

Research Article

Green Synthesis of Silver Nanoparticles Using *Polyalthia longifolia* Leaf Extract along with D-Sorbitol: Study of Antibacterial Activity

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Synthesis of silver nanoparticles (AgNPs) using *Polyalthia longifolia* leaf extract as reducing and capping agent along with D-sorbitol used to increase the stability of the nanoparticles has been reported. The reaction is carried out at two different concentrations (10^{-3} M and 10^{-4} M) of silver nitrate, and the effect of temperature on the synthesis of AgNPs is investigated by stirring at room temperature (25°C) and at 60°C. The UV-visible spectra of NPs showed a blue shift with increasing temperature at both concentrations. FT-IR analysis shows that the biomolecules played an important role in the reduction of Ag⁺ ions and the growth of AgNPs. TEM results were utilized for the determination of the size and morphology of nanoparticles. The synthesized silver nanoparticles are found to be highly toxic against Gram-positive bacteria than Gram-negative bacteria.

1. Introduction

An important area of research in nanotechnology is the synthesis of nano silver particles. Silver has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms [1]. Antibacterial activity of the silver-containing materials used in medicine to reduce infections in burn treatment [2] and arthroplasty [3], as well as to prevent bacteria colonization on prostheses [4], catheters [5], vascular grafts, dental materials [6], stainless steel materials [7], and human skin [8]. Silver nanoparticles also exhibit a potent cytoprotective activity towards HIV-infected cells [9]. Because of such wide range of applications, numerous synthetic methods have been developed [10]. Biological routes of nanoparticles synthesis using microorganism [11–13], enzyme [14] and plant or plant extract [15–21] have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [22]. It can also be suitably scaled up for large-scale synthesis of nanoparticles. Specific surface area is relevant for catalytic

reactivity and other related properties such as antimicrobial activity in silver nanoparticles.

Polyalthia longifolia is a lofty evergreen tree, native to India, commonly planted due to its effectiveness in alleviating noise pollution. Methanolic extract of *Polyalthia longifolia* have yielded 20 known and 2 new organic compounds, some of which show cytotoxic properties [23]. Here in, we report for the first time synthesis of silver nanoparticles using aqueous extract derived from *Polyalthia longifolia* leaves with D-sorbitol and their catalytic and antibacterial activity of the synthesized NPs is described.

2. Experimental

The *Polyalthia longifolia* leaves were collected from University of Madras Campus located at Chennai, India. All the chemicals were obtained from Aldrich and experiments done in triplicates. Double-distilled water was used for the experiments. Fresh leaves of *Polyalthia longifolia* were collected, washed thoroughly with double-distilled water, and incised into small pieces. About 4 g of finely cut *Polyalthia longifolia* leaves were weighed and transferred into

a 250 mL beaker containing 40 mL double-distilled water, mixed well, and boiled for 2 min. The extract obtained was filtered through Whatman number 1 filter paper, and the filtrate was collected in 250 mL Erlenmeyer flask and stored at 4°C for further use.

Aqueous solution of 10^{-3} M and 10^{-4} M silver nitrate (AgNO_3) and 10^{-2} M of D-sorbitol was prepared and used for the synthesis of silver nanoparticles. 3 mL of extract and 1 mL of D-sorbitol were added to 40 mL of AgNO_3 solution. The effect of temperature on the synthesis of silver nanoparticles was carried out at room temperature (25°C) and 60°C. The silver nanoparticles synthesized using *Polyalthia longifolia* leaf extract was tested for antimicrobial activity by agar well diffusion method against pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative), and *Staphylococcus aureus* (Gram positive). The pure cultures of bacteria were subcultured on nutrient agar medium. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 10 mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 50 μL of nanoparticle solution was poured onto each well on all plates. After incubation at 37°C for 24 hours, the different levels of zone of inhibition of bacteria were measured.

The bioreduction of Ag^+ ion in solution was monitored using UV-visible spectrometer (Techomp 8500 spectrometer). Further characterization was done using FTIR (Bruker tensor 27) spectrometer. The extract was centrifuged at 5000 rpm for 30 min and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifuging and redispersing process was repeated three times. Finally, the dried form of extract was palletized with KBr and analyzed using FTIR. The morphology of the AgNPs was examined using transmission electron microscopy (JEOL 3010 TEM). The films of the samples were prepared on a carbon coated copper grid by dropping a small amount of the sample and then allowing it to dry.

3. Results and Discussion

The time of addition of extract into the metal ion solution was considered as the start of the reaction. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [15]. As the *Polyalthia longifolia* leaf extract was mixed in the aqueous solution of the silver ion complex and D-sorbitol, initially the color changed from watery to yellowish brown due to the reduction of silver ion. The reduction rate is found to increase with the reaction temperature [24]. For 10^{-3} M solution the addition of 3 mL of extract to the reaction mixture, the reaction completed by 1.30 h, 1 h while 10^{-4} M solution the reaction completed by 1 h, 40 min at 25°C and 60°C, respectively.

