SILVER NANOPARTICLES FROM *JUSTICIA ADHATODA* LEAF EXTRACT FOR ITS ANTIPATHOGENIC EFFICACY

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Abstract : Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize. The synthesis, characterization, and application of biologically synthesized nanomaterials have become an important branch of nanotechnology. Research in nanotechnology highlights the possibility of green chemistry pathways to produce technologically important nanomaterials. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are often toxic and flammable. Silver nanoparticles are the metal of choice as they hold the promise to kill microbes effectively. The present study describes a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM silver nitrate solution through the a plant extract. The appearance of brown colour indicates the synthesis of silver nanoparticles. Nanoparticles were characterized using UV-Vis absorption spectroscopy and SEM analysis. UV-Vis spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at 450nm. SEM analysis showed the average particle size of 50-70nm and spherical shape of the silver nanoparticles. Further studies on the silver nanoparticles showed that it has the antibacterial activities. Antipathogenic activity study was carried out by disc diffusion methods on pathogenic organisms such as Escherichia coli, Proteus vulgaricus, Klebsiellapneumoniae, Pseudomonas aeruginosa. Zone of inhibition was observed by disc diffusion methods and among these four pathogens, Klebsiellapneumoniae and Escherichia coli showed the maximum activity.

Keywords: Silver nanoparticles, Antipathogenic, Plant extract

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INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity bio molecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics (Jain *et al.*, 2009). Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nanoscale stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications (Mritunjai*et al*., 2008). The importance of bactericidal nanomaterials study is because of the increase in new resistant strains of bacteria against most potent antibiotics and has promoted research in the well known activity of silver ions and silver-based compounds, including silver nanoparticles. This effect was size and dose dependent and was more pronounced against gram-negative bacteria than gram-positive organisms (Mritunjai*et al*., 2008).

Silver nanoparticles are one of the most promising nanomaterials due to their antibacterial capacity and economical manufacturing. Dissolved silver has been known for decades to be an efficient bactericide, binding to DNA and disrupting cell replication. Silver nanoparticles have become a promising material for their potential use as an alternative bactericide combating antibiotic resistant strains, a major hazard in hospitals. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendliness. Plant extracts have been found to be cost effective and environment friendly for the large scale synthesis of nanoparticles. The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Jain *et al*., 2009).

Silver compounds have been used as antimicrobial compounds for coliform found in waste water. Silver nanoparticles, nanodots or nanopowder are spherical or flake high surface area metal particles having high antibacterial activity are used in wound. In the area of water purification, nanotechnology offers the possibility of an efficient removal of pollutants and germs. Silver nanoparticles are the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes, whereby silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation. Silver ions and silver-based compounds are highly toxic to microorganisms showing strong biocidal effects on as many as 16 species of bacteria including *E. coli.* Thus, silver ions, as an antibacterial component, have been used in the formulation of dental resin composites and ion exchange fibers and in coatings of medical devices (Sondi*et al.*, 2004).

The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. Silver containing materials can be employed to eliminate microorganisms on textile fabrics or they can be used for water treatment (Parashar*et al.,* 2009). Silver ions have been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. Some forms of silver have been demonstrated to be effective against burns, severe chronic osteomyelitis, urinary tract infections and central venous catheter infections (Feng*et al*., 2000). The bactericidal effect of silver ions on micro-organisms is very well known; however, the bactericidal mechanism is only partially understood. It has been proposed that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. Experimental evidence suggests that DNA loses its replication ability once the bacteria have been treated with silver ions. Other studies have shown evidence of structural changes in the cell membrane as well as the formation of small electron-dense granules formed by silver and sulphur. Silver ions have been demonstrated to be useful and effective in bactericidal applications, but due to the unique properties of nanoparticles nanotechnology presents a reasonable alternative for development of new bactericides (Mritunjai*et al*., 2008). Silver nanoparticles exhibit a broad size distribution and morphologies with highly reactive facets. The major mechanism through which silver nanoparticles manifested antibacterial properties is by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. The antibacterial effect of nanoparticles is independent of acquisition of resistance by the bacteria against antibiotics. However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity of nanoparticles towards human cells before proposing their therapeutic use (Mritunjai*et al*., 2008).

The present study concentrating on the synthesis of silver nanoparticles from *Justiciaadhatoda*leaf extract by using 1mMsilver nitrate at different concentrations. The efficacy of the silver nanoparticle were screened to check their antipathogenic properties. The synthesized particles were characterized by SEM analysis.

