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CHLOROPHYTUM COMOSUM-MEDIATED IRON NANOPARTICLES: AN ECO-FRIENDLY APPROACH FOR ANTIMICROBIAL AND DYE DEGRADATION APPLICATIONS

KAYANI REZ[1*], RUSTAM A[1], SALEEM U[1], BILAL M[1], BAKHTIAR M[1], JAMSHAID S[1], SOHAIL M[2], FAROOQ K[3], KHAN S[4], REHMAN B[2*]

[1]Department of Allied Health Sciences, Molecular Biology Laboratory, Iqra National University (INU) Peshawar, 25000 Peshawar, Pakistan
[2]Department of Health and Biological Sciences, Iqra National University (INU) Peshawar, 25000 Peshawar, Pakistan
[3]Department of Pharmacy, Capital University of Science and Technology Islamabad, 4550 Islamabad, Pakistan.
[4]School of Resources and Environmental Engineering, East China University of Science and Technology Shanghai, 200237, China
**Correspondence Author Email Address: ridakayani04@gmail.com; bushraismael.ismael@gmail.com*

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Abstract *Green synthesis techniques are becoming more and more popular in nanotechnology because of their many advantages, which include great efficiency, affordability, and environmental friendliness. Here, iron nanoparticles (FeNPs) were prepared using the methanolic extract of Chlorophytum comosum leaf. The results were spherical and amorphous FeNPs, with a particle size of around 50 nm, as validated by SEM. Energy Dispersive X-ray Analysis EDAX revealed the presence of Iron (Fe) in the sample. The peaks in the FTIR spectrum of aqueous extract of C.comosum at 3367.15cm, 2076.08cm, 2162.58cm, 1996.39cm, 2021.91cm, 475.58cm, 456.57cm,456.58cm, 418.05cm, 430.34cm, 410.21cm were OH, Carbohydrates proteins and polyphenols, Silicon Compound, Alkene conjugated triple bond, Aromatics, Silicon Compounds and Cycloalkanes. Methyl Orange (MO) was successfully removed from the aqueous solution by the synthesized FeNPs. Using ultraviolet-visible (UV-Vis) spectroscopy, it is simple to monitor the concentration of MO while using FeNPs in the presence of H_2O_2. After 6 hours, the synthesized FeNPs showed an 83% MO degradation efficiency. Additionally, several Gram-positive and Gram-negative bacteria as well as fungus species including Aspergillus were used to test the antibacterial and antifungal properties of these FeNPs. The results indicate that FeNPs have a bactericidal effect on both Gram-negative Pseudomonas aeruginosa (the zone of inhibition is 11mm to 16mm) and Gram-positive Staphylococcus epidermidis (the lowest zone of inhibition is 12mm and the greatest is 18mm). Additionally, the range of Penicillium's zone of inhibition is 12 mm to 17 mm, while the lowest and greatest zones of inhibition against Aspergillus Niger are 17 mm and 20 mm, respectively. The substantial bactericidal effect of these INPs on both Gram-positive and Gram-negative bacteria as well as on fungi was demonstrated by the results of their antibacterial and antifungal activity. All things considered, ecologically friendly FeNPs can be a good option for several scientific domains, especially the elimination of organic dyes and the eradication of germs and fungus.*

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Keywords: *iron nanoparticles (FeNPs); Chlorophytum comosum; Methyl Orange (MO) degradation; SEM; ultraviolet-visible (UV-Vis) spectroscopy; EDAX; FTIR*

Introduction

Plants have been applied in diverse fields in biological, clinical, and medicinal science. In recent years, plant extracts, as templates for nanoparticle synthesis have become popular among researchers, and different parts of the plant like leaf extract, stem, root, flower, seeds, etc. are employed for synthesizing nanoparticles. Biomarkers are used in several fields of bioengineering sciences (Khatami *et al*., 2019). The various bio-activities of the Secondary metabolites generated in medicinal plants can be regarded as potent markers in the synthesis of nanoparticles. Metal NPs including gold, iron, nickel, copper, and silver NPs are used in different scientific sectors including medicine, physics, and chemistry (Cao *et al*., 2021) therapeutic use of metal NPs has been documented against several bacterial, viral, and fungal pathogens (Akbari *et al*., 2020). Green syntheses or green methods were more specifically defined as the synthesis of metal NPs using plants or plant parts or using plant extracts only and an alternative to chemical and physical ones. Green synthesis is a new growing area in both the interdisciplinary fields of biotechnology and

nanotechnology and yields huge amounts of economic and environmental gains. However, green synthesis has some limitations, including longer process time and repeatability issues; however, this approach is very much needed to prevent the formation of unwanted or toxic byproducts during the construction of robust, sustainable, and environmentally friendly synthesis techniques. The development of Green synthesis methods for Nanoparticles (NPs) has focused on the reduction of wastes produced and the application of environmentally friendly processes (Gopalakrishnan *et al*., 2021).

