Research Guru
Online Journal of Multidisciplinary Subjects (ISSN : 2349-266X)
www.researchguru.net
Volume-11, Issue-2(September, 2017)

IMPACT FACTOR:3.021

PP:264to274

Silver Nanoparticles: Antibacterial Activity of Biosynthetically prepared Silver Shells

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Abstract

Nanotechnology plays an increasingly crucial role in many key technologies of the new millennium. The application of nanoscale materials and structures, usually ranging from 1 to 100nm, is an emerging area of nanoscience and nanotechnology. Manipulating matter atom by atom and creating features on the atomic or nano scale is now a proven technology and there is an ever growing catalogue that utilizes nanotechnology. Among them Silver Nanoparticles (AgNPs) have gained much attention because of their unique physical, chemical and biological properties. AgNPs displays a broad spectrum of bactericidal and fungicidal activities, which have made them remarkably popular in the diverse range of consumer products and because of their broad range of applications many synthetic routes have been developed for the synthesis of AgNPs. Recently, AgNPs synthesis is among the most interesting scientific areas of enquiry and there is a growing attention to produce nanoparticles using environmentally friendly methods (green synthesis). In the present study, biosynthesis of AgNPs using plant extract and fungi has been carried out and their characterization has been done using Transmission Electron Microscope. This biosynthetically synthesized AgNPs were subjected to antibacterial susceptibility tests and it showed positive activity against various organisms such as: Escherichia coli; Staphylococcus aureus; Proteus vulgaris; Bacillus cereus; Vibrio cholerea; Corynebacterium amycolatum by means of Disc Diffusion and Cup Borer Methods.

Keywords: Silver Nanoparticle; *Mentha sachalinensis; Adhatoda vasika*; *Aspergillus niger*, Transmission Electron Microscope.

Introduction

The fast expansion of the global nanotechnology market involves a higher environmental and human exposure to nanomaterials. Silver nanoparticles (AgNPs) are today one of the most commonly used nanomaterials both in fundamental medical sciences as well as clinical practices. These nanoparticles are incorporated into many commercial products including clothing/textiles, furniture, household appliances, cosmetics and even children toys[1]. Recently, it has been suggested that AgNPs in some circumstances may have substantial genotoxic effects and some authors indicated that AgNP exposure in some cells may lead to the process of programmed cell death (apoptosis) [2-9]. This research paper focuses on the findings regarding the antibacterial effect of the synthesized AgNPs. AgNPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and nonlinear optical behavior [10-11]. These properties make them of potential value in inks [12], microelectronics [13], and medical imaging [14]. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity [15] that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value [16]. Nanosilver can be used in a liquid form, such as a colloid (coating and spray) or contained within a shampoo (liquid) and can also appear embedded in a solid such as a polymer master batch or be suspended in a bar of soap (solid). Nanosilver can also be utilized either in the textile industry by incorporating it into the fiber (spun) or employed in filtration membranes of water purification systems. In many of these applications, the technological idea is to store silver ions and incorporate a time-release mechanism [16-17]. Advances in nanomedicine can be made by engineering nanoparticles that are capable of targeted delivery of drugs. Promoting nanotechnology for diagnosis, prevention and treatment is the focus of the recently developing multifunctional nanotechnology. Many synthetic routes that have been developed to synthesize AgNPs due to the applications found tremendously in wide range of fields. Among the synthetic routes includes 1) chemical reduction [18,19], 2) thermal decomposition [20], 3) electrochemical [21], 4) sonochemical [22], 5) photochemical [23], 6) microwave [24], 7) radiation assisted process [25,26] and currently by 8) green synthesis [27,28]. Chemical reduction method is widely used to synthesize AgNPs because of its readiness to generate AgNPs under gentle conditions and its ability to synthesize AgNPs on a large scale [29]. However, these chemical synthesis methods employ toxic chemicals in the synthesis route which may have adverse effect in the medical applications and hazard to environment. Therefore, preparation of AgNPs by green synthesis approach has advantages over physical and chemical approaches as it is environmental friendly, cost effective and the most significant advantage is that conditions of high temperature, pressure, energy and toxic chemicals are not required in the synthesis protocol [30]. Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells [31-35]. Several studies propose that AgNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell [36]. Smaller Ag NPs having the large surface area available for interaction would give more bactericidal effect than the larger AgNPs [36]. It is also possible that AgNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria [37]. Ag-NPs have been known for its inhibitory and bactericidal effects in the past decades [38]. Antibacterial activity of silver containing materials can be applied in medicine for reduction of infections on the burn treatment [39,40], prevention of bacteria colonization on catheters [41,42] and elimination of microorganisms on textile fabrics [43,44] as well as disinfection in water treatment [45]. Besides that, AgNPs were also being reported in the literature to exhibit a strong cytoprotective activity towards human immunodeficiency virus (HIV) infections[46]. Also, AgNPs at different µg/L concentrations results in numerous physiological and biochemical responses indicative of silver toxicity. Toxicity data of AgNP vary considerably, reflecting species sensitivity differences, characteristics of the different Ag-NP formulations and the water chemistry conditions used in the different studies [47].

