

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Chemical Engineering

Research Article.....!!!

Received: 13-02-2012; Accepted: 25-02-2012

ENVIRONMENTAL FRIENDLY BIOSYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES BY USING *SENNA SAIMEA* PLANT LEAF AQUEOUS EXTRACT

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ABSTRACT

Keywords:

Silver Nanoparticle, *Senna siamea*, biosynthesis, antibacterial activity

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The exploitation of various plant materials for the biosynthesis of nanoparticles is considered a green technology because it does not involve any harmful chemicals. In this work, biosynthesis of silver nanoparticles was achieved using AgNO₃ by aqueous leaf extract from *Senna siamea*. The color changes in reaction mixture were observed during the incubation period, because of the formation of silver nanoparticles enables to produce particular color due to their specific properties. The nanoparticle formation was confirmed by UV-Visible spectroscopy and Transmission Electron Microscopy analysis. FTIR measurement was carried out to identify binding property which is responsible for capping and efficient stabilization of the nanoparticles. Based on the findings, it seems very reasonable to believe that this greener way of synthesizing silver nanoparticles is not just an environmentally viable technique but it also opens up scope to improve their antibacterial properties.

1. INTRODUCTION

Synthesis of nanomaterials with the desired quality and properties is one of the key issues in current nanotechnology. Today, the “green” synthesis of metallic nanoparticles has received increasing attention due to the development of eco-friendly technologies in materials science. Well-dispersed and ultrafine metal nanoparticles, especially transition metals, have great interest due to their distinctive physicochemical and thermodynamic properties, which have made them suitable for use in various fields, such as catalysis, optics, and medicine. The synthesis of nanoparticles with a controlled shape and size is one of the most promising research areas. The excellent properties of some materials strongly depend on crystallographic and morphological characteristics. The silver nanoparticles have several important biological applications such as Antimicrobial activity [1], Anticancer activity [2], Drug delivery [3], Biosensor [4], Bio-labeling [5]. The silver nanoparticles are capable of purifying drinking water; degrading pesticides and killing human pathogenic bacteria [6]. Recent studies of micro and macroorganisms in the synthesis of nanoparticles are a new and exciting area of research with considerable potential for development [7, 8]. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration. Recently, many plants are used for the nanoparticle synthesis. It can be advantageous over other biological processes because it eliminates elaborate process of maintaining cell cultures and can be synthesis in large scale. Many researchers reported that nanoparticles can be synthesized using plant extracts at rates and shapes are reasonable and comparable to these of chemical methods. [9-10]. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process (12). The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds.

Here in, we report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous extract of *Senna siamea* leaf. Further these biologically synthesized nanoparticles were found highly toxic against different human pathogens. Our study in total, thus, highlights a novel greener synthesis of Ag-NPs which not only have environmental advantages but also showed better antibacterial properties than those synthesized through conventional methods. We postulate that possibly some organic molecules present in the leaf extract reduce the Ag⁺ to Ag-NPs and may actually stabilize them in the solution as well. These molecules may also help to anchor the green silver nanoparticles to bacterial cell membranes in a better way which eventually helps them

to achieve better antibacterial properties. Such a way these silver nanoparticles showed good inhibitory effect on some of the human pathogens. So far, there is no previous report on the synthesis of nanoparticles using leaf extract of *Senna siamea*.

2. MATERIALS AND METHODS

2.1 Preparation of leaf extract

Senna siamea plant leaves were washed, dried and cut into fine pieces and were crushed into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 μm). The filtrate was further filtered through 0.6 μm sized filters.

2.2 Biosynthesis of nanoparticles

Leaf extract and AgNO_3 (1×10^{-3} M) stock solutions were prepared by using sterile demonized triple distilled water and the subsequent dilutions were made from this stock solution. The time of addition of leaf extract into the metal ion solution was considered as the start of the reaction. All the procedures were followed in sterile condition.

3. CHARACTERIZATION OF NANOPARTICLES

3.1 UV Spectroscopic analysis

UV-Visible spectroscopy measurement was carried out on JASCO V 550 spectrophotometer operated at a resolution of 1 nm.

3.2 FTIR Analysis

To identify the possible biomolecules responsible for the reduction and stabilization of silver and gold nanoparticles, Fourier Transform-Infrared spectra were recorded using Perkin-Elmer FT-IR spectrophotometer at a resolution of 4 cm^{-1} . The silver and gold nanoparticle solutions were dried and ground with KBr to obtain a pellet for the purpose of FT-IR analysis.

