

Synthesis of silver nanoparticles from skin of *Labeo rohita* and their application of biocide

Iram Manzoor^{1*} and Bushra Safeer¹

1. Department of Zoology, Women University of Azad Jammu and Kashmir, Bagh-Pakistan

*Corresponding author e-mail: iram.manzoor408@gmail.com

SUMMARY

Since ancient times, silver has been used to treat wounds, and a few bacterial infections as silver nitrate, silver sulfadiazine and metallic silver. However, due to the introduction of a few anti-toxins, the use of these silver mixtures has decreased dramatically. Because of its ability to balance metals into nano-size, which significantly modifies the synthetic, physical, and optical characteristics of metals, nanotechnology is having massive consequences in the twenty-first century. The current study successfully incorporated silver nanoparticles from *Labeo rohita* fish skin and scales, which are bio-composites of well organized amino acids and collagen filaments. The silver nanoparticles were applied to contemplate the movement of antibacterial against the few human microorganisms. Silver nanoparticles show most noteworthy possibility towards managing the growth of microbes and show biofilm arrangement.

Keywords: Nanoparticles, Fish, Bacteria, Antimicrobial, Nanotechnology, Biofilm

Citation: Manzoor, I., B. Safeer. 2022. Synthesis of silver nanoparticles from skin of *Labeo rohita* and their application of biocide. Journal of Wildlife and Ecology. 6: 129-140.

Received: August, 2022; **Accepted:** September, 2022

INTRODUCTION

NANOTECHNOLOGY

Nanotechnology is a field of science which deals with materials having structural characters of atoms and large materials with one dimension lying in nano range. This technology deals with the materials having size below 100nm. In 2006, Food and Drug Administration (FDA) defined nonmaterial very simply as those particles which having the size smaller than micrometers and show special characters (Patil *et al.*, 2012). According to the recent research it is to be said that the most active areas of research fields of life sciences. This field also deals with many other technologies like recombinant DNA technology, protein technology. Some of the nanoparticles are used for manufacturing of small constructive things (Duran, 2005). Due to the some unique properties nowadays there is a great intention towards metal nanoparticles. Mostly they are synthesized from noble metals (silver, lead, gold, platinum). Among the nanotechnology, the most important one are silver nanoparticles (Parashar *et al.*, 2009).

APPLICATION OF NANOTECHNOLOGY

The most important application of nanotechnology is to produce new group of catalyst which is recognized as nano-catalysts. Some important improvements are made in this field on the basis of their composition, particle size and structure are being made in this field contributing to the production and comprehensive understandings of the natural world (composition, particle size, and structure) (Jae and Beom, 2009).

SILVER NANOPARTICLES

Nanoparticles are particles that exist on a nanometer scale (Afzalipour, 2020). From the many of nanoparticles silver nanoparticles are more efficient and important type. Silver ions and silver based compounds are very lethal to small organisms, including some main species of bacteria. This quality of silver makes the silver best choice in the field of medicine. There are so many important applications of silver nanoparticles but from some of these the activity against small organisms is important one. Exact process for killing of bacteria is not well known but has been assumed that the silver nanoparticles works by breaking down the cell or by stopping the transfer of cell (Inbakandan *et al.*, 2012).

SYNTHESIS OF SILVER NANOPARTICLES

Nanoparticles may synthesize by chemical, physical and biological means. By physical method nanopartioicles may also synthesize using evaporation–condensation process at atmospheric pressure using tube furnace. By using evaporation–condensation method different type of nanoparticles has been synthesized (Sun *et al.*, 2001; Inbakandan *et al.*, 2012; Suresh *et al.*, 2012; Singh *et al.*, 2014; Rather *et al.*, 2016). But this process in not so much beneficial because it consumes more energy takes a large space and requires a lot of time. In chemical synthesis some chemical compounds are used which help in reduction to AgNPs but this is so adverse method due to the toxicity of chemical compounds. By using different parameters like temperature, pH, reducing and stabilizing agent, concentration, type of precursor nucleation and growth of NPs may governed (Evanoff and Chumanov, 2005). Nanoparticles may likewise be combining organically. Biogenic union of silver nanoparticles is arranged under base up approach. A few strategies have been utilized to integrate silver nanoparticles, including compound decrease, microwave-helped blend, ultrasonic-helped decrease, electrochemical decrease, layout technique, photograph instigated or photograph reactant decrease, light decrease, miniature emulsion strategy, and biochemical decrease (Sanchez *et al.*, 2012).

