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Utilizing *Chlorophytum comosum* Leaf Extract for Eco-friendly Synthesis of Iron-Based Nanoparticles: Assessing Their Efficacy in Methyl Orange Dye Degradation and Antimicrobial Activities

Mansi Kushwaha, Shivani Singh*

Department of Chemistry, Miranda House, University of Delhi, Delhi-110007, India
*Corresponding author: shivani.singh@mirandahouse.ac.in

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Abstract The utilization of plant extracts for environmentally friendly nanoparticle synthesis has garnered significant attention from researchers due to its clean, safe, cost-efficient, and eco-conscious approach to crafting nanomaterials. In the present study, eco-friendly synthesis of iron nanoparticles by using *Chlorophytum comosum* leaf extract have been explored with special emphasis on its potential as Fenton-like catalyst for degrading methyl orange dye. Noticeable was almost complete disappearance of the dye's color, achieving an impressive 77% degradation efficiency within a mere 6 hours. Additionally, exploration of its antimicrobial properties revealed a potent effect against Staphylococcus aureus. This investigation highlights the straightforward green synthesis of Fe nanoparticles, showcasing their dual benefits in dye degradation and potent antibacterial action.

Keywords: *Chlorophytum cosmosum, Green synthesis, Fenton like catalyst, Antimicrobial properties, Methyl orange dye*

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1. Introduction

One of the most cutting-edge and promising realms of investigation in contemporary medical science is nanotechnology. Nanoparticles exhibit enhanced characteristics, including size, distribution, and morphology, when compared to larger particles constituting bulk materials [1]. The synthesis of nanoparticles can be achieved through three distinct methodologies: physical, chemical, and biological [2]. Despite the various approaches available, there is a growing demand for the development of high-yield, non-toxic, cost-effective, and environmentally friendly procedures. Consequently, the biological approach to nanoparticle synthesis becomes imperative. Biological molecules possess unique attributes that facilitate highly controlled and hierarchical assembly, rendering them ideal for establishing a dependable and eco-friendly process for metal nanoparticle synthesis [1]. Biologically synthesized nanomaterials hold tremendous potential across diverse domains, encompassing medical treatment, diagnostic tools, the creation of surgical nanodevices, and the manufacturing of commercial products. Among various biological materials, plant biomass or extracts offer distinct advantages over other microorganisms for nanoparticle synthesis. Plant-mediated biosynthesis of metallic nanoparticles occurs through the presence of biomolecules such as proteins, vitamins, amino acids, enzymes, polysaccharides, and organic acids like citrates in the plant biomass [2].

A wide array of nanoparticles, including gold (Au) [3], titanium dioxide (TiO_2) [4], manganese-doped zinc oxide (ZnO) [5], zinc ferrite [6], and iron nanoparticles (Fe-NPs), have been successfully employed for dye degradation applications.[7] Owing to their versatile properties, such as high catalytic activity and abundant surface functional groups, Fe-NPs have been extensively investigated for the degradation and elimination of organic pollutants. Recently, Fe-NPs have been utilized as heterogeneous Fenton-like catalysts for the removal and degradation of various organic contaminants in aqueous environments. The Fenton reagent, consisting of Fe^{2+}/Fe^{3+} and hydrogen peroxide (H_2O_2), rapidly oxidizes diverse organic pollutants by generating highly reactive hydroxyl radicals (OH^-). Among these pollutants, azo dyes pose particular concern due to their widespread use in various industries, resulting in their resistance to conventional biological and physicochemical treatments [8].

The synthesis of Fe-NPs can be readily accomplished using sodium borohydride ($NaBH_4$) as a reducing agent.

