Biosynthesis of Silver Nanoparticles from Aqueous Leaf Extract of Synedrella nodiflora under Sunlight Irradiation and Screening of its Antibacterial Activity

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ABSTRACT

The field of nanotechnology and nanoscience is the most currently an area of intense scientific interest for researchers in modern materials science. However there are many chemical as well as physical methods, green synthesis of nanomaterials is the most emerging method of synthesis. We report the synthesis of antibacterial silver nanoparticles (AgNPs) using aqueous leaf extract of medicinal herb Synedrella nodiflora (L.) Gaertn (Family: Asteraceae) at direct sunlight and monitored by UV-Vis spectroscopy. Both leaf extract and resulting AgNPs is subjected to antibacterial study against five pathogenic bacterial strains such as Bacillus megaterium, Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa and Salmonella typhimurium. AgNPs exhibited higher antibacterial efficacy than leaf extract and found to be more effective against B. megaterium and S. aureus.

KEYWORDS: Synedrella nodiflora; Asteraceae; Aqueous leaf extract; Ag-nanoparticles; Antibacterial activity.

Introduction

Nanomaterials synthesis and their characterization is an emerging growing field of nanotechnology from the past two decades, due to their numerous applications in science and technology including physics, chemistry, biology and medicine for mankind. With the development of several chemical-synthetic techniques, the concern for environmental contaminations is also heightened as the chemical synthesis protocols need some toxic chemicals for synthesis (Song and Kim, 2008). Most of the physical methods deal with enormous consumption of energy to maintain the high pressure and temperature employed in the synthesis procedures. In the present scenario, synthesis of nanoparticles through biological methods is a good alternative over the chemical and the physical methods as they are both environment friendly and economic and tremendous interest in the biomedical applications of nanoparticles (NPs) (Rout et al., 2012; Zaheer and Rafiuddin, 2013). Their applications is not only limited to the preliminary research stage but also in clinical stages. In particular, silver nanoparticles (AgNPs) are increasingly being investigated as tools for antibacterial and antifungal (Rout et al., 2012), anti-inflammatory (Singh et al., 2007), radio imaging, retinal neovascularization (Bhattacharya and Mukherjee, 2008; Kalishwaralal et al., 2010), antiviral, antioxidant (Hakkim et al., 2007) and novel cancer therapeutics, capitalizing on their unique properties to enhance potential therapeutic efficacy (Bhattacharya and Mukherjee, 2008; Kalishwaralal et al., 2010). Notably silver oxide nanoparticles exhibit antitumor properties in transplanted Pliss lymphosarcoma tumor models when administered by intravenous injection in the form of aqueous dispersions (Rutberg et al., 2008). Perhaps to inhibit the function, AgNPs can interact with sulfur-containing compounds as well as with phosphorus-containing compounds like DNA inside the bacterial membranes (Zaheer and Rafiuddin, 2013). The diameters of AgNPs are normally smaller than 100 nm and contain 20-15000 silver atoms (Lok et al., 2007; Simi and Abraham 2007). When cells or tissue are being exposed to AgNPs, the active surface of AgNPs would be significantly large compared to silver compounds, and thereby exhibiting remarkably unusual physicochemical properties and biological activities (Yen et al., 2009).

The biological activity of the synthesized nanoparticles always depends on the starting capping agent for stabilization of the nanoparticles and also with its concentration. There are several matrixes for the biosynthesis of AgNPs has been reported till date using microorganisms include bacteria (Hebbalalu et al., 2013),
fungi (Kim et al., 2012), enzyme (Hebbalalu et al., 2013), and also medicinal plants (Chandran et al., 2000; Huang et al., 2007; Bar et al., 2009; Vijayaraghavan et al., 2012). Shankar et al., 2004 reported that the synthesis of pure metallic nanoparticles of silver by reduction of Ag+ ion using *Azadirachta indica* leaf broth. However, little has been carried out about engineering approaches such as rapid nanoparticles synthesis using plant extracts and size control of the synthesized nanoparticles (Shankar et al., 2004). There are quite a few reports are available for the rapid biosynthesis of AgNPs using plant extract as the stabilizing agent. Different plant extracts have been in use for this process (Rajasekharreddy et al., 2010). However the uses of edible plants are in tremendous demand for the biomedical applications of AgNPs.

