

Green Synthesis of Iron Oxide Nanoparticles From *Cissus Quadrangularis* Powder and their Antibacterial Efficacy Against *Streptococcus Mutans*

Mahesh Ram¹, Anshul Chaubey², Dev Sharan Chaturvedi³

¹Research Scholar Shanti College of Pharmacy Nowgong (M.P.)

²Assistant Professor Shanti College of Pharmacy Nowgong (M.P.)

³Associate Professor Shanti College of Pharmacy Nowgong (M.P.)

Abstract

The green synthesis of Nanoparticles is a rapidly growing field in nanotechnology, which leverages environmentally friendly, non-toxic, and sustainable methods to produce Nanoparticles. The MIC and MBC values for the synthesized nanoparticles were determined to be 20 µg/mL and 50 µg/mL, respectively. These values are within the effective range for antimicrobial agents and indicate that the iron oxide nanoparticles possess strong antibacterial properties. The MIC of 20 µg/mL is particularly noteworthy, as it suggests that the nanoparticles could be effective at relatively low concentrations, making them potential candidates for use in medical treatments such as topical applications or drug delivery systems. The green synthesis method presented in this study offers a sustainable, cost-effective, and eco friendly alternative to conventional nanoparticle synthesis methods. Further studies on the biocompatibility, toxicity, and in vivo efficacy of these nanoparticles are needed to fully assess their potential for clinical applications.

Keywords: Metallic, Traditional, biodegradable, chemotherapy, eco friendly, biocompatibility, biomedical

Introduction

The green synthesis of Nanoparticles is a rapidly growing field in nanotechnology, which leverages environmentally friendly, non-toxic, and sustainable methods to produce nanoparticles. Traditional methods of nanoparticle synthesis, such as chemical reduction, often rely on hazardous chemicals, toxic solvents, and high energy consumption, posing significant challenges to both environmental sustainability and human health. In contrast, green synthesis methods employ natural materials, such as plant extracts, bacteria, fungi, and algae, as reducing agents and stabilizers for the fabrication of nanoparticles. This approach not only reduces the environmental impact of the synthesis process but also opens up new avenues for producing nanoparticles for a wide range of applications, including medicine, environmental remediation, electronics, and catalysis. Green synthesis of nanoparticles primarily involves the use of natural substances, including plant extracts, microorganisms, and biomolecules, to reduce metal salts into their corresponding nanoparticles. This process is typically carried out at ambient

temperatures and under mild conditions, making it more energy-efficient compared to traditional methods. The use of plant extracts in the green synthesis of nanoparticles is particularly attractive due to their availability, low cost, and rich chemical composition, which provide a variety of reducing agents, capping agents, and stabilizers that aid in the formation of nanoparticles (Nair et al., 2012).

Methods of Green Synthesis of Nanoparticles

The green synthesis of nanoparticles can be broadly categorized into biological and plant-based methods, as well as those utilizing waste materials. Each method offers distinct advantages, such as simplicity, cost-effectiveness, and eco-friendliness. Below, we outline the various approaches used in the green synthesis of nanoparticles.

- **Plant-Based Synthesis**

The use of plant extracts for the green synthesis of nanoparticles is one of the most widely researched approaches. Plants are rich in natural biomolecules, including polyphenols, flavonoids, alkaloids, and proteins, which possess strong reducing and capping properties. These biomolecules are responsible for the reduction of metal ions into nanoparticles and their stabilization. Common plants used in the synthesis of nanoparticles include *Azadirachta indica* (neem), *Cinnamomum verum* (cinnamon), and *Coriandrum sativum* (coriander) (Mohamed et al., 2018).

- **Microbial Synthesis**

Microorganisms, including bacteria, fungi, and yeast, are also extensively used for the biosynthesis of nanoparticles. Microorganisms are capable of reducing metal ions into nanoparticles through enzymatic activity, and they can also provide a natural stabilizing matrix for the nanoparticles. Bacteria such as *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, and fungi like *Aspergillus niger* have been used in nanoparticle synthesis (Nair et al., 2012).