3.3 X-Ray Diffraction analysis

XRD measurement was carried out on a Philips PW 1830 instrument operating at a voltage of 40 KV and at a current of 30 mA with $\text{Cu K}\alpha$ radiation.

3.4 TEM Analysis

A drop of the solution nanoparticles is placed on copper-grid precoated formvar film and the solvent was evaporated under vacuum. The Grids were observed under Transmission electron microscope.

3.5 Antibacterial assays

The stock solutions of different concentrations were prepared. The leaf extract, silver nanoparticles, from the stock solution 25 μL , 75 μL and 100 μL of concentrations were immediately dispensed into each agar wells of culture inoculated in muller hinton agar

(MHA) plates using sterilized micropipette. Bacterial pathogens were obtained as follows: Two-gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and four gram negative (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*) were used.

4. RESULTS AND DISCUSSION

The present work was focused on the development of a biosynthetic method for the production of silver nanoparticles using green chemistry. For the silver nanoparticle formation at constant concentration of AgNO_3 solutions with different amount of leaf extract in aqueous medium. Under the continuous stirring conditions, after 5 hours, the yellow color of AgNO_3 solution turned to light brown color indicates the formation of silver nanoparticles.



Fig.1. Aqueous solution of AgNO_3 with *Senna siamea* aqueous leaf extract and synthesized silver nanoparticles from pale yellow to dark brown color

After the addition of leaf extract to the aqueous AgNO_3 , the solution color changed from colorless to pale yellow and followed to dark brown color which is indicative of the formation of silver nanoparticles. UV-vis spectroscopy is one of the important techniques to ascertain the formation and stability of metal nanoparticles in aqueous solution.

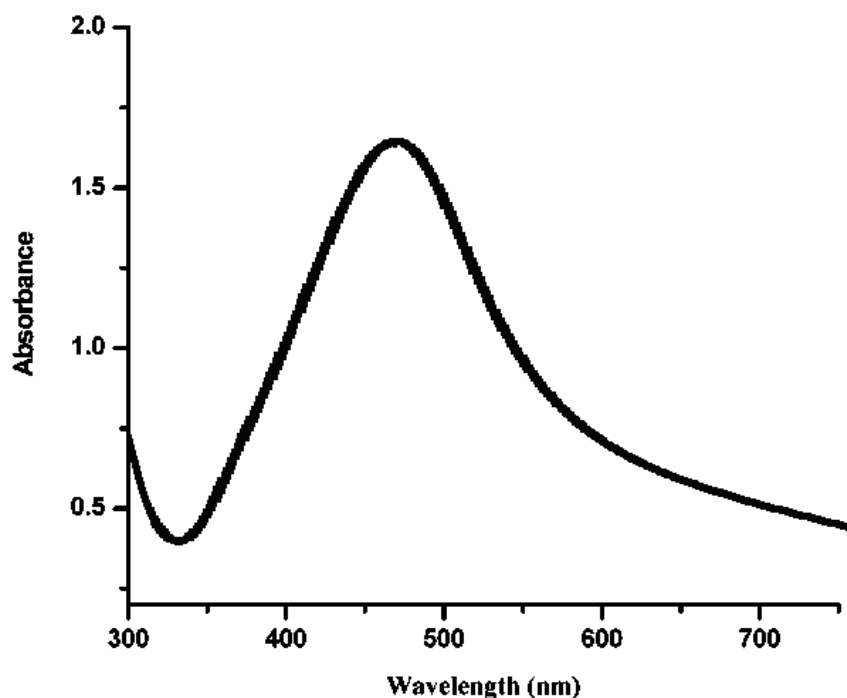


Fig. 2 UV Spectrum of Silver nanoparticles

Fig 2 shows the stacked UV-vis spectra of silver nanoparticles formed from the reaction aqueous AgNO_3 with 6 mL of leaf extract at various time intervals. The appearance of a surface plasmon resonance band at about 470 nm after 5 h of the reaction, which increased in intensity as a function of time of reaction and it attained maximum at 5 h of incubation.

