Biosynthesis of Silver nanoparticles using *Drimia indica* and exploring its antibacterial profiling

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Abstract:

Biological synthesis reflects as an eco-friendly, nontoxic and easy method of nanoparticle preparation. Present investigation deals with biosynthesis of silver nanoparticles using *Drimia indica* leaf extract. Initially the synthesized silver nanoparticles were characterized and confirmed by UV- Vis spectroscopy, X-ray diffraction (XRD) and SEM. The synthesized nanoparticles when used for determination of antibacterial activity, by Microtitre Broth Dilution method exhibited remarkable activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*.

Key Words: *Drimia indica*, *Klebsiella*, *Pseudomonas*, *Escherichia*, Microtitre Broth Dilution.

1. INTRODUCTION

Nanotechnology is the most immerging branch of Science and Technology. It is one of the most promising technologies applied in all areas of Science. Apart from its tremendous applications in Pharmaceuticals, textiles industries, electronics etc, it is ecologically sound and cost effective. Metal nanoparticles have received global attention due to their enormous applications in the biomedical and physiochemical fields. Nanoparticles aqueduct the space between bulky materials and molecular structures (Thakkar *et al*., 2010). Synthesizing metal nanoparticles using plants has been recognized as green and efficient way for further exploiting plants as convenient nanofactories (Singh *et. al.*, 2016). Nanoparticles with antimicrobial activity are advantageous in reducing acute toxicity, lowering cost and overcoming resistance as compared to other prevalent antibiotics (Pal *et al*., 2007 and Weir *et al*., 2008).

Antibiotics are used as antimicrobial agents in medical field. Higher doses of antibiotics causes harm to human cells and forms cancerous cells and mutations. Use of nanoparticles as antimicrobial agents is increasing day-by-day. The over doses of antibiotics harms human cells, causes paralysis with more disabilities.

Continued use of antibiotics for treatment of an array of infections has imposed the danger of developing antibiotic resistance. Widespread bacterial infection treatment regime involves higher initial doses of antibiotics followed by gradual lowering down the doses, which requires a longer span of time. A scenario of increased initial doses is practiced regularly which has developed antibiotic resistance for a specific microorganism or a set of microorganisms. Treatment plans for dreadful infections having selective antibiotics for use are compromised due to development of antibiotic resistance, as single antibiotic is used for treatment of such infections. Control measures may be possible in case of broad spectrum antibiotic used or in organisms that are non-selective to antibiotics. On the other hand there are infections which need specific/ selective antibiotics for cure. If resistance for single known antibiotic is developed in organisms, the treatment lines may go unattended or may fail totally. There is a need of promising interventions of multifaceted alternative products to antibiotics which can play vital role in the field of medical science.

Biogenic nanoparticles are used as replacement over antibiotics to target the microbes (Wang *et al*., 2017). Recently, researchers have showed the antimicrobial potential of biogenic nanoparticles on microorganisms. The use of nanoparticles as antimicrobial agents is increasing exceptionally in the field of medical science (Heather., 2017).

Through nanotechnology, highly medicinal plants can be directly used for nanoparticle synthesis. The biologically synthesized nanoparticles have helped in target-oriented drug delivery, which has lowered down the drug doses with increased efficacy (Patra *et al.*, 2018). The plant based synthesis of nanomaterials can be explored and used for human welfare.

Drimia indica (Asparagaceae) is a highly medicinal plant. Is is commonly known as Indian squill or Rankanda or jangali pyaz in India. It is pear-shaped, onion like, scaly bulb that grows up to 30 cm in diameter (Pretorius *et al*., 2005 and Crespo *et al*., 2020). *Drimia indica* contains cardiac glycosides, quinones, resins, saponins and steroids (Chittoor et al., 2012 and Pandey *et al*., 2014). It possesses antiprotozoal, hypoglycemic, anticancer, antidiabetic and antimicrobial properties. (Aswal *et al*., 2019 and Manganyi *et al*., 2021).

2. MATERIAL AND METHODS

2.1 Materials

The leaves of *Drimia indica* were collected from Dev Dari, Ambheri, District: Satara, State: Maharashtra (GPS– 17.605087º, 74.279927º). Chemicals used were purchased from Thomas Bakers (C) Pvt. Ltd.

2.2 Preparation of Plant extract

For the preparation of leaf extract, fresh leaves of *Drimia indica* were washed thoroughly under tap water and bolted dry. Leaves (20 g) were boiled into 100 ml of distilled water at 100 ℃ for 15 minutes. Extract was passed through Buchner's funnel and volume of filtrate was adjusted to 100 ml with distilled water.

2.3 Synthesis of Silver Nanoparticles

Leaf extract (20%) was taken into burette and 3mM aqueous solution of $AgNO_3$ was taken in conical flask. The flask was placed on magnetic stirrer with hotplate at 60℃. Leaf extract was added drop wise into conical flask containing 3mM $AgNO_3$ solution with continuous stirring. Colorless $AgNO_3$ solution turned yellowish brown which indicated the formation of silver nanoparticles after reduction of silver ions. After 24 hours the reaction mixture was centrifuged at 10000 RPM. The pellet was taken into beaker and washed with alcohol for 2-3 times followed by washes with distilled water. The pellet was dried in microwave oven and further used for characterization process.

