INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 22, No. 5, p. 17-24, 2023
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Rapid biosynthesis of highly stabilized nano silver by *Andrographis paniculata* leaf extract; A prioritized medicinal plant and its germicidal activity

M. Revathy[1], C. Aswathy[1], S. Amutha[1], E. Amutha, T. Madhumitha[1], E. Pushpalakshmi[1], R. Venkateshwari[1], S. Rajaduraipandian[2], G. Annadurai[*1]

[1]*Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, India*

[2]*Sri Paramakalyani College, Manonmaniam Sundaranar University, Alwarkurichi, India*

Article published on May 09, 2023

Key words: Silver nanoparticles, Biosynthesis, *Andrographis paniculata*, Characterization, Antibacterial activity

Abstract

The present research explains a rapid and environmental friendly route for the synthesis of highly stabilized silver nanoparticles using the *Andrographis paniculata* leaf extract. The biosynthesized silver nanoparticles were characterized by using UV-visible spectrophotometer, Fourier Transform Infrared spectroscopy, X-ray diffraction, and Scanning electron microscope. The UV- visible spectrum exhibited surface Plasmon resonance band at 421 nm signifies the presence of silver nanoparticles in the reaction mixture. The XRD technique revealed the synthesized nanoparticles were crystalline in nature as well as possess face centered cubic geometry. FTIR studies were carried out to investigate the functional groups responsible for the silver nanoparticle reduction in the range 4000 – 400cm^{-1}. EDAX analysis displayed the elemental composition in the sample. SEM analysis confirmed the synthesized particles are polydispersed in nature. The inhibition zone gradually increased with increase in silver nanoparticle concentration. Further the silver nanoparticle exhibited an effective germicidal activity against both Gram positive and Gram negative organisms by using disc diffusion method. The *in-vitro* antimicrobial assay demonstrated the results of maximum inhibition zone with the (36±0.334) in *Lactobacillus* sp and minimum zone with (15±0.318) in *Streptococcus* sp. Because of the potent antimicrobial activity it might be concluded that silver nanoparticles were efficiently utilized as an effective antibacterial compounds.

***Corresponding Author:** G. Annadurai ✉ gannadurai@msuniv.ac.in

Introduction

Nanoscience and nanotechnology is a promising area of nanoscale structures and materials appliances typically ranging from 1 – 100 nm and at these range materials physical and chemical characteristics are drastically distinct from those in bulk materials because of quantum effects (Sharma *et al*., 2009; Matos *et al*., 2011). Due to their distinctive properties as well as growing use for several applications in nanomedicine, silver nanoparticles have attracted considerable importance among the rising nanoproducts. Silver in the silver nitrate or silver sulfadiazine form has been utilized for the healing of bacterial infections connected along with burns and injuries due to its antimicrobial properties for a long time (Lok *et al*., 2007). Several physical, chemical and biological techniques have been improved for the silver nanoparticle synthesis and it has a low yield as well as complex to synthesize nanoparticles with a definite size (Malik *et al.,* 2010).

Traditional approaches similar to physical and chemical such as lithography, laser ablation, pyrolysis, chemical vapor deposition, electrodeposition, sol-gel techniques for nanoparticle synthesis appear to be costly and harmful. Additionally the process includes numerous reactants such as sodium borohydride, potassium bitartrate, methoxypolyethylene glycol, hydrazine as well as it involves stabilizing agents sodium dodecyl benzyl sulfate, polyvinyl pyrrolidone to inhibit the metallic nanoparticles agglomeration. Though numerous techniques are obtainable for the nanoparticle synthesis, there is an essential to expand easy, cost effective, and ecofriendly methods. Hence it is vital to find an alternative biological process for metal nanoparticle synthesis with control particle size and shape for several biomedical applications (Gurunathan *et al*., 2009; Gurunathan *et al.,* 2013). Plant, plant products, algae, fungi, yeast, bacteria and viruses are a huge collection of biological reserves may possibly utilized for the nanoparticle synthesis and the period needed for the absolute reduction is lesser in biological methods. Biosynthesized nanoparticles are quickly available in solution with high stability, density and depend upon the compounds like alkaloids, tannin, steroids, phenol, saponins, and flavonoids in the plant extract we assume whether the proteins, polysaccharides or secondary metabolites are able to reduce the Ag^{+} to Ag^{0} state and develop silver nanoparticles (Singhal *et al.,* 2011). *Andrographis paniculata* is frequently recognized as king of bitter belongs to the family Acanthaceae indigenous to India and Srilanka. The leaves and root were utilized predominantly for therapeutic purposes in traditional ayurvedic and siddha medicine in India and several other countries. Extract of this plant revealed antifungal, antityphoid, antioxidants, anti-snake venom, anti-inflammatory and antipyretic properties. *Andrographis paniculata* extract contains two major chemical components acquired from the whole plant such as diterpenoids and flavonoids 7, 2', 3'- tetramethoxyflavonone as well as 5- hydroxy-7, 2', 3'-trimethoxyflavone which are expected to be responsible for the major bioactivities of this plant (Aliyu *et al.,* 2009; Puri *et al*., 1996; Tang *et al.,* 1992).

