Biosynthesis of Silver Nanoparticles using Different Bacteria and Optimization of the Process Parameters using *Proteus vulgaris*

R. Narayanan, S. Jiji, K. Kadirvelu, N. Gopalan, K. Sekhar

Abstract: ***In recent years, biosynthesis of nanoparticles has gained significant interest over chemical and physical synthesis, because of their eco-friendly unique properties and applications. In the present study, an attempt was made to synthesize silver nanoparticles (AgNPs) by optimizing the process variables using various bacterial species to get the consistency in the size and shape of the nanoparticles. The bacterial species used were Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Salmonella paratyphi, Yersinia entero, Pseudomonas aeruginosa, Shigella flexneri, Agrobacterium tumefaciens and Bacillius thuringinesis. Different process variables including time, temperature and silver nitrate concentrations were optimized to obtain the uniform size and shape of AgNPs. Among the different bacterial species studied, Proteus vulgaris was found to be the most suitable one for the proposed application. Spectroscopy and electron microscopic characterizations reveal that the biosynthesized AgNPs were uniform in size with spherical form and particles size ranging from 5-10 nm. In order to know the utility of the biosynthesized AgNPs, cytotoxic effects and antibacterial activities were undertaken using RAW-264.17 cells and pathogenic bacterial cultures respectively. Results of the antibacterial studies reveal that, bio-synthesized AgNPs were capable of inhibiting the growth of tested bacterial species at a concentration of 10-30 µg/ml. The cytotoxic studies with RAW-264.17 cells further reveal that AgNPs had shown significant anti-cell proliferation effect against the studied cells with a concentration of 10-50 µg/ml. Based on the studies it is concluded that the established method in the present study is a viable alternative for cumbersome chemical synthesis of AgNPs with significant antimicrobial properties. The AgNPs obtained can be used in the preparation of different antiseptic formulations.***

Keywords: ***Bacteria: Silver-nanoparticles: optimization: Electron microscopy: antimicrobial activity: anti-cancerous activity.***

I. INTRODUCTION

Nanotechnology is one of the emerging fields which has significantly influenced science, economy and everyday life. Nanotechnology deals with the development of structures by biological, physical or chemical methods which possess at least one dimension in the size range of 1 to 100 nm [1]. This technology involves a unique combination of scientists from different fields including physicists, chemists, engineers and biologists.

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Many interesting nano devices are useful in biomedical field especially for the improved cancer detection, diagnosis and treatment [2, 3]. The outbreak of emerging and reemerging infectious diseases is becoming a constant burden on global economies and public health. The main reasons for the outbreaks of these infectious pathogens are the growth of population and urbanization along with poor water supply and environmental hygiene [4]. Comprehensive treatments of environments containing infectious pathogens using advanced disinfectant nanomaterials have been proposed for prevention of the outbreaks of pathogenic microorganisms. Among these nanomaterials silver nanoparticles (AgNPs) with unique properties of high antimicrobial activity have attracted much interest from scientists and technologists to make nanosilver based disinfectant products [5]. Silver nanoparticles may eventually offer treatment of various diseases. Their extremely large surface area permits the coordination of a vast number of ligands [6, 7]. The properties of silver AgNPs applicable to human treatments are under investigation in laboratory and animal studies, assessing potential efficacy, toxicity, and costs. Although AgNPs have shown enormous applications in the medical field they have also shown their effectiveness in other applications for e.g., mineralization of pesticides [8, 9]. Studies have shown that AgNPs have been employed to carry out the degradation of organophosphorus compounds which are widely used as pesticides [11, 12]. The AgNPs can be synthesized by biological, physical or chemical methods [13, 14, 15]. The physical methods are initially used to give a low yield. Chemical methods use various chemical agents to reduce metallic ions to nanoparticles. This comprises certain drawbacks as there will be use of toxic chemicals and generation of hazardous byproducts [31]. In the medical aspects, applications of nanoparticles increased tremendously only when the biological approach for nanoparticle synthesis came into focus. Though there is a large platform for the green synthesis of nanoparticles, the most commonly preferred way is the bacterial synthesis, as they are easy to handle, and genetic manipulation is also possible [33, 34, 35]. Biological way of synthesizing nanoparticles is eco friendly in nature, when compared to other methods [31, 32, 36]. Microbes are used for development of manufacturing techniques that are more environment friendly than chemical process and sophisticated methodologies [20, 21]. Recognizing the importance of developing eco-friendly nanoparticle synthesis methods many researchers have turned to biological synthesis by microorganisms [20, 21, 22]. Synthesis of Silver nanoparticles by microbes is due to their defence mechanism. The resistance caused by the bacterial cell for silver ions in the environment is responsible for its

