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BIOFABRICATION OF GOLD NANOPARTICLES USING LEAF EXTRACT OF *FICUS BENGHALENSIS* AND THEIR CHARACTERIZATION

R. M. TRIPATHI[A,B], ARCHANA SHRIVASTAV[A,]* AND B. R. SHRIVASTAV[C,D]

[A]Department of Microbiology, College of Life Sciences, Gwalior - 474 009 (M.P.), India.
[B]School of Science in Biotechnology, Jiwaji University, Gwalior - 474 011 (M.P.), India
[C]Department of Surgical Oncology, Cancer Hospital & Research Institute, Gwalior - 474 009 (M.P.), India.
[D]Department of Surgery, G. R. Medical College, Palace Road, Gwalior - 474009 (M.P.), India

ABSTRACT

We report here the biofabrication of gold nanoparticles (AuNPs) using leaf extract of *Ficus benghalensis* (a national tree of India). This method is rapid, cost effective, non toxic and eco-friendly and these features are making it more attractive for biomedical application. Aqueous solution of *F. benghalensis* leaf extract was used as reducing and capping agent in the synthesis of AuNPs. UV–Vis spectroscopy was used to monitor the quantitative formation of AuNPs. UV-Vis spectrum of the reaction solution showed a peak at 527 nm corresponding to the surface Plasmon resonance of gold nanoparticles. Particle size distribution was analysed by Dynamic light scattering (DLS). Further investigation was carried out by Fourier Transform Infrared Spectroscopy (FTIR), provides confirmation for the presence of proteins as capping agent, which support in enhancing the stability of AuNPs. The morphology and particle size was confirmed by Transmission Electron microscopy (TEM) which showed formation of well-dispersed AuNPs in the range of 17-50 nm with spherical in shape.

KEYWORDS- ***Gold Nanoparticles, Biosynthesis of AuNPs, Ficus benghalensis, TEM, DLS and FTIR***

ARCHANA SHRIVASTAV
Department of Microbiology, College of Life Sciences, Gwalior - 474 009 (M.P.), India

Corresponding author

INTRODUCTION

Nanotechnology is the application of science and technology to manipulate the matter at atomic and molecular scale. It is the ability to build micro and macro materials and products with atomic precision.[1,2] Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging,[3] sensing,[4] targeted drug delivery,[5] gene delivery,[6] and artificial implants.[7] A variety of preparation routes have been reported for the preparation of metallic nanoparticles.[8] A wide variety of physical,[9] chemical,[10] and biological[11,12] process results in the synthesis of nanoparticles, some of them are novel and others are quite common. Nature has devised various process for the synthesis of nano scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials.[13] The use of environmentally benign materials like plant leaf extract,[13,14] bacteria,[15,16] fungi[17] and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.[18] An important area of research in nanotechnology is the biosynthesis of metallic nanoparticles due to their unusual optical, catalytic, chemical, photochemical and electronics properties[19-23] and have a high specific surface area and a high fraction of surface atoms as compared to bulk. For these reasons metallic nanoparticles have found uses in many applications in different fields as catalysis,[24] electronics,[23] and photonics.[15]
In this paper, we report here the biofabrication of pure metallic gold nanoparticles by reduction of Au^{3+} to Au with the aqueous extract of *F. benghalensis* and their characterization. Leaf extract is used in this method as reducing and capping agent for the synthesis of nanoparticles.

MATERIALS AND METHODS

Plant material and preparation of Extract

We have used leaf extract of *F. benghalensis* (fig-1) as reducing and capping agent which is taken from Garden, Gwalior (M.P.), India. Chloroauric acid ($HAuCl_4$) was purchased from Qualigens fine chemicals, Mumbai, India. *F. benghalensis* leaf weighing 25g were thoroughly washed in deionised water, dried, cut into fine pieces and was crushed into 100 ml sterile deionized water in 500 ml of Erlenmeyer flask and then boiling the mixture for 10 min. Subsequently mixture was filtered through Whatman No.1 filter paper.

Figure 1
Leaves of Ficus benghalensis (a national tree of India)

Synthesis of Gold Nanoparticles

For the reduction of Au^{3+}, we prepare 50 ml 0.1mM chloroauric acid in deionised water and 10 ml of leaf extract of *F. benghalensis* was added drop wise with constant stirring at 80˚-90˚C temperature. After 5 min a colour change is observed due to reduction of Au^{3+} to Au (fig-2). The reduction of Au^{3+} was observed by measuring the UV–Vis spectra of the solution.

