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Research Article

CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED IRON NANOPARTICLES FROM *PTEROCARPUS MARSUPIUM* BARK

Aruna L. Hugar [1], Ramesh L. Londonkar [2]*

[1]Faculty, Biopharmaceutical and Nanobiotechnology Laboratory, Department of Biotechnology, Gulbarga University, Kalaburagi-585106, Karnataka, India

[2]Professor, Department of Biotechnology, Gulbarga University, Kalaburagi-585106, Karnataka, India

*Corresponding Author Email: londonkarramesh53@gmail.com

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ABSTRACT

Nanotechnology has emerged as a state-of-the-art and cutting-edge technology with multifarious applications in a wide array of fields. Physical and synthetic processes are traditionally utilized for nanoparticles formation, anyway because of constraints of these techniques; nowadays, the main focus of research has been moved towards the improvement of safer and pure green synthesis methods. In recent years, the development of efficient green chemistry methods for synthesis of metal nanoparticles has become a major theme of researchers. One of the most considered methods is production of metal nanoparticles using cost efficient and environment favoring techniques like green synthesis. In this study, a very cheap and simple method was used to obtain the iron nanoparticles (FeNPs) using *Pterocarpus marsupium* bark. The iron nanoparticles were characterized by UV-Vis spectrophotometer, Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD). The antibacterial activity was studied against *Staphylococcus aureus* and *Shigella dysenteriae* by using well-diffusion method. The UV-VIS spectrum of iron nanoparticles revealed a characteristic peak at 435nm. SEM study has showed a spherical shaped FeNPs and size of FeNPs were in the range of 180nm-233nm. XRD photograph indicated FeNPs were in crystalline face-centered cubic structure and the size of 16-17nm, further confirming the result of SEM. The FeNPs nanoparticles have shown a potential antibacterial activity when compared with their standards. Therefore, this simple, low cost and greener method for development of nanoparticles may be valuable in environmental, biotechnological and biomedical applications.

KEYWORDS: *Pterocarpus marsupium*, Iron nanoparticles (FeNPs), XRD, SEM, *S. aureus*, *S. dysenteriae*.

INTRODUCTION

Nanoparticles are submicron moieties ranging from 1-100nm in size, made of inorganic or organic materials having many novel properties compared with the bulk materials. [1] They have a large surface area to volume ratio which is their most important feature responsible for the widespread use of these nanomaterials. FeNPs are of great interest for researchers from a broad range of disciplines, including magnetic fluids, data storage, catalysis and bio applications. They have shown extensive interest in research due to their significant functions in delivery of drug, malignancy treatment, analysis of wastewater and MRI (Magnetic Resonance Imaging).[2] Metal nanoparticles are widely synthesized using physical and chemical processes, but these production methods are usually expensive, labor-intensive and potentially hazardous to the environment. Therefore, green synthesis has advanced over the physical and chemical methods, as it is cost operative, atmosphere friendly, easily scaled up for large scale synthesis and free of toxic chemicals. [3] With the antioxidant or reducing properties of plant extracts, they are usually responsible for the reduction of metal compounds into their respective nanoparticles. [4]

Plant mediated biological synthesis of nanoparticles has gained importance only in the recent years. Microbes take a longer time in condensing the metal ion than plant extracts. Based on the type of plant and phytochemicals concentration, synthesis of nanoparticles takes very less time. [5] Different nanoparticles have been synthesized from various plants having enormous applications in various sectors. Among them, *Pterocarpus marsupium* mediated iron nanoparticle biosynthesis and characterization is the first to be reported based on literature survey. However, *P. marsupium* bark mediated synthesis of silver [6], gold [7] and copper [8] nanoparticles have been reported. It is also known by the names of Red Kino Tree (English), Bijasal (Hindi) and Rakht honne (Kannada). [9] Traditionally, the plant material has been used as a cooling agent with an external application for headache, inflammations, as antipyretic, anti-helminthic, aphrodisiac, mental aberrations and ulcers. The heart wood, leaves, flowers and bark of the plant have useful medicinal properties. [10] The wood and bark of the tree is useful for diabetic patients. [11] The plant contains iso-flavonoids, terpenoids and phenolic compounds with strong antimicrobial, antioxidative, anti-inflammatory, antidiabetic and anticancer activities. [12] Different pieces of bark are used as antacid, anti-diarrheal, for toothache treatment and diabetes. [13] With these vast biological applications on plant, the current research work has been designed.

MATERIALS AND METHODS

Collection of plant material

Pterocarpus marsupium bark was collected from Kalaburagi district, Karnataka, during the month of June 2015-2017. The plant selected for our study was identified and authenticated as *Pterocarpus marsupium* belonging to the family Fabaceae after submission of the herbarium. A voucher specimen-UASB: 4552 was deposited in the herbarium of GKVK botanical garden at UAS, Bengaluru.