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nanoparticles synthesis [23]. The silver ions in nature are highly toxic for the bacterial cells. So their cellular machinery helps in the conversion of reactive silver ions into stable silver atoms. The first evidence of the synthesis comes from *Pseudomonas stutzeri* AG259, a bacterial strain that was originally isolated from silver mine [17, 24, 25]. The silver nanoparticles with their unique chemical and physical properties are proving as an alternative for the development of new antibacterial agents. The burn wounds treated with silver nanoparticles show faster healing and better cosmetic appearance [26]. Many mechanisms involving various biomolecules have been proposed for the biosynthesis of nanoparticles, but the most accepted mechanism involves nitrate reductase-dependent reduction of silver ions. Nitrate reductase is an enzyme that is co-factored by nicotinamide adenine dinucleotide (NADH) and is capable of reducing silver ions to silver nanoparticles. Basically, it is an enzyme that is responsible for the conversion of nitrate in the nitrogen cycle. This mechanism has been clearly studied in *Bacillus licheniformis* as it secretes many NADH-dependent enzymes in which the effect of α- NADH-dependent nitrate reductase on silver ions also has been clearly studied [17]. The enzyme nitrate reductase converts nitrate to nitrite and will transfer an electron to silver ions (Ag+) to form free silver. However, the synthesis would take place only at lower concentration of $AgNO_3$ and at higher concentrations it would lead to cell destruction [23].

Studies have shown that psychrophilic bacteria namely *Phaeocystis antarctica, Pseudomonas proteolytica, Pseudomonas meridiana, Arthrobacter kerguelensis, and Arthrobacter gangotriensis* and mesophilic bacteria like *Bacillus indicus* and *Bacillus cecembensis* have been used to synthesize AgNPs. The synthesis and stability of AgNPs appeared to depend on the temperature, pH, or the species of bacteria from which the supernatant was used. It was observed that the *Arthrobacter kerguelensis* supernatant could not produce AgNPs at the temperature where *Phaeocystis antarctica* could synthesize silver NPs [27]. Size, shape and the stability of the nanoparticles will vary according to the species used for the synthesis. Comparing to the other synthesis technologies like usage of plant extracts and chemical precursors, cell free supernatant showed more efficient size reduction and mono dispersion in the synthesis. However, there is a need for the consistency, reproducibility and optimization of the process parameters for any kind of synthesis using microbial organisms.

In the present study various bacterial species including *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Salmonella paratyphi, Yersinia entero, Pseudomonas aeruginosa, Shigella flexneri, Agrobacterium tumefaciens and Bacillius thuringinesis* were used for the bio-synthesis of silver nanoparticles. Different process variables including time, temperature and silver nitrate concentration were optimized to obtain the uniform size and shape of AgNPs.

II. MATERIALS AND METHODS

2.1 Bacterial growth media and other chemicals used in present study

Silver nitrate ($AgNO_3$), Nutrient broth, Nutrient Agar was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India.

2.2 Bacterial strains and growth conditions

Pure cultures of bacteria including *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Salmonella paratyphi, Yersinia entero, Pseudomonas aeruginosa, Shigella flexneri, Agrobacterium tumefaciens and Bacillius thuringinesis* were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), India. Bacterial cultures were grown in sterilized nutrient agar medium under aerobic conditions at 37ºC for 24 Hours. The isolated colony of each culture was sub cultured into sterilized nutrient broth media and incubated for 24 hours at 37ºC in orbital shaker at 120 rpm. Glycerol stocks of the cultures were further maintained at - 80^0 C.

2.3 Biosynthesis of Silver Nanoparticles (AgNPs)

The 24 hour old bacterial cultures grown in nutrient broth media were centrifuged to separate the bacterial cells from culture media and supernatants were collected separately into sterile flasks. In order to achieve uniformly grown and high yielding AgNPs, several parameters including temperature, time and concentration of the substrate were optimized. To both the pellets and the supernatants different concentrations (0.5mM, 1mM, and 2mM) of silver nitrate solutions were added in the ratio of 1:1 in sterile condition to determine the optimum concentration. Then the conical flasks were incubated in orbital shaker in dark. The effect of temperature and time were studied by doing the trials at three different temperatures (20°C, 40°C and 60°C) and time intervals (0, 4, 8, 24, 48 and 72 h). A control was also maintained throughout the experiments. Like the supernatants, the pellets were also subjected to the addition of $AgNO_3$. The pellets were brought into solution using double distilled water and made up to 50 ml. After the synthesis of the AgNPs the flasks were centrifuged and the supernatants were collected. Then the supernatants were dried using various methods like freeze drying, evaporation and vacuum drying for characterization.

The synthesized silver nanoparticles were characterized using Ultra Violet Visible (UV-Vis) spectroscopy, X-Ray Diffraction analysis (XRD), High Resolution Transmission Electron Microscope (HR-TEM), Energy Dispersive Spectroscopy (EDS) and Dynamic Light Scattering (DLS) Technology.

