SILVER NANOPARTICLES DERIVED FROM *BUTEA MONOSPERMA* AS ANTIMICROBIAL AGENTS

K. P. Ghoshal* and S. S. Chourasia[1]
*Head, Department of Botany, M. B Patel College of Arts, Commerce and Science, Deori 441901(M.S)
[1]Head, Department of Chemistry, M. B. Patel College of Arts Commerce and Science, Deori 441901 (M.S.)
**Corresponding Author*. E *Mail address*: kalpanabanerjee56@gmail.com

ABSTRACT:
Synthesis of metal nanoparticles form the plants or plant extracts has emerged as an important alternative to the chemical method. The biological approach to the synthesis of nanoparticles has many advantages such as non-elaborative process, no multiple purification steps, no need of intracellular synthesis and does not require maintenance of microbial cell cultures. In the present study, an attempt has been made to develop a simple rapid procedure for bio reduction of silver nanoparticles (AgNPs) using bark extract of Butea monosperma. The synthesised silver nanoparticles have been evaluated for their antimicrobial activity against various microorganisms. Characterization of the synthesised nanoparticles was determined using UV-VIS spectroscopy and the antimicrobial activities were determined using the disc diffusion assay method. Silver nanoparticles showed absorption peak at 478nm in aqueous medium in UV-VIS spectrum. The formed silver nanoparticles showed good antimicrobial activity against various microorganisms Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Streptococcus pneumonia. Present investigation confirms the potential of Butea monosperma for synthesis of silver nanoparticles by rapid reduction of silver ions (Ag+ to Ago). The present study gives a simple, rapid and economical route for the synthesis of AgNPs using bark extract of Butea monosperma in aqueous medium. The antimicrobial activities of the synthesized AgNPs were compared against the standard antibiotics. Silver nanoparticles were found to be more potent than the antibiotics, even at small concentrations... Thus the present study offers a rapid, simple, cost effective and green method for synthesis of microbial active silver nanoparticles.

Key words: Silver nanoparticles, Butea monosperma, UV-VIS spectroscopy, Antimicrobial activity

INTRODUCTION

The modern era has witnessed the emergence of the nanotechnology as basis of many technological innovations in the 21st century. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials (Mazur, 2004). These special and unique properties could be ascribed to their small sizes and large surface areas. For these reasons, metallic nanoparticles have found applicability in many applications in different fields, such as electronic,s photonics, and catalysis.

Preparation of silver nanoparticles has attracted particularly considerable attention due to their diverse properties and uses like electrical conductivity (Chang and Yen, 1995) antimicrobial and antibacterial activities (Baker et. al., 2005; Shahverdi et. al., 2007), DNA sequencing (Cao et. al., 2001)and surface-enhanced Raman scattering (SERS) (Matejkaet. al., 1992

Many techniques of synthesizing silver nanoparticles, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents (Liz-Marzan and Lado-Tourino, 1996), thermal decomposition in organic solvents (Esumi et.al., 1990) chemical reduction and photoreduction in reverse micelles (Pileni, 2000; Sun et.al., 2001) and radiation chemical environmentally friendlyprocesses for nanoparticle synthesis that do not use toxic chemicals have come into existence. Biological methods of nanoparticle synthesis using microorganisms (Klaus et. al., 1999; Nair and Pradeep, 2002; Konishi and Uruga, 2007), enzymes (Willne ret. al., 2006), fungus (Vigneshwaranet. al., 2007) and plants or plant extracts (Jae and Beom, 2009) have been suggested as possible eco friendly alternatives to chemical and physical methods. Recent research reported that silver nanoparticles have been synthesized using various natural products like green tea *Camellia sinensis* (Vilchiset.al., 2008), *Azadiracta indica* leaf broth (Shankar et.al., 2004) natural rubber (Abu et.al., 2007), Aloe vera plant extract (Chandran et.al., 2006) Latex of Jatropacureas (Bar et.al., 2009) etc.

Butea monosperma (Lam) Taub (*Butea frondosa*) commonly known as Palas in Sanskrit belonging to family Fabeceae is a traditionally used medicinal plant. Seeds, leaves bark, flowers all have medicinal properties. Besides it has been reported to have antibacterial, antifungal properties also.

In the present study for the first time the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous bark extract of *B. Monosperma* were prepared. Further these green synthesized silver particles were explored for their potential against different pathogenic bacteria.

