Synthesis of Silver Nanoparticles by Green method Using *Vernonia anthelmintica* and its Antibacterial activity.

Tarannum RashidPatave[1] *, Dr. Aquil-ur Rahim Siddiqui[2]

[1] *Y.B.Chavan College of Pharmacy, affiliated to Dr. Babasaheb Ambedkar Marathwada University, India.*
[2] *Shree Bhagwan College of Pharmacy, affiliated to Dr. Babasaheb Ambedkar Marathwada University, India.*

* Corresponding Author. E-mail: tarannumpatave@gmail.com(T.P.); Tel. +91-742-092 65 65.

ABSTRACT:

In the modern years there is enlarged use of silver nanoparticles (SNPs) in industrial and in the field of medicine practice significantly, still their high risk and lethal outcome have not been studied considerably. The aim behind this research work was to incorporate silver nanoparticles (AgNPs) using Vernonia anthelmintica seeds extract and to assess its antibacterial activity. AgNPs were properly synthesized by green method and evolution of AgNPs was firmed by optical color change and UV (ultraviolet) spectroscopy. Synthesized AgNPs were decontaminated and characterized by using Silver Content Estimation-Inductive Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES),Zeta potentiometry, X-ray diffraction (XRD), and transmission electron microscopy (TEM). UV peak at 453.5 nm confirmed the formation of AgNPs. XRD studies disclosed crystalline nature of AgNPs. ICP-AES studies showed the presence of silver in colloidal dispersion and images were recorded by using HRTEM. Synthesized AgNPs were found to be potent against Staphylococcus aureus, Pseudomonasaeruginosa, E. coli and MRSA. However, these SNPs are cytotoxic in nature and could obey as a superior antibacterial for therapy of bacterial infections in this period of multiplr drug defience.

KEYWORDS: Antibacterial activity; *Vernonia anthelmintica;* silver nanoparticles (AgNP)

1. INTRODUCTION

The synthesis of nanoparticles has acquired considerable curiosity due to the extending need to begin environmental friendly technologies in substance synthesis. Out of number of metallic nanoparticles silver is feasibly the most widely acknowledged for its use in the branch of technology [1–3]. By using plant extracts or by using microorganisms are green methods for synthesis of AgNPs. By using Microorganism is very costly methodand needs advanced laboratory, skilled personnel for handling, and also difficulty in refining of AgNPs from matrix. With regard this, take exception to microorganism ; use of plant extract gives new enviromentally sound approach[4].The utilization of plants can also be appropriately scaled up for huge scale synthesis of nanoparticles in a controlled means according to their dimension, form and dispersity. Besides, in synthesis of Nanoparticles, the use of plant extract is more favourable than other processes.The literature survey which have demonstrated that synthesis of AgNPs by using plants like Alfalfa Sprouts [5], Jatropha curcas [6] , Aloe vera [7], Cymbopogon flexuos [8], green tea [9], neem leaf broth [10], natural rubber [11], starch [12], aloe vera plant extract [13], lemongrass leaves extract [14,15] leguminous shrub [16], and Emblica officinalis [17].After synthesis, specific particle characterization is needed, because the physicochemical properties of a particle could have a notable effect on their biological assets. In order to mark the safety issue to use the full potential of any nano material in the purpose of nanomedicines, or in the health care industry, it is needed to characterize the prepared nanoparticles before application.

Vernonia anthelmintica seeds belongs to family Asteraceae, is an annual herb distributed throughout India. It possesses antibacterial, antifungal, antiinflamatory, anticancer, antioxidant activities [18]. In this study, we have reportedthatby the use of aqueous extract of *Vernonia anthelmintica* seeds synthesizes the silver nanoparticles in the solution of silver nitrate by reduction mechanism and its antibacterial activity.

2. MATERIALS AND METHODS

2.1 Plant Extract Preparation and its Phytochemical Studies

Thoroughly washed, grinded powder of plant material (10 g) was mixed with 100ml deionised water and boiled for 10min. By using Whattman Filter Paper it was filtered and filtrate used for synthesis of Silver nanoparticles. The remaining extract was used for Phytochemical studies like existence of carbohydrate, protein, flavonides, phenols, tannins and Antioxidants [19].