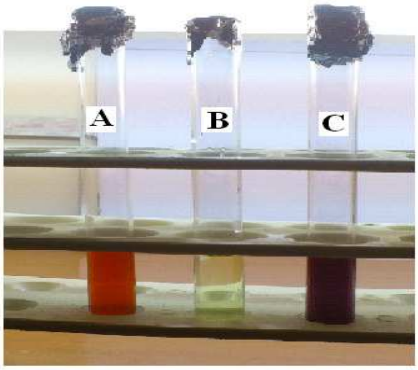

Figure 2
A) F. benghalensis leaf extract, B) Solution of 0.1 mM of Chloroauric acid, C) After reaction between extract and Chlorouric acid

Characterization UV-Vis Spectroscopy

Bio-reduction of Au^{3+} in the solution was monitored by Ultraviolet-visible spectroscopy (UV-1601 pc shimadzu spectrophotometer) or ultraviolet-Visible spectrophotometry (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. It utilizes light in the visible and adjacent (near-UV and near-infrared) range. Color change of solution due to chemical reaction affects the absorption in the visible region. The reaction mixture was scanned from 400 to 650 nm wave lengths.

Dynamic Light Scattering (DLS)

DLS is used to determine the size distribution of particles in suspension. Radiating a monochromatic light beam, like laser, onto a solution with spherical particles in Brownian motion causes a Doppler Shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. DLS (Zetasizer, Malvern) will measure the diffusion coefficient of the particle and using the autocorrelation function to compute the sphere size distribution and give a description of the particle's motion in the medium.

Fourier Transform Infrared (FTIR) Spectroscopy

Biofabricated nanoparticles were centrifuge at 10000 rpm for 30 min for FTIR analysis. The supernatant was discarded and pellet was washed with deionised water. This process was repeats three times to get rid of the free proteins or enzymes that are not participated in the capping of AuNPs. The pellet was scanned on Perkin-Elmer FTIR spectrum in transmittance mode at a resolution of 2 cm^{-1}.

Transmission Electron Microscopy (TEM)

Morphology and size of AuNPs were analyzed by Transmission electron microscopy (TEM) (Philips CM-10). A thin film of the sample was prepared onto a carbon coated copper grid and a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen. The image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film.

RESULTS AND DISCUSSION

UV-VIS Spectroscopy Analysis

It is well known that the gold nanoparticles exhibit ruby red colour in water due to surface Plasmon resonance.[12] The reduction of metal ions during exposure to the leaf extract of *F. benghalensis* may be easily followed by UV–vis spectroscopy. The reduction of pure Au^{3+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 min after diluting a small aliquot of the sample into deionized water. UV-Vis spectrum analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu) and we observed that reduction of the metal ions occurs rapidly, the reduction of Au^{3+}ions is complete within 10 minutes after addition of broth to the metal ion solution. Surface Plasmon resonance spectra for AuNPs are obtained at 527 nm (fig-3).

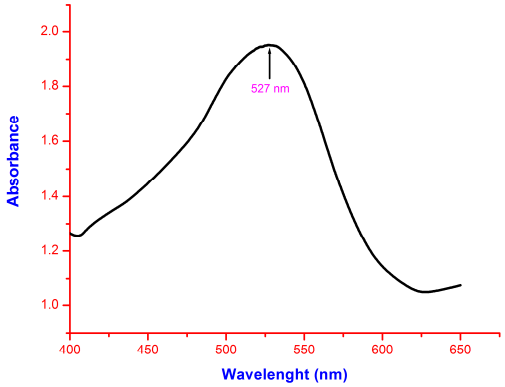

Figure 3
UV-Vis absorption spectra of biofabricated gold nanoparticles

Dynamic Light Scattering

Dynamic light scattering (DLS) is used to measure hydrodynamic size, size distribution and aggregation effects of colloidal samples. DLS is used in the nanotechnology research for the accurate and fast size measurement of nanoparticles made of different materials. Here, dynamic Light scattering (Zetasizer, Malvern) technique was used to determine the size distribution profile of nanoparticles in suspension. The reaction mixture was analyzed by DLS and found average mean diameter 45 nm (fig-4).

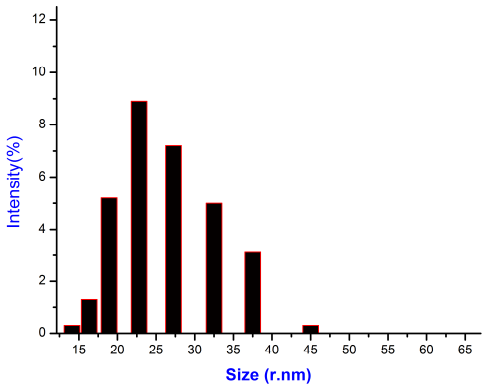

Figure 4
DLS showing the size distribution of gold nanoparticles in the suspension

TEM analysis of Gold nanoparticles

Transmission electron microscopy (TEM) analysis was done using Philips CM-10 TEM machine. TEM micrograph recorded from drop-coated films of the gold nanoparticles synthesized by treating chloroauric acid solution with *F. benghalensis* leaf extract. TEM micrograph has depicted that AuNPs are spherical with the range of 17-50 nm (fig-5).

