ejpmr, 2017,4(4), 537-545

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PREPARATION, SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES BY FISH SCALES OF *CATLA CATLA* AND THEIR ANTIBACTERIAL ACTIVITY AGAINST FISH PATHOGEN, *AEROMONAS VERONII*

D. Vineela, S. Janardana Reddy* and B. Kiran Kumar

Department of Fishery Science and Aquaculture Sri Venkateswara University, Tirupati – 517502.

***Corresponding Author: S. Janardana Reddy**

Department of Fishery Science and Aquaculture Sri Venkateswara University, Tirupati – 517502.

Article Received on 10/02/2017 **Article Revised on 02/03/2017** **Article Accepted on 22/03/2017**

ABSTRACT

Silver has been in use since time immemorial in the form of metallic silver, silver nitrate, silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. But due to the emergence of several antibiotics the use of these silver compounds has been declined remarkably. Nanotechnology is gaining tremendous impacts in the present century due to its capability of modulating metals in to their nanosize, which drastically changes the chemical, physical and optical properties of metals. From the results, unveil that the synthesized silver nanoparticles were characterized by SEM, UV-VIS, and FT-IR spectroscopy. In the present work successfully synthesized *AgNPs* from the fish scales of *Catla catla* which are biocomposites of high ordered collagen fibres, hydroxyapatite and amino acids. *AgNPs* synthesis was confirmed by colour change, wave range of UV-VIS, FT-IR spectroscopy and the shape and size of the nanoparticles has made a remarkable comeback as potential antimicrobial agents. The synthesized silver nanoparticles were used to study the Antibacterial activity against the fish pathogen *A.veronii* by the application of 100µl of 1 M silver nanoparticle. Silver nanoparticles show greatest potentiality towards controlling the growth of bacteria.

KEYWORDS: Silver nanoparticles, *Aeromonas veronii*, *Catla catla*, Fish Scales, Synthesis, SEM analysis, UV-VIS, FT-IR, Antibacterial activity, Zone of Inhibition.

INTRODUCTION

It is well known that fish is a predominant constituent of human diet and one of the quality animal proteins available to millions across the world. Fish serves as a vital health food owing to its higher protein, beneficial fat and various micro nutrients. Moreover, during the past several decades fisheries and aquaculture are subsidized to global food security, poverty alleviation, rural livelihoods, employment and income generation (Biplab Sarkar, 2012).

Fish disease is one of the major threats to the feasible development of aquaculture generating loss of millions of dollars annually. *Aeromonas veronii* can grow in both aerobic and anaerobic conditions and causes a diversity of diseases in both and human populations. There are disproving views on whether the microbe is a primary cause of diseases or an strategic one causing diseases to vertebrate hosts that are immune compromised and stressed (Nielsen, 2001). The pervasive nature of the bacteria in aquatic environments provides significant opportunity for animals, mainly fish and amphibians to contact and ingest organisms (Seshadri, 2006).

Aeromonas veronii is a Gram-negative, rod-shaped bacterium found in fresh water and in association with animals (Hickman-Brenner et al., 1988). It can be a pathogen of humans and a beneficial symbiont of leeches. In humans *A. veronii* can cause diseases ranging from wound infections and diarrhea to septicemia in immune compromised patients. Humans treated with medicinal leeches after vascular surgery can be at risk for infection from *A. veronii* and regenerally placed on prophylactic antibiotics (Whitaker et al., 2009), frequently ciprofloxacin is used but there have been reports of resistant strains prominent to infection. In Leeches, this bacterium is reflection to function in the digestion of blood, provision of nutrients, or preventing other bacteria from growing (Patel et al., 2012).

Water is one of the transmittal routes of many microorganisms that cause various endemic diseases in aquatic organisms; thus, many disease conservation methods are depend on water disinfection of water. The main cause of economic loss in aquaculture is diseased fish, followed by omycete (water molds) infections (Meyer, 1991). Therefore, reducing fish diseases is decisive to the future success of the aquaculture industry,

and in this regard, nanotechnology may overture some new solutions.

Richard Feynman (1965) was a Nobel Prize winner and a brilliant physicist, in 1959 he introduced the concept of Nanotechnology, when he gave a talk titled "***There is a plenty of Rooms at the bottom***". Norio Taniguchi coined the name "***Nanotechnology***" in 1974.

