ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

A comparitive study on *in vitro* susceptibility, synthesis and characterization of green and chemical mediated iron nanoparticles

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ABSTRACT

Nanobiotechnology has developed the modern and novel approach in medical field leads to various unfolds functions of the plant extract in the synthesis of iron nanoparticles (INPs). Biosynthesis of nanoparticles was carried out by green technology and chemical mediated method that are the more biocompatible and environmentally benign. The present study was aimed to synthesis of INP by using aqueous solution of *Erythrina variegate* plant extract for green for green method and polyethylene glycol for chemical mediated. INP were further characterized and confirmed by UV-Visible, Scanning Electron Microscope, XRD technique. Antimicrobial test against laboratory bacterial species and thermal stability of INPs at various temperatures were carried out for both methods. Green mediated INPs were exhibited highest antimicrobial activity of *Staphylococcus aureus* and *Bacillus Pumilis.* The green mediated method has more effective and stable than chemical method on the basis of thermal stability and antimicrobial activity.

KEY WORDS: Iron Nanoparticles, Chemical synthesis, Green synthesis, Scanning Electron Microscope, XRD and Antimicrobial activity.

1. INTRODUCTION

Nanotechnology is the science of engineering materials and systems on a molecular scale. Its function to medicine, nanomedicine has enabled the expansion of nanoparticles drug delivery vehicles. Nanostructures range from 1-100 nm in dimension. Nanotechnology is a steadfast and ecofriendly process for the production of nanoscale particles. Nanosize results in precise physicochemical characteristics of high surface area to volume ratio, resulting in high reactivity. Iron based magnetic nanoparticles magnetite (Fe_3O_4) have received numerous attentions in potential applications in biomedical application. Diverse synthesis techniques have been developed, most commonly used is chemical reduction of a metal salt in solution and crystallization of zero-valence metal particles. Green synthesis provides progression over chemical and physical method as it is cost efficient, easily scaled up for large scale synthesis. A wide range of biological sources like microorganisms and plants can be used for nanoparticle synthesis. .In prehistoric era it has been known that very low concentration of as-synthesized nanoparticles was sufficient to display effective antimicrobial activity as compared to earlier reports.

Here we reported the synthesis of nanoparticles through the reduction of ferric ions present in the ferric chloride ($FeCl_3$) by green mediate of *Erythrina variegate* plant leaf extract and chemical mediated by poly ethylene glycol (PEG).The objective of the present work was comparison of green synthesis INPs using selected leaf extract of *Erythrina variegate* and chemical method of INPs further to evaluate their thermal stability and anti-microbial activity.

2. MATERIALS AND METHODS

2.1. Green synthesis:

2.1.1. Preparation of plant extract: *Erythrina variegata* leaves were collected from local region and washed in distilled water, then dried with water absorbent paper. 20g of leaves was cut into small pieces and crushed in mortar and pestle dispensed in 100ml of distilled water. The extract was filtered by Whatman filter paper and the filtrate was collected in a clean and dried conical flask and stored at room temperature.

2.1.2. Synthesis of Iron Nanoparticles by green method: The precursor and reducing agent were mixed in a clean sterilized flask in 1:1 proportion. For the reduction of Fe^+ ions 5ml of filtered plant leaves extract mixed to 5ml of freshly prepared 0.1M of $FeCl_3$ solution with constant stirring (Remi mechanical stirrer, Mumbai) at 50-60^{o}C.Within the particular time the color changes from light green to black color obtained by nanoparticles synthesis. Then it can be centrifuged at 8000 rpm for 15 min .The supernatant was discarded and the pellet was washed with distilled water. Then clean pellet was collected and dried it by hot air oven.