UV-vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions [25]. Figure 1 shows the UV-vis spectra which are recorded after the completion of the reaction. For 10^{-3} M solution, the silver nanoparticles have absorbance peak at 451 nm and 435 nm, and 10^{-4} M solution has peak at 425 nm and 422 nm

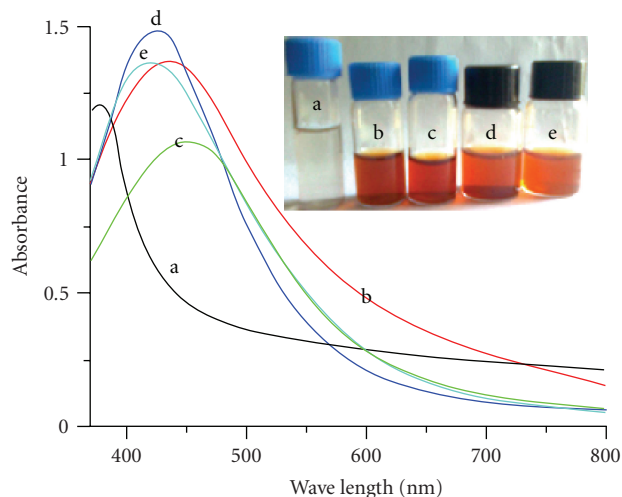


FIGURE 1: UV-vis absorption spectrum of (a) *Polyalthia longifolia* leaf extract, biosynthesized silver nanoparticles of different concentration (10^{-3} M and 10^{-4} M) at (b and d) 25°C, (c and e) 60°C.

for reaction at 25°C and 60°C, respectively. The frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [24]. Supposing the same particle shape, medium dielectric constant and temperature, the mean diameter of the nanoparticles strongly influence the SPR band in aqueous solution [25]. The spectrum shows the blue shift with raising temperature. This blue shift indicates the reduction of mean diameter of the biogenic silver nanoparticles [24, 26, 27].

FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bioreduced silver nanoparticles synthesized by *Polyalthia longifolia* leaf extract along with D-sorbitol. Figure 2(b) represents the FTIR spectrum of D-sorbitol and shows bands at 2938 cm^{-1} (C-H stretching in alkanes) and 1645 cm^{-1} (C=O stretch of carbonyls). Figure 2(a) represents the FTIR spectrum of the leaf extract and shows peaks at 1637 , 1418 , and 1063 cm^{-1} . These peaks are known to be associated with the amide I arise due to carbonyl stretch in proteins (1637 cm^{-1}), -C-C- stretch (in ring) aromatic (1418 cm^{-1}) [28], and C-N stretching vibration of amine (1063 cm^{-1}) [29], respectively. Proteins present in the extract can bind to AgNP through either free amino or carboxyl groups in the proteins [30]. Experimentally, D-sorbitol does not have the potential to reduce the silver ions in the solution, but it may cap the formed silver nanoparticles through electrostatic attraction or bind to the protein groups in the extract via hydrogen bond and increase the stability of the silver nanoparticles. It indicates that the functional groups in biomolecules are mainly responsible for the reduction of silver ions.

The silver nanoparticles are spherical in shape and are not aggregated in solution with raising temperature (Figure 3). This is due to the binding force between the AgNPs and the capping molecules that may get decreased with increasing temperature even though the size of the