A number of novel antibiotics were exploited in the previous decades; none have recovered action against multidrug resistant bacteria and due to the growing pervasiveness of microbial resistance has made the public health organization a major concern in the recent world (Mohanty *et al.,* 2012). The main objective of the current study was to improve an easy and ecofriendly method for the silver nanoparticle synthesis and characterization by employing *Andrographis paniculata*. After that aim of this analysis engaged the germicidal activity of green synthesized silver nanoparticles against both Gram positive and Gram negative organisms. Improving silver nanoparticles as an innovative origination of antimicrobial agents could be a desirable and cheaper means to overwhelm the multi-drug resistance difficulties noticed with bacteria.

Materials and methods

Andrographis paniculata leaves were collected from Manonmaniam Sundaranar University Campus Tirunelveli (District), Tamil Nadu, India. All the

culture media, Nutrient broth, Silver nitrate as well as Mueller Hinton Agar were obtained from Hi media laboratories, Mumbai.

Biosynthesis and characterization of silver nanoparticle

The collected fresh leaves were washed thrice with tap water thoroughly and twice with double distilled water in order to eliminate dust particles and other associated pollutants. About 10 grams of surface sterilized leaves were finely chopped into small slices and boiled with 100mL of double distilled water for about 10 -15 minutes at 60°C as shown Fig.1. Then the boiled extracts were filtered using Whatmann no 1 filter paper. For reduction of Ag^{+} 10mL of leaf broth was added to 100mL of 1mM $Agno_3$ solution and incubate at room temperature. The reduction of silver ions was examined by visual examination and determining the absorbance of the reaction mixture in a range of wavelengths ranging from 300-700 nm^{-1} via UV- visible spectrophotometer (Perkin Elmer Lambda double beam UV-Spectrophotometer).

The change of color from yellow to dark brown color within 1 minute revealed the silver nanoparticle synthesized from the leaves. After 8 h of incubation the pellet formed was centrifuged at 10,000 rpm for 5 minutes and suspension was repeated three times to make certain the absolute separation of nanoparticles. After drying the purified nanoparticles were suspended in Millipore water and stored in a freezer for further investigation.

Then the dried nanoparticle was further analyzed by XRD (Philips PW 1830). The functional group present in the nanoparticle was scrutinized by the SHIMADZU instrument along with the sample as KBR pellet in the wavenumber region of 500-4,000cm^{-1}. Morphological characterization was performed by using Scanning Electron microscopy (Philip Modelcm, 200).

Antibacterial activity of biosynthesized silver nanoparticles

The biologically synthesized silver nanoparticles were investigated for antibacterial activity by agar well diffusion method against both Gram positive and Gram negative organisms such as *Escherichia coli, Enterococcus, Streptococcus, Klebsiella, Pneumoniae, Serratia, Planomicrobium* and *Lactobacillus*. Bacterial pure cultures were maintained on Mueller Hinton agar plates. Every strain was swabbed evenly onto the individual plates utilizing sterile cotton swabs and using gel puncture 8mm diameter cavity were made on nutrient agar plates.