2.1.3. Chemical synthesis: 8mM of ferrous sulphate (2.224g) and 4mM of sodium thiosulphate (0.992g) was mixed with 10ml of distilled water. The polyethylene glycol solution was added with them, kept in magnetic stirring at 50-60^{0}C. Stirring 5ml of sodium hydroxide solution was added to the beaker. The solution is transfer into the Teflon chamber of autoclave and kept under 150^{0}C temperature. Then the samples were kept in the incubation period of 6 hours. The entire solution was washed with ethanol and kept in drying.

2.2. UV–Vis Spectra Analysis: The reduction of the Fe^{+} ions by green and chemical mediated INPs were characterized by UV-visible spectroscopy monitored by sampling the aqueous component (2.0 mL) and measuring the UV-VIS spectrum of solutions. The UV-VIS spectra of these samples were measured on a UV-118 spectrophotometer (Perkin Elmer Lambda EZ201) operated at a resolution of 1 nm. The bio-reduction of Fe^{+} ions in aqueous solution was monitored by UV-VIS spectra of the solution between 100 nm – 400 nm. Distilled water was used to adjust the baseline.

2.3. XRD measurement: The Fe^{+} nanoparticles was purified by repeated centrifugation of green and chemical mediated synthesized suspensions at 10,000 rpm for 20 min followed by re dispersion of the pellet of iron nanoparticles in de ionised water and again centrifuged in same way. The freeze dried of above pellet nanoparticles was analysed by XRD, the crystalline domain size were determined by freeze dried pellet nanoparticles through XRD.

2.4. Scanning Electron Microscope analysis: After the preparation of the INPs nanoparticles by green and chemical mediated suspensions of nanoparticles in water were used for Scanning electron microscope analysis done by fabricating a drop of INP suspended in water onto a clean electric Stubs then allowing water to completely evaporate. Scanning Electron Microscope observations were carried out in Indian Institute of Technology, Chennai.

2.5. Antibacterial Activity

2.5.1. Human pathogens: Most common human pathogens such as *E.Coli, Staphylococcus, Lactobacillus, Bacillus megaterium, Bacillus Pumilis* and *Zymomonas mobilis* were collected from Kamban Arts and science college, Tiruvannamalai, India for the antibacterial susceptibility study.

2.5.2. Antibacterial determination by well diffusion method: Antibacterial activity of Iron nanoparticle was evaluated by disc method followed by Bauer et al. Twenty five microlitre of Iron nanoparticle was in Scanning electron microscopeinated with commercially accessible sterile empty disc (Hi-media Laboratory Private Limited, Mumbai, India) with the dimensions of 6mm diameter for antibacterial assay. Sterile MHA plates was prepared and swabbed with overnight broth cultures test pathogens (108 cells). Iron nanoparticle in Scanning electron microscopeinated disc was placed at the middle of the plate aseptically. Triplicates were maintained test pathogens to obtain mean value. The disc in Scanning electron microscopeinated with culture supernatant (25µl/disc) was used as a negative control to compare the antibacterial efficacy. All the inoculated plates were incubated at 37°C for 24 hours. Following incubation the various levels of zone of inhibition were measured and recorded in mm diameter for test pathogen.

3. RESULT AND DISCUSSION

3.1. Thermal stability of Green and chemical mediated INPs: 1ml of green and chemical mediated synthesized iron nanoparticles suspension were taken in 1.5 ml centrifuge tubes and kept in various temperatures such as 25˚C, 50˚C temperature, fridge (8˚C) and cold storage (0˚C) for 4 weeks and the UV absorbance were taken. The results were shown in Fig1, 2.

3.2. Characterization technique:

3.2.1. UV- Visible spectra: UV-Vis spectral analysis was done by using UV-Vis spectrophotometer Systronics 118 (Perkin Elmer Lambda EZ201) at the range of 100-400nm. The absorption peaks at 230 nm for green synthesized iron nanoparticles and 350 nm for chemically synthesized iron nanoparticles were observe. The excitation of surface Plasmon vibrations in the FeNPs solution, similar to the characteristics UV-Visible spectrum of metallic iron was recorded and the graph shown in fig 3.