MATERIALS AND METHOD

All chemicals used in the experiment were of highest purity and obtained from Merk and Hi-media laboratories Pvt. Ltd Mumbai, India. The bacterial culture of *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Streptococcus pneumonia* were obtained from National Center for cell science (NCCS) Pune. Antibiotics (Vancomycin and Erythromycin) were purchased from Hi-media Mumbai, India. Bark of *B. monosperma* was collected locally from taluka Deori, Dist. Gondia city, Maharashtra. Bark extract was prepared in Department of Botany M. B. Patel College, Deori and Synthesis of Silver Nanoparticles and its stability in aqueous colloidal solution was confirmed using UV-VIS spectra analysis.

(The UV-VIS spectral analysis was done by using UV-VIS spectrophotometer (Shimadzu UV- 2450). The reduction of pure Ag^{+} ions was monitored by measuring the UV-VIS spectrum of the reaction medium at room temperature operated at a resolution of 1nm. The reduction of silver ions was confirmed by qualitative testing of supernatant obtained after centrifugation with a pinch of NaCl and further its antimicrobial activities were evaluated.

ANTI-BACTERIAL STUDY

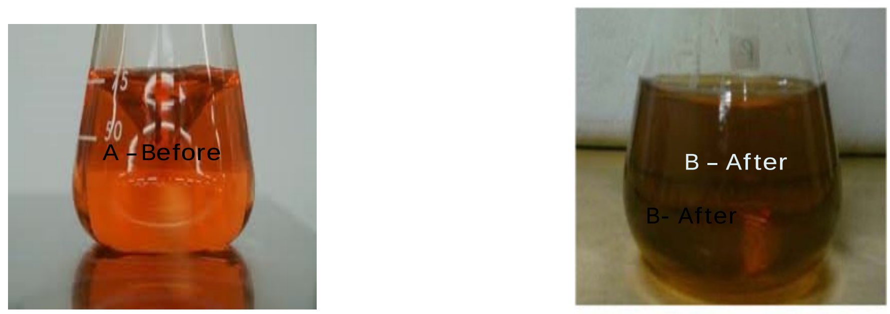
The antibacterial assay was performed by standard disc diffusion method. Nutrients were used to cultivate bacteria. The media was autoclaved and cooled. The media was poured in petri discs and was kept for 30 minutes for solidification. After 30 minutes, the fresh overnight cultures of inoculums (100µ1) of four different organisms were spread on nutrient agar plates. Sterile paper discs made of Whatman filter paper, 5mm diameter dipped in different concentration of aqueous solution of silver nanoparticle such as 0.2mM, 0.4mM, and 0.8mM along with two standard antibiotics containing disc were placed in each plates. The cultured agar plates were incubated at 37°C for 24 hr. After 24 hr of incubation the zone of inhibition was measured in millimeter

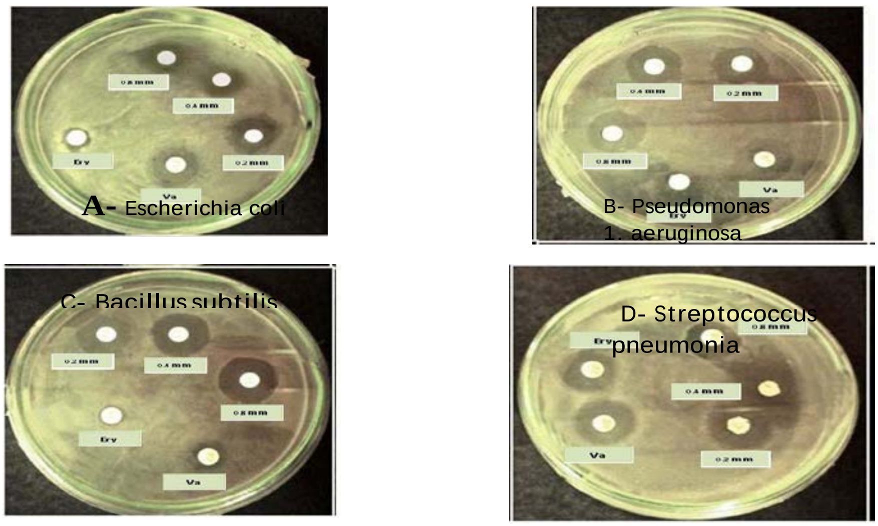
RESULTS

Biosynthesized silver nanoparticles were studied for antimicrobial activity against pathogenic microorganisms by using standard zone of inhibition. The effect of different concentration such as 0.2mM, 0.4mM and 0.8mM of silver nanoparticles on bacteria was performed. A clear inhibition zone treated with silver nanoparticles was observed. (Table 1.) The standard antibiotics like vancomycin, erythromycin shows smaller zone of inhibition as compared to the nanoparticles treated discs. (Plate.1)