Preparation of bark extract

Freshly collected bark was washed thoroughly, shade dried and then powdered to required particle size. 20gms of bark powder was boiled in 100mL double distilled water for 20minutes at 80˚C. After boiling, the extract was filtered using Whatmann No.1 (25µm pore size) filter paper. The filtrate was collected and stored at 4˚C for further use.

FeNPs Synthesis

For the synthesis of iron nanoparticles, the bark extract was mixed with 0.1mM ferric chloride solution in 1:2 proportions. The whole mixture was kept at room temperature for 24 hours. The bioreduction of iron ions was monitored by periodic sampling by UV spectrophotometer. The solution was centrifuged at 10,000rpm for 20minutes, consequently dispersed in double distilled water to remove any unwanted materials with constant stirring at 50-60° C. Sediment NPs were given three washing with double distilled water at 10,000rpm for 15minutes and the pellet was used for characterization.

CHARACTERIZATION OF SYNTHESIZED IRON NANOPARTICLES [14]

UV-Visible Spectroscopy analysis

The bioreduction of Fe^{+} ions in solution was monitored by UV-Visible spectrophotometer (Shimadzu UV–VIS 2450) at room temperature in the range 200-800nm.Double distilled water was used as reference. The reaction mixture was diluted with deionized water and used for analysis.

X-ray Diffraction Analysis

The crystalline structure of the biosynthesized iron nanoparticles was investigated through X-ray diffraction technique using X-ray powder diffractometer. The iron nanoparticles dispersion was placed on a glass slide and the solution (ethanol) was allowed to evaporate, to get a thin film of iron nanoparticles. This thin film was subjected to X-ray diffraction operating between 10° and 80° with the scanning rate of 2° per minute.

SEM analysis

SEM is a surface imaging method, capable of resolving different particle sizes, size distributions, nanomaterial shapes and surface morphology of the synthesized particles at nanoscales. [15] Purified FeNPs were sonicated for 15 minutes to make it uniform distribution and a drop of this solution was loaded on carbon-coated copper grids and solvent was allowed to evaporate under infrared light for 30 minutes. The synthesized nanoparticles were examined by scanning electron microscope (JOEL JSM) to know the shape and size of the particles.

ANTIBACTERIAL ACTIVITY

Disc Diffusion Method

The bacterial isolates *Staphylococcus aureus* and *Shigella dysenteriae* was grown in nutrient broth for 18 hours and standardized to 0.5 McFarland standards (106 CFU/mL). The nutrient agar plates were prepared by pouring 20mL of molten media into sterile petriplates.0.1% inoculum suspension was swabbed uniformly, and the inoculum was allowed to dry for 5 minutes. Wells were punched using a sterile 6mm cork borer. Different concentrations (25µg/mL, 50µg/mL) of synthesized iron nanoparticles were added into the wells, incubated at 37°C for 24 hours. The effects were compared with standard chemotherapeutic agent Amikacin (30mcg) and Piperacillin (30mcg) for *S. aureus* and *S. dysenteriae* respectively. Antibacterial activity was assayed by measuring the diameter of inhibition zone formed around the well using standard (Hi-Media) scale. The experiment done in triplicates and the average values were calculated for antibacterial activity. [16]

RESULTS AND DISCUSSION

Characterization of synthesized silver nanoparticles

In the typical synthesis of iron nanoparticles, *P.marsupium* bark extract was added slowly into $FeCl_3$ solution at room temperature. After adding the extract into $FeCl_3$ solution, the yellow color aqueous solution of $FeCl_3$ turned to greenish black indicating the synthesis of iron nanoparticles as shown in **Figure-1**, which is a preliminary confirmation of FeNPs formation. UV-Visible spectroscopy analysis was done in the range of 200-800 nm and the maximum absorbance was observed at 324nm region depicted in **Figure-2** for the formation of iron oxide nanoparticles due to the excitation of surface plasmon vibrations. The lambda maxima of synthesized iron oxide nanoparticles were quite similar to those reported for Fe_2O_3. [17]

UV-Visible Spectroscopy analysis

Figure-1: Sample tubes containing Ferric chloride solution (A), *P. marsupium* bark extract (B) and FeNPs solution (C)

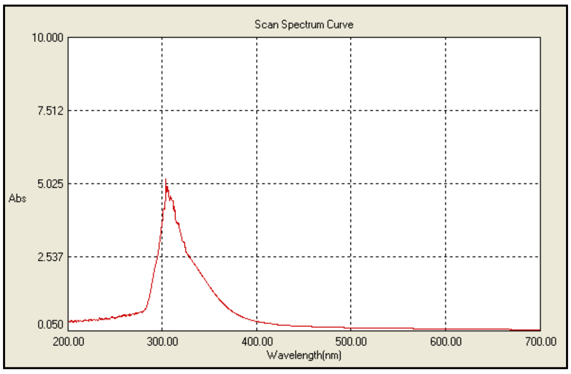

Figure-2: UV–Vis absorption peak of biosynthesized FeNPs.