Nanomedicine is a fastly flourishing attitude of nanotechnology (Freitas, 2005), Nanotechnology is an ingenious technology and many promising applications are starting to appear in the area of agriculture sciences, notably aquaculture, where the antimicrobial properties of assured nanomaterials are of precise interest (Li et al., 2008). Nanotechnology is able to detect measure, manipulate and manufacture things at the nanometer scale. A nanometer (nm) is an SI (System International Units) unit of length 10^{-9} or a distance of one-billionth of a meter (Mongillo, 2007). These new materials are manufactured to have exclusive physical or chemical properties which emerge from their small size, shape, surface area, conductivity or surface chemistry and have found diverse applications in textiles, electronics, engineering and medicine (Smith et al., 2007).

Nanoparticles can be used in feed production technology, also nanofiltration can be used filtration of treatment water which is used seafood processing. Although nanotechnology intensifies the quality and availability of products, some materials emerging from nanotechnology may pose a risk to aquatic environment.

Recently, various inorganic antibacterial and antifungal materials consist or silver have been developed and some of them are existed in commercial use (Johari et al., 2014b; Kawahara et al., 2000; Wang et al., 2007). Silver is well known to have a wide antibacterial spectrum and be relatively safety (Cho et al., 2005). One of the antimicrobial silver species that has yet received little consideration is nanometer sized silver particles (Mohan et al., 2007).

In the present study a cleaner, cheaper and environment friendly method for generation of self assembled silver nanoparticles employing a simple irradiation technique using aqueous extract of the fish scales, which is considered as a waste material of fish, is used for the synthesis of silver nanoparticles.

MATERIALS AND METHODS

Preparation of Fish Scales extract

10ml of 0.1M aqueous solutions of silver nitrate is added in different concentrations of fish scale extracts (10%, 20%, 30 and 40%). The fish scales are grinded and heated at 70^0c for 10min followed by slow cooling at room temperature and then stabilize the solution for 3 days. After 3 days brown sediment were formed at bottom indicating the presence of silver nanoparticles and then centrifuged and filtered. Finally the residues are washed with double distilled water to remove unbound polymers to yield silver nanoparticles (*AgNPs*). Then the extract was filtered through double Whattman filter paper for two times, collected and stored in refrigerator (Tanur Sinha et al., 2014).

Table – 1: Chemical composition of Fish scales of *Catla catla*

Compositions	**2gms - fish scales**
Type-I collagen fibres	47–88%
Hydroxyapatite	42-75%
Aminoacids 1050	
a. glycine	23%,
b. proline	14%
c. hydroxyproline	12%
d. glutamic acid	10%
e. alanine	9%
f. arginine	8%
g. aspartic acid	6%
h. lysine	4%,
i. serine	3%
j. leucine	3%
k. valine	2%
l. phenyl alanine	2%
m. threonine	2%
n. isoleucine	1%,
o. hydroxylysine	1%
p.methionineand histidine	<1%
q. tyrosine	<0.5%

Preparation of Silver nitrate solution

Initially 0.787 g silver nitrate was dissolved in 100 ml distilled water.

Synthesis of nanoparticles

10% of fish scale extract was mixed with silver nitrate solution in 1:9 proportions and kept at room temperature for 72 hrs for the development of reddish brown colour.

Source of organism and composition of growth media

Nutrient Agar Medium preparation

1.5g of nutrient agar medium was mixed with 100ml distilled water and two drops of antibiotic was added. The conical flask was cotton plugged and autoclaved at 15 l b/inch^2 pressure and 121^{0}C for 15 min.

Inoculation

After cooling the agar medium, bacteria *Aeromonas veronii* subculture obtained from the Department of Microbiology, Sri Venkateswara University, Tirupati. The culture is inoculated with a needle to the solidified agar medium and was kept in 30^{0}C temperature in incubator for 48 hrs.

Antibacterial Activity

The antibacterial activity of *AgNPs* were carried out by Agar diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The *AgNPs* were poured in to wells with different concentrations (10µl, 20 µl, 40 µl, 60 µl, 80 µl and 100 µl) in the nutrient agar plate and kept for incubation at 37^{0}C for 24 hours.

UV-Visible spectral analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-visible spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. UV-visible analysis was done by using UV-VIS spectrophotometer **(Nanotrope, Model No- 8000).** UV-VIS spectroscopic analysis was done at DST-PURSE, Sri Venkateswara University, Tiruapti.

CHARACTERIZATION OF SILVER NANOPARTICLES

Scanning Electron Microscope (SEM) study

The solution of fish scale extract in each beaker was dried and sent for scanning electron micrograph (SEM). The SEM characterization was carried out using a scanning electron microscope **(Carlzeiss, Model No-EVO15).** SEM analysis was done in the Central Institute Lab, Department of Physics, Sri Venkateswara University, Tiruapti.