However, conventional $NaBH_4$-based synthesis raises concerns due to the corrosive and flammable nature of $NaBH_4$. As a result, green synthesis of iron nanoparticles has emerged as an approach that imparts steric stabilization to prevent Fe-NP aggregation and addresses the issues associated with the conventional use of sodium borohydride as a reducing agent. [9]

Given the exceptional features and multifunctional applications of nanoparticles, the plant extract method was selected for Fe-NP synthesis. The obtained Fe-NPs were subsequently employed for decolorizing an azo dye solution to evaluate their dye degradation efficacy (see Figure 1). In this study, we explored the potential of *Chlorophytum comosum*, known for its rich content of phenols, flavonoids, and saponins capable of reducing metal ions and stabilizing resulting NPs, in the synthesis of Fe-NPs [8], *Chlorophytum comosum* has been extensively studied for phytoremediation purposes in indoor air pollution, and it has also been utilized for synthesizing Fe-NPs as a novel dye-removing material [10]. Additionally, we assessed the antimicrobial activity of the prepared Fe-NPs using the standard microdilution technique.

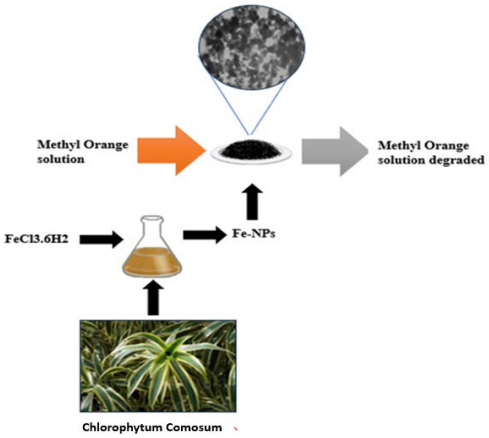

Figure 1. Synthesis of Fe-NPs and its dye degradation performance

Synthesis nanoparticles: a green approach

A diverse range of biological resources, including microorganisms (such as bacteria, yeast, fungi, algae, and viruses) and plants, can be harnessed for nanoparticle synthesis [11]. While microbial protocols have been developed over time through the collective efforts of many researchers, the significance of plant-mediated biological synthesis of nanoparticles has only recently gained prominence [12]. The advantage of plant extracts over microbes lies in their ability to rapidly reduce metal ions, often achieving synthesis within minutes to hours, as opposed to the longer timescales required by microorganism-based methods. [13] Moreover, microbial nanoparticle synthesis necessitates stringent aseptic conditions, demanding trained personnel and increasing scaling-up costs, which serves as a notable drawback [14]. These factors, coupled with the ready availability of plants in nature, establish plant-mediated nanoparticle synthesis as a preferred biological resource over microbes.

To date, iron nanoparticles, including nZVI and iron oxide variants, have predominantly been synthesized using various plant extracts. Plant extracts serve as both stabilizing agents and cost-effective reducing agents. The synthesis of magnetic nanoparticles can be achieved through hydrothermal processes or at room temperature by mixing plant extracts with metal salt solutions in fixed ratios [15].

Green synthesis of Fe-NPs has garnered substantial attention due to its environmentally friendly and cost-efficient nature. Iron-based nanoparticles (OT-FeNP) have been synthesized in various ways, utilizing extracts from a range of sources including Oolong tea [12], *Mangifera indica* (mango leaves), *Syzygium aromaticum* (clove buds), *Azadirachta indica* (neem), *Camellia sinensis* (black tea), *Camellia sinensis* (green tea leaves), *Coffea arabica* (coffee seeds), *Rosa indica* (rose leaves), *Murraya koenigii* (curry leaves), among others.

Table 1. Different leaves and seeds whose extract can be used in green synthesis of Fe-NPs

S.No.	Scientific name	Common name	Image	Reference
1.	*Mangifera indica*	Mango leaves		[16]
2.	*Syzygium aromaticum*	Clove buds		[17]
3.	*Azadirachta indica*	Neem		[18]

S.No.	Scientific name	Common name	Image	Reference
				
4.	*Camellia sinensis*	Black tea		[19]
5.	*Camellia sinensis*	Green tea leaves		[19]
6.	*Coffea arabica*	Coffee seeds		[20]
7.	*Rosa indica*	Rose leaves		[21]
8.	*Murraya koenigii*	Curry leaves		[22]

In this instance, iron nanoparticles were synthesized using *Chlorophytum comosum* leaf extract.

