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Green synthesis of silver nanoparticles using leaf extract and fruit pulp of *Azadirachta indica*

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Abstract

Nanoparticles mostly silver and gold have unique properties which help in their diverse applications like molecular diagnostics, as therapeutic agents, in medical devices for several medical procedures. The major methods used for their synthesis are the physical and chemical methods. These methods are expensive, not environment friendly and also may lead cytotoxicity. To overcome this problem, in present study the green synthesis method for the synthesis of silver nanoparticle was adopted using aqueous leaf extract and fruit pulp of *Azadirachta indica* (Neem) as a feasible alternative. In this method both extracts acted as a reducing as well as stabilizing agent that reduced silver nitrate ($AgNO_3$) and also acted as a protecting agent for nanoparticles. The formation of nanoparticles was assured by characterization with UV-vis spectroscopy, scanning electron microscopy (SEM). The absorbance of silver nanoparticles with neem aqueous leaf exract and neem fruit pulp was read at 450nm and 430nm respectively. SEM image shows that the nanoparticles synthesized by using neem aqueous leaf extract were spherical in shape with 28nm–41nm dimensions. The antimicrobial activity of as synthesized silver nanoparticles was tested against various bacteria like Gram positive, Gram negative forms and plant pathogens. The results confirmed that the tested bacteria were inhibited as indicated by higher zones of inhibition. These non-toxic nanoparticles synthesized in a simple, ecofriendly and cost effective manner by green synthesis may be suitable for the formulation of new types of bactericidal agents or therapeutic agents.

Keywords: Antimicrobial activity, *Azadirachta indica*, Green synthesis, Reducing agent, Scanning electron microscopy, Silver nanoparticles

Introduction

Synthesis of silver nanoparticles has attracted considerable attention owing to their diverse properties like catalysis (Shiraishi and Toshima, 2000), magnetic and optical polarizability, electrical conductivity (Chan and Yen, 1995), antimicrobial activity (Srinivas and Naga Padma, 2016b), and purification of water (Bindhu and Umadevi, 2014). A number of techniques are available for the synthesis of silver nanoparticles namely ion sputtering, chemical reduction (Sre *et al*., 2015), which are nether cost effective nor ecofriendly as they involve the use of hazardous chemicals, have high energy requirements,

and are also purified with difficult and wasteful methods (Ahmed and Ahmad, 2016). Thus, there is an urgent need for non toxic and eco-friendly silver nanoparticles synthesis technology. Green synthesis of nanoparticles provides advance over other methods as they are simple, one step, cost-effective, environment friendly (Mittal *et al*., 2014). Microorganisms (Quester *et al*., 2013) and plants (Mittal *et al*., 2013) have been reported to bioreduce metal ions to Ag, Au and Pd nanoparticles today. The bacterium *Pseudomonas stutzeri* AG259, an isolate from silver mine reduce the Ag^+ ions to form nanoparticles of well defined size and distinct morphology (Joerger *et al*., 2000). In addition, eukaryotic organisms such as fungi like *Verticillum sp* have also been used to synthesis of nanoparticles (Mukherjee *et al*., 2001). Synthesis of nanoparticles utilizing plants or parts of plants could prove advantage over other biological process by eliminating the elaborate process of maintaining the microbial culture. Hence, many researchers synthesized AgNPs by using plants extracts of marigold flower (Padalia *et al*., 2015), *Melia dubia* (Ashok kumar *et al*., 2013) and *Ocimum tenuiflorum* (Logeswari *et al*., 2012). Considering the vast potentiality of plants as sources for the synthesis of silver nanoparticles, in present study we synthesized AgNPs using aqueous leaf extract and fruit pulp of *Azadirachta indica* as both reducing and stabilizing agent . The antimicrobial activity of as synthesized silver nanoparticles was also studied against different bacteria and found to be significant considerably.

Materials and methods

Preparation of plant extracts

Fresh leaves and fruits of *Azadirachta indica* were thoroughly washed with tap water first and then with distilled water to remove debris and other contaminants. These thoroughly washed leaves and fruits were air dried. For extract preparation 15 gm of cut leaves and fruit pulp were boiled in 100 ml ultra pure water for 20 min and filtered through Whatman filter paper No.1 after cooling. The filtered extracts of both cut leaves and fruit pulp were used for the synthesis of silver nanoparticles.