*Synedrella nodiflora* (L.) Gaertn. (Family: Asteraceae) is a herb, commonly known as Cinderella weed, node weed, pig grass, pig grass, Synedrella (Chopra et al., 1956). The herb is adapted to many environments. The plants appear in moist soil and along roads and foot trails (Holm et al., 1997). Erect herb to 50-90 cm tall, branched dichotomously, leaves ovate or elliptic, mostly 5-15 cm long and 2-9 cm wide, crenate-serrate, acute, abruptly narrowed at base to the 1-5 cm long petiole mostly pubescent on both surfaces, usually 3-nerved; heads solitary or several together, subsessile or pedunculate, cylindric-campanulate, 10-20-flowered, 8-10 mm long; involucral bracts 2-seriate, usually only 4 or 5, nearly 1 cm long; all corollas yellow, about 3.5-4 mm long; ligules bifid; achenes of 2 kinds, those of ray-florets unwinged, those of disc-florets unwinged, often puberulent, all nearly black, 4-5 mm long, awned (Stone, 1970). It is particularly well adapted to the partial shade found under jute and plantation crops like tea, coffee, bananas, cacao, and rubber (Forestieri et al., 1996). Plants appear in moist soil and along roads and foot trails. The species is largely available at and around Santiniketan, West Bengal, India. *S. nodiflora* showed strong antioxidant potential, anti-feedant efficiencies, anti-inflammatory effect and analgesic and antipyretic activity (Forestieri et al., 1996; Wijaya et al., 2011). The crude plant extract was also reported to possess insecticidal property. In order to search for potential lead compounds from the plant concerned the challenges have been taken (Martin and Gopalkrishnan, 2005).

In the present communication we described a simple one step method for the rapid synthesis of AgNPs by reducing Ag+ using the aqueous leaf extract of *S. nodiflora* at direct sunlight condition. The synthesized nanoparticles were then qualitative characterized by UV-Vis spectroscopy. Leaf extract of *S. nodiflora* acts in dual both as reducing agent as well as capping agent here. We also combine here its inherent pharmacological activities with the enhanced antibacterial activities of the prepared AgNPs against some known pathogenic strains.

**Materials and Methods**

**General experimental procedure**

Fresh leaves of medicinal plant *S. nodiflora* were collected during the period between the last week of February, 2009 at and around Santiniketan, West Bengal, India, and identified with the help of Taxonomical Division, Botany Department, Visva-Bharati. Voucher specimen is preserved in the department of chemistry of our college under university of Burdwan. The water used as the solvent was previously subjected to deionization, followed by double distillation (first time in alkaline KMnO4). Fresh leaves of the plant were washed thoroughly with double-distilled water for several times to make it free from dust and were then cut into small pieces. Silver nitrate (AgNO3) (Sigma-Aldrich, Bangalore, India) were used as the source of Ag (I) ion required for the synthesis of Ag nanoparticles. UV-Vis absorption spectra were recorded on a Shimadzu UVPC-3101 spectrophotometer.

**Preparation of plant extract**

10 gm of fresh leaves of *S. nodiflora* were washed thoroughly with double-distilled water and were then cut into small pieces. These finely cut pieces were then mixed with 100 ml doubled-distilled water and the mixture was boiled for a period of 10 minutes and then filtered after cooling. This filtrate has been used as mother solution in our investigation.

**Synthesis of AgNPs and evaluation of reducing potential of the extract**

5 ml of aqueous extract of *S. nodiflora* leaves was added to 45 ml of 10−3 M silver nitrate solution so as to make its final volume 50 ml. The solution was allowed to react at room temperature under sunlight. Periodic sampling after 30 minutes was carried out to monitor the formation of AgNPs.