Materials and Methods

Preparation and Characterization of silver nanoparticles

In the present study, 100 g of leaves of two plants; *Mentha sachalinensis, Adhatoda vasika* were collected and air dried for 10 days, kept in the hot air oven at 600°C for 24-48 h and then the leaves were ground to fine powder. 20 g of dried powder was placed in 100 mL of distilled water in a conical flask and then kept on the rotary shaker for 24 h. Then, it was filtered through four layer of muslin cloth and centrifuged. The supernatant was collected and this crude extract was diluted with 5% Dimethyl Sulfoxide (DMSO) and stored at 4°C in air tight black bottles for further use. Plant extracts were added to 1mM of Silver nitrate ($AgNO_3$). The solution was heated at 65°C on a hot plate magnetic stirrer for 90 minutes. The appearance of colour change exhibited the formation of AgNPs. Extracts from various plants may act both as reducing and capping agents in AgNPs synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides, and vitamins [48,49]. For synthesis of AgNPs using fungi; the fungi was grown in Sabouraud's broth (Peptone-10g; Glucose-40g; NaCl-5g; Agar agar-20 g in 1000mL D/W having pH-5.2) and flasks containing broth were incubated in an incubator shaker at 18000 rpm at 37ºC. After three days of incubation, mycelia was separated by centrifugation and washed thrice with deionised water. The washed mycelia were suspended in deionised water and the aqueous filtrate obtained was added to 100 mL of $AgNO_3$.

By Transmission Electron Microscopy the size and shape of the AgNPs was determined at SICART, Anand. The sample was prepared by putting 1-2 drops of sample suspension to a clean 300 mesh carbon supported copper grid operating at 200KV.

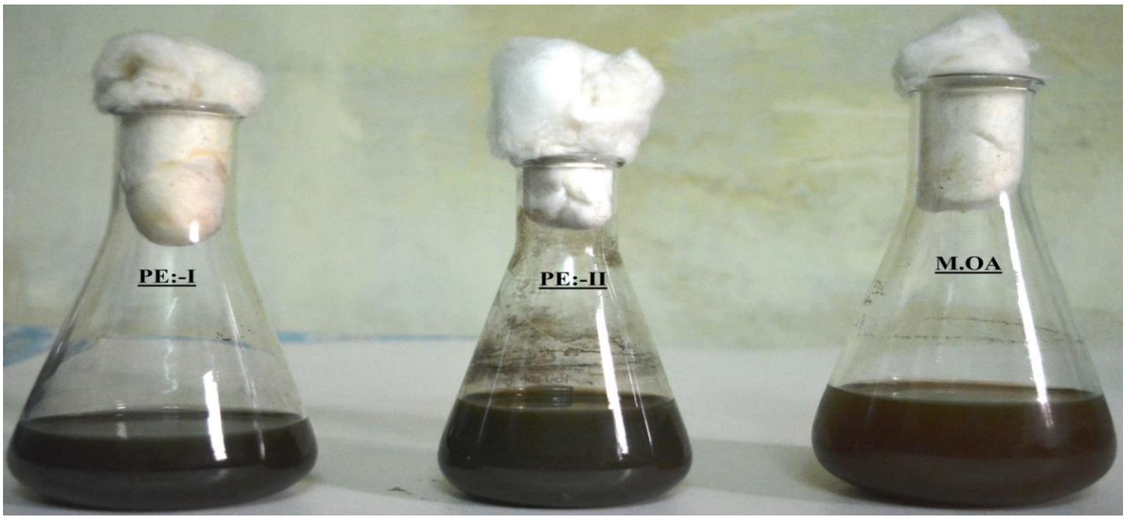
There are numerous techniques to perform antibacterial and antimicrobial susceptibility tests. The techniques include 1) agar disk diffusion, 2) broth dilution, 3) agar dilution and 4) E- test method (modification of the disk diffusion and the agar dilution method) [50]. Agar disk diffusion is a traditional and routine method for antimicrobial susceptibility tests [51]. Here in this study, the antibacterial activity has been tested by using two different methods: Disc Diffusion method and Cup Borer method. Bactericidal effects were observed in *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris, Vibrio cholerea and Corynebacterium amycolactum.*