2.2 AgNPs Synthesis

1mM silver nitrate solution was prepared using deionized water. And in that prepared plant extract filtrate in different volume ratios (10:1, 10:2, 10:3, 10:4, and 10:5) was added to form silver nanoparticles. Generation of AgNP's was observed by optcal color change from yellowish to dark brown and confirmed by UV spectroscopy.

2.3 AgNPs Purification

Purification of AgNP's were carried out by using high speed centrifuge at 17000 rpm, 4^0C, for 20 min. After centrifugation, settled AgNPs were collected.

To affirm the purity of synthesized AgNP's, the UV spectrum was taken before and after centrifugation. Presence of pure silver metal was also confirmed by EDAX analysis. Purified AgNPs were stored for characterization.

2.4 Characterization of AgNPs

2.4.1 Surface Plasmon Resonance (SPR) Studies

UV–Vis spectrophotometer(S-ican) was used to confirm the presence of SPR peak after each synthesis. This peak is the indicator for the presence of AgNPs. The samples were examined in wavelength range of 200–700 nm.

2.4.2 Dynamic Light Scattering Studies

To determine the zeta potential,particle size, and particle size distribution of colloidal dispersionof AgNPs the Malvern Zetasizer was used. The nanoparticles were accordinglydiluted using deionized water and analyzed.

2.4.3 X-Ray Diffraction

Philips PRO expert diffractometer was used to record X-ray diffraction data of AgNP's.

2.4.4 Transmission Electron Microscopy (TEM)

TEM images were recorded using Hitachi (H-7500, Japan)120 kV equipped with CCD camera. Samples were spottedonto a carbon-coated copper grid and scanned through1,50,000_to 3,00,000_magnification to get clear images.

2.4.5 Silver Content Estimation-Inductive Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

ICP-AES (SPECTRO ARCOS, Germany) was used for the analysis. 300ml AGNPs reaction mixture was centrifuged and purified AGNPs was dispersed in 1ml of distilled water. From this 1 ml of stock solution, 50 µl was digested (nitric acid and HCl) and diluted upto 5ml. These diluted samples were analyzed for silver content.

2.5 Antibacterial Activity

Agar well diffusion assay method was used to study antibacterial activity of AgNPs synthesized by different conditions. Antibacterial activity was performed against four microorganisms, viz, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and MRSA. MullerHinton agar media was prepared, sterilized, and aseptically transferred to petri plates. These petri plates were kept for solidification of media and later kept in incubator overnight to confirm that the petri plates are free from contamination.These petri plates were then inoculated with test organisms and wells were prepared for loading test samples.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by serial dilution technique [20]. Accordingly, the lowest concentration of AgNPs showing growth inhibition (visual observation)was considered as the MIC whereas the lowest concentration of AgNPs that showed zero growth on Mueller Hinton (MH) agar plates after spot inoculation and incubation for 24 h was recorded as MBC. Assay was performed in triplicate with appropriate controls (medium without inoculum and medium without AgNPs) [21].

3. RESULT & DISCUSSION

3.1 Preparation of Plant Extract and Preliminary Phytochemical Screening

It was observed that the aqueous solution of plant extract was dark yellowish in color. Preliminary phytochemical screening revealed that the plant consists of flavonoids, saponins, proteins, carbohydrates, and phenolic compounds.

3.2 Synthesis of AgNPs

AgNPs were prepared by addition of plant extract to the solution of silver nitrate. After formation of AgNPs, visual color change was observed from yellowish to dark brown as shown in Figure 1. Formation of AgNPs was confirmed by UV spectroscopy which showed a characteristic peak at 453.5 nm (Figure 2).Volume ratio of silver nitrate (1mM) to plant extract was fixed at 10:3. It was found that synthesis of AgNPs was completed in 4 h.

3.3 Purification of AgNPs

AgNPs were successfully purified by repeated centrifugation at 17000 rpm for 20 min. After centrifugation, the pellets were dispersed in deionized water to check the purity of synthesized AgNPs. Purity of AgNPs was confirmed by UV spectrophotometry where all other peaks except the characteristic peak of AgNPs were absent as shown in Figures 2.

