are commonly used in green synthesis [10–13]. Iron oxide nanoparticles are one of the most widely used metal oxides due to the simplicity of their synthesis and their ability to remove a large number of pollutants, alone or in combination with other adsorbents [14].

Iron oxide nanoparticles have several applications including drug targeting in cancer therapy [15], antioxidant [16], anticancer [17, 18], antimicrobial [19–22], dye removal and degradation [23, 24]. The reports have shown that they have high efficiency, wide distribution ranges and low cost in removing pollutants in treatment processes [25]. Iron oxide nanoparticles are recognized as compatible with biological systems and have demonstrated multiple biomedical applications. Various reports have been published on the development of synthesis methods for iron nanoparticles, including chemical reduction, vapor–solid growth techniques, sol–gel processes, chemical co-precipitation, and hydrothermal synthesis. However, these methods produce toxic by-products from chemical reactions and require special equipment and high temperatures. Green synthesis is a simple, economical, and environmentally friendly method that reduces environmental hazards [26]. Biological synthesis, or green synthesis, is the process of making nanomaterials made up of biodegradable materials and environmentally friendly structures without toxic inputs and outputs [27]. Plant-mediated syntheses have an important place in green synthesis.

Plantago lanceolata, a species of the Plantaginaceae family, contains biologically active components such as alkaloids, flavonoids, and terpenoids. In *P. lanceolata*, the predominant groups include iridoids, hydroxycinnamic acids, luteolin, and glycosides (verbascoside) [28]. These groups tend to bind to metal ions, prevent agglomeration, and stabilize them in nature [27]. At the same time, these groups impart antioxidant, antimicrobial, anticancer activities, and photocatalytic activities to *P. lanceolata*. The genus *Plantago* has been studied in many aspects due to its rich content. Only the *Plantago major* species of this content-rich genus has been studied for the green synthesis of iron oxide nanoparticles [29]. The difference between these two studies is the synthesis method and the use of different dyes for removal.

In light of all this information, in this study, unlike previously published studies, *P. lanceolata* extract was used to prepare magnetite PLE@FeNPs. In addition, for the first time, adsorption isotherm and kinetic studies were conducted to understand the nature of CR adsorption with PLE@FeNPs. In addition, iron element was used to give magnetic properties to the material. Therefore, the separation of the magnetic nanoparticles from the system was easy and effective. This feature is important in terms of reducing material loss in the applicable area. Thus, the leakage of the dye retained in the system was prevented. There is no study in the literature that combines experiments such as biological activities (antioxidant, antimicrobial, anticancer) and dye degradation activities of supermagnetic iron oxide nanoparticles of *P. lanceolata* L. Thus, it has advantages such as the recyclability of the structures given to the living ecosystem and being active with dye removal, not leaving

any waste in the system, and being reusable with regeneration. At the same time, this research focuses on the development of nanoparticles with strong bioactive properties and the investigation of sustainable and environmentally friendly methods for dye degradation.

Materials and Methods

Chemicals

All chemicals were obtained from Sigma-Aldrich and Merck. Chemicals used in the present study are as follows: iron chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, % 99 Merck), iron chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, % 97 Sigma-Aldrich), sodium hydroxide (NaOH, Merck), hydrochloric acid (HCl, Merck), Congo red ($\text{C}_{32}\text{H}_{22}\text{N}_6\text{Na}_2\text{O}_6\text{S}_2$, Sigma-Aldrich), ethanol ($\text{C}_2\text{H}_5\text{OH}$, Merck), methanol (CH_3OH , Merck), hydrogen peroxide (30.0%, H_2O_2 , Merck), dimethyl sulfoxide ($\text{C}_2\text{H}_6\text{OS}$), sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, sodium nitrite (NaNO_2), aluminum chloride (AlCl_3), DPPH ($\text{C}_{18}\text{H}_{12}\text{N}_5\text{O}_6$, Sigma-Aldrich), ferrozine ($\text{C}_{20}\text{H}_{12}\text{N}_4\text{Na}_2\text{O}_6\text{S}_2$, Merck), sulfuric acid (H_2SO_4 , Merck), monosodium phosphate (NaH_2PO_4 , Merck), and ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, Merck) Butylhydroxyanisole (BHA).

Collection of Plant Material

Plantago lanceolata L. leaves were collected from the Aksaray/Aksaray University gardens 38°19'37"N, 33°58'42"E in November and December 2019. Verified by Dr. Yakup YAPAR from the Botany Department of Bingöl University. The Flora of Turkey conducted the taxonomic identification of the plant. The collected leaves were separated from the branches, washed with water, and dried in the shade.

Methods

Extraction of *P. lanceolata* Plant (PLE)

First, the leaves of the dried *P. lanceolata* plant were grounded. Then, the grounded plant was weighed to 35 g, and 350 mL of distilled water was added on (to a ratio of 1/10). The prepared plant solution was kept at 20 °C on a magnetic stirrer for approximately 24 h. The plant solution was then removed from the magnetic stirrer and filtered. Due to evaporation, the volume was reduced to around 250 mL, which became overconcentrated; therefore, distilled water was added to bring the solution back up to 350 mL.

Iron Nanoparticle (FeNP) Synthesis by *P. lanceolata* (PLE@FeNPs)

Initially, 100 mL of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.01 M) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.01 M) were prepared. Then, they were mixed and heated at 60 °C on a magnetic stirrer. After the temperature of this mixture was fixed, 200 mL of plant extract (PLE/water = 1:10) was added and mixed at 200 rpm. After 30 min, 20 mL of NaOH (2 M) was slowly dripped into the mixture. Following the dripping process, the mixture was stirred for another 40 min (60 °C, 200 rpm). Then, the nanoparticles, which precipitated at room temperature, were washed three times with 200 mL water and twice with 200 mL ethanol to remove chlorine residues. Subsequently, PLE@FeNPs were dried in a glass petri dish at 40 °C.

Characterization

The physical and chemical characteristics of the synthesized magnetite PLE@FeNPs were analyzed using UV–visible absorption spectroscopy (UV–Vis) (JASCO V-650), scanning electron microscopy (SEM) (JEOL JSM 6510, 15 kV), X-ray spectroscopy (EDX) mounted on a Hitachi S800, transmission electron microscopy (TEM) (Hitachi HighTech HT7700), X-ray diffraction spectroscopy (XRD) (Rigaku Ultima IV), and Fourier transform infrared spectroscopy (FT-IR) (Perkin Elmer). The particle size distribution was established with a Zetasizer (Malvern Zetasizer Nano ZSP).

Adsorption Experiments

Congo red (CR) adsorption experiments were performed using synthesized PLE@FeNPs. The effects of pH, dye concentration, adsorbent concentration, and adsorption temperature on CR adsorption were investigated. After the adsorption process, dye concentrations in the solutions were determined by measuring absorption at 538 nm. First, dye removal was studied at different pH levels. A solution of Congo red ($\text{C}_{32}\text{H}_{22}\text{N}_6\text{O}_6\text{S}_2\text{Na}_2$, formula weight = 696.65) was prepared by mixing 200 ppm of Congo red (28.7 mmol) with 100 mL of distilled water at ambient temperature. Other concentrations were prepared by diluting this stock dye (200 ppm) solution. For each concentration, the pH range of 2–8 was studied. For Fenton reaction, 10.0% H_2O_2 solution (v/v) was prepared by diluting 30.0% H_2O_2 solution with deionized water, and 1 mL of 10.0% H_2O_2 solution was added to all test sets. Then, 200- μL samples were taken from each set before and after the adsorption process and transferred to a 96-well plate. The absorbance measurements at 538 nm were recorded.

The adsorption capacity and removal efficiency of the biosorbent at equilibrium were calculated using Eqs. (1) and (2), respectively:

$$q_e = \frac{C_i - C_e}{W} \times V \quad (1)$$

$$\text{Adsorption (\% removal efficiency)} = \frac{C_i - C_e}{C_i} \times 100 \quad (2)$$

where q_e is the amount of substance adsorbed per gram by the adsorbent at equilibrium, C_i is the initial concentration, C_e is the concentration at equilibrium, V is the solution volume, and W is the adsorbent weight.