In nutrient agar and blood agar plates three wells each for different sample were made and 25µL of AgNP solution was poured using micropipette. Plates were incubated for 24 h at 37ºC. After incubation period, zone of inhibition was measured in cm. Similarly, in disc diffusion method paper discs were used and plates were incubated at 37ºC for 24h. After incubation period, zone of inhibition was measured in cm.

Results and Discussion

As the plant extract and microbial filtrate was added to the $AgNO_3$ solution, it started giving brown colour due to reduction of silver ion; which indicated the formation of AgNPs, which is indicated in Plate:1. It is generally accepted that UV–Visible spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous solution. Figure: 1-3 shows the UV–Visible spectra recorded from the reaction medium after 90 minutes. The absorption spectra of silver sol consists a single sharp surface plasmon resonance band at 400 nm and 300nm for plant extract and microbial filtrate respectively. The Ag-nanocrystals that emerged in the images have variety of shapes: spherical, triangle and irregular. In agreement with the UV-Vis spectrophotometric observations, the TEM images reveal that Ag-nanocrystals are polydisperse and roughly spherical of diameter ranging from 3-23 nm.

Plate :1



PE:-I: ***Mentha sachalinensis***
PE:-II: ***Adhatoda vasika***
M.OA:- ***A.niger***
[PE:- Plant Extract; M.OA:- Microorganism. ***A.niger*****]**

Spectral Analysis Using UV-Visible Spectrophotometer

Fig:1: Plant Extract Synthesized AgNPs(*Mentha sachalinensis*)

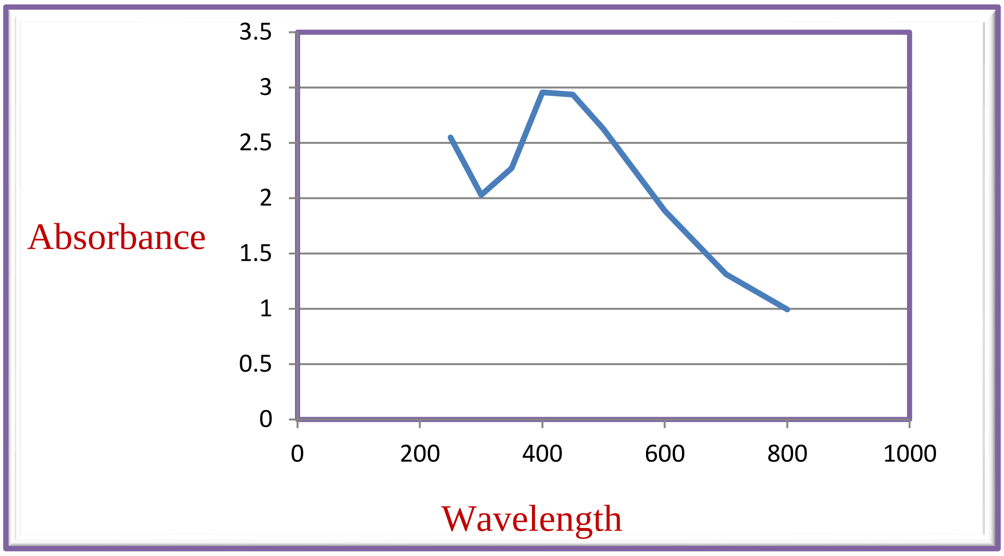


Fig-2: Plant Extract Synthesized AgNPs(*Adhatoda vasika*)