Different concentrations of Ag Nps were poured onto each well on all plates using sterile micropipette. Incubate the plates at 37°C for 24 hours for the zone formation around the well and inhibition zone was measured in millimeter as well as recorded.

Results and discussion

The color change of the reaction mixture from yellow into intense brown color within few seconds was noticed by the visual observation in extract of *Andrographis paniculata* leaf extract incubated with 100mL of 1mm silver nitrate solution shown in Fig.2 (A to C) as shown Table.1.

The appearance of yellowish brown color in aqueous extract of silver nanoparticles was an effect of surface Plasmon vibrations Krishnaraj *et al.* (2010). Similar reports have confirmed that the surface resonance peak of silver nanoparticles occurs around this region (Zahir and Rahuman, 2012).

Thus the biosynthesis of silver nanoparticles was more rapid using *Andrographis paniculata* leaf extract when compared to other plant leaves (Song and Kim, 2009; Bar *et al*., 2009; Bankar *et al.* 2009; Dwivedi and Gopal, 2010; Dubey *et al.* 2010; Geethalakshmi and Sarada, 2010). It was examined in the Fig.3 that the surface Plasmon resonance peak obtained at 421.79 nm initially signifying the reduction of silver nitrate into silver nanoparticle. The reduction of silver ions into silver nanoparticles initiated at the beginning of the reaction as well as the completion process takes place nearly 1 minute at room temperature suggesting the very rapid biological synthesis of silver nanoparticles was noticed in the *Andrographis paniculata* extract.

Phytochemical investigation

Table 1. Preliminary Phytochemical analysis of aqueous extracts of *Andrographis paniculata*.

Phytochemical constituents	Results
Proteins	+
Carbohydrates	+
Tannins	+
Alkaloids	+
Flavanoids	+
Steroids	-

Visual Identification

Fig. 1. Photograph of *Andrographis paniculata*.

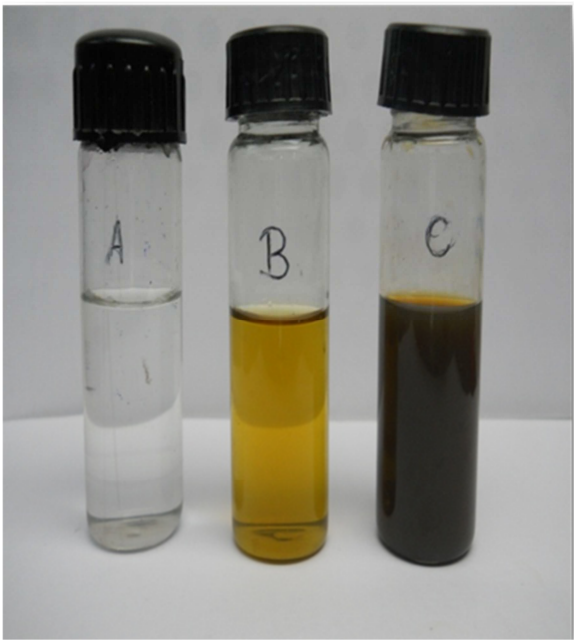

Fig. 2. A-$AgNo_3$ Solution B – Plant Extract C- Initial color change of silver nanoparticle.

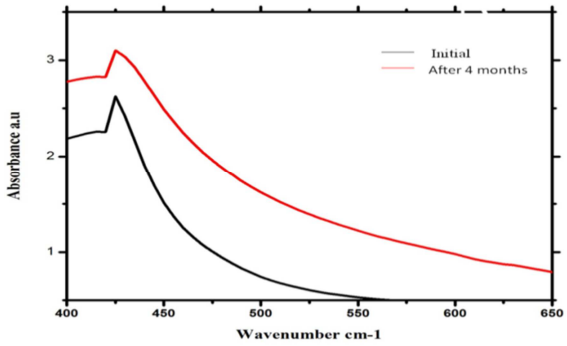

Fig. 3. UV–Visible spectrum of biosynthesized silver nanoparticle using *Andrographis paniculata*.