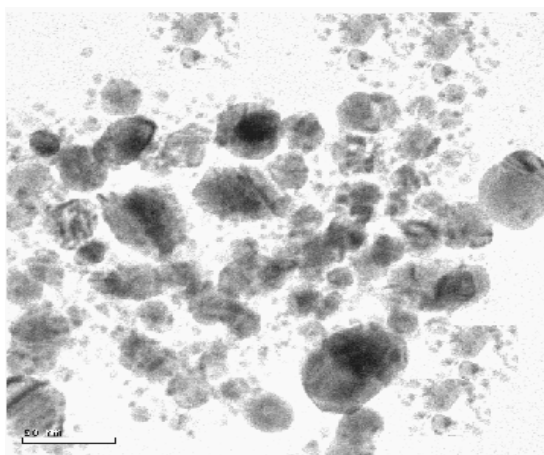


Fig. 3 TEM Image of Silver Nanoparticles

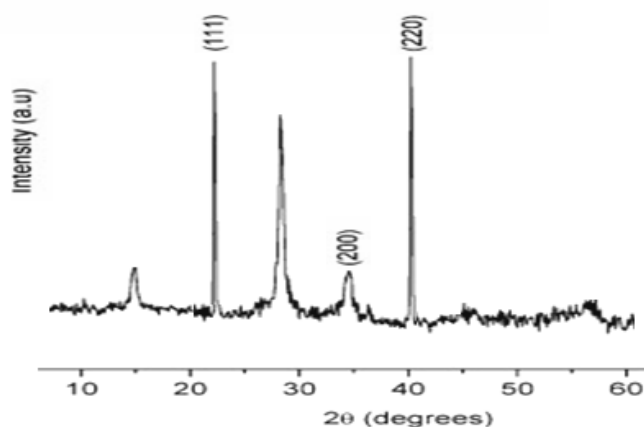


Fig 4 XRD analysis of silver nanoparticles

The morphology and size of as-formed silver nanoparticles were further determined by TEM images. TEM image has shown individual silver particles as well as a number of aggregates. The morphology of the silver nanoparticles was predominately spherical and aggregated into larger irregular structure with no well-defined morphology observed in the micrograph (fig.3). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (leaf extract). Further more, the difference in size is possibly due to the fact that the nanoparticles are being formed at different times. Fig 4 shows a representative XRD pattern of the silver nanoparticle synthesized by the leaf extract after completion of the reduction. The XRD pattern thus clearly shows that the silver nanoparticles were essentially crystalline. Thus, the result was consistent with previous TEM results. And also Fig. 4 represent the XRD pattern of silver nanoparticle, the additional peak indicates the biomass of leaf extracts.

The wave number or frequency (cm^{-1}) of absorption band or peak assigned to the type of vibration, intensity and functional groups of the silver nanoparticles synthesized using *Senna siamea* leaf extract. Different functional groups were involved in reduction of silver ions to silver nanoparticles were shown in fig 5. The peaks in the region of 3452 to 3150 cm^{-1} were assigned to O-H stretching of alcohol and phenol compounds stretching of alkanes, respectively. The peaks at the region of 1643 to 1397 cm^{-1} correspond to -C-N- stretching vibration of the amine or -C-O- stretching of alcohols, ethers, carboxylic acids, esters and anhydrides.