Green synthesis of silver nanoparticles

Silver nitrate solutions (0.005M, 0.01M and 0.02M) were prepared in distilled water separately. Then 2.5 ml, 5 ml and 7.5 ml of plant extracts of both leaf and fruit pulp were added to different concentrations of silver nitrate at room temperature. The transparent colorless solution was converted to the brown color, indicating the formation of silver nanoparticles.

Characterization of synthesized silver nanoparticles

UV-vis spectroscopy

The reduction of pure Ag^+ ions was monitored by measuring the UV-vis spectrum of the reaction. UV-vis absorption spectrum of the sample (silver nanoparticles prepared from 2.5 ml, 5 ml and 7.5 ml of leaf extract and fruit pulp) was done in an Electronics India-371 UV-vis spectrophotometer in the wavelength range from 300 to 500 nm to determine absorption maxima. The measurements of silver nanoparticles synthesis under different conditions like variation of concentration of leaf extract and fruit pulp, Molarity of silver nitrate solution and incubation time were taken at a particular wave length that gave absorption maxima.

Scanning electron microscopy (SEM)

Scanning electron microscope was also used to observe the size, shape of the synthesized nanoparticles (Silver nanoparticles prepared from 0.01M silver nitrate solution with 5 ml of leaf extract). Scanning electron microscopy study was observed on a

Hitachi-S-3700N at an accelerating voltage of 30Kv.

Assay for antibacterial activity of silver nanoparticles

Gram positive organisms like *Staphylococcus aureus*, *Bacillus sp*, Gram negative organisms namely *Escherichia coli*, *Klebsiella sp* and plant pathogens like *Xanthomonas sp* and *Erwinia sp* were tested for antibacterial activity of the green synthesized silver nanoparticles by agar well assay method. The zones of inhibition were measured after 24 hours at 37^0C.

Results

Azadirachta indica leaf extract and fruit pulp were used for green synthesis of silver nanoparticles as both reducing and a stabilizing agent. The reduction of silver ions into silver nanoparticles during exposure to the leaf extract and fruit pulp is indicated by color change that could be read spectrophotometrically using UV-vis spectrophotometer. Silver nanoparticles exhibit brown color in aqueous solution due to the excitation of surface plasma vibrations in silver nanoparticles (Veerasamy *et al*., 2011). Silver nanoparticles prepared from leaf extract and fruit pulp (5 ml of both leaf extract and fruit pulp with 0.01M silver nitrite) exhibit a sharp emission peak at 450 nm and 430nm respectively and this remained stable even after 60 days indicating stability of the green synthesized silver nanoparticles (Figure-1a and 1b). This also indicated that there was almost no agglomeration of silver nanoparticles.

Further absorbance of silver nanoparticles synthesized from different concentration of silver nitrate and leaf extract and fruit pulp extract were observed at 450nm and 430nm respectively (Figures 2a-2f). The results indicated that as concentration of silver nitrate and reducing agent (leaf extract and fruit pulp) increased optical density also simultaneously increased.

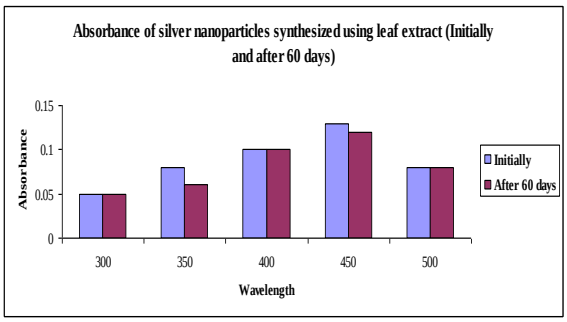

Figure 1a: Absorbance of silver nanoparticles prepared using *Azadirachta indica* leaf extract initially and after 60 days.

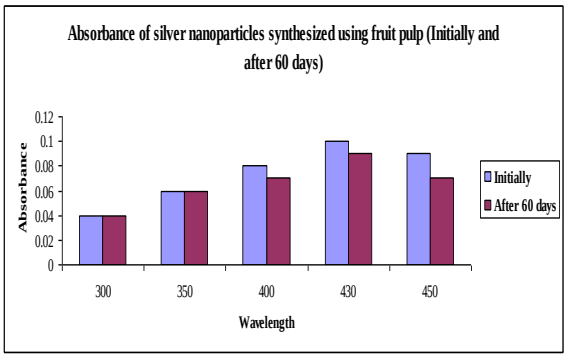

Figure 1b: Absorbance of silver nanoparticles prepared using *Azadirachta indica* fruit pulp extract initially and after 60 days.

