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Biosynthesis and characterization of Magnetic (Fe3o4) Iron oxide nanoparticles from a red seaweed *gracilaria edulis* and its antimicrobial activity

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Abstract

In this study an eco-friendly method was established for extracellular synthesis of Fe3O4iron oxide nanoparticles using the extracts of red macro-algae *Gracilaria edulis* and also examined for its antimicrobial activity against various bacterial and fungal pathogens. The unexplored *Gracilaria edulis* extract was found to be capable in green synthesis of Fe3O4Iron oxide nanoparticles and their characteristics were studied using UV-visible spectrophotometer, SEM, EDX, XRD and FT-IR. The synthesised Fe3O4 Iron oxide nanoparticles were naturally stable, cubic shaped and in the size range of 20nm - 26 nm. The phytochemicals present in the seaweed has a main role as a reducing agent that assists to the biosynthesis of Fe3O4 iron oxide nanoparticles with enhanced antimicrobial property. Effective growth of inhibition of cells was observed to be more in *p.Aerogenosa* (Bacteria), *A.nidulans* and *C.albicans* (Fungi) in antimicrobial activity. Thus, this naturally stabilised iron oxide nanoparticles with herbal properties can be used in various biological applications.

Keywords: *Green synthesis, Fe3O4 Iron oxide nanoparticles, Gracilaria edulis, Antimicrobial activity.*

1 Introduction

Nano particles exhibit many interesting properties of materials in the form of nanosized particles. Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of Nano particles (Andreescu *et al.*, 2007). Though physical and chemical ways are a lot of in style for Nano particle synthesis, the utilization of hepatotoxic compounds limits their applications. Green nanotechnology has attracted a lot of attention and includes a wide range of processes that reduce or eliminate toxic substances to restore the environment. Green synthesis of nanoparticles makes use of environmental friendly, non-toxic, and safe reagents (Mahnaz Mahdavi *et al.*, 2013.)

Super paramagnetic iron oxide nanoparticles with appropriate surface chemistry can be used for numerous *in* V*ivo* applications, such as, tissue repair, detoxification of biological fluids, hyperthermia, drug delivery, and MRI contrast enhancement. All of these biomedical applications require the nanoparticles that have high magnetization values, a size smaller than 100 nm, and a narrow particle size distribution. Such magnetic nanoparticles can bind to drugs, proteins, enzymes, antibodies, or nucleotides and can be

directed to an organ, tissue, or tumour using an external magnetic field (Sophie Laurent *et al.*, 2008)

Seaweeds are the group of marine macro algae that lives in marine water environment. *Gracilaria edulis* is a predominant red seaweed found in the coastal regions of Mandapam, Tamilnadu. It is an important source for the agar extraction and other related products. It possesses several biomedical properties such as antiviral, antifungal, antiprotozoal, anti-tumour, anti-inflammatory, anti-oxidant and cytotoxic effects. (Ganesapandian and Kumaraguru, 2008). (Satyajithpatra *et al*., 2013) reported that ethanolic extract of G. edulis induces caspase-mediated cell death and inhibits the growth in Paul Ehrlich pathology growth cells in vivo and in vitro .Recently, there are reports in which algae are being used as a bio factory for the synthesis of metallic nanoparticles.Biosynthesis of nanoparticles using algal extract is more advantageous over other biological processes such as bacteria and fungi, because it eliminates the cell culture maintaining process, and it is also more suitable for large-scale production of nanoparticles (Ramaramesh *et al*., 2014). In view of the above, the present study was mainly focused on synthesizing and characterizing the Fe3O4 Iron oxide nanoparticles using the extracts of *G. edulis* and to evaluate its antimicrobial efficacy.

2 Materials and methods

2.1Collectionof*G.Edulis* and preparation of the extract

The healthy samples of *Gracilaria edulis* (fresh material) were collected along the coast of Mandapam (Lat. 09° 17′ N; Long. 79° 08′ E), Tamil Nadu, India. After thorough washing with seawater and manual sorting to remove epiphytes, the fresh biomass was exhaustively washed with tap water followed by distilled water. Fresh alga (10 g) was mixed in 50 mL of sterile distilled water and chopped into fine pieces of approximately 1 mm. The mixture was then boiled by microwave oven irradiation for 10 min. Then, the extract was filtered through Whatman no.1 filter paper, and the filtrate was used for further study. Iron oxide chemical was obtained from Merck, Mumbai, India.