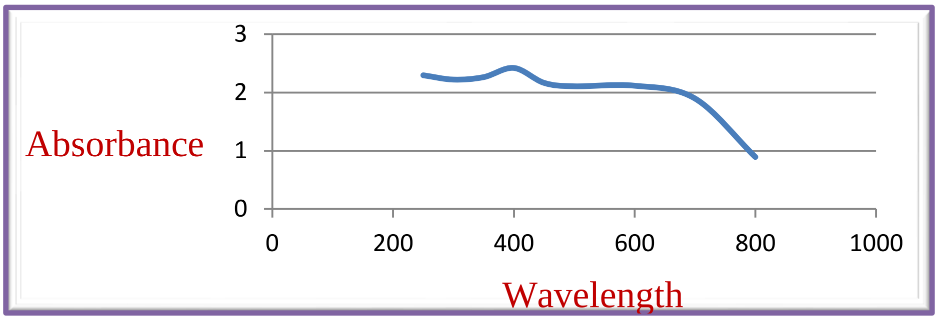


Fig-3: *A.niger* synthesized AgNPs

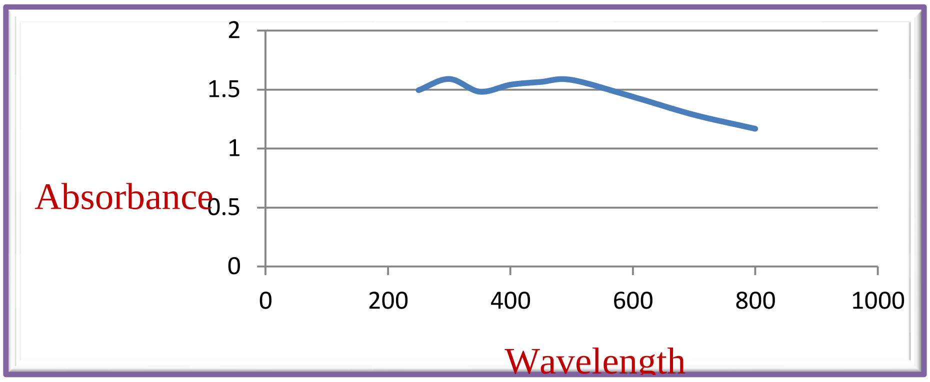


Plate-2:Plant Extract synthesized AgNPs(*Mentha sachalinensis*)

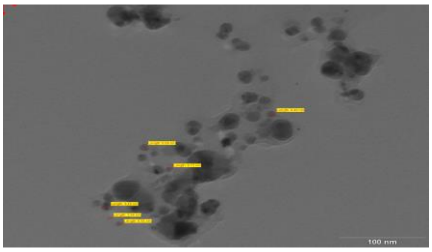


Plate-3: Plant Extract synthesized AgNPs(*Adhatoda vasika*)

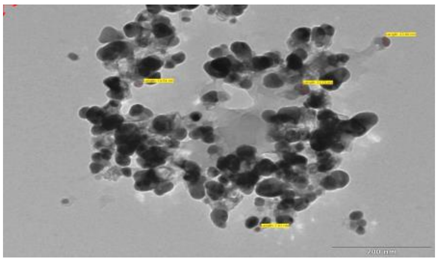


Plate-4: Microbially *A.niger* synthesized AgNPs

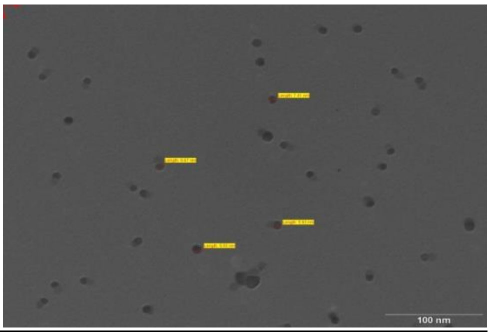
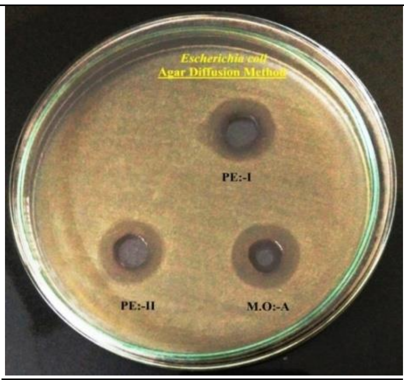