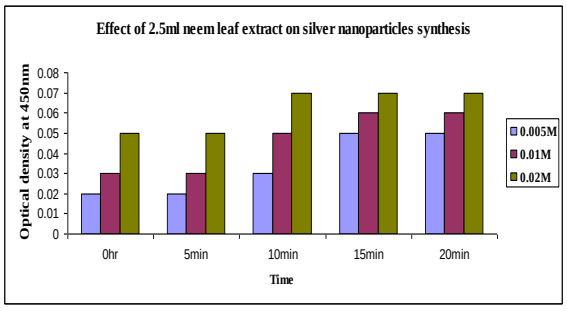

Figure 2a: Effect of 2.5ml *Azadirachta indica* (neem) leaf extract on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

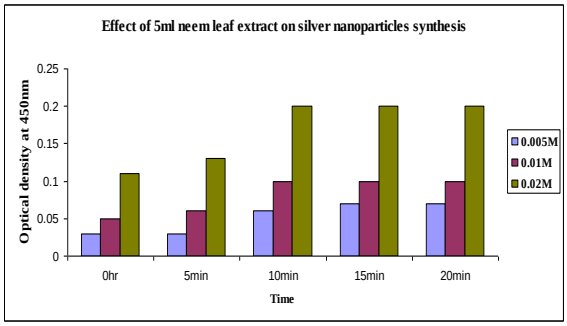

Figure 2b: Effect of 5ml *Azadirachta indica* (neem) leaf extract on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

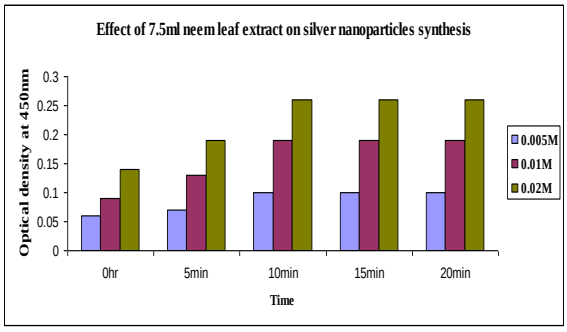

Figure 2c: Effect of 7.5ml *Azadirachta indica* (neem) leaf extract on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

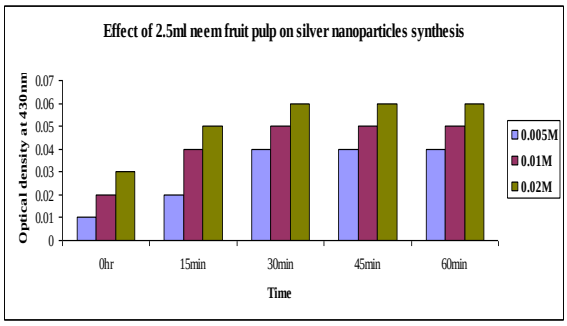

Figure 2d: Effect of 2.5ml *Azadirachta indica* (neem) fruit pulp extract on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

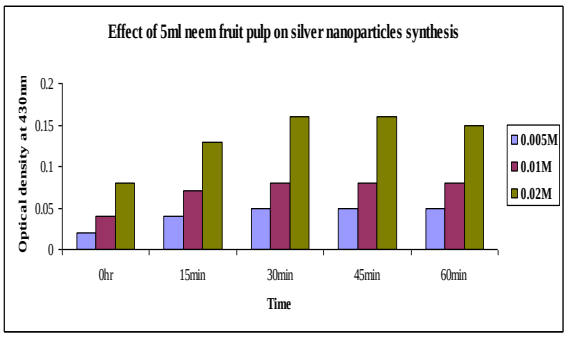

Figure 2e: Effect of 5ml *Azadirachta indica* (neem) fruit pulp extract on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

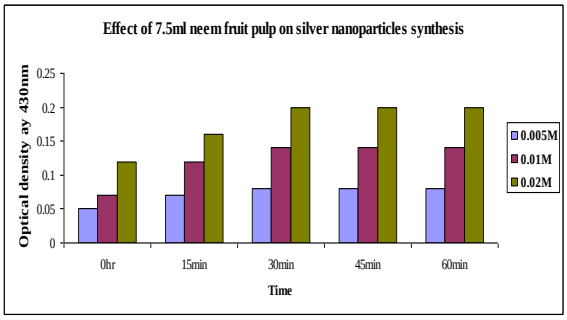

Figure 2f: Effect of 7.5ml *Azadirachta indica* (neem) fruit pulp extract on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