2.4 Characterization of Silver Nanoparticles

2.4.1 Visible interpretation

There was an immediate colour change to pale yellow after the addition of silver nitrate, in the case of synthesis using the bacterial strain *Proteus vulgaris.* Then the colour of the sample started changing from pale yellow to brown from 4 hours after the addition of silver nitrate precursor. The colour change was observed during the time intervals from 0 to 72 h.

2.4.2 UV–Vis spectroscopy:

The bio reduction of Ag ions in aqueous extract was monitored using UV–Visible spectroscopy. After dilution

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of a small aliquot (0.1ml) of the sample to 10 times with double distilled water, the absorbance was measured using a Hitachi double beam spectrophotometer. The scan was run from 300 to 800 nm wavelength at room temperature. Double distilled water was used as the reaction blank.

2.4.3 DLS analysis of particle size and zeta potential:

The average particle size along with its polydispersity index (PdI) and the zeta potential (ZP) of the nanoparticles were analysed by photon correlation spectroscopy and laser Doppler anemometry respectively using a Zetasizer Nano ZS (Malvern Instruments, UK).

2.4.4 Energy Dispersive spectroscopy:

Energy Dispersive spectroscopy (EDS) is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. The elemental analysis was performed using EDS (EDAX Genesis instrument) which is an attachment to the scanning electron microscopy (SEM).

2.4.5 High Resolution Transmission Electron Microscopy (HRTEM)

The morphological examination of the nanoparticles was performed by High Resolution Transmission Electron Microscopy (HRTEM) on a JEOL (JEM-2100) instrument with an acceleration voltage of 80kV after evaporating the solvent from a drop of the suspension containing AgNPs on a carbon-coated copper TEM grid. HRTEM experiments were performed to characterize size and shape of bio-reduced AgNPs. Purified AgNPs were solicited for 15 minutes to make a uniform distribution and a drop of this solution was loaded on carbon-coated copper grids and solvent was allowed to evaporate. The particle size distribution of the AgNPs obtained from TEM images was evaluated using Image J 1.45s software.

2.5 In-vitro cytotoxic potential of AgNps

2.5.1 Cell Viability Assay

The cell viability assay was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide dye (MTT) reduction assay which was performed to determine the cytotoxic effect of the AgNPs at various concentrations. Briefly, RAW 254.7 cells ($5x10^3$) were seeded into individual 96-well plates with various concentrations of AgNPs and incubated at humidified environment with 5% CO_2 at 37^{o}C for 24 h. After incubation the cells were treated with various concentrations of AgNPs and were incubated as mentioned earlier. After 48 h of incubation the culture media was aspirated and 10 µL of MTT (5 mg/mL in phosphate-buffered saline (PBS)) dye was added along with 90 µL of serum free culture medium and incubated at 37^{o}C for 4 h. The resulting formazan was dissolved in 100µL of DMSO with gentle shaking at 37°C, and absorbance was measured with an ELISA microplate reader (Biotek, Germany) at 550 – 600 nm. Concentrations of AgNPs showing a 50% reduction in cell viability (i.e., IC50 values) were then calculated.

2.5.2 Membrane Integrity

Cell membrane integrity of RAW 254.7 cells was evaluated by determining the activity of lactate dehydrogenase (LDH) leaking out of the cell. The LDH assay is based on the release of the cytosolic enzyme, LDH, from cells with damaged cellular membranes. In cell culture, the course of AgNPs induced cytotoxicity was followed quantitatively by measuring the activity of LDH in the supernatant. Briefly, cells were exposed to various concentrations of AgNPs for 24h, then 100µl per well of each cell-free supernatant was transferred in triplicates into wells in a 96-well, and 100µl of LDH assay reaction mixture was added to each well. After 3h incubation under standard conditions, the optical density of the color generated was determined at a wavelength of 490 nm using a microplate reader.

2.5.3 Half Maximal Inhibitory Concentration (IC50):

The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular substance is needed to inhibit a given biological process. In the present study the IC50 was determined after carrying out the MTT and LDH assay.

2.6 Screening of antibacterial activity of silver nanoparticles:

The synthesized AgNPs was tested for antibacterial activity by well diffusion method and Minimum Inhibitory Concentration (MIC) against clinically isolated Gram positive and Gram negative microorganisms like *S. aureus*, *E. coli* and *P.aeruoginosa*. MIC is defined as the lowest concentration of the nanoparticles that inhibited the visual growth of the test cultures. The pathogenic cultures were subcultured into peptone broth and incubated at 37˚C to attain 10^5-10^6 CFU/ml using MacFarland's standard and were used in further experiments.