Fourier Transform Infrared Spectroscopy (FT-IR) study

Infrared photograph was recorded by Fourier transform infrared spectroscopy (Bruker, Ettilngen, ALPHA, interferometer (ECO-ATR). FT-IR analysis was carried out Central Institute Lab, Department of Chemistry, College of Engineering, Sri Venkateswara University, Tiruapti.

RESULTS AND DISCUSSION

Synthesis of Silver nanoparticles

In according to the base line report, it is well known that the silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of Surface Plasmon vibrations in silver nanoparticles (Shreya medda et al., 2015). But in our investigation, the silver nanoparticles usually exhibited reddish brown colour in aqueous solution, due to excitation of Surface Plasmon Resonance (SPR) in the silver nanoparticles after incubation (Thirumurugan et al., 2011). The appearance of reddish brown colour in the reaction vessels suggested the formation of silver nanoparticles. Silver Nitrate is used as reducing agent as silver has an identical properties such as good conductivity, catalytic and chemical stability.

It is well known that aqueous $AgNO_3$ is not a stable chemical and can be decomposed easily either by heating or by UV light illumination (Kundu, 2013). Under such conditions, Ag(0) is the sole product after releasing oxygen and nitrogen dioxide and in such case the Ag(0) formed non-uniform micron-size Ag particles in the absence of any stabilizing agent is very much essential for the synthesis of *AgNPs*. Here fish scale extract serves as both reducing as well as stabilizing agent.

Fish scales are biocomposites of high ordered, Type-1 collagen fibres (41-84%) and Hydroxyapatite $Ca_{10}(OH)_2(PO_4)_6$ (Onozato, 1980; Zulberberg et al., 1988; Ikoma et al., 2003). Type-1 collagen is a heterotrimeric copolymer composed of two α_1 (1) and one α_2 (1) polypeptide chains, containing approximately 1050 amino acids each. Each polypeptide chain has a conformation of a left handed, polyproline-II-type helix, which are twisted together into a right handed coil, a triple helix, which represents a quaternary structure, being stabilized by numerous hydrogen bonds and intra molecular vaderwaals interactions (Brinckmann et al., 2005) as well as by some covalent bonds (Harkness, 1961) and further associated into right handed micro fibrils and fibrils, being further assembled into collagen fibres with unusual strength and stability.

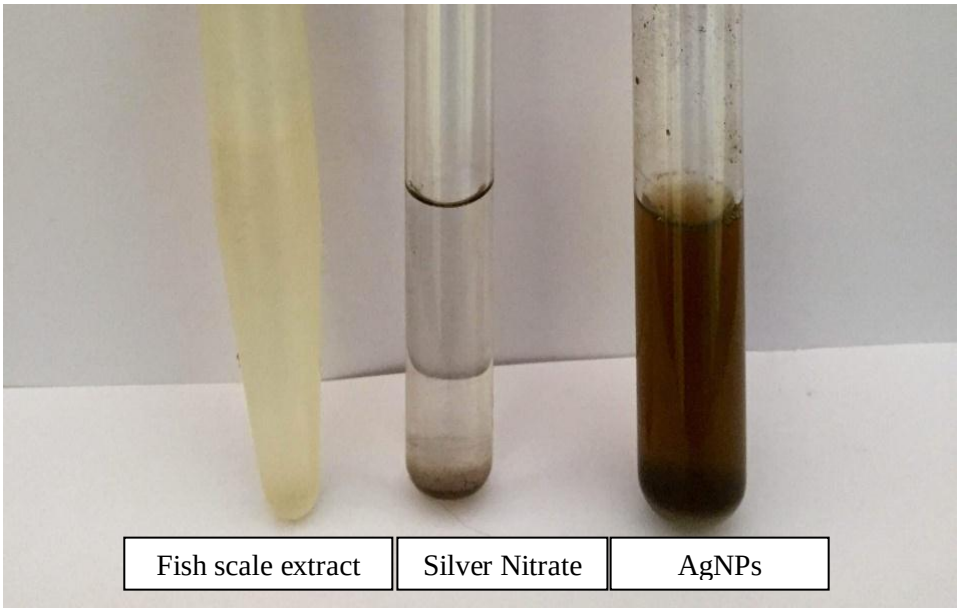

Fig-2 Change of colour was observed after 72hours

Fig-2 represents the right side test tube indicates the synthesized silver nanoparticles clearly indicates colourless to reddish brown within 72hrs of incubation at room temperature.

Normally *AgNPs* were synthesized by the raw material such as plant, microbes, haemolymph, etc. But it is the first approach synthesizing the *AgNPs* in the scales of fresh water edible fish, ***Catla catla***.