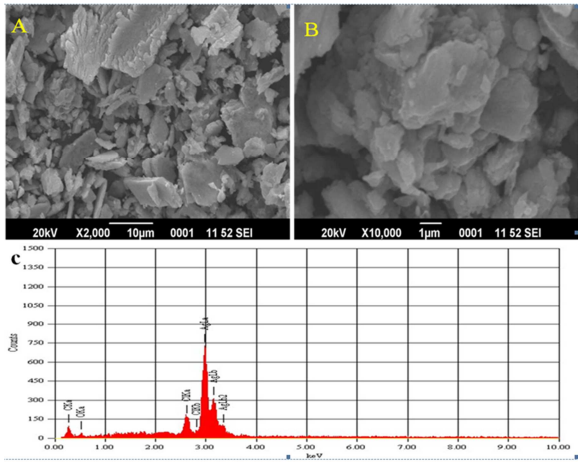

Fig. 4. A & B. SEM images of different magnification of biosynthesized silver nanoparticle using *Andrographis paniculata* C: EDAX Spectrum of biologically synthesized silver nanoparticles.

Fig. 4 (A and B) displays characteristic SEM representations which were confirmed at various magnifications from drop coated films of silver nanoparticles fabricated by silver nitrate solution treated with *Andrographis paniculata* leaf extract. SEM analysis revealed the occurrences of large sized silver nanoparticles detected might be assigned to the small sized nanoparticle aggregation. EDX spectrum revealed in the Fig. 4C exhibited the silver occurrence in the dried nanopowder and furthermore proved the silver nanoparticle, carbon and oxygen presence indicating that the nanoparticles should be capped by the prevalence of organic components in the plant extract. Silver nanoparticles are recognized as antimicrobial agents whereas the existence of plant bioorganic capping material upon the silver nanoparticle allows them to revealed antibacterial

activity (MubarakAli *et al.,* 2011; Prabhu and Poulose 2012). The silver substance in particles was obtained to be 68.6%.

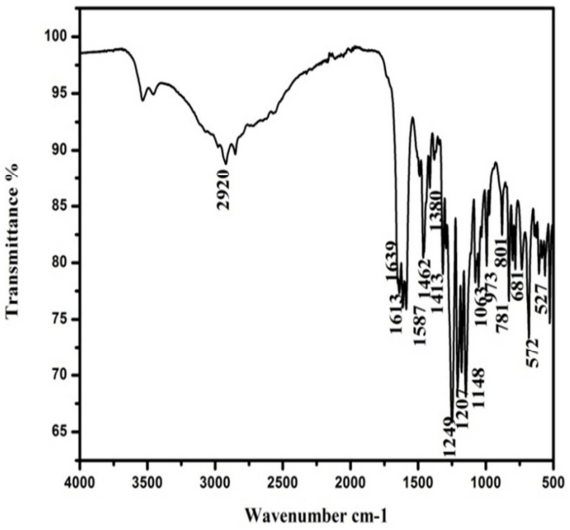

Fig. 5. FTIR Spectrum of biosynthesized silver nanoparticles synthesized by *Andrographis paniculata.*

Fourier transform infrared spectroscopy analysis was performed to detect the potential biomolecules which is responsible for the reduction, capping as well as the stabilization of the silver nanoparticles. The band which appeared at 2920cm^{-1} corresponding to C-H stretching of the functional group alkanes. The bands at 1639cm^{-1} and 1618cm^{-1} are because of N-H bending of primary amines and C-C stretching of aromatics. The IR bands observed at 1462cm^{-1} might be ascribed to C-H ending of the functional group alkanes. The band appeared at 1249cm^{-1} and 1207cm^{-1} corresponds to C-O stretching of alcohols, carboxylic acids, esters, ethers and C-N stretching of aliphatic amines respectively. The bands obtained at 1148cm-1 and 1063cm-1 are due to the C–H stretching of alkyl halides and C–N stretching of aliphatic amines. The absorption peak at around 973cm^{-1} might be assigned to the =C–H bending of the functional group alkenes. FTIR analysis also displays peak at 801cm^{-1} and 781cm^{-1} which arises due to N–H stretching of primary and secondary amines as well as C–Cl stretching of alkyl halides. The band at 691cm^{-1} corresponds to –C ≡ C–H: C–H bending of alkynes. The peaks obtained at 572cm^{-1} and 527cm^{-1} was ascribed to the C–Cl stretching and C–Br stretching of functional group alkyl halides. FTIR Spectrum results exhibited the prominent peaks along with distinctive 2920cm^{-1}, 1639cm^{-1}, 1618cm^{-1},1462cm^{-1}, 1249cm^{-1}, 1207cm^{-1}, 1148cm$^{-1,}$ 1063cm^{-1}, 973cm^{-1},801cm^{-1} ,781cm-1, 691cm^{-1}, 572cm^{-1} and 527cm^{-1} values as shown in Fig.5. The occurrences of functional group had a strong binding capability with silver indicating the formation of a layer enveloping nanoparticles of silver as well as performing as a capping agent to inhibit agglomeration and impart stability to the medium. FTIR results prove the potential proteins presence act as influential reducing and stabilizing agents.

