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A Facile Green Synthesis of Silver Nanoparticles using Soap Nuts

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ABSTRACT

The ecofriendly synthesis of nanoparticles through various biological means, helps to explore various herbs for their ability to synthesis nanoparticles. In this study, soap nuts-which are the part and parcel in daily life of Indians from several centuries, are used to synthesize silver nanoparticles. The synthesized nanoparticles were characterized under UV-Visible spectrophotometer, SEM, TEM and EDX. The antibacterial activity of silver nanoparticles was performed on various gram positive and gram negative bacteria. The silver nanoparticles showed more inhibitory activity on pathogenic gram positive than gram negative bacteria.

Key words: Soap nuts, saponins, green synthesis, silver nanoparticles, antimicrobial activity

INTRODUCTION

Green synthesis of nanoparticles is an easy, efficient and eco friendly approach, where most researchers are looking at the ecofriendly and green synthesis of nanoparticles paves the way for the researchers around the world to explore the ability of various herbs in synthesizing nanoparticles (Savithramma *et al*., 2011). Silver nanoparticle are synthesized from various parts of the herbal plants like bark of Cinnamom (Sathishkumar *et al*., 2009), Neem leaves (Tripathi *et al*., 2009), Citrus limon (Prathna *et al*., 2011), Tannic acid (Sivaraman *et al*., 2009) and various plant leaves (Song and Kim, 2008). Reports showed that tea and coffee extracts has the ability to synthesize silver and palladium nanoparticles (Nadagouda and Varma, 2008).

Silver nanoparticles (AgNPs) have been recently used for a wide range of applications including health and household products even though an understanding of their mechanistic action in human is not fully explored.

The effect of nanoparticles on the biological functions or the relative toxicity is not well-understood and becomes the major public concerns. This study determined the *in vitro* effect of gold and silver nanoparticles as the two most frequently used metallic nanomaterials for therapeutics and diagnostic on the microsomes containing wild-type cDNA expressed human CYP450 enzymes CYP1A2, 2C9, 2C19 and 3A4. Results demonstrated that all of the CYP450s activities were down-regulated by metallic nanoparticles. These findings suggest the inhibition of oxidation based biological process by penetration of metallic nanosized particles across the microsomal membrane (Sereemaspun *et al*., 2008).

Biological organsims like bacteria (Kalimuthu *et al*., 2008) fungi and actinomycete (Sastry *et al*., 2003) are also used to synthesize silver nanoparticles. The synthesis of metal nanoparticles using biological systems is an expanding research area due to the potential applications in nanomedicines. Nanoparticles synthesized by chemical method are not eco-friendly (Savithramma *et al*., 2011). From the time immemorial, soap nuts are used as a natural shampoo and also for other miscellaneous purposes. Soap nuts are the fruits of plants with genus *Sapindus*. Soap nut or soap berry are the common names for the fruits of these plants. The genus *Sapindus* has different species distrubuted all around Asia and some parts of America. *Sapindus tumorossi* and *Sapindus trifoliatus* are the species dominant in Northern and southern parts of India. The rich literature revealed that the soap nuts consist in majority of a compound called Saponins which are natural surfactants. These plant glycosides have been used for washing for thousands of years by native peoples in Asia as well as Native Americans (Austin and Honychurch, 2004). Soapnuts are being considered for commercial use in cosmetics and detergents as wellas many other products.

Silver nanoparticles are found to have wide applications in various areas like optical receptors (Schultz *et al*., 2000), bio-labelling (Hayat, 1989) sensors (Dubas and Pimpan, 2008), bio active materials (Blaker *et al*., 2004), signal enhancers in SERS based enzyme Immunoassay (Chen *et al*., 2009). Silver nanoparticles are known for their antimicrobial activity (Morones *et al*., 2005). Silver is an effective antibacterial agent with low toxicity which is important especially in the treatment of burn wounds. Given its broad spectrum activity, silver nanoparticles have been the focus of increasing interest and are being used as an excellent candidate for therapeutic purposes (Bellantone *et al*., 2000).