The minimum inhibitory concentration (MIC) of biosynthesized AgNPs were determined by micro dilution method [30]. Briefly, synthesized AgNPs were suspended in double distilled water and different concentrations were prepared. Test bacterial cultures were diluted to contain approximately 105–106 CFU/ml with sterile media and were added to 96 well microtitre plate (Nunc, USA). If a zone of inhibition was observed around the well after the incubation period, then a positive result was concluded. Tests were performed in triplicate, and mean values of zone diameter were recorded.

2.7 Statistical analysis

Data were expressed as mean ± SE of a minimum of 3 replicates and all the experiments were repeated twice. Statistical differences between control and target groups for all experiments were determined using Student's *t*-test with two way anova at $p \leq 0.05$ significance level.

III. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles (AgNPs)

When the silver nitrate was added to the cell free supernatant of the various cultures, only the supernatant obtained from *Proteus vulgaris* showed a distinct brown

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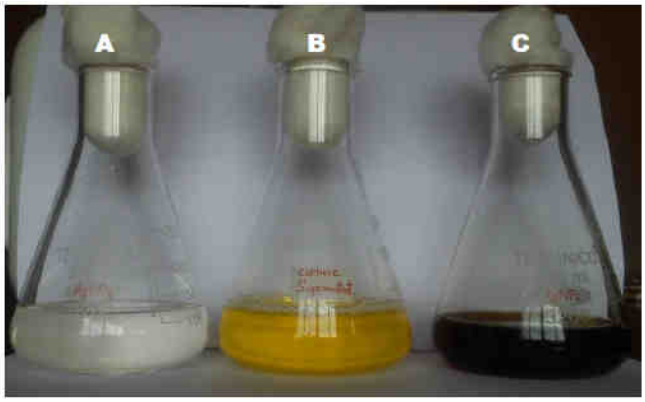
colour change after 2 h which is due to the reduction of silver ions and formation of silver nanoparticles (Fig 3) and the colour change is because of surface plasmon vibrations in the particles [26]. So far there are no reports on the use of *Proteus vulgaris* for the synthesis of silver nanoparticles. The supernatant from *Escherichia coli, Klebsiella pneumonia, Salmonella paratyphi, Yersinia entero, Pseudomonas aeruginosa, Shigella flexneri, Agrobacterium tumefaciens and Bacillius thuringinesis* did not show a distinct change from pale yellow to brown even after 4 h though there were changes after 24h (Fig 1).



a) Silver nitrate (AgNO3), b) Culture Supernatant of Proteus vulgaris c) Culture Supernatant of Proteus vulgaris with Silver Nitrate

Figure 1: Synthesis of silver nanoparticles using Culture Supernatant of *Proteus vulgaris*

The studies have shown that the silver nanoparticles UV absorbance will be in the range of 420-435nm. The maximum absorbance of the nanoparticles was obtained in 24 h culture at 432nm range (Fig 2). The absorbance from UV-Visible spectroscopy showed a distinct absorbance for the bio synthesized AgNPs using *Proteus vulgaris* and most of the supernatants obtained from all the other bacterial species used for the synthesis didn't show any absorbance at 432nm except the one obtained using *Pseudomonas aeruginosa* which showed a slight absorbance (Figure 2).

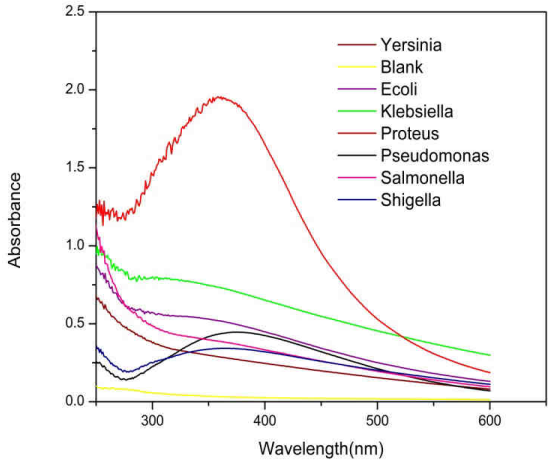


Figure 2: Optimization of Bacterial strains for the Bio-Synthesis of Nano particles

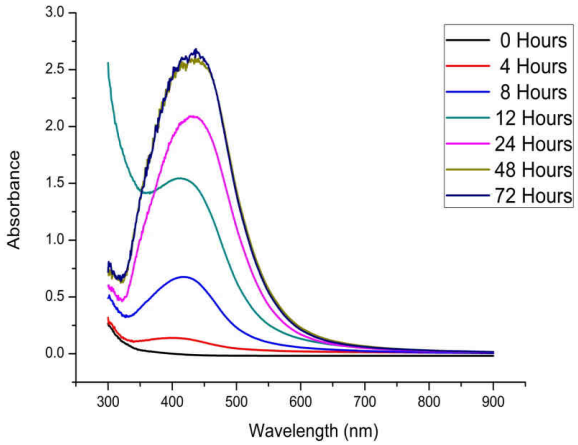
Pellets of the strains did not show any silver nanoparticles formation. Hence based on the absorbance values it was decided to go ahead with the bio synthesis of AgNPs using in order to streamline and optimize the process parameters. Further confirmation on formation of silver nanoparticles in cell free supernatant was observed in DLS and HR-TEM. Particles were Spherical shaped and the size distribution was in the range of 5-10 nm. Colour mapping showed the presence of silver nanoparticles by the coloured representation.