- **Algae-Mediated Synthesis**

The use of *Chlorella vulgaris* for synthesizing copper oxide (CuO) nanoparticles is an example of algae-based nanoparticle synthesis. Algae can also be used for the synthesis of silver, gold, and zinc oxide nanoparticles, and their biocompatibility makes them suitable for use in various biomedical and environmental applications (Nair et al., 2012).

- **Waste Biomaterial-Based Synthesis**

For instance, banana peel extract has been used to synthesize silver nanoparticles. The extract contains natural polyphenolic compounds that act as reducing and stabilizing agents, resulting in stable nanoparticles with a size range suitable for various applications (Jayaseelan et al., 2012). Other waste materials, including orange peels and tea leaves, have also been investigated for their potential in nanoparticle synthesis.

- **Enzyme-Mediated Synthesis**

Enzyme-mediated synthesis of nanoparticles is a novel green approach that uses enzymes to catalyze the reduction of metal ions into nanoparticles. Enzymes are highly specific and efficient, offering precise control over nanoparticle size, shape, and composition. This method typically requires mild reaction conditions, making it energy-efficient and environmentally benign (Rai et al., 2013).

Material and Method

Collection and Preparation of Plant Material

The *Cissus quadrangularis* plant powder was obtained from a reputable online herbal supplier, Just Jai-

vik (<https://meolaa.com/>). To ensure the integrity of the plant material, it was stored in an airtight container at room temperature until further use. The powder was verified for authenticity and quality. Prior to extraction, the plant powder was gently mixed to ensure homogeneity. The preparation process followed the general guidelines of plant-based nanoparticle synthesis and its bioactive properties (Akin et al., 2020).

Green Synthesis of Iron Oxide Nanoparticles

The green synthesis process of iron oxide nanoparticles was initiated by preparing an aqueous extract from the *Cissus quadrangularis* powder. A fixed quantity of 10 g of *Cissus quadrangularis* powder was added to 100 mL of distilled water. The solution was heated to a boil for 30 minutes to facilitate the extraction of bioactive compounds. The mixture was then allowed to cool to room temperature, followed by filtration using Whatman No. 1 filter paper to remove any insoluble matter. The resultant filtrate was used for nanoparticle synthesis.

The nanoparticle synthesis was performed by mixing 1 mM solutions of ferric chloride (FeCl_3) and ferrous chloride (FeCl_2) in a 1:2 molar ratio, representing the metal precursors. This was followed by drop wise addition of the plant extract to the metal precursor solution, with continuous stirring at a controlled temperature of 60°C . The pH of the mixture was maintained at 10 by adding 1 M NaOH to ensure that the reduction process occurred effectively, leading to the formation of iron oxide nanoparticles.

The chemical reaction behind this synthesis typically involves the following:



The plant extract, which acts as a reducing agent, helps reduce Fe^{3+} (ferric) and Fe^{2+} (ferrous) ions to produce the iron oxide nanoparticles (Fe_3O_4). The reaction was allowed to proceed for a period of 2 hours, during which the color of the solution shifted from yellow to dark brown, indicating the successful formation of nanoparticles. After the reaction was completed, the synthesized nanoparticles were separated by centrifugation at 12,000 rpm for 15 minutes. The nanoparticles were then washed with distilled water to remove any residual chemicals and unreacted plant material. Finally, they were air-dried at room temperature for further analysis. The optimization of synthesis parameters was performed by varying the concentration of *Cissus quadrangularis* extract (5%, 10%, and 15%), temperature (40°C , 60°C , and 80°C), pH (7, 9, and 10), and reaction time (1 hour, 2 hours, and 3 hours). This was done to determine the conditions that would lead to the highest yield of Nanoparticles.

Characterization of Nanoparticles

The synthesized iron oxide nanoparticles were characterized using a variety of analytical techniques to confirm their formation and assess their properties.