Table 1: Comparative Biological activities of the various bioactive agents

Bioactive agent	**Zone of inhibition (Diameter, mm)**				
		E. coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Streptococcus pneumonia*
Agnanoparticle	0.2mM	2.5	3.2	3.1	3.2
	0.4mM	3.4	4.2	3.3	3.4
	0.8mM	4.5	4.4	4.3	3.8
Erythromycin(10mcg /disc)		nil	nil	0.6	4.1
Vancomycin(10mcg /disc)		0.8	nil	0.8	3.8





DISCUSSION

The present study deals with the synthesis of silver nanoparticles using bark extract of *B. monosperma* and aqueous Ag^+ ions. Comparative studies were carried out to study the rate of bio reduction of silver ions. The approach appears to be cost effective alternative to conventional methods of assembling silver nanoparticles. Formation and stability of

silver nanoparticles in aqueous colloidal solution was confirmed using UV-VIS spectral analysis. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibration in silver nanoparticles (Krishnaraj et. al., 2010 and Nogmov et. al., 2006). As the *B. monosperma* bark extract was mixed with aqueous solution of the silver nitrate , it started to change the colour from yellowish brown to dark reddish brown due to reduction of silver ions, which indicated the formation of silver nanoparticles. It is generally recognized that UV-VIS spectroscopy could be used to examine size and shape controlled nanoparticle s in aqueous suspension (Shrivastavs et. al., 2009). Absorption spectra of silver nanoparticles formed in the reaction media has a strong absorbance peak at 478 nm and broadening of peak indicated that the particles are poly dispersed. Silver nitrate which is readily soluble in water has been exploited as an antiseptic agent for many decades. It is being used as a safe inorganic antibacterial agent since centuries and is capable of killing about 650 microorganisms that causes diseases. Silver has been described as being 'oligodynamic' that is, its ions are capable of causing a bacterostatic (growth inhibition) or even a bactericidal (antibacterial) impact. It has also having ability to exert a bactericidal effect at minute concentration (Panacek et. al., 2006). The exact mechanism of the anti-bacterial effect of silver ions was partially understood. Literature survey reveals that the bactericidal behaviour of nanoparticles is attributed to the presence of electronic effect that are brought about as a result of change in local electronic structure of the surface due to smaller sizes. These effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surface. Silver in ionic form strongly interact with thiol group of vital enzyme and inactivates them. Shrivastava et. al., (2007) studied antibacterial activity against *E. Coli, S. aureus and S. typhi.* They have reported that the effect was dose dependant and was more pronounced against gram negative organisms than gram positives ones. They have found that the major mechanisms through which silver nanoparticles manifest antibacterial property was either by anchoring or penetrating the bacterial cell wall or modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues. The antibacterial efficacy of the biogenic silver nanoparticles reported in the present study may be ascribed to the mechanism described above but it still remains to clarify the exact effect of the nanoparticles on important cellular metabolism like DNA, RNA and protein synthesis.

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for the synthesis of silver nanoparticles. We have demonstrated first time the synthesis of silver nanoparticles using bark extract of *B. monosperma* through efficient green methodology, avoiding the presence of hazardous and toxic solvents. The green synthesized silver nanoparticle s using bark extract of *B. monosperma* shows excellent antimicrobial activity. The present study showed simple, rapid and economical route to synthesize silver nanoparticles.

REFERENCES

1) Abu Bakar NHH, Ismail J, Abu Bakar M. (2007): Synthesis and characterization of silver nanoparticle in natural rubber. Mater Chem. Phys. 104:276-283.

2) Bar H, Bhui D K, Sahoo G P, Sarkar P, De S P, Misra A. (2009): Green synthesis of silver nanoparticles using latex of Jatrophacurcas. Colloids Surf A PhysicochemEng Asp 339:134-139.

3) Baker, C., Pradhan, A., Pakstis, L., Pochan, D. J. and Shah, S. I. (2005): Synthesis and antibacterial properties of silver nanoparticles. J.

Nanosci. Nanotechnol.5:224-249.

4) Cao, Y.W., Jin, R. and Mirkin C.A. (2001): DNA- modified core-shell Ag/Au nanoparticles. J. Am. Chem. SOC. 123:7961-7962.

5) Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M(2006): Synthesis of gold and silver nanoparticles using Aloe Veraplant extract. J. Biote chnol. Prog. 22:577-583.

6) Chang, L.T. and Yen, C.C. (1995): Studies on the preparation and properties of conductive polymers. VIII. Use of heat treatment to prepare metalized films from silver chelate of PVA and PAN. J. Appl. Polym. Sci. 55:371-374.

