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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *PHYLLANTHUS NIRURI* LEAF EXTRACT AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT
There is an increasing commercial demand for nanoparticles due of their wide applicability in various areas such as electronics, catalysis, chemistry, energy, medicine etc. Traditionally metallic nanoparticles are synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. Hence to overcome this limitation, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles. In the study we synthesized nanoparticles from 5 mM $AgNO_3$ solution through *Phyllanthus niruri* leaf extract as reducing agent as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy, and SEM. SEM analysis showed the average particle size of 30-40 nm as well as revealed their structure. Further these biologically synthesized nanoparticles were found to be highly toxic against different bacterial spp. In addition to this effect of $AgNO_3$ concentration and temperature was also carried out in synthesis of these nanoparticles.

Keywords: Silver nanoparticles, green synthesis, *Phyllanthus niruri*.

INTRODUCTION

Clusters of atoms with a size upto 100nm are called as nanomaterials. Nanotechnology is the most emerging area of research these days due to its immense potential and applications and is making an impact in all spheres of human life. Various applications of nanoparticles and nanomaterials are rising rapidly [1-3]. Silver particles have incredible applications in biomolecular detection and diagnostics [4], antimicrobials and therapeutics [5-7], Catalysis [8] and micro-electronics [9]. A large number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions [10], chemical and photochemical reactions in reverse micelles [11], thermal decomposition of silver compounds [12], radiation assisted [13], electrochemical [14], sonochemical [15], microwave assisted process [16] and recently via green chemistry route [17-19]. The use of environmentally benign materials like plant extract [20,21], bacteria [22], fungi [23] and enzymes [24] for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Silver has been in use since time immemorial. However the use of silver for medicine or local antibacterial agent was not recognized until the nineteenth century. But due to the emergence of several antibiotics the use of these silver compounds has been declined remarkably. Metallic silver in the form of silver nanoparticles has made a remarkable comeback as a potential antimicrobial agent. The antimicrobial property of silver has been investigated and employed more extensively than any other inorganic antibacterial agent and is recognized to have inhibitory effect on microbes present in medical and industrial process [25-27]. It is also useful in medical industry [28]. They have large surface area to volume ratio [29]. Specific surface area is relevant for catalytic activity and other related properties such as antimicrobial activity in silver nanoparticles. Their unique size dependent properties make these materials superior and indispensable as they show unique physical, chemical and biological properties. In the present study we have used *Phyllanthus niruri* leaf extract for synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate. Further these biologically synthesized nanoparticles were found highly toxic against different pathogenic bacteria and fungi.

MATERIALS AND METHODS

Plant material and preparation of the extract

Green *Phyllanthus niruri* leaves were used to make the aqueous extract. *Phyllanthus niruri* leaves weighing 5g were thoroughly washed in distilled water, cut into fine pieces and were boiled into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 μm).

Synthesis of silver nanoparticles

5 mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Phyllanthus niruri* leaf extract was added into 90 ml of aqueous solution of 5 mM Silver nitrate for reduction into Ag+ ions and kept at room temperature for 4 hours.

Various concentrations of Silver nitrate (AgNO3) were used, but 5 mM proved to be the best. Additionally various ranges of temperature were also used to study the effect of temperature, but room temperature proved to be the best.

UV-Vis Spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 4 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer, UV-2450 (Shimadzu).

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done at Advanced Instrumentation Research Facility (AIRF), JNU, Delhi. The sample was prepared by placing a drop of very fine suspension of nanoparticles (in water) over a cut glass and allowed to air dry.

Antibacterial assays

The antibacterial assays were done on *Escherichia coli* and *Pseudomonas aeruginosa* by standard disc diffusion method. Briefly Luria Bertani (LB) agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (100 μl) of each culture were spread on to LB agar plates. Sterile paper discs of 3mm diameter (containing 50mg/liter silver nanoparticles) along with four standard antibiotic containing discs were placed in each plate.

Antifungal Assays

The antifungal assays were done on *Aspergillus flavus* by food poisoning method. Potato dextrose agar (PDA) medium was used in the study. The medium of each Petri dish contains 30 ppm silver nanoparticles were inoculated each alone at the centre with 5mm Inoculum disc of each pathogenic fungus and incubated at 25 °C for 7 days. The medium with Inoculum disc of each fungus but without silver nanoparticles served as control.