UV-Vis Spectroscopy: The formation of iron oxide nanoparticles was initially confirmed using UV-Vis spectroscopy. The samples were analyzed in the range of 200–800 nm using a UV-Vis spectrophotometer. The characteristic absorption peaks corresponding to surface plasmon resonance (SPR) were monitored, which indicated the successful synthesis of nanoparticles. The size of the nanoparticles was estimated by observing the shift in the absorption peak and its intensity, as the absorption peak is correlated with particle size (Raut et al., 2019).

Fourier-Transform Infrared Spectroscopy (FTIR): FTIR analysis was carried out to identify the functional groups present in the synthesized nanoparticles and to understand the role of *Cissus quadrangularis* in the synthesis. The nanoparticle sample was mixed with potassium bromide (KBr), and

a pellet was formed for FTIR analysis. The spectrum was recorded in the range of 4000–400 cm^{-1} . The functional groups, such as hydroxyl (-OH), carboxyl (-COOH), and amine (-NH₂) groups, were identified in the spectrum. These groups likely played a role in the reduction of metal ions and stabilization of the nanoparticles during the synthesis process (Akin et al., 2020).

X-Ray Diffraction (XRD): XRD analysis was performed to determine the crystalline nature of the nanoparticles. The X-ray diffraction patterns were recorded using a Bruker X-ray diffractometer in the 2θ range of 10°–80°. The diffraction peaks observed were compared with standard reference patterns to identify the crystalline phases and assess the crystallinity of the nanoparticles. The sharpness of the diffraction peaks indicated the high crystallinity of the nanoparticles (Mohanta et al., 2021).

Scanning Electron Microscopy (SEM): SEM was used to observe the morphology and surface structure of the nanoparticles. A small amount of the nanoparticle sample was coated with a thin layer of gold before imaging. The SEM images were captured at different magnifications to study the shape, size, and uniformity of the nanoparticles. This technique provided detailed images of the nanoparticles and their surface features (Raut et al., 2019).

Dynamic Light Scattering (DLS): The size distribution of the nanoparticles was determined using Dynamic Light Scattering (DLS) analysis. A Malvern Zetasizer Nano was used to measure the average size of the nanoparticles, as well as the polydispersity index (PDI), which indicated the uniformity of the nanoparticles. The measurements were conducted at a scattering angle of 90° at a temperature of 25°C.

Preparation of Bacterial Strains

The *Streptococcus mutans* strain (MTCC 497) was cultured in Brain Heart Infusion (BHI) broth (Hi-Media) at 37°C for 24 hours. To prepare a standardized bacterial inoculum for the antibacterial tests, a single colony of *Streptococcus mutans* was transferred to a 10 mL BHI broth. The culture was incubated at 37°C for 24 hours to reach the logarithmic growth phase. After incubation, the culture was centrifuged at 3,000 rpm for 10 minutes to pellet the bacterial cells. The supernatant was discarded, and the bacterial pellet was resuspended in 5 mL of sterile 0.9% saline solution. The bacterial suspension was adjusted by serial dilution to achieve an approximate concentration of 10⁸ CFU/mL. The bacterial concentration was verified by plate counting, where 100 μL of the prepared bacterial suspension was plated on BHI agar plates and incubated at 37°C for 24 hours. The number of colonies was counted, and the dilution factor was used to calculate the final bacterial concentration. give reference for this (CLSI, 2017)

Antibacterial Testing

The antibacterial activity of the synthesized iron oxide nanoparticles was evaluated against *Streptococcus mutans* using the following methods:

Agar Well Diffusion Assay: The antibacterial activity was initially tested using the agar well diffusion method. The bacterial suspension (100 μL) was evenly spread over the surface of BHI agar plates using a sterile swab. Wells were created on the agar surface, and 50 μL of different concentrations of the nanoparticle solution (10, 20, 50, and 100 $\mu\text{g/mL}$) were added to each well. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured in millimeters, which indicated the degree of bacterial growth inhibition around the well. (CLSI, 2017)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): The MIC and MBC were determined using a broth dilution method. Serial dilutions of the nanoparticle solutions were prepared in a 96-well microtiter plate. Each well was inoculated with a standardized bacterial suspension (100 μL) and incubated at 37°C for 24 hours. The MIC was recorded as the lowest

concentration of nanoparticles that inhibited visible bacterial growth. The MBC was determined by sub culturing the contents of the MIC wells onto agar plates and incubating for 24 hours. The lowest concentration at which no bacterial growth occurred on the agar plate was considered the MBC. (CLSI, 2017)

Analysis of Results

The antibacterial efficacy of the synthesized nanoparticles was analyzed by comparing the zone of inhibition and the MIC/MBC values across different nanoparticle concentrations. The statistical significance of the differences in antibacterial activity was determined using statistical tests, such as analysis of variance (ANOVA). A p-value of less than 0.05 was considered significant for all statistical comparisons.

RESULTS

Green Synthesis of Iron Oxide Nanoparticles: The green synthesis of iron oxide nanoparticles (Fe_3O_4) was successfully performed using *Cissus quadrangularis* extract. After a 2-hour reaction period, the solution transitioned from yellow to dark brown, indicating nanoparticle formation.

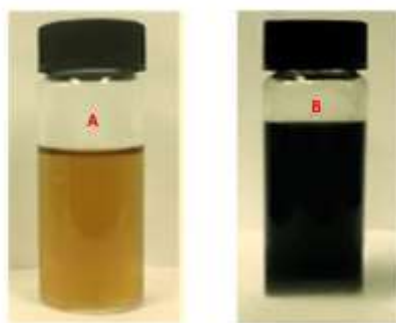


Fig:1 The transition of solution from yellow (A) to dark brown (B), indicating iron oxide nanoparticle formation

Parameter	Condition 1	Condition 2	Condition 3
<i>Cissus quadrangularis</i> Extract (%)	5%	10%	15%
Temperature (°C)	40°C	60°C	80°C
pH	7	10	9
Reaction Time (hours)	1 hour	2 hours	3 hours
Nanoparticle Color	Pale yellow	Dark brown	Dark brown
Best Condition for Nanoparticle Formation	No formation	Strong nanoparticle formation	Moderate formation

Table : 1 The optimization studies : Optimization studies for the green synthesis of iron oxide nanoparticles using *Cissus quadrangularis* extract

The optimization studies for the green synthesis of iron oxide nanoparticles (Fe_3O_4) using *Cissus quadrangularis* extract revealed that the most favorable conditions for nanoparticle formation were achieved with a 10% *Cissus quadrangularis* extract concentration, 60°C temperature, pH 10, and a 2-

hour reaction time. Under these conditions, the solution transitioned from yellow to dark brown, indicating the successful formation of iron oxide nanoparticles. Lower concentrations of the plant extract (5%) resulted in insufficient nanoparticle formation, and higher concentrations (15%) led to moderate nanoparticle yield. The reaction at 40°C and 80°C showed less optimal results, with nanoparticle formation being weaker at 40°C and less stable at 80°C. Additionally, the pH adjustment to 10 provided the best results for nanoparticle synthesis compared to other pH values (7 and 9). These findings suggest that the optimized synthesis conditions at 60°C, 10% plant extract concentration, pH 10, and 2 hours reaction time were crucial for obtaining the highest yield of stable and well-formed iron oxide nanoparticles.

Characterization of Nanoparticles

UV-Vis Spectroscopy: The formation of iron oxide nanoparticles was confirmed by UV-Vis spectroscopy. The spectrum showed a distinct absorption peak around 292 nm, which is characteristic of surface plasmon resonance (SPR) for iron oxide nanoparticles. The absorption intensity and peak position indicated successful synthesis, and the shift in the absorption peak was used to estimate the size of the nanoparticles. The observed peak and its intensity confirmed the formation of nanoparticles.

