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Research Article

Evaluation of Antibacterial Activity and Cytotoxicity of Green Synthesized Silver Nanoparticles Using *Hemidesmus Indicus* R.Br.

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Abstract

In this work, Silver nanoparticle with potent antimicrobial activity was produced by plant mediated green synthesis of silver nitrate. Characterization of silver nanoparticle revealed that nanoparticles were crystalline in nature. TEM analysis confirmed the spherical shape of nanoparticle billow 20 nm of size. X-ray diffraction analysis reveals that the silver nanoparticles are crystals. Investigation of the antibacterial properties of silver nanoparticles against gram positive S. aureus and gram negative P. aeruginosa offered a minimum inhibition concentration of 12.5 µg/ml for both the cases. In addition, silver nanoparticles were able to decrease the viability of both PA-1 cell line (derived from human ovary Teratocarcinoma), and A549 (human non-small cell lung alveolar epithelial cell line) in a dose dependent manner.

Keywords: Green synthesis; silver-nanoparticle; Antimicrobial; cytotoxic

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1. Introduction

The term "Nano" comes from the Greek *word dwarf* generally elaborates the particle of size roughly in the range of 1 to 100 *nanometers. The particles* due to their size offer several unique or improved properties due to its size, distribution and morphology. The nanoscience and nanotechnology is the most active discipline all around the world and considered as the fastest growing technological-revolution the human history has ever seen [1]. Metallic nanoparticles prepared from noble metals such as Gold, Silver, Platinum and Lead exhibits antibacterial properties along with increased chemical activity due to their large surface to volume ratios and crystallographic surface structure [2]. Among the noble metals, silver (Ag) is the metal of choice in the application field of catalysts, staining pigments, solar cells surface coatings, photonics, biological sensing, electronics and surface-enhanced Raman scattering detection biological

systems, living organisms and medicine and antimicrobial [3]. The increasing tendency of microbial infections, rapid emergence of drug-resistant to recent antibiotics and quick evolution through mutation are of great threats to control of microbial infection. Looking the reorganization of antimicrobial action of silver against many bacterial strains, synthesis of silver nanoparticle for antimicrobial application is of recent focus.

Although several synthesis methods are available to produce silver nano particle, biological synthesis of silver nanoparticle of size 1 to 100 nanometers (nm) [4] has become a preferred choice due to the difficulties regarding the processing (use of hazardous chemicals, low material conversion, high energy requirement) and purification of silver nanoparticles in other methods [5]. Biological synthesis offers a time bound, easy, hazardous component free protocol [6] in the wide application need silver nanoparticles, for addressing the problems of versatile field [7-11] of science and technology.

Water soluble phyto-constituents like terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids, flavones, organic acids, and employ natural reduction of silver ion. Besides, *in situ* capping and stabilizing of nanoparticles with desired morphology and size are also assigned with this process.

In this current research, we discuss the biosynthesis of Ag nanoparticles using the commercially economic and abundantly available *Hemidesmus indicus R.Br.* root extract with several phyto-constituents and established therapeutic importance [12-14]. The synthesized nanoparticle is characterized and applied for checking anti microbial and cytotoxicity study.