Green Synthesis Using *Chlorophytum comosum* Leaf Extract

Chlorophytum comosum, a perennial lily-like plant adorned with petite white flowers, storage roots, and gently curving leaves radiating from a central rosette, has been a globally cherished houseplant, especially its various cultivars. The selection of *Chlorophytum comosum* as a model plant for Fe-NP synthesis stems from its rich content of reductants, notably polyphenolic compounds [8].

Preparation of the Leaf Extract

To craft a range of leaf extract concentrations (5–20% wv^{-1}), dried leaves were subjected to heating at 800°C for one hour in deionized water (DI). The resulting extract suspension was subsequently cooled to room temperature, vacuum-filtered through 0.2-mm filter paper, and then centrifuged (at 2000 rpm) for five minutes to remove any remaining particles. The clear extract was sealed in polypropylene tubes and stored at -200°C in a refrigerator [8].

Synthesis of Fe-NPs

For Fe-NP synthesis, different concentrations of leaf extract (5%–20% wv^{-1}) were employed. Typically, under an inert atmosphere (N_2), 1 ml of 0.1 M $FeCl_3{\cdot}6H_2O$ (27 gL^{-1}) was added to 9 ml of 5% leaf extract at pH=6. This mixture was then poured into a 25-mL round-bottom flask and vigorously stirred using a magnetic stirrer (1000 rpm) at room temperature for 24 hours. Subsequently, the reaction medium underwent centrifugation (at 10,000 rpm for 10 minutes) to separate the resulting black pellets. These pellets were washed with deionized water three times, dried in a vacuum oven at 50°C for 12 hours, and stored in a tightly sealed container filled with an inert gas (N_2) within a desiccator for further analysis [8].

Characterization of Fe-NPs

The characterization of Fe-NPs encompassed various techniques. Transmission electron microscopy (TEM, Philips CM 10, 100 kV) was employed for visual analysis and morphological examination. A particle size analyzer (Microtrac S3500) was utilized to measure the particle size. X-ray powder diffractometry (Siemens D5000, 2Θ range of 10–80 with a 2 min-1 scan rate) was used to analyze composition and crystallinity. Energy-dispersive X-ray spectroscopy (EDX, Bruker, India) was employed to determine the elemental composition. FT-IR spectroscopy (Bruker, Vertex 70, 4000-400 cm-1) was conducted for further analysis. Magnetic characteristics were assessed using a vibration sample magnetometer (VSM, Microsense EZ9) at room temperature, with a field sweeping range of -10 to +10 kOe. Finally, thermogravimetric analysis (TGA, 209 F3 Tarsus) was used to quantify and identify any coated organic compounds on the surface of Fe-NPs [8].

TEM analysis revealed that nearly all particles exhibited a spherical shape, with a size below 100 nm, occasionally forming aggregates. The hydrodynamic diameter, assessed by PSA, indicated a particle size distribution ranging from approximately 100 to 1000 nm, with an average particle size of 246 nm. It's noteworthy that the PSA-measured particle size distribution of the Fe-NPs differed from that obtained through TEM, owing to a biological coating and the aggregation tendency of NPs in aqueous media. As observed in the TEM image, the Fe-NPs were enveloped by a biological coating originating from the extract, which was a factor not considered in the PSA method. The size distribution of Fe-NPs displayed high instability and a pronounced aggregation propensity due to their low surface charge [8].

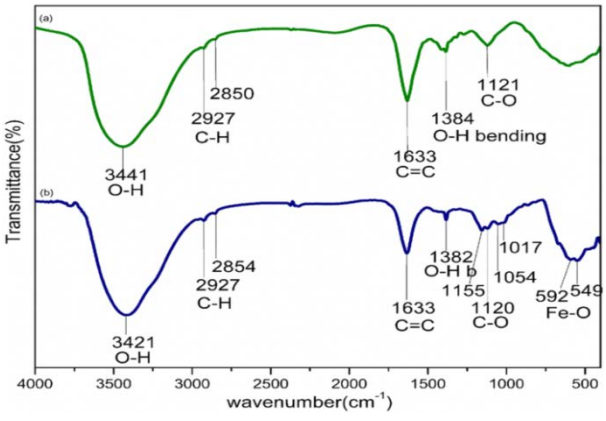

Figure 2. FT-IR spectra of green synthesized Fe-NPs [23]