**Preparation of solutions**

Total volume = 50 ml in a 100 ml conical flask

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Solution Composition</th>
</tr>
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<tbody>
<tr>
<td>3%</td>
<td>45 ml 0.001 (M) AgNO3 solution + 3.5 ml H2O + 1.5 ml mother solution</td>
</tr>
<tr>
<td>5%</td>
<td>45 ml 0.001 (M) AgNO3 solution + 2.5 ml H2O + 2.5 ml mother solution</td>
</tr>
<tr>
<td>7%</td>
<td>45 ml 0.001 (M) AgNO3 solution + 1.5 ml H2O + 3.5 ml mother solution</td>
</tr>
<tr>
<td>10%</td>
<td>45 ml 0.001 (M) AgNO3 solution + 5 ml mother solution</td>
</tr>
</tbody>
</table>

![Fig. 1. Photograph of the plant: Synedrella nodiflora L.](image)
15%: 42.5 ml 0.001 (M) AgNO₃ solution + 7.5 ml mother solution.
20%: 40 ml 0.001 (M) AgNO₃ solution + 10 ml mother solution.

Analysis of bioreduced silver nanoparticles: UV-Vis spectroscopy

To observe the optical property of biosynthesized silver nanoparticles, samples were periodically analyzed for UV-Vis spectroscopic studies (Shimadzu UVPC-3101) at room temperature operated at a resolution of 1 nm between 200 and 600 nm ranges.

Microorganisms

The bacterial strains used in this study were procured from Microbial Type Culture Collection (MTCC), Institute of microbial technology, Chandigarh, India. Five pathogenic bacterial strains including three Gram-positive and two Gram-negative bacteria, were used for determining the preliminary antibacterial activity of biosynthesized silver nanoparticles. Bacillus megaterium MTCC 1684, Staphylococcus aureus MTCC 96, Listeria monocytogenes MTCC 657 were used as Gram-positive bacteria and Salmonella typhimurium MTCC 98 and Pseudomonas aeruginosa MTCC 741, were used as Gram-negative bacteria.

Antimicrobial spectrum

In order to examine the antibacterial activity of the AgNPs on selected bacteria, the Kirby-Bauer agar disc diffusion method (Bauer et al., 1966) was used for determination of inhibition zone diameters. Known amounts of test sample (0-1000 mg/disk) were applied to cellulose paper disks. The disks were placed on the surface of prespreaded lawns of the test microorganisms, followed by incubation for 24 hr. The diameter of the inhibition zones appearing around the disks was measured for the evaluation of the antimicrobial activities. Filter paper discs saturated with AgNPs, S. nodiflora leaf extract and silver nitrate solution were placed onto these plates with the help of sterile forceps and incubated at 37 °C. A disc soaked in sterile distilled water acted as controls for the experiment.

Results and Discussions

UV-Vis absorbance studies

Formation of AgNPs by reduction of silver nitrate during exposure to S. nodiflora leaf extract can be easily monitored from the change in colour from pale yellow, yellow, light-red to wine-red with reaction time of the reaction mixture (Figure 2). These color changes arise because of the excitation of surface plasmon vibrations with the silver nanoparticles (Mulvaney, 1996). Biosynthesis of AgNPs was carried out with different concentration (3%, 5%, 7%, 10%, 15% and 20%) of aqueous leaves extract of S. nodiflora with 10⁻³ M AgNO₃ solution. UV-Visible spectra in Figure 3 showed the formation AgNPs at different concentration after 30 min. Out of the all different concentration of aqueous leaf extract, the maximum satisfactory results observed with 20%. All data are shown in Table 1. UV-Vis absorbance of reaction mixture was taken for 20% of S. nodiflora leaf extract after 1 min to till 60 min (Figure 4). Figure 4 results shown that the surface plasmon resonance (SPR) of silver nanoparticles produced a peak centered near 428.7 nm and increased their absorbance value with time to time. It was also observed that bioreduction of silver ions into nanoparticles started at the start of reaction and bioreduction was completed at almost 60 min indicating rapid biosynthesis of silver nanoparticles.
Fig. 2. Optical Image of step by step colour change for the formation of AgNPs by 20% aqueous leaf extract of *Synedrella nodiflora* at different times.