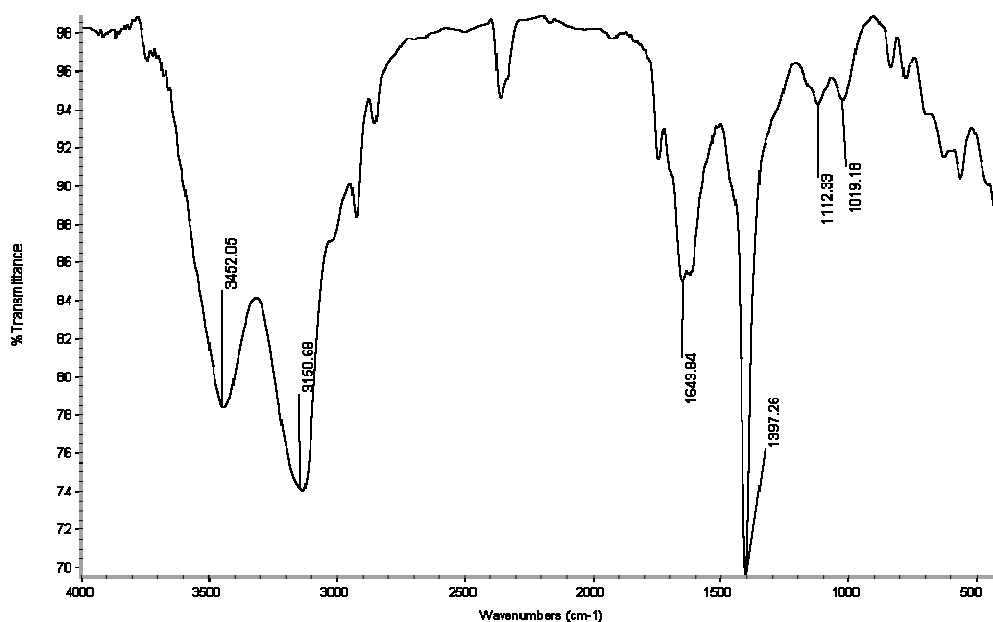


Fig.5. Fourier Transform Infrared Spectroscopy spectrum of silver nanoparticles

The stronger ability of leaf extract to bind metal indicating that the biomolecules could possibly form a layer covering the metal nanoparticles (*i.e.*, capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. Further the nanoparticles synthesis by green route is found highly toxic against human pathogenic bacteria at different concentrations. This may be due to capping agent bio molecule which is present in the leaf extract of *Senna siamea*. Optimal concentration that is a concentration which demonstrates a five log reduction in the test conditions were 25 μ L, 75 μ L and 100 μ L of silver nanoparticles for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. In the present study, although higher concentration (200 μ L) of silver nanoparticles seems to decrease the number of *Bacillus subtilis*. But longer contact time doesn't seem to cause significant decrease in the number of this bacterium so it sounds that this product is not an effective disinfectant on spores. It showed the slight negative effect on antibacterial activity of silver nanoparticles. Thus Silver nanoparticles exhibit a broad size distribution and morphologies with highly reactive facets.

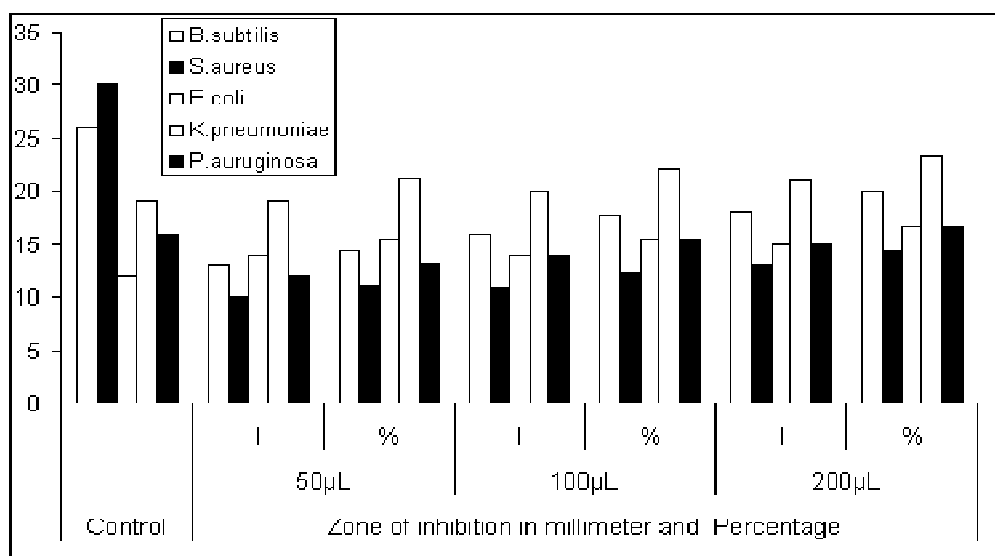


Chart.1.Antibacterial activity of Silver Nanoparticles against five human pathogenic Bacteria.

5. CONCLUSION

This green chemistry approach towards the synthesis of nanoparticles has many advantages such as, anticancer, wound healing and drug delivery applications. Achievement of such a nature friendly synthesis of silver nanoparticles, contributes to a raise in the efficiency of synthetic procedures using environmentally benign natural sources. The effect is dose dependent and is more pronounced against gram negative organisms than gram-positive ones.

However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity of nanoparticles towards human cells before proposing their therapeutic use.

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