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Silver Nanoparticles from a Plant *Echinacea Purpurea* 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 *Echinacea purpurea* (cone flower) 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 spread plate, pour plate on *Escherichia coli* and disc diffusion methods on pathogenic organisms such as *Escherichia coli, Proteus vulgaricus, Klebsiella pneumoniae, Pseudomonas aeruginosa.* Compared to spread plate, pour plate method showed the maximum antibacterial activity. Zone of inhibition was observed by disc diffusion methods and among these four pathogens, *Klebsiella pneumoniae* and *Escherichia coli* showed the maximum activity.

KEYWORDS: *Silver nanoparticles, Antipathogenic, Plant extract*

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INTRODUCTION

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. Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. 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).

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. 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). 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). 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 a plant *Echinacea purpurea* (cone flower) extract by using 1mM silver 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.

MATERIALS AND METHODS

Preparation of *Echinacea purpurea* 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 Whatman No.1 filter paper. 1mM aqueous solution of silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles.1mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. Here 5 different concentrations of samples were prepared. 10 ml of papaya fruit 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 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 spectral analysis was done by using UV-Vis spectrophotometer. UV-Vis spectra readings were taken for the plant sample. Scanning Electron Microscopic (SEM) analysis was carried out by 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 of the synthesised silver nanoparticles was determined using *Escherichia coli, Proteus vulgaricus, Klebsiella pneumoniae, Pseudomonas aeruginosa* by spread plate, pour plate, and standard disc diffusion method. Nutrient broth was used to cultivate bacteria. 10 test tubes were taken for the preparation of sample and control was also taken. To each test tube, 10ml nutrient broth was added and then 1ml to 10ml silver nanoparticles was added to each test tube respectively. To all the test tube, a loop full of organism was added. Then 20ml of molten agar is allowed to cool and the growth media was added to each petriplate and allowed to solidify. After solidification of agar, 0.1ml of sample was transferred to each agar petriplate with a sterile micropipette and spread throughout over the agar with an L-shaped glass rod by keeping the plate over the spread plate strand. Then these plates were allowed to incubate in an inverted position for 24 hours at 37°C. 10 test tubes were taken for the preparation of sample and control was also taken. To each test tube, 10ml nutrient broth was added and then 1ml to 10ml silver nanoparticles was added to each test tube. To all the test tube, a loop full of organism was added.1ml of the sample was transferred to each of the sterile petriplate with a sterile pipette. Approximately 20ml of the molten agar was allowed to cool and the growth media was added to each petriplate. The plates were allowed to solidify and incubated for 24 hours at 37°C. 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, Klebsiella pneumoniae, Pseudomonas aeruginosa.*

RESULTS AND DISCUSSION

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. When the *plant* 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 UV-Vis spectra reading indicated that the *plant* 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 Antibacterial activity of silver nanoparticle was studied using *Escherchia coli,Proteus vulgaricus, Klebsiella pneumoniae, Pseudomonas aeruginosa.* Study was conducted using spread plate, pour plate, disc diffusion method. Spread plate was carried out using pathogenic *Escherichia coli*. 10ml silver nanoparticles added sample showed less number of colonies which indicates that when the concentration of silver nanoparticles added increases, number of bacterial colonies grown on plate becomes gradually declined Similarly pour plate was carried out and 10ml silver nanoparticles added plate showed only 5 colonies and 9ml added showed 10 colonies. Compared to spread plate, pour plate shows the maximum antibacterial effects. 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 *Klebsiella pneumoniae* and *Escherichia coli*. 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. 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. 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 approzach addressed in the present study for the synthesis of silver nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible.

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