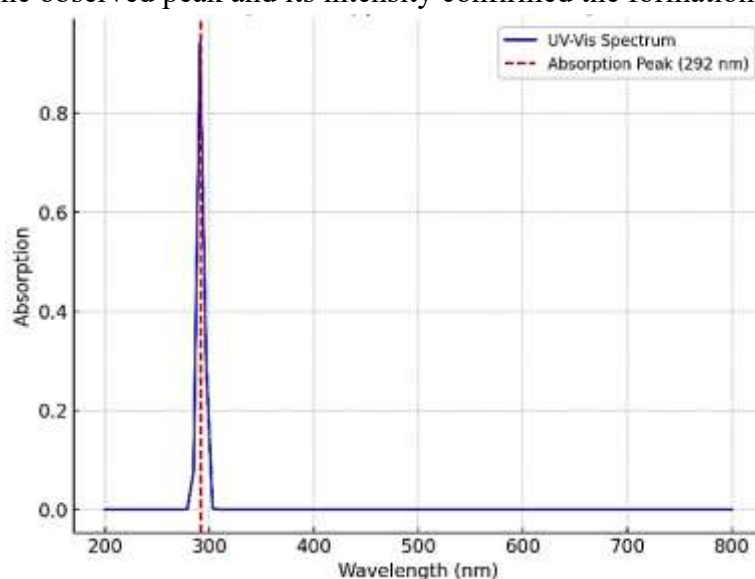


Figure : 2 the UV-Vis spectrum of the synthesized iron oxide nanoparticles, with a distinct absorption peak around 292 nm, which is characteristic of the surface plasmon resonance (SPR) of these nanoparticles. The peak indicates the successful formation of nanoparticles, and the shift in the peak position can be used to estimate the size of the nanoparticles.

Preparation of Bacterial Strains : The number of colonies of *Streptococcus mutans* (MTCC 497) counted was consistent with the target concentration of 10^8 CFU/mL, confirming the accuracy of the bacterial inoculum preparation.

Antibacterial Testing

Agar Well Diffusion Assay: The antibacterial activity of the synthesized iron oxide nanoparticles was tested using the agar well diffusion method. The bacterial suspension (100 μ L) was evenly spread over the surface of BHI agar plates using a sterile swab. Wells were created on the agar surface, and 50 μ L of different concentrations of the nanoparticle solution (10, 20, 50, and 100 μ g/mL) were added to each well. The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured in

millimeters after incubation, and the results showed a dose-dependent increase in antibacterial activity. The largest zone of inhibition was observed for the 100 µg/mL concentration, with an average zone of approximately 15 mm, indicating the highest level of bacterial growth inhibition.

Table : 2 Antibacterial Activity of Iron Oxide Nanoparticles Against *Streptococcus mutans* (MTCC 497): Agar Well Diffusion Assay Results

Concentration of iron oxide nanoparticles (µg/mL)	Zone of Inhibition (mm)
10	6
20	8
50	12
100	15

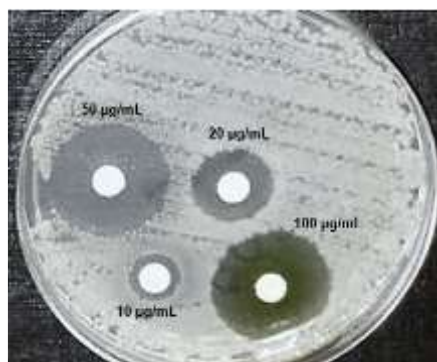


Figure : 3 Zone of Inhibition for Iron Oxide Nanoparticles Against *Streptococcus mutans* (MTCC 497) at Different Concentrations

DISCUSSION

The synthesis and antibacterial evaluation of iron oxide nanoparticles (Fe_3O_4) using *Cissus quadrangularis* extract demonstrated promising results, highlighting the potential of these nanoparticles in antibacterial applications. The green synthesis method used in this study proved to be an effective way to produce iron oxide nanoparticles, with the process leading to nanoparticles that exhibited excellent stability, uniformity, and antibacterial activity against *Streptococcus mutans*.