2.2 Green synthesis of G. Edulis Fe3O4-NPs

The FeCl3 (0.1mol/l) solution was added to the seaweed extract in a 1:1 volume ratio. PH were adjusted to 11.0 by using 0.1N NaOH. Fe3O4 Nanoparticles were obtained with the reduction process. The mixture was stirred for 60mins and then allowed to stand at room temperature for another 30mins. The obtained colloidal suspensions were then centrifuged and washed several times with ethanol and then dried at 40°C under vacuum to obtain the Fe3O4-Nanoparticles. Seaweed extract have the best reduction capability against ferric chloride when compared to other parts of the plants that is observed by the external colour change. After the visual confirmation test the Fe3O4 -NPs were synthesised by using the above procedure for further characterisation.

2.3 Characterisation of Fe3O4-NPs

Characterisation techniques help us to understand the specific properties of the substance or nanocrystals to be studied in an accurate rapid manner which is reliable to understand the measured values. The synthesised Fe3O4-NPs were subjected to various characterisation studies to understand the specific properties such as optical, structural, morphological, elemental composition, particle size, functional groups studies which could be made precisely using sophisticated techniques such as UV-VIS spectroscopy (SHIMADZU 3600 UV-Vis NIR model), XRD (PAN analytical X'Pert Pro instrument with Cu Kα1 radiation of wavelength (l) of 1.5406 (°A)), SEM, EDS (FEI-QUANTA 200) and FT – IR (SHIMADZU FTIR 8400S) instruments. These techniques were helpful to verify our method is well optimised and meeting the requirements.

2.4 Evaluation of antimicrobial activity of Fe3O4-NPs

The following bacterial pathogens namely *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* and fungal pathogens *Aspergillus. niger*, *Aspergillus. flavus*, *Aspergillus. oryzae*, *Candida.albicans,and Aspergilus. nidulans* were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The in vitro antimicrobial activity of Fe3O4 nanoparticles with and without stabilizing agents against the pathogenic bacteria and fungi was screened by agar well diffusion method. Pure culture was sub cultured overnight in nutrient broth at 37^{o}C. Pathogens were seeded on Muller Hinton agar media for bacteria and potato dextrose agar for fungi using sterilized cotton swabs. Wells of 6 mm diameter were made on agar plates using sterile gel puncture. Using a micropipette 50 µl of nanoparticle suspension was introduced into each well on all the plates. Tetracycline was used as appositive control for bacteria and amphotericin for

fungi. Different concentrations of the sample were maintained (10, 20, 30, 40, 40, µg) for both bacteria and fungi .Plates were incubated for 24hr at 37°c (bacteria) and for 48hr at room temperature for fungi. After incubation, the presence of inhibition zone around the sample loaded well was observed and their diameters (mm) were measured using measuring scale (Gottesman *et al*., 2011). Each nanoparticle was tested in triplicate with broad spectrum antibiotic gentamycin (bacteria) and Amphotericin (Fungi) (10 mcg/disc) as a standard.

3 Results and discussion

3.1 Characterisation of Fe3O4-NPs

Visual Inspection

The fine brick red precipitate was visually observed in the reaction mixture which indicated the rapid formation of Fe3O4 nanoparticles (Fig. 1). Brick red precipitate was appeared immediately after the first drop of reducing agent into iron solution.

Fig 1: Synthesized Fe3O4 iron oxide nanoparticles from the seaweed *Gracilaria edulis*

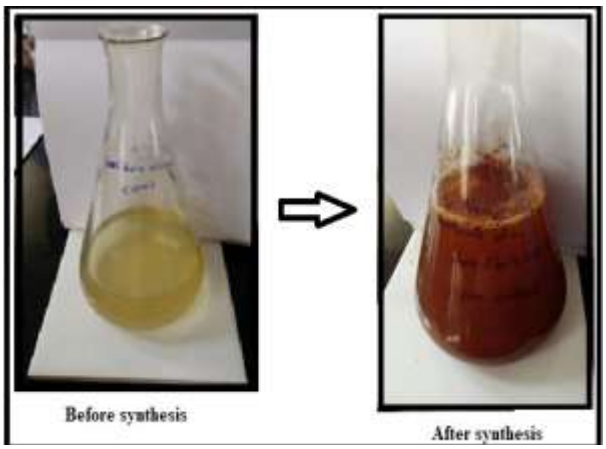

Fig1a: Conversion of Fe3O4 iron oxide nanoparticles.

