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Synthesis and Antimicrobial Efficacy of Mulberry Leaves Extract Mediated Iron Nanoparticles Against Bacterial Strains

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Abstract

Due to easy availability, non toxic and devoid of use of hazardous chemical substances in green approach in the present study, synthesis and characterization and the antimicrobial efficacy of environment friendly Iron nanoparticles (FeNPs) using M*ulberry* leaf extract is investigated.

Characterization of synthesized Iron nanoparticles is done using UV spectroscopy, XRD, DLS and TEM. The color shift from colorless to colored after mixing plant leaves extract to aqueous solution of Ferric chloride indicates capping, reducing, and stabilizing nature of leaf extract.

Transmission electron microscopy analysis confirmed that Iron nanoparticles were spherical in shape having range 8 ±1.4 nm.

Antimicrobial efficiency of synthesized Iron nanoparticles was tested against *Salmonella typhi*, *Bacillus cereus*, and *Pseudomonas aeruginosa* bacterial strains employing well diffusion method.

Keywords: Mulberry, Secondary metabolites, Iron Nanoparticles, Antimicrobial activity

INTRODUCTION

Different approaches based on chemical, physical and green techniques have been reported for the synthesis of nanoparticles. Some of the common reported chemical methods are hydrothermal, microemulsion, sonochemical, co-precipitation, electrochemical [1-3]. In the case of physical methods the most popular are arc-discharge and physical vapor condensation [4, 5]. When plant leaves, fruits, herb, algae, bacteria, yeast, fungi and proteins etc are used as stabilizing and reducing agents for nanoparticle synthesis, the approach is termed as green synthesis [6, 7]. The aforementioned green reagents reduce and stabilize metallic ions like Au^{+}, Ag^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Cr^{3+}, Al^{3+}etc. to their nanostate by generating phytochemicals like poly phenols, flavonoids, catechols, catechin, epigalloctedin etc. The advantage of such an approach is use of non toxic chemical compounds in the place of toxic chemicals normally used like sodium borohydride and hydrazine etc. Phytochemicals are known to act as good stabilizing and functionalizing agents by actively chelating the reduced metallic ions with carboxylic, hydroxyl or phosphate groups etc. present in them.

It has been further reported in literature [8-10] that terpenoids present in geranium leaves and eugenol present in cinnamon leaves reduced Ag^{+} into Ag nanoparticles. Glucose present in plants also reduced Ag^{+} and Au^{+} ions to its metallic nanoparticles. Different amino acids like lysine, cystine, arginine show

good stabilizing ability for Ag^{+}. It shown that polyphenols extracted from tea leaves act as reducing agent for Fe^{3+} and $Fe.^{2+}$

Metallic nanoparticles have found applications in high density recording media, catalysis, targeted drug and gene delivery agent, substrate in cancer treatment, hyperthermia agent, waste water treatment agent and in magnetic resonance imaging etc. due to their bio-compatibility and biodegrability and microwave absorption property [11-14].

Nanoparicles have been used for mitigation of microbial toxicants which includes those synthesized using green techniques which have been shown to be less toxic. (Mallikarjuna. N et al., 2010) synthesized nanoparticles of iron using polyphenols extracted from tea leaves as well as $NaBH_4$ [15]. Their assessment employing MTS and LDH assays using cell lines of human keratinocyte cells indicated that the former was less toxic in comparison. (Erick et al., 2014) synthesized Silver nanoparticles using *Tridex procumbens* which showed anti microbial property against *Escherilic coli and Vibrio cholera* [16]. Copper nanoparticles using *Nerium oleander* leaves which showed anti bacterial activity against *Klebsiella pneumoniae, Salmonella typhi and Bacillus subtilis*. (Lokina.S et al., 2013) synthesized gold nanoparticles using Grape fruit which showed good anti cancer activity against HeLa cell lines [17]. (Naseem.T et al., 2015) synthesized iron nanoparticles using Lawsonia inermis and Gardenia jasminoides which showed anti microbial activity against *Staphylococcus aureus, Salmonella enterica, and Proteus mirabilis* [18]. (Khalil et al., 2014) synthesized silver nanoparticles using *olive* which showed anti microbial property against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [19].