A fast rate of nanoparticle synthesis was observed, and thus the use of plants over microorganisms for the biosynthesis of metal nanoparticles through greener and safer approaches. In the subsequent sections, we have explained the synthesis process of iron nanoparticles based on color changes, changes in pH, changes in UV-Vis absorbance, and the size of the particle formed after reduction (Dhuper *et al.*,2012). A large number of disciplines have used metallic nanoparticles in a rather active manner. The use of innovations based on nanostructured materials has been applied in several fields, including chemistry and pharmaceuticals, under the close connections between forms, dimensions, and compositions of metallic nanoparticles, as well as their physicochemical and optical characteristics. These three approaches are being used for the development of nanomaterials using physical chemical and biological techniques (Lee & Jun, 2019). Iron the most common of the transition metals and the fourth most abundant metal on earth is the framework of most of our modern Built environment. Iron as a nanoparticle has therefore been somewhat left unexplored in favour of its oxides and other metals such as cobalt, nickel, gold, and platinum. Iron on the other hand has a lot to bring in at the nanoscale such as very efficient magnetic and catalytic characteristics. Some recent work has been done to exploit iron and it seems that this line of work is picking up momentum (Huber, 2005). *C. comosum* (spider plant) is among 120 plant species that have been tested on phytoremediation of pollutants in indoor air (Gawronska *et al*.,2013) There is a wealth of literature on particulate matter uptake from the air by outdoor-growing plants, a possible reason being the upsurge of the pollutant's detrimental effect on human health and the environment. Nevertheless, there is scant information on plant efficiency in cleaning up the particulate matter (PM) of indoor air (Gawrońska & Bakera., 2015). To date, relatively little is known about the therapeutic effects of the plant C.comosum. It is proved that the leaves of the plants have high sorption characteristics concerning such intercalation with carbon monoxide, trichloroethylene, phenols, and other reagents. When it examined the enzymatic hydrolysate chemical composition of *C. comosum* it was discovered ornithine monohydrochloride had disintoxicational and hepatoprotective activity. These facts enable us to regard this hydrolysate as a biologically active substance exhibiting hepatoprotective activity which is why authors can stay focused on the investigation of the action of the hydrolyzation and the regenerative capabilities of the mammalian liver (David *et al.*, 2013). The present work also discusses the antitumor activity of the Chlorophytum genus. The majority of investigations have used in vitro systems, the preparations of which are extracts or fractions of herbs, or isolated compounds. In general, butanol extract of C.comosum roots inhibited the proliferation and caused apoptosis in four investigated cell lines, of which three were leukemic. As it corresponds to the concentration of reagents used in the study, the extract's anti-proliferative effect on T-cell leukemia CCRF-HSB-2 cells was 7- to 8-fold higher than that of Actinomycin D (Rzhepakovsky *et al.*,2022). This study used *C. comosum*-based iron nanoparticles for the degradation of methyl orange dye in contaminated water and also checked its antibacterial and antifungal activity.

Methodology

Collection of Plant

Recent study we collected fresh *C.comosum* plants from an agriculture farm in Peshawar, Pakistan, and then confirmed by the Botany department at Peshawar University.

Plant Grinding

C. comosum plants were completely shade-dried and then ground in the Microbiology Lab of Abasyn University Peshawar.

Preparation of Plant Extract

A total 150 grams of powder of plants were soaked in 1000ml Methanol for 15 days at room temperature. After that, the extract was filtered through watman filter paper.

Green synthesis of Iron Nanoparticles (FeNPs)

We dissolved 8.1gm Fecl3 powder in 500 ml distilled water (0.1M) in a conical flask which also contains 40 ml plant extract and firmly stirred using a magnetic stirrer for 1 hour and left the extract overnight at room temperature to precipitate black pellets. The black pellets were separated by leaving the reaction media in a centrifuge (10,000 rmp for 10 minutes). Next, the pellets were washed with Distilled water thrice and then the pellets were placed in an oven at 50℃ for 24hrs.

Formula: x = 0.1 x 500 x 162/1000 = 8.1

Characterization of biosynthesized Iron nanoparticles (FeNPs)

To characterize green synthesized iron nanoparticles, we used various types of spectroscopic techniques. The green synthesized nanoparticles were confirmed by measuring their UV–vis spectroscopy. EDX, FT-IR, and SEM were used for further confirmation of nanoparticles (Alharbi *et al*., 2022).