2.4 Antibacterial Assay

The bacterial cultures were sub cultured on liquid nutrient broth and they were incubated at 37℃ for 24 hours in incubator with continuous stirring. The characterized nanoparticles were used for determination of antimicrobial potential by Microtitre Broth Dilution method on spectrophotometer against pathogenic bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. The antibacterial activity of silver nanoparticles was measured by calculating percent inhibition and minimum inhibitory concentration (MIC).

3. Result and Discussion

3.1 UV visible spectroscopy:

Synthesis of nanomaterials and their applications are having great importance in modern era. Silver nanoparticles were formed by gradual reduction of silver ions during reaction with *Drimia indica* plant extract. The reaction mixture showed the color change from colorless to light brown to dark brown colored solution.

The initial formation of silver nanoparticles was determined by UV-visible spectroscopy. The percentage of transmittance light radiation determines when light of certain frequency is passed through the samples. The spectrophotometer analysis records the intensity of absorption (A) or optical density (O.D) as a function of wavelength. The reaction mixture was taken in quartz cuvette and exposed to UV visible radiation (200-800 nm) to MultiscanSky_1530-00496C

spectrophotometer. The synthesized nanoparticles were well dispersed in the solution and were stable for long time by showing brown color to reaction mixture. The absorbance peak has already been recorded for nanoparticles which ranged from 2 to 100 nm in size. (Ravindra *et al*., 2014). The absorbance maxima recorded at 421 nm clearly indicated the formation of silver nanoparticles (Fig.1).

3.2 SEM:

The morphology and size of silver nanoparticles derived from *Drimia indica* leaf extract were characterized by Scanning Electron Microscope (Fig. 2). The image magnification was 10,000 X. From scanning electron microscope we obtained the average size of silver nanoparticles between 50-80 nm. The nanoparticles were spherical in shape and agglomerated. These synthesized nanoparticles were highly stable in nature.

3.3 XRD:

To investigate the crystalline nature and structure of AgNPs, the sample was analyzed on BRUKER- D8 ADVANCE machine at CFC, Shivaji University, Kolhapur. The X-ray diffraction pattern obtained for the silver nanoparticles synthesized from *Drimia indica* leaf extract showed five distinct diffraction peaks (Fig. 3) of 2θ values of 38.11° (111), 44.30°(200), 64.22° (220), 77.51 ° (311)and 81.47° (222) with the assigned planes indicated in the braktes. The free crystalline nature of synthesized nanoparticles was explained on the basis of JCPDS file. Analysis was done by using Origin Pro 8 software.

3.4 Antibacterial assay:

The antibacterial activity of silver nanoparticles synthesized from *Drimia indica* leaf extract was tested on pathogenic bacteria like *Pseudomonas aeruginosa* [ATCC2021], *Klebsiella pneumonia* [ATCC2075] and *Escherichia coli* [ATCC2065]. Bacterial cultures were maintained in liquid nutrient broth. Calculation of inhibition activity of synthesized nanoparticles on selected pathogenic bacterial cultures was done using different concentrations of AgNPs. Antibacterial assay was carried out in 96-well microtiter plate. The assay volume was set to 300 µl which contained 260 µl nutrient broth, 20 µl bacterial culture and 20 µl AgNPs. The AgNPs were used in varied concentrations (25 µl/ml, 50 µl/ml and 100 µl/ml from mg/ml stock). Replacement of 20 µl AgNPs with 20 µl distilled water in assay mixture served as negative control. The experiment was performed in triplicates. The 96-well microtitre plate was incubated for 12 hours. The readings were recorded after time interval of every 30 minutes in MultiscanSky_1530-00496C spectrophotometer. Graph was plotted which indicated sigmoid growth curve. Similar assay was repeated for all bacteria under study. The lowest concentration which inhibits the growth of bacterial strain is taken as MIC of the AgNPs for the pathogenic bacteria. Broad spectrum antibiotic streptomycin (positive control) in different concentrations (25 µl/ml, 50 µl/ml and 100 µl/ml) was used to calculate the MIC values for bacterial strains against streptomycin.

When AgNPs were tested for antimicrobial activity, *Pseudomonas aeruginosa* showed MIC value of 25 µl/ml, *Klebsiella pneumoniae* showed 25 µl/ ml and *Escherichia coli* showed 50 µl/ ml (Table 1).

Pseudomonas aeruginosa inhibition percentage (Fig. 4) was recorded to be highest (68%) at 100 µl/ml concentration of streptomycin while it was found to be lowest (45%) at 25 µl/ml Streptomycin concentration. The highest inhibition percentage (65%) at 100 µl/ml and lowest (43%) at 25 µl/ml was recorded for *Klebsiella* pneumoniae. While in case of *Escherichia coli* antibiotic Streptomycin showed highest inhibition percentage (69%) at 100 µl/ml and lowest inhibition percentage (47%) at 25 µl/ml (Fig. 4).