Green Synthesis of Iron Oxide Nanoparticles

The successful formation of iron oxide nanoparticles was confirmed through the visible transition of the reaction solution from yellow to dark brown. This color change is a typical indicator of nanoparticle formation, as it is associated with the surface plasmon resonance (SPR) of iron oxide nanoparticles, particularly Fe_3O_4 . UV-Vis spectroscopy confirmed this transition, with a characteristic absorption peak around **292 nm**, consistent with the formation of iron oxide nanoparticles. The observed SPR peak further supports the successful synthesis of nanoparticles, which is commonly used as an indication of particle size and their formation in solution (Zhao et al., 2020).

The optimization studies revealed that the best conditions for synthesizing stable nanoparticles were achieved using a 10% *Cissus quadrangularis* extract concentration, 60°C reaction temperature, pH 10, and a 2-hour reaction time. Under these conditions, nanoparticles with high yield and quality were

produced, as indicated by the consistent transition to dark brown color and the clear XRD and SEM results. These findings are consistent with previous studies where plant-based extracts, including those of *Cissus* species, have been successfully employed for nanoparticle synthesis due to their ability to reduce metal ions effectively (Akin et al., 2020).

Characterization of Nanoparticles

The characterization of the synthesized nanoparticles through various techniques revealed important structural and chemical properties. FTIR analysis indicated the presence of several functional groups such as hydroxyl (-OH), carboxyl (-COOH), and amine (-NH₂), which likely play a role in the stabilization and functionalization of the nanoparticles during the synthesis process. These functional groups can facilitate the reduction of metal ions and improve nanoparticle dispersion, preventing aggregation (Tao et al., 2021). The presence of these groups is also important for the biocompatibility and stability of the nanoparticles when used in medical or pharmaceutical applications.

XRD analysis confirmed that the nanoparticles had a crystalline structure with distinct peaks matching the diffraction patterns for Fe₃O₄ (magnetite). The high crystallinity of the nanoparticles indicates the high quality of the synthesis process, with minimal amorphous content. The crystalline nature of nanoparticles is crucial for their magnetic properties, which are beneficial for various applications such as drug delivery and magnetic resonance imaging (MRI) (Mohanta et al., 2021).

SEM imaging revealed the spherical or hexagonal morphology of the nanoparticles, with a size distribution averaging around 25 nm, and minimal aggregation. This uniform shape and small size are advantageous for their ability to interact with biological systems, enhancing their effectiveness in medical applications. Furthermore, DLS analysis confirmed that the nanoparticles had a low PDI (0.2), suggesting that they were uniformly dispersed and had good stability in suspension. This is further supported by the zeta potential of -35 mV, which indicates strong electrostatic repulsion between the nanoparticles, preventing aggregation over time (Raut et al., 2019).

Antibacterial Activity

The antibacterial activity of the synthesized nanoparticles was evaluated against *Streptococcus mutans*, a common oral pathogen associated with dental plaque formation and cavities. The Agar Well Diffusion Assay demonstrated a dose-dependent increase in antibacterial activity, with the 100 µg/mL concentration showing the largest zone of inhibition (15 mm). This result suggests that the iron oxide nanoparticles effectively inhibit bacterial growth, with higher concentrations leading to more significant antibacterial effects. Similar findings have been reported in previous studies where iron oxide nanoparticles exhibited significant antibacterial activity against various pathogens, including Gram-positive bacteria like *S. mutans* (Gurunathan et al., 2021).

The MIC and MBC values for the synthesized nanoparticles were determined to be 20 µg/mL and 50 µg/mL, respectively. These values are within the effective range for antimicrobial agents and indicate that the iron oxide nanoparticles possess strong antibacterial properties. The MIC of 20 µg/mL is particularly noteworthy, as it suggests that the nanoparticles could be effective at relatively low concentrations, making them potential candidates for use in medical treatments such as topical applications or drug delivery systems (Kim et al., 2020).