UV-Vis spectrophotometry

The bio reduction of metallic element ions in aqueous solutions was monitored by measurement UV/Vis spectra (Fig2). UV/Vis spectral analysis was done at a wavelength vary of 300-800 nm to check the absorption spectra of green synthesized Fe3O4NPs and also the absorption peaks were discovered at 300-400 nm ranges due to the excitation of surface Plasmon vibrations in Fe3O4NPs as has been reported earlier (Kaviya *et al*., 2011). Effect of precursor salt solution on nanoparticles synthesis revealed that 5mM concentration of Fe3O4 resulted in maximum nanoparticles synthesis with the absorption peak around 410 nm (Fig. 2). Therefore, the selected algae are very efficient in biosynthesis of Fe3O4-NPs.

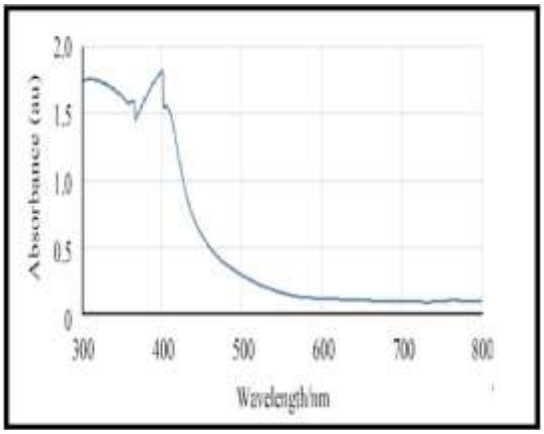

Fig.2: UV absorption spectra of *Gracilaria edulis* Fe3O4 mediated nanoparticles

X-Ray Diffraction

The X-ray diffraction spectrum of the Fe3O4iron nanoparticles is illustrated in (Fig.3). The information obtained from the spectrum indicated that the iron was mainly in its Fe3O4 state, characterized by basic reflection appearing at 2Ø value of 44.80^{o} and additional peaks at 30.95^{o} and 36.00^{o} (Kang *et al*., 2003). The obtained broad peak 44.80^{o} revealed that the existence of an amorphous phase of iron and represented bcc (body-centred cubic crystal) Fe3O4 lattice plane (110) of Fe3O4 (Huang *et al*., 2005).

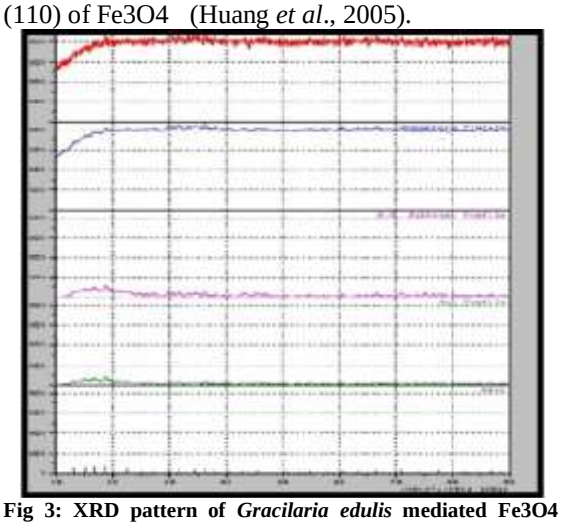

Fig 3: XRD pattern of *Gracilaria edulis* mediated Fe3O4 iron oxide nanoparticles

(Elumalai *et al*., 2010) reported that the Fe3O4 state is characterized by the basic reflection appearing at 2Ø value 44.90^{o} and additional peaks at 65.22^{o} and 82.50^{o}. These peaks represent bcc Fe3O4 lattice planes (110), bcc Fe3O4 (200), and bcc Fe3O4 (211) respectively. The crystalline size of the nanoparticles was calculated using Scherrer's formula and the size was found to be 24.87nm. The result obtained in the present experiment is in confirmation with the findings of (Khani *et al*., 2013).

Scanning Electron Microscopy

Formation of Fe3O4-NPs and its morphological dimensions were studied using the SEM. The study demonstrated that the average size of the NPs were in the range of 20nm -100nm and also exhibits the formation of cube shape of iron nanoparticles as shown in the (Fig. 4).The cube shaped nanoparticles formation was induced by chloride, potassium compounds present in the sample. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three dimensional appearance useful for understanding the surface structure of a sample (Kanagasubbulakshmi *et al.*, 2017). In this micrograph, the Fe3O4nanoparticles didn't seem as distinct particles, however type abundant larger nerve fibre flocs resulted in chain like aggregates whose size reached micron scale. The aggregation was attributed due to the magnetic attractive forces between the particles. This chain like Nano iron aggregation is also observed by other researchers (Huang *et al*., 2007).