The highest inhibition percentage of Ag NP's against *Pseudomonas aeruginosa* (Fig.5) was observed to be 54% at AgNPs concentration 100 µl/ml and lowest 39% at 25 µl/ml. In case of *Klebsiella pneumoniae* highest inhibition percentage (49%) was observed at 100 µl/ml of concentration Ag NP's and lowest was 35% at 25 µl/ml. While, highest percentage inhibition for *Escherichia coli* was 51% at 100 µl/ml and lowest was 37% at 25 µl/ml concentration of AgNPs (Fig. 5).

Thus, while studying the antibacterial activity of *Pseudomonas aeruginosa*, highest inhibition percentage (68%) was recorded when Streptomycin (100 µl/ml) was used and when Streptomycin was replaced with same concentration of AgNPs, the highest inhibition percentage recorded was 54 %. In case of *Klebsiella pneumoniae*, highest inhibition percentage (65 %) was recorded in Streptomycin (100 µl/ml). The same concentration of AgNPs exhibited 49 % inhibition. Highest inhibition percentage (69 %) was recorded in *Escherichia coli* for Streptomycin (100 µl/ml). Highest inhibition percentage 51 % was recorded for *Escherichia coli* when Ag NP's (100 µl/ml) was used.

The results depicted that the role of antibiotic was remarkable in inhibition of growth in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. Comparative results avoiding antibiotic and replacing it with biologically synthesized Ag NP's showed the presence of inhibition activity in all the bacteria tested. Although inhibition activity of AgNPs was lower than that of antibiotic, the AgNPs were able to show antibacterial activity. Further research needs to be refined by designing the protocol in which higher concentrations must be tried to determine antibacterial activity. However, high doses of AgNPs have advantage over higher doses of antibiotics as AgNPs are ecofriendly, nontoxic and biologically synthesized. Biologically synthesized nanoparticles can be used as better options over hazardous antibiotics by using them as antibacterial agents.

Conclusion

AgNPs of *Drimia indica* has shown remarkable antibacterial effect as compared to control in present study. The concentration was directly proportional to antibacterial activity. It clearly revealed that the inhibition activity of AgNPs is completely dose dependent and it inhibits

the pathogenic bacteria. Thus, they can be widely used as medicines for bacterial diseases. This will eventually help to investigate the problems and mutations caused by high doses of antibiotics.

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Author contribution SP has designed and conceived the experiments. PK has carried out he experiments and have written the manuscript. MN helped in SEM analysis. SP has analyzed the data and refined the manuscript. All authors have read the manuscript and approved it.

Conflict of interest Authors declare no conflict of interest.

References

Awwad Akl M., Nida M. Salem, Amany O. Abdeen (2012). Biosynthesis of Silver Nanoparticles using *Olea europaea* Leaves Extract and its Antibacterial Activity. *Nanoscience and Nanotechnology*. **2(6):** 164-170.

Bar Harekrishna, Dipak Kr. Bhui, Gobinda P. Sahoo, Priyanka Sarkar, Sankar P. De, Ajay Misra (2009). Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. *Colloids and Surface A: Physicochem. Eng. Aspects.* 339: 134-139.

Chittoor M.S., Binny A.J.R., Yadlapalli S.K., Cheruku A., Dandu C., Nimmanapalli Y. (2012). Anthelmintic and antimicrobial studies of *Drimia indica* (Roxb.) Jessop. bulb aqueous extracts. *J. Pharm. Res.* **5:**3677–3686.

Crespo, M.B. Martínez-Azorín, M.; Alonso, M.Á. (2020). The identity of Drimia purpurascens, with a new nomenclatural and taxonomic approach to the “Drimia undata” group (Hyacinthaceae = Asparagaceae subfam. Scilloideae). *Plant Syst. Evol*. 306: 1–18.

Emma Weir , Antoin Lawlor , Aine Whelan and Fiona Regan (2008). The use of nanoparticles in anti- microbial materials and their characterization. *Analyst*.**133:** 835-845.

Ericka Rodríguez-León , Ramón Iñiguez-Palomares, Rosa Elena Navarro , Ronaldo Herrera-Urbina , Judith Tánori , Claudia Iñiguez-Palomares and Amir Maldonado (2013). Synthesis of

silver nanoparticles using reducing agents obtained from natural sources (*Rumex hymenosepalus* extracts) Nanoscale Research Letters. **8:** 318.

Govingdaraju K., S. Tamilselvan, V. Kiruthiga and G. Singaravelu (2010). Biogenic silver nanoparticles by Solanum torvum and their promising antimicrobial activity. *Journal of Biopesticides.* 3(1): 394-399.

Hamouda Ragaa A., Hussein Mervat H., Abo-elmagd Rasha A. and Bawazir Salwa S. (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica. In : Scientific reports, nature research*. **9:** 13071.