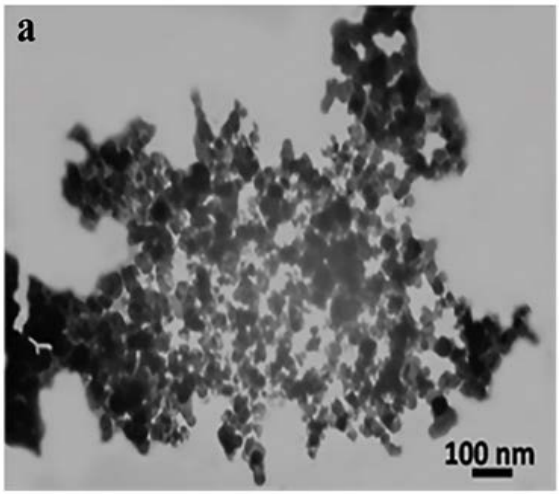

Figure 3. TEM micrographs of the green synthesis Fe-NPs [8]

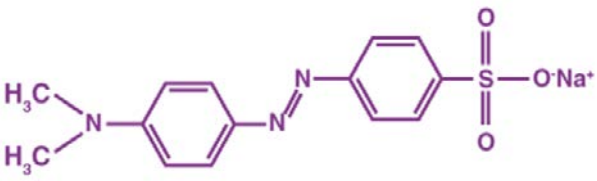

Figure 4. Structure of methyl orange

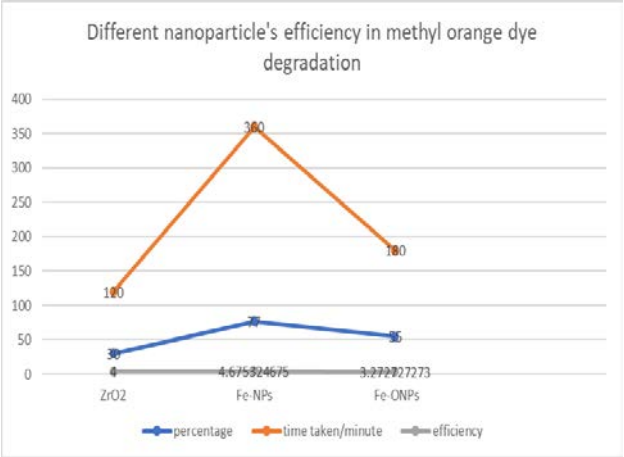

Figure 5. Dye degradation comparison by ZrO_2, Fe-NPs, Fe-ONPs

Methyl Orange undergoes oxidation and decolorization through a Fenton-like reaction in which Fe-NPs act as a source of ferrous ions (Fe^{2+} ions). The combination of Fe-NPs and H_2O_2 generates free hydroxyl radicals (OH^-) [10]. These radicals facilitate the decomposition of the azo bond (-N=N-) in Methyl Orange, resulting in the decolorization of the dye in contaminated aqueous environments [24,25]. The catalytic function of Fe-NPs in the Fenton-like process is depicted in Figure 5, demonstrating the efficient degradation of Methyl Orange by Fe-NPs within a span of 6 hours.

Green synthesized zero valent Fe-NPs also show significant efficiency in Methyl Orange degradation; however, non Fe-NPs such as ZrO_2 [26], and ZnO nanocomposites [27] demonstrated lower Methyl Orange degradation efficiency.

Comparably the synthesized Fe-ONPs degraded 55% of the initial Methyl Orange concentration during the first 3 hours. Then reduction in color degradation continued at

the fourth hour and no significant reduction occurred in the next 2 hours. As reported by Muthukumar and coworkers, green synthesized Fe-NPs have shown better Methyl Orange degradation than chemically synthesized Fe-NPs [28].

