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Full Length Research Paper

Biosynthesis of silver nanoparticles and its antibacterial activity using seaweed *Urospora* sp.

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In the present research work, biosynthesis of silver nanoparticles and its activity on bacterial pathogens were investigated. Silver nanoparticles were rapidly synthesized using *Urospora* sp. and the formation of nanoparticles was observed within 30 min. The results recorded from UV–vis spectrum, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and High Resolution Transmission Electron Microscopy (HRTEM) support the biosynthesis and characterization of silver nanoparticles. From HRTEM analysis, the size of the silver nanoparticles was measured 20 to 30 nm. Further, the antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against the pathogens namely: *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

Key words: *Urospora* sp, silver nanoparticles, biosynthesis, antibacterial activity.

INTRODUCTION

In the recent years, biologically synthesized nanoparticles are of considerable interest in the area of biology and medicine due to their unique particle size and shape dependent physical, chemical and biological properties (Sun et al., 2008; Ko et al., 2007). Bionanotechnology has emerged as the integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for synthesis of nanomaterials. Elemental silver and silver salts have potential to kill the pathogens and are also used as disinfectants in health care from time immemorial, even before the advent of synthetically manufactured organic medicines such as penicillin (Williams et al., 1999). At present, silver nanoparticles (Ag-NPs), as an anti-microbial agent, is gaining greater demand in medical applications as emergence of antibiotic-resistant bacterial strains in public health care is on the increase (Goldmann et al., 1996; Chastre, 2008). Silver components have been proven as an effective tool for retarding and preventing the bacterial infections and they also exhibit wound healing activity. In addition, silver is known to exhibit oligodynamic effect because of its ability to exert bactericidal activity at minute concentrations (Tien et al., 2009). Synthesis of nanoparticles through biological method is a good, environment friendly and economically alternative method. There is a very little literature on the extra cellular biosynthesis of Ag-NPs using plants and pure compounds from plants (Song and Kim, 2008; Gilaki, 2010). Specifically, there is relatively little work done so far on the extra cellular synthesis of Ag-NPs by using seaweeds (Govindaraju et al., 2009).

The bioreduction of $AgNO_3$ using green seaweed *Urospora* sp. extract was implemented in this study. Seaweed has various phytochemicals including carbohydrates, alkaloids, steroids, phenols, saponins and flavonoids (Mansuya et al., 2010). The reduction of silver nanoparticle from seaweed is a green chemical method that demonstrates good antibacterial activity (Govindaraju et al., 2009). These bioinorganic materials can exhibit exquisite hierarchical ordering from the nanometer to macroscopic length scales. Seaweed is available throughout the year and easily accessible to harvest. *Urospora* sp is green algae and this species inhabits hard substrates in the middle–upper intertidal and the splash zone and is daily exposed to extreme changes in

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environmental conditions. Studies have assured that biomolecules like protein, phenols, flavonoids and some phytochemicals has ability to reduce the ions to the nanosize and also play an important role in the capping of the nanoparticles for its stability (Vedpriya et al., 2010).

The present study aimed at bioreduction of $AgNO_3$ and characterizes the nanoparticles using green seaweed *Urospora* sp. and evaluates its bactericidal activity against some clinically important pathogens.

MATERIALS AND METHODS

Sample collection

Seaweeds were collected from rocky shore of South East coast of India, Mandapam (Lat 9° 45' N; Long 79° 15' E), and Tamil Nadu. The collected seaweeds were washed with sea water and with distilled water to remove the dust and soil. The cleaned samples were preserved in a clean zip lock polythene bags. The bags were stored under refrigeration at 8°C for further process.

Synthesis of silver nanoparticles

The collected seaweeds were washed twice with fresh water and distilled water. Cleaned samples were shade dried still clearance of moisture content and dried seaweeds were powdered with the help of mortar and pestle. Seaweed powder was added to the 100 ml deionized water with constant stirring and $AgNO_3$ was added to the obtained solution to get a final concentration of 1 mM and the solution was kept in a magnetic stirrer at 70°C in dark condition at constant stirring. Thereafter, the solutions were centrifuged at 5000 × *g* for 20 min to get a clear solution of silver nanoparticles. The change in color from colorless to brown color was taken for visible confirmation of formation of silver nanoparticles. Then, the centrifuged sample was subjected to further characterization.