APPLICATION OF SILVER NANOPARTICLES

AgNPs have been widely used as antibacterial and anticancer specialists, as well as for biomedical applications in the medical care business, as a result of their inherent cytotoxicity. The surface charges of AgNPs influence the amount of toxicity to cells. AgNPs with a positive surface charge are more likely to remain on the tissue surface or luminal side of the vein for a longer period of time, which is an important route for administering anticancer agents. AgNPs' inherent cytotoxic potential has been used against several types of cancer cells, including hepatocellular carcinoma, lung and

bosom illness, and cervical carcinoma. AgNPs have a variety of fascinating features (Korbekandi *et al.*, 2012).

CONTAGIOUS DISEASES

Infectious maladies, otherwise called contagious sicknesses or transmittable ailments involve clinically clear ailment (i.e., trademark clinical signs or symptoms of ailment) coming about because of the disease, presence and development of pathogenic natural operators in an individual host life form.

BACTERIAL PATHOGEN

Pathogenic microorganisms are microscopic organisms that can cause ailment. Albeit most microbes are innocuous or frequently advantageous, some are pathogenic, with the quantity of species assessed as less than a hundred that are believed to cause irresistible sicknesses in people. On the other hand, a few thousand animal varieties exist in the human stomach related framework. Pathogenic bacteria contribute to other widely relevant diseases, such as pneumonia, which may be caused by microscopic organisms such as *Streptococcus* and *Pseudomonas*, and foodborne illnesses, which can be caused by germs such as *Shigella*, *Campylobacter*, and *Salmonella* uncleanness (Mohanpuria *et al.*, 2008; Prasad *et al.*, 2011). *Pseudomonas stutzeri* AG259 was used by Joerger *et al.* (2000) to incorporate Ag nanoparticles with sizes less than 200 nm. Microscopic organisms were grown in darkness (Mathanraj *et al.*, 2009; Schroll *et al.*, 2010).

FISHES

It is notable that fish is a transcendent constituent of human eating routine and one of the quality creature proteins accessible to millions over the world. Fish fills in as an essential wellbeing food inferable from its higher protein, useful fat and different miniature supplements. Besides, during the previous quite a few years fisheries and hydroponics are financed to worldwide food security, destitution easing, provincial occupations, work and pay age (Sarkar, 2012).

LABEO ROHITA

Labeo rohita is a freshwater fish belongs to the family crypnidae, have quite elongated bilaterally symmetrical body. They have the vaulted body from upper side of body than the lower side. Scales present on whole body of *Labeo rohita* except head region (Sarkar, 2012). Rohu is a fresh water fish. It is viewed as a rich wellspring of vitamin C, which is fundamental for keeping up a decent wellbeing. It keeps ailments like cold and hack under control and forestalls different maladies identified with it (Chashoo *et al.*, 2012).

CHEMICAL COMPOSITION OF SKIN AND SCALES OF FISHES

The fishes belongs to family crypnidae have the skin composed of 54% moisture, 43% protein, 1.6% fat and 0.35% ash. The skins of these fishes contain a collagen protein (Jena *et al.*, 1998).

OBJECTIVES

- To prepare and characterize of silver nanoparticles using fish (*Labeo rohita*) skin.
- To know the antibacterial impact of biogenic synthesized silver nanoparticles using skin of *Labeo rohita*.

MATERIALS AND METHODS

The work has been completed in the “Microbial Biotechnology Laboratory, Department of Zoology, Women University Bagh Azad Kashmir Pakistan”.

CHEMICALS, GLASSWARES, EQUIPMENT

Every single manufactured substance and reagents are gotten from Sigma Aldrich (Switzerland), Merck (Germany), and Sigma Aldrich (Germany), “37°C shaker (N-BIOTEK)”, “37°C hatchery (Advantage lab GmbH)”, “steam sterilizer autoclave”, “laminar stream (ESCO IESTRP-CCOO2.2, Singapore)”, “Weighing machine (SHIMADZU ELECTRONIC BALANCE KYOTO JAPAN)”, legitimate adjustment (SARTORIUS GMBH GOTTINGEN, Germany), “STURDY APEX GROUP (SA-300VL)”, and “UV-vis spectrophotometer (T80, PG instruments Ltd)” were used.