RESULTS AND DISCUSSION

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [30]. In this case, after mixing *Phyllanthus niruri* leaf extract in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion; which indicated formation of silver nanoparticles. It is generally recognized that UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions [31]. Figure 1 show the change in color and figure 2 shows the UV-Vis spectra recorded from the reaction medium after 4 hours. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 440 nm, broadening of peak indicated that the particles are polydispersed.

Moreover, different concentrations of AgNO3 (1.5 and 10mM) were tried and 5 mM proved to be the best for particles synthesis (data not shown).. Additionally, different temperatures were also tried (4, 10, 25, room temperature, 50, 75 C), but room temperature proved to be the best (data not shown).The biosynthesized silver nanostructure was further demonstrated and confirmed by scanning electron microscope (Figure 3). Average size of the particles synthesized was 30-40 nm with size range 10 to 50nm with cubic and hexagonal shape. The SEM image showing the high density silver nanoparticles synthesized by the *Phyllanthus niruri* leaf extract further confirmed the development of silver nanostructures.

Further the nanoparticles synthesized by green route are found highly toxic against pathogenic bacteria and fungi (Table 1). Antibacterial effects of Ag nanoparticles obeyed a dual action mechanism of antibacterial activity, i.e., the bactericidal effect of Ag+ and membrane-disrupting effect of the polymer subunits. The present study showed a simple, rapid and economical route to synthesized Silver nanoparticles.

Table 1. Effect of silver nanoparticles on human pathogens

Name of Pathogen	Disease	Zone of Inhibition (mm)	
Bacteria		Food poisoning method (50 ppm Ag NPs)	
		Treated	**Control**
Escherichia coli	Cholecystitis, Bactremia, Cholangitis, Diarrhea	16± 0.5	43± 0.2
Pseudomonas syringae	Urinary tract infection, Ventilor associated Pneumonia	13± 0.5	41± 0.4
Fungi		**Food poisoning method (30 ppm Ag NPs)**	
		Treated	**Control**
Aspergillus flavus	Mycotoxicosis	11± 0.3	29± 0.2

Figure 1. Photographs of (A) *Phyllanthus niruri* extract (B) leaf extract + $AgNO_3$ (C) $AgNO_3$ with *Phyllanthus niruri* extract after 4 hrs of incubation

A

B

C

Figure 2. UV-Vis absorption spectrum of silver nanoparticles synthesized by treating aqueous AgNO3 solution with plant extract after 4 hrs

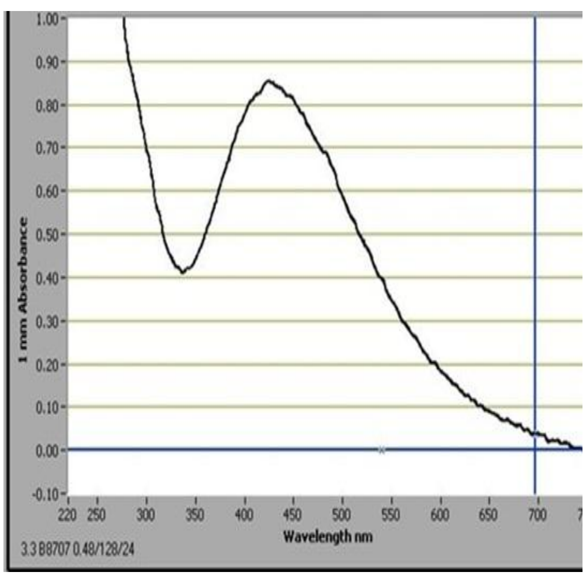
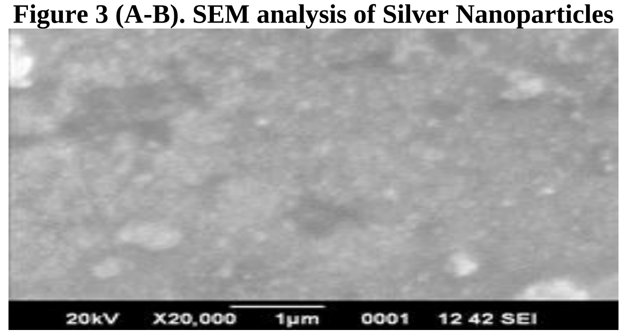
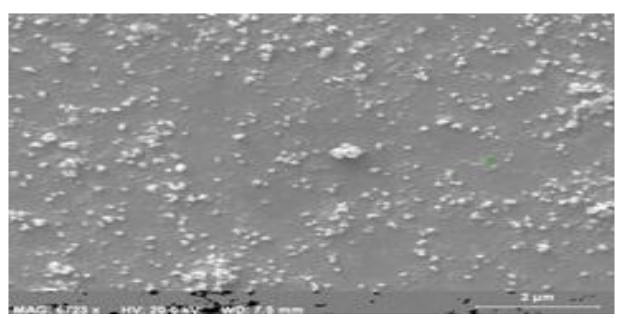