MATERIALS AND METHODS

Chemicals

The fresh leaves of *Mulberry* plant were collected from campus of Government College for Women, Gurawara, Rewari, Haryana,India and analytical grade anhydrous $FeCl_3$ was purchased from Hi-Media. The bacterial strains of *Bacillus cerus* (MTCC- 1272), *Pseudomonas aeruginosa* (PAO-1) and *Salmonella enteric typhi* (MTCC- 734) were used for assessing antimicrobial efficacy of synthesized Iron nanoparticles

Glassware and Apparatus

All glassware (beakers, conical flasks, burette, measuring cylinder, funnel etc.) were purchased from Borosil, India. Whatman No 1 filter paper, eppendorfs tubes, eppendorf stand, microtips, micropipettes, spatula, falcon tubes, sterile plastic petri dishes, cotton swabs, laminar hood, autoclave, incubator etc. were used in experimental process.

Preparation of *Mullberry* Plant Extract

Fresh leaves were thoroughly washed twice with simple tap water then with distil water and dried over an absorbent sheet at room temperature. Dry leaves weighing about 2.4 gm were refluxed with 100 ml of distilled water in 250 ml round bottom flask for 2 hours. The broth was brought to room temperature and filtered with Whatman filter paper number 1. The extracts were centrifuged for five minutes to remove heavy biomaterials. The centrifuged plant extracts were stored at $4^{O}C$ for iron nanoparticles synthesis.

Synthesis of Iron nanoparticles

This preparation of bio mediated Iron nanoparticle is almost similar to the chemically mediated procedure used commonly for the synthesis of FeNPs. In green synthesis of nanoparticles secondary metabolaties present in plant extract itself acts as reducing as well as stabilizing agent therefore in the

procedure followed varying amount of plant extract was added to varying amount of 1 mM solution of $FeCl_3$ **(**see Table 2**)** and stirred for 1 hr. The solution after mixing turned to dark blue colour after 2-3 min, indicating the initial formation of Iron nanoparticles. The resulting solutions were centrifuged to separate out the solid nanoparticles which were further washed with ethanol before further characterization.

Anti Microbial activity

Antimicrobial activity of the mulberry extract mediated synthesized Iron nanoparticles evaluated against *Salmonella typhi*, *Bacillus cereus*, and *Pseudomonas aeruginosa* bacterial strains.

Preparation of Nutrient agar and Nutrient broth

For the culturing and growth of all microorganisms Nutrient agar media was used while Nutrient broth was used for incubation and standardization of microorganisms. 28 gm and 13 gm of nutrient agar and Nutrient broth were dissolved in 1 liter of distilled water respectively followed by sterilization by autoclaving at 121°C for 15 minutes. The agar media was then dispended and solidify in sterilized plastic Petri plates

Antimicrobial Activity Evaluation

Antibacterial activity was studied employing well-diffusion method against pathogenic organism (*Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi).*[63]**.** The pure bacterial culture was sub cultured on nutrient agar. A number of wells of 8mm diameter were made on nutrient agar using gel puncture. The bacterial strain was swabbed uniformly onto the individual plates using sterile cotton swabs.100 µl of iron nanoparticles solution was poured into each well. After incubation at 37°C for 24 hours, the levels of zone of inhibition were measured and expressed in units of mm as shown in Figure 5 and Table 2.

Characterization of Synthesized Iron Nanoparticles

The synthesised FeNPs were characterised by their characteristic peak known to occur in the range 200-300 nm [**29**] employing UV spectrometry. The surface functionalization of synthesized FeNPs was analyzed by absorption spectra of Fourier transform Infrared spectroscopy (FTIR) in the range 500-4000 cm^{-1}. The size, shape and morphology of synthesized nanoparticles were determined with the help of TEM and XRD techniques.