Characterization of silver nanoparticles

UV–Vis analysis

Color formation in the supernatant was monitored both by visual inspection and absorbance measurements using double beam UV–Vis spectrophotometer. After complete reduction, the reaction mixture was treated with NaCl to precipitate unreacted Ag ions and the precipitate was removed by filtration through Whatman filter paper No. 1. Silver nanoparticles were concentrated by repeated (4 to 5 times) centrifugation of the reaction mixture at 10,000 rpm for 10 min. The supernatant was replaced by distilled water each time and subjected to UV–Vis analysis. Then, the sample was analyzed in UV- visible spectrophotometer PerkinElmer (Lamda-25) from 200 to 600 nm (Gajbhiye et al., 2009).

Transmission electron microscopy

The TEM sample of silver nanoparticles synthesized using sea weed extract were prepared by placing drop of the reaction mixture over carbon coated copper grids and allowing the acetone to evaporate. High-resolution transmission electron microscope (HR-TEM) micrographs of the sample were taken using JEOL, JEM-3010 operated at an accelerating voltage of 80 kV (Ankamwar et al., 2005).

FTIR and XRD analyses

The biologically synthesized silver nanoparticles solution was kept in lyophilizer for freeze drying. The freeze dried powder was subjected for FTIR analysis using KBr. X ray diffraction (XRD) measurements of the bioreduced silver solution drop-coated onto glass substrates were done on a Siefert X-diffractometer instrument operating at a voltage of 80 kV and a current of 30 mA (Chandran et al., 2006).

Antimicrobial assay

Anti-pathogenic activity of reduced silver nanoparticles was determined by agar well diffusion method (Kora et al., 2009). Media and glassware used were sterilized in an autoclave at 121°C for 20 min. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis* were used for antibacterial study. Bacterial suspension was prepared by growing a single colony overnight in nutrient broth and by adjusting the turbidity to 0.5 McFarland standards (Kora et al., 2009). Mueller Hinton agar (MHA) plates were spread with 0.1 ml (10^5 to10^6 bacteria per ml) of this bacterial suspension and 25, 50 and 100 µl of silver nanoparticles were added to the prepared wells with a diameter of 5 mm and control well was filled with distilled water. These plates were incubated at 37°C for 24 h in a bacteriological incubator and the zone of inhibition (ZOI) was measured by subtracting the well diameter from the total inhibition zone diameter. Three independent experiments were carried out with each strain.

RESULTS AND DISCUSSION

Several approaches have been implicated to obtain a better synthesis of silver nanoparticles such as physical, chemical and biological methods. Recently, synthesis of silver nanoparticles using plant extracts is getting more popular (Li et al., 2007; Song et al., 2009) as it is eco-friendly. Silver nanoparticles were formed by the reduction of Ag^+ into Ag° with the addition of seaweed extraction to the solution of 1 mM $AgNO_3$. The colorless solution of $AgNO_3$ turned into dark brownish yellow color indicating the formation of silver nanoparticles. The formation of silver nanoparticles was monitored by UV-vis absorption spectra at 200 to 600 nm where an intense band was clearly detected at 430 nm (Figure 1). This band was identified as a “surface Plasmon resonance band” and ascribed to the excitation of free electrons in the nanoparticles. The shape of the band was symmetrical, suggesting uniform dispersal of spherical shape nanoparticles (Travan et al., 2009).