3.1.1 Optimization of Time:

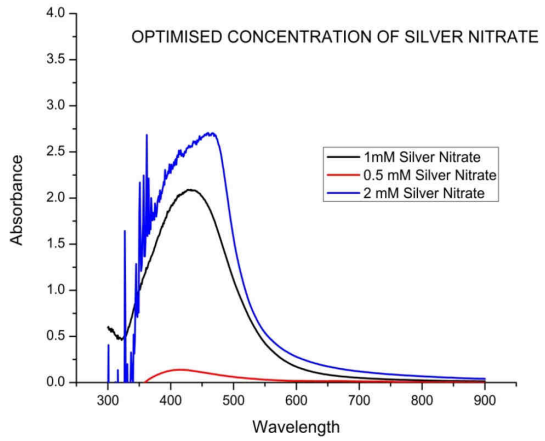
Among the study of time points from 4 to 72h, it was found that 24 h of incubation showed maximum intensity of the color change and the change in λ maximum from 413 to432 nm was observed. Upon increasing the interaction time up to 72 h, the λmax value did not vary significantly (Fig 3a).

3.1.2 Optimization of Concentration:

It was found that the silver nanoparticles formed by using 0.5 mM concentration of $AgNO_3$ had shown an absorbance of 0.138 which was very low compared to the AgNPs formed using 1 mM and 2 mM $AgNO_3$ (Fig 3b) and also it indicates that the formation is very slow. When we compared the absorbance peaks of AgNPs formed by using 1 mM and 2 mM concentration of $AgNO_3$, we found that the absorbance peak of AgNPs obtained from 1 mM concentration was quite stable where as the one obtained using 2 mM peak was broad.



(a) Optimization of time



(b) Optimization of Concentration

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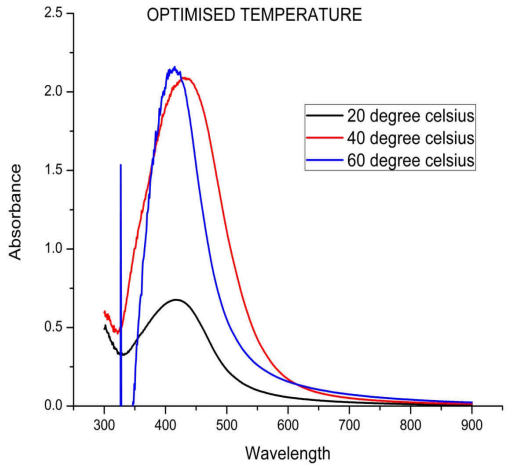

(c) Optimization of temperature

Figure 3: UV-Visible spectra analysis for the optimization of parameters for the Bio synthesis of AgNPs using Proteus *vulgaris*

3.1.3 Optimization of Temperature:

Studies on the effect of temperature from 20ºC to 60ºC (Fig 3c) on AgNPs synthesis showed that at 20ºC the UV absorbance was very less even after 24 h of incubation which indicates that the formation of AgNPs is slow. Synthesis at 60ºC showed broad peak with instability. The synthesis carried out at 40ºC showed a stable and significant increase in absorbance peak. This indicates the stable formation of silver nanoparticles which needed to be further characterized using other techniques.

Table 1 UV- Vis spectral data of silver colloid solution produced under different conditions

S. No	Variable conditions	Wavelength (nm)	Absorbance (λ max)
Effect of Concentration (mM)			
1	0.5	420	0.138
2	1	432	1.921
3	2	451	2.873
Effect of Temperature (°C)			
4	20	422	0.675
5	40	432	1.934
6	60	412	2.145
Effect of Time Intervals (hrs)			
8	4	405	0.139
9	8	419	0.676
10	12	413	1.545
11	24	432	1.928
12	48	435	2.609
13	72	435	2.673

3.2 UV- Vis spectral characterization:

From the stable absorbance peak at 432 nm showing the presence of AgNPs it was determined that the optimum parameters for the bio synthesis of AgNPs using *Proteus vulgaris* are a. 1:1 ratio of 1mM $AgNO_3$ b. 24 h reaction time and c. 40ºC (Fig 4) using cell free supernatant.Table 1 shows the absorbance values obtained during the optimization at various different reaction conditions.

