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Green Synthesis of Silver Nanoparticles and Their Antimicrobial Activity against Gram Positive and Gram Negative Bacteria

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Abstract

In the present work, synthesis of silver nanoparticles has been done using a particular variety of medicinal plant extract. The green synthesis of silver nanoparticles was done by the bioreduction of silver nitrate using different concentrations of plant extract taken from Azadirachta indica (Indian Neem). UV-visible studies were conducted to confirm the formation of silver nanoparticles. The peak showing the standard surface plasmon resonance wavelength in UV-visible studies confirm the formation of silver nanoparticles. Later, the antimicrobial activity of the synthesized silver nanoparticles was tested using both gram positive as well as gram negative bacteria i.e. Staphylococcus aureus and Escherichia coli, respectively. The zone of inhibition increased with the increase in the concentration of silver nanoparticles. These studies are quite useful as it shows the utility of green nanotechnology for the synthesis of silver nanoparticles without any toxic residuals and by-products. Further, efficient antimicrobial activity of the synthesized silver nanoparticles proves the application potential of green synthesis in the area of nano-medicine.

Keywords: Green Synthesis, Silver Nanoparticles, Antimicrobial Activity.

1. Introduction

Silver nanoparticles have unique optical, electrical, and thermal properties that play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering. In chemical reduction process, we get silver nanoparticles plus toxic residuals which are not desirable for any kind of biomedical application. So alternative methods are always searched on for the synthesis of silver nanoparticles without any toxic by-products. It is quite interesting that silver nanoparticles can also be synthesized from bacteria [Nanda and Saravanan, 2009] fungus [Ahmad et al, 2003; Balaji, et al, 2009] and plants [Jha, et al, 2009; Nabikhan et al, 2010; Krishnaraj et al, 2010]. The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nanobiotechnology research. Biosynthesis of nanoparticles from green synthesis is advantageous over chemical and physical methods as it is a cost effective and environmental friendly method and it is not necessary to use high pressure, energy, temperature and toxic chemicals. Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticle synthesis by microorganisms. In this work, we report the biosynthesis of silver nanoparticles using a plant extract of *Azadirachta indica* (Indian Neem). Their antimicrobial property has also been reported against gram positive and gram negative bacteria.

2. Materials and Methods

Silver nanoparticles were synthesized from *Azadirachta indica* (Neem) leaves and were then tested for their antimicrobial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria. Fresh and healthy leaves of *Azadirachta indica* were collected from Yamunanagar (Haryana, India). 20 grams of fresh leaves of the plant were collected and washed with distilled water 3-4 times to remove the dust particles and were dried at 50°C for 5-10 minutes in hot air oven. Leaves were chopped into small pieces and mixed into 100 ml distilled water separately. Mixture was stirred at 60°C for 10 minutes in water bath. It was allowed to cool and filtered with Whatman filter paper no. 1. The filterate of the samples were stored at 4°C for further experiments. 15ml of the prepared extract was added to 45ml of aqueous $AgNO_3$ (0.1M solution) at room temperature. The mixture was stirred continuously for 5-10 minutes. The reduction was completed after 24 hours with the appearance of brownish-black colour which confirms the formation of silver nanoparticles. The synthesized silver nanoparticles were analyzed for UV-Visible spectroscopic studies after the time duration of 24 hrs. For antimicrobial studies, the

bacterial strains used in this study were *Escherichia coli* and *Staphylococcus aureus.* Both of these were collected from Department of Medical Microbiology and Biochemistry of Maharishi Markandeshwar University, Mullana, (Ambala) India. Bacterial cultures were maintained on Nutrient Agar plates and Slants. They were subcultured and subsequently stored at 4°C. The strains were inoculated in the nutrient broth (Peptone, 10 grams; beef extract, 3 grams; NaCl, 3 grams; DW, 1000ml; pH 7.0) and incubated at 37°C for 24 hrs.

3. Results & Discussions

The synthesized silver nanoparticles showed the following absorption spectrum at the wavelength range of 300nm – 800nm. The surface plasmon resonance peak at around 425nm confirmed the formation of silver nanoparticles as shown in Figure1. The silver nanoparticles were tested for their antimicrobial property against gram positive *(Staphylococcus aureus)* and gram negative *(Escherichia coli)* and the solvent taken for silver nanoparticles was chloroform. The results of antimicrobial activity are shown in Table-1 and Table-2.

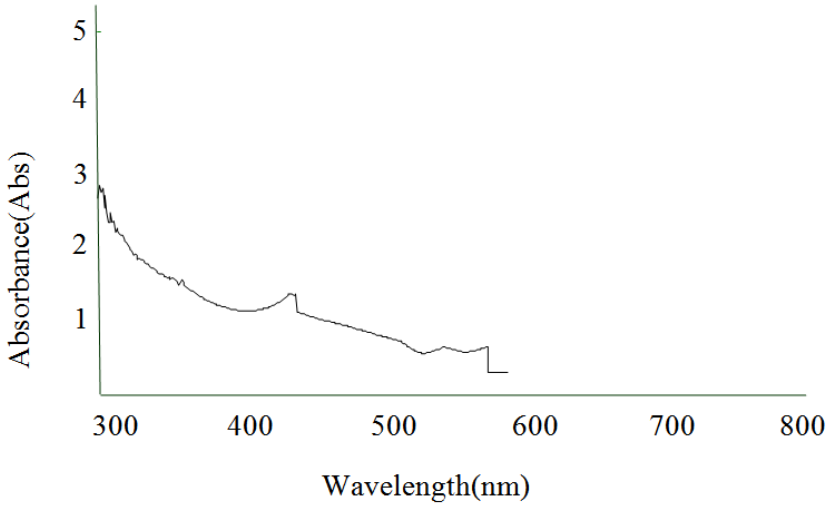

Figure 1: Absorption spectrum for silver nanoparticles.

Table 1: Antimicrobial activity of silver nanoparticles against gram positive bacteria (Staphylococcus aureus).

SERIAL NO.	PLANT	CONCENTRATION 50µg/ml	CONCENTRATION 100µg/ml
1	Azadirachta indica	10mm (ZOI)	13mm (ZOI)

Table 2: Antimicrobial activity of silver nanoparticles against gram negative bacteria (Escherichia coli).

Serial No.	Silver Nanoparticles	Concentration 50µg/ml	Concentration 100µg/ml
1	Azadirachta indica	12mm (ZOI)	15mm (ZOI)

Zone of inhibition (ZOI) observed with different concentrations of nanoparticles produced in chloroform against *S. aureus* and *E. coli* is around 10 mm and 12 mm for 50µg/ml and 13 mm and 15mm for 100µg/ml, respectively. Though not significant, but they provide a considerable insight to provide a new way to synthesize nanoparticles and simultaneously enhance its antimicrobial property as the antimicrobial results of nanoparticles show more effective results than that of the plant leaf extracts. There are numerous concerning features which are still on the verge of experimental verification and are yet to be explored.

4. Conclusion

The present study is regarding the green synthesis of silver nanoparticles and their antimicrobial activity against gram negative and gram positive bacteria *i.e. Echerichia coli* and *Staphylococcus aureus.* It is confirmed that silver nanoparticles are capable of rendering high antimicrobial efficacy and hence has a great potential in the field of medicine. Control experiments in which silver nitrate was taken as control showed no zone of inhibition against any bacteria.

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