INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Research Article

DETERMINATION OF ANTIMICROBIAL AND ANTICANCER ACTIVITY OF BIOLOGICALLY SYNTHESISED SILVER AND ZINC NANOPARTICLE

M. RAJAMANI, V.RAMYA AND S.CHANDRASEKAR*

Department of Microbiology, Sri Krishna Arts and Science College, Coimbatore, Tamilnadu, India.
Corresponding Author: sekar_biotech@yahoo.com

Abstract

Silver and zinc nanoparticles were synthesized by cell free filtrates of *Bacillus subtilis* and *Streptomyces* PDS1 respectively. The synthesized nanoparticles were characterized by UV visible spectra, FTIR and Scanning electron microscopy. Antibacterial activity of synthesized nanoparticles against