Hamzah Basil Mohammaed and Senthil Kumar R (2015). ANTIBIOTIC SENSITIVITY OF GOLD AND SILVER NANOPARTICLES AGAINST ENTEROCOCCAL PATHOGENS. *Asian J Pharm Clin Res*.8 (3): 201-203.

Heather K. Allen (2017). Alternatives to Antibiotics: Why and How. *NATIONAL ACADEMY OF MEDICNE*. https://doi.org/10.31478/201707g

Jayanta Kumar Patra, Gitishree Das, Leonardo Fernandes Fraceto, Estefania Vangelie Ramos Campos, Maria del Pilar Rodriguez-Torres, Laura Susana Acosta-Torres, Luis Armando Diaz-Torres, Renato Grillo, Mallappa Kumara Swamy, Shivesh Sharma, Solomon Habtemariam & Han-Seung Shin (2018). Nano based drug delivery systems: recent developments and future prospects. *Journal of Nanobiotechnology*. **16 (71).**

Kaviya S., J. Santhanalakshmi and B. Viswanathan (2011). Green Synthesis of Silver Nanoparticles Using Polyalthia longifolia Leaf Extract along with D-Sorbitol: study of Antibacterial Activity. *Journal of Nanotechnology.*1-6.

Khan Ibrahim, Khalid Saeed and Idrees Khan (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*. **12:** 909-931.

Koparde Shital B. and D. K. Gaikwad (2018). ANTIBACTERIAL POTENTIAL OF GREEN SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES AND BARK EXTRACT OF SIMAROUBA GLAUCA. *Int. Res. J. Pharm.* **9 (4):** 42-45.

Koparde Shital B. and D. K. Gaikwad (2019). Integrated Management of Linseed Blight by Green Silver Nanoparticles Synthesized from Fruit and Leaves Extract of *Morinda citrifolia* L. *Indo Global Journal of Pharmaceutical Sciences*. **9(2):** 73-76.

Linlin Wang Chen Hu, and Longquan Shao (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int J Nanomedicine* **12:** 1227–1249.

Madira Coutlyne Manganyi, Gothusaone Simon Tlatsana, Given Thato Mokoroane, Keamogetswe Prudence Senna, John Frederick Mohaswa, Kabo Ntsayagae, Justine Fri and

Collins Njie Ateba. (2021). Bulbous Plants Drimia: “A Thin Line between Poisonous and Healing Compounds” with Biological Activities. *Pharmaceutics.*13, 1385, 2 -20.

Mallikarjuna K., G. Narasimha, G.R. Dillip, B. Praveen, B. Shreedhar, C. Sree Lakshmi, B.V. S. Reddy, B. Deva Prasad Raju. (2011). Green synthesis of Silver Nanoparticles using *Ocimum* leaf extract and their characterization. *Digest Journal of Nanomaterials and Biostructures*. **6 (1):** 181-186.

Marx, J. Pretorius, E., Espag, W.J., Bester, M.J. (2005). Urginea sanguinea: Medicinal wonder or death in disguise? Environ. Toxicol. Pharmacol. 20: 26–34.

Muthusamy Govarthanan, Thangasamy Selvankumar Koildhasan Manoharan Rajiniganth Rathika Kuppusamy Shanthi Kui-Jae Lee Min Cho Seralathan Kamala-Kannan Byung-Taek Oh (2014). *International Journal of Nanomedicine*. **9:** 1593–1599.

Pandey D., Gupta A.K. (2014). Antimicrobial activity and phytochemical analysis of Urginea indica from Bastar district of Chhattisgarh. *Int. J. Pharm. Sci. Rev. Res.* **26:**273–281.

Parveen Asra, Roy Aashis S. and Rao Srinath (2012). Biosynthesis and characterization of silver nanoparticles from *Cassia auriculata* leaf extract and in vitro evaluation of antimicrobial activity. *International Journal of Applied Biology and Pharmaceutical Technology*. **3(2):**222-228.

Prasad Ram and Vyshnava Satyanarayana Swamy (2013). Antibacterial Activity of Silver Nanoparticles Synthesized by Bark Extract of *Syzygium cumini*. *Journal of Nanoparticles.* 1-6.

Priyanka Singh , Yu-Jin Kim , Dabing Zhang , Deok-Chun Yang rends (2016). Biological Synthesis of Nanoparticles from Plants and Microorganisms. *Trends Biotechnol*. **34(7):**588-599.

Shiv Shankar S., Absar Ahmad and Murali Sastry (2003). Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles. *Biotechnol. Prog.* 19: 1627- 1631.

Singaravelu G., J. S. Arockiamary, V. Ganesh Kumar, K. Govindaraju (2007). A novel extracellular synthesis of monodisperse gold nanoparticles using marine algae, *Sargassum wightii Greville*. *Colloids and Surfaces B: Biointerfaces*. **57:** 97-101.