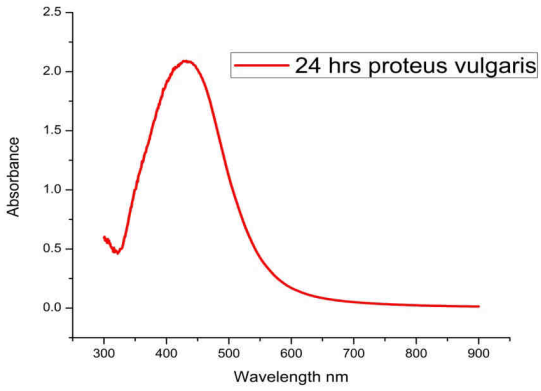

Fig 4: UV-Vis absorbance of the bio synthesized silver nanoparticles using *Proteus vulgaris* in optimized conditions

3.3 DLS analysis of particle size and zeta potential:

The particle size analysis showed that the silver nanoparticles synthesized in the bio reduction process using cell free supernatant of *Proteus vulgaris* were extensively distributed in the solution. The particle size of the silver nanoparticles was approximately 25-50 nm and zeta potential indicates the degree of repulsion between the particles [28]. Silver nanoparticles showed zeta potential of -6.33 mv which indicates the presence of repulsion and absence of agglomeration among the particles (Fig 5).

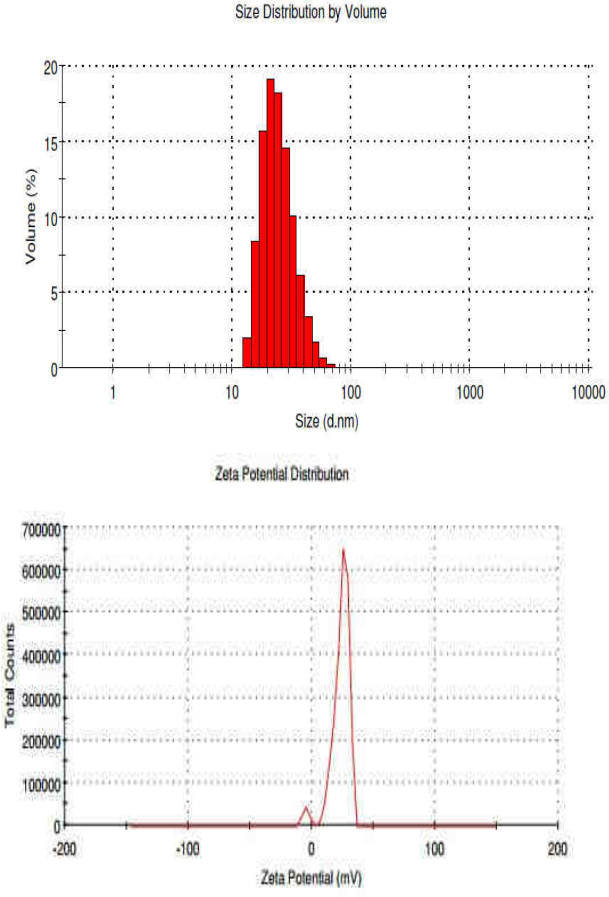

Figure 5: DLS results of size distribution and zeta potential

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The z Average size obtained was 26 nm. The DLS measured size is slightly bigger as compared to the particle size measured from HR-TEM micrographs, which could be explained as the dynamic light scattering (DLS) measures the hydrodynamic radius which is an indirect measurement.

3.4 Energy Dispersive Spectroscopy:

Chemical analysis of the produced AgNPs was accomplished by means of EDS, which confirmed both the existence of the AgNPs. Metallic silver nanocrystals generally show typical optical observation peak due to surface plasmon resonance [26]. The EDS spectra also proved that the Ag nanoparticles are in metallic form, with no formation of silver oxide in them and free from any other impurities. The EDS results showed the presence of silver nanoparticles with the percentage of 62.14 % (Fig 6). Colour mapping of EDS confirms the uniform distribution of silver nanoparticles.