FTIR analysis of silver nanoparticles

FTIR analysis were carried out to identify the possible biomolecule responsible for the reduction of the silver ion

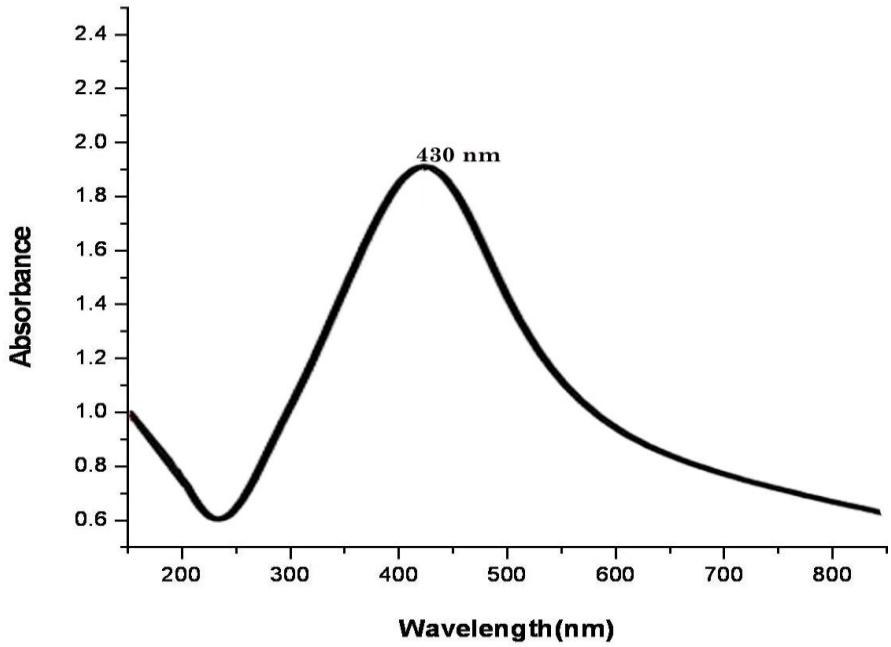

Figure 1. UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Urospora* sp extraction.

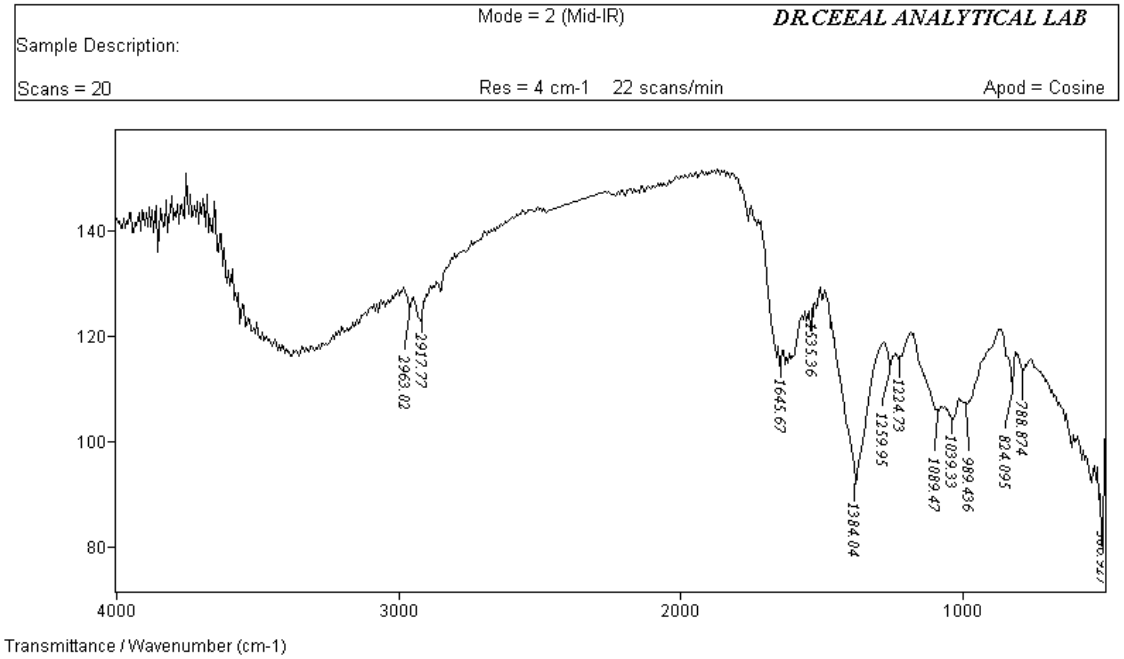

Figure 2. FTIR spectrum of *Urospora* Sp.

