International Journal of Biotechnology and Biomedical Sciences
p-ISSN 2454-4582, e-ISSN 2454-7808, Volume 2, Number 1; January-June, 2016 pp. 6-8

http://www.krishisanskriti.org

Antibacterial Properties of Silver Nanoparticles Synthesized from Litchi Chinensis Fruit Peel Extract

Darshan Singh[1] and Ritu Gupta[2]

[1,2]Department of Chemistry, Daulat Ram College, University of Delhi
E-mail: [1]darshnachem2004@gmail.com

Abstract—*The antimicrobial activity of silver nanoparticles (SNPs) against bacillus subtilis and E. coli was investigated. Bacteriological tests were performed in Luria–Bertani (LB) medium on solid agar plates loaded with different concentrations of silver nanoparticles. Synthesized silver nanoparticles showed an effective bactericide. Various mechanisms have been reported for the antibacterial properties of silver nanoparticles. Silver nanoparticles damage the cell wall of the bacteria and get accumulated in the bacterial membrane, thereby killing bacteria. The nontoxic SNPs were prepared by a simple and cost-effective green method and may be suitable for the formulation of new types of bactericidal agents.*

Keywords*: Litchi Chinensis fruit peel, Silver nanoparticles, E. coli, B. subtilis*

1. INTRODUCTION

Now days, metal and metal oxide nanoparticles such as silver, gold, copper, iron oxide and zinc oxide nanoparticles have gained the attention of scientists because of their extensive application to new technologies in medicine, chemistry, biotechnology, electronics, textile and engineering [1]. These nanoparticles possess superior property as compared to their bulk structure because of significantly improved physical, chemical and biological properties. Although nanoparticles can be synthesized using various physicochemical methods [2] but these methods suffer from many disadvantages. Chemical synthetic methods lead to the absorption of some toxic chemical on the surface that may have adverse effect in the medical applications. Their synthesis using nontoxic and environmentally benign biological methods has attracted special attention if they are intended for invasive applications in medicine. Several routes have been developed for biological or biogenic synthesis of nanoparticles from salts of the corresponding metals [3-7]. Microorganisms, whole plants, plant tissue and fruits, plant extracts and marine algae [8-9] have been used to produce nanoparticles. It has many advantages such as ease with which the process can be scaled up, economic viability etc. Phytochemicals in the plants extract like essential oils (terpenes, eugenols, etc), polyphenols and carbohydrates, contain active functional groups, such as hydroxyl, aldehyde and carboxyl units which may play important role for reduction of silver salt to silver nanoparticles. Silver is traditionally well-known antimicrobial material. Nanosized silver is expected to show better antimicrobial characteristics due to their larger specific surface area. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds [10]. It is considered that it reacts with proteins by combining the -SH groups of enzymes leading to the inactivation of the proteins [9]. Antibiotic resistance is the world's major public healthcare problem. Some antibacterial agents are highly irritating and toxic hence, there is a need to find out the ways to formulate new types of safe and cost-effective biocidal materials. SNPs play a vital role in nanobiotechnology as biomedicine against drug-resistant bacteria. Development of reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. One method that shows immense potential is the biosynthesis of nanoparticles using biological waste plant products such as fruit peel.

In the present article, we report the green synthesis of silver nanoparticles using litchi chinensis fruit peel extract. Aqueous extract of peel acts as a bio-reductant and stabilizer for silver nanoparticles. Litchi chinensis peel contains significant amount of polyphenolic compounds such as gallic acid, anthocyanin, procyanidine B-2, epicatechin etc. [11]. Synthesized SNPs were then screened for their antibacterial activity.

2. MATERIAL AND METHODS

2.1. Materials

Silver nitrate ($AgNO_3$, 99.995%) was purchased from Merck India. Litchi chinensis fruit peel waste was collected from homes. It was washed with deionized water and then dried in sunlight for 10-15 days. All glassware was washed and rinsed with deionized water, followed by subsequent drying.