2. Experiment

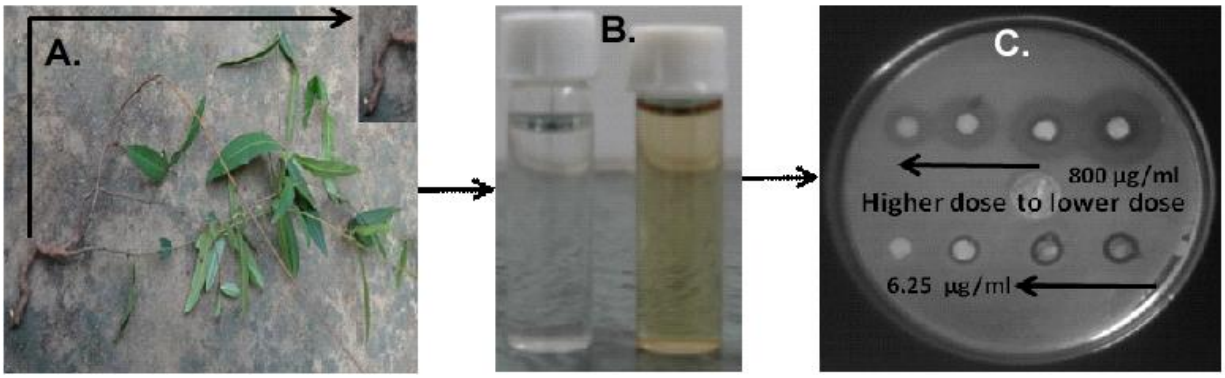

Figure 1 *Hemidesmus indicus R.Br.* mediated synthesis of silver nanoparticles and its antibacterial activity. A. Shows the plant and the root. B. Shows the synthesis of nano particles. C. Shows the antimicrobial property.

2.1 Plant material and preparation of the Extract

Roots (Fig 1A) from matured *Hemidesmus indicus R.Br.* were collected from East Medinipur District of West Bengal, India. Ten grams of fresh roots were thoroughly washed in distilled water and cut into small pieces. Then roots were dispensed in 100ml of sterile distilled water and boiled for one hour at 80 ℃. The extract was filtered before synthesis starts.

2.2 Synthesis of Silver Nanoparticles

Silver Nanoparticles were synthesized by the reduction of Silver nitrate by water soluble phytochemicals of *Hemidesmus indicus R.Br.* 1mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and 10 ml of plant extract was mixed with 90 ml of this prepared silver nitrate solution. The mixture was then placed in a water bath maintained at 90 ℃ for 1 hour. A color change was observed (Fig 1B).

2.3 UV-Vis Spectra analysis

Surface plasmon resonance of AgNPs due to reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the solution after diluting a small aliquot of the sample with distilled water. UV-Vis spectral analysis of the reaction solutions were carried out at room temperature on UV-Vis spectrophotometer (Shimadzu) in the scanning spectra between 200-700 nm at the resolution of 1 nm.

2.4 X-ray diffraction measurements

The synthesized silver nanoparticle was purified by repeated centrifugation at 5000 rpm for 20 min followed by re-dispersion of the pellet of silver nanoparticles into 10 ml of deionized water. The formation and quality of compounds were checked by XRD technique. The dried mixture of Ag-NPs was checked for the determination of the formation of Ag-NPs by a X'Pert Pro x-ray diffractometer (PAN analyticalBV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ- 2 θ configuration.

2.5 TEM analysis

Silver nanoparticles were furthermore, characterized by Transmission Electron Microscopy (TEM). Initially, the sample was sonicated for 15 min. A drop of synthesizes silver solution was loaded on carbon-coated copper grids, and solvent was allowed to evaporate under vacuums drier for 4 hours. TEM measurements were performed on JEOL model JEM 2100 instrument operated at an accelerating voltage at 200 kV.

2.6 Antimicrobial tests

Two human pathogenic strains - gram positive *Staphylococcus aureus and* gram negative *Pseudomonas aeruginosa* were collected from MTCC-Chandigarh. These strains were cultured according to their specifications.

Minimum inhibitory concentration of the antibiotic as well synthesized nanoparticle was evaluated as mentioned by Clinical and Laboratory Standard Institute (formerly known as National Committee for Clinically Laboratory Standards) [15]. The concentration was further checked in plates. Bacterial inoculum size of 10^5cfu/ml was plated. Synthesized nanomaterial was diluted to a final concentration of 0.50 µg/ml. The plates were kept at 37 ℃ for 18 hour. Bactericidal activity with respect to zone of inhibition was also carried out. Each experiment was repeated at least 3 times.

2.7 Cytotoxicity study on PA-1, and A549 cancer cell lines

PA-1 is a cell line derived from human ovary Teratocarcinoma, whereas A549 is human non-small cell lung alveolar epithelial cell line. These two cell lines were maintained in DMEM, supplemented with 2mM L-glutamine, 1% penicillin-streptomycin, and 10% FCS. Cells were grown at 37C in a humidified chamber containing 5% CO_2.