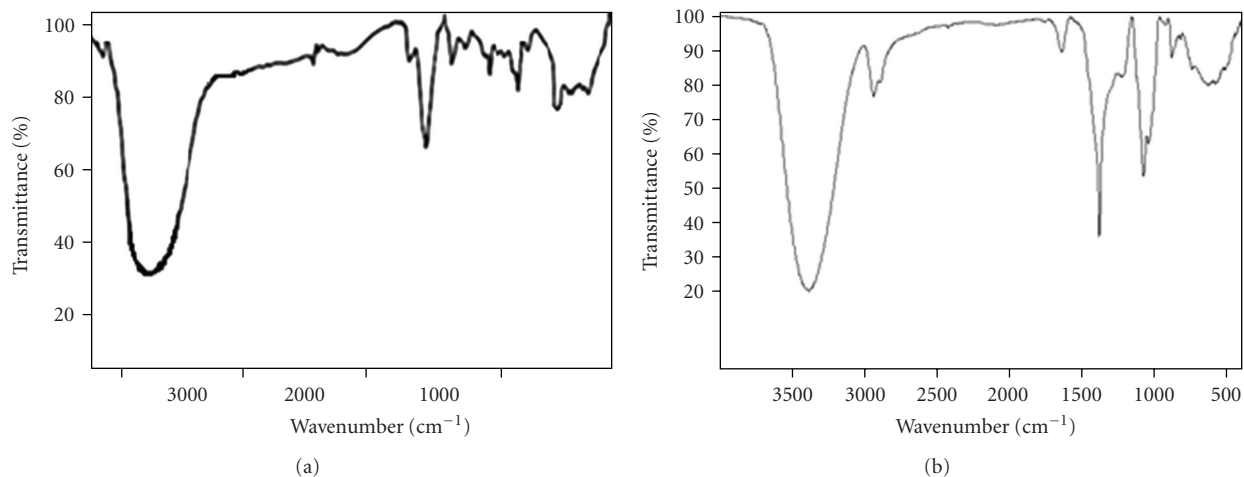


FIGURE 2: FTIR spectrum of (a) *Polyalthia longifolia* leaf extract and (b) D-sorbitol.

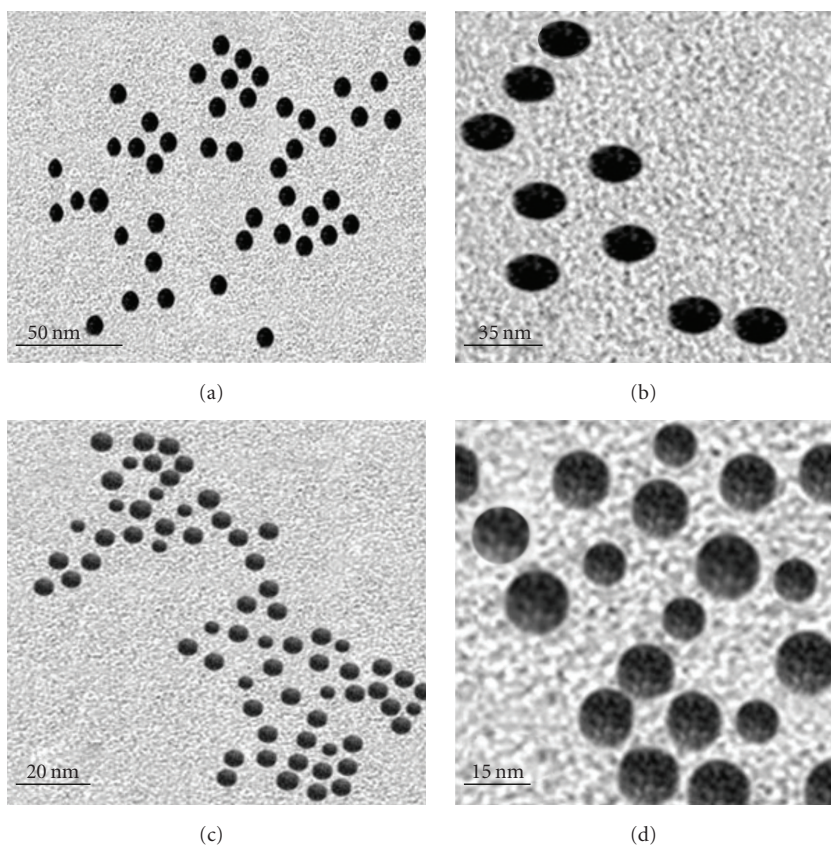


FIGURE 3: HRTEM image of the biosynthesized silver nanoparticles showing various particle sizes at (a and c) 25°C, (b and d) 60°C.

nanoparticles is reduced. In the 10^{-3} M, the size of the synthesized nanoparticle is 50 nm and 35 nm at 25°C and 60°C, respectively. Similarly, in the case of 10^{-4} M, the size of the synthesized nanoparticle is 20 nm and 15 nm at 25°C and 60°C, respectively.

The biologically synthesized silver nanoparticles exhibited excellent antibacterial activity against the bacterial pathogens *Staphylococcus aureus* (Gram positive), *Escherichia*

coli, and *Pseudomonas aeruginosa* (Gram negative) [31]. It has been reported that antibacterial effect was size and dose dependant and was more pronounced against Gram-negative bacteria than Gram-positive bacteria. But the present study clearly indicates that the synthesized silver nanoparticles have good antibacterial action against Gram-positive organism than Gram-negative organisms (Figure 4 and Table 1). The antimicrobial activities of colloidal silver particles are