Plate-5: Zone of inhibition against *E.coli*



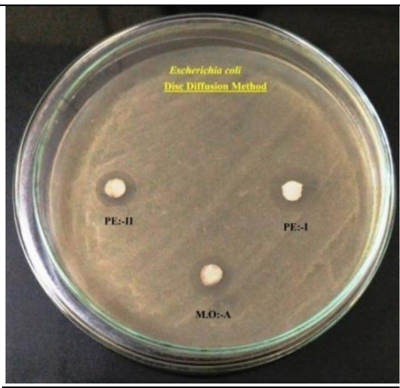
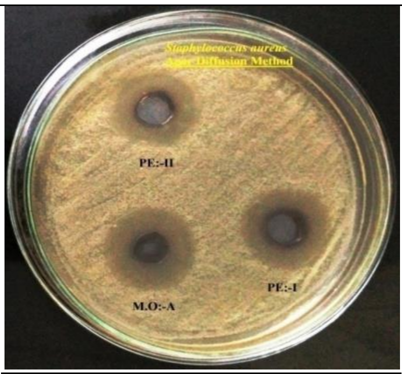


Plate-6: Zone of inhibition against *S.aureus*



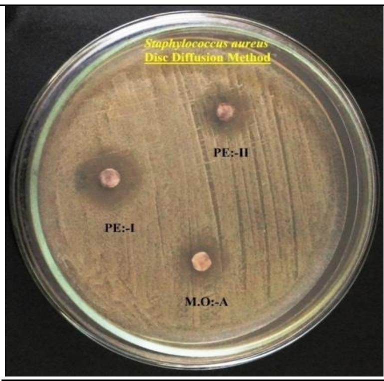
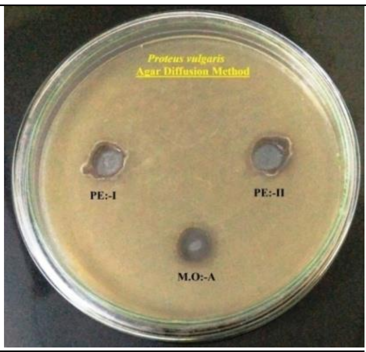


Plate-7: Zone of inhibition against *P.vulgaris*



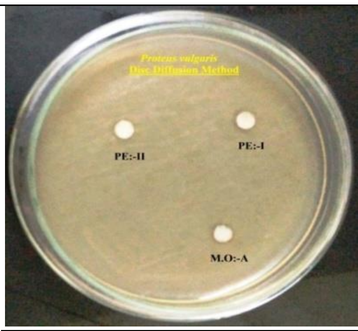
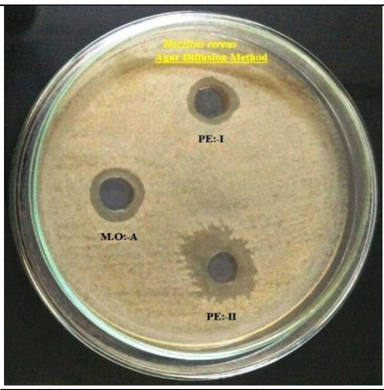


