ISSN 2277-7199

Original Article

Green synthesis, characterization and antimicrobial activity of Silver Nanoparticles by using *Sterculia foetida* L. young leaves aqueous extract

Shivakumar Singh P1 and Vidyasagar G M1 *

1Medicinal plants and microbiology Research Laboratory, Department of Post Graduate Studies and Research in Botany, Gulbarga University, Gulbarga-585 106, Karnataka. India.

*Prof. G.M. Vidyasagar, gmvidyasagar@rediffmail.com.

Received 02 January 2014; accepted 14 January 2014

Abstract

This is a first and novel report on extracellular silver nanoparticles synthesis using young leaf aqueous extract of *Sterculia foetida* as a reducing agent. The synthesised AgNPs characterised by using spectral analysis like UV-visible spectroscopy, Transmission Electron Microscopy, X-ray diffraction studies, energy dispersive X-ray, Fourier transform infrared spectroscopy. Antimicrobial activity of AgNPs was performed by agar well diffusion technique. The appearance, size, and shape of the silver nanoparticles were understood by UV-visible spectroscopy, transmission electron microscopy. The X-ray diffraction studies, energy dispersive X-ray analysis indicate that particles are crystalline in nature. Fourier transform infrared spectroscopy analysis revealed that the nanoparticles are covered with bio-moieties on their surface. As can be seen from our studies, the bio functionalized silver nanoparticles thus produced have shown admirable antimicrobial effect. The synthetic procedure involved is eco-friendly, simple and hence high range production of the same can be considered for using them in many pharmaceutical applications.

Key words: *Sterculia foetida*, Green synthesis, Silver nanoparticles, TEM, Antimicrobial activity.

1. Introduction

Nanotechnology deals with the production and stabilization of various types of nanoparticles [1]. In order to obtain nanoparticles in large quantities within a short period, physical and chemical procedures are used [2]. Biologically synthesized silver nanoparticles (Ag-NPs) have wide range of applications because of their remarkable physical and chemical properties. Generally, metal nanoparticles are synthesized and stabilized through chemical and mechanical methods [3,4], electrochemical techniques [5], photochemical reactions in reverse micelles [6] and now a days via green chemistry method [7]. The use of green chemistry is an increasing interest of the synthetic procedure for nanoproducts, which are targeted as potential applications in the fields of catalysis in chemical reactions [8], medicinal [9,10], biolabelling [11], microelectronic [12], information storage [13] and optoelectronic devices [14]. At present, several groups of researchers concentrating on biomimetic approaches such as plant or plant leaf extracts, nuts, microorganisms and yeast to synthesize the metal nanoparticles called as “green chemical or phytochemical” approach[15-18].

*Sterculia foetida* is a traditional medicinal plant of India which is a source of secondary metabolites, it is also well known for its phenolic content [19], as also for its antibacterial [20-22] and antioxidant [23] activities as well. So far, there have been no reports on the synthesis of nanoparticles by using leaf extract. Therefore, the present report is on the synthesis of extracellular formation of AgNPs at room temperature using the aqueous extract of young leaves of *Sterculia foetida* as a simple, low cost and reproducible method.

![Fig. 1 Sterculia foetida](Image)
2. Materials and Methods

2.1 Collection of material
Fresh young leaves of Sterculia foetida were collected from botanical garden of Gulbarga university campus. Silver nitrate (AgNO₃) is procured from High Media Laboratories. Solutions were prepared with triply distilled water.

2.2 Preparation of the extract
25 g of young leaves were washed repeatedly with distilled water, so as to remove any organic impurities present on it and cut into fine pieces. The pieces of Sterculia foetida leaves are then taken into 1000 ml beaker containing 500 ml double distilled water and were exposed to microwave for 180°C to suppress the enzymes present in the solution. The raw extract obtained was filtered twice with Whatman filter paper No. 42 (pore size 0.45 µm and 0.22 µm sized). The resultant filtrate is nothing but extract of the S. foetida leaves used for the reduction of Ag⁺ to Ag₀. The extract was treated with silver nitrate solution of concentration 10⁻¹ M.