3.2.2. Scanning Electron Microscope: Scanning electron microscope (Jeol version 1.1 JSM 6360, Japan) examines microscopic structure by scanning the surface of materials with higher resolution and greater depth of field. Magnification in a SCANNING ELECTRON MICROSCOPE can be controlled over a range of up to 6 orders of magnitude from about 10 to 500,000 times. The particles prepared by Green method were spherical and has smooth surface and chemically prepared iron nanoparticles were rod and crystalline in nature. The SEM results shown in fig4, 5.

3.2.3. XRD analysis: A thin film of the Fe nanoparticles was made by dipping a glass plate in the solution and carried out X-ray studies. The diffraction pattern was recorded by Co–Kα1 radiation with a wavelength of 1.78 Å. The scanning was done in the region of 20˚ to 90˚for 2θ at 0.02˚/min and the time constant was 2 s. The crystalline nature of Fe nanoparticles was confirmed from the X-ray diffraction analysis. The broadening of the Bragg peaks indicates the formation of nanoparticles. In addition to the Bragg peaks representative of Fe nanocrystals, additional, and yet unassigned, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the Iron nanoparticles. XRD results shown in fig 6.

3.3. Antibacterial activity: Antibacterial activity was carried out by well diffusion method for synthesized INPs and various antibiotic agents against six laboratory bacterial strains. Zone of inhibition(mm) of chemical and green mediated iron nanoparticles, others plant extract, tetracycline, bacitracin and chloramphenicol were shown in

Table1.The maximum zone of inhibition was obtained in *Staphylococcus aureus*, *Bacillus Pumilis* for plant mediated synthesis compare to Chemical mediated synthesis Fig.7, 8.

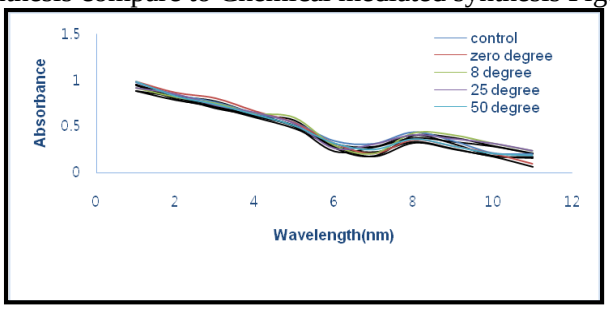


Figure.1.Determination of thermal stability for Chemical mediated INPs

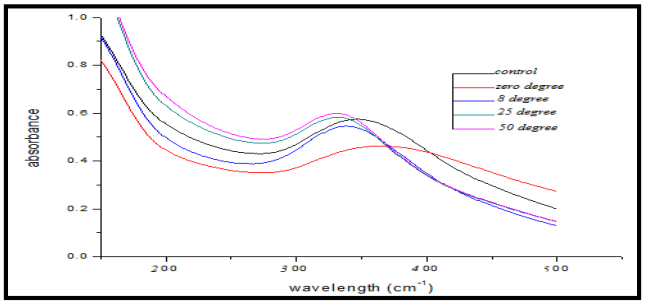


Figure.2.Determination of thermal stability for green mediated INPs

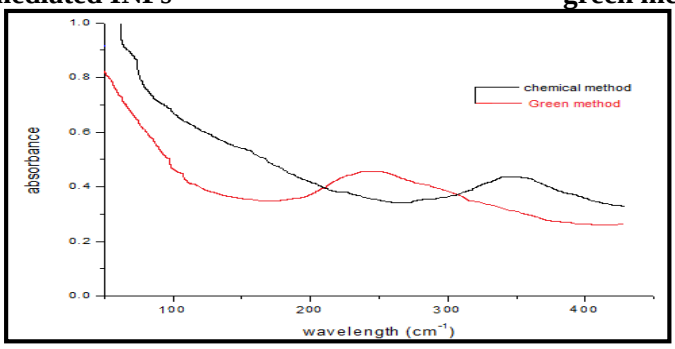


Figure.3.UV-Visible spectra for green and chemical mediated INPs

Figure.4.Scanning Electron Microscope analysis images for Green method (a,b)