3.4 Characterization of AgNPs

UV–visible spectrophotometer gives the idea about the shape and size of particle which mainly governs optical and electronic properties of AgNPs and consequently affects its biomedical applications[22] . In the present research, it was used to confirm the formation and stability of AgNPs in aqueous colloidal dispersion. Change in color from yellowish to dark brown color was observed due to surface plasmon vibrations in AgNPs [23] which was indicated by UV–visible peak at 453.5 nm (typical band of AgNPs). Broadening of UV–visible peak suggests that synthesized AgNPs were polydisperse in nature [24]. The stability of synthesized AgNPs was confirmed by measuring shift in wavelength at an interval of one week for 90 days. There was no significant change in the wavelength of AgNPs on storage, which suggests that the AgNPs did not aggregate and were stable during this period. The reason for stability was attributed to capping of AgNPs with phytochemicals used for synthesis of AgNPs. Soluble phytochemicals like flavonoids, polyphenols, tannins, and proteins encapsulate AgNPs generating negative charge on surface. Nano-silver bearing negative charge exhibit Brownian moments and are well dispersed in dispersion medium leading to formation of stable silver colloid [25].

Particle size, zeta potential and polydispersity index (PDI) of AgNPs were 102.2 ± 0.6 nm,-30.1± 1.41 and 0.27± 0.002 respectively. Particle size of AgNPs (102.2 ± 0.6 nm) measured by zeta sizer corresponds to hydrodynamic diameter of particles which is not the actual diameter of nanoparticles but is always greater than the actual diameter of nanoparticles. Hydrodynamic diameter is the diameter of the particle along with the coated phytochemicals which are mainly responsible for stability of nanoparticles whereas actual diameter of the particle is represented by TEM. Due to coating of phytochemicals like flavonoids and polyphenols onto AgNPs, hydroxyl groups of these phytochemicals generate negative charge onto these nanoparticles which was confirmed by negative zeta potential value (-30.1± 1.41). Stability of the AgNPs is directly proportional to the magnitude of zeta potential. PDI of synthesized AgNPs (0.2 ± 0.002) was below 0.4 which suggests that the nanoparticles were almost monodisperse in nature.XRD was used to confirm the crystalline or amorphous nature of particles [26]. In the present study, XRD spectrum showed the presence of peaks as per Bragg's reflection from (111) and (200) planes of face center cubic (FCC) crystal structure corresponding to the 2θ value of 38.092,and 44.22 which was under control with the standard values of JCPDS No.: 04- 0783 for silver (Fig. 3). Analysis of XRD spectrum of synthesized AgNPs with standard values confirmed that AgNPs were nanoclusters in nature. In addition to the typical Bragg's peak representative of FCC AgNPs, additional peaks were also observed suggesting that the crystallization of phytoconstituents appeared on the surface of the AgNPs.

A TEM image representing the morphology of AgNPs at 300,000×magnification propose that AgNPs were construct to be polymorphic showing triangular, hexagonal, deformed spherical and rod model morphologies (Fig. 4a). From TEM images, it could be concluded that phytochemicals which are used as a green source for synthesis of AgNPs have control on particle size and morphology. (Figure 4b) shows the selected area electron diffraction (SAED) pattern obtained from Ag NPs.

3.5. Silver Content Estimation-Inductive Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

ICP-AES showed colorless compound when samples were treated with Nitric acid and confirmed that the silver is entirely solubilized in solution. The concentration of synthesized AgNPs quantified by using ICP-AES was found to be 426.38 µg/mL.

3.6 Antibacterial Activity

MIC was ascertained by optical observation method in which the lowest concentration of AgNPs prevetns visible growth in tubes when compared to control one (growth organism).Inoculation of all concentrations

were equal to or higher than MIC was carried out on Muller Hinton agar plates and incubated for 24hrs. The concentration of prepared AgNPs which showed zero colony forming units (CFU) was recorded as MBC (Figure 5). The MICs were 13.32 µg/ml for MRSA, 26.64 µg/ml for S. aureus, 59.29 µg/ml for E. coli, 13.32 µg/ml for PA while the MBCs were 26.64 µg/ml for MRSA, 59.29 µg/ml for S. aureus, 106.59 µg/ml for E. coli, and 26.64 µg/ml for PA. Effect of AgNPs against E. coli, PA, SA and MRSA certifies its vigorous anti-microbial activity against all organisms causes infections.