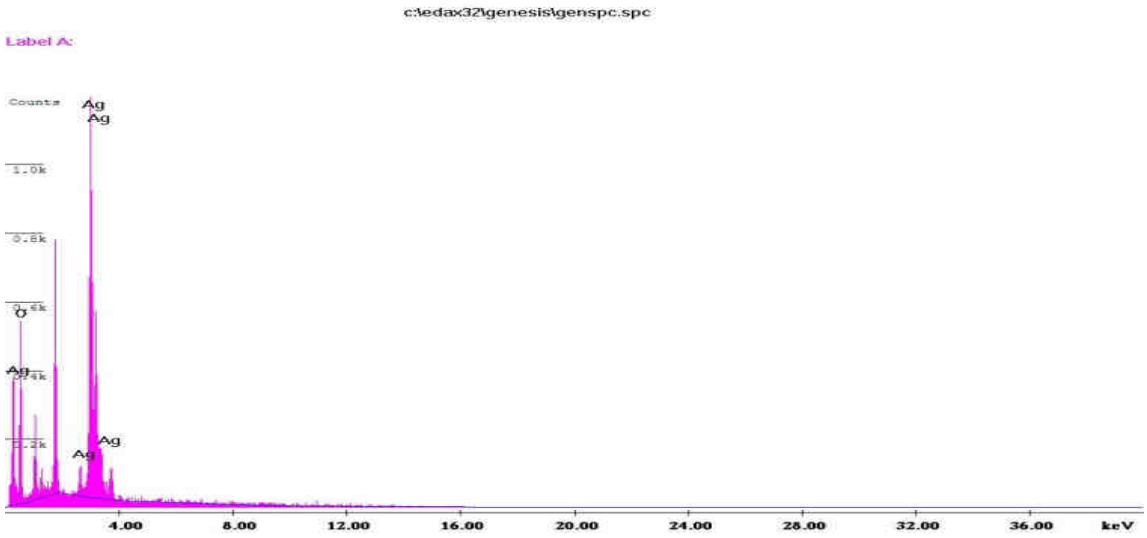

Figure 6: EDS elemental analysis of AgNPs

3.5 HRTEM image analysis:

HRTEM analysis was used to measure the size and shape of the AgNPs formed and the images of the TEM visualization are shown in (Fig 7). Synthesized nanoparticles showed spherical shape and showed a large distribution of size ranging from 5-10 nm. There was no visible agglomeration observed between the nanoparticles

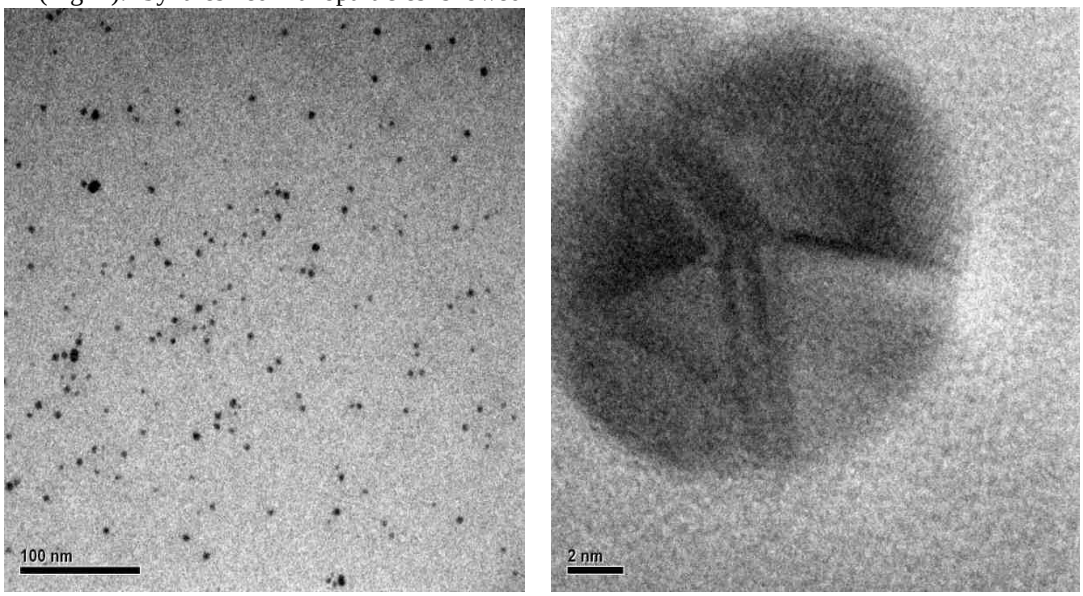

Figure 7: HRTEM images of synthesized AgNPs

3.6. Cytotoxicity effect of AgNPs in RAW- 264.7 cells:

The cell viability assay is one of the important methods for toxicology analysis which explains the cellular response to toxic materials, and it can provide information on cell death, survival, and metabolic activities [37]. The cells were treated with various concentrations (0–50 µg/mL) of AgNPs for 24h, and the results suggest that AgNPs were able to reduce the cell viability of RAW- 264.7 cells in a dose dependent manner. After 24h of treatment AgNPs were found to be cytotoxic to the cells at concentrations of 10µg/mL and higher. Cytotoxic potential of the biosynthesized AgNPs against RAW- 264.7 (mouse macrophage cells) revealed that bio reduced AgNPs have

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shown significant cytotoxic effect against tested cancerous cell line.