and capping agent of bioreduced silver nanoparticles synthesized by the seaweed *Urospora* sp. FTIR spectral analysis showed an array of absorbance bands in 600 cm^{-1}. The spectral bands were interpreted for identification of functional moieties of organic compounds adhering to the silver nanoparticles. The FTIR spectrum of seaweed (Figure 2) shows peaks at 2917 cm^{-1}, 1645cm^{-1}, and 1039 cm^{-1} shows the presence of hydrogen bonded hydroxyl (-OH) group, carbonyl (C=O) and alcoholic group (C-O) respectively.

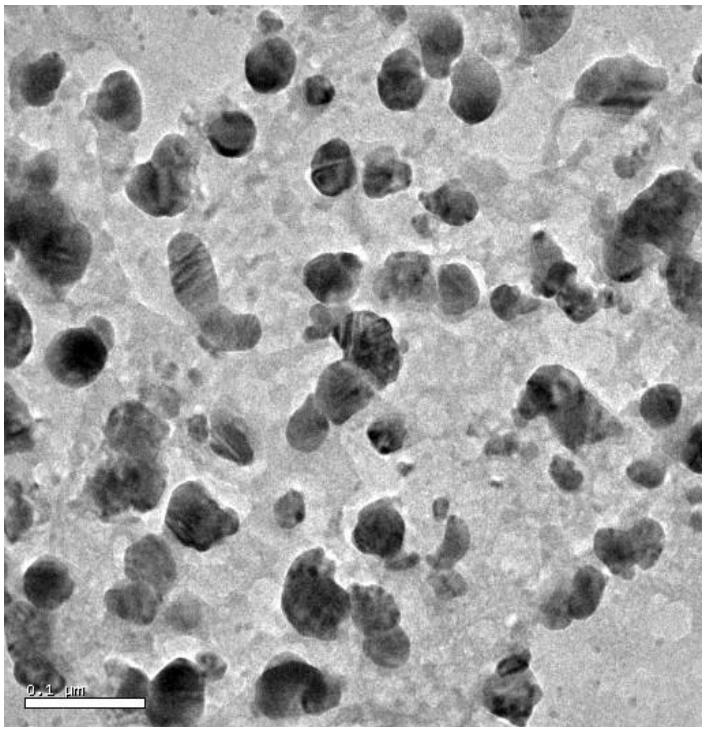

Figure 3. HRTEM image for silver nanoparticles, synthesized using *Urospora* sp.

HRTEM analysis for silver nanoparticle

The silver nanoparticle size, morphology and distribution was analysed by TEM. The size of the nanoparticles influenced the antimicrobial properties (Panacek et al., 2006). In all these spectra, there were no peaks located around 335 and 560 nm, indicating the complete absence of nanoparticles aggregation (Kora et al., 2009; Mohan et al., 2007). The size of the nanoparticle was around 20 to 30 nm. A similar result was recorded by Jain et al. (2009) using papaya fruit as reducing as well as capping agent. Spherical shaped nanoparticles were observed (Figure 3).

XRD analysis for silver nanoparticles

XRD is a widely used technique to estimate the size of nanoparticles in the range between 1 to 100 nm, because of the commonly used x-ray's wavelength. X-ray diffractogram of the biosynthesized nanosilver exhibited Bragg reflection corresponding to face centered cubic (fcc) type bulk silver. X-ray diffractogram of the biosynthesized nanosilver exhibited Bragg reflaction due to (111), (200), (220), (311) and (222) corresponding to fcc type bulk silver (Anil Kumar et al., 2007). The diffraction peaks were broadened around their base indicating that the silver nanoparticle was in nanosizes (Figure 4). XRD analysis showed three distinct diffraction peak at 35.1°, 38.41° and 46° at 2θ angel. The average grain size of the silver nanoparticle that formed bioreduction process was determined.