Figure 3 (A-B). SEM analysis of Silver Nanoparticles



A



B

CONCLUSION

Nanoparticles have unique physical properties (size, charge, biocompatibility, solubility) and can be manipulated to increase various properties. In the present study, the bio-reduction of aqueous Ag+ ions by the *Phyllanthus niruri* leaf extract has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. But the capabilities of *Phyllanthus niruri* leaf extract as a capping and reducing agent is not tested and not well defined. In the present study we found that *Phyllanthus niruri* can be also good source for synthesis of silver nanoparticles along with gold nanoparticles [32,33].

This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents. The reduction of silver ions and stabilization of the silver NPs was thought to occur through the participation of Leaf proteins and metabolites. Most importantly, the reaction was simple and convenient to handle, and it is believed that it has advantages over other biological syntheses.

Hence, it can be concluded that other than toxic chemical synthesis, plants (*Phyllanthus niruri*) also have ability to perform synthesis of nanoparticles which is cost effective and environment friendly technique. These nanoparticles which are synthesized using green chemistry can be used in the future for a variety of applications.

REFERENCES

1. Jahn W. (1999). Chemical aspects of the use of gold clusters in structural biology. *Journal of Structural Biology*, 127, 106-112.
2. Naiwa HS. (2000). Hand Book of Nanostructural Materials and Nanotechnology, Academic Press New York, 1-5.
3. Murphy CJ. (2008). Sustainability as an emerging design criterion in nanoparticle synthesis and applications. *Journal of Materials Chemistry*, 18, 2173–2176.
4. Schultz S, Smith DR, Mock JJ and Schultz DA. (2000). Single-target molecule detection with nonbleaching multicolor optical immunolabels. Proceeding of the national academy of sciences USA, 97, 996-1001.
5. Rai M, Yadav A and Gade A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advance*, 27, 76–83.
6. Elechiguerra JL, Burt JL, Morones JR, et al. (2005). Interaction of silver nanoparticles with HIV-1. *Journal of Nanobiotechnology*, 3, 6.
7. Panneerselvam C, Ponarulselvam S and Murugan K. (2011). Potential Anti-plasmodial Activity of Synthesized Silver Nanoparticle using *Andrographis paniculata* Nees (Acanthaceae). *Archives of Applied Science Research*, 3(6), 208-217.
8. Crooks RM, Lemon BI, Sun L, Yeung LK and Zhao M. (2000). Dendrimer-Encapsulated Metals and Semiconductors: Synthesis, Characterization, and Applications. *Topics in Current Chemical*, 212, 82-135.
9. Gittins DI, Bethell D, Nichols RJ, and Schiffrin DJ. (2000). Diode-like electron transfer across nanostructured films containing a redox ligand. *Journal of Material Chemistry*, 10, 79–83.
10. Goia DV and Matijevic E. (1998). Preparation of nanodispersed metal particles. *New Journal of Chemistry*, 22, 1203-1215.
11. Taleb A, Petit C and Pileni MP. (1997). Synthesis of Highly Monodisperse Silver Nanoparticles from AOT Reverse Micelles: A Way to 2D and 3D Self-Organization. Chemistry of Materials, 9, 950-959.
12. Esumi K, Tano T, Torigoe K and Meguro K. (1990). Preparation and characterization of bimetallic palladium-copper colloids by thermal decomposition of their acetate compounds in organic solvents. *Chemistry of Materials*, 2, 564-567.
13. Henglein A. (2001). Reduction of Ag (CN) 2- on silver and platinum colloidal nanoparticles. *Langmuir*, 17, 2329-2333.
14. L. Rodriguez-Sanchez, Blanco MC and Lopez-Quintela MA. (2004). Electrochemical synthesis of silver nanoparticles. *Journal of Physics Chemistry B*, 104, 9683-9688.
15. Zhu JJ, Liu SW, Palchik O, Koltypin Y and Gedanken A. (2000). Shape-Controlled Synthesis of Silver Nanoparticles by Pulse Sonoelectrochemical Methods. *Langmuir*, 16, 6396-6399.
16. Pastoriza-Santos and Liz-Marzan LM. (2002). Formation of PVP-Protected metal nanoparticles in DMF. *Langmuir*, 18, 2888-2894.
17. Begum NA, Mondal S, Basu S, Laskar RA, and Mandal D. (2009). Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of black tea leaf extracts. *Colloids and Surfaces B: Biointerfaces*, 71(1), 113-118.
18. Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP and Misra A. (2009). Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 339, 134–139.
19. Song JY and Kim BS. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess* and *Biosystems Engineering*, 32, 79–84.