Sonali Aswal, Ankit Kumar, Ruchi Badoni Semwal, Ashutosh Chauhan, Abhimanyu Kumar, Jörg Lehmann, and Deepak Kumar Semwal (2019). *Drimia indica*: A Plant Used in Traditional Medicine and Its Potential for Clinical Uses. *Medicina (Kaunas).* **55(6):** 255.

Sukdeb Pal, Yu Kyung Tak, Joon Myong Song (2020). Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*. *ASM journal*. **73:** 6.

Thakkar Kaushik N., Mhatre Snehit S. and Parikh Rasesh Y. (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine.* **6:** 257- 262.

Vanaja Mahendran and Gurusamy Annadurai (2013). *Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. *Appl Nanosci*. **3:** 217–223.

Wiglusz Rafal J. , Anna Kedziora, Anna Lukowiak, Wlodzimierz Doroszkiewicz, and Wieslaw Strek (2012). Hydroxyapatites and Europium(III) Doped Hydroxyapatites as a Carrier of Silver Nanoparticles and Their Antimicrobial Activity. *Journal of Biomedical Nanotechnology*. **8:** 1–8.

Yasin Sohail , Lin Liu and Juming Yao (2013). Biosynthesis of Silver Nanoparticles by Bamboo Leaves Extract and Their Antimicrobial Activity. *Journal of Fiber Bioengineering and Informatics* **6(1**): 77–84.

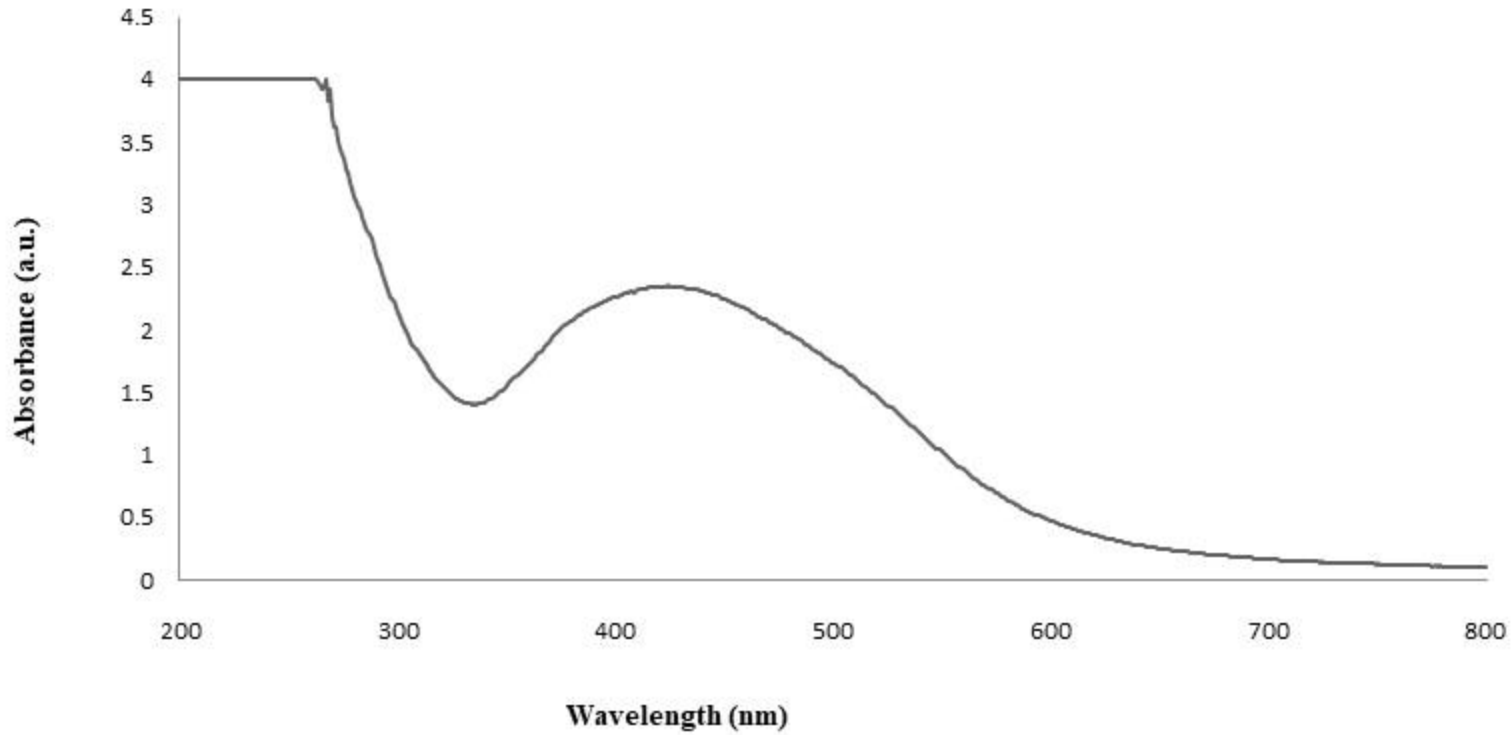

Fig. 1 UV-vis absorption spectrum of Silver nanoparticles.