Dye removal assay

In this procedure, 10 mg of prepared FeNPs and 1 ml H_2O_2 (optimum concentration) were added to a 25 ml

separated flask containing 8 ml of 25 mg concentration of MO and stirred with 150 rpm temperature at 50℃. The MO concentrations were determined at various times using a UV-Vis spectrophotometer. The ability to decompose H_2O_2 will also be checked as a sample without containing the NPs.

Biological assays

Antibacterial activity

Anti-bacterial potentials of C.comosum plant extract and green synthesized INPs were determined by the well diffusion method. To study the antibacterial properties of NPs, five organisms, both the Gram-negative organisms including Escherichia coli, *Pseudomonas aeruginosa*, and *Salmonella* and the Gram-positive organisms including Staphylococcus aureus and *Staphylococcus epidermidis* were chosen. The culture ability of bacteria was performed on Mueller–Hinton agar (MHA) at 37℃ for 18-24 hrs.

Antifungal activity

Antifungal activities of C.*comosum* plant extract and green synthesized FeNPs were identified by the well diffusion method. To examine the antifungal activity of FeNPs, two identified fungi species were utilized. Potato dextrose agar (PDA)was used for the culturing of fungal species at 28℃ for 2-3 days.

Data Analysis

The data analysis was performed using MS Excel Office and the mean and standard deviation of different parameters.

Results

Characterization of FeNPs synthesized from *C. comosum* leaf extract.

In the recent study, FeNPs were synthesized using plant-mediated synthesis through *C.comosum* leaf extract and characterized using UV vis, SEM, and FTIR spectroscopy. The kinetic behavior of FeNPs was analyzed in a UV-visible spectrophotometer to characterize FeNPs. The samples' scanning range, was set at 200 to 500 nm. UV–visible spectroscopy analysis indicated that the sample had absorbed energy at a wavelength of 360 nm which can be compared to a typical peak value for FeNPs. In addition, the absorption peak at 360nm without another peak shows the high purity of the nanoparticles as shown in Figure 1.

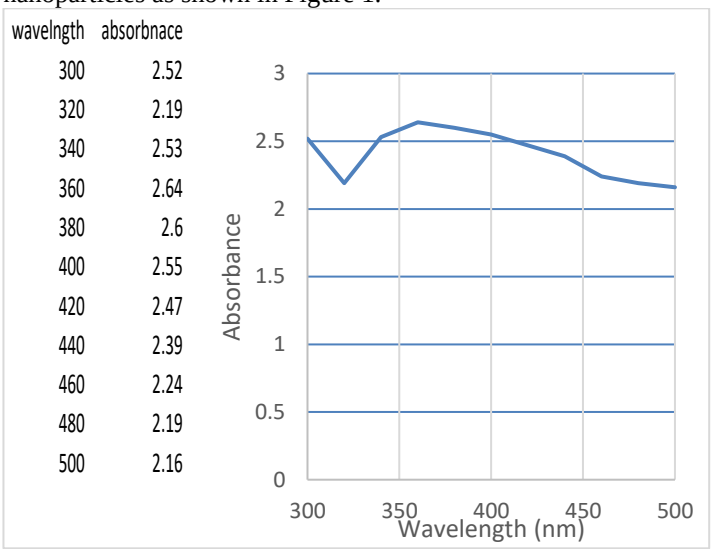


Figure 1. UV-Vis spectrum of synthesized FeNPs

Scanning Electron Microscope (SEM) was used to examine the surface morphology and to estimate the obtained structural rectangle, triangle, radial hexagonal, rod, and spherical shapes. SEM technique used in the present study revealed the particle size and external morphology of the FeNPs as shown in. The SEM analysis of iron nanoparticles was performed, and the results showed the average size of the iron nanoparticles was determined to be approximately 50 nm, with a relatively narrow size distribution. The nanoparticles exhibited a spherical morphology, and no significant agglomeration was observed. Additionally, the SEM images (Figures 2 &3) revealed a high degree of uniformity in the size and shape of the nanoparticles, indicating a successful synthesis process. The analysis also indicated that the iron nanoparticles displayed a smooth surface, suggesting a well-defined crystalline structure. Overall, the SEM analysis provided valuable insights into the physical characteristics of the iron nanoparticles, validating their potential applications in various fields, such as catalysis and magnetic materials.