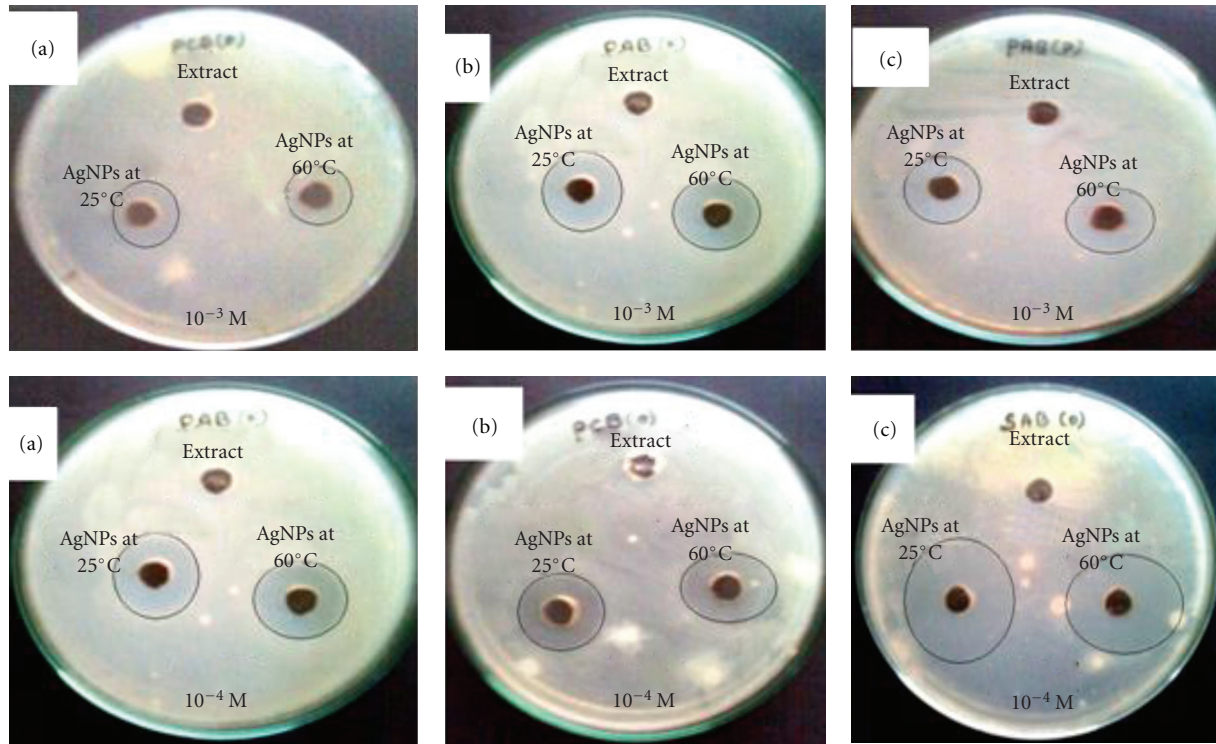


FIGURE 4: Zone of inhibition of silver nanoparticles against (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, and (c) *Staphylococcus aureus*.

TABLE 1: Zone of inhibition (mm) of biologically synthesized silver nanoparticles against bacterial pathogens.

S. NO	Test organism	10 ⁻³ M AgNPs synthesized at		10 ⁻⁴ M AgNPs synthesized at	
		25°C	60°C	25°C	60°C
(1)	<i>Escherichia coli</i>	7.3	7.7	7.3	8
(2)	<i>Pseudomonas aeruginosa</i>	8.3	9	8.8	9.5
(3)	<i>Staphylococcus aureus</i>	14	16	14.6	16.4

influenced by the dimensions of the particles. The smaller particles lead to the greater antimicrobial effects [32]. The effect of antibacterial activity is higher in the case of silver nanoparticles synthesized at 60°C compared to 25°C because of being smaller in size [31, 33].

It is necessary to emphasize that the tested silver nanoparticles have bactericidal effects resulting not only in inhibition of bacterial growth but also in killing bacteria. Experiments conducted using the scanning tunneling electron microscopy (STEM) and X-ray energy dispersive spectrometer (EDS) showed that silver nanoparticles not only at the surface of cell membrane, but also inside the bacteria [34]. This suggests the possibility that the silver nanoparticles may also penetrate inside the bacteria and cause damage by interacting with phosphorus and sulfur containing compounds such as DNA [35]. The exact of inhibition of bacterial growth reported in

this study is dependent on the concentration and number of nanoparticles in medium.

4. Conclusions

Silver nanoparticles were synthesized by *Polyalthia longifolia* leaves extract along with D-sorbitol. The spectroscopic characterization from UV-visible, FTIR, and TEM supports the stability of the biosynthesized nanoparticles. The nanosilver was found to have wider antimicrobial activity in Gram positive than Gram negative organisms. We believe that the silver nanoparticle has great potential for applications in catalysis, biomedical, and pharmaceutical industries.

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