EDX - Energy Dispersive X-ray Spectroscopy

(Fig.5) shows the EDX spectrum of synthesized iron nanoparticles. The elemental profile of Fe3O4nanoparticles confirmed the presence of elemental iron signal at 6.4 keV. The elemental analysis revealed that iron was in highest proportion (72.11%) followed by oxygen (20.66%) and chlorine (7.23%) in nanoparticles mass (Table 1). The additional peak for oxygen was due to the adsorbed oxygen or facile oxidation of the particles and the presence of chlorine peak was due to the application of chlorine during the synthesis process. Similar elemental composition of Fe3o4 nanoparticles is observed by (Suntornchot *et al*., 2010).

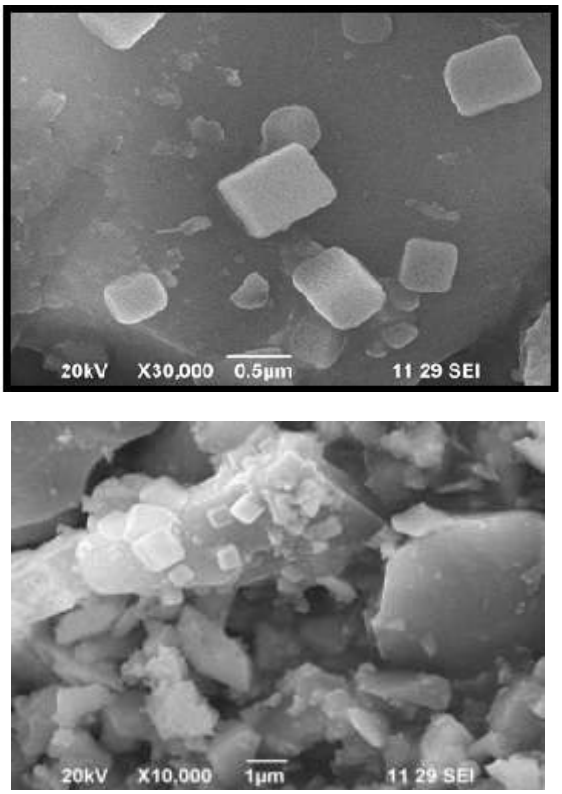

Fig 4: SEM imaging of *Gracilaria edulis* mediated Fe3O4iron oxide nanoparticles

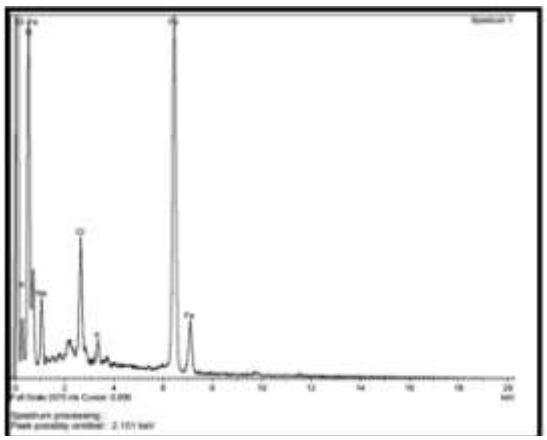

Fig 5: EDX - Energy Dispersive X-ray Spectroscopy pattern of *Gracilaria edulis* mediated Fe3O4 nanoparticles

Table: 1 EDX analysis of Fe3O4 nanoparticles

Element	Weight (%)	Atomic weight (%)
Fe	72.11	41.67
O	20.66	41.67
Cl	7.23	16.66
Total	100	100

Fourier-transform infrared spectroscopy

The FTIR bands of G. edulis aqueous extracts (Fig.6) showed the strong absorption band at

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3446.94cm^{-1} indicate the presence of amine group in the seaweed extract which participated in the reaction, due to N-H stretching vibration. The strong band at 1516.11cm^{-1}in spectrum suggests the C=O stretching amide functional group. The band at 1383.02cm^{-1} signify N=O stretching vibrations of nitro compound. The formation of Fe_3O_4 is characterized by two absorption peaks at 574cm-1 and 421cm^{-1}which correspond to the Fe-O bond in magnetite (Nunes *et al.*, 2006). The presence of various bands in FTIR ascertained the fact that the nanoparticle complex has a variety of functional groups which arose from the various phytochemicals present in the seaweed extract. These phytochemicals response to the reduction of iron salt to iron oxide nanoparticles.