3.7. Impact of AgNPs on Membrane Integrity:

Lactate dehydrogenase (LDH) is a soluble cytosolic enzyme that is released into the culture medium following loss of membrane integrity resulting from either apoptosis or necrosis. LDH activity, therefore, can be used as an indicator of cell membrane integrity and serve as a general means to assess cell viability by measuring plasma membrane permeability. Cayman's LDH Cytotoxicity Assay Kit was used for the measurement and the results show that cell membrane integrity in RAW- 264.7 cells was affected in a dose dependent manner by AgNPs of 10nm size. Lee et al. [38] observed that the LDH level was elevated when cells were cultivated for 48 h in the culture medium containing AgNPs at 100 µg/ml. In the LDH assay, it was evident that as the concentration of the AgNPs increased, cells became progressively more cytotoxic, leading to a higher absorbance reading in the LDH assay and a decrease in absorbance in the MTT assay with a concurrent decrease in the percentage of viable cells. The inverse relationship between the LDH and the MTT cell viability results adds support to the accuracy of the data.

3.8. Determination of IC50 Values of AgNPs:

To determine the cytotoxic effect of particular concentration, the half maximal inhibitory concentration (IC50) was calculated as the concentration required to inhibit the growth of tumor cells in culture by 50% compared to the untreated cells. The AgNPs at 10.7µg/ml decreased the viability of RAW- 264.7 cells to 50%, and hence this was determined as the IC50. Longer exposures resulted in additional toxicity to the cells.

3.9. Minimum Inhibitory Concentration for determining the Antimicrobial activity

The antimicrobial activity of biosynthesized AgNPs tested against gram positive and gram negative pathogenic bacteria such as *S. aureus*, *E. coli* and *P. aeruginosa*. The results are presented in Table 2 as the average values of zone of inhibition radii and Minimum Inhibitory Concentration (MIC). Disc diffusion test results indicate that the maximum zone of inhibition against *E. coli* is 15.6 mm, while *P. aeruginosa* requires 12.2 mm and *S. aureus* requires 7.5 mm. These results support the findings of Ingle et al. [39] which suggest that AgNPs exhibit significant antibacterial activity against *E. coli* and multidrug-resistant bacteria.

Table 2: Antibacterial activity of biosynthesized silver nanoparticles using *Proteus vulgaris*

Pathogens	***Proteus vulgaris* supernatant**		**$AgNO_3$**		**Synthesized Silver Nanoparticles**	
	Disk Diffusion Assay (mm.dia)	MIC (µg/ml)	Disk Diffusion Assay (mm.dia)	MIC (µg/ml)	Disk Diffusion Assay (mm.dia)	MIC (µg/ml)
Escherichia coli	-	-	14.8 ± 1.08	8 ± 0.00	15.6 ± 0.64	10 ± 0.00
Pseudomonas aeruginosa	-	-	12.8 ± 1.09	11± 0.00	12.2 ± 1.76	11 ± 0.00
Staphylococcus aureus	-	-	11.2 ± 0.78	15± 0.00	7.5 ± 0.66	25.8 ± 0.00

Table 2 shows the MIC values of AgNPs for different bacteria. *E. coli* (10 µg/ml) was observed as the most sensitive bacteria, followed *P. aeruginosa* (11 µg/ml) and *S. aureus* (25.8 µg/ml) exhibited the highest MIC value. MIC values for *E. coli* and *S. aureus* recorded in this study are higher than those observed by Vertelov et al. (1 µg/ml for *E. coli* and 5 µg/ml for *S. aureus*) [41] and lower than those reported by Chudasama et al. (100 µg/ml for *E. coli* and 350 µg/ml for *S. aureus* [40]. The results of the present study were in agreement with the previous results [42] where they studied the antibacterial activity of green synthesized AgNPs against test pathogens. In general, upon interaction of bacterial cells silver ions from AgNPs which are released into the bacterial cells lead to the bactericidal activity.

IV. CONCLUSION

In the present study a simple, reliable methodology for the synthesis of silver nanoparticles was developed using *Proteus vulgaris* as a bio-reducing agent. The developed method is a viable alternative for cumbersome chemical syntheses of AgNPs with significant biological activity. The cytotoxic and antimicrobial activities of biosynthesized AgNPs against RAW 254.7 cells as well as Gram positive and Gram negative bacteria were assessed. The present study revealed that the potential cytotoxic effect of biologically synthesized AgNPs in RAW 254.7 cells by inhibiting growth of cells leading to a high absorbance of LDH. The antibacterial activity of synthesized AgNPs showed effective inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Also these AgNPs may find a utility in the biodegradation of organophosphorus compounds usually present in contaminated soil and other farming lands which lead to ground water pollution. Thus, outcome of this study would be useful in developing nano-materials in biomedical and nanotechnology industries.

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Conflict of Interest: None

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