The size and morphology of synthesized nanoparticles (prepared from 0.01M 5 ml *Azadirachta indica* leaf extract) was determined by scanning electron microscope (Figure-3). SEM image indicates that the nanoparticles prepared from 0.01M silver nitrate with 5 ml *Azadirachta indica* leaf extract was spherical in shape with 28nm-41nm diameter. The formation of large size particles may also occurs due to increase in concentration of silver nitrate solution (Kondow and Mafune, 2000). The silver nanoparticles solution (prepared from 0.01M silver nitrate with 5 ml leaf extract) showed excellent antibacterial activity against *Staphylococcus aureus*, *Bacillus sp* (Gram positive), *Escherichia coli*, *Klebsiella sp*

(Gram negative), *Xanthomonas sp* and *Erwinia sp* (Plant pathogens) by showing zone of inhibition around the cavities with bacteria growth plate. The radial diameter of the inhibitory zones of *Staphylococcus aureus, Bacillus sp, Escherichia coli, Klebsiella sp, Xanthomonas sp* and *Erwinia sp* were 24mm, 22mm, 19mm, 16mm, 20mm and 18mm respectively (Figures-4-7).

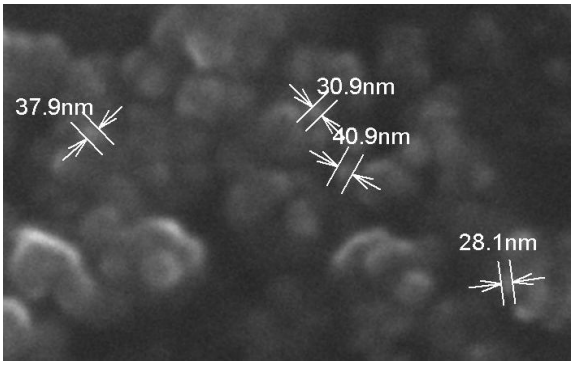

Figure 3: SEM image of silver nanoparticles synthesized from 0.01M silver nitrate with 5 ml leaf extract.

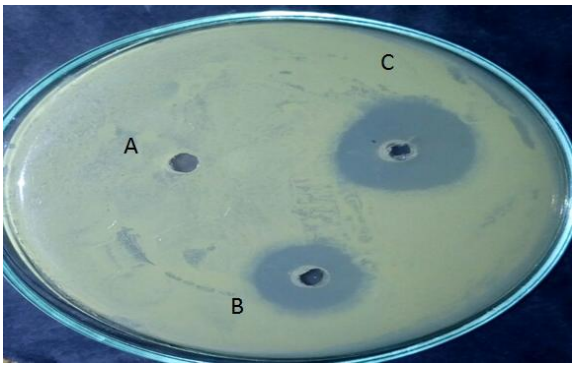

Figure 4: Antibacterial activity of silver nanoparticles against *Staphylococcus aureus* (Gram positive) assayed by the agar well method. The cavity-A contained sterilized water, cavity-B contained silver nitrate solution and cavity-C contained silver nanoparticles.

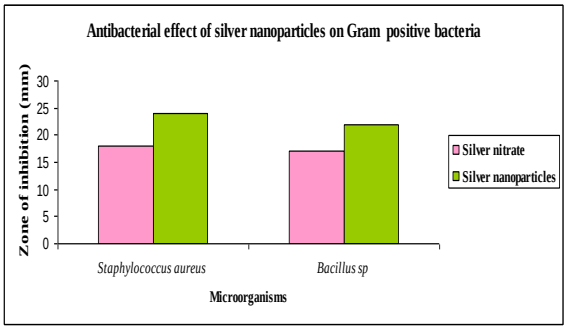

Figure 5: Antibacterial effect indicated by zone of inhibition (mm) of silver nitrate (0.01M) and silver nanoparticles synthesized from silver nitrate (0.01M) with 5 ml leaf extract.