2.2 Preparation of peel extract

The sun dried litchi chinensis fruit peel was powdered and stored in containers for the synthesis of SNPs. The powered peel was boiled in 50 ml deionized water for 30 min. After boiling, the color of the aqueous solution changed from colorless to yellow color. The aqueous extract was separated by filtration with Whatmann No. 1 filter paper and then centrifuged at 1,200 rpm for 10 min to remove heavy biomaterials. The prepared peel extract was stored at room temperature to be used for biosynthesis of silver nanoparticles from silver nitrate.

2.3 Synthesis of silver nanoparticles

3mM aqueous solution of silver nitrate was prepared in 100 ml deionized water. 10 ml aqueous extract of peel extract was slowly added to 10 ml silver nitrate solution (3mM) with constant stirring. No change in color of the solution was observed. The solution was then microwaved for 1 minute. There was a slight change in color and then the solution was heated in microwaved for 3 min. The intense brown color change after 3 min indicated the formation of silver nanoparticles.

2.4 Characterization

The bioreduction of Ag^+ ion in aqueous solution was monitored with the help of UV-2600 series Shimadzu spectrophotometer. Fourier transform infrared (FT-IR) spectra for litchi chinensis fruit peel powder and silver nanoparticles were obtained in the range 4,000 to 400 cm^{-1} with a Shimadzu FT-IR spectrophotometer using KBr pellet method. All TEM images were obtained using a JEOL model 1200 EX instrument with an accelerating voltage of 120 kV. TEM samples were prepared by drop casting of nanoparticle dispersion onto carbon-coated copper TEM grid, followed by air drying at ambient conditions. TEM samples were stored in a desiccator and imaged shortly after collection.

2.5 Anti-microbial study

The antibacterial activity of silver nanoparticles was carried out on human pathogenic E. coli and bacillus subtilis by standard disc diffusion method. LB broth/agar medium was used to cultivate bacteria. Fresh overnight inoculum (100µl) of each culture was spread on to LB agar plates. Sterile paper discs of 5mm diameter (containing 25µg/ml silver nanoparticles) along with standard antibiotic containing discs were placed in each plate. The plates containing bacterial and silver nanoparticles were incubated at 37°C. The plates were then examined for zones of inhibition. The clear area was appeared around the disk, the diameters of such zones was measured using meter ruler and expressed in millimetre.

3. RESULTS AND DISCUSSIONS

3.1 UV-Visible Spectral analysis

The formation of silver nanoparticles from litchi chinensis fruit peel extract was analyzed by UV-Visible spectrophotometer. After 3 min. microwave heating, the color of the solution changed from colorless to brown. Characteristics surface plasmon absorption band of silver nanoparticles was observed at 371 nm after 3 min microwave heating.

3.2 FT-IR

The FT-IR spectrum was recorded to identify the possible biomolecules responsible for the reduction of Ag^+ ions to SNPs by litchi chinensis fruit peel extract. The FT-IR spectrum showed a broad band at 3390 $cm^{-1}$and other peaks were obtained at 1665 cm^{-1}, 2938 cm^{-1} respectively. The band at 3390 cm^{-1} was due to phenolic hydroxyl group. The band at 2938 cm^{-1} has been assigned to stretching vibrations of alkanes. The peak around 1665 cm^{-1} are due to the amide I bonds of proteins/enzymes [12]. The intense bands observed at 710, 1053, 1125 and 1399 cm^{-1} have been assigned to alcohols and phenolic groups,

3.3 High Resolution transmission electron microscopy (HRTEM) analysis

High resolution transmission Electron Microscopy (HRTEM) technique was used to study the morphology of silver nanoparticles synthesized using litchi chinensis fruit peel extract. The dried silver nanoparticles were mounted on a copper coated grid. TEM images were taken randomly and spherical nanoparticles with a size ranging from 20 nm to 50 nm were observed.