COLLECTION AND IDENTIFICATION OF RAHO

The Raho (*Labeo Rohita*) were collected from region Gujranwala, the locale of Pakistan and identified by using book fishes of Pakistan. Concentrate was set up by utilizing the adjusted technique. The fish was finely pounded applying “mortar” and “pestle”. The concentrate was made up to “100mL” utilizing twofold refined “Milli-Q” water. At that point extricates was alienated through “Whatman NO. 1” channel paper to separate the “tissue rubbles” and get a pure concentrate.

PREPARATION OF FISH SKIN AND SCALES EXTRACT

The fish skin and scales are dried then grinded and heated at “700c for 30m” in followed by slow cooling at room temperature and stabilize the solution for “3 days”. After that brown sediment were formed at bottom indicating the presence of silver nanoparticles and then centrifuged for 30 minutes and filtered. Then the collected nanoparticles are stored in refrigerator.

SYNTHESIS OF NANOPARTICLES

The “50 ml of fish scale extract” was mixed with “silver nitrate solution” in “0.04 proportions” and kept at room temperature and then heated for the development of reddish brown colour.

CHARACTERIZATION OF SILVER NANOPARTICLES

The decrease of pure “Ag⁺ particles” was checked by estimating the UV-noticeable range of the response medium at 5 h in the wake of weakening a little aliquot of the example into refined water. UV-noticeable investigation was finished by utilizing UV-VIS spectrophotometer (Nanotrope, Model No-8000). UV-VIS spectroscopic investigation was done at DST-PURSE, Sri Venkateswara University, Tiruapti (Sinha and Ahmaruzzaman, 2014).

COMPOSITION OF GROWTH MEDIA NUTRIENT AGAR MEDIUM PREPARATION

1.5g of nutrient agar medium was mixed with 150ml distilled water. The conical flask was cotton plugged and autoclaved at 121 °C for 30 min.

INOCULATION

After cooling the agar medium the culture is inoculated with a needle to the solidified agar medium and was kept in 30°C temperature in incubator for 24 hrs.

ANTIBACTERIAL ACTIVITY

The antibacterial action of AgNPs was completed by Agar dispersion strategy. Supplement agar medium plates were arranged, cleaned and set. After hardening bacterial societies were cleaned on these plates. The AgNPs were poured in to wells supplement agar plate and saved for brooding at 37°C for 24 hours. The zone of inhibition of bacterial growth was calculated by measuring its diameter through scale in millimeter after 24 hours. The result sensitivity test were represent as “zero” for no sensitivity, “1-5mm” for low sensitivity, “5-10mm” for moderate sensitivity and “10-25mm” for high sensitivity.

BIOFILM ESSAY

The antibiofilm development was evaluated using a precious stone violet inspection with minor adjustments (Toole, 2011). Microorganisms were advanced in “Borosilicate tubes (Minitex, USA)” with each chamber containing “30 mg/ml of DHAqu” and “DHAgNPs” and “2 ml” of supplement stock medium before being placed in an incubation environment at 37°C for overnight. As a control, “chloramphenicol” and “silver nitrate” were used. So-called supplement stock was collected for negative control. The next day, the stock media was removed, and the connected cells were recolored with “0.1% diamond violet”. These compartments were then agonized for “10 to 15 minutes: at room temperature before being flushed with water, which eliminated tone and additional unattached cells. These biofilms were then separated in “30%” acidic destructive and incubated for “10 to 15 minutes” at room temperature. A spectrophotometer was used to evaluate precious stone violet at “550 nm” separation. As a clear, acidic damaging (30%) water was employed.

RESULTS AND DISCUSSION

SYNTHESIS AND CHARACTERIZATION OF LRAgNPs

Synthesis of LRAgNPs

The current examination was completed of readiness of AgNPs from the fish skin and scales extract of *Labeo rohita*. We built up a straightforward convention for blend and synthesis of AgNPs from the fish skin and scales concentrate of *Labeo rohita* and examined their biocide. The *Labeo Rohita* silver nanoparticles were synthesized by utilizing skin and scales of fish. Silver nitrate is utilized as lessening operator as silver has indistinguishable properties, for example, reactant, compound security and great conductivity. The reduction cycle began when an aqueous concentration of *Labeo Rohita* (LRAqu) was combined together with the silver particles. Before the AgNO₃

addition, the LRAqu separation had a yellowish hue, but after bubbling for 15-20 minutes, the hue changed to a dull earthy hue, demonstrating the development of silver nanoparticles. LRAgNPs (Figure 1) applying LRAqu eliminate shade shift from yellowish to dim earthy colored hue demonstrates LRAgNPs production. SPRP produced the earthy colored shading scheme.