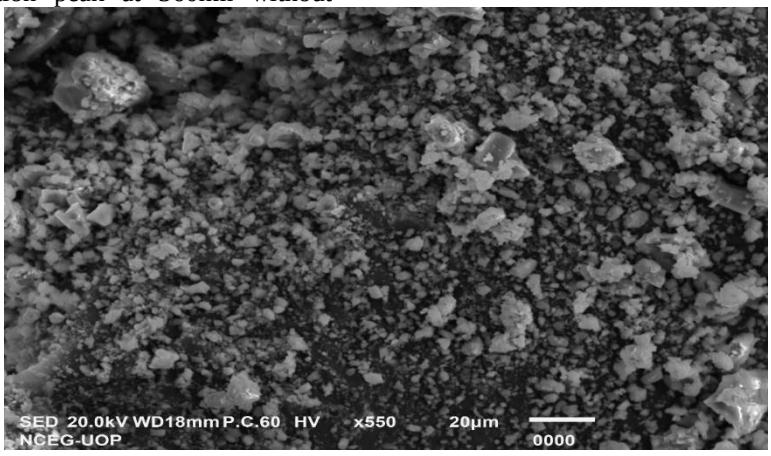
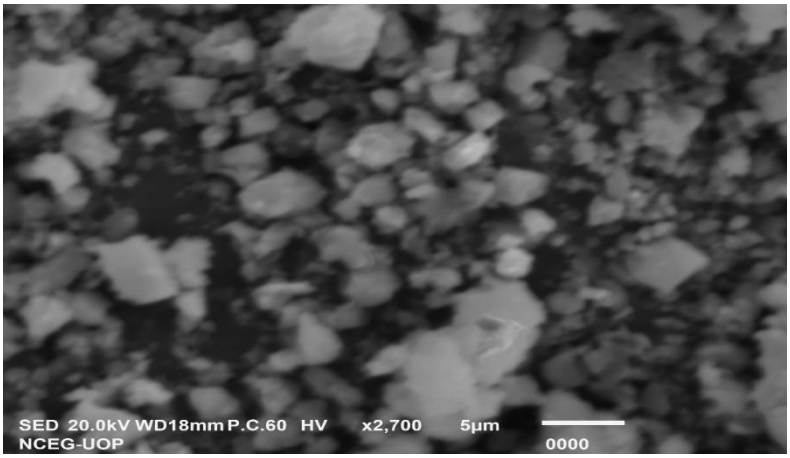


Figure 2. SEM of FeNPs



Figur 3. SEM of FeNPs

Energy Dispersive X-ray Analysis (EDAX) was used to measure the elemental composition of samples. The EDAX revealed that the required segment is present. Iron (Fe) is present in the sample as shown in Fig 04. The graph also displayed the presence of Carbon (C), Oxygen (O), Chloride (Cl), and Sodium (Na).

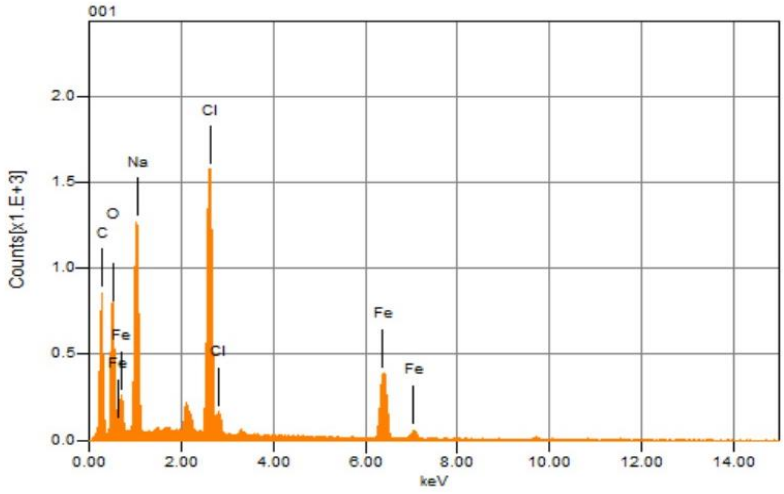


Figure 4. EDAX Spectrum of synthesized FeNPs.

Furthermore, the presence of biomolecules in the leaf extract of *C.comosum* which was responsible for the reduction and stabilization of the synthesized nanoparticles can be identified using FTIR spectroscopy. The FTIR spectrum of the leaf extract of *C. comosum* mediated synthesized FeNPs is shown in Fig 05.