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MATERIALS AND METHODS

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Preparation of plant extracts

Sample weighing 25g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed with 100 ml sterile distilled water and filtered through WhatmanNo.1 filter paper. 1mM aqueous solution of Silver nitrate ($AgNO3$) was prepared and used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

Five different concentrations of samples were prepared. 10 ml of *J.adhatoda*leaf extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate for reduction into Ag^+ ions and kept at room temperature for 5 hours. Similarly 20ml, 30ml, 40ml, 50ml of plant extract was taken and to this amount, 80ml, 70ml, 60ml, 50ml of silver nitrate was added and kept for incubation.

UV-Vis spectra analysis

UV-Vis spectroscopy is a valuable tool for the structural characterization of silver nanoparticles. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectra readings were taken for the plant sample.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was carried out at National Institute of Interdisciplinary Science and Technology (NIIST), Thiruvananthapuram using SEM (Jeol model

JSM-5600 LV), (Sputter cutting Jeol model 1200). Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

Antibacterial activity study

Antibacterial activity of the synthesised silver nanoparticles were determined using *Escherichia coli, Proteus vulgaricus, Klebsiellapneumoniae, Pseudomonas aeruginosa* by disc diffusion method.

Antibacterial activity by Disc diffusion method

Antibacterial activity of the synthesized silver nanoparticles was determined using agar well diffusion method. Approximately 20ml of the molten and cooled nutrient agar was poured in sterilized petridishes. Sterile paper discs (containing silver nanoparticles) were placed in each plate. 4 petriplate was prepared in similar way for the four bacteria namely, *Escherichia coli, Proteus vulgaricus, Klebsiellapneumoniae, Pseudomonas aeruginosa.*

RESULTS AND DISCUSSION

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. The present study is a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO3 solution through the plant extract. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

Synthesis of silver nanoparticles from *Justiciaadhatoda*leaf extract.

When the *Justiciaadhatoda*leaf extract was mixed with 1mM silver nitrate after 5 hours of incubation, it started to change the colour from watery to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles and the colour change is due to excitation of surface plasmon vibrations in silver nanoparticles. It is generally recognized that UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions. UV-Vis spectra reading indicated that the *Justiciaadhatoda*leaf extract added at different concentrations showed maximum absorption values at 450nm and the maximum absorption peak at 450nm was observed in the 60+40 concentrations (Fig.1). SEM analysis showed the average particle size of 50- 70nm and spherical shape of the silver nanoparticles (Fig.2).

Antibacterial activity of Silver Nanoparticles

Antibacterial activity of silver nanoparticle was studied using *Escherchiacoli,Proteusvulgaricus, Klebsiellapneumoniae, Pseudomonas aeruginosa.* Disc diffusion method was carried against 4 pathogenic organisms. Zone of inhibition was measured after 24hr of incubation. The number of bacterial colonies grown on agar plates as a function of the different concentration of silver nanoparticles was gradually declined when the concentration of nanoparticles increased. Antibacterial study by disc diffusion method showed the efficiency of silver nanoparticles. Maximum zone of inhibition was observed against *Klebsiellapneumoniae* and*Escherichia coli* (Fig.3). Results clearly demonstrate that newly synthesized silver nanoparticles are promising antimicrobial agent against the pathogens employed. The mechanism of the bactericidal effect of silver colloid particles against bacteria is not very well-known. Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles. Several studies demonstrated that using the Scanning Tunnelling Electron Microscopy (STEM) and the X-ray Energy Dispersive Spectrometer (EDS) it was found out that the silver nanoparticles are not only at the surface of cell membrane, but also inside the bacteria. This then suggests the possibility that the silver nanoparticles may also penetrate inside the bacteria and fungi, causing damage by interacting with phosphorus- and sulphur-containing compounds such as DNA. Silver tends to have a high affinity to react with such compounds (Govindaraju*et al*., 2010). One more possibility would be the release of silver ions from nanoparticles, which will have an additional contribution to the antimicrobial properties of silver nanoparticles. Currently, the increase of bacterial resistance to anti microbial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Gram-positive and Gram-negative bacteria (Govindaraju*et al*., 2010).The green chemistry approach addressed in the present study for the synthesis of silver nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible.

Fig 1 Graph showing the maximum absorbance at 450 nm for 60+40 concentrations.