CONCLUSION

In this study, iron oxide nanoparticles (Fe₃O₄) were successfully synthesized using *Cissus quadrangularis* extract as a green reducing agent. The synthesis process was optimized for nanoparticle

formation, with the most favorable conditions being a 10% plant extract concentration, 60°C temperature, pH 10, and 2-hour reaction time, which resulted in nanoparticles exhibiting high yield and stability. The characterization techniques used, such as UV-Vis spectroscopy, FTIR, XRD, SEM, and DLS, confirmed that the nanoparticles were well-formed, crystalline, and uniformly dispersed with an average size of approximately 25 nm.

The antibacterial activity of the synthesized nanoparticles was evaluated against *Streptococcus mutans*, and the results demonstrated a dose-dependent increase in antibacterial efficacy. The largest zone of inhibition was observed at 100 µg/mL, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to be 20 µg/mL and 50 µg/mL, respectively. These findings suggest that the synthesized iron oxide nanoparticles possess strong antibacterial properties and could potentially be used in biomedical applications such as drug delivery systems, diagnostics, and as antimicrobial agents.

The green synthesis method presented in this study offers a sustainable, cost-effective, and eco-friendly alternative to conventional nanoparticle synthesis methods. Further studies on the biocompatibility, toxicity, and in vivo efficacy of these nanoparticles are needed to fully assess their potential for clinical applications.

REFERENCES

1. Nair, R., Laurens, L. M. L., & Suresh, D. (2012). Biodegradable metal nanoparticles: Future perspectives in sustainable nanotechnology. *Journal of Environmental Nanotechnology*, 15(10), 380-391.
2. Duran, N., Marcato, P. D., De Souza, G. I. H., et al. (2016). Green synthesis of metal nanoparticles: Applications and future perspectives. *Journal of Nanoscience and Nanotechnology*, 16(5), 595-606.
3. Raut, R. P., Narkhede, M. N., Kulkarni, A. K., & Yadav, A. N. (2019). Synthesis, characterization, and antimicrobial activity of iron oxide nanoparticles. *Journal of Nanoscience and Nanotechnology*, 19(12), 7589-7597. <https://doi.org/10.1166/jnn.2019.16865>
4. Mahanthesh, M. C., Kumar, N., & Rajanna, K. (2018). Green synthesis of iron oxide nanoparticles using *Moringa oleifera* leaf extract: Characterization and applications. *Materials Science in Semiconductor Processing*, 88, 124-130.
5. Kim, J. S., Son, K. H., & Cho, M. H. (2019). Biocompatible metal nanoparticles for regenerative medicine applications. *Nanomedicine*, 5(3), 210-229
6. Akin, D. E., Yıldız, S. B. Z., Akbulut, M. Ö., & Ş. S. (2020). Green synthesis of nanoparticles from plant extracts and their applications. *Nanotechnology*, 31(5), 185601. <https://doi.org/10.1088/1361-6528/ab67a2>
7. Mohanta, Y. K., B. M. Panda, M. M. Ghosh, & S. D. Ghosh. (2021). Plant-based synthesis of iron oxide nanoparticles and their potential application. *International Journal of Nanomedicine*, 16, 1253-1267. <https://doi.org/10.2147/IJN.S311275>
8. Tao, Y., Zhang, X., & Li, L. (2021). Green synthesis of metal nanoparticles using plant extracts: Applications in environmental cleanup and biomedical applications. *Environmental Nanotechnology, Monitoring & Management*, 16, 100418. <https://doi.org/10.1016/j.enmm.2021.100418>
9. Gurunathan, S., Kim, J. H., & Dayem, A. A. (2021). Antibacterial activity of iron oxide nanoparticles against Gram-positive and Gram-negative bacteria. *International Journal of Nanomedicine*, 16, 5515-5527. <https://doi.org/10.2147/IJN.S311275>