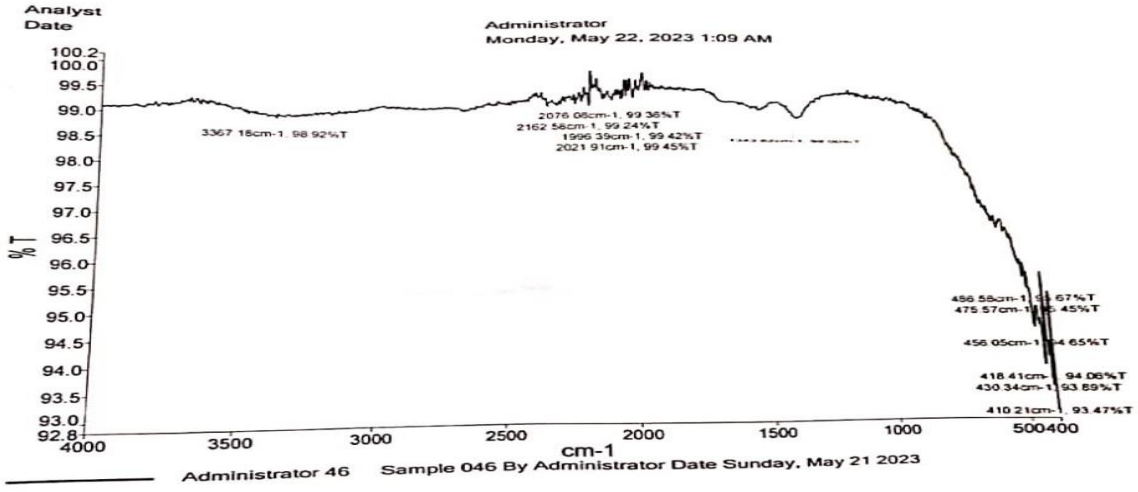


Figure 5. FTIR Spectrum of FeNPs

In the current study, the peaks in the FTIR spectrum of aqueous extract of *C.comosum* at 3367.15cm, 2076.08cm, 2162.58cm, 1996.39cm, 2021.91cm, 475.58cm, 456.57cm,456.58cm, 418.05cm, 430.34cm, 410.21cm OH, Carbohydrates proteins and polyphenols, Silicon Compound, Alkene conjugated triple bond, Aromatics, Silicon Compounds and Cycloalkanes.

Characterization of Methyl Orange Degradation

In the current study, during the above process, H_2O_2 has no activity even 6 hrs without FeNPs while with FeNPs MO was degraded and the color vanished almost completely with 83% within 6 hrs. The spectral band of MO after künctiron1ation with the aqueous mixture of FeNPs and H_2O_2 has been red shifted from 480 nm to 500 nm highest peak of FeNPs was observed around 480 nm with an absorbance value of 0.0484 after 15 minutes of incubation of the aqueous mixture of FeNPs and H_2O_2.

The synthesized FeNPs removed 66% of the MO initial concentration in the first 3 hrs. The reduction in color degradation was noticed up to the fourth hour and there was no considerable decrease in the subsequent two hours.

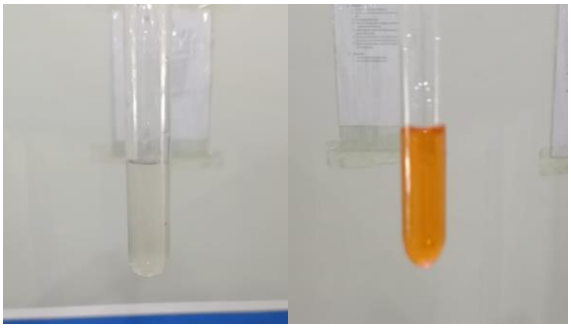


Figure 6. Methyl Orange dye without FeNPs. Methyl Orange dye with FeNPs

Anti-Bacterial activity of methanolic extract of *C.comosum* Plant.

In the current research study, three different concentrations 50 ul, 75 ul, and 100 ul were used to evaluate the activity of methanolic extract against identified bacterial organisms. such as (*Staphylococcus epidermidis, Pseudomonas aeruginosa, Staphylococcus aureus, E.coli* and *Salmonella).*It was observed that the activity at 50 ul concentration against *Pseudomonas aeruginosa* (10mm), *Staphylococcus epidermidis* (11mm), the zone of inhibition at 75 ul concentration against *Pseudomonas aeruginosa* (12 mm), *Staphylococcus epidermidis* (14 mm) and the zone of inhibition at 100ul concentration against *Pseudomonas aeruginosa* (15 mm), *Staphylococcus epidermidis* (17 mm). The plant extract shows resistivity against *Staphylococcus aureus, E. coli,* and *Salmonella* shown in Fig. 7