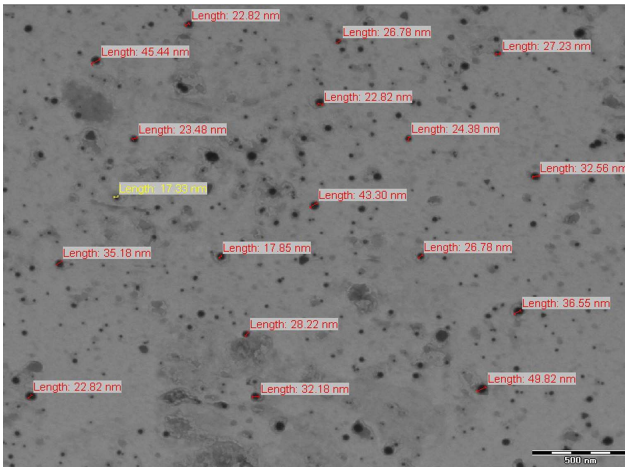

Figure 5
TEM micrograph of biofabricated gold nanoparticles

Fourier Transform Infrared (FTIR) Spectroscopy

It is one of the vital tools for understanding the involvement of surface functional biological groups in metal interactions. Fourier transform infrared spectroscopy analysis was used for the identification of possible biomolecules which can be responsible for reduction and stabilization of nanoparticles synthesized by the leaf extract. The dispersed nanoparticles were gone through FTIR analysis (Figure 7). The FTIR spectrum of nanoparticles shows peaks at 3423 cm^{-1} (presence of amide group characteristic of proteins/enzymes which can be responsible for the reduction of gold metal ions as well as presence of –OH group in the extract which is broad and showing strong intermolecular hydrogen bonding), 2634 cm^{-1} and 2476 cm^{-1} (Carboxylic Acid), 2098 cm^{-1} (Alkynyl C≡C) 1645 cm^{-1} (carbonyls of ketones, aldehydes and esters) and 692 cm^{-1} (C-C bonding). From this we can conclude that *F. benghalensis* leaf extract having proteins/enzymes can reduce the metal ions and it also contains reducing sugars like flavanones which are present on the surface of nanoparticles and provided stability (fig-6).

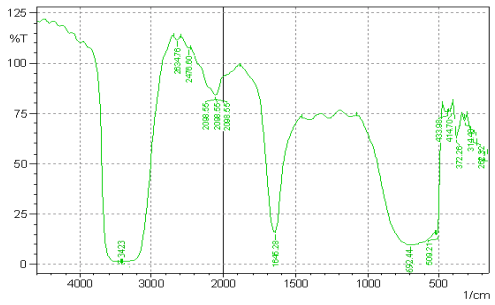

Figure 6
FTIR spectra of gold nanoparticles synthesized by reduction of Au^{3+} ions by F. benghalensis leaf broth.

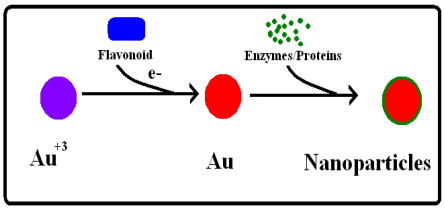

Figure 7
Schematic representation of mechanistic approach of biofabrication of gold nanoparticles

Mechanism of Biofabrication of AuNPs

One of the authors of this article (R.M.Tripathi) has already described the mechanism of biological synthesis of nanoparticles using *F. benghalensis* leaf extract. It contains high level of water soluble antioxidants, poly-phenols (Flavonoids) which is a well acquire scientific conformity emanating from scientific examination.[25] Hydroxyl and ketonic groups are found in phenolic compounds which are able to bind with metals and show chelate effect.[26] Moran J.F et al. reported that the general chelating capability of phenolic compounds are perhaps correlated to the high nucleophilic property of the aromatic rings rather than to precise chelating groups within the molecule.[27] Molecular species of active oxygen can directly scavenge through phenolic compounds like Flavonoids.[26] Flavonoids have antioxidant ability means donate electrons or hydrogen atoms (fig-7). Flavonoids and other biochemical agents which are present in the *F. benghalensis* leaf extract are not only source of reducing the AuNPs but also provide the anti-agglomeration ability to nanoparticles. Further detailed mechanism of biological synthesis of nanoparticles is under study.

CONCLUSION

The present study demonstrated the biofabrication of gold nanoparticles using *F. benghalensis* leaf extract. This leaf extract was found appropriate for the synthesis of gold nanoparticles with spherical, stable and having size ranges from 17-50 nm. The synthesized nanoparticles were characterized through UV-Vis spectroscopy, Dynamic light scattering, Transmission Electron microscopy and Fourier Transform Infrared Spectroscopy. Plant leaf extract is used here as reducing and capping agents. Leaf extract has flavonoids and enzymes/proteins which play a key role in biofabrication of AuNPs. Flavonoids have ability to reduce the Au^{3+} to Au and enzymes/proteins give the stability to nanoparticles. The biofabrication of nanoparticles using biological materials like *F. benghalensis* is a challenging substitute to chemical synthesis, because this novel biofabrication is a rapid, cost effective, non toxic and eco-friendly.

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