2.3 Synthesis of Silver nanoparticles from Sterculia foetida leaf extract
The aqueous solution of 1 mM silver nitrate (AgNO₃) was prepared and used for the synthesis of silver nanoparticles. 10 ml of Sterculia foetida young leaf extract was added into 490 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag⁺ ions and kept for incubation for 5 min at room temperature. The filtrate acts as reducing and stabilizing agent for 1 mM of AgNO₃.

2.4 Characterization

2.4.1 UV-Visible spectra analysis
The formation of AgNPs is verified by using UV–visible spectrophotometer at 5704SS ELICO operated at 1 nm resolution with optical length of 10 mm. UV–visible analysis of the reaction mixture was observed for a period of 300s.

2.4.2 X-ray-diffraction (XRD) analysis
This analysis carried out for crystallinity, films of colloidal AgNPs formed on Si(III) substrates by drop coating were used for X-ray-diffraction (XRD) study. The data were obtained using Rigaku X-Ray Diffractometer (Japan), operated at 30 kV and 20 mA electric power with Cu Ka(I = 1.54 Å⁻¹).

2.4.3 Transmission Electron Microscopy (TEM) analysis
The transmission electron microscopy (TEM) images were obtained using Technai-20 Philips instrument operated at 190 kV. Sample for this analysis were prepared by Rapid Biosynthesis of Silver Nanoparticles Using Sterculia foetida 109 coating of aqueous AgNPs drops on carbon coated copper grids, kept for 5 min; the extra solution was removed using blotting paper. The film of TEM grid is exposed to IR light for drying.

2.4.4 Atomic Force Microscope (AFM) analysis
The images of atomic force microscope (AFM) were collected under ambient conditions on a Veeco-Innova scanning probe microscope etched Si–nano probe tips (RTESPA-M) were used for the same.

2.4.5 Fourier Transforms Infra-Red Spectroscopy (FTIR) analysis
The powder sample of AgNPs was prepared by centrifuging the synthesized AgNPs solution at 10,000 rpm for 20 min. The solid residue formed is then washed with deionised water to remove any unattached biological moieties to the surface of the nanoparticles, which are not responsible for biofunctionalization and capping. The resultant residue was then dried completely and the powder obtained was used for FTIR measurements carried out on a Nicolet iS5 FTIR with diamond ATR (Attenuated Reflectance Technique).

3. Antimicrobial activity of AgNPs synthesised from S. foetida aqueous leaf extract

3.1 Test microorganisms: Four fungi such as, Trichophyton rubrum, Trichophyton tonsurans, Microsporum gypseum and Candida albicans and three bacterial strains such as, Staphylococcus aureus, Escherichia coli and Bacillus subtilis were used in the present study. All the tested strains were obtained from M.R.M.C medical college, Gulbarga, Karnataka, India. These test cultures were grown in SDB, nutrient broth (Himedia, M002) at 37°C and maintained on nutrient and potato agar slants at 4°C.

3.2 Agar-well diffusion method: The assay was conducted by agar well diffusion method. About 15 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. The bacterial strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of test strain was spread over the medium using a sterilized glass spreader. Using flame sterilized borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations (80 μl, 60 μl, 40 μl, 20 μl) of AgNPs solution were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37°C. After incubation for 48h, the plates were observed for zones of inhibition. The diameter of zone of inhibition was measured and expressed in millimetres. AgNO₃ solution and plant aqueous extract was used as negative control. The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium and incubated at 37°C for 18h.

4. Results

Synthesis of Ag nanoparticles using Sterculia foetida leaf extract (Green synthesis)
For the synthesis of silver nanoparticles, 2 ml of leaf extract was added to 250 ml of 1 mM AgNO₃ solution. The solution turned colourless to brown within 30 minutes. Ag nanoparticles exhibit light yellowish colour in aqueous solution due to excitation of surface plasmon resonance. On mixing the extract with aqueous solution of the Ag ion complex, a change in the colour from colourless to brown was observed. It was due to the reduction of Ag⁺ which indicates the formation of Ag nanoparticles shown in Figure 2. A visible colour change from transparent to yellow within 15 min indicates the formation of silver nanoparticles which was confirmed by UV-visible analysis. Further, the colour change to dark orange-brown is due to increased concentration as well as growth of silver nanoparticles. After 30 minutes, there was no significant colour change, which is evidence for the completion of reduction reaction.
Fig. 2 Synthesis of silver nanoparticles using *Sterculia foetida* aqueous young leaves extract treating with AgNO3 solution at room temperature