Figure 1: Preliminary synthesis of LRAgNPs using aqueous extract of LRAqu

Characterization of LRAgNPs

Ultraviolet–visible spectroscopy is easier, simplest and very fast technique for the characterization of nanoparticles. UV-vis spectroscopy is an important technique to check stability and characterization of synthesized silver nanoparticles (Sastry *et al.*, 2012). Silver nanoparticles have optical characteristics that allow them to make contact with light wavelengths. In silver nanoparticles, two bands are present: the valence band and the transfer band, which are close to one other and allow electrons to travel freely. These liberated electrons collided with the light wave, resulting in the surface Plasmon resonance (SPR) absorption band. Absorption of silver nanoparticles

depends on particle size and medium. The nanoparticles with sizes ranging from 2 to 100 nm produce a peak with surface Plasmon (Tomaszewska *et al.*, 2013).

ANTIBACTERIAL EFFECT

Using an agar well dispersion test, the flow research was started to analyse the base inhibitory convergences of naturally blended nanoparticles (LRAgNPs) and its watery concentrate (LRAqu) against four human infection producing microscopic organisms (Table 1). The current study found that LRAgNPs, as opposed to LR aqu, had a significant bactericidal effect against all tested bacterial pathogens.

Table 1: Antibacterial activity from LRAqu and LRAgNPs.

Test sample pathogens	LRAqu	LRAgNPs	DMSO	Choramphenicol (10g)
<i>Staphylococcus aureus</i>	0	12 ±1	—	15+0
<i>Klebsiella pnemoniae</i>	0	13±1	—	21.3+3.21
<i>Pseudomonas aeruginosa</i>	17±1	15±1	—	25+5
<i>Proteus mirabilis</i>	0	11±1	—	17.3+0.57

Zone of inhibition stated as:

1. Zone(0) for no sensitivity
2. (1-5mm) for low sensitivity
3. (5-10mm) for moderate sensitivity
4. (10-25mm) for high sensitivity

In the flow research LRAgNPs indicated greater affectability against pathogenic microbes when contrasted with LRAqu. The bactericidal proficiency of the combined AgNPs was concentrated against a sub refined strain of four microorganisms utilizing agar well dispersion strategy, the zone of hindrance were watched. LRAgNPs indicated bigger zone of restraint against pseudomonas aeuroginosa which is recorded as (15±1). LRAqu indicated highest zone of restraint against pseudomonas aeuroginosa which is recorded as (17±1). LRAgNPs demonstrated moderate zone of restraint against Klebsiella pneumonia which is recorded as (13±1). LRAgNPs indicated highest zone of restraint against Staphylococcus aureus which is recorded as (12±1). LRAqu showed no zone of inhibition Against human pathogenic bacteria (Staphylococcus aureus and proteous mirabilis) and LRAgNPs showed zone of inhibition against proteous mirabilis which is recorded as (11±1). AgNPs was successfully synthesized by biological method from fish skin and scales. The Surface Plasmon Resonance (SPR) property of synthesized nanoparticles was studied by UV-Vis spectroscopy. The antibacterial activity of silver nanoparticles shows the significant antibacterial activity against human pathogens (Figure 2 and Table 1).