3.4 Antimicrobial study

The silver nanoparticles synthesized using litchi chinensis fruit peel extract were screened for their antimicrobial activity against two human pathogens i.e. Escherichia Coli and bacillus subtilis by disc diffusion method. The zone of inhibition against these bacteria is shown in table 1.

Table 1: Antibacterial activity of Litchi chinensis fruit peel mediated silver nanoparticles

Samples	Concentration (µg/mL)	Zone of inhibition (mm)
E.coli	25	19.0
	50	22.0
	75	23.5
Bacillus subtilis	25	18.0
	50	20.0
	75	22.7

The minimum concentration of SNPs required to inhibit the growth of E.coli and bacillus subtilis bacteria was 25µg/mL.

Inhibition against bacteria increased with increasing the concentration of silver nanoparticles. The bactericidal effect of nanoparticles is due to their small size and high surface to volume ratio which makes them to interact closely with microbial membranes [13]. Silver nanoparticles also destabilized the outer membrane and causes rupture of the plasma membrane so causing depletion of intracellular ATP [14].

4. CONCLUSIONS

It has been demonstrated that aqueous extract of litchi chinensis fruit peel is capable of producing silver nanoparticles and these nanoparticles show good stability in solution. These were characterized by various spectroscopic techniques. Synthesized SNPs showed superior antibacterial activity towards E.Coli and bacillus subtilis pathogens. It is an environmental friendly process for the production of silver nanoparticles and completely free from toxic solvents and chemicals.

5. ACKNOWLEDGEMENT

We are thankful to the Principal and staff of Daulat Ram College, University of Delhi for the encouragement and support. We express our sincere thanks to the director, USIC, University of Delhi for providing instrumentation technique necessary this research work.

REFERENCES

[1] D. Mandal, M.E. Bolander, D. Mukhopadhyay, G. Sarkar, P. Mukherjee, The use of microorganisms for formation of metal nanoparticles and their applica- tion, Appl. Environ. Microbiol. 69, 2006, 485–492.

[2] Cao G. Nanostructures and nanomaterials: synthesis, properties and applications. London: Imperial College Press; 2004.

[3] Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, Misra A. Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids Surf A 339, 2009, 134–9.

[4] Dhillon GS, Brar SK, Kaur S, Verma M. Green approach for nanoparticle biosynthesis by fungi: current trends and applications. Crit Rev Biotechnol 32, 2012, 49–73.

[5] Duran N, Seabra AB. Metallic oxide nanoparticles: state of the art in biogenic syntheses and their mechanisms. Appl Microbiol Biotechnol 95, 2012, 275–88.

[6] Duran N, Marcato PD, De Souza GIH, Alves OL, Esposito E. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J Biomed Nanotechnol 3, 2007, 203–8.

[7] Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res 10, 2008, 507–17

[8] Singaravelu G, Arockiamary J, Kumar VG, Govindaraju K. A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, Sargassum wightii Greville. Colloids Surf B Biointerfaces 57, 2007, 97-101.

[9] Rajesh S, Raja DP, Rathi JM, Sahayaraj K. Biosynthesis of silver nanoparticles using Ulva fasciata (Delile) ethyl acetate extract and its activity against Xanthomonas campestris pv. malvacearum. J Biopest 5, 2012, 119–28.

[10] M. Ip, S. L. Lui, V. K. M. Poon, I. Lung, A. Burd J. Medical Microbiol. **55**, 2006, 59–63.

[11] Ref Li W, liang H, Zhang MW, Molecules, Dec 17, 2012, 14954.

[12] Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobialagent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275, 2004, 177–82.

[13] Morones JR, Elechiguerra JC, Camacho A, Holt K and Kouri JB, "The bactericidal effect of silver nanoparticles" *Nanotechnology,* 16, 2005, 2346.

[14] Lok C, "Proteomic analysis of the most of antibacterial action of silver nanoparticles" *J Proteomic Res,* 5, 2006, 916.