When reviewing the literature, it is observed that a large number of adsorption isotherms have been developed: Freundlich, Dubinin-Radushkevich, Temkin, Langmuir, Flory–Huggins, Hill, Redlich–Peterson, Toth, Koble–Corrigan, Khan, and Radke–Prausnitz isotherms. However, the most commonly used adsorption models to explain the interaction between adsorbate and adsorbent are Freundlich, Temkin, and Langmuir adsorption models. With the help of these equations, the surface properties of the adsorbent and the relationship between adsorbent and adsorbed material can be defined. Kinetic data were modeled using the pseudo-first-order (PFO) model and the pseudo-second-order (PSO) model [30].

Determination of Biological Activities of PLE@FeNPs

Antioxidant Activity

For the determination of antioxidant activity, 1 mg/mL of PLE@FeNPs and 1 mg/mL of *P. lanceolata* extract were used. The experiments were repeated three times. Total phenol content was calculated per kilogram as gallic acid equivalents using the method of Folin and Ciocalteu [31]. Total flavonoid content [32], total antioxidant capacity [33], DPPH radical effect [34], and metal chelating activity [35] of PLE@FeNPs and *P. lanceolata* were determined. We transferred all samples to a 96-well plate and measured absorbance in ELISA for antioxidant activity.

Cytotoxic Activity

A549 (human lung cancer cell line) and SH-SY5Y (human neuroblastoma cell line) cells used in the study were obtained from Bingöl University, Department of Molecular Biology and Genetics. The cells were stored at –80 °C prior to use.

Cytotoxic activities of PLE@FeNPs and plant extract of *P. lanceolata* L were studied against A549 and SH-SY5Y cell lines using MTT assay. The cells were cultured in Dubelco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), streptomycin, and penicillin

(100 µg/mL). The cells were maintained in humidified CO₂ incubator with 5% CO₂ and 95% humidity for 48 h. The cells were seeded in 96 well tissue culture plates along with different concentration of PLE@FeNPs (30–1000 µg/mL) and incubated for 72 h. After 72 h of incubation, 20 µL of MTT was added into the each well and incubated at 37 °C for 4 h, and absorbance values at 450–690 nm were measured.

DNA Fragmentation Activity

The DNA fragmentation assay was performed based on the slight modification of Tartik et al. [36]. After PLE@FeNPs (1 mg/mL) treatment, cells were collected and washed with PBS. Afterwards, the cells were centrifuged at 4000 rpm for 3 min. The obtained pellet was suspended with DNA lysis buffer and incubated on ice for an hour and DNA isolation was performed following the procedure supplied with content (Biospeedy, Turkey). DNA concentration was measured at 260 nm using a spectrophotometer (Shimadzu, Japan). DNA was run on a 1.5% agarose gel (containing 3 mg/mL ethidium bromide in 1×TAE buffer of pH 8.5) at 90 V for 45 min, and bands were visualized using a UV transilluminator (BioRad USA).

Antimicrobial Activity

Microorganisms used for antimicrobial activity were obtained from Refik Saydam Public Hygiene Center of Turkish Health Ministry. The organisms used in the study were gram-positive (+) microorganisms *Staphylococcus aureus* (ATCC 29213); gram-negative (−) microorganisms *Klebsiella pneumoniae* (EMCS) and *Escherichia coli* (ATCC 25922), and yeast *Saccharomyces cerevisiae*. Antimicrobial activities were measured by ELISA reader.

Antimicrobial activities of both nanoparticles and plant extracts were studied by microdilution approach in 96-well plate. The bacteria cultures were incubated overnight in Mueller Hinton Broth and diluted as Mc Farland 0.5 ($1-2 \times 10^5$). Following that, the cells were mixed with serially diluted PLE@FeNPs and plant extract of *P. lanceolata* L. with the final concentration ranging from 0.15625 to 5 mg/mL. After incubation at 35.5 °C for 24 h, the optical density at 538 nm was determined, and MIC values were calculated.

Statistical Analysis

All experiments were performed in triplicate. Statistical analysis was carried out GraphPad Prism 5.01 software and comparable data groups were evaluated by one-way ANOVA; $p < 0.05$ was considered significant.

Results and Discussion

Characterization

The formation of PLE@FeNPs was examined and confirmed via UV–vis spectroscopy to evaluate the signature surface plasmon resonance (SPR) bands. UV-spectrum 200–1100 cm^{−1} field scanning of PLE@FeNPs. The UV–vis absorption analysis revealed the broad band at around 352 nm (Fig. 1a). A single-SPR band indicates the formation of spherical nanoparticles, while presence of two or more SPR bands indicates formation of anisotropic molecules [37]. There is a possibility that the different absorptions may be due to the various components of the plant extracts. The bands observed around 204 nm and 352 nm were due to the surface plasma resonance created by iron electrons (Fig. 1a).

FT-IR analysis was performed to determine the surface chemistry of *P. lanceolata* L. plant extract and PLE@FeNPs, and functional groups present in the active ingredients were identified (Fig. 2b). In the FT-IR analysis of PLE@FeNP, peaks with values of 983 cm^{−1}, 1146 cm^{−1}, 1463 cm^{−1}, 1578 cm^{−1}, 1726 cm^{−1}, 2932 cm^{−1}, and 3444 cm^{−1} were obtained. The peak of the broad and dense band formed by organic compounds with hydroxyl (OH) groups in the plant extract occurred at 3444 cm^{−1} [38, 39]. The 2932 cm^{−1} band indicated the presence of aliphatic hydrocarbons with CH and CH₂ groups [40]. The band at 1726 cm^{−1} originated from the C–O, C–H, and C=C groups of aromatic compounds [41]. The band at 1578 cm^{−1} formed due to C=C aromatic ring stretching vibration [40]. It has been observed that bands of C–H, C–O, and C=C groups of water-soluble organic compounds are formed at 1463 cm^{−1}, 1146 cm^{−1}, and 983 cm^{−1} [42]. The bands formed indicated that the organic groups cover the surface of the nanoparticles and keep them stable. The presence of functional groups (aromatics, phenols, amines, hydroxyl groups, carboxyl groups, carbonyl groups, and aliphatic hydrocarbon groups) in the structure of the *P. lanceolata* extract was confirmed based on the observed bands [40, 43].

The crystal structure and phase of PLE@FeNPs were characterized by XRD (Fig. 1c). XRD spectrum of the nanoparticles revealed the presence of distinct diffraction peaks at $2\theta = 38^\circ, 44.9^\circ, 47.1^\circ, 54.9^\circ, 68.9^\circ, 73.9^\circ, 81.7^\circ$, and 93.4° . The obtained peaks were determined to be the characteristic of magnetite [44, 45]. The indexes corresponding to these peaks are (220), (311), (222), (400), (422), (511), (440), and (620), respectively. These are the peaks of iron oxide in the crystal structure, and these peaks are the magnetite (Fe₃O₄) form of the synthesized nanoparticles. This analysis fits the PLE@FeNP reference model (JCPDS: 00–001–1111/ cubic). The JCPDS code confirms that PLE@NPs is Fe₃O₄ [46].

Fig. 1 **a** UV–vis absorption spectrum of PLE@FeNPs. **b** FT-IR spectra of PLE@FeNPs. **c** XRD graph of PLE@FeNPs. **d** TEM analysis image of PLE@FeNPs and particle size distribution histogram. **e** Zeta potential plot of PLE@FeNPs. **f** SEM and EDX analysis results of PLE@FeNPs image

