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Biosynthesis and characterization of iron oxide nanoparticles using *Eichhornia crassipes* leaf extract and assessing their antibacterial activity

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ABSTRACT

The study elaborated the processes of biogenic iron oxide nanoparticle (FeNPs) formation via green chemistry approach and analyzed their antibacterial activity. The biosynthesized iron oxide nanoparticles (FeNPs) were characterized by UV-visible spectroscopy, FT-IR (Fourier transform infrared spectroscopy), X-ray Diffractometer (XRD), EDX (Energy dispersive X-ray spectroscopy) and SEM (Scanning electron microscopy). The synthesized nanoparticles were rod shaped. The antibacterial activity was determined for *Eichhornia* mediated iron oxide nanoparticles (FeNPs). The highest zone of inhibition was observed at 100 μg/ml concentration of *Eichhornia* mediated iron oxide nanoparticles against *Staphylococcus aureus* and *Pseudomonas fluorescens*. The iron oxide nanoparticles (FeNPs) showed good antibacterial activity and may be used in medicinal fields.

1. Introduction

Nanotechnology is one of the fast growing emerging fields in modern science. Nanotechnology can be defined as the exploitation of substance by certain physical, chemical or biological processes to produce materials in nanoscale level (1–100 nm) with specific properties (Sridhara et al., 2012). Nanocrystalline particles have a tremendous application in various fields such as catalysis, cosmetics, paints, agriculture, drug delivery, photonic crystals analysis, food, coatings, health care and material science (Rai and Ingle, 2012; Padil and Cernik, 2013). In recent years, the development of efficient green chemistry approach attracted the attention of researchers to find out the non toxic method for the synthesis of nanoparticles. The metal oxide nanoparticles are synthesized using eco-friendly materials such as plant tissue (Shameli et al., 2012), plant extracts (Parsons et al., 2007) and other parts of living plants (Jain et al., 2005). Iron oxide nanoparticles have attracted the great interests due to their unique physiochemical properties. The synthesis of iron oxide nanoparticles by plants through green chemistry approach is a novel technique to overcome the limitations of other conventional methods. In this green synthesis method, the biomolecules in plant act as a reducing and capping agents (Wang et al., 2014a, b). The Fe_2O_3 nanoparticles are synthesized from various plant extracts like *Melaleucane sophila* (Kumar et al., 2013), *Camellia sinensis* leaves (Hoag et al., 2009), *Eucalyptus globules* (Madhavi et al., 2013), *Terminalia chebula* (Wang et al., 2014a, b),*Tridax procumbens* (Senthil and Ramesh, 2012) etc.

Bacterial resistance of different antibiotics is a severe clinical problem in public health. Therefore, researchers are focused to find the new antibiotics from different sources. Current development in nanotechnology provided an attractive method for synthesizing different antimicrobial agents (Lalitha et al., 2012).

Eichhornia crassipes is one of the aquatic weed. It belongs to Pontederiaceae family which is resistant to all the challenges of eradication methods. It contains secondary metabolites such as phenols, sterols, flavonoids, terpenoids, anthoquinones and phenalenone compounds (Vanathi et al., 2014).

Herein we report a facile synthesis of iron oxide nanoparticles (FeNPs) and evaluating their antibacterial activity by the well diffusion method.

2. Materials and methods

2.1. Materials

E. crassipes were collected from Ukkadam Lake, Coimbatore, India (10°90′N, 77°01′E). Experimental chemicals were obtained from Sigma-

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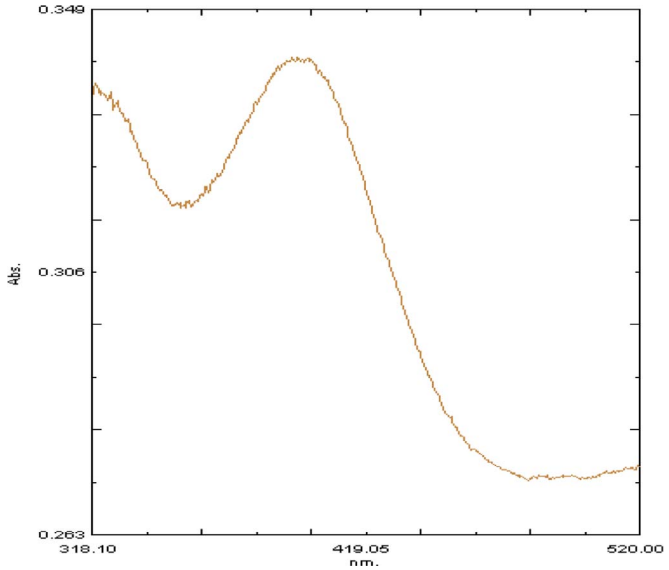

Fig. 1. UV-Vis analysis of *Eichhornia* mediated FeNPs.

Aldrich Chemicals, India. Glass wares were submerged overnight in acid solutions and washed with tap water and distilled water. Bacterial pathogens (*E.coli*, *Pseudomonas fluorescens, Pseudomonas aeruginosa, Staphylococcus aureus* and *Proteus vulgaris*) were acquired from the Department of Microbiology, Karpagam University, Coimbatore, Tamil Nadu, India.