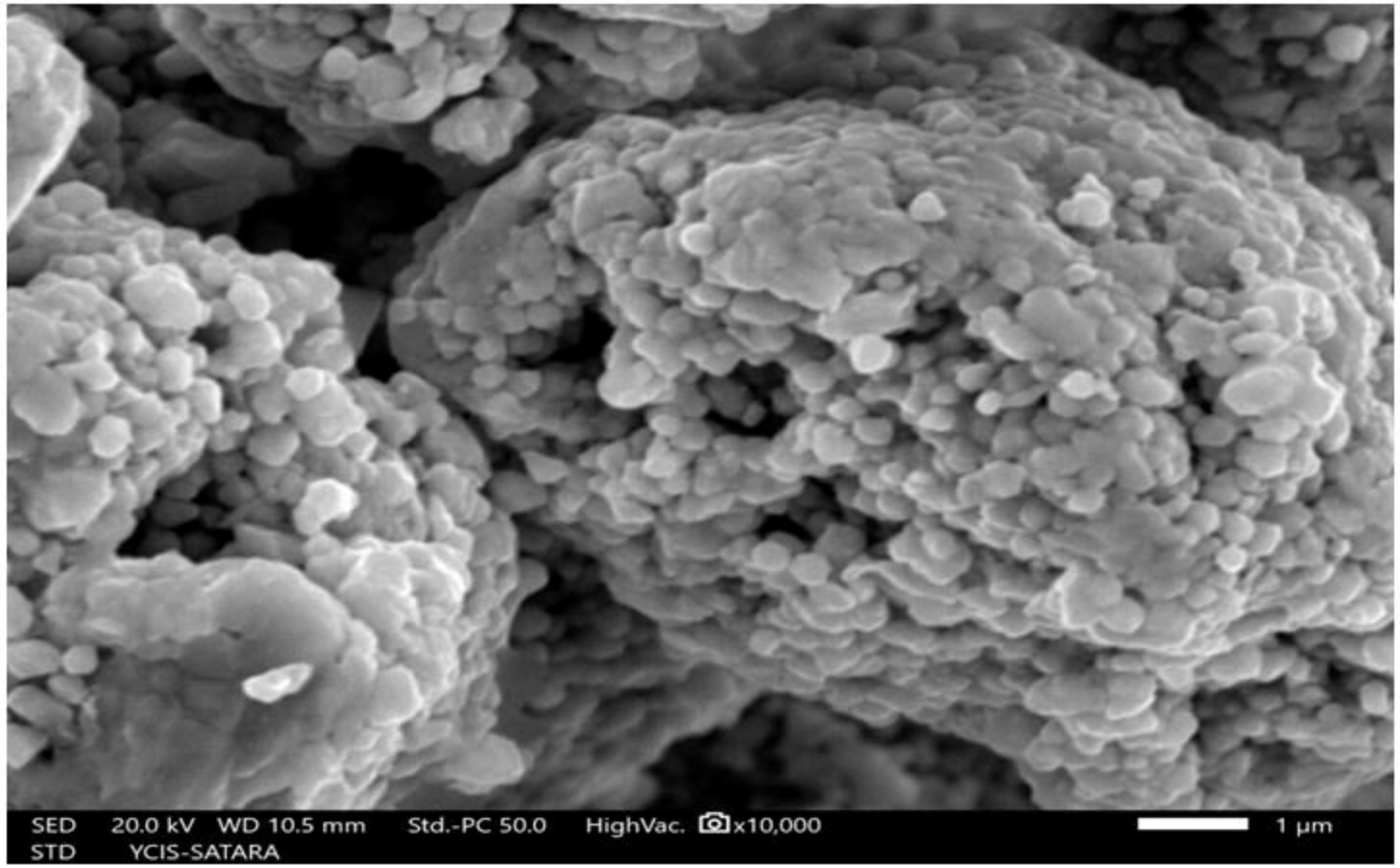

Fig. 2 SEM micrograph of Silver nanoparticles.