3.7 Cytotoxicity Evaluation of AgNPs

Cytotoxicity evaluation ofAGNPs disclosed ≥90% cell viability at all concentrations. Absence of toxicity of *Vernonia anthelmintica* seeds stabilized AgNPs even at higher concentrations confirms its innocuous nature (Figure 6).

4. CONCLUSION

In the current study we revealed that *Vernonia anthelmintica* due to its antioxidant activity could be used productively as a green source for synthesis of AgNPs. Clear zone of inhibition propose that silver nanoparticle synthesize from plant extract could be effectively used as an antibacterial agent.

ACKNOWLEDGEMENT

Authors wish to acknowledge Y.B. Chavan College of Pharmacy, Aurangabad and Dr. Prabhakar Kore Basic Science Research Center, KLE University, Belgaum for providing required facilities for research. Authors are also thankful to SAIF, Panjab University, Chandigarh for TEM analysis.

Conflict of interest statement: The authors declared no conflict of interest.

5. REFERENCES

[1] Jadhav K, Dhamecha D, Dalvi B, Patil M. Green synthesis of silver nanoparticles using Salacia chinensis: characterization and its antibacterial activity. Particulate Science and Technology. 2015;33(5):pp 445-55.

[2] Jadhav K, Deore S, Dhamecha D, Hr R, Jagwani S, Jalalpure S, Bohara R. Phytosynthesis of silver nanoparticles: characterization, biocompatibility studies, and anticancer activity. ACS Biomaterials Science & Engineering. 2018;4(3): pp 892-9.

[3] Rajeshwari H R , Dinesh Dhamecha , Satveer Jagwani , Dhanashree Patil , Satisha Hegde , Raksha Potdar,et al. Formulation of thermoreversible gel of cranberry juice concentrate: Evaluation, biocompatibility studies and its antimicrobial activity against periodontal pathogensMaterials Science and Engineering: C.2017; (75) :pp 1506-1514 .

[4] Jadhav K, Dhamecha D, Bhattacharya D, Patil M. Green and ecofriendly synthesis of silver nanoparticles: characterization, biocompatibility studies and gel formulation for treatment of infections in burns. Journal of Photochemistry and Photobiology B: Biology. 2016;155: pp 109-15.

[5] Jorge L. Gardea-Torresdey , Eduardo Gomez, et al .Alfalfa Sprouts: A Natural Source for the Synthesis of Silver Nanoparticles *Langmuir* 2003;*19: pp* 1357-1361.

[6] Harekrishna Bar, Dipak Kr. Bhui, Gobinda P. Sahoo, et al, Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas* Colloids and Surfaces A: Physicochem. Eng. Aspects. 2009; 348:pp 212–216.

[7] Chandran, S., M. Chaudhari, R. Pasrichaa, A. Ahmed, and M. Sastry. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnology Progress. 2006;22:pp 577–583.

[8] Shankar, S., A. Rai, A. Ahmad, and M. Sastry. Controlling the optical properties of lemongrass extract synthesized gold nanotriagles and potential application in infra absorbing optical coatings. Chemistry of Materials. 2005; 17:pp 566–572.

[9] A.R. Vilchis-Nestor, V. Sanchez-Mendieta, M.A. Camacho-Lopez, R.M. Gomez- Espinosa, M.A. Camacho-Lopez, J.A. Arenas-Alatorre, Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract, Mater. Lett. 2008;62 : pp 3103–3105.
[10] S. Shiv Shankar, A. Rai, A. Ahmad, M. Sastry, Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth, J. Colloid Interf. Sci. 2004; 275: pp 496–502.