UV-VIS Spectra Analysis of synthesized Silver nanoparticles

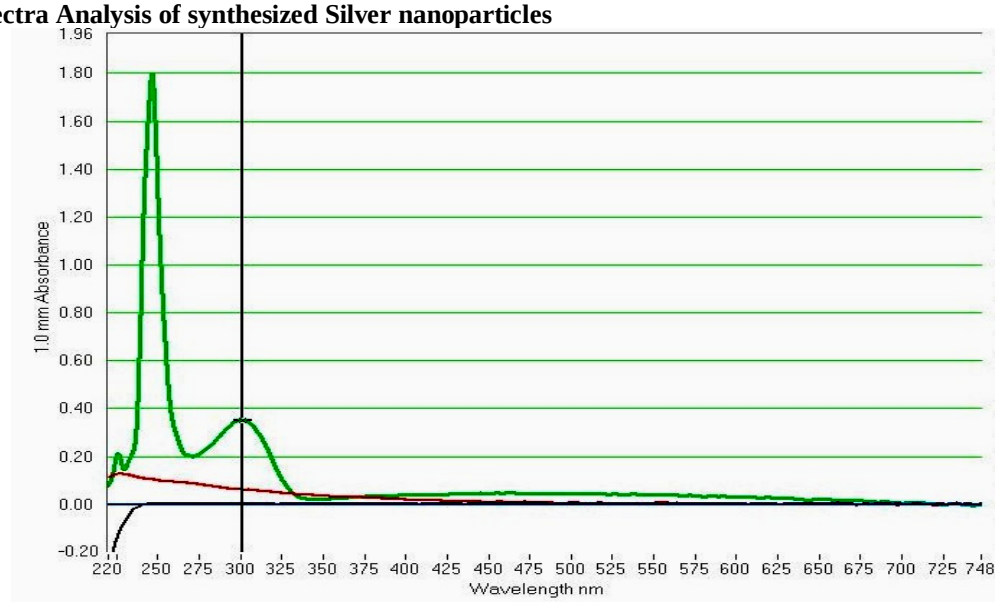

Fig-3 represents the Corresponding UV-VIS absorption spectrum of *AgNPs* recorded.

The spectra of *AgNPs* showed maximum absorption at 300nm to the Surface Plasmon Resonance (SPR) of the formed silver nanoparticles. Earlier reports showed that silver nanoparticles may grow in a process involving rapid bio-reduction and that they strongly influence the SPR in the water extract (Huang et al., 2007).

Fourier Transform Infrared Spectroscopy (FT-IR) study

FT-IR spectra show the biosynthesized silver nanoparticles and carried out to identify the possible interaction between protein and silver nanoparticles. The results of FT-IR analysis for *AgNPs* is depicted in Fig-4 spectra of *AgNPs* showed transmission peaks at 3,329 cm^{-1}, 2,124 $cm^{-1}$and 1,637 cm^{-1} respectively.

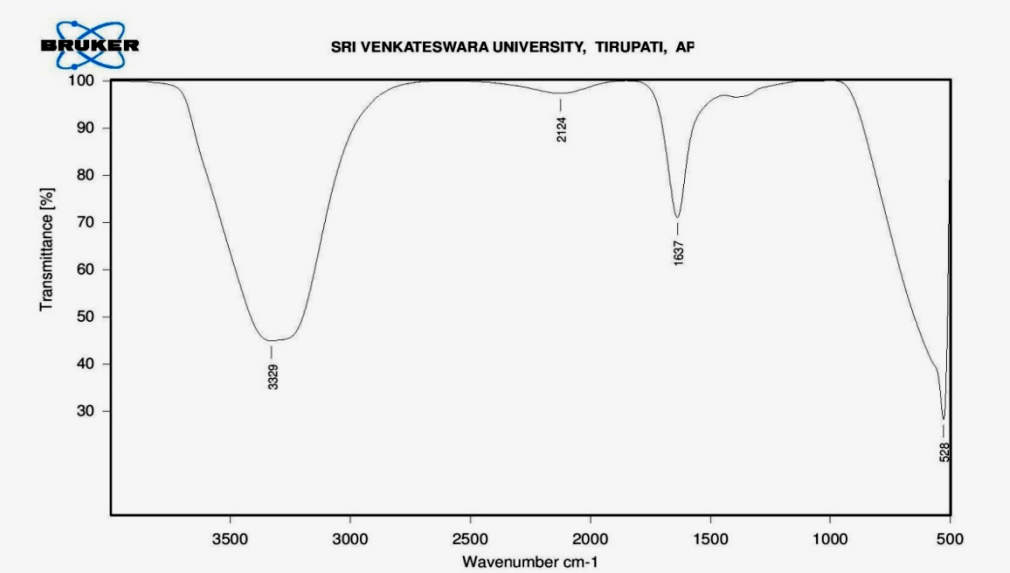

Fig- 4: FT-IR Analysis spectra of *AgNPs* showed various transmission peaks.