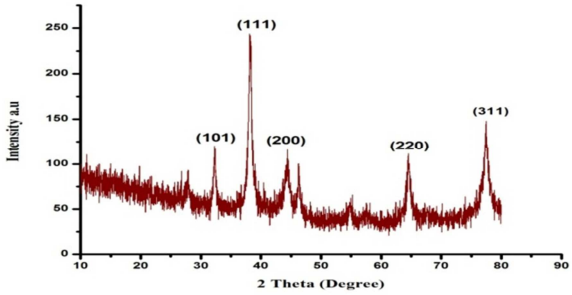

Fig. 6. XRD Spectrum of biosynthesized silver nanoparticles using *Andrographis paniculata*.

X-ray diffraction analysis was performed to verify the crystalline nature of the silver nanoparticles and the pattern displayed the Bragg reflections which can be indicated on the basis of the face centered cubic arrangement of silver. The X-ray diffraction analysis obviously illustrates the biosynthesized silver nanoparticles were crystalline in nature shown in the Fig.6. Silver nanoparticles have revealed five distinct intense peaks (101) (111), (200), (220), (311) in the entire spectrum of 2θ values of 32.32°, 38.29°, 44.42°, 64.61°, 77.49° ranging from 20 to 80 at room temperature. Additionally unassigned peaks are also detected intimating that the crystallization of the bioorganic phase arises on the nanoparticle surface (Sathyavathi *et al.,* 2010). The peak intensity reveals the high degree of crystallinity of the biosynthesized silver nanoparticles using *Andrographis paniculata*. The widening of XRD peaks all around their bases revealed that the silver nanoparticles were in nanorange (Ahmad and Sharma 2012). The size of the crystallite was assessed by using Scherrer formula from the extent of the XRD peaks considering which they are free from non-uniform strains.

$$D = 0.94 \lambda / \beta \cos\theta$$

Where, D represents average crystalline domain size, λ signifies the wavelength of X-ray, β denotes full width at half maximum (FWHM) and θ implies the diffraction angle. FWHM from a large grained Si sample was adjusted to remove additional instrumental broadening (Faghri Zonooz *et al.*, 2011).

Mechanism of Antibacterial activity of Silver nanoparticles

In human cell the toxicity of silver ions is low, thus the silver dependent nanoparticles gained more attention. It is also a long term biocide along with thermal stability as well as low instability (Williams *et al.,* 1989; Berger *et al.,* 1976). Because of the reality another advantageous effect of utilizing silver is which pathogen cannot alter to evade its antibacterial activity as shown in Fig. 7 and Table. 2.

The improvement of resistance capacity to antibacterial silver would be particularly unusual for the reason that an organism would have to endure instantaneous mutations in each significant function in a particular generation to break its effect. Spontaneous mutation is uncommon happening in simply 1 for each 10^5 divisions, thus the possibility of multiple dependent mutations takes place in the similar generation of microorganism is tremendously slight (Gibbins, 2003). From the results obtained the zone of inhibition by AgNps synthesized from *Andrographis paniculata* leaf extract revealed maximum inhibition for *Lactobacillus* sp and maximum inhibition for *Streptococcus* sp. Zone of inhibition in diameter was recorded in table 1. Silver nanoparticles are highly toxic in gram positive bacteria than the gram negative bacteria because of the variation in the bacterial cell wall.