Antimicrobial activity

The antibacterial activity of silver nanoparticles were analysed by well diffusion assay. The 23 mm clear inhibitory zone appeared around 100 µl silver nanoparticles against *S. aureus* after incubation for 24 h followed by *Bacillus subtilis* (20mm), and *E. coli* (18mm), suggesting that synthesized nanoparticles showed phenomenal bactericidal effect (Figure 5). This observation is in excellent agreement with earlier studies (Cho et al., 2005; Sharma et al., 2009; Xu et al., 2006, 2009).

This study clearly demonstrates that the bactericidal effect depends on the concentration of the silver nanoparticles. The result demonstrates that the zone of clearance increased according to concentration of silver nanoparticles in all microbes. The mechanism of the bactericidal effect of silver colloid particles against bacteria is not very well-known (Panacek et al., 2006). Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that

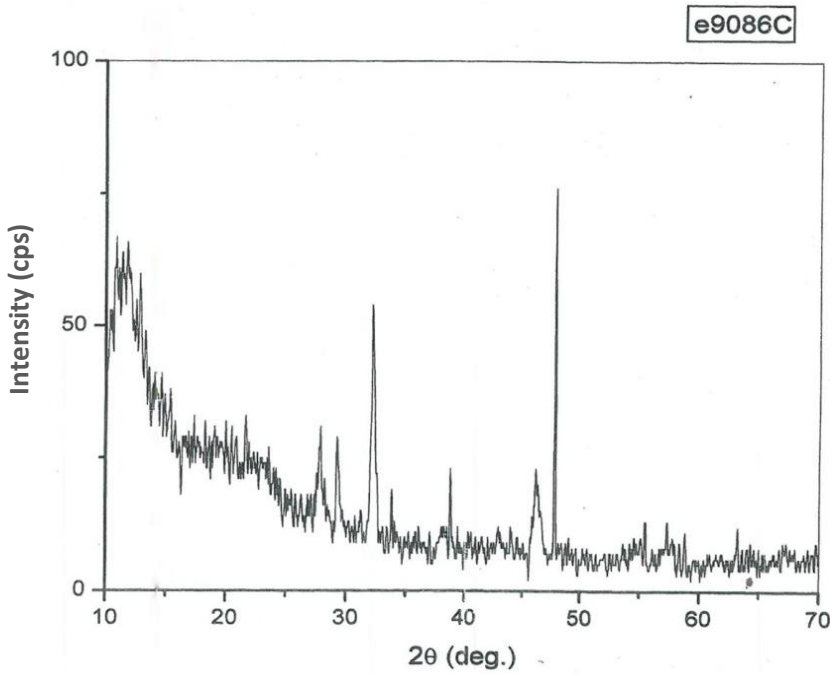

Figure 4. XRD patterns recorded from drop-coated films of silver nanoparticles on glass substrates.