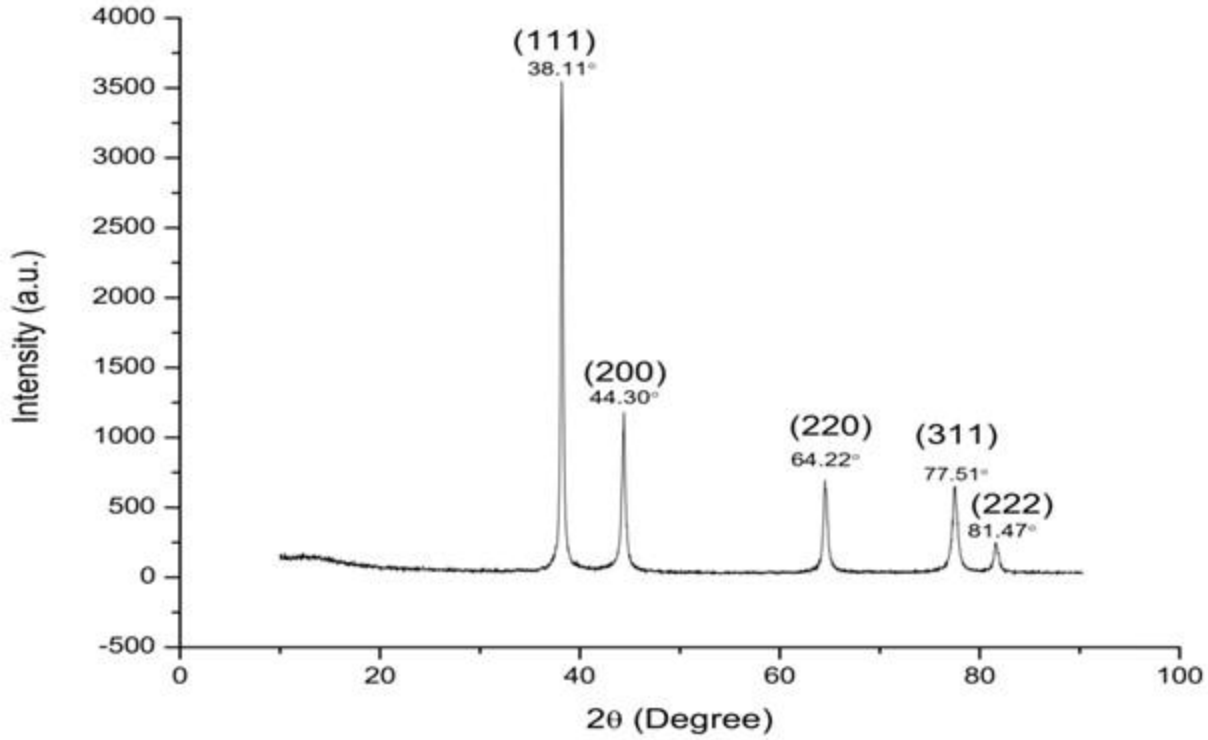

Fig. 3 X-ray diffraction pattern of Silver nanoparticles

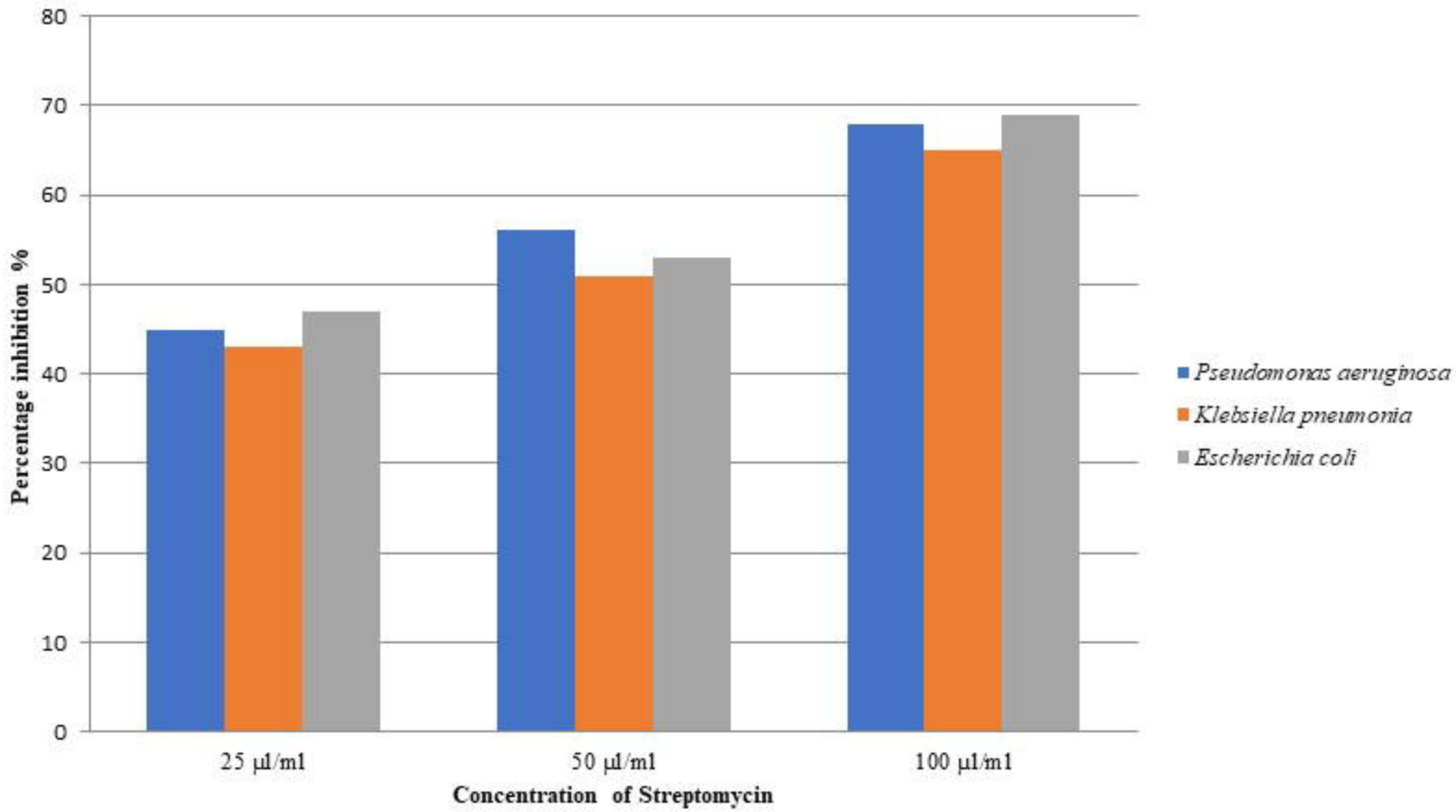

Fig. 4 Percentage inhibition