The peak at 2,124 cm^{-1} indicates primary amines, the peak at 3,329 cm^{-1} corresponds to O-H bonded phenols and alcohols in *AgNPs* while the peak at 1,637 cm^{-1} in *AgNPs* corresponds to involvement of nitriles (-C=N) groups.

Absorption band at 1,637 cm^{-1} suggested the presence of Amide group raised by the carbonyl stretch of proteins. IR Spectroscopic studies confirmed that carbonyl group amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nanoparticles, and acting as capping agent to prevent agglomeration and provide stability to the medium.

Our results confirmed the presence of possible proteins acting as reducing and stabilizing agents and indicated that the carbonyl group of proteins adsorbed strongly to metals (Garg, 2012; Shreya medda et al., 2015).

Scanning Electron Microscope (SEM) Study

After the Synthesis of silver nanoparticles the synthesized *AgNPs* are taken for centrifugation at 3000 rpm for 15 min (Fig-5**).**

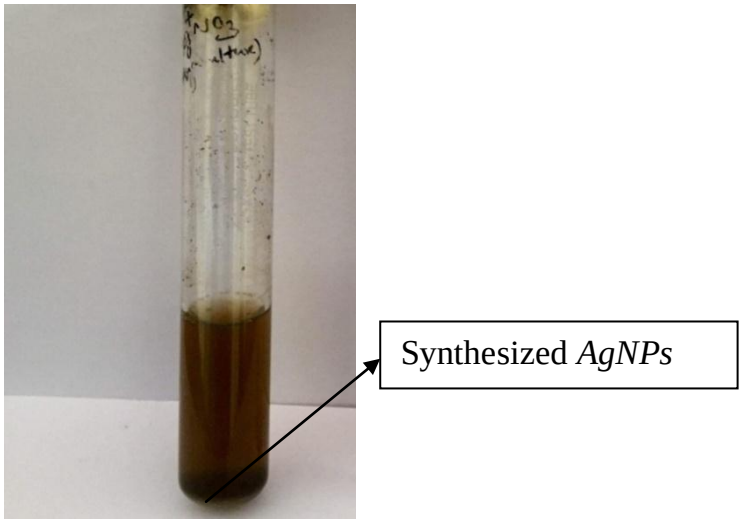

Fig-5 showing synthesized *AgNPs*

After centrifugation the supernatant and pellet were collected. The pellet was redisperesed in deionized water to get rid of any uncoordinated biological molecules. The supernatant was collected and stored in refrigerator for further use. The pellet is sun dried (Fig-6) for 5 hours and powdered from *AgNPs* (Fig-7) are taken in to test tube to undergo SEM analysis.

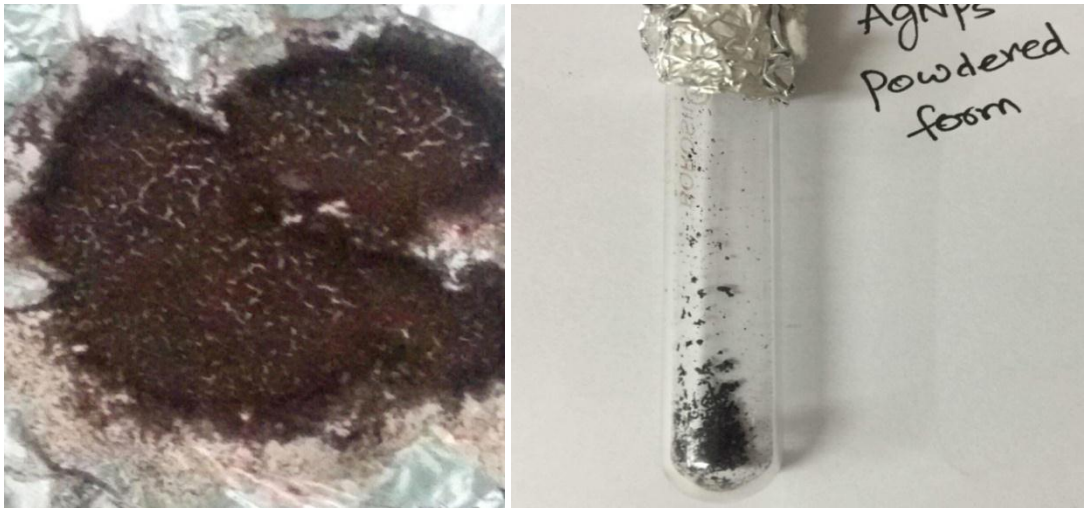

Fig-6&7: showing sun dried Pellet and powdered form *AgNPs*

Scanning Electron Microscopy (SEM) analysis provided the morphology size details of the nanoparticles (Fig-8), shows high density *AgNPs* synthesized from fish scales more confirmed the presence of silver nanoparticles.