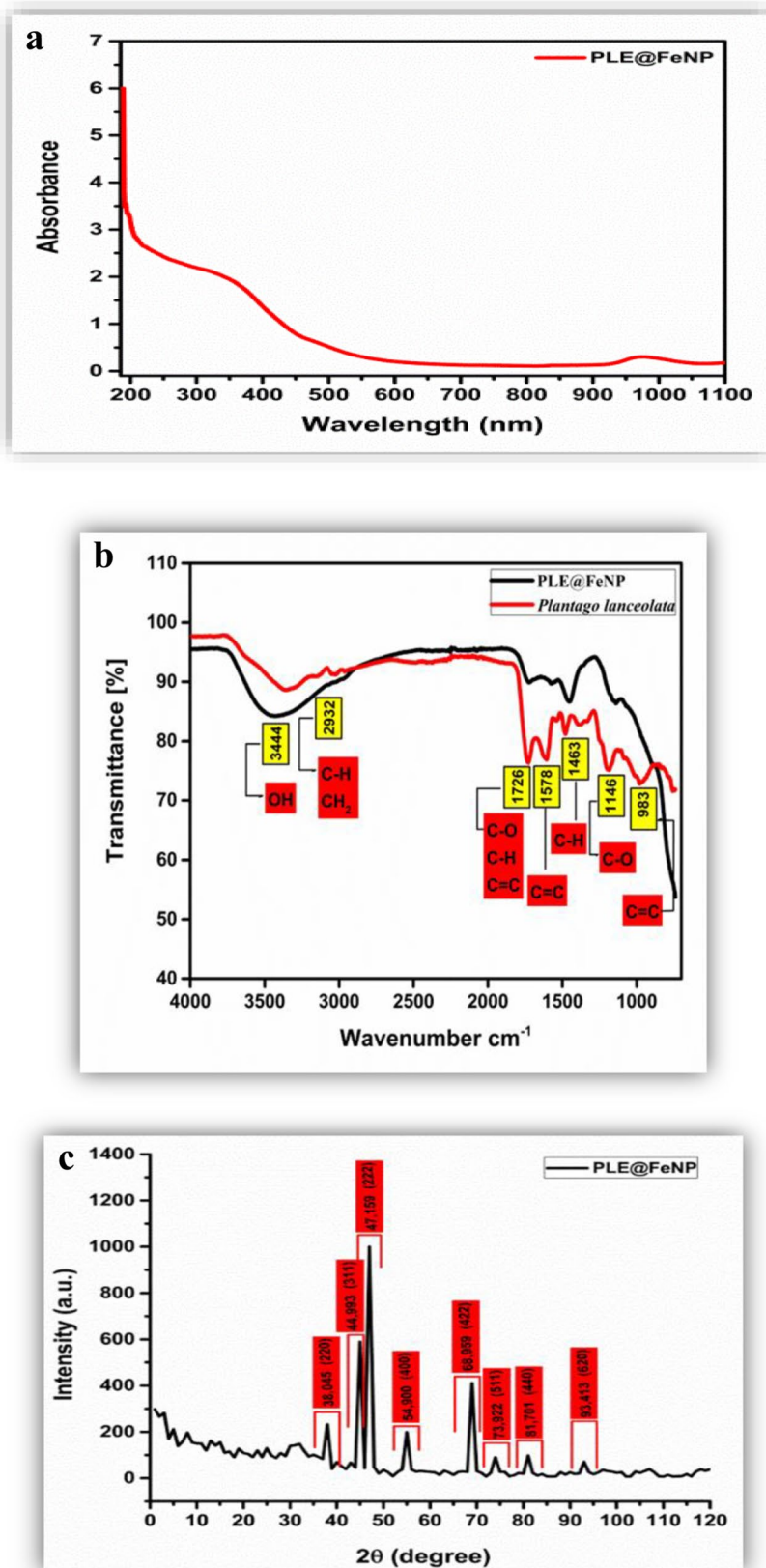


Fig. 1 (continued)

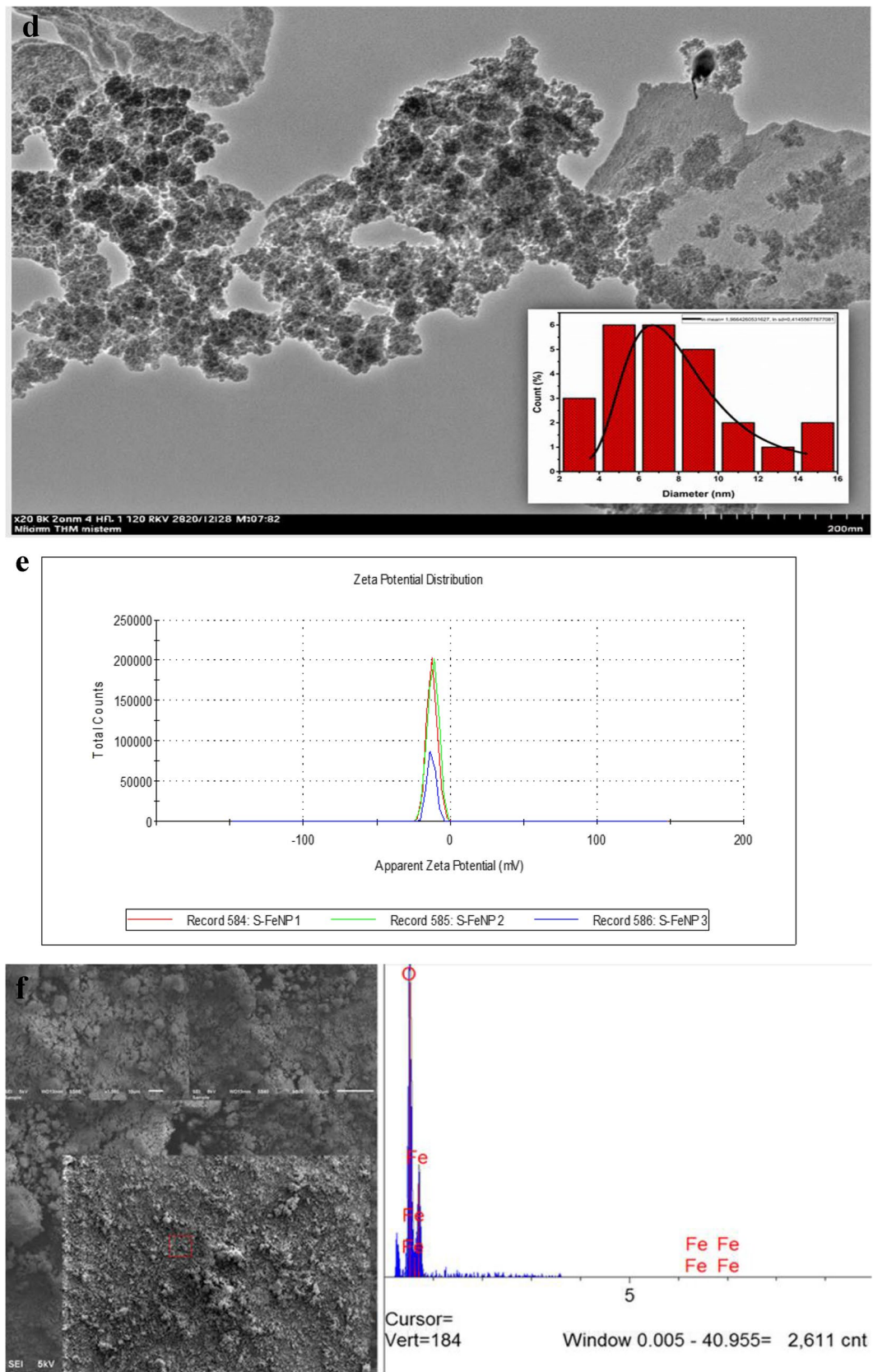
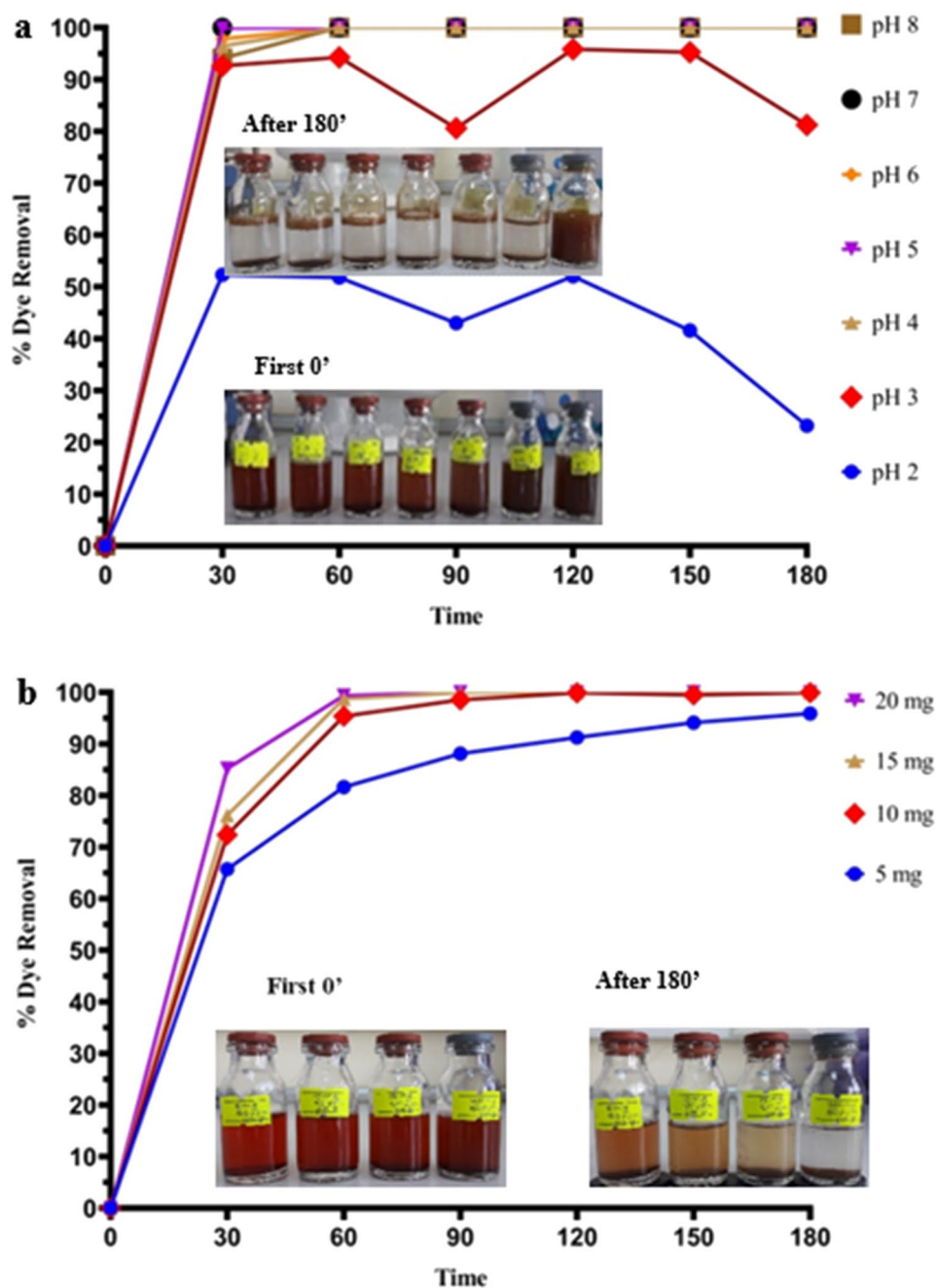