Plate-8: Zone of inhibition against *B.cereus*



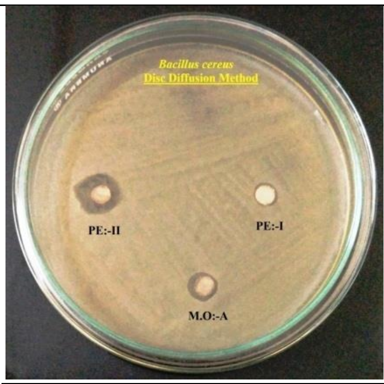


Plate-9: Zone of inhibition against *V.cholerea*

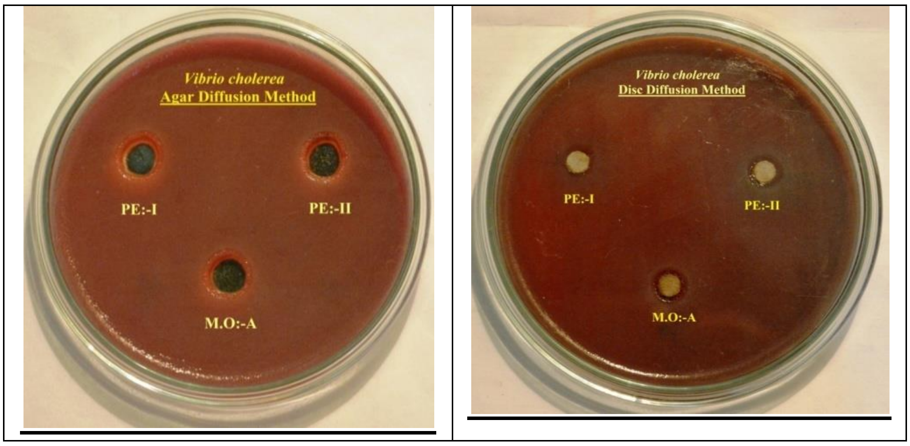
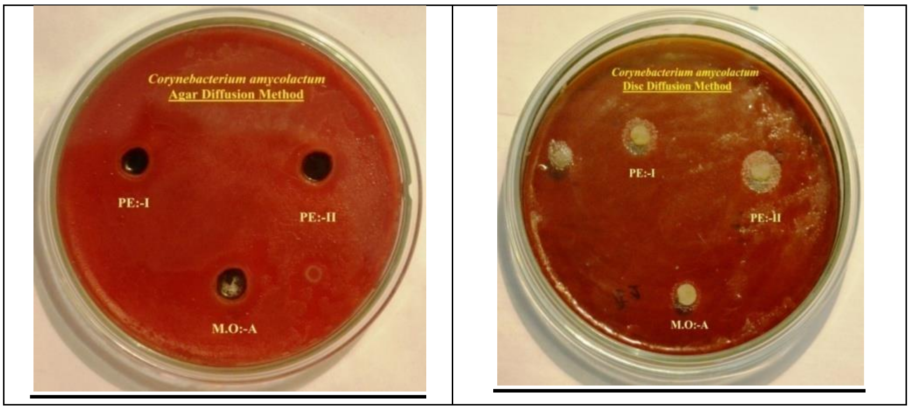


Plate-10: Zone of inhibition against *C.amycolatum*



Reduction of silver ion into AgNPs during exposure to plant extract as well as microbial filtrate was observed as a result of colour change. AgNPs exhibits dark-yellowish brown colour in the aqueous solution due to the surface plasmon resonance. UV-Vis spectroscopy of the colloidal solution of AgNPs has been recorded and the absorbance peak at 400 nm and 300 nm of plant extracts and *A. niger* filtrate respectively, indicates that the particles are polydispersed in nature. The TEM images showed relatively spherical shaped nanoparticles formed with the diameter range 3-23 nm. The most potent effect, of the synthesized AgNPs was observed against *E.coli*; *S. aureus; Vibrio cholerea* as well as *Corynebacterium amycolatum.*

Conclusion

AgNPs were synthesized using plant extract and microbial filtrate. Characterization was done using UV-Vis spectra and TEM. The TEM images revealed that the Ag-nanocrystals are polydispersed and roughly spherical of diameter ranging from 3-23 nm. Synthesized AgNPs showed antibacterial activity against *Escherichia coli; Staphylococcus aureus; Proteus vulgaris; Bacillus cereus; Vibrio cholerea* and *Corynebacterium amycolatum.* Biosynthetic methods using plant extract and microbial filtrate have shown a great potential in AgNP synthesis. A progress in this area will give new green paths in the development of controlled shape and size AgNPs. Custom designed biomolecules can then be made to synthesize AgNPs, which in turn will fill the gap between biological synthesis and biometric synthesis. Also, further studies are needed to understand the exact role of free radicals in the antimicrobial activity of nanoparticles and the mechanisms of antimicrobial properties in the particles.