Table 2. Antibacterial Activity Zone of inhibition in diameter Values.

SL	Pathogens	AgNps (50 µl) Zone of inhibition in mm
1.	*Bacillus cereus*	18 ± 0.167
2.	*Lactobacillus* sp	36 ± 0.334
3.	*E.coli*	22 ± 0.145
4.	*Enterococcus* sp	22 ± 0.067
5.	*Klebsiella planticola*	34 ± 0.116
6.	*Planomicrobium*	20 ± 0.145
7	*Streptococcus*	15 ± 0.318

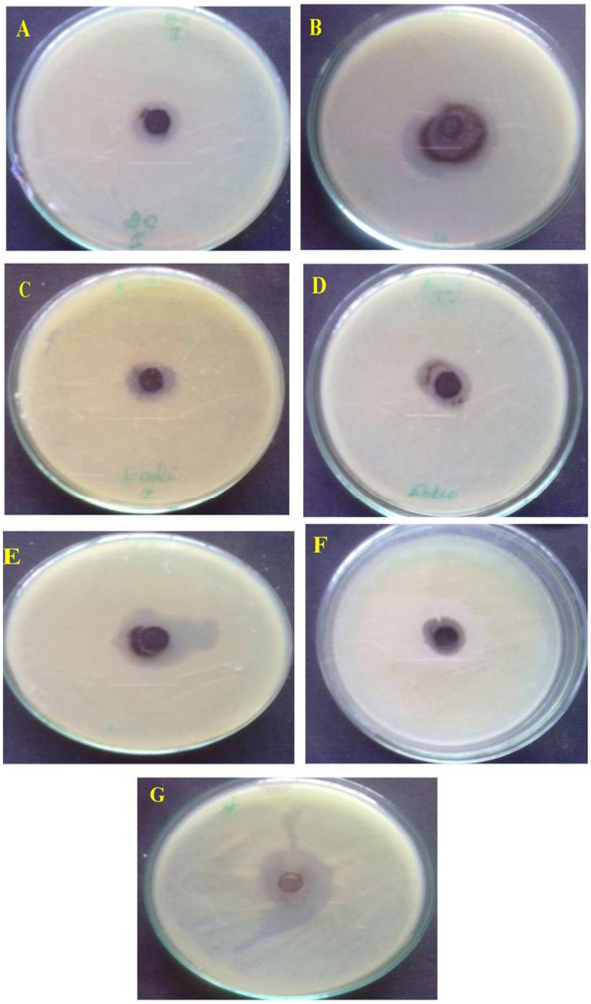

Fig. 7. Antibacterial activity of silver nanoparticles synthesized using *Andrographis paniculata* extract A- *Bacillus cereus,* B- *Lactobacillus*, C- *E. coli*, D- *Enterococcus*, E - *Klebsiella,* F- *Planomicrobium*, G- *Streptococcus*.

Conclusions

The bio reduction of aqueous Ag^+ ions by the leaf extract of the *Andrographis paniculata* has been verified. The size of the nanoparticle ranges 40 – 50 nm correspondingly, and polydispersed with crystalline nature. EDX analysis revealed the elemental composition of the silver nanoparticles. Functional groups present in the plant extracts responsible for the silver ion reduction process were analyzed by FT-IR. The reduction of the metal ions through leaf extracts leading to the development of highly stabilized nanoparticles of absolutely definite dimensions. In the present investigation we identified that leaves can be a potential source for silver nanoparticle synthesis.

This green chemistry method regarding the silver nanoparticles has several advantages such as stability more than 3 months, consistency, process simplicity and environmental friendly. Appliances of non-toxic nanoparticles with antibacterial activity in wound healing and other pharmaceutical applications makes this process effective for the broad synthesis of nanomaterials.

References

Ahmad N, Sharma S. 2012. Green synthesis of silver nanoparticles using extracts of *Ananas comosus*. Green Sustain Chem **2,** 141-147.

Aliyu AB, Musa AM, Sallau MS and Oyewale AO. 2009. Proximate Composition, Mineral Elements and Anti-nutritional Factors of *Anisopus mannii* N. E. Br. (Asclepiadaceae). Trends Appl. Sci. Res **4(1),** 68-72.