Fig. 2 **a** pH scanning (pH 2–8) with 15 mg of PLE@FeNPs at 25 °C and 50 ppm dye concentration. **b** Congo red dye removal pH:8 with 5,10, 15, and 20 mg of PLE@FeNPs and 50 ppm dye concentration



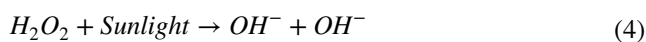
TEM images showed that most of the PLE@FeNPs were spherical, and histogram calculations show the average size of the nanoparticles obtained in the TEM images was 8 nm (Fig. 1d). At 120 kV $\times$ 60 k magnification, the bar size was scaled to 200 nm, and the calculation was determined by the Image J program. The morphological structure of PLE@FeNPs was investigated using TEM (Fig. 1d). The thin layer surrounding the PLE@FeNPs supports the presence of plant metabolites in the *P. lanceolata* extract that reduces Fe ions to nanoparticles.

The electrical charge of the synthesized PLE@FeNPs was determined by zeta potential analysis (Fig. 1e). The zeta potential, a measure of the charge on the surface of nanoparticles, indicates the electrical potential of moving nanoparticles [47]. The zeta potential showed that PLE@FeNPs have an electrical charge of -12.2 mV. This value indicates that PLE@FeNPs are moderately stable [48, 49]. A negative value may occur due to the adhesion of organic compounds to the surface of iron nanoparticles [50].

The sizes and morphological structures of PLE@FeNPs were examined using SEM (Fig. 1f). The functional groups in the plant extract used are effective in the formation of this morphological structure [51]. The material surface had irregular shapes and partial agglomerates. Particle clustering occurred due to the magnetic interface formed by the presence of Fe, which is a component of the nanoparticles, and these clusters can also result from the accumulation of small-sized nanoparticles. The TEM analysis confirms this situation, showing that their size is 8 nm. The formed agglomerations were created by particle clusters held together by electrostatic interactions. Additionally, the morphological structure of PLE@FeNPs had rough surface characteristics that provided more area for the adsorption of the dye. Elements in the structure of PLE@FeNPs were determined using EDX (Fig. 1g). Elemental analysis of PLE@FeNPs demonstrated the presence of Fe and O in the sample, confirming the high purity of the synthesized PLE@FeNPs [50]. In the EDX analysis, it was determined that iron oxides were formed in the presence of high oxygen content.

Dye Degradation Mechanism

PLE@NPs can be used as an effective material for the degradation of pollutants with pollutants. The mixture of PLE@NPs and H_2O_2 can produce free hydroxyl radicals ($OH^\bullet$) that can break the azo bond ($-N=N-$) in CR and cause the color of the dye solution to lighten [52]. Organic degradation is highly associated with OH^- formation, as OH^- -produced from H_2O_2 is the main oxidant of the photo-Fenton process [53]. In the photo-Fenton process, in addition to Fenton reactions, hydroxyl radical formation occurs with the following reactions shown in Eqs. (3) and (4) [54–56]. Possible dye absorption mechanism of PLE@NPs is given in Fig. S1.



Dye Degradation Studies

The performance of PLE@NPs was investigated for the removal of industrial CR dye using a Fenton-like mechanism. Experiments were conducted at different concentrations (20, 50, 100, 200–100 ppm), varying amounts of PLE@NPs (catalyst) (5, 10, 15, 20 mg), and pH levels (2 to 8). The study conducted the adsorption process during daylight hours. Samples were taken from the upper phase of the samples at 30-min intervals in a temperature-controlled and shaking system (Stuart orbital incubator S1600). The study was concluded after 180 min. Samples were taken from the upper phase at 30-min intervals and centrifuged at 4500 to examine dye degradation.

The Effect of pH Values on the Color Removal of CR

In the evaluation of dye removal studies, the effects of pH on adsorptive removal of CR by PLE@FeNPs were performed in the pH range 2–8. There was over 90% removal in all dye concentrations and pH ranges studied (except pH 2). Results of scanning performed at 25 °C with 15 mg adsorbent and 50 ppm dye concentration in the range pH 2–8 showed the highest dye removal occurred at pH 8 (Fig. 2a). In a previous study, highest dye removal activity was observed at pH 3 in studies performed with Fenton reaction as the highest production of H_2O_2 was obtained at this pH [57]. The removal of CR using synthesized PLE@FeNPs is strongly affected by pH as pH greatly influences to the charging behavior of PLE@FeNPs. However, when the pH is above 3.5, the system is controlled by $Fe(OH)_3$ [58]. In Table S1, the effectiveness at different pH values is given for Fe^{3+} (ferric) species. In our study, the highest dye removal results were obtained at pH 8. Under this condition, the removal of the pollutant is mainly due to the coagulation or sorption process, not the Fenton process. In contrast, Fe^{3+} ions control the balance [59]. A comparison of the effects of different conditions on the adsorption removal of various reactive dyes by nanoparticles is summarized in Table S1.

Since CR is an isoelectric point of 3.58, it is completely negatively charged at high pH. The main dye removal mechanism between pH 7–10, which we have mentioned above, is due to sorption. In this case, we consider that together with an electrostatic interaction where the positive charges of Fe^{2+} and Fe^{3+} attract the dye molecules towards the ions, porosity in the nanoparticles and the role of plant surfactants are helping to hold the dye molecules, and thereby, some of them are degraded. It is possible that the adsorption by the iron nanoparticle system and the associated functional groups occurs by electrostatic attraction due to the groups that migrate over the plant to the iron nanoparticle at pH 8 (Fig. 2b). In the study carried out for CR dye removal of iron nanoparticles, 90% dye removal was achieved at a dose of 500 mg of $150 \mu g L^{-1}$ 500 mg of dye initial concentration at 25° temperature [60]. In our study, this removal percentage was obtained at higher dye concentration and much lower adsorbent dose.