7) Esumi, K., Tano, T., Torigoe, K. and Meguro, K. (1990): Preparation and characterization of biometallic Pd-Cu colloids by thermal decomposition of their acetate compounds in organic solvents. J. Chem. Mater. 2: 564-567.

8) Hengle in, A. (1993): Physicochemical properties of small metal particles in solution: 'Microelectrode' reactions, chemisorptions, composite metal particle s, and the atom-to- metal transition. J. Phys. Chem. B. 97: 5457-5471.

9) Hengle in, A. (2001) Reduction of $Ag(CN)_2$ on silver and platinum colloidal nanoparticle s. Langmuir. 17: 2329-2333.

10) Jae, Y. S. and Beom, S.K. (2009): Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng. 32:79- 84.

11) Klaus, T., Joerger, R., Olsson, E. and Granqvist, C.G. (1999): Silver-based crystalline nanoparticles, microbially fabricated. J. Proc. Natl. Acad. Sci. USA. 96: 13611-13614.

12) Krishnaraj C, Jagan E G, Rajasekar S, Selvakumar P. Kalaichelvan P T, Mohan N. (2010): Synthesis of silver nanoparticles using Acalyphaindica leaf extract and its antibacterial activity against water borne pathogens. Colloids surface Biointerfaces.76: 50-56.

13) Konishi, Y. and Uruga, T. (2007): "Bioreductive deposition of platinum nanoparticles on the bacterium Shewanella algae. J. Biotechnol. 8: 648-653.

14) Liz-Marzan, L.M. and Lado-Tourino, I.(1996): Reduction and stabilization of silver nanoparticle s in ethanol by nonionic surfactant. Langmuir. 12: 3585-3589.

15) Matejka, P., Vlckova, B., Vohlidal, J., Pancoska, P. and Baumuruk, V. (1992): The Role of Triton X-l00 as an adsorbate and a molecular spacer on the surface of silver colloid: A surface-enhanced Raman scattering study. J. Phys. Chem. 96:1361-1366.

16) Mazur, M. (2004): Electrochemically pre pared silver nanoflakes and nanowire s. Electrochemistry Communications. 6: 400-403.

17) Nair, B. and Pradeep, T. (2002): coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. Cryst.Growth Des. 2:293-298.

18) Nogmov m a, Zhu G, Bahoura M, Adegoke J,Amall C, Ritzo B . A. (2006): The effect of grain and absorption on surface plasmon in metal nanoparticle s. ApplPhys B. 86: 455-460

19.}Panacek A, Kvitek L, Prucek r, Kolar M, Vecerova R, Pizurova N. (2006): Synthe sis, characterization and their antibacterial activity. J phys chemistry 110:16248-16253

19) Pileni, M.P. (2000): Fabrication and physical properties of self-organized silver nanocrystals. Pure Appl. Chem.72: 53-65.

20) Shankar SS, Rai A, Ahmad A, Sastry M. (2004): Rapid synthesis of Au, Ag and bimetallic Au core-Ag shell nanoparticles using neem(Azadirachtaindica) leaf broth. J ColloidInterface Sci 275:496-502.

21) Shahverdi, A.R., Mianaeian, S., Shahverdi, H.R., Jamalifar, H. and Nohi, A.A. (2007): Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. Process Biochem. 42: 919-923.

22) Shrivastava S, Bera T, Roy A, Singh G, Ramchandrarao P, Dash D. (2007): Characterization of enhanced antibacterial effect of novel silver nanoparticles. Nanotechnology.18: 225103.

23) Shrivastava S, Dash D. (2009): Applying nanotechnology to human health. J Nanotechnol. 12: 240-243.

24) Sun, Y.P., A torngitjawat, P. and Meziani, M.J. (2001): Preparation of silver nanoparticles via rapid expansion of water in carbondioxidemicroemulsion into reductant solution. Langmuir. 17: 5707-5710.

25) Vigneshwaran, N., Ashtaputre, N.M., Varadarajan, P.V., Nachane, R.P., Paraliker, K.M. and Balasubramanya, R.H. (2007): Biological synthesis of silver nanoparticles using the fungus Aspergillus flavus. Mater.Lett. 61: 1413-1418.

26) Willner, I., Baron, R. and Willner, B. (2006): Growing metal nanoparticles by Enzymes. J. Adv. Mater. 18: 1109-1120.

27) Vilchis-Nestor A R, Sanchez-M V, Camacho-Lopez M A, Gomez-Espinoza R M. (2008): Solventless synthesis and optical properties of Au and Ag nanoparticles using Camellia sinensis extract. Mater Lett . 62:3103-3105