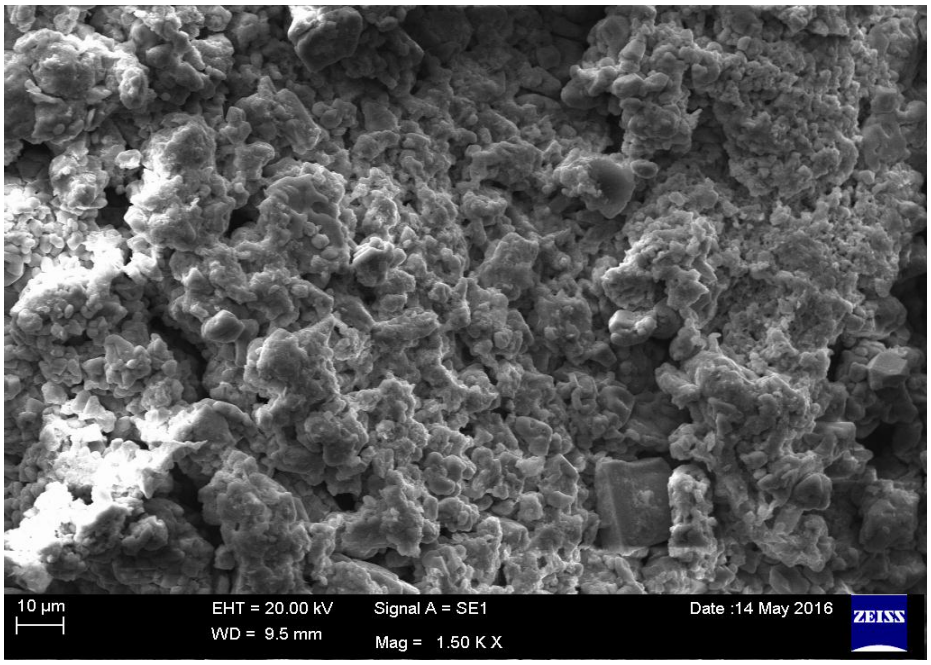

Fig-8: SEM of Synthesized Nanoparticles

Fig-8 showed that the silver nanoparticles are spherical, triangular, rectangular and cubical in shape with uniform distribution. However, on most occasions, agglomeration of the particles was observed probably due to the presence of weak capping agent which moderately stabilized the nanoparticles (Nethra, 2012).

The image in Figure-8 reveals the presence of agglomerated nanoparticles were in the range of 218.6-265.3nm; however the average size of an individual particle is estimated to be 50nm. Increasing of Silver nitrate concentration favours the silver ions reduction which leads to clustering of *AgNPs* and formation of larger agglomerates.

It is believed that the mechanism for the formation of *AgNPs* followed two steps: In the first step, when $AgNo_3$ solution is added to the fish scale extract, formed an instantaneous gelatin-Ag^+ complex where the positively charged Ag (I) ions were initially bound with the negatively charged group of gelatin via electrostatic interaction that can be evidenced by the shift of the absorption of UV-Visible spectra by 5-6 nm as well as dramatic increase in the absorption intensity as compared to the absorption band of pure gelatin (Sinha et al., 2014).

In the second step the heating of the solution mixture for 10min, so that the formed complex starts reducing ultimately self assembled *AgNPs* were formed after

cooling for 72 hours at room temperature (Sinha et al., 2014). It is believed that the reduction mechanism of all the silver ions followed the autocatalytic growth route. It is concluded that the gelatin itself acted as reducing agent as well as stabilizing agent for the formation of silver nanoparticles.

Antibacterial Activity of *AgNPs* by Agar Well Difussion Method

The mechanism of the bactericidal effect of silver nanoparticles is not very well known. It is believed that cellular proteins become inactive proteins after treatment with silver nanoparticles. For many decades, silver has been known for its antimicrobial prospective and its speculated that the *AgNPs* exert their effects by inhibiting enzymatic respiratory system of microbes, alteration of DNA replication and interaction with S-H bonds of proteins leading to inactivation (Guzman et al., 2008).