The Effect of Initial Dye Concentration Change on Biosorption

The effect of dye concentration (20, 50, 100, 200 ppm) on dye degradation was investigated in the presence of sunlight and Fenton's reagent at pH:8. And the maximum degradation was achieved at a dye concentration of 50 ppm. All doses of PLE@NPs demonstrated effective dye removal at the studied concentrations of 20 and 50 ppm. However,

we observed a decrease in the dye removal percentage at higher dye concentrations, even at the lowest dose of PLE@NPs (5 mg). The increasing concentration of the dye shows that the ratio of accessible active regions of the low-dose PLE@NPs to the dye molecules decreases. The increased dye molecule adsorption on the surface of PLE@NPs and a decrease in light intensity may cause a reduction in the dye removal rate. It has been reported that the efficiency of color removal gradually decreases with an increase in the initial dye concentration, and this situation has been interpreted as the interaction between the dye and OH [57]. The increase in dye concentration (10–200 mg/mL) leads to an increase in the amount of adsorbed CR per unit mass of PLE@FeNPs; therefore, with an increase in dye concentration (q_e , 30.11–111.32 mg/g), more dye molecules are transferred to the adsorbent surface.

Effect of Adsorbent Dose

The present investigation assessed the degradation of CR dye and PLE@NPs by altering the adsorbent dose to 5, 10, 15, and 20 mg of PLE@NPs, within a 50 ppm concentration of CR dye and a volume of 10 mL. The studies were performed under circumstances of pH 8. The degradation of CR dye and PLE@NPs escalated concurrently with the augmentation of adsorbent dosage. With the increase in adsorbent dosage, both the surface area and active sites expanded, resulting in a greater percentage of CR adsorption. The condition has peaked at 15 mg (Fig. 2b). There was 99% dye removal in the presence of 15 mg PLE@FeNPs in 50 ppm dye concentration at 25 °C. In another study, adsorption efficacy of iron oxide nanoparticles for CR is significantly superior at 97% compared to commercial α -Fe₂O₃. This situation has been ascribed to the extensive surface area offered by the nanoparticle (60). In our study, 99.9% dye removal was achieved in a shorter time (180 min) at a lower adsorbent dose. The increase in dye concentration (10–200 mg/mL) leads to an increase in the amount of adsorbed CR per unit mass of PLE@FeNPs; therefore, with an increase in dye concentration (q_e , 30.11–111.32 mg/g), more dye molecules are transferred to the adsorbent surface. As the amount of PLE@FeNPs increased during the reaction, the surface area increased, thus, in the active and adsorptive regions. Therefore, dye removal increases depending on the adsorbent dose increase [61]. Zeta potential measurement is of great importance to understand aggregation processes as it prevents aggregate formation. It is thought that the adsorption capacity is 136.79 mg/g as a result of the particles becoming aggregates due to the low zeta potential value (−12.2 mV) (Table 1).

Adsorption Isotherms, Adsorption Kinetics, and Thermodynamic Analysis

There are studies on isotherm and kinetic models for the removal of CR dye from wastewater with nanoparticles produced by green synthesis approaches [60, 65, 67]. When the Congo red dye removal results using the biosorbent (PLE@FeNP) are compared in terms of correlation coefficients according to isotherm modeling, it could be concluded that the Langmuir isotherm ($R^2=0.96$, Fig. 3a) is the best among the mean values of R^2 , followed by the Freundlich isotherm ($R^2=0.95$, Fig. 3b, Table S2). The Langmuir model assumes that CR adsorption on PLE@FeNPs occurs predominantly as a monolayer with a homogeneous dye molecule distribution on the surface; all adsorbed substances have the same adsorption energy and do not move on the solid surface. On the other hand, Freundlich isotherm is an experimental model used to describe multilayered adsorption. This model assumes that possible interactions occur between adsorbed groups in heterogeneous surface regions with different energies. In addition, the Langmuir separation factor (R_L value) indicates the shape of the isotherm. In the current study, R_L was determined to be 0.17 (Table S2). According to McKay [68], R_L values between 0 and 1 indicate appropriate absorption; that is, the process is favorable. The high values of K_F and n in the Freundlich isotherm indicate easy separation of iron nanoparticles from wastewater and high adsorption capacity. The fact that the n value, which is related to the distribution of ions bound to the adsorbent surface, is positive for the adsorption of the nanoparticle indicates that the adsorption is favorable (Table S2).

Kinetic analyses were performed to obtain the rate of biosorption, which determines the contact time required for biosorption of CR with iron nanoparticles. Kinetic analyses were examined according to the pseudo-first-order (Fig. 3d) and pseudo-second-order models (Fig. 3e). The biosorption kinetics of the dye material used in our study was observed to be more compatible with the pseudo-second-order kinetic model ($R^2=0.99$, Fig. 3d) at 25 °C. When the model that best represents the adsorption kinetics is the pseudo-second-order kinetic model, the step that controls the rate of the adsorption process is the adhesion to the adsorbent surface. In addition, the estimated maximum adsorption capacity for CR adsorption in the current study ($q_{e\text{ cal}}=79.11$; $q_{e\text{ exp}}=77.10$ mg/g) was slightly higher than the experimental maximum adsorption capacity (Table S2).

Thermodynamic analyses determine the nature of the adsorption process (exothermic/endothermic). They help to distinguish the chemical and physical reactions during adsorption [69]. Evaluations of the thermodynamics showed that the reaction occurs spontaneously ($\Delta G=-26.6$ kJ mol^{−1}), and it is exothermic ($\Delta H=-35.3$ kJ mol^{−1}); the results are given in Fig. 3f and Table 2. A value of $\Delta H^\circ < 0$ indicates

Table 1 Comparison of degradation efficiency of various FeNPs and reactive dyes

Plant nanoparticle	Experimental adsorption conditions	Dye	Adsorption capacity (mg/g)	Reference
GT-FeNP Green tea	<i>pH (no pH control)</i> <i>Initial concentration 50 mg L⁻¹</i> <i>at room temperature</i> <i>5 min (80% dye removed)</i>	Methylene blue (MB) Methylene orange (MO)	–	[62]
FONPs <i>P. pterocarpum</i>	<i>pH (no pH control)</i> <i>Initial concentration 50 mg L⁻¹</i> <i>220 min (90% dye removed)</i> <i>at 150 rpm</i> <i>Dosage: 50 mg</i>	Methylene blue (MB)	–	[63]
FeNPs (<i>T. foenum-graecum</i>)	<i>pH (no pH control)</i> <i>Initial concentration 25.0 mg L⁻¹</i> <i>90 min (95% dye removed)</i> <i>Dosage: 30 mg</i>	Methylene orange (MO)	–	[64]
Iron oxide (-Fe ₂ O ₃)	<i>pH (no pH control)</i> <i>Initial concentration of 100 mg L⁻¹</i> <i>At room temperature</i> <i>300 min (97.9% dye removed)</i> <i>Dosage: 60 mg</i>	Congo red (CR)	253 mg/g	[65]
TW-FeNPs (waste tea)	<i>pH 8.0, 25 °C</i> <i>Initial concentration of 10 mg L⁻¹</i> <i>300 min (98% dye removed)</i> <i>Dosage: 0.1 g, at 100 rpm</i>	Phenol red (PR)	227.3 mg/g	[66]
FeNPs (<i>Datura inoxia</i>)	<i>pH 2.0</i> <i>35 °C</i> <i>Initial concentration of 10 mg L⁻¹</i> <i>75 min (98% dye removed)</i> <i>Dosage: 0.1 g</i> <i>At 150 rpm</i>	Methylene orange (MO)	81.967 mg/g	[61]
Iron CNPs	<i>pH 7.0</i> <i>25 °C</i> <i>Initial concentration of 150 µg L⁻¹</i> <i>25 min (90% dye removed)</i> <i>Dosage: 0.5 g</i>	Congo red (CR)	43.10 (µg/g)	[60]
PLE@FeNPs (<i>P. lanceolata</i>)	<i>pH 8.0</i> <i>25 °C</i> <i>Initial concentration of 50 mg L⁻¹</i> <i>180 min (99% dye removed)</i> <i>Dosage: 20 mg</i> <i>At 150 rpm</i>	Congo red (CR)	136.79 mg/g	Present study

that this process is exothermic and low temperatures are in favor of adsorption, while negative ΔG° values indicate that adsorption occurs spontaneously [70, 71].

Cell Proliferation Analysis

The cytotoxic effects of PLE@FeNPs and *P. lanceolata* extract at different concentrations were studied against A549 and SH-SY5Y cell lines (Fig. 4a and b, respectively). The A549-cell line was studied due to the beneficial effects of the *P. lanceolata* plant on lung diseases. Despite the extensive use of iron oxide nanoparticles in industrial fields, there is still a lack of information regarding their neurotoxic effects on the nervous system. To determine the neurotoxic effects

of iron oxide on the neuroblastoma cell line, the SH-SY5Y cell line was examined.