References

1. Seltenrich, N. //(2013) *Environ. Health Perspect*. 121**:** 220.
2. Asare, N., Instanes, C., Sandberg ,W.J., Refsnes, M., Schwarze ,P., Kruszewski, M. and Brunborg ,G. (2012) // *Toxicology* 291 **:** 65.
3. Demir, E., Vales,G., Kaya, B., Creus, A. and Marcos, R. (2011) // *Nanotoxicology* 5 **:** 417**.**
4. Kim, H.R., Kim, M.J., Lee, S.Y., Oh ,S.M. and Chung, K.H. (2011) // *Mutat. Res*. 726 **:** 129.
5. X. Li, L. Xu, A. Shao, G. Wu and Hanagata ,N. (2013) // *J. Nanosci. Nanotechnol*. 13 **:** 161.
6. Govender,R., Phulukdaree, A., Gengan, R.M., Anand, K. and Chuturgoon, A. A. (2013) // *J. Nanobiotechnology* 11 **:** 5.
7. Kim ,A.S., Chae, C.H., Kim, J., Choi,J.Y. Kim, S.G. and Baciut, G. (2012) // *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 113 : 789.
8. Lee,Y.S., Kim,D.W. ,Y.H. , Oh, J.H. S. Yoon, Choi, M.S., Lee, S.K., Kim, J.W., ` Lee K. and Song, C. W. (2011)// *Arch. Toxicol.* 85 : 1529.
9. Satapathy, S.R., Mohapatra,P. R. Preet, D. Das, B. Sarkar, T. Choudhuri, M.D. Wyatt and Kundu, C.N. (2013) // *Nanomedicine (Lond*) 8 : 1307.
10. Capek, I., (2004) Preparation of metal nanoparticles in water-in-oil (w/o) microemulsions. *Adv Colloid Interface Sci*. 110 : 49-74.
11. Frattini, A., Pellegri, N., Nicastro, D., Sanctis, (2005) OD. Effect of amine groups in the synthesis of Ag nanoparticles using aminosilanes. Mater Chem Phys. 94 :148–52.
12. Zhang Z, Zhang X, Xin Z, Deng M, Wen Y, Song Y. (2011)Synthesis of monodisperse silver nanoparticles for ink-jet printed flexible electronics. *Nanotechnol*; 22(42) :425-601.
13. Thirumalai Arasu V, Prabhu D, Soniya MJ. (2010) Stable silver nanoparticle synthesizing methods and its applications. *J Biosci Res*;1 :259-70**.**
14. Prabhu S, Poulose EK. (2012**)** Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Nano Lett*;8 **:**1–10.
15. Ahamed M, AlSalhi MS, Siddiqui MKJ: (2010) Silver nanoparticle applications and human health. Clin Chim Acta, 411: 1841–1848. 10.1016/j.cca.2010.08.016
16. García-Barrasa J, López-de-luzuriaga JM, Monge M: (2011) Silver nanoparticles: synthesis through chemical methods in solution and biomedical applications. Cent Eur *J Chem,* 9: 7–19. 10.2478/s11532-010-0124-x

17. Tran QH, Nguyen VQ, Le AT: (2013) Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. *Adv Nat Sci: Nanosci Nanotechnol*, 4: 033001. 10.1088/2043-6262/4/3/033001
18. Shameli K, Ahmad MB, Zargar M, Yunus WMZW, Ibrahim NA, Shabanzadeh P, Moghaddam MG: (2011) Synthesise and characterization of silver/montmorillonite/chitosanbionano composites by chemical reduction method and their antibacterial activity. *Int J Nanomed*,6: 271–284.
19. Shameli K, Ahmad MB, Yunus WMZW, Ibrahim NA, Darroudi M: (2010) Synthesis and characterization of silver/talc nanocomposites using the wet chemical reduction method. *Int J Nanomed*, 5:743–751.
20. Navaladian S, Viswanathan B, Viswanath RP, Varadarajan TK: **(**2007) Thermaldecomposition as route for silver nanoparticles. *Nanoscale Res Lett*, 2:44–48.
21. Rodriguez-Sanchez L, Blanco MC, Lopez-Quintela MA: (2000) Electrochemicalsynthesis of silver nanoparticles. *J PhysChem B*, 104: 9683–9688.
22. Perelshtein I, Applerot G, Perkas N, Guibert G, Mikhailov S, Gedanken A(2008):Sonochemical coating of silver nanoparticles on textile fabrics (nylon,polyester and cotton) and their antibacterial activity. *Nanotechnology*, 19: 245-705.
23. Mallick K, Witcomb MJ, Scurrell MS: (2004) Polymer stabilized silver nanoparticles:A photochemical synthesis route.*J Mater Sci*, 39 : 4459–4463.
24. Yin H, Yamamoto T, Wada Y, Yanagida S: (2004) Large-scale and size-controlledsynthesis of silver nanoparticles under microwave irradiation. *MaterChemPhys*, 83 : 66–70.
25. Shameli K, Ahmad M, Yunus WMZW, Rustaiyan A, Ibrahim NA, Zargar M, Abdollahi Y: (2010) Green synthesis ofsilver/montmorillonite/chitosanbionanocomposites using the UV irradiation method and evaluation ofantibacterial activity. *Int J Nanomed*, 5 **:**875–887.
26. Shameli K, Ahmad MB, Yunus WMZW, Ibrahim NA, Gharayebi Y, Sedaghat S: (2010) Synthesis of silver/montmorillonitenanocomposite using γ-irradiation. *Int J Nanomed*, 5 :1067–1077.
27. Darroudi M, Ahmad MB, Abdullah AH, Ibrahim NA: (2011)Green synthesis andcharacterization of gelatin-based and sugar-reduced silver nanoparticles. *Int J Nanomed*, 6: 569–574.
28. Raveendran P, Fu J, Wallen SL: (2003) Completely “Green” Synthesis andStabilization of Metal Nanoparticles. *J AmChemSoc*, 125: 13940–13941.
29. Vigneshwaran N, Nachane RP, Balasubramanya RH, Varadarajan PV: (2006) A novel one-pot‘green’ synthesis of stable silver nanoparticles using solublestarch. *Carbohyd Res*, 341 :2012–2018.
30. Sharma VK, Yngard RA, Lin Y: (2009)Silver nanoparticles: Green synthesis and their antimicrobial activities. *Adv Colloid Interfac*, 145: 83–96.
31. Nino-Martinez N, Martinez-Castanon GA, Aragon-Pina A, Martinez-Gutierrez F, Martinez-Mendoza JR, Ruiz F. (2008) *Nanotechnology*, 19(6) :065711/1-065711/8.