Silver nitrate(AgNO3) solution, B) *Sterculia foetida* aqueous young leaf extract, C) Formation of AgNPs

**UV–VIS spectra Analysis**

UV–VIS spectroscopy analysis is an important technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. Silver nanoparticles are known to exhibit a UV–Visible absorption maximum in the range of 400–500nm [24]. In this report the formation of AgNPs was initially confirmed using UV–Visible spectroscopy due to Surface Plasmon Resonance phenomenon-SPR [24]. The evidence of SPR was shown in Fig. 3. One narrow absorption band was observed at 420-440nm which is a characteristic of mono dispersed AgNPs.

**XRD analysis**

The XRD analysis of synthesised AgNPs using young leaves of *Sterculia foetida* was recorded and typical XRD pattern is shown in Fig.4. The peaks are indexed as (111), (220), (311) and (222) plans of *S. foetida* silver by comparing [24] with JCPDS data. This may be due to the formation of crystalline bio-organic compounds/proteins that are present in the *S. foetida* leaf broth. The detailed investigations on this crystalline phase existing with the silver nanocrystals are in progress.

**TEM analysis**

Transmission electron microscopic analysis carried out at IIT Mumbai revealed the particle size ranges from 30 to 50 nano-meters and shape from spherical to irregular. Of the total particles, 20% particles were of 30nm, 30% were of 40nm and remaining 50% particles were of 50nm size.

**AFM Study**

The AFM analysis is a powerful tool to understand the morphology of bio functionalized Ag NPs particles. The synthesised AgNPs further confirmed by AFM measurements. The three-dimensional analysis of synthesised nanoparticles was made on tapping mode technique developed especially for studying biofunctionalization. Figure 6 shows the AgNPs bio functionalized having organic layer which consist of lot of organic moieties at the surface. Therefore, the shape of randomly distributed nanoparticles can be predicted as spherical and irregular.

**FTIR analysis**

FTIR measurement was carried out to identify the possible bio molecules in *Sterculia foetida* leaf extract responsible for capping leading to efficient stabilization of the AgNPs (fig.4). The IR spectrum of silver nanoparticles manifests prominent absorption band located 1616, 44 cm−1. The strong band at 1386,30 cm−1 may result from the N-H stretching vibration and can be assigned as absorption bands of C=H, -O-H, -S-H, -N=C=N, -C=O and -S=O.
stirigated and exhibited particles in plants extracts. This can, phases.

Reduction of silver ion into silver nanoparticles during application of nanoparticles that are beneficial for mankind. helps to increase the interest in the synthesis and

The zones of 35.00mm & 23.00mm, respectively.

Two negative controls i.e.,

antimicrobial activity was directly proportional to the

AgNPs of Sterculia foetida of 400

The production of the silver nanoparticles synthesized from the aqueous extract of Sterculia foetida leaves was evaluated through spectrophotometer at a wavelength range of 400-500 nm and observed a characteristic peak for Sterculia foetida AgNPs at 420 nm for the extract and AgNO3 mixture, which confirmed the formation of silver nanoparticles. This is similar to the characteristic peaks of the silver nanoparticles prepared by Geranium leaf [27]. The result obtained in this investigation is very much interesting in terms of the identification of potential forest plants for the synthesis of silver nanoparticles.

Table. 1 Antimicrobial activity of AgNPs synthesised from aqueous young leaves extract of Sterculia foetida

<table>
<thead>
<tr>
<th>Dermatophytic fungi and bacterial strains</th>
<th>Zone of inhibition in mm at different conc. of Ag NPs</th>
<th>Young leaf aqueous extract</th>
<th>1mM AgNO3 Sol*</th>
<th>Standard K&amp;S (1000 μg/ml conc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T. rubrum</strong></td>
<td>14.00 09.00 06.00 04.00 - - 22.00</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><strong>T. tonsurans</strong></td>
<td>10.00 07.00 06.00 05.00 - - 23.00</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><strong>M. gypseum</strong></td>
<td>13.00 10.00 08.00 05.00 - - 20.00</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><strong>C. albicans</strong></td>
<td>14.00 11.00 09.00 08.00 - - 23.00</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>22.00 18.00 15.00 10.00 -- 32.00</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><strong>B. subtilis</strong></td>
<td>24.00 20.00 15.00 10.00 -- 35.00</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>25.00 22.00 18.00 11.00 - - 30.00</td>
<td>- -</td>
<td>- -</td>
<td></td>
</tr>
</tbody>
</table>