Moreover , some of the green synthesized NPs like Ag [29] and ZnO [30] showed higher Methyl Orange degradation efficiency, which can be related to their physicochemical properties like band gap, morphology, size and surface coating materials[28]. Overall, green synthesis of Fe-NPs can lead to great results in azo dyes degradation. [8]

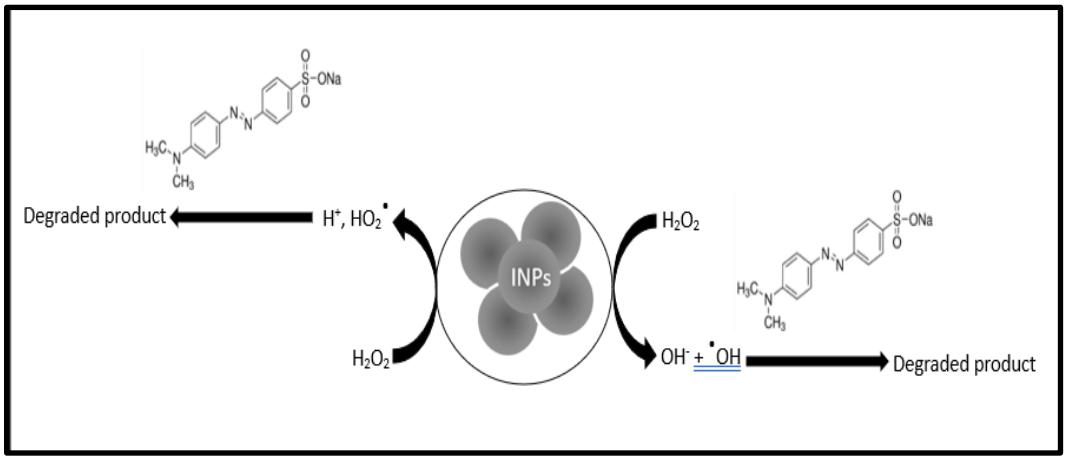

Figure 6. Fe-NPs Fenton-like mechanism and MO degradation

Application of Fe-NPs

Fe-NPs exhibit a unique form of magnetism known as superparamagnetism, making them exceptionally versatile. Their adjustability renders them particularly intriguing for various applications. The requirement for high coercivity is crucial in magnetic recording media, a primary practical application of Fe-NPs. Conversely, in transformer core materials, very low coercivity is essential. Fe-NPs also excel as catalysts in coal liquefaction. Their biomedical applications encompass magnetic separation and labelling of biological materials, targeted drug delivery, and aiding in MRI contrast enhancement. Moreover, they contribute to heavy metal removal and selectively heating cancerous tumors.

Fe is an essential nutrient for all organisms, and Fe-NPs have been explored as potential micronutrients for methanogenic bacteria in anaerobic digestion (AD) [31] of organic waste, which serves as a valuable energy source [32]. In biogas production, the supplementation of micronutrients not only stimulates methane production but also enhances process stability. Among various additives, Fe-NPs have proven effective in improving methane yield, reducing odours (e.g., H_2S), and facilitating chemical oxygen demand (COD) removal during AD [33]. Researchers, such as Suanon et al. [34], have investigated the influence of Fe-NPs on methane production during sewage sludge AD.

Kadar et al. demonstrated that synthetic nano-Fe outperformed EDTA–Fe in laboratory cultures within generic growth media, suggesting that nanoparticulate forms of the metal may be more bioavailable to microalgae. Nano-sized materials exhibit superior chemical and physical properties compared to their bulky or atomic counterparts, owing to phenomena like the small object effect, mesoscopic effect, surface effect, and quantum size effect [35].

Zhang et al. (2010) revealed that when zero-valent iron (ZVI) was introduced into an anaerobic reactor, it not only served as an electron donor but also created an enhanced anaerobic environment, improving reactor performance for wastewater treatment. This finding provided direct evidence that ZVI promoted methanogen growth, enabling the reactor to achieve greater COD removal in a short hydraulic retention time and at low temperatures. Nanotechnology has evolved into an attractive option in environmental science and engineering. This discussion encompasses the use of iron nanoparticles as Fenton-like catalysts, their role in methyl orange dye degradation, and their medicinal properties [36].