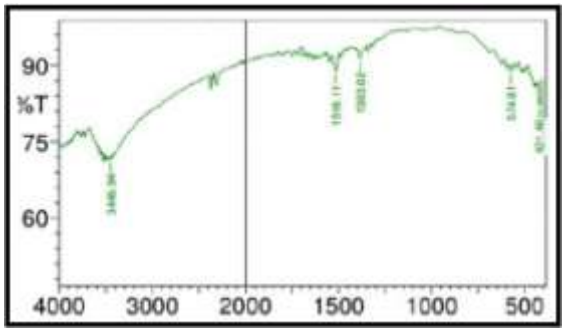

Fig 6: Fourier Transform Infrared (FTIR) spectrum of *Gracilariaedulis* mediated Fe3O4iron oxide nanoparticles

4 Evaluation of antimicrobial activity of Fe3O4-NPs

In this study, among the different bacterial pathogens used, antibacterial activity of the Fe3O4nanoparticles exhibited maximum activity in *Pseudomonas aeruginosa* (15 ± 0.5 mm), while the lowest activity was observed in *k.pneumoniae* (3±0.5 mm) (F 7a). Antifungal activity of the present study reveals that maximum inhibitory activity was observed with *Aspergillus nidulans* (16 ± 0.5 mm) and minimal inhibition activity was observed with *Aspergillus oryzae* (12 ± 0.5 mm). (Fig 7b). Similar phenomenon has been reported for the antibacterial effect of iron oxide nanoparticles prepared by (Saba A. Quasy *et al.*, 2012). The bactericidal effect of iron oxide nanoparticles may be due to their smaller size (Changha lee *et al*. 2008). The inactivation of *E. coli* by iron oxide nanoparticles could be because of the penetration of the small particles (sizes ranging from 10 to 80 nm) into *E. coli* membranes, leading to oxidative stress and causes interruption of the cell membrane. The significance of study showed that the saturation of the media with the iron nanoparticles results in loss of oxygen which may be due to the Fenton's reaction.

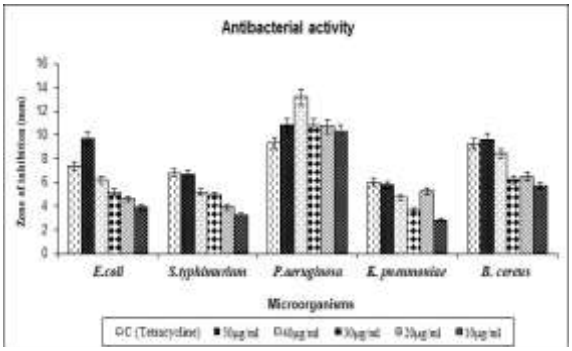

Fig 7a: Antimicrobial activity of Fe3O4-NPs against bacterial pathogens

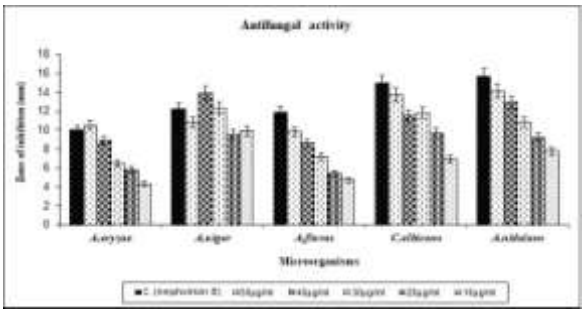

Fig 7b: Antimicrobial activity of Fe3O4-NPs against fungal pathogens

5 Conclusion

A critical need in the field of Nanotechnology is the development of reliable and eco-friendly processes for synthesis of metal oxide nanoparticles. In this study, we have succeeded in synthesizing Fe_3O_4 nanoparticles from a red seaweed *Gracilaria edulis* by bio reduction of Ferric chloride solution with a green method. The process does not require any harsh chemicals. The synthesized nanoparticles characterized using UV-Vis, FT-IR, XRD, EDX and SEM, Confirmed that the size of nanoparticles (20-26nm) and shape (cube). Bio synthesized Fe3O4iron oxide nanoparticles also showed enhanced antimicrobial property against various bacterial and fungal pathogens. This Biosynthesized naturally stabilised Fe_3O_4 nanoparticles with antimicrobial properties can be used in various biomedical applications.

Acknowledgement

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