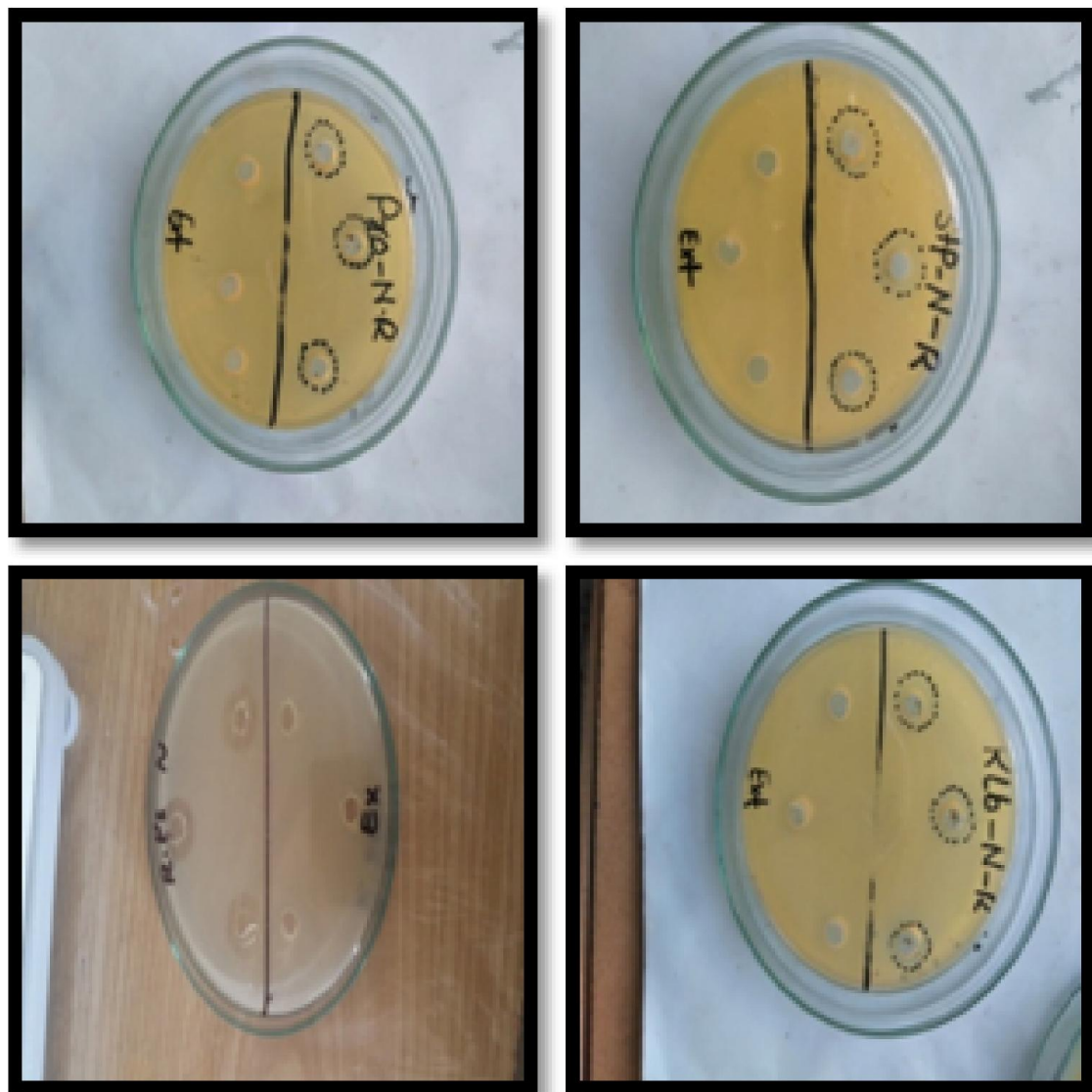


Figure 2: Zone of inhibition with LRAqu and LRAgNPs against bacterial pathogens

Duran *et al.*, (2010) advised that the naturally synthesized silver nanoparticles showed great antibacterial activity against *A. hydrophila* (fish pathogen). The biologically synthesized silver nanoparticles showed more prominent antibacterial activity using the animals' skin. It is seen that the synthesized silver nanoparticles displayed great antibacterial activity against *Aeromonas hydrophila* by the well diffusion method. The inhibitory impact of silver nanoparticles is a total impact of numerous unmistakable instruments.

ANTIBIOFILM ACTIVITY

The effect of LRAqu and LRAgNPs on biofilm development was investigated in the current study using a crystal violet assay (Figure 3). In the recent research result exposed that tested bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *staphylococcus aureus* and *proteus mirabilis*) were able to form biofilm in the

presence of LRAqu. The tested bacteria (*Staphylococcus aureus* and *pseudomonas aeruginosa*) were unable to form the biofilm in the presence of LRAgNPs but the tested bacteria (*Klebsiella pneumonia* and *proteus mirabilis*) formed the thin layer. The results indicated that LRAgNPs had a greater capacity to minimise biofilm development than LRAqu, although the complete tested group had a high ability to inhibit biofilm formation at substantial land (Figure 4 and Table 2).