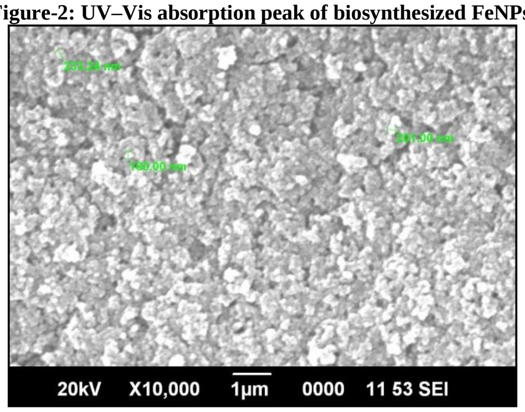

Figure-3: SEM image of biosynthesized FeNPs from *P. marsupium* bark extract

Formation of FeNPs and its morphological dimensions were studied using the SEM. The study demonstrated that the average sizes of the FeNPs were in the range of 180nm-233nm as represented in **Figure-3** and it was found to be spherical in shape. Similar phenomenon was reported in the previous studies. [18]

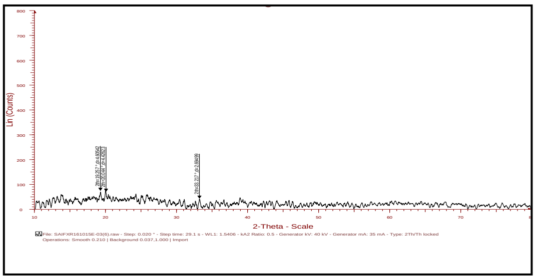

Figure-4: XRD diffractogram of biosynthesized iron nanoparticles

The XRD patterns showed that the synthesized FeNPs are crystalline in nature with face-centered cubic phase. The spectra were recorded in a Phillips Xpert Pro Diffractometer running at 40 kV and 30 mA. The diffracted intensities were recorded and the calculation was performed with the help of instanano.com site which showed the presence of nanoparticles size of 1st peak was 16.84nm, 2nd peak was 16.86nm and 3rd peak was 17.32nm as depicted in **Figure-4**. These results were in good agreement with work reported by Veeramanikandan *et al*., (2017). [19]

ANTIBACTERIAL ACTIVITY

Disc Diffusion Method

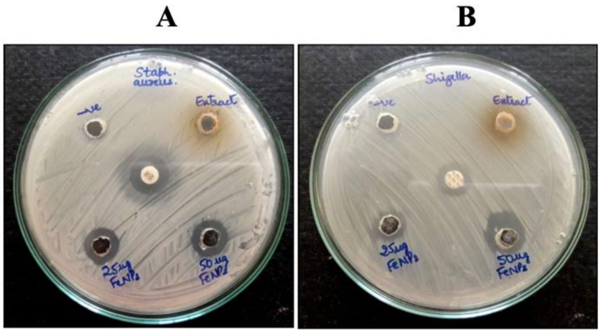

Figure-5: Antibacterial activity of FeNPs and *Pterocarpus marsupium* bark extract against A. *Staphylococcus aureus*, B. *Shigella dysenteriae* respectively

The maximum zone of inhibition was observed with *S. aureus* (13mm) and *S. dysenteriae* (13mm) at 50µg/mL concentration of FeNPs, whereas zone of inhibition were 9mm for *S. aureus* and 6mm for *S. dysenteriae* at 25µg/mL concentration of FeNPs which was comparable with *P. marsupium* bark extract (5mm) as represented in the **Figure-5**. The negative control has showed no zone of inhibition. Hence, it is indicated as good antimicrobial activity against the human pathogens as compared to extract. Since the herbal property based functional groups of the plant covers surface of NPs it leads to the activation of antimicrobial properties. Less size of NPs further helps to the penetration of NPs inside the cell wall and leads to cell death.

CONCLUSION

In the present study, the green synthesis method of nanoparticle biosynthesis represents an easy, eco-friendly and cost-effective process to prepare FeNPs by reduction of ferric chloride solution with *P. marsupium* extract that is alternative to conventional chemical and physical methods. This would be suitable for developing at large scale production. Therefore, this cheap, green synthesis of nanoparticles is very important in biomedical, environmental and biotechnological applications.

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