[11] N.H.H. Abu Bakar, J. Ismail, M. Abu Bakar, Synthesis and characterization of silver nanoparticles in natural rubber,Mater. Chem. Phys. 2007;104:pp 276–283.
[12] N. Vigneshwaran, R.P. Nachane, R.H. Balasubramanya, P.V. Varadarajan, A novel one pot 'green' synthesis of stable silver nanoparticles using soluble starch, Carbohyd. Res. 2006; 341:pp2012–2018.
[13] S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, Synthesis of gold nanotriangles and silver nanoparticles using Aloevera plant extract, Biotechnol. Prog. 2006;22:pp 577–583.
[14] S.S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmad, M. Sastry, Biological synthesis of triangular gold nanoprisms, Nat. Mater. 2004;3:pp 482–488.
[15] S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings, Chem. Mater. 2005; 17:pp 566–572.
[16] N.C. Sharma, S.V. Sahi, S. Nath, J.G. Parsons, J.L. Gardea-Torresdey, T. Pal, Synthesis of plant-mediated gold nanoparticles and catalytic role of biomatrix embedded nanomaterials, Environ. Sci. Technol. 2007; 41:pp 5137–5142.
[17] Ankamwar, B., C. Damle, A. Ahmad, and M. Sastry. Biosynthesis of silver and gold nanoparticles using Embellica officinalis fruit extract, their phase transfer and transmetallation in an organic solution. Journal of Nanoscience and Nanotechnology.2005.;5:pp 1665–1671.
[18] Manvar M. N., T R Desai, Vernonia anthelmintica Willd.: An Overview on Phytopharmacological Properties . ResearchGate.net Review article June 2015.
[19] Khandelwal, K. R. 2008. Practical pharmacognosy, 8th ed. Nirali Prakashan, Pragati Books Pvt. Ltd.: India.pp 220.

[20] Andrews, M. Jennifer, Determination of minimum inhibitory concentrations, J. Antimicrob. Chemother. 2001 :48: pp 5–16.
[21] J. Jain, S. Arora, M. Jyutika, R. Pratibha, S. Khandelwal, M. Kishore, Paknikar, Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use, Mol. Pharm. 2009;6: pp 1388–1401.

[22] S. Pal, T. Yu, J. Song, Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli, Appl. Environ. Microbiol. 2007;73: pp 1712–1720.

[23]Krishnaraj, E. Jagan, S. Rajasekar, P. Selvakumar, P. Kalaichelvan, N. Mohan, Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens, Colloids Surf. B: Biointerfaces . 2010;76 :pp 50–56.
[24] P. Patel, P. Agarwal, S. Kanawaria, S. Kachhwaha, S. Kothari, Plant-based synthesis of silver nanoparticles and their characterization, nanotechnology and plant sciences, Springer International Publishing, 2015;pp 271–288.
[25] H. Wang, X. Qiao, J. Chen, S. Ding, Physicochemical and engineering aspects: preparation of silver nanoparticles by chemical reduction method, Colloids Surf. A . 2005;256 : pp 111–115.
[26]Jadhav K, Rajeshwari HR, Deshpande S, Jagwani S, Dhamecha D, Jalalpure S, Subburayan K, Baheti D. Phytosynthesis of gold nanoparticles: Characterization, biocompatibility, and evaluation of its osteoinductive potential for application in implant dentistry. Materials Science and Engineering: C. 2018;93: pp 664-70.

FIGURES

Figure 1. Optical Colour changes after formation of AgNPs.