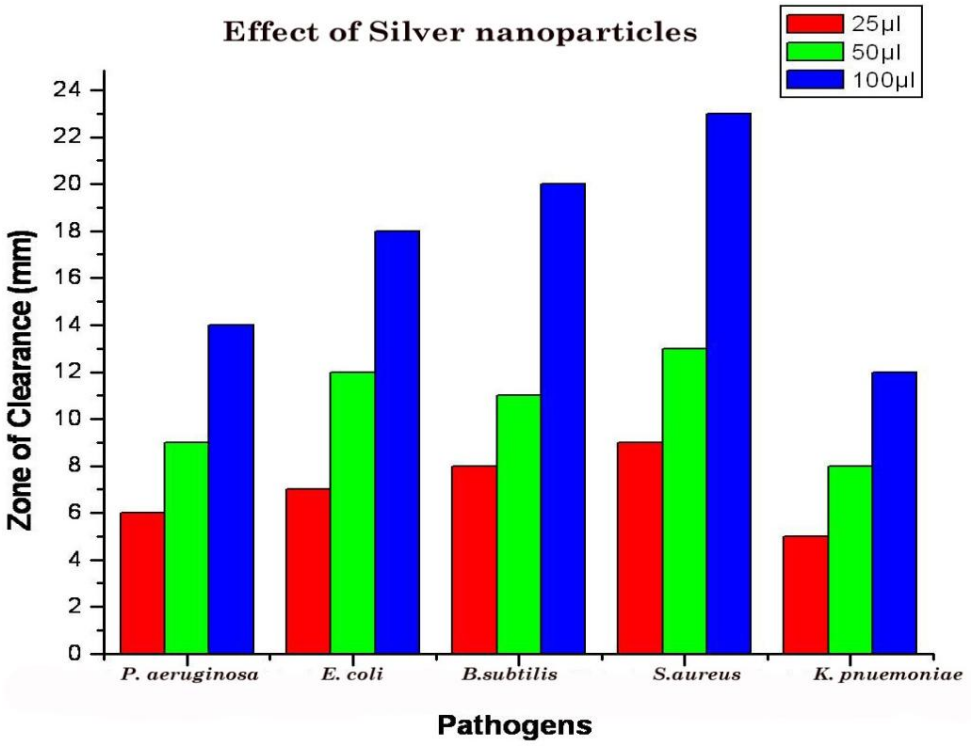

Figure 5. Anti-pathogenic activity of nanoparticles.

the binding of the particles to the bacteria depends on the surface area available for interaction. Some nanoparticles penetrate into the cell and bind with DNA interrupting some gene expression necessary for important

metabolism. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles (Panacek et al., 2006). Morones et al. (2005) reported presence of silver nanoparticles not only at the surface of cell membrane, but also inside the bacteria using the Scanning Tunneling Electron Microscopy (STEM). This suggests the possibility that the silver nanoparticles may also penetrate inside the bacteria and fungi, causing damage by interacting with phosphorus- and sulphur-containing compounds such as DNA. One more possibility would be the release of silver ions from nanoparticles, which will have an additional contribution to the antimicrobial properties of silver nanoparticles. Currently, the increase of bacterial resistance to antimicrobial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Gram-positive and Gram-negative bacteria. Dealing with bacterial resistance will require precautions that lead to prevention of the emergence and spreading of multi-resistant bacterial strains and the development of new antimicrobial substances (Panacek et al., 2006).

To the best of our knowledge, this is the first report on the synthesis of silver nanoparticles using extract of *Urospora* sp. Presently, silver nanoparticles are finding a variety of applications starting from biological tagging to electronic devices (Rao et al., 2003).

Conclusion

In conclusion, this study describes the synthesis of silver nanoparticles using seaweed. Formations of silver nanoparticles were confirmed by UV-vis spectroscopy and FTIR. The spherical shape was observed using HRTEM. The nanoparticles formed were small in size (10 to 20 nm) and well dispersed. The method described in this study for synthesis of silver nanoparticles is environment friendly and well compatible for pharmaceutical and other biomedical applications. The antibacterial effect of silver nanoparticles showed greater bactericidal effect on the bacterium *S. aureus*. The silver nanoparticles with proven antibacterial property may find applications in pharmacology.

REFERENCES

Anil Kumar S, Abyaneh MK, Gosavi Sulabha SW, Ahmad A, Khan MI (2007). Nitrate reductase mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnol. Lett., 29: 439-445.

Ankamwar B, Damle C, Ahmad A and Sastry M (2005). Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. J. Nanosci. Nanotechnol., 5: 1665-1671.

Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M (2006). Synthesis of gold nanotriangles and silver nanoparticles using aloe vera plant extract. Biotechnol. Prog., 22: 577-583.

Chastre J (2008). Evolving problems with resistant pathogens. Clin. Microbiol. Infect., 14: 3-14.

Cho KH, Park JE, Osaka T, Park SG (2005). The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim. Acta, 51(5): 956-960

Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M (2009). Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine: Nanotechnol. Biol. Med., 5: 382-386

Gilaki M (2010). Biosynthesis of Silver nanoparticles using plant extracts. J. Biol. Sci., 10(5): 465-467.

Goldmann DA, Weinstein RA, Wenzel RP, Tablan OC, Duma RJ, Gaynes RP, Schlosser J, Martone WJ (1996) Strategies to prevent and control the emergence and spread of antimicrobial-resistant microorganisms in hospital. A challenge to hospital leadership. J. Am. Med. Assoc., 275: 234-240.