Exponentially growing cells was harvested from the culture flasks by trypsinization and then resuspended in fresh medium. The suspended cells of 5000 cells/well was dispensed into a 96-well micro-plate and be incubated for 24hrs. Then various concentrations of NP were used. Lung cancer and ovarian cancer cell lines (A549 & PA-1) was treated with the drug for different concentration for 48 hrs. Each experiment was conducted in triplicate. The cell viability in the microplate was determined using the MTT (3-(4, 5-dimethylthiazol-2-yle) 2, 5-diphenyl-tetraloziumbromide) after incubation [16]. MTT was added to each well 5mg/ml concentration. After incubation for 4 hrs, the cells from each well were solubilized with 100 µl DMSO for determination of optical density at 570 nm.

3. Results

Aqueous root extract of *Hemidesmus indicus R.Br.* was used for the preparation of silver nanoparticles from 1 mM silver nitrate solution. Reaction at

water bath showed remarkable change of color from watery to brown and pink, due to the reduction of metal ions which indicated the formation of silver nanoparticles.

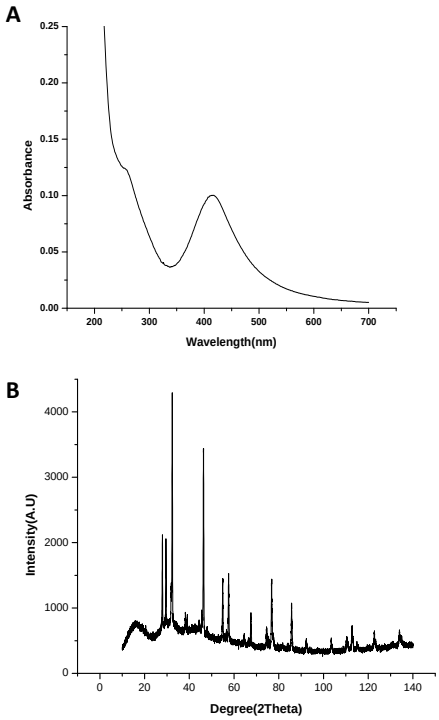

Figure 2 (A) UV–visible spectra of green synthesized silver nanoparticle from the root extract of Hemidesmus indicus R.Br. (B) XRD pattern of green synthesized silver nanoparticle from the root extract of Hemidesmus indicus R.Br.silver nanoparticles.

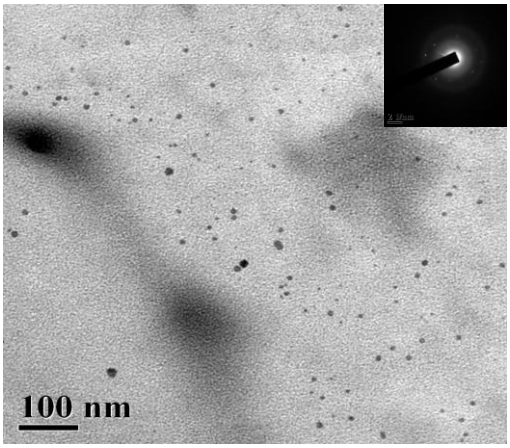

Figure 3 TEM images of nanoparticles.

3.1 UV-visible spectroscopy

Figure 2A shows the absorption spectra of synthesized silver nanoparticles. Silver nanoparticles exhibit an intense absorption peak due to the surface plasmon excitation which describes the collective excitation of conduction electrons in a metal. The absorption spectrum of brown silver colloids showed the absorption band with a maximum of ~ 430 nm, indicating the presence of amorphous or roughly spherical Ag nanoparticles and TEM imaging confirmed this (Fig. 3). A minimum at ~320 nm corresponds to the wavelength at which the real and imaginary parts of the dielectric function of silver almost vanish [17]. Only one surface plasmon resonances band is expected in the absorption spectra of spherical nanoparticles, whereas solution of anisotropic particles could give rise to two or more surface plasmon resonances bands depending on the shape of the particles [18]. Increase in the number of surface plasmon resonances peaks or small shoulders generally results decrease in the symmetry of the nanoparticle [17]. Synthesized silver nanoparticle showed single peak with band width approx 100 nm signifies colloids produced by chemical reduction are rather large and that their size distribution is not widely spread.

It was quite interesting that no precipitation was observed even after several days. That is possible due to *in situ* capping and stabilization of silver nanoparticle by the phyto-constituents.