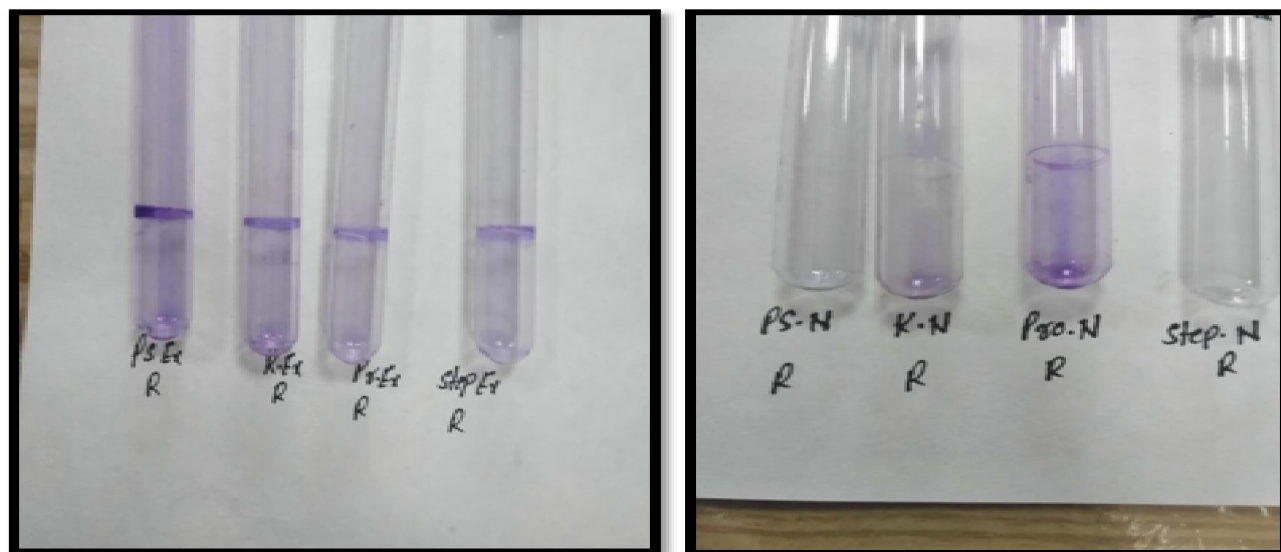


Figure 3: Antibiofilm shown by LRAqu and LRAgNPs.