of Bacterial species using antibiotic Streptomycin

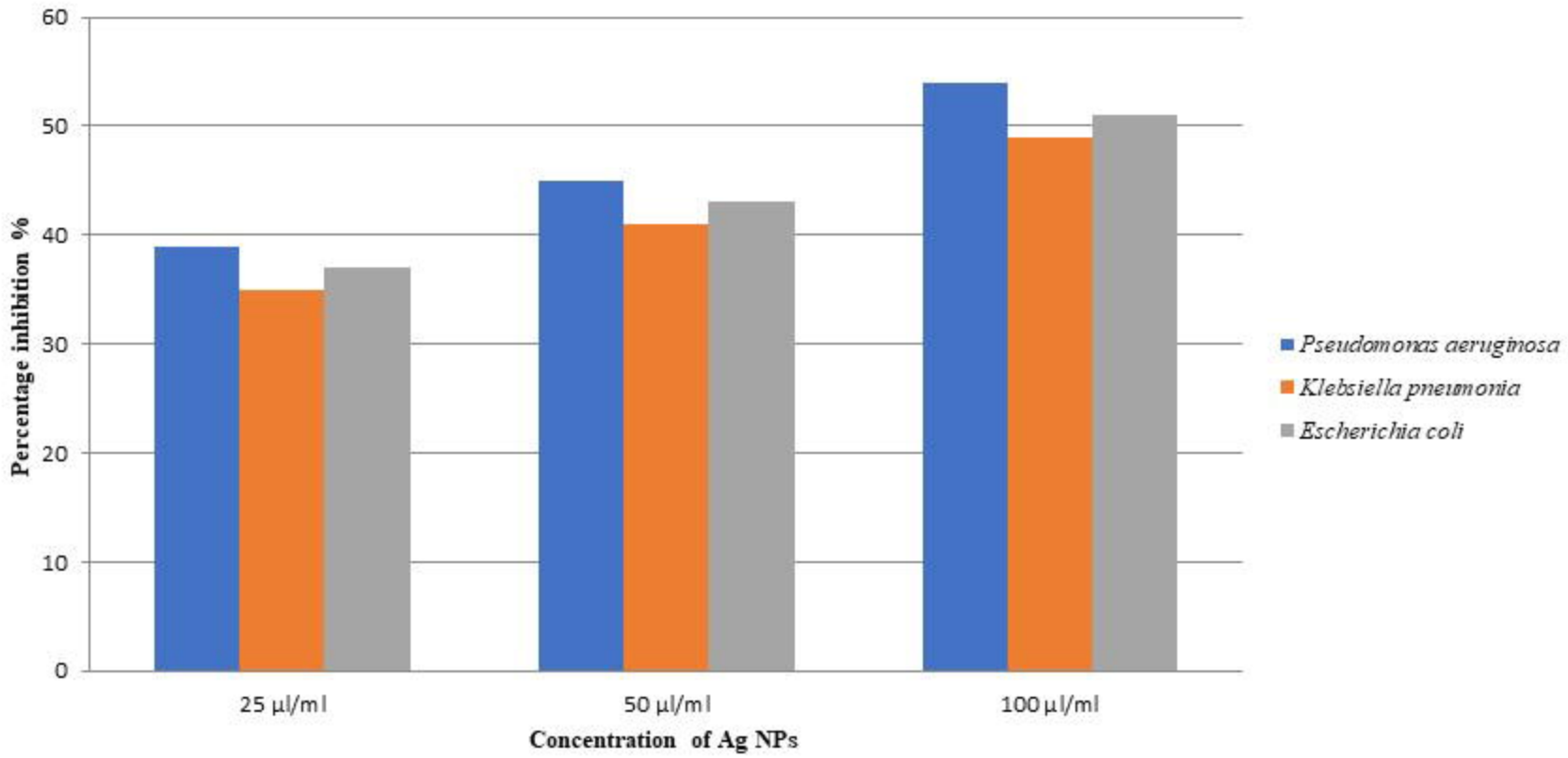

Fig. 5 Percentage inhibition of Bacterial species using AgNPs of *Drimia indica*