Govindaraju K, Kiruthiga V, Ganesh Kumar V and Singaravelu G (2009). Extracellular synthesis of silver nanoparticles by a marine alga, *Sargassum wightii*, *Grevilli* and their antibacterial effects. J. Nanosci. Nanotechnol., 9: 5497-5501.

Jain D, Daima HK, Kacggwaga S and Kothari SL (2009). Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Dig. J. Nanomater. Biostruct., 4(3): 557-563.

Ko SH, Park I, Pan H, Grigoropoulos CP, Pisano AP, Luscombe CK, Frechet JMJ (2007) Direct nanoimprinting of metal nanoparticles for nanoscale electronics fabrication. NanoLetters, 7: 1869-1877.

Kora AJ, Manjusha R, Arunachalam J (2009). Superior bactericidal activity of SDS capped silver nanoparticles: synthesis and characterization. Mater. Sci. Eng., C 29: 2104-2109.

Li S, Shen Y, Xie A, Yu X, Qiu L, Zhang L, Zhang Q (2007). Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. Green Chem., 9: 852-858.

Mansuya P, Aruna P , Sridhar S, Kumar JS and Babu S (2010). Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from Gulf of Mannar Region. J. Exp. Sci., 1(8): 23-26.

Mohan YM, Raju KM, Sambasivudu K , Singh S, Sreedhar B (2007). Preparation of acacia-stabilized silver nanoparticles: A green approach. J. Appl. Polym. Sci., 106: 3375-3381.

Morones J, Elechiguerra J, Camacho A, Holt K, Kouri J,Ramirez J, Yacaman M (2005). The bactericidal effect of silver nanoparticles. Nanotechnology, 16: 2346-2353.

Panacek A, Kvitek L, Prucek R, Kolar M, Vecerova R, Pizurova N, Sharma V, Nevecna T, Zboril R (2006). Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J. Phys. Chem., B 110: 16248-16253.

Rao CNR, Kulkarni GU, John Thomas P, Ved Varun Agrawal, Gautam UK, Ghosh M (2003). Nanocrystals of metals, Semiconductors and oxides: Novel synthesis and applications. Curr. Sci., 85: 1041-1045.

Sharma VK, RA Yngard and Y Lin (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci. 145: 83-96.

Sharma VK, Yngard RA, Lin Y (2009). Silver nanoparticles:green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci., 145: 83-96.

Song JY, Kim BS (2008). Biological synthesis of bimetallic Au/Ag nanoparticles using Persimmon (*Diopyros kaki*) leaf extract. Korean J. Chem. Eng., 25(4): 808-811.

Song KC, Lee SM, Park TS and Lee BS (2009). Preparation of colloidal silver nanoparticles by chemical reduction method. Korean J. Chem. Eng., 26: 153-155.

Sun C, Lee JSH, Zhang MQ (2008). Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Delivery Rev., 60: 1252-1265.

Tien D, Tseng K, Liao C, Tsung T (2009). Identification and quantification of ionic silver from colloidal silver prepared by electric spark discharge system and its antimicrobial potency study. J. Alloy Compd., 473: 298-302.

Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, Scarpa T, Semeraro S, Turco G, Gennaro R, Paoletti S (2009). Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity. Biomacromolecules, 10: 1429-1435.

Vedpriya A (2010). Living Systems: eco-friendly nanofactories. Dig J. Nanomater. Bios., 5(1): 9-21.
Williams DH, Bardsley B (1999). The vancomycin group of antibiotics and the fight against resistant bacteria. Angew. Chem. Int. Ed., 38: 1172-1193.
Xu X, Yang Q, Wang Y, Yu H, Chen X, Jing X (2006). Biodegradable electrospun poly (L-lactide) fibers containing antibacterial silver nanoparticles. Eur. Phys. J., D 42: 2081-2087.
Xu K, Wang J, Kang X, Chen J (2009). Fabrication of antibacterial monodispersed Ag–SiO2 core–shell nanoparticles with high concentration. Materials Lett., 63: 31-33.