These *AgNPs* are also involved in formation of reactive oxygen species thereby inhibiting respiratory enzymes and proteins leading to physiological malfunctioning responsible for morality of *A.veronii.*

Silver nanoparticles are also involved in formation of reactive oxygen species thereby inhibiting respiratory enzymes and proteins leading to physiological malfunctioning responsible for mortality of *A.veronii*. The ionic silver strongly interacts with thiol group of vital enzymes and inactivates the enzyme activity. Earlier workers report that Silver inhibits phosphate uptake and exchange in *Escherichia coli* and causes efflux of accumulated phosphate as well as of mannitol, succinate, glutamine and proline (Duran et al., 2010).

Fig-9: Zone of Inhibition with *AgNPs*

The bactericidal efficiency of the synthesized *AgNPs* was studied against a sub cultured strain of *A.veronii* using agar well diffusion method, the Zone of inhibition were observed. It is obvious from our results that DNA loses its replication ability, once the bacteria have been treated with silver ions, and their stability in the medium as colloid, which modulated the phosphotyrosine profile of the pathogen proteins and arrest its growth.

In conclusion, Silver nanoparticles with antibacterial activity against fish pathogens can become an asset for fishery and aquaculture industry as a potential alternative to antibiotics. AgNPs was successfully synthesizing by biological method from fish scales. The Surface Plasmon Resonance (SPR) property of synthesized nanoparticles was studied by UV-Vis spectroscopy and the peak of the spectra was found to be at 300 nm and the SEM studies shows the presence of agglomerated nanoparticles were in the range of 218.6-265.3nm; however the average size of an individual particle is estimated to be 50nm.The Physicochemical characteristics of silver nanoparticles are done by FT-IR. The antibacterial activity of silver nanoparticles shows the significant antibacterial activity against fish pathogen *A.veronii*.

ACKNOWLEDGEMENT

D. Vineela is highly grateful to the Department of Science and Technology, New Delhi for sanctioning the INSPIRE Fellowship. Authors are grateful to Departments of Physics, Chemistry and DST-PURSE, Sri Venkateswara University, Tirupati for the scientific support.

REFERENCES

1. Biplab Sarkar., Arabinda Mahanty., Surya Praksh Netam., Snehasish Mishra., Niltpala Pradhan., Mrinal Samanta. (2012). Inhibitory role of Silver nanoparticles against important fish pathogen, *Aeromonas hydrophila*, International Journal of Nanomaterials and Biostructures, 2(4): 70-74.
2. Nielsen, ME., Hoil, L., Chmidt, AS., Qian, D., Shimada, T., Shen, JY., Larsen, JL. (2001). Is *Aeromonas hydrophila* the dominant motile Aeromonas species that causes disease outbreaks in