RESULTS AND DISCUSSION

UV-visible Studies

The optical absorption spectra of synthesized Iron nanoparticles were studied in the range 200-800nm using a UV-Vis Jasco V550 spectrometer. Figure 1 shows the UV-Vis spectra to ascertain synthesis of FeNPs using Mulberry leaves extract. A colour shift from colourless to coloured observed after mixing plant leaves extract in aqueous solution of ferric chloride ascertain the synthesis ofnanoparticles. The two characteristic absorbance peaks in the range 200 nm -300 nm of Iron nanoparticles synthesized using *Mulberry leaves* extract are shown in Figure 1. It has been reported in literature that surface Plasmon peak related to Iron nanoparticles having size in nano range occurs in the range 200-300 nm. Figure 1 and Table 1 shows the surface Plasmon peak around 204 and 266 nm in the present work establishes the nano size of the synthesized iron nonoparticle.

Table 1:- Details of optical spectrometric characterisation of FeNPs synthesized by varying the amount of mulberry plant leaf extract.

Sample no	Volume of 0.01M $FeCl_3$ solution (ml)	Volume of Plant Extract (ml)	Characteristic Absorbance peak for FeNPS in UV-visible Spectrometery (200-270nm)
1	5	1	62 nm
2	5	3	204 nm, 266 nm
3	5	5	204 nm, 266 nm
4	5	7	204 nm, 266 nm
5	5	9	203 nm , 266 nm
6	5	10	205 nm, 267 nm

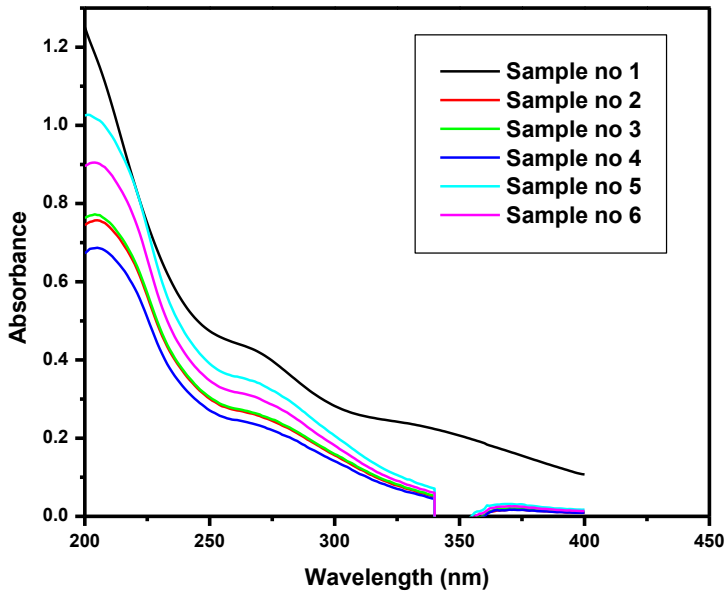

Figure 1. UV spectra of Fe nanoparticles synthesized using Mulberry leaves extract

X-Ray Diffraction (XRD) Analysis

The amorphous/crystalline phase of synthesized iron nanoparticles was determined using D/Max-2500 X-ray diffractometer in the range 10-90°. Figure 2 shows the XRD spectrum (X-Ray diffraction) of iron nanoparticles synthesized using *Mulberry* extract peaks were observed at 24.32°, 27.4°, 33.3°, 35.78°, 49.62° and 54.24° indicating their crystalline nature.

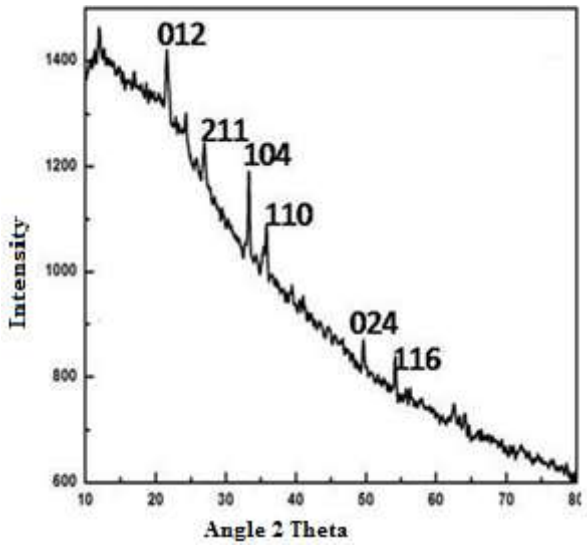

Figure 2. XRD spectra of Iron nanoparticles synthesized using Mulberry extract.