20. Singh A, Jain D, Upadhyay MK, Khandelwal N and Verma HN. (2010). Green synthesis of silver nanoparticles using *Argemone mexicana* leaf extract and evaluation of their antimicrobial activities. *Digest Journal of Nanomaterials and Biostructures*, 5(2), 483-489.
21. Das J, Paul Das M and Velusamy P. (2013). *Sesbania grandiflora* leaf extract mediated green synthesis of antibacterial silver nanoparticles against selected human pathogens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 104, 265–270.
22. Saifuddin N, Wong CW and Yasumira AAN. (2009). Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation. *E-Journal of Chemistry*, 6(1), 61-70.
23. Bhainsa KC and D'Souza SF. (2006). Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. *Colloids and Surfaces B: Biointerfaces*, 47, 160–164.
24. Willner B, Basnar B and Willner B. (2007). Nanoparticle–enzyme hybrid systems for nanobiotechnology. *The FEBS Journal*, 274 (2), 302–309.
25. Jose RM, Jose LE, Alejandra C, Katherine H, Juan BK, Jose TR, Miguel JY. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16, 2346–2353.
26. Lok C, Ho C, Chen R, et al. (2007). Silver nanoparticles: partial oxidation and antibacterial activities. *Journal of Biological Inorganic Chemistry*, 12, 527–534.
27. Asghari G, Varshosaz J, Shahbazi N. (2014). Synthesis of silver nanoparticle using Portulaca oleracea L. extracts. *Nanomedicine Journal*, 1(2), 94-99.
28. Ip M, Lui SL, Poon VKM, Lung I and Burd A. (2006). Antimicrobial activities of silver dressings: an in vitro comparison. *Journal of Medical Microbiology*, 55, 59–63.
29. Song HY, Ko KK, Oh LH and Lee BT. (2006). Fabrication of Silver Nanoparticles and Their Antimicrobial Mechanisms. *European Cells and Materials*, 11(1), 58.
30. Shankar SS, Rai A, Ankamwar B, Singh A, Ahmad A and Sastry M. (2004). Biological synthesis of triangular gold nanoprisms. *Nature Materials*, 3, 482.
31. Wiley BJ, Im SH, McLellan J, Siekkinen A and Xia Y. (2006). Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. *The Journal of Physical Chemistry B*, 110, 15666.
32. Singh A, Sharma MM and Batra A. (2013). Synthesis of gold nanoparticles using *Phyllanthus niruri* leaf extract using green chemistry. *Journal of Optoelectronics and Biomedical Materials*, 5(2), 27-32.
33. Pasca RD, Mocanu A, Cobzac SC, Petean I, Horovitz O and Cotisel MT. (2014). Biogenic Syntheses of Gold Nanoparticles Using Plant Extracts. *Particulate Science and Technology: An International Journal*, 32(2), 131-137.