Bankar A, Joshi B, Kumar AR, Zinjarde S. 2009. Banana peel extract mediated novel route for synthesis of silver nanoparticles. Colloid Surf A Physicochem Eng Aspect **368,** 58-63.

Bar H, Bhui DK, Gobinda SP, Sarkar PM, Pyne S, Misra A. 2009. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Physicochem Eng. Aspects **348,** 212-216.

Berger TJ, Spadaro JA, Chapin SE, Becker RO. 1976. Electrically generated silver ions: Quantitative effects on bacterial and mammalian cells. Antimicrob Agents Chemother **9,** 357-358.

Dubey SP, Lahtinen M, Sillanpaa M. 2010. Green synthesis and characterization of silver and gold nanoparticles using leaf extract of *Rosa rugosa*. Colloid Surf A Physicochem Eng Aspect **364,** 34-41.

Dwivedi AD, Gopal K. 2010. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. Colloid Surf A Physicochem Eng. Aspect **369,** 27-33.

Geethalakshmi E, Sarada DV. 2010. Synthesis of plant-mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their antimicrobial activities. Int. J. Eng. Sci. Tech. **2,** 970-975.

Gibbins B. 2003. The antimicrobial benefits of silver and the relevance of micro lattice technology. J. Inorg Biochem. **101,** 291-296.

Gurunathan S, Han JW, Eppakayala V, Jeyaraj M, Kim JH. 2013. Cytotoxicity of biologically synthesized silver nanoparticles in MDA-MB-231 human breast cancer cells. Biomed. Res. Int. **2013,** 535-796.

Gurunathan S, Kalishwaralal K, Vaidyanathan R, Deepak V, Pandian SRK, Muniyandi J, Hariharan N, Eom SH. 2009. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloid Surf B **74,** 328-335.

Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B: Biointerfaces **76,** 50-56.

Lok CN, Hocm, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Checm. 2007. Silver nanoparticles: Partial oxidation and antibacterial activities. J. Biol Inorg Chem **12,** 527-534.

Malik MA, O'Brien P, Revaprasadu N. 2002. A simple route to the synthesis of core/shell nanoparticles of chalcogenides. Chem Mater **14,** 2004-2010.

Matos RA, Cordeiro TS, Samad RE, Vieira JrND, Courrol LC. 2011. Green synthesis of stable silver nanoparticles using *Euphorbia milii* latex. Colloid Surface A **389,** 134-137.

Mohanty S, Mishra S, Jena P, Jacob B, Sarkar B, Sonawane A. 2012. An investigation on the antibacterial, cytotoxic and antibiofilm efficacy of starch-stabilized silver nanoparticles. Nanomed: Nanotechnol Biol. Med. **8,** 916-924.

MubarakAli D, Thajuddin N, Jeganathan K, Gunasekaran M. 2011. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Coll Surf B **85,** 360-365.

Prabhu S, Poulose EK. 2012. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett **2,** 1-10.

Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. 1996. Immunostimulant Agents from *Andrographis paniculata*, J. Natural Prod **56(7),** 995-999.

Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. 2010. Biosynthesis of Silver Nanoparticles Using *Coriandrum sativum* Leaf Extract and Their Application in Nonlinear Optics, American Scientific Publishers **3(2),** 138-143(6).

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

Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. 2011. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nanoparticle Res. **13,** 2981-2988.

Song JY, Kim BS. 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extract. Bioprocess Biosyst Eng **32,** 79-84.

Tang W, Eisenbrand G. 1992. Chinese Drugs of Plant Origin, Chemistry, Pharmacology and Use in Traditional and Modern Medicine, Springer Verlag, Berlin pp. 97-103.

Williams RL, Doherty PJ, Vince DG, Grashoff GJ and Williams DF. 1989. The biocompatibility of silver. Crit Rev. Biocompat **5,** 221-243.

Zahir AA, Rahuman AA. 2012. Evaluation of different extracts and synthesized silver nanoparticles from leaves of *Euphorbia prostrata* against *Haemaphysalis bispinosa* and *Hippobosca maculate*. Vet. Parasitol **187,** 511-520.