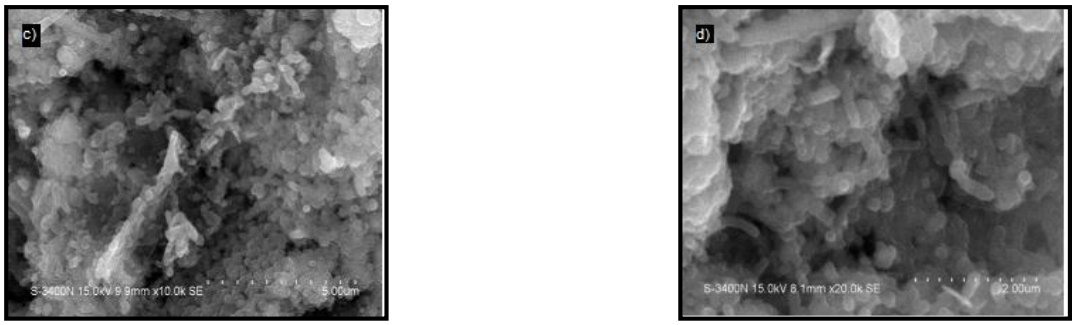


Figure.5.Scanning Electron Microscope analysis images for Chemical method(c,d)

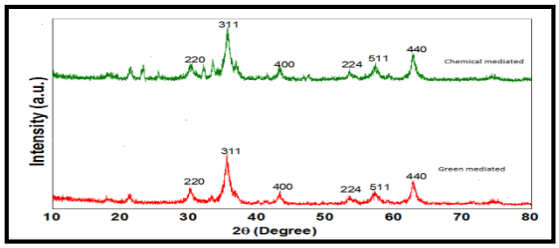
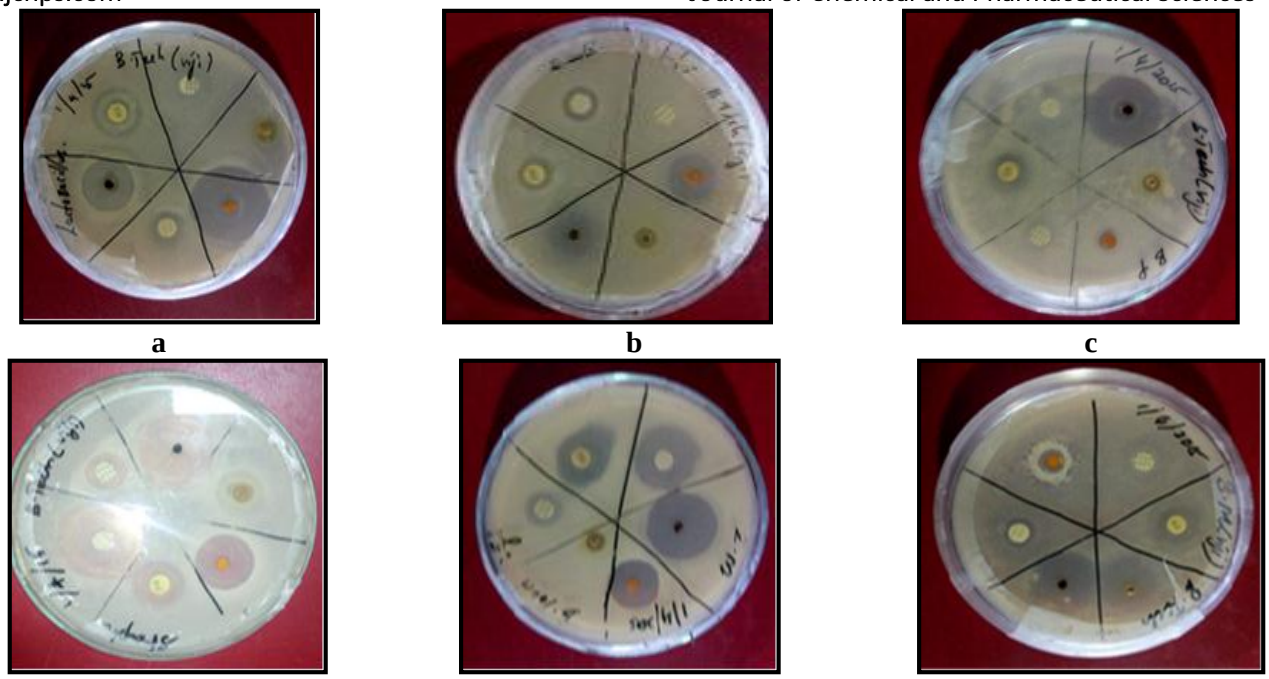