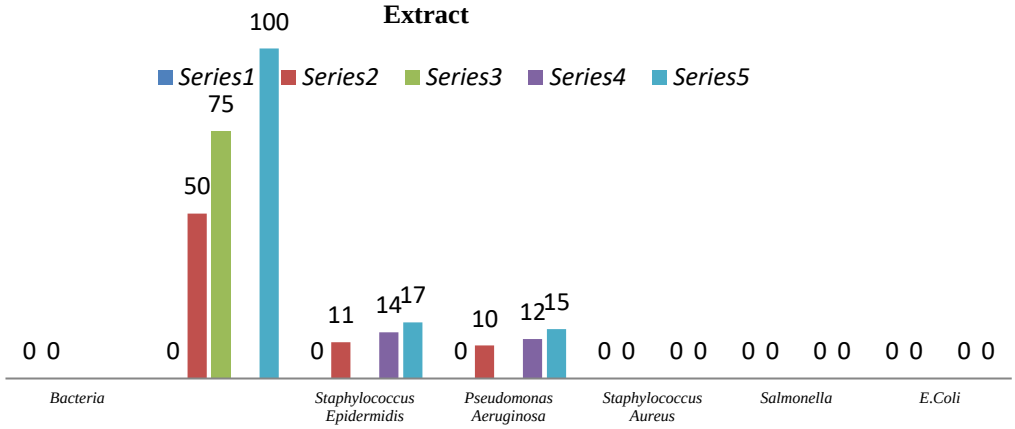


Figure 7. This graph shows the antibacterial activity of *C.comosum* extract.

Antibacterial activity of green synthesized FeNPs from leaf extract of *C.comosum*.

In the current research study, three different concentrations (50 ul, 75ul, and 100ul) were used to evaluate the activity of green synthesized FeNPs against identified bacterial organisms. such as (*Staphylococcus epidermidis, Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* and *Salmonella).* It was observed that the activity at 50 ul concentration against *Pseudomonas aeruginosa* (11mm), *Staphylococcus epidermidis* (12mm), the zone of inhibition at 75ul concentration against *Pseudomonas aeruginosa* (14mm), *Staphylococcus epidermidis*(15mm) and the zone of inhibition at 100ul concentration against *Pseudomonas aeruginosa* (16mm), *Staphylococcus epidermidis* (18mm). While the plant extract shows resistivity against *Staphylococcus aureus, E. coli* and *Salmonella* (Fig8)

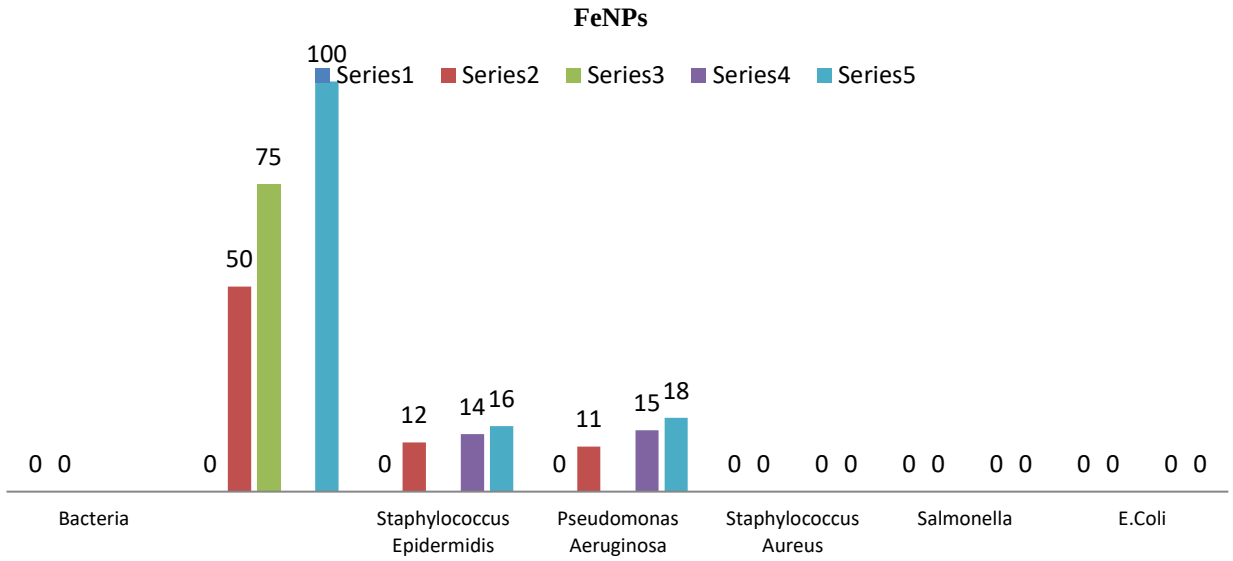


Figure 8. This graph shows the antibacterial activity of FeNPs

Anti-fungal activity of methanolic extract of *C.comosum*.

In the current research study, three different concentrations 50ul, 75ul, and 100ul were used to evaluate the activity of methanolic extract against identified fungal species such as *(Aspergillus Nigar* and *Penicillium).* It was observed that the activity at 50 ul concentration against *Aspergillus Nigar* (10mm) and *Penicillium* (11mm), the zone of inhibition at 75ul concentration against *Aspergillus Nigar* (11mm) and *Penicillium*(14mm), and the zone of inhibition at 100ul concentration against *Aspergillusnigar*(14mm) and *Penicillium* (15mm) (Fig. 9).