Dynamic Light Scattering (DLS) Studies

The correlation between amounts of mulberry leaves extract and the size distribution of synthesized FeNPs was studied using dynamic light scattering technique. Figure 3 shows the effect of concentration of mulberry leaf extract on the size distribution of FeNPs synthesized from the mulberry plant leaf extract. It shows that as we increase the concentration of mulberry plant leaf extract the size of the FeNPs keeps on decreasing. It is further observed that in the case of sample 6 which was prepared using 5 ml of 0.01 mM of salt precursor and 10 ml of mulberry plant leaf extract the most probable size of synthesized nanoparticle was minimum i.e. around 10 nm.

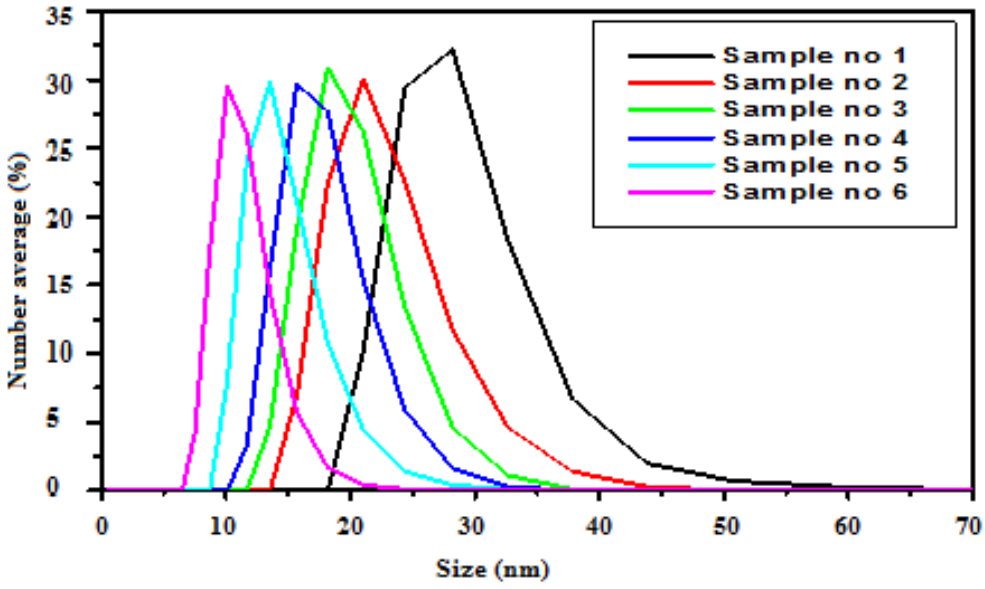

Figure 3:- Size distribution by number plot showing the effect of concentration of the mulberry plant leaf extract.

TEM Studies

Figure 4.shows the morphology and size distribution of the Iron naoparticles (sample 6) synthesized fro-

m the mulberry plant leaf extract. The TEM image shows that FeNPS have a mean size of 8 ±1.4 nm and these corroborates the results as obtained in DLS studies. It was further observed that the shape of the synthesized FeNPs was nearly spherical.