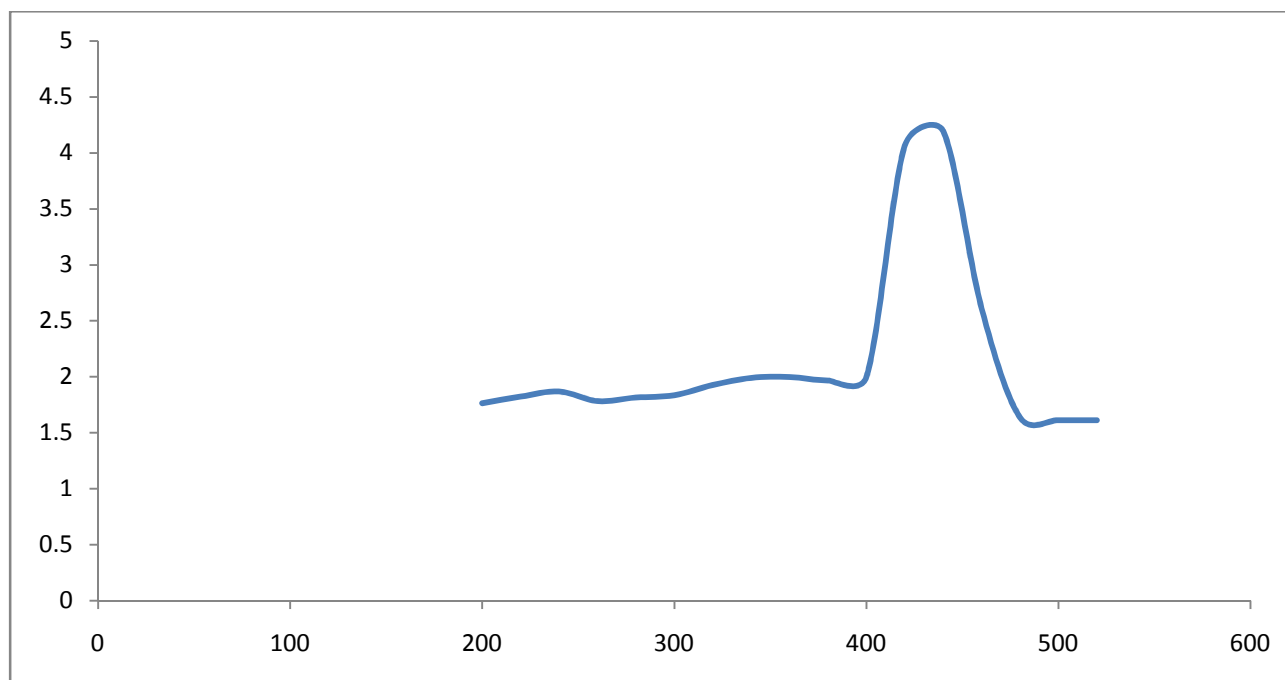


Figure 4: Graphical representation of antibiofilm activity of LR AgNPs and LR Aqu and chloramphenicol against bacterial pathogen.

Table 2: Mean and standard deviation of LRAqu and LRAgNPs

Test organism	LRAqu Mean $\pm$ S.D	LRAgNPs Mean $\pm$ S.D	Pathogen	Chloramphenicol
<i>Staph</i>	0.144 $\pm$ 0.0063	0.044 $\pm$ 0.003	1.45 $\pm$ 0	0.12 $\pm$ 0
<i>Pseudo</i>	0.176 $\pm$ 0.004	0.026 $\pm$ 0.004	1.45 $\pm$ 0	0.023 $\pm$ 0
<i>Proteus</i>	0.080 $\pm$ 0.058	0.055 $\pm$ 0.003	1.05 $\pm$ 0	1.430 $\pm$ 0
<i>Kleb</i>	0.126 $\pm$ 0.005	0.053 $\pm$ 0.057	1.542 $\pm$ 0	0.027 $\pm$ 0

Sriram *et al.* (2010) recommended that AgNPs have been utilized to restrain the action of biofilms. In the current examination, the portion subordinate capacity of AgNPs to hinder the movement of biofilms framed by the human microorganism's *P. Aruginosa*, and *S. pneumoniae* was resolved under in vitro conditions. These outcomes demonstrated that, for all the tried bacterial strains, the organically incorporated AgNPs restrained the action of biofilms. Chaudhari *et al.* (2012) revealed that AgNPs got from various creatures indicated improved majority extinguishing activity against *S. aureus* biofilm and anticipation of biofilm arrangement, and they proposed that AgNPs may be associated with killing these sticky substances accordingly forestalling biofilm development.

REFERENCES

- Afzalipour, R., S. Khoei., S. Khoe., S. Shirvalilou., N. J. Raoufi., M. Motevalian., and M. Y. Karimi., (2020). Thermosensitive magnetic nanoparticles exposed to alternating magnetic field and heat-mediated chemotherapy for an effective dual therapy in rat Glioma model. *Nanomedicine: Nanotechnol, Biology and Medicine*, 10(23): 19-23.
- Chashoo, I., A. D. Kumar and Z. A. Bhat. (2012). Antimicrobial studies of *Sambucus wightiana* Wall. ex. Wight and Arn. *J. Pharo*. 5(5): 2467-2468.
- Chaudhari, K.R., A. Kumar, V.K.M. Khandelwal, M. Ukawala, A.S. Manjappa, A.K. Mishra, J. Monkkonen, R.S.R. Murthy. 2012. Bone metastasis targeting: a novel approach to reach bone using Zoledronate anchored PLGA nanoparticle as carrier system loaded with Docetaxel. *Journal of controlled release*. 158: 470-478.
- Duran, N., P.D. Marcato, R.D. Conti, O.L. Alves, F. Costa, M. Brocchi. 2010. Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. *Journal of the Brazilian Chemical Society*. 21: 949-959.
- Evanoff, D. D., G. Chumanov., (2005). Synthesis and optical properties of silver nanoparticles. 5(3): 5-8.

