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Green Nanoparticles From *Butea Monosperma* And Their Antimicrobial Activities

K. P. Ghoshal

Dept. of Botany, M. B Patel College of Arts, Commerce and Science, Deori (M.S)
*Corresponding Author. E Mail address:kalpanaghoshal71@gmail.com

ABSTRACT

Biosysthesis (Green synthesis) of nanoparticles by plant extract is currently under exploitation. The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolved into an important branch of nanotechnology. In the present study silver nanoparticles(AGNPs) using bark extract of *Butea monosperma*were prepared and its antimicrobial activities against various microorganism were evaluated. *Butea monosperma* have strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions(Ag+ to Ag0) and the formed AgNPs showed a strong antimicrobial potential against *Escherischia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Streptococcus pneumonea*. The results were compared with the effects of antibiotics, and were were found to be more potent than antibiotics. It was also observed that the growth rate was strongly inhibited by small concentration of nanoparticles.

KEY WORDS: Silver nanoparticles, *Butea monosperma*, UV-VIS spectroscopy, Antimicrobial activity

INTRODUCTION

Now a day's nanotechnology is expected to be the 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 attributed to their small sizes and large surface areas. For these reasons, metallic nanoparticles have found uses in many applications in different fields, such as catalysis, photonics, and electronics. 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) (Matejka*et. 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 reduction (Henglein, 1993, 2001) have been reported in the literature. Most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. Since noble metal nanoparticles are widely applied to areas of human contact (Jae and Beom, 2009**)** there is a growing need to develop environmentally friendly processes for nanoparticle synthesis that do not use toxic chemicals. Biological methods of nanoparticle synthesis using microorganisms (Klaus *et. al*., 1999; Nair and Pradeep, 2002; Konishi and Uruga, 2007), enzymes (Willner*et. al*., 2006), fungus (Vigneshwaran*et. al*., 2007) and plants or plant extracts (Jae and Beom, 2009) have been suggested as possible ecofriendly alternatives to chemical and physical methods. Recent research reported that silver nanoparticles have been synthesized using various natural products like green tea *Camellia sinensis* (Vilchis*et.al*., 2008), *Azadiractaindica* leaf broth(Shankar *et. al*., 2004**)** natural rubber (Abu *et. al*., 2007), Aloeavera 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.

MATERIAL AND METHODS

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 pneumonea*were obtained from

NationalCenter for cell science (NCCS) Pune. Antibiotics(Vancomycin and Erythromycin) were purchased from Hi-media Mumbai, India. Bark of *B. monosperma* was collected locally fromtaluka 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 activies were evaluated.

ANTI-BACTERIAL ACTIVITY 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 (100µl) 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 millimetre.

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)

DISCUSSION

The synthesis of nanoparticles is in the lime light of modern nanotechnology. Biosynthesis of nanoparticles by plant extract is currently under exploitation. The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolved into an important branch of nanotechnology. 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 bioreduction 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 nanoparticles 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 polydispersed.

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 behaviours 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 signalling 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 nanoparticles using bark extract of *B.monosperma* shows excellent antimicrobial activity. The present study showed simple, rapid and economical route to synthesize silver nanoparticles.

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Table 1

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**

Figure 1. Colour changes before (A) and after (B) the process of reduction of Ag^{+} to Ag nanoparticles.

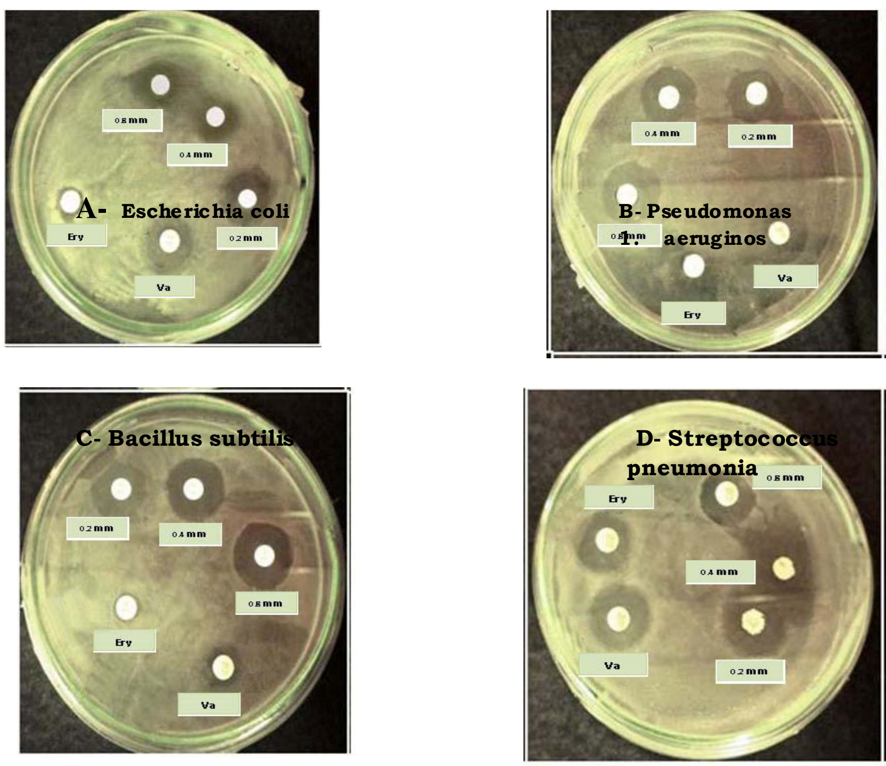

Photo Plate 1 :- Images of antibacterial activities of discs of different concentration of Ag nanoparticles(0.2mM,0.4mM and 0.8mM) and other antibiotics on A. *E coli* B. *Pseudomonas aeruginosa*C.*Bacillus subtilis*D. *Streptococcus pneumonia* N= nanoparticles, VA= Vancomycin, E= Erythromycin