2.2. Preparation of leaf extract

Five gram of fresh (*E. crassipes)* leaves were taken and cleaned with distilled water. Leaves were ground into fine powder using mortar and pestle by demineralized water and boiled with 100 ml of distilled water for 10 min. Then the extract was filtered through filter paper (Whatman filter paper 1) and stored in refrigerator at 4 °C for further studies (Mrinmoy et al., 2008).

2.3. Synthesis of iron oxide nanoparticles

Ferrous sulphate solution was prepared using deionized water. 50% of leaf extract was used. 0.1 M of Ferrous sulphate was prepared using 100 ml of deionized water. To this precursor solution and 100 ml of plant extract (50%) were mixed. 10 ml of NaOH (0.1 M) was added drop wise to mixture of solution (precursor solution and plant extract) under continuous stirring. The mixture was stirred at 55 °C for 2 h. After that, the supernatant was discarded and the pellet was dried in hot air oven. A brownish black color powder was obtained and it was kept in the sterile bottles for the further investigation (Madhavi et al., 2013).

2.4. Analysis of iron oxide nanoparticles

The optical wavelength of FeNPs was analyzed using UV absorption spectroscopy (UV-2450, Shimadzu). The presence and phase purity of the synthesized *Eichhornia* mediated FeNPs were characterized by X-ray Diffractometer (Perkin-Elmer spectrum). Fourier Transform Infrared Spectroscopy (Perkin-Elmer 1725x) was done to identify the functional group present in the synthesized iron oxide nanoparticles. Morphology and size of the particles were analyzed using Scanning Electron Microscope (SEM) (Model JSM 6390LV). Further, metal elements components (Iron) were measured by Energy Dispersive X-Ray (EDX) spectrometer (RONTEC's EDX system, Model QuanTax 200, Germany) (Rajeshwari et al., 2014).

2.5. Assessment of antibacterial activity

Antibacterial activity of phyto-mediated FeNPs was determined by well diffusion method (Bauer et al., 1966). The bacterial cultures were maintained in nutrient broth. 100 µl of bacterial culture was spread over nutrient agar plates using sterile L-rod. The well of 5 mm size was punctured on plates with help of gel puncture (sterile) and various concentrations (25, 50, 75 and 100 µg/ml) of the phyto-mediated FeNPs were added into the well. Tetracycline was used as a positive control. The bacterial cultured plates were incubated for overnight at 37 °C. After incubation period, the zone of inhibitions was assessed in millimeter.

3. Results and discussions

3.1. Analysis of iron oxide nanoparticles

3.1.1. UV-Vis, FT-IR and XRD analysis

Broad absorption spectrum at 379 nm was observed in UV- vis spectra for *Eichhornia* mediated FeNPs (Fig. 1). The band gap energy of synthesized FeNPs was estimated using formula $E = hc/\lambda$ (h - Plank's constant, c - velocity of light and λ – wavelength). The band gap value of iron oxide nanoparticle was found to be 3.27 eV. Mahdavi et al. (2013) reported that using UV- vis analysis of two broad spectrum peaks at 402 and 415 nm showed for *Sargassum* mediated FeNPs. FT-IR analysis helps to find the functional groups that attached on the outside of the FeNPs (Fig. 2a). The synthesized iron oxide nanoparticles have peaks at 1400 and 1587 cm^{-1} refer to N-H bending, 3136 cm^{-1} refers to phosphorous compound. The peak at 621 cm^{-1} indicates the C-H group and a peak at 2382 cm^{-1} denotes the nitriles group. The band at 1114 cm^{-1} corresponds to C-O-C stretch group (Rajeshwari et al., 2014). Rajiv et al. (2013) synthesized zinc oxide nanoparticles from *Parthenium* leaf extract. They reported that the peaks between 1500 and 1514 cm^{-1} indicated the N-H bending mode (amide II); 1371 cm^{-1} referred monosubstitued alkynes; 941 cm^{-1} and specified the C-C-O, C= C and C-O vibrational stretching. The FT-IR spectrum of *Eichhornia* plant extract shows in Fig. 2b. The plant extract has many functional groups such as = c- H bending (817 cm^{-1}), C-H group (1033 cm^{-1}), C=C group (1620 cm^{-1}), Aromatic over tone region (1936 cm^{-1}), nitriles group (2927 cm^{-1}), C- H group (2924 cm^{-1}) and monomeric carboxylic acid group (3556 cm^{-1}). The functional group of plant extract has been transfer during the nanoparticle synthesis.

X -Ray Diffraction was done to prove the phase of FeNPs. The XRD peaks at 2θ values match to the crystal planes of 30° = 220, 37° = 311, 42° = 400, 54° = 422, 58° = 511, 63° = 440 of *Eichhornia* mediated iron oxide nanoparticles (Fig. 3). The analyzed diffraction peaks were matched well with the standard magnetite XRD patterns using JCPDS (card number 19–0629). The obtained peaks were broad due to nano size effect of synthesized particles. Yew et al. (2016) reported the similar results about the XRD of FeNPs.