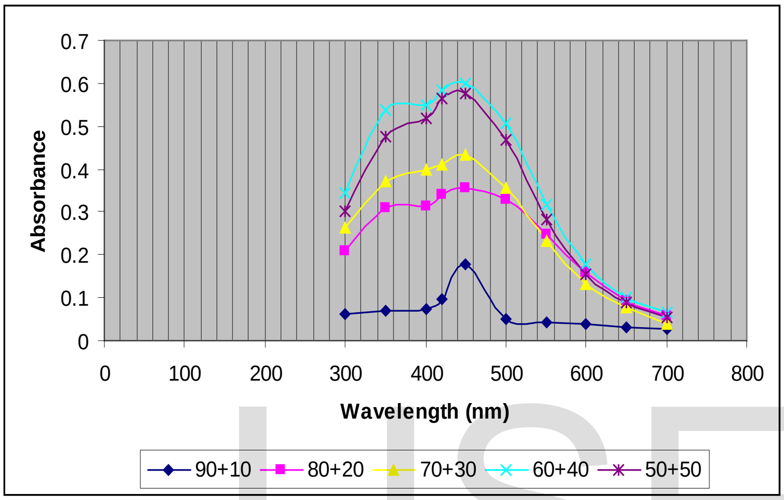
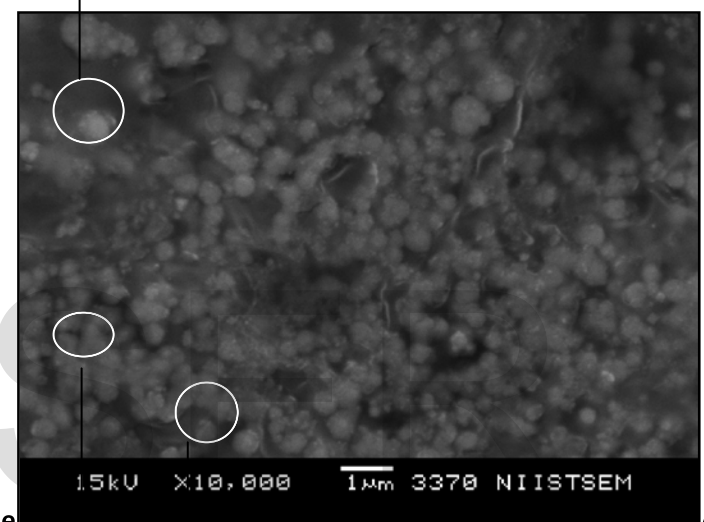


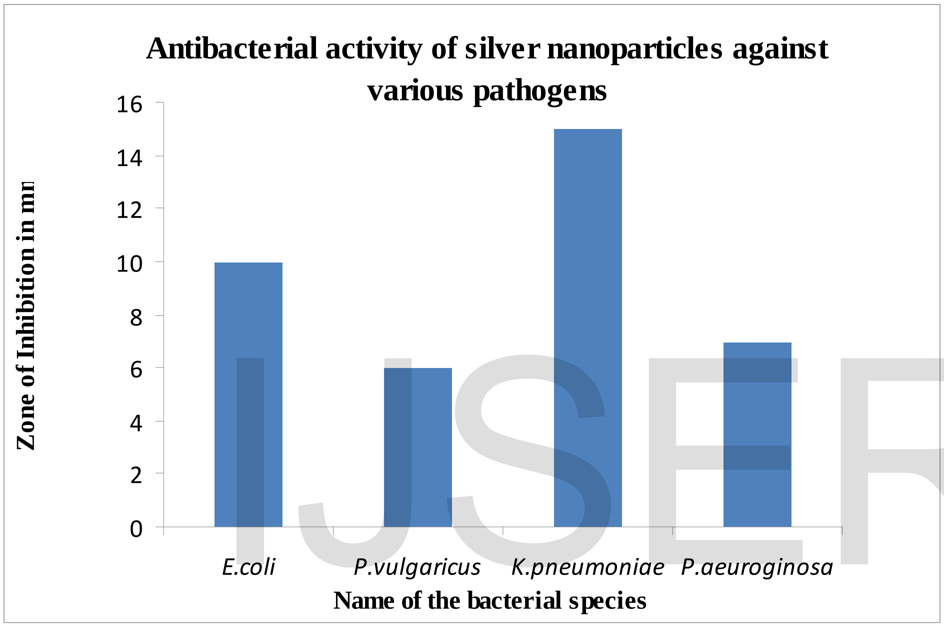
Fig : SEM image of Ag nanoparticle synthesised by the *plant extract*



Ag nanoparticle **Ag**

nanoparticle

Fig: 3 Antibacterial activity of silver nanoparticles



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