Figure.6.XRD pattern of green and chemical mediated INP



a **b** **c**

d **e** **f**

Figure.7.a)*Lactobacillus planetarium* b) *E.Coli* c) *Bacillus pumilis* d) *Staphylococcus aureus* e) *Zymomonas mobilis* and f) *Bacillus megaterium.*

Table.1.Zone of inhibition (mm) of chemical and green mediated iron nanoparticles are wide when compared to others such as plant extract, tetracycline, bacitracin and chloramphenicol

Organisms	*Lacto Bacillus*	*Zymomonas mobilis*	*Bacillus megaterium*	*Staphylococcus aureus*	*E.coli*	*Bacillus pumilis*
Plant Extract	06	16	16	06	06	07
Chemical Mediated Iron Nanoparticles	29	18	21	18	15	08
Green Mediated Iron nanoparticles	19	24	21	23	02	23
Bacitracin	NIL	11	07	11	NIL	07
Tetracycline	16	17	13	13	11	14
Chloramphenicol	1.2	2.0	0.8	2.5	1.2	1.2

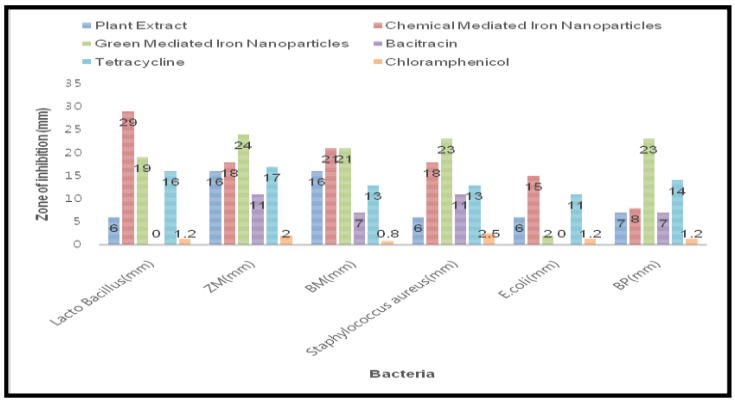


Figure.8.Zone of inhibition (mm) comparison of antimicrobial activity for green and chemical mediated iron nanoparticles

4. CONCLUSION

Iron nanoparticles were successfully prepared by green synthesis method using aqueous leaf extract of *Erythrina variegata* and chemical method. Thermal stability shown the Green method iron nanoparticles were more stable in various temperatures than chemically synthesized iron nanoparticles. SEM and XRD results showed that the particles prepared by green method were spherical and has smooth surface and chemically prepared iron nanoparticles were rod and crystalline in nature. The antibacterial activity of green synthesized iron nanoparticles is

higher than chemical mediated iron nanoparticles. The results proved that green synthesis is more effective than chemical method for synthesis of iron nanoparticles.

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