PLE@FeNPs and *P. lanceolata* extracts reduced cell viability 71.8% and 65.6%, respectively in A549 cancer cell line at 500 µg/mL concentration, and 84.4% and 82%, respectively, at 1000 µg/mL concentration. The viability of A549 cancer cells decreased with an increasing concentration. The cytotoxic effect of PLE@NPs may be due to some polyphenolic compounds that pass from the leaves of *P. lanceolata* to the aqueous fraction. The FT-IR measurement indicates that these groups have migrated from the leaves to the nanoparticle system (Fig. 1b). In a study, it was reported that the extract of *P. lanceolata* completely inhibited MCF-7 cancer cells [72]. It has shown

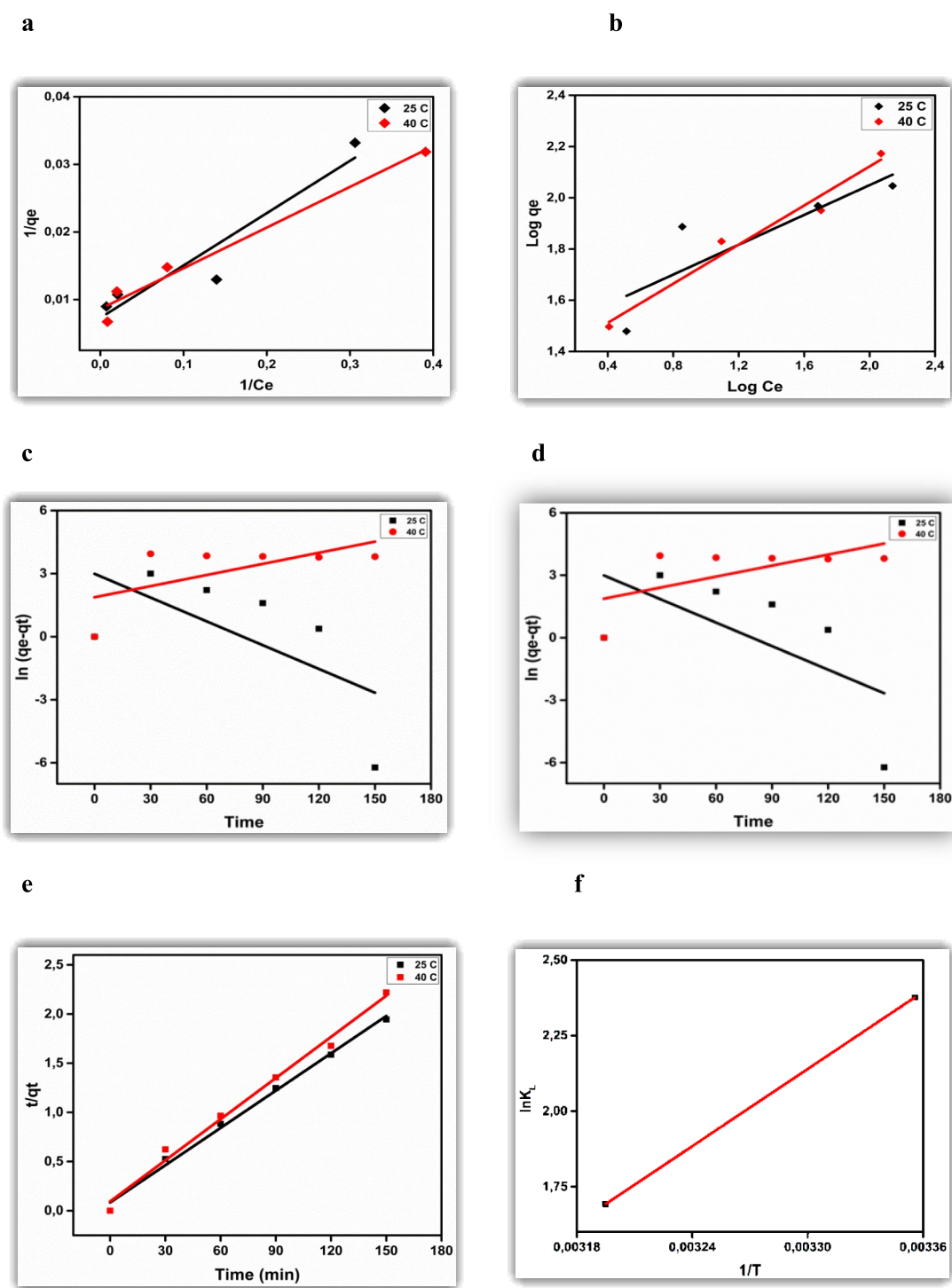


Fig. 3 Plots of **a** Langmuir isotherm, **b** Freundlich isotherm, **c** Temkin isotherm, **d** pseudo-first-order, and **e** pseudo-second-order plots for CR removal. **f** Graph showing the relationship between $\ln K_L$ and $1/T$ for CR removal with PLE@FeNPs

Table 2 Thermodynamic parameters of CR adsorption onto the PLE@FeNPs

Adsorbent	Temp (K/°C)	K_L	ΔG° (KJ mol ⁻¹)	ΔH° (KJ mol ⁻¹)	ΔS° (KJ ⁻¹ mol ⁻¹)	R^2
PLE@FeNPs	298/25	10.765	-26.671	-35.3422	-98.8414	1.000
	313/40	5.435	-14.140			

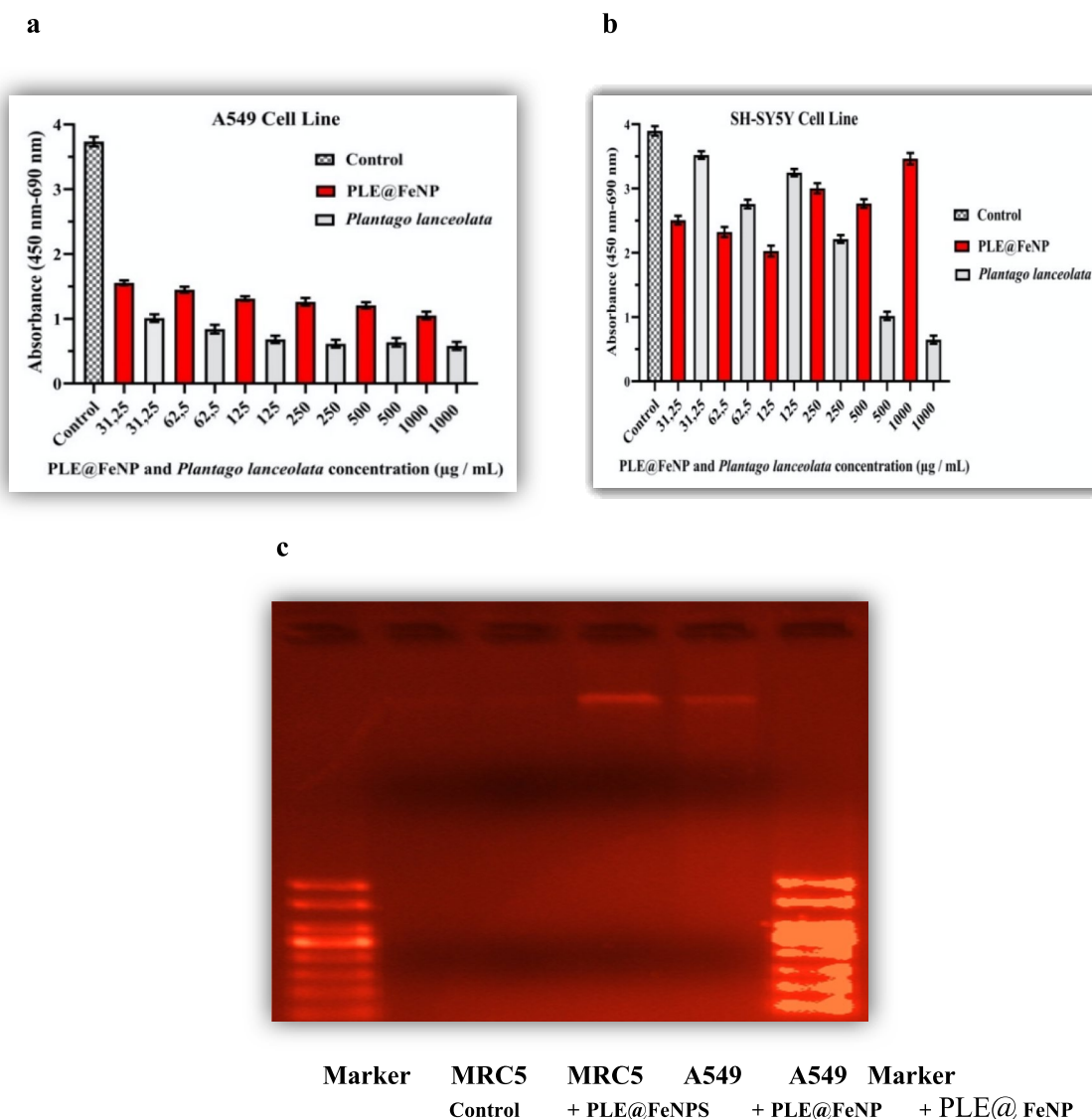


Fig. 4 **a** Graphs showing viability in cell lines A549, **b** SHSY5Y after treatment with PLE@FeNPs and *P. lanceolata* plant water extract. Cell survival rates at concentrations obtained with serial dilu-