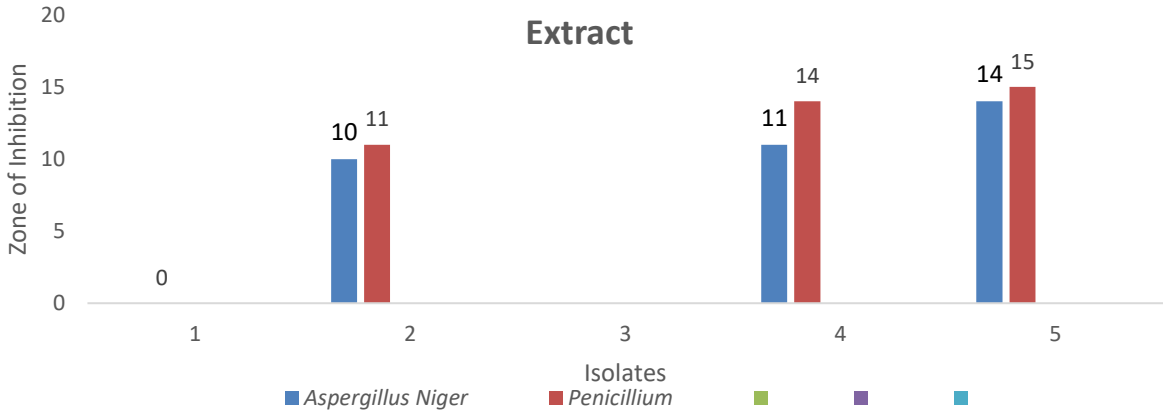


Figure 9. This graph shows the antifungal activity of *C.comosum* Extract

Anti-fungal activity of green synthesized FeNPs from leaf extract of *C.comosum.*

In the current research study, three different concentrations (50 ul, 75ul, and 100ul) were used to evaluate the activity of green synthesized FeNPs against identified fungal species. Such as (*Aspergillusnigar* and *Penicillium).* It was observed that the activity at 50 ul concentration against *Aspergillus nigar* (17mm) and *Penicillium* (12mm), the zone of inhibition at 75ul concentration against *Aspergillus nigar* (18mm) and *Penicillium* (15mm), and the zone of inhibition at 100ul concentration *Aspergillus nigar* (20mm) and *Penicillium* (17mm) as shown in Fig. 10.

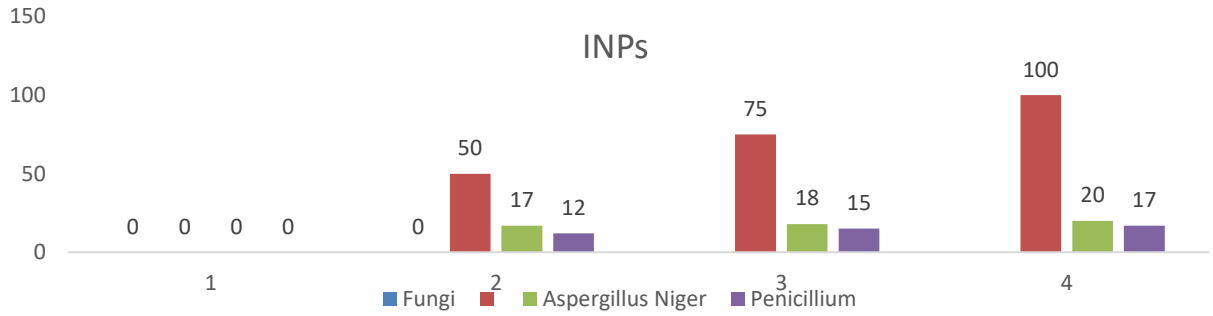


Figure 10. Antifungal activity of FeNPs

Discussion

Green synthesis of iron nanoparticles which is an economical and ecofriendly treatment process is getting important these days Saif *et al*., (2016). Due to their applications in environmental remediation technologies, FeNPs are becoming important. In this research different part of plants was employed to prepare green synthesized FeNPs. Plant extracts when reacted with ferric chloride (FeCl3) solution provide us with FeNPs Shah *et al.*, (2014). In the present investigation, FeNPs were synthesized using the leaf extract of *C.comosum* and then various spectroscopic tools like UV, visible spectroscopy, SEM, EDAX, and FTIR spectroscopy were employed for the characterization of FeNPs. From UV-visible spectroscopy, the Nature of the energy absorbed was detected to be at 460nm which was a peak value for FeNPs. Besides, green synthesized iron nanoparticles from aqueous leaf extract of Vitex leucoxylon actualized the highest absorption peak at 395 nm in ultraviolet-visible spectroscopic analysis. Moreover, other researchers noticed that variations in the absorption peak of FeNPs were in the range of 200-500nm. One of the possible sources of this variability could be the type of manufacturing process used; chemical, physical, or biological method, and due to such; there is variation in the morphology of FeNPs Nahari *et al.*, (2022).