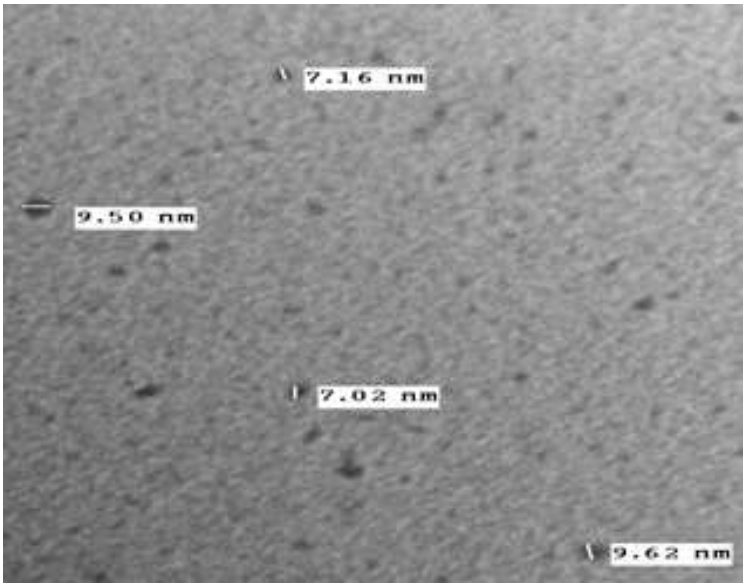

Figure 4:- TEM image of synthesized FeNPs (sample 6)

Antimicrobial Efficacy of FeNPs

Figure 5 shows the antibacterial activity of synthesized FeNPs against *Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi.* employing well-diffusion method where as Table 2 shows the average zone of Inhibition (mm) against different strains. The bacterial strains were challenged by 70 µL of iron nanoparticles of iron having size 8 ± 1.4 nm and concentration 1176 ng which was obtained using XRF technique. The zones of inhibition were measured after incubation of the culture at 37°C for 24 hours. The preliminary studie shows that FeNPs synthesized using Mulberry leafextract are effective as antimicrobial agent only in the case of *Pseudomonas aeruginosa and Salmonella typhi.*

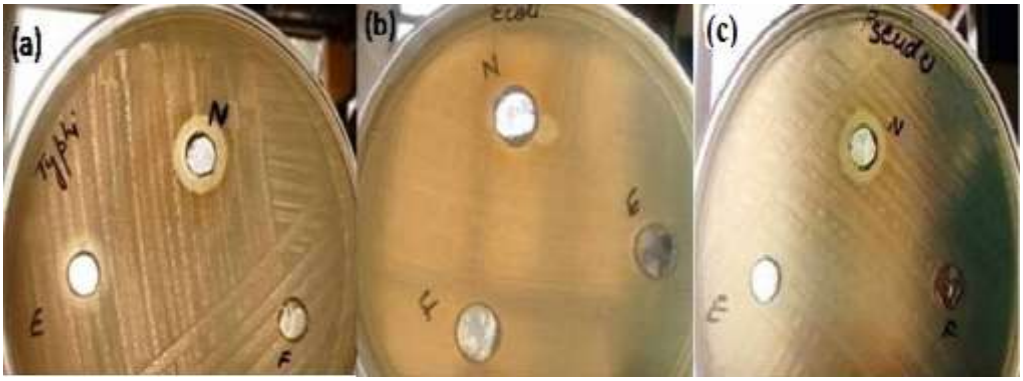

Figure 5. Antimicrobial activity of FeNPs synthesized (a) *Salmonella typhi.* (b) *Escherichia coli* (c) *Pseudomonas aeruginosa*. where as E – pure extract, F- $FeCl_3$ solution and N- nanoparticles.

Table 2: Magnitude of Zone of inhibition of synthesized FeNPs against different microbes.

Sr. No.	Name of the microbe	Zone of inhibition (mm)
2	*Pseudomonas aeruginosa*	4 mm
3	*Escherichia coli*	Nil
4	*Salmonella typhi.*	6 mm

Conclusion

The size of FeNPs synthesized in the present work showed strong dependence on the amount of mulberry leaf extract used as reducing agent/stabilising agent. The FeNPs having size of 8 ± 1.4 nm and Fe concentration of 1716 ng showed antimicrobial activity against *Pseudomonas aeruginosa* and *Salmonella typhi* except *E. Coli.*

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