tions in the range of 1000–31.25 µg/mL are given, **c** DNA fragmentation analysis of A549 and MRC-5

a similar effect in A549 cell line as well. For chemotherapy, nanoparticles must be absorbable by cells at a sufficiently high rate and extent. It has been suggested that the size of the particles plays an important role in their adhesion to biological cells and their interactions with them. PLE@NPs have shown a significant level of cytotoxicity in the A549 cell line. This situation indicates that PLE@NPs are of an appropriate size (8 nm) to pass through the cell. The concentration and size of nanoparticles play an important role in inducing toxicity. Nanoparticles interact with NADPH oxidases in the cell membrane and mitochondria to form superoxide anions. This causes cytotoxicity by activating redox reactions [51]. While *P. lanceolata* extract showed 83.3% and 73.8% neurotoxicity

against SH-SY5Y cell line at 1000 µg/mL and 500 µg/mL concentrations, respectively, the neurotoxic effect was lower at other concentrations tested. However, the results of the study conducted with MTT showed that PLE@NPs nanoparticles are not toxic in SH-SY5Y cell lines. The main reason for the widening gap between the neurotoxic activity of the extract and the increasing concentration in SHSY-5Y neuroblastoma cell lines is that the extract showed almost the same results between 31.5 and 125 µg/mL. However, at concentrations above 125 µg/mL, the increase in the amount of oxygen present in the nanoparticle system's structure may have triggered cell division as a factor providing energy metabolism necessary for the proliferation of neuroblastoma cells.

Nanoparticles can be transmitted to the blood system through the digestive system in the body and then reach the liver, brain, and other organs. Therapeutic drugs can be administered to the brain and other organs through nanoparticles [18]. There was no study investigating the effect of PLE@FNPs on neuroblastoma cell line in the literature. The lack of significant cell toxicity [73] in a different study examining the neurotoxicity effect of MgO nanoparticles on the SH-SY5Y cell line coincides with the results of our study. The entry of nanoparticles into cancer cells is influenced by their size and shape, which allow them to penetrate cells through pores [7].

DNA Fragmentation Assay

The DNA fragmentation assay was carried out to examine the impacts of PLE@FeNPs on A549 cells. The results of our study demonstrated that PLE@FeNPs cleaved nuclear DNA of human A549 cells by forming ladder pattern as illustrated in Fig. 4c. The control cells (MRC5) have not shown any fragmented DNA.

Antioxidant Activity and Total Phenolic and Flavonoid Content

Total DPPH radical scavenging, metal chelation, and total antioxidant of the water extract obtained from *P. lanceolata* are given in Fig. 5a and b, respectively. DPPH removal activity of *P. lanceolata* plant extract was calculated as $78.86 \pm 0.65\%$, and DPPH removal activity of PLE@FeNPs was calculated as $89.79 \pm 0.21\%$, respectively. According to BHA, the DPPH activity of PLE@NPs and *P. lanceolata* was found to be significant ($p < 0.05$). Phenolic and flavonoid compounds are strong radical scavengers and have high reducing power [74]. Thus, the high rate of DPPH radical scavenging of *P. lanceolata* can be attributed to the presence of these compounds in the extract. PLE@FeNPs also achieved a high rate of radical removal. This could be interpreted as the fact that 983, 1578, and 1726 cm^{-1} peaks in the FT-IR spectrum of PLE@FeNPs originate from C = C ring polyphenol (Fig. 2b). Organic groups on the surface of nanoparticles were effective in radical removal. Many studies have shown that iron nanoparticles have a high DPPH radical scavenging activity [75, 76].

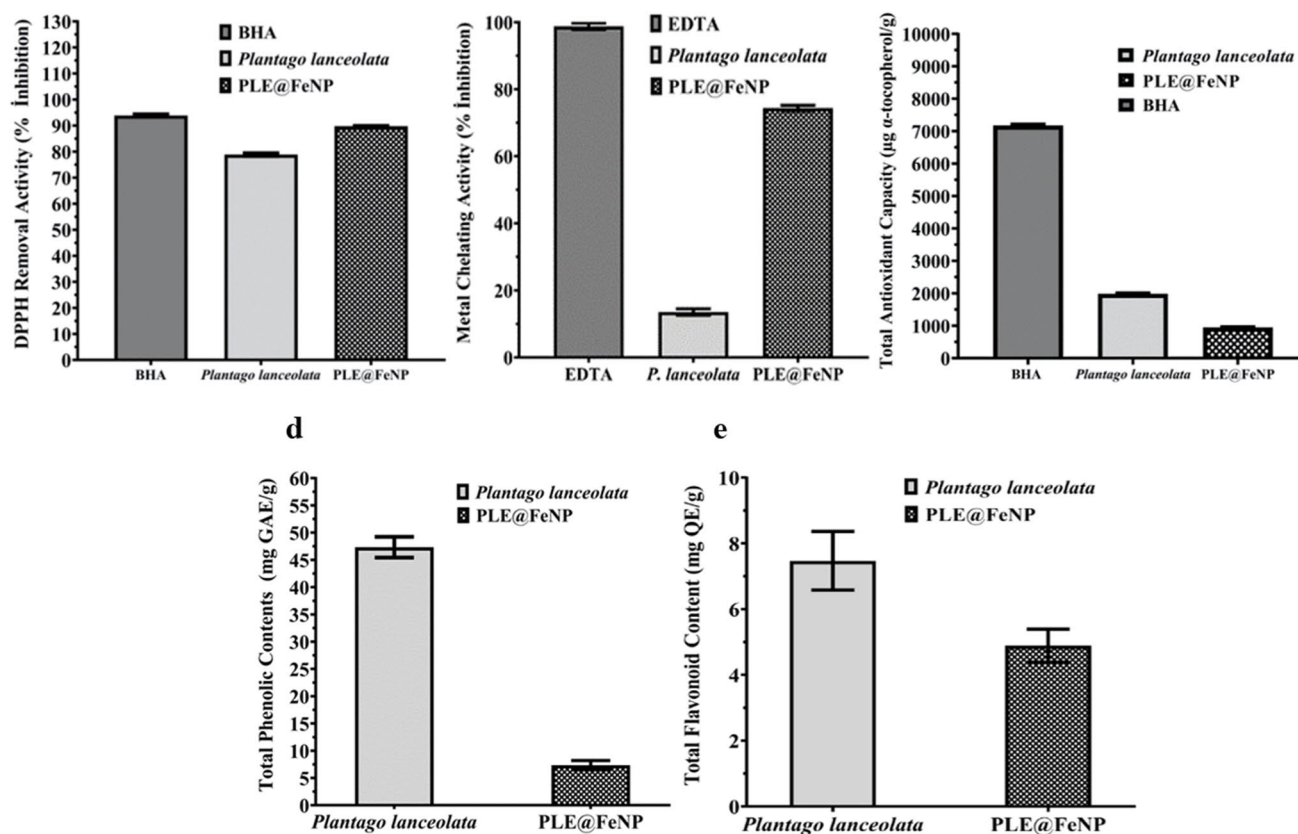


Fig. 5 **a** DPPH removal activity (% removal), **b** metal chelating activity (% removal), **c** Total antioxidant activity (µg α-tocopherol/g), **d** total phenolic content (mg gallic acid/g), and **e** total flavonoid content (mg quercetin/g) (data mean ± SD of the studies; $n = 3$, p -value < 0.05)