32. Alt V, Bechert T, Steinrücke P, Wagener M, Seidel P, Dingeldein E, et al. (2004)*Biomaterial*, 25 :4383.
33. Russell AD, Hugo WB. (1994) *Prog Med Chem*, 31:351.
34. Lee HY, Park HK, Lee YM, Kim K, Park SB. (2007) *Chem Commun*:2959.
35. Jeong S, Yeo S, Yi S. (2005) *J Mater Sci*, 40: 5407.
36. Kvitek L, Panacek A, Soukupova J, Kolar M, Vecerova R, Prucek R, et al. (2008) *J Phys Chem C*, 112: 5825.
37. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri J, Ramirez JT, et al. *Nanotechnology*, 16 (2005)2346.
38. Chou WL, Yu DJ, Yang MC, (2005) *Polym Adv Technol*, 16: 600–607.
39. Ulkur E, Oncul O, Karagoz H, Yeniz E, Celikoz B, (2005) 31 :874–877.
40. Parikh DV, Fink T, Rajasekharan K, Sachinvala ND, Sawhney APS, Calamari TA,Parikh AD: (2005) *Text Res J*, 75 :134–138.
41. Rupp ME, Fitzgerald T, Marion N, Helget V, Puumala S, Anderson JR, Fey PD: (2004) *J Infect Control*, 32: 445–450.
42. Samuel U, Guggenbichler JP: Prevention of catheter-related infections: (2004) *J AntimicrobAgents*, 23: 75–78.
43. Yuranova T, Rincon AG, Bozzi A, Parra S, Pulgarin C, Albers P, Kiwi(2003) *J: PhotochemPhotobiolA*, 161:27–34.
44. Jeong SH, Yeo SY, Yi SC: (2005) *J Mater Sci*, 40:5407–5411.
45. Chou WL, Yu DG, Yang MC(2005): *PolymAdvTechnol*, 16:600–607.
46. Sun RW, Chen R, Chung NP, Ho CM, Lin CL, (2005) Che CM:*ChemCommun*, 40:5059–5061.
47. Chandani, D., Gaurav S. (2014) *J. International Journal of Sciences & Applied Research*, 1(2): 58-63.
48. Collera-Zuniga O, Jimenez FG, Gordillo RM.(2005) *Food Chem*, 9: 109.
49. Jagadeesh BH, Prabha TN, Srinivasan K.(2004) *Plant Sci*, 167**:**1263
50. Baker CN, Stocker SA, Culver DH, Thornsberry C. (1991) *J ClinMicrobiol*, 29: 533–538.
51. Erfani Y, Rasti A, Mirsalehian A, Mirafshar SM, Ownegh V. (2011): *J Microbiol Res*,5: 608–611.