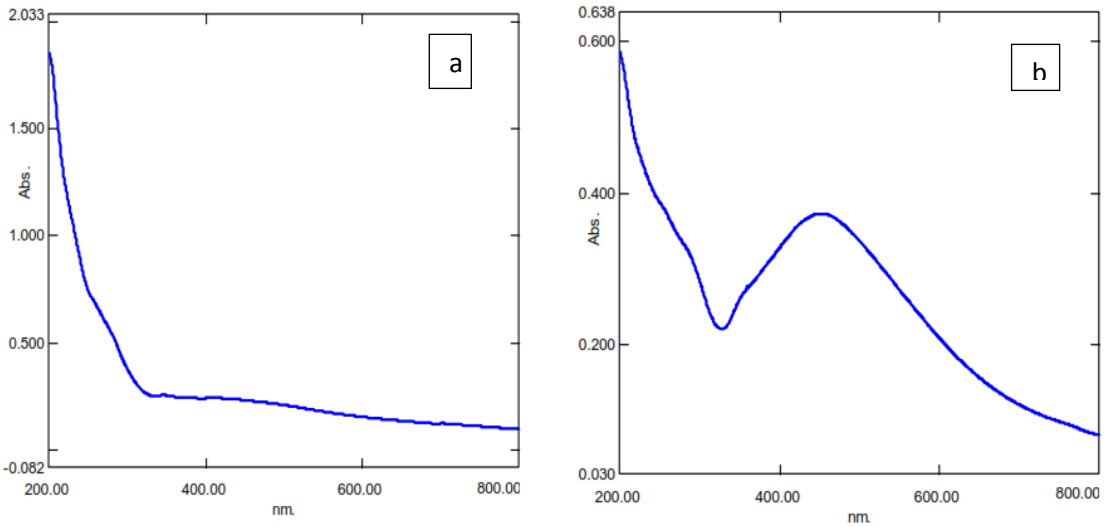

Figure 2. UV–visible spectrum of VA (a) before centrifugation (b) after centrifugation showing characteristic peak of *Vernonia anthelmintica* mediated silver nanoparticles.

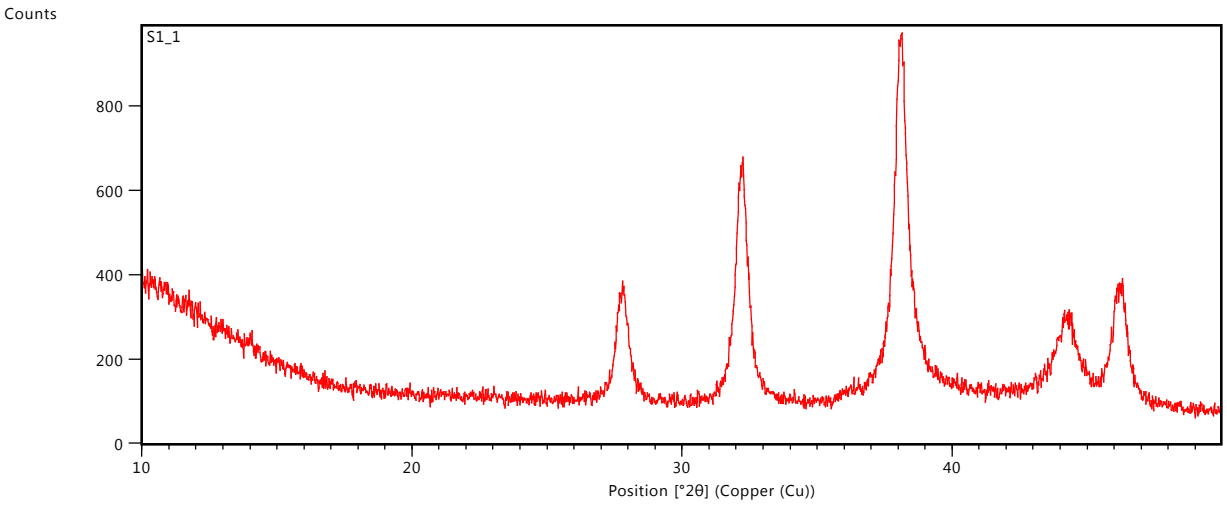

Figure 3. X-RD spectrum of synthesized AgNPs.