aquaculture production in the Zhejiang Province of China?, Dis. Aquat. Organ, 46: 23-29.
3. Seshadri, R., Joseph, SW., Chopra, AK., Sha, J., Graf, J., Haft, D., Wu, M., Ren, Q., Rosovitz, MJ., Madhupu, R., Tallon, L., Kim, M., Jin, S., Vuong, H., Colin Stine, O., Ali, A., Horneman, AJ., Heidelberg, JF. (2006).Genome Sequence of *Aeromonas hydrophila* ATCC 7966T, Jack of All Trades. J. Bacteriol, 188(23): 8272-8282.
4. Hickman-Brenner, F.W., MacDonald, K.L., Steigerwalt, AG., Fanning,GR.,. Brenner, DJ., Farmer, JJ. (1988). *Aeromonas veronii*, a new ornithine decarboxylase-positive species that may cause diarrhea, Journal of Clinical Microbiology, 25(5): 900-906.
5. Whitaker, I.S., Rao, J., Izadi, D., Bulter, PR. (2004). Historical Article: *Hirudo medicinalis*: ancient origins of, and trends in the use of medicinal leeches throughout history, Br. J. Oral Maxillofac. Surg, 42(2): 133-137.
6. Patel Ketan M., Svestka Michael., Sinkin., Jeremy., Ruff, Paul. (2012). Ciprofloxacin -resistant *Aeromonas hydrophila* infection following leech therapy: A case report and review of the literature, Journal of Plastic, Reconstructive and Aesthetic Surgery, 66(1): 20- 22.
7. Meyer, FP. (1991). Aquaculture disease and health management, J. Anim. Sci, 69: 4201- 4208.
8. Freitas, RA.(2005). Current status of Nanomedicine and Medical Nanobiotics, J. Comput. Theor. Nanosci, 2: 1-25.
9. Li, Q., Mahendra, S., Lyon, DY., Brunet, L., Liga, MV., Li, D., Alvarez, PJ. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. Water. Res. 42(18): 4591–4602.
10. Mongillo, F J. (2007). Nanotechnology, 101. Greenwood Press, Westpost.
11. Smith, C J., Shaw, BJ., Handv, RD. (2007). Toxicity of single walled carbon nanotubes to rainbow trout, (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies and other physiological effects, Aquat. Toxicol, 82: 94 - 109.
12. Johari, S.A., Sourinejad, I., Barsch, N., Saed Moocheshi, S., Kaseb, A., Nazdar, N. (2014b). Does physical production of nanoparticles reduce their ecotoxicity? A case of lower toxicity of AgNPs produced by laser ablation to zebrafish (*Danio rerio*), International Journal of Aquatic Biology, 2(4): 188-192.
13. Kawahara, K., Tsurdua K., Morishita, M., Uchida, M. (2000). Antibacterial effect of silver zeolite on oral bacteria under anaerobic conditions, Dental Materials, 16: 452-455.
14. Wang, S., Hou W., Wei, L., Jia, H., Liu, X., Xu, B. (2007). Antibacterial activity of nano -SiO_2 antibacterial agent grafted on wool surface, Surface and Coatings Technology, 202: 460-465.
15. Cho, K.H., Park, J.E., Osaka, T., Park, S.G. (2005). The study of antimicrobial activity and preservative effects of nanosilver ingredient, Electrochemical Acta, 51: 956-960.
16. Mohan, Y.M., Lee, K., Premkumar, T., Geckeler, K.E. (2007). Hydrogel networks as nanoreactors: A novel approach to Silver nanoparticles for antibacterial applications, Polymer, 48: 158-164.
17. Shahverdi, AR., Fakhimi, A., Shahverdi, HR., Minaian, S. (2007). Synthesis and effect of Silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Esherichia coli*, Nanomed. Nanotechnol. Biol. Med, 168-171.
18. Tanur Sinha, M., Ahmaruzzaman, AK,. Archita Bhattacharjee. (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 Part A: Molecular and Biomolecular Spectroscopy, 413 - 423.
19. Shreya Medda., Amita Hajra., Uttiya Dey., Paulomi Bose., Naba Kumar Mondal.(2015). Biosynthesis of silver nanoparticles from *Aloe vera* leaf extract and antifungal activity against Rhizopus sp. and Aspergillus sp, Appl. Nanosci, 5: 875- 880.
20. Thirumurugan, G., Satya Veni, V., Ramachandran, S., Seshagiri Rao, J., and Dhanaraju, MD. (2011). Superior wound healing effect of topically delivered silver nanoparticle formulation using eco-friendly potato plant pathogenic fungus: synthesis and characterization, J. Biomed. Nanotechnol, 7: 659-666.
21. Kundu, S. (2013). Formation of Self-assembled Ag Nanoparticles on DNA chains with Enhanced Catalytic Activity, Cell Tissue Res, 201: 303-316.
22. Zylberberg, L., Bereiter-Hahn, J. and Sire, J Y. (1988). Cytoskeletal Organization and Collagen Orientation in the Fish Scales, Cell and Tissue Research, 253: 597-607.
23. Ikoma, T., Kobayashi, H., Tanaka, J., Walsj, D., Mann, S. (2003). Physical properties of type I collagen extracted from fish scales of *Pagrus major* and *Oreochromis niloticas*, Int J. Biol. Macromol, 32: 199-204.
24. Brinckmann, J., H. Notbohm, PK., Mueller. (2005). Collagen Primer in Structure, Processing And Assembly, Springer, 1-252.
25. Harkness, RD. (1961). Biological functions of collagen, Biol.Rev, 36: 399 - 463.
26. Huang, JL., Li, QB., Sun, DH., Lu, Yh., Su, YB., Yang, X., Wang, HX., Wang, YP., Shao, WY., He, N., Hong, JQ., Chen, CX. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf, Nanotechnol, 18: 1-11.
27. Garg, S. (2012). Rapid biogenic synthesis of Silver nanoparticles using black pepper (*Piper nigrum*) corn extract, Int. J. Inno. Biol. Chem. Sci, 3: 5 - 10.
28. Nethra, DC., Sivakumar, P., Renganathan, S. (2012). Green synthesis of silver nanoparticles using Datura metel flower extract and evaluation of their

antimicrobial activity, Int. J. Nanomater Biostruct, 2(2): 16-21.
29. Guzman, M G., Dille, J., Godet, S. (2008). Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity, World Acad. Sci. Eng. Technol, 43: 357-364.
30. Duran, N., Marcato, PD., Conti, RD., Alves, O L., Costa, FTM., Brocchi, M. (2010). Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action, J. Braz. Chem. Soc, 21(6): 949-959.