Fig. 7. FTIR spectrum of bio functionalized AgNPs stretching vibration. These are derived from water soluble compounds such as, flavonoids, alkaloids and polyphenols present in leaves. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles [25].

Antimicrobial activity of AgNPs of Sterculia foetida leaf extract

The Ag NPs of S. foetida leaves at 80 μl/well showed maximum antibacterial activity against E. Coli, (25.00 mm), followed by Bacillus subtilis (24.00mm) and minimum of 22.00 mm activity against Staphylococcus aureus. Similarly, against fungi the maximum activity of 14.00 mm was recorded against T rubrum and C albicans followed by 13.00 mm against M. gypseum and minimum of 10.00mm activity against T. tonsurans. The antimicrobial activity was directly proportional to the concentration of AgNPs.

Two negative controls i.e., Plant aqueous extract and AgNO3 solution did not show any activity against any tested strains. Streptomycin sulphate and Ketoconazole used as standards against bacteria & fungi showed the inhibition zones of 35.00mm & 23.00mm, respectively.

5. Discussion

The development of easy, reliable and eco-friendly method helps to increase the interest in the synthesis and application of nanoparticles that are beneficial for mankind. Reduction of silver ion into silver nanoparticles during exposure to the plant extracts could be followed by colour change. In our study within 30 minutes the colour change was observed at room temperature. Silver nanoparticles exhibited dark redish-brown colour in aqueous solution due to the surface plasmon resonance phenomenon [26]. The synthesized silver nanoparticles using plant extract at 3 h of incubation were reported to have flavanoid and terpenoid constituents from leaf extract and they might be the surface active molecules stabilizing the nanoparticles.

Interestingly, in the present study, the silver nanoparticles were synthesized rapidly within 30 min of incubation period making it one of the fastest bio reducing methods to produce silver nanoparticles by Sterculia foetida with no further significant change in colour.

The frequency and width of the surface plasmon absorption are depends on the metal nanoparticles detecting the surface plasmon resonance phenomenon [26]. The synthesized silver nanoparticles using plant extract at 3 h of incubation were reported to have flavanoid and terpenoid constituents from leaf extract and they might be the surface active molecules stabilizing the nanoparticles.

In present study the silver nanoparticles prepared by Geranium leaf [27]. The result obtained in this investigation is very much interesting in terms of the identification of potential forest plants for the synthesis of silver nanoparticles.

The frequency and width of the surface plasmon absorption are depends on the metal nanoparticles detecting the presence of silver nanoparticles in plants extracts. This can be achieved by using XRD to examine the diffraction peaks of the plant. In present study the X-ray pattern of synthesized silver nanoparticles matches the FCC structure of the bulk silver and there was no obvious other phases found in the XRD patterns. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by the S. foetida are spherical in nature.

In this study, the application of AgNPs as antimicrobial agent was investigated and exhibited better activity against dermatophytic fungi and bacteria. However, the antimicrobial effect was dose-dependent.

6. Conclusion

The green synthesis of silver nanoparticles using S. foetida extract was shown to be rapid and produce particles of crystallographic spherical shapes. Following the addition of S. foetida broth to the silver nitrate solution, silver nanoparticles began to form within 15 minutes and the
reaction completed in 2 h. It was also found that the increasing broth concentration increases the rate of reduction in particle size along with their agglomeration. The synthesized particles size range 30-50nm and were spherical in shape, as shown by the TEM analysis. The particles also tended to aggregate which suggest that they are useful in applications as antimicrobial agent.

Acknowledgments

Authors are thankful to IIT Mumbai for TEM analysis, JNTU Hyderabad for FTIR analysis, also thankful to USIC and Dept of Physics Gulbarga University Gulbarga for XRD analysis.

References