The total antioxidant activity of the water extract of *P. lanceolata* and PLE@FeNPs was $31,290.91 \pm 190.07$ and 3624.24 ± 150.04 μg α -tocopherol/g, respectively (Fig. 5c). The total antioxidant activity of *P. lanceolata* plant extract was higher than that of PLE@FeNPs. PLE@FeNPs showed strong metal chelating potential compared to EDTA as a reference. This arises from the synergy between iron metals and functional groups. The metal chelating activity of the *P. lanceolata* plant extract was calculated as $13.52 \pm 0.97\%$ and for PLE@FeNPs at $74.39 \pm 0.78\%$. The ranking for metal chelating activity was EDTA > PLE@FeNPs > *P. lanceolata*, respectively. According to EDTA, the metal chelation activity of PLE@NPs and *P. lanceolata* was found to be significant ($p < 0.05$). Bahadori et al. [74] reported that the best iron chelation was in the water extract (95%) in their study, where they calculated the metal chelating activity of different fractions of *P. lanceolata*. The total phenolic and total flavonoid contents of the water extract obtained from *P. lanceolata* are given in Fig. 5d and e, respectively. The total phenolic content of *P. lanceolata* water extract and PLE@FeNPs was calculated as 47.32 ± 1.90 and 7.35 ± 0.85 mg GAE/g, respectively, and total flavonoid content was 7.47 ± 0.89 and 4.89 ± 0.50 mg QE/g, respectively. PLE@FeNPs were observed to have a lower phenolic and flavonoid content than the *P. lanceolata* plant extract. The phenolic and flavonoid contents found in the plant extract are in agreement with previous studies [77]. Beara et al. [78] reported phenolic contents ranging from 38 to 70 mg GAE/g and flavonoid content ranging from 6 to 12 mg QE/g for five different *Plantago* species. Since phenolic and flavonoid compounds have functional properties and an important role in the green synthesis of nanoparticles, it may be useful to determine their contents. The presence of phenolic and flavonoid compounds may correspond to the extracts used in the biosynthesis of nanoparticles [79].

Antimicrobial Activity

Minimum inhibition concentration (MIC) values of *P. lanceolata* L. plant extract and PLE@FeNPs are shown in Table 3. MIC values for microorganisms tested with *P. lanceolata* extract are as follows: *K. pneumoniae* EMCS, 0.3125 mg/mL; *E. coli* ATCC® 25,922TM, 5 mg/mL; *S. cerevisiae*; and *S. aureus* ATCC® 29,213TM, 0.625 mg/mL (Fig. S3). In studies which determined the MIC value of

P. lanceolata extract on gram-positive *Streptococcus* species, the MIC values for *P. lanceolata* extract were obtained at concentrations of 0.5–1 mg/L, although their sensitivity varied significantly [80]. On the other hand, in the study of Sharifa et al. [81], which determined the effectiveness of *P. major* extracts in gram-positive and negative bacteria, the researchers reported that the *P. major* extract caused significant changes in the cell wall structure of gram-positive bacteria, whereas its effectiveness was low in gram-negative bacteria. In our study, the most resistant strain was determined to be gram-negative *E. coli* with a concentration of 5 mg/mL. The MIC values for microorganisms tested with PLE@FeNPs was 0.625 (mg/mL) for *K. pneumoniae* EMCS, *E. coli* ATCC® 25,922TM, and *S. cerevisiae*; it was 0.3125 (mg/mL) for *S. aureus* ATCC® 29,213TM. In this study, it is clear that the nanoparticle's high antioxidant activity contributes to a better antimicrobial profile. This contribution may be related to the content of phenolic compounds as well as the presence of iron in the nanoparticle. Smaller sizes can easily pass through bacterial protective barriers and reach the depths of the microorganism to cause greater harm. In the study, the antimicrobial efficacy of PLE@NPs can be attributed to their very small size. Small PLE@NPs (8 nm) penetrate cell walls more effectively, increasing antibacterial activity. FeO₄ nanoparticles, produced via the green route, generate more reactive oxygen species, contributing to their antibacterial properties [5]. Some other studies reported that nanoparticle concentration has a significant effect on the inactivation of microorganisms [82, 83]. It has been suggested in the literature that nanoparticles slow the mechanism of antimicrobial activity in two ways. The first mechanism of action is disturbances in the cell structure by the accumulation of nanoparticles in the cytoplasm, and the second mechanism of action is the interaction of the nanoparticles with oxygen radicals by passing into the cell after binding to the cell wall, subsequently creating oxidative stress [84]. The most important mechanism of action of antibiotics on microorganisms is that they kill microorganisms as a result of damage to DNA, protein, and cell membrane [85]. On the other hand, nanoparticles of 10–80-nm size penetrate the cell wall surface and interact with the oxygen inside the cell, causing oxidative stress. As a result, microorganisms are killed [86]. According to the results of the TEM analysis, the interaction of nanoparticles of 8 nm average size with the cell wall and the oxygen inside the cell showed

Table 3 Minimum inhibitory concentration (MIC) values for antimicrobial activity

	Microorganism	PLE@FeNP (MIC)	<i>P. lanceolata</i> (MIC)
A	<i>Klebsiella pneumoniae</i> ; EMCS	0.625 (mg/mL)	0.3125 (mg/mL)
B	<i>Escherichia coli</i> ; ATCC® 25,922 TM	0.625 (mg/mL)	5 (mg/mL)
C	<i>Saccharomyces cerevisiae</i> ATCC® 76,521 TM	0.625 (mg/mL)	0.625 (mg/mL)
D	<i>Staphylococcus aureus</i> ; ATCC® 29,213 TM	0.3125 (mg/mL)	0.625 (mg/mL)

a high efficiency on the oxidative stress of microorganisms. While iron oxide nanoparticles obtained by green synthesis from *Carica papaya* showed moderate antibacterial activity against bacterial strains of *Klebsiella* spp., *E. coli*, *Pseudomonas* spp., and *S. aureus* at 50 mg/mL [87], in the present study, an effective antimicrobial activity was observed at very low concentrations (0.3125 mg/mL) (Table 3 and Fig. S3). Analyses of the antibacterial properties of PLE@FeNPs showed that the greatest effect was observed in *S. aureus* compared to other microorganisms [79] and attributed the antimicrobial activity they obtained against similar microorganisms to the small size of the nanoparticles. Iron oxide nanoparticles are considered compatible with biological systems and have numerous biomedical applications. It has been reported that microorganisms have decreased their resistance capacity against iron oxide nanoparticles. This supports the use of iron oxide nanoparticles in biological systems for this purpose, but their side effects in the system should also be questioned [88].

Conclusion

The textile industry's wastewater is significantly more polluting than other industrial sectors, primarily due to dyes in composition. In this study, the removal of CR dye in wastewater with PLE@NPs generated through green synthesis was explored and the results were achieved. The morphology of the adsorbent was investigated using XRD, SEM, BET, zeta potential, and EDX. In the study, the best results were obtained at a pH of 8 and a temperature of 25 °C, which are close to the natural temperature and pH range of water. Additionally, in all dye concentrations studied above 25 °C and pH 6, the removal rate was over 90%. Therefore, the variation of the water's pH between 6 and 8 will not significantly change the removal percentage. These data were obtained in daylight without the need for an additional heat source. Additionally, Fenton's reagent can be used as an effective material for the degradation of pollutants. The mixture of PLE@NPs and H₂O₂ can break the azo bond (–N=N–) in CR, causing the color of the dye solution to lighten. This situation has made them potentially useful for environmental remediation. Because in this mechanism, as a result of the interaction of nanoparticles with the dye, the structure of the CR dye is degraded and the nanoparticles can be reused in the system. PLE@FeNPs showed bactericidal and antioxidant activities. The cytotoxicity experiments demonstrated that PLE@FeNPs possessed significant cytotoxic effects in human lung cancer (A549) cell line. DNA fragmentation result also supports the cytotoxicity of PLE@FeNPs on A549 cell line. In the SH-SY5Y cell line, which we used to evaluate the neurotoxicity of PLE@NPs, very little neurotoxic effect was observed. Overall, these results urged

further applications of PLE@FeNPs in CR dye removal and biological applications. The synthesis of PLE@FeNPs using *P. lanceolata* plant extract with the green synthesis method has been offered as an alternative to traditional methods, considering the advantages such as low cost, non-toxicity, environmental friendliness, and abundance of iron and the *P. lanceolata* plant.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval Ethics approval not required.

Conflict of Interest The authors declare no competing interests.

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