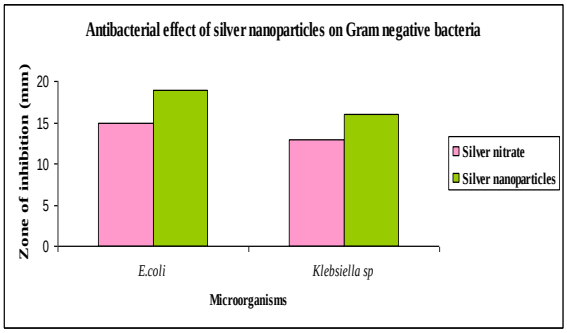

Figure 6: Antibacterial effect indicated by zone of inhibition (mm) of silver nitrate (0.01M) and silver nanoparticles synthesized from silver nitrate (0.01M) with 5 ml leaf extract.

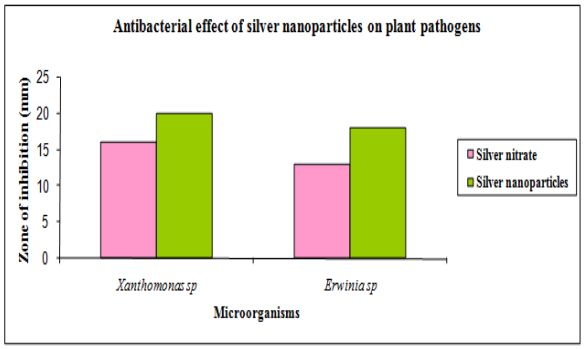

Figure 7: Antibacterial effect indicated by zone of inhibition (mm) of silver nitrate (0.01M) and silver nanoparticles synthesized from silver nitrate (0.01M) with 5 ml leaf extract.

Discussion

Azadirachta indica has been used as a household remedy against since ages for treatment of various ailments and infections (Omoja *et al*., 2011). Leaf extract and fruit pulp of *Azadirachta indica* were used as both reducing and stabilizing agent in preparation of metal nanoparticles. Due to surface plasmon resonance AgNPs prepared from leaf extract and fruit pulp absorb radiation maximally at 450nm and 430nm respectively. The surface plasmon resonance transition is responsible for the striking brownish coloration of silver nanoparticles (Shankar *et al*., 2003). Synthesized nanoparticles were stable even after 60 days, this reveals that the AgNPs are well capped with leaf extract and fruit pulp extract. SEM image indicate the synthesized silver nanoparticles (prepared from 0.01M silver nitrate with 5 ml leaf extract) were spherical in shape with 28nm–41nm diameter. Low concentration of silver nanoparticles is attractive due to its non-toxicity to the human body, its broad spectrum antibacterial action (Baker *et al*., 2005), and also due to its bactericidal action against multiresistant bacteria like Methicillin-resistant *Staphylococcus aureus* (MRSA), as well as multi drug resistant *Pseudomonas aeruginosa* (Lara *et al*., 2010). Silver nanoparticles interact with a wide range of metabolic process with in microorganisms, resulting in inhibition of growth, loss of infectivity leading to cell death, but this mainly depends on size, shape and concentration of AgNPs (Asharani *et al*., 2009). Positive charge on the Ag^+ ion is crucial for its antimicrobial activity through the electrostatic attraction between the negatively charged cell membrane of the microorganisms and the positive charged nanoparticles. The mode of action of silver nanoparticles was also found to be similar to that of Ag^+ ion, however, the effective concentrations of AgNPs and Ag^+ ion were at nanomolar and micro molar levels. Synthesized silver nanoparticles showed significant antibacterial activity against *Staphylococcus aureus*, *Bacillus sp* (Gram positive), *Escherichia coli*, *Klebsiella sp* (Gram negative), *Xanthomonas sp* and *Erwinia sp* (Plant pathogens), as indicated by their inhibition zones indicating their potential use as effective antimicrobial compounds against drug resistant bacteria.

Conclusion

A critical need in the area of nanotechnology is the development of reliable and eco-friendly process for preparation of metallic nanoparticles. In the present study a simple, one-step, cost effective and eco-friendly method of green synthesis of silver nanoparticles using neem leaf extract and fruit pulp was developed. Only 10 min was required for the conversion of silver ions into AgNPs at room temperature, without the involvement of any hazardous chemical and also the synthesized AgNPs remained stable for 60 days without agglomeration. The synthesized silver nanoparticles showed significant antibacterial activity against different bacteria including plant pathogens. These non-toxic nanomaterials which can be prepared by green synthesis may have in future valuable application as antimicrobial agents to combat drug resistant bacteria.

Acknowledgments

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