**TABLE 1**

<table>
<thead>
<tr>
<th>No of Observations</th>
<th>Concentration</th>
<th>Wavelength $\lambda_{\text{max}}$ (nm)</th>
<th>Absorbance (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3%</td>
<td>439.9</td>
<td>0.276</td>
</tr>
<tr>
<td>2</td>
<td>5%</td>
<td>433.0</td>
<td>0.389</td>
</tr>
<tr>
<td>3</td>
<td>7%</td>
<td>434.1</td>
<td>0.392</td>
</tr>
<tr>
<td>4</td>
<td>10%</td>
<td>438.0</td>
<td>0.407</td>
</tr>
<tr>
<td>5</td>
<td>15%</td>
<td>433.0</td>
<td>0.482</td>
</tr>
<tr>
<td>6</td>
<td>20%</td>
<td>428.7</td>
<td>0.707</td>
</tr>
</tbody>
</table>

Fig. 3. UV-Visible spectra of the different concentration of aqueous *Synedrella nodiflora* leaf extract (Plant extract) with $10^{-3}$ M AgNO$_3$ after 30 min.
Antibacterial studies

Anti-bacterial screening test was carried out for various bacterial species by observing inhibition zone formation. Antibacterial potential of AgNPs is known for many years (Raut et al., 2009). In our study, the AgNPs synthesized using aqueous leaf extract of *S. nodiflora* exerted significant antibacterial activity on the tested three Gram-positive bacteria viz. *B. megaterium*, *S. aureus* and *L. monocytogenes* and two Gram-negative bacteria viz. *P. aeruginosa* and *S. typhimurium* to determine the inhibition zone formed by paper disk method (Bauer et al., 1966) and after 24 hours in controlled condition the plates are visualized. Figure 5 shows the zones of inhibition of the tested bacterium against AgNPs, silver nitrate, *S. nodiflora* leaf extract and distilled water as control. According to the diameter of the inhibition zone formed, the results are noted in the form of '+' or '+++' sign, which has been shown in the Table 2. For all the bacterial strains, no zone of inhibition was observed for control as well as silver nitrate solution. A very small but noticeable zone of inhibition was observed for *S. nodiflora* leaf extract. Bioreduced silver nanoparticles showed considerable growth inhibition zone when studied with agar plate diffusion method (Figure 5). Among these bacteria, *B. megaterium* and *S. aureus* developed better inhibition zones against AgNPs. Coupling of inherent property of *S. nodiflora* extract with that of silver nanoparticles has really proved to be useful to minimize the dose that requirements to be administered for total microbial reduction.
### TABLE 2

Values of zones of inhibition obtained by disc diffusion method.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Distilled water (control)</th>
<th>Silver nitrate solution</th>
<th>Water diluted SNE (dilution factor 1 : 1)</th>
<th>SNE</th>
<th>AgNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. megaterium</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

*— no inhibition zone; ‘+’ inhibition zone up to 8 mm; ‘++’ inhibition zone up to 12 mm; ‘++’ inhibition zone 15 mm or more; SNE: Synedrella nodiflora leaf extract

### Conclusions

This study demonstrates in brief the green synthesis of small-sized Ag nanoparticles by sunlight irradiation method. The rapid reduction, relatively smaller-sized particles of Ag ions using *S. nodiflora* leaf extract provides several advantages in the direction of biogenic process and also denotes the superiority over the chemical synthesis in providing green, environmentally safer method of nanoparticle production. Most importantly, the reaction is simple and easy to handle, and it is believed that it has advantages over other biological syntheses. Formation of Ag nanoparticles with *S. nodiflora* leaf extract also reveals that the medicinal plants contains polyphenolic compounds and have antibacterial activity against five pathogenic bacteria tested. It is cleared that among these five pathogenic bacteria, *B. megaterium* and *S. aureus* showed greater inhibitory activity than the other three. This work will surely boost up present-day researchers to do more work regarding isolation, characterization, bioactivity and other related study of the isolated phytochemicals from this important medicinal plant so as to explore this medicinal plant to a great extent.

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