In the current study, the SEM analysis of the synthesized iron nanoparticles was conducted, where iron nanoparticles were estimated to be of average size of 50 nm with relatively small variation. The synthesized nanoparticles possess a spherical shape and no sign of aggregation. Further, the SEM images showed that the size, shape, and surface of the nanoparticles were fairly uniform and the nature of the smooth surface pointed toward the well-defined crystalline structure of the nanoparticles confirming that a successful synthesis process was achieved. Whereas Vilardi *et al*., (2019) observed that the investigation on the surface morphology and distribution of the synthesized FeNPs by the TEM technique exposed that nearly all particles are spherical and are less than 100 nm in size with certain extents of agglomeration. In the present study, the EDX analysis of iron nanoparticles was done and the result indicates, C=38.77, O=27.41, Na=12.56, Cl=10.18, Fe=11.07 percent. Ardakini *et al.*, (2021) pointed out, the FeNPs- EDX analysis where the authors identified the elements in the FeNPs, whereas. The composition obtained from the EDX analysis was composed of 25.1% C, 36.75% O, 7.22% N, and 30% Fe. Apart from Fe peaks, the presence of other peaks pointed to the presence of other organic compounds responsible for the synthesis and stabilization of FeNPs.

FT-IR spectroscopy was also used to identify functional groups of the active component. In FT-IR spectrum of FeNPs, the vibration of Fe-O stretching was observed at 516.9 cm 1 and that vibrate in the leaf extract was not observed Njagi *et al.*, (2011). In the present investigation, the feasibility of the decolorization of methyl orange dye was traced through a series of experiments that involved the use of FeNPs. In the past study, the percentage reduction of methyl orange when using FeNPs was 77 percent in 6hrs and the spectral band of MO which is 465nm was shifted to 490nm within 15 mins of interaction between FeNPs and aqueous H2O2 Shahwan *et al*., (2011). The antibacterial properties of biosynthesized FeNPs were investigated on *S. epidermidis* and *S. aureus* (Gram +ve), *E. coli*, *P. aeruginosa*, and *Salmonella* (Gram –ve bacterial pathogens). The FeNPs had very high bactericidal activity against Staphylococcus epidermidis and Pseudomonas aeruginosa than that of other gram-positive and gram-negative bacteria. Previous studies explored, the antibacterial activity of FeNPs on Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *Enterococcus faecalis*). The outcomes were more pronounced on *S. aureus* as compared with other bacteria (Wang *et al.*, 2017). In the current study, we apply the *C.comosum* plant extract and plant-mediated FeNPs to two identified fungus species. The effect was more pronounced on *Aspergillus Niger* and *Penicillium.*

Conclusion

In this research, green synthesized FeNPs using *chlorophtum comosum* leave extract were investigated. We investigate the prepared FeNPs ability to degrade MO, inhibit bacteria, and have antifungal properties. In this approach, the only reducing and stabilizing agent introduced to the iron salt precursor is water-dispersed *C.comosum* leaf extract. For this reason, the current technique for preparing amorphous FeNPs is environmentally

friendly. MO was used as a model azo dye contaminant to examine the dye removal ability of H_2O_2-catalyzed FeNPs towards the degradation of organic contamination. The greatest MO decomposition efficiency (83%) was seen after 6 hours, according to the results. The antibacterial activity of these NPs was carried out against several Gram-positive and Gram-negative microorganisms. In light of the research conclusions, FeNPs demonstrated a bacteria-killing effect both on Gram-negative Pseudomonas aeruginosa and on Gram-positive Staphylococcus epidermidis. The bactericidal effect was significant on both gram-negative and gram-positive organisms as indicated by antibacterial activity results generated. Additionally, the range of *Penicillium's* zone of inhibition is 12 mm to 17 mm, while the lowest and greatest zones of inhibition against *Aspergillus Niger* are 17 mm and 20 mm, respectively. All things considered, ecologically friendly FeNPs can be a good option for several scientific domains, especially the elimination of organic dyes and the eradication of germs and fungi.

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Declaration

Data Availability statement

All data generated or analyzed during the study have been included in the manuscript and its supporting file.

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Conflict of interest

The authors assure that there were no financial relationships involved that could be perceived as a conflict of interest.

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Ethics approval

Not applicable.

AutAuthhor's Contributions

REZM did the experiments and wrote the manuscript. AR, US, MB, MB, SJ, and MS conceived the study and design, KF, SK and BR reviewed the manuscript. All the authors participated in the experimentation & optimizations

Consent for Publication

Not applicable