Exploiting Fe-NPs as Fenton-Like Catalysts for the Degradation of Methyl Orange Dye

The Fenton reaction, first proposed by Henry J. Fenton in the late 19th century, has become a cornerstone of advanced oxidation processes (AOPs) [37]. Among the methods for degrading organic pollutants in moderately concentrated aqueous solutions, the Fenton reaction, involving the generation of hydroxyl radicals from H_2O_2, stands out [38]. Azo dyes, in particular, pose significant challenges due to their extensive use in various industries and subsequent resistance to biological and physicochemical treatments [39].

To evaluate the potential of the synthesized Fe-NPs for oxidative degradation of Methyl Orange, they were employed as heterogeneous Fenton-like oxidants in an aqueous solution (see Figure 6). During the dye removal process, H_2O_2 showed no significant activity even after 6 hours. In contrast, when Fe-NPs were utilized, Methyl Orange underwent degradation, and its color almost completely vanished, achieving a remarkable 77% efficiency after 6 hours. Notably, the spectral band of Methyl Orange exhibited a redshift from 465 nm to 490 nm after just 15 minutes of incubation with the aqueous mixture of Fe-NPs and H_2O_2. This shift in the absorption peak position may be attributed to Methyl Orange protonation and the formation of azonium ions [40,41].

Medicinal Applications of Fe-NPs: Antimicrobial Properties

The antibacterial potential of biologically synthesized Fe-NPs was investigated against a selection of Gram-

positive and Gram-negative bacteria, with their minimum inhibitory concentrations (MIC values) listed in Table 2. Notably, even at the lowest concentrations, Fe-NPs exhibited a remarkable inhibition of bacterial growth. This inhibitory effect was particularly pronounced against Staphylococcus aureus in comparison to other bacteria (as detailed in Table 2), a phenomenon attributed to the complex cell wall structure of Gram-positive bacteria [42]. Reports also indicate a higher antimicrobial efficacy of Fe-NPs against Gram-positive bacteria in contrast to Gram-negative counterparts.

Various factors contribute to the bactericidal mechanisms of metallic oxide NPs. It appears that oxidative stress induced by reactive oxygen species (ROS), including superoxide radicals (O^{2-}), hydroxyl radicals (OH^{-}), H_2O_2, and singlet oxygen, plays a pivotal role in the antibacterial activity of these NPs. These ROS can inflict damage on bacterial DNA and proteins, binding to and permeating the bacterial cell wall, leading to structural alterations in the cell membrane, cell death, and increased membrane permeability [8]. The collective action of ROS production, cell wall penetration, gene regulation alterations, and metabolite binding concurrently weakens bacterial defense mechanisms against these interactions [43].

Table 2. MIC of synthesized nanoparticles

Bacteria	MIC (μg/mL)
Staphylococcus aureus	6
Escherichia coli	17
Pseudomonas aeruginosa	9
Enterococcus faecalis	8

2. Conclusion

In summary, this review paper delved into the green synthesis of Fe-nanoparticles utilizing *Chlorophytum comosum* leaf extract, highlighting its environmentally friendly approach. This method yielded amorphous Fe-NPs with a diminutive particle size of less than 100 nm. Notably, the process relied solely on a water dispersion of *Chlorophytum comosum* leaf extract, serving both as a stabilizing and reduction agent in conjunction with the iron salt precursor.

The investigation extended to the assessment of Fe nanoparticles for their dye degradation capabilities, using azo-dye as a model contaminant. The findings revealed a remarkable efficiency, with the highest rate of Methyl Orange (MO) degradation reaching 77% within a span of 6 hours. Additionally, the antimicrobial potential of these iron nanoparticles was explored against both Gram-negative and Gram-positive bacteria, with particularly notable effectiveness observed against Staphylococcus aureus.

Collectively, the environmentally friendly synthesis of iron nanoparticles presented in this review bears significant promise across scientific domains. Its applications span from dye degradation to medicinal uses, underpinned by its remarkable antimicrobial properties.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this review article.

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