- Inbakandan, D., G. Sivaleela., D. M. Peter., R. Kiurbagaran., R. Venkatesan., and S. A. Khan., (2012). Marine sponge extract assisted biosynthesis of silver nanoparticles. *Materials Letters*, 87(2)66-68.
- Jae, Y. S. and S. k. Beom., (2009). Rapid Biological Synthesis of Silver Nanoparticles, *Bioprocess Biosyst.*, 32(1)79-84.
- Jena, J., P. Aravindakshan, S. Chandra, H. Muduli, S. Ayyappan. 1998. Comparative evaluation of growth and survival of Indian major carps and exotic carps in raising fingerlings. *Journal of Aquaculture in the Tropics*.
- Joerger, T., R. Joerger, E. Olsson, C.-G. Granqvist. 2001. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. *TRENDS in Biotechnology*. 19: 15-20.
- Korbekandi, H., S. Iravan.i., S. Abbasi., (2012). Optimization of biological synthesis of silver nanoparticles using *Lactobacillus casei* subsp. *casei*. *J. Chem. Tech Biotechnol*, 87(7)932-937. *Lancet*, 366(9498), 1736-1743.
- Mathanraj. S., S. Sujatha, K. Sivasangeetha and S. C. Parija. (2009) Antimicrobial studies of animal. *J.Nanobiotech*. 15(4)18-24.
- Mohanpuria, P., N.K. Rana, S.K. Yadav. 2008. Biosynthesis of nanoparticles: technological concepts and future applications. *Journal of nanoparticle research*. 10: 507-517.
- Parashar, U. K., S.P. Saxena., A. Srivastava., (2009). Bioinspired synthesis of silver nanoparticles. *Dig. J. Nanomat. Biostruct.*, 4(2)159–166.\
- Patil, R. S., M. R. Kokate and S.S. Kolekar. (2012). Bioinspired synthesis of highly stabilized silver nanoparticles using *Ocimumtenuiflorum* leaf extract and their antibacterial activity. *Spectrochimica Acta Part A: Mol and BiomolSpectros.*, 3(91-98): 234-238.
- Prasad R., R. Pandey.,I. Barman., (2011) .Engineering tailored nanoparticles with microbes: quo vadis. *Wiress Nanomed. Nanobiotechnol*. 8(3)316–330.
- Rather, M. A., I. A Bhat., N. Sharma., A. Gora, and R. Sharma., (2016). Silver Nanoparticles: A Vital Fish Antimicrobial Agent. *World aquaculture.*, 57(11)65-69.
- Sanchez, V. C., A. Jachak., R.H. Hurt., A.B Kane, (2012). Biological interactions of graphene-family nanomaterials: an interdisciplinary review. *Chemical research in toxicology*, 25(1)15-34.
- Sarkar B., (2012). Application of nanotechnology on aquaculture. *Fishing chime*, 30(9) 21-25.
- Sastry, M. V., S. R. Patil.,R. Sainkar. (1998). Electrostatically controlled diffusion of Schroll, C. K.B. Barke., K.A. Krogfelt., C. Struve., (2010). Role of type 1 and type 3
- Singh, R., S. K Sahu., and M. Thangaraj., (2014). Biosynthesis of silver nanoparticles by marine invertebrate (Polychaete) and assessment of its efficacy against human pathogens. *J. of Nanopart.*, 53(17)54-59.
- Sinha, M., A.K. Ahmaruzzaman., (2014). Biomimetic synthesis of silver nanoparticles using the fish scales of *Labeo rohita* and their application as catalysts for the reduction of aromatic nitro compounds, *Spectrochimica Acta*. 4(9)41-45.

- Sun, Y., Y. Xia., (2001). Shape-controlled synthesis of gold and silver nanoparticles. *Science*, 298(3) 2176–2179.
- Suresh, A. K., D. A. Pelletier., W. Wang., J. L Morrell-Falvey., B. Gu., M. J. Doktycz., (2012). Cytotoxicity induced by engineered silver nanocrystallites is dependent on surface coatings and cell types. *Langmuir* 28,(3) 27–35
- Tomaszewska, E., K. Soliwoda, K. Kadziola, B. Tkacz-Szczesna, G. Celichowski, M. Cichomski, W. Szmaja, J. Grobelny. 2013. Detection limits of DLS and UV-Vis spectroscopy in characterization of polydisperse nanoparticles colloids. *Journal of Nanomaterials*. 2013.
- Toole, G. A. 2011. Microtitre dish biofilm formation assay', *J. visualized Exp.* 3(47): 2437-2437.

Competing interests: Authors have declared that no competing interests exist.

Funding: Authors have no source of funding for this work.

Authors' contributions: Manzoor has designed this project, collected data and written this article; while Manzoor and Safeer have critically analyzed this article and approved as final.