3.2 TEM Imaging

A drop of sonicated sample of synthesized silver nanoparticles was loaded on carbon-coated copper grids. After evaporate under vacuums drier, the shapes and sizes of biosynthesized silver nanoparticles was evaluated using transmission electron microscopy (TEM). It is obvious that silver nanoparticles formed in this bio-reduction process are very fast and mostly particles are spherical in shape (Fig. 3). According to the size distribution, the prevailing size was of 13 nm although particles below 13 nm also seen. A few larger particle of 18-19nm of size were also observed. That can be said as a symmetric nanoparticle with billow 20 nm

range, that was proposed in UV result. Selected area electron diffraction (SAED) analysis revealed the crystalline nature and faces centered spherical structure of silver nanoparticles.

3.3 X-RD studies

X-raydiffraction pattern of biogenic synthesized silvernanoparticles was used to index Bragg's reflections on the basis of the fcc structure. The XRD pattern of the AgNPs is shown in the Figure 2B. Various Bragg reflections clearly indicated the presence of (111), (200), 220, 311 sets of lattice planes. According to it, it can be said that they can be indexed as face-centered-cubic (FCC) structure of silver. Hence it is clear that AgNPs formed using *Hemidesmus indicus R.Br. root extract* was crystalline in nature. In addition to the Bragg peaks representative of FCC AgNPs, some additional yet unassigned peaks are also observed exploring crystallization of bioorganic phase on the surface of the nanoparticles.

3.4 Antimicrobial Activity

Aqueous root extract of *Hemidesmus indicus R.Br.* mediated silver nanoparticles have offered high antimicrobial activity. The minimum inhibition concentration of synthesized silver nanoparticle is 12.5 µg/ml for both gram positive *S. aureus* and gram negative *P. aeruginosa* (Fig 1C). Antibacterial activity of silver ions released from metallic bulk silver or from nanoparticle surfaces is thought to be due to the interaction with the thiol groups in bacterial proteins or interfere with DNA replication [19-20]. The respiratory chain in bacteria can also be affected by Silver ions. The toxicity of silver nanoparticle may also arise directly from physical processes caused by nano-objects, like disruption of cell membrane and penetration of nanoparticles into the cytoplasm [21].

3.5 Cytotoxicity study

The in vitro cytotoxicity effects of silver nanoparticles were tested against two different cancer cell lines of different origin-A549, and PA-1 by MTT assay. The silver nanoparticles were able to decrease the viability of PA-1 cells in a dose dependent manner as shown in Figure 4. Rate of survival of A549 is superior than PA-1.

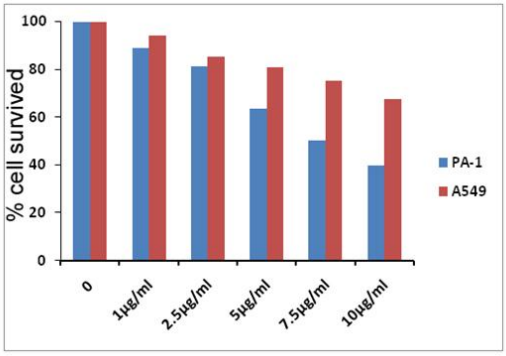

Figure 4 Cytotoxicity study of newly synthesized nanoparticles with two cell lines of different origin-PA-1; and A549.

Conclusions

Several routes of biological synthesis protocol are adopted [22]. Plant mediated synthesis protocol is most advantageous among them as it don't require any microbial culture. Here we have synthesized silver nano particles of billow 20nm of size reducing silver nitrate by root extract of *Hemidesmus indicus R.Br*. These particles are monodispersed and spherical in nature. Color change along with spectra peak at 430 nm was due to the surface plasmon resonance of silver nanoparticles. Silver nanoparticles were further characterized by UV-VIS, TEM, XRD studies. Characterization results nanoparticle having size of billow 20nm and existed crystalline fcc structure. Investigation of the antibacterial properties of silver nanoparticles against gram positive *S. aureus and* gram negative *P. aeruginosa* offered minimum inhibition concentration of 12.5 µg/ml for both the cases. Silver nanoparticles were able to decrease the viability of both PA-1 cell line (derived from human ovary Teratocarcinoma), and A549 (human non-small cell lung alveolar epithelial cell line) in a dose dependent manner. Further studies on the mode of action of silver nanoparticles are required to fully evaluate its potential for use as an antimicrobial drug to control the emergence of bacterial infection.

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