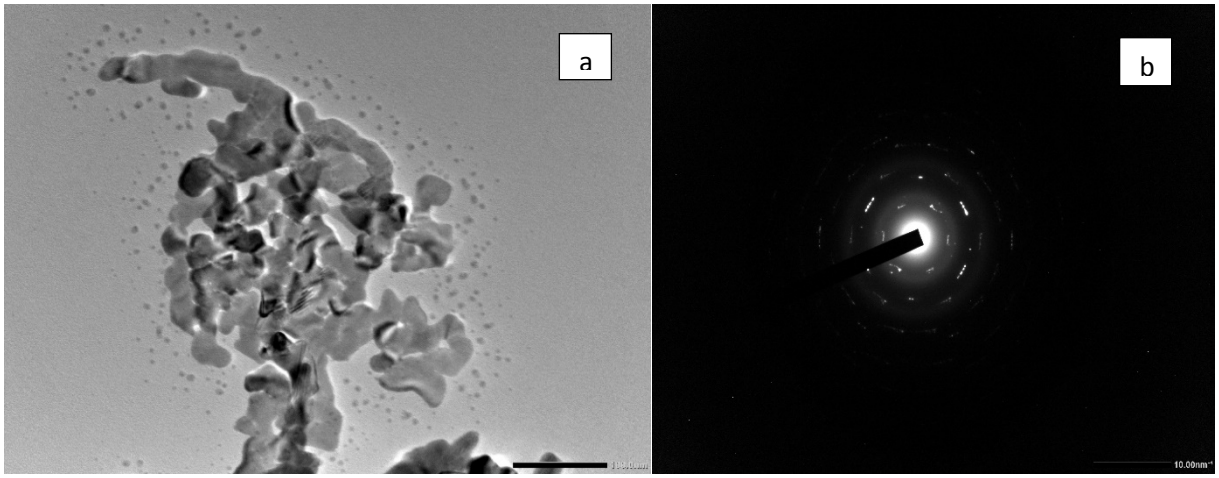

Figure 4a.TEM image of synthesized AgNPs , b. SAED pattern of AgNPs.

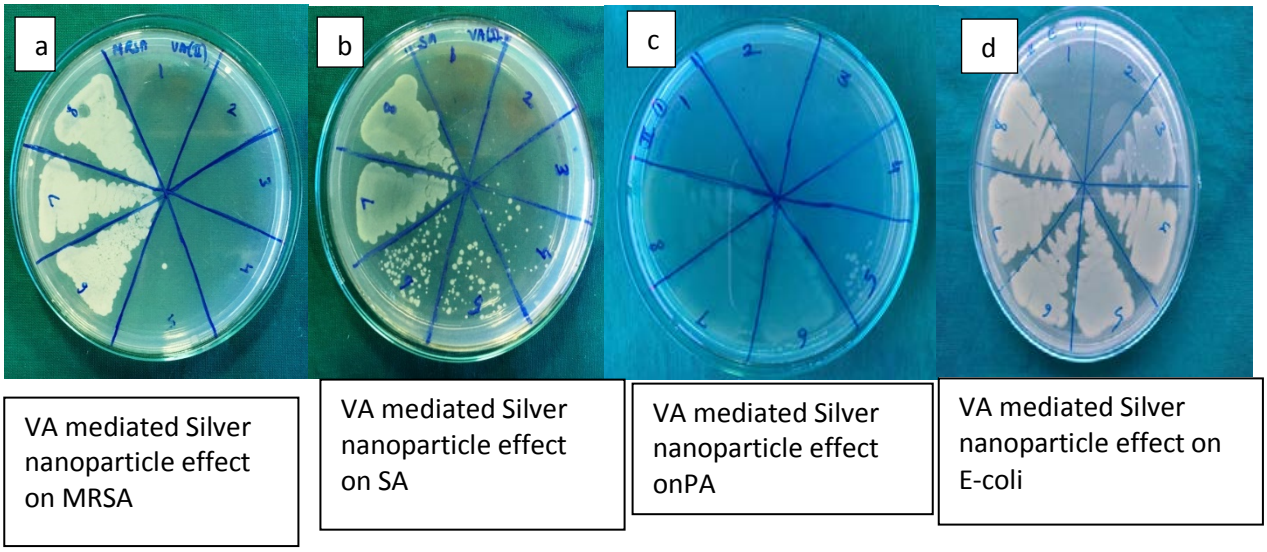

Figure 5. Antibacterial activity of AgNP against a) P. aeruginosa, b) S. aureus and c) methicillin resistant S. aureus & d) E. Coli

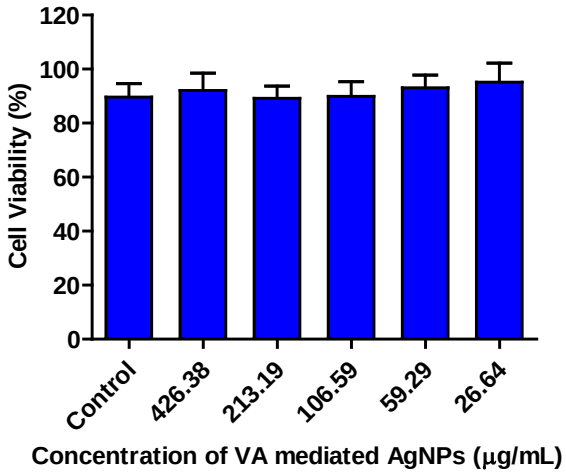

Figure 6. Cytotoxicity studies of synthesized AgNPs against L929 mouse fibroblast cell lines.