In the present study, silver nanoparticles are synthesized by using Soap nuts. The silver nanoparticles are characterized with various characterization techniques. The antibacterial activity of the silver nanoparticles was done against various pathogenic gram positive and gram negative bacterial strains.

MATERIALS AND METHODS

Soap nuts were purchased from the stores. Silver nitrate was purchased from Fisher scientific, Mumbai, India. The plain antimicrobial disks were purchased from Merck, Mumbai, India. Hi-media Muller-Hinton agar and Luria broth were purchased from Merck chemicals, Mumbai, India. The bacterial strains used in the present study were obtained from the Department of Microbiology. All the glass ware used was of borosil make. The glassware was washed with con. HCl, rinsed with distilled water and kept in hot air oven at 100°C until use.

Synthesis of nanoparticles: Ten gram of soap nuts in 100 mL millipore water were taken in a 250 mL beaker and stirred for overnight on a magnetic stirrer. The mixture was filtered through the Whatmann No.1 filter study. The filterate was stored under cooling conditions when not used. Five milliliter of the filtrate was mixed into 1 mM $AgNO_3$ in 50 mL Millipore water. The solution was heated to 80°C until a color change appears.

Characterization of silver nanoparticles: The formation of the silver nanoparticles was monitored under UV-Visible spectrophotometer (Perkin Elmer) by withdrawing aliquotes of 3 mL at regular time intervals at a wavelength range of 200-800 nm. The particle morphology was obtained from the Scanning Electron Microscope (Zeiss). The elemental analysis was performed in the Energy Dispersive X-ray Spectroscopy (Oxford Instruments) which is an attachment to the

Scanning Electron Microscope. The particle size and shape were examined by placing the colloidal silver nanoparticles on a carbon coated copper grid, air dried and imaged under Transmission Electron Microscope (Hitachi 7500) at 80 kV operation voltages.

Antibacterial activity: The bacterial strains *Staphylococcs aureus, Serratia marcescens, Escherichia coli, Pseudomonas aeruginosa* are used to perform the antibacterial acitivity for the synthesized silver nanoparticles. The antibacterial activity against both gram positive and gram negative bacterial strains was done by kirby-bauer method. Briefly, Overnight cultures of the bacterial strains were prepared in Luria broth. Eighty micro litter of the as synthesized colloidal silver nanopaticles were given to the empty antibacterial discs (Merck chemicals). One hundred microlitter of the active bacterial cultures were spreaded using a sterile swab (Merck chemicals) over a Muller-Hinton agar plates. The discs with nanoparticles were placed over the spreaded bacterial cultures in the petri dishes. The plates were incubated for overnight at 37°C.

RESULTS AND DISCUSSION

The formation of silver nanoparticles was evidenced with the change in color of the soap nut extract after addition of $AgNO_3$ from the pale/light yellowish brown color to the dark reddish brown color.

The formation of silver nanoparticles was monitored through UV-Visible spectrophotomer at time intervals of 0, 0.5, 1 and 3 h, respectively (Fig. 1). The spectrum obtained at 3rd h shows the absorption maximum as 425 nm. The intensity of the peak with respect to the height increases gradually with increase of time. The UV-visible spectrum shows the formation of silver nanoparticles as the peak maxima 425 nm is characteristic to silver nanoparticles. The specific characteristic peak for silver nanoparticles is due to the surface plasmon resonance. The nanoparticles which are smaller than the wavelength of light can produce a coherent resonance waves at a particlular absorbance wavelength which is in the visible range for silver nanoparticles. The absorbance wavelenth varies with particle size and shape (Kerker, 1985; Sosa *et al*., 2003). From the Fig. 1 it is evident that a slight shift of peak is observed from 0.5 hr to 1 and 3 hrs. The formation of nanoparticles can be concluded from the height of the peak at particular time interval. The peak height gradually increases from 0 to 3 h which shows the gradual formation of nanoparticles say in number and size. The shift of the peak is the blue shift reflects the formation of smaller nanoparticles.

The SEM microgrphs (Fig. 2) shows the surface morphology of the silver nanoparticles. From the images, the nanoparticles are appearing to be aggregated. The surface of the aggregates is rough. The particles are more or less spherical with sizes in the range 20-30 nm (approx).

The presence of silver was confirmed from the Ag peak obtained from the EDX spectrum as shown in Fig. 3.

The size and shape of the silver nanoparticles were examined clearly under Transmission Electron Microscope at 80 kV of operation voltage. The images show that the particles are dispersed and roughly spherical. This shows the formation of polydispersed nanoparticles (Fig. 4). The particle size histogram (Fig. 5) of the nanoparticles calculted and plotted from the TEM micrographs show that the particles were with sizes of 6-35 nm. The average size of the nanoparticles was found to be 18 nm.

The antibacterial activity for silver nanoparticles was done with various gram positive bacterial strains like *staphylococcus aureus*, *serratia marcescens* and gram negative bacterial strains such

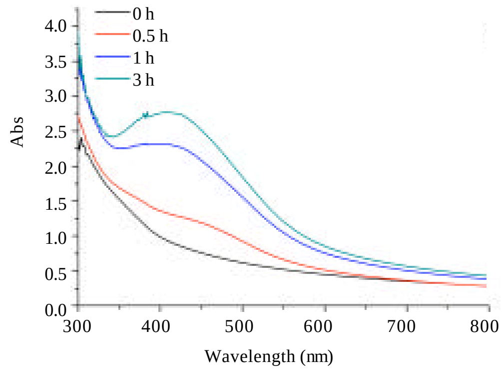

Fig. 1: UV-Visible spectrum of Silver nanoparticles

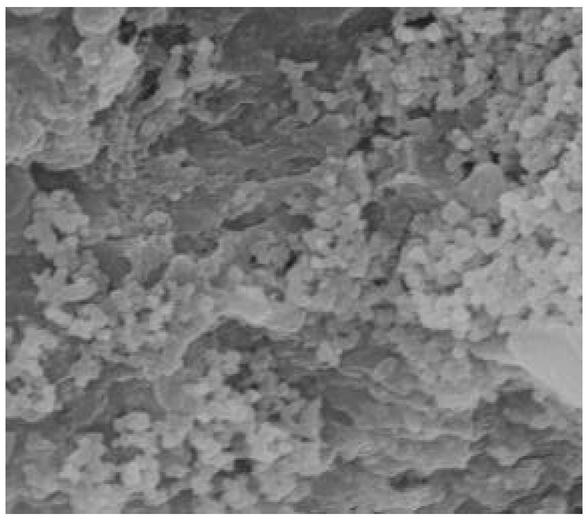

Fig. 2: SEM image showing silver nanoparticles

Table 1: Antimicrobial activity of silver nanoparticles on various pathogenic gram positive and gram negative bacterial strains

Organism	Zone of inhibition (cm)
Staphylococcs aureus	1.4
Serratia marcescens	1.6
Escherichia coli	1.2
Pseudomonas aeruginosa	1.1

as *Escherichia coli*, *Pseudomonas aeruginosa*. The silver nanoparticles showed the inhibition zone of 1.4 and 1.6 nm for gram positive strains *staphylococcus aureus*, *serratia marcescens* and 1.2 and 1.1 cm for negative strains *Escherichia coli*, *Pseudomonas aeruginosa,* respectively (Table 1).

From the data it is inferred that the synthesized silver nanoparticles were more effectvie against gram positive bacterial strains than the gram negative bacterial strains. Similar reults were obtained by Maliszewska and Sadowski (2009). Silver nanoparticle interactions with bacteria are and localized on the membrane of E. coli cells. It has also been reported that Ag^+ ions uncouple the

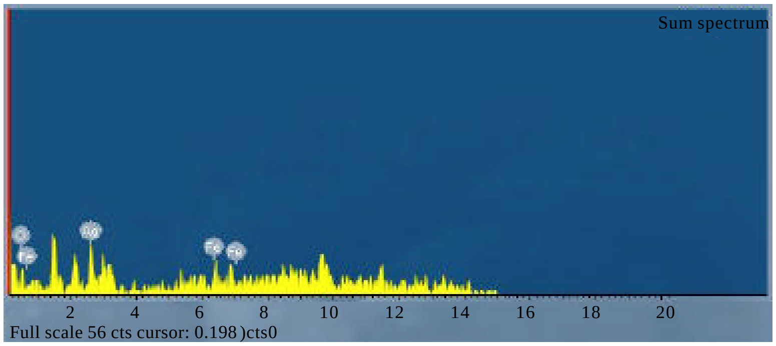

Fig. 3: EDX spectrum of silver nanopartilces

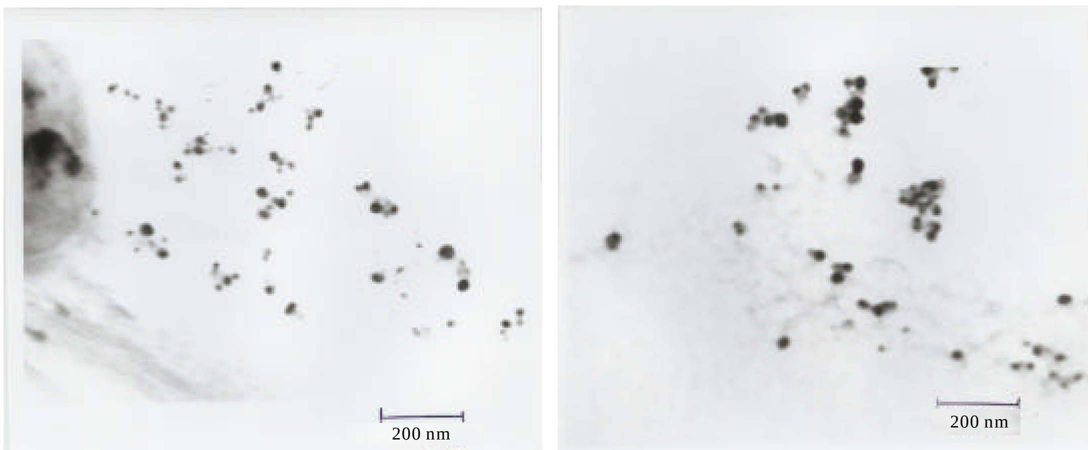

Fig. 4: TEM micrographs showing polydispersed silver nanoparticles

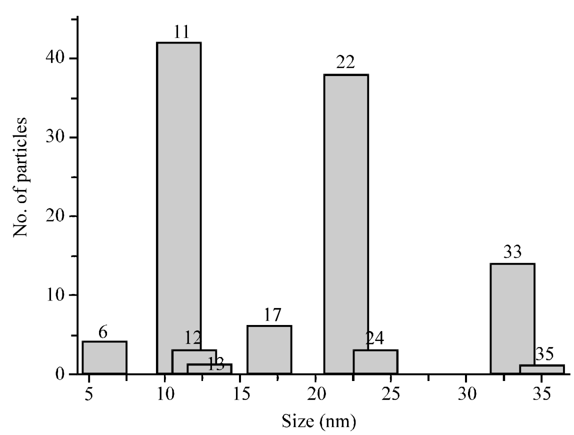

Fig. 5: Particle size histogram of silver nanoparticles

respiratory chain from oxidative phosphorylation or collapse the proton-motive force across the dependent on the size and shape of the nanoparticles (Panacek *et al*., 2006; Morones *et al*., 2005; Pal *et al*., 2007). Morones *et al*. (2005) reported that silver nanoparticles were preferentially bound cytoplasmic membrane (Holt and Bard, 2005). In addition to that, the pitting of the silver nanoparticles caused in the bacterial cell wall is also responsible for the death of bacteria. The actual bactericide mechanism of silver nanoparticles is not well known. Some researchers agree that silver release Ag^+ ions and they interact with the thiol groups of bacterial proteins affecting the replication of DNA (Marini *et al.*, 2007).

CONCLUSION

Silver nanoparticles were synthesized using soap nuts. The nanoparticles produced were of polydispersed with size range from 6-35 nm. The silver nanoparticles are proved to be effective against both gram positive and gram negative bacteria. Saponins might be the possible reason for the formation of silver nanoparticles. Finding of the exact reason for the formation of silver nanoparticles with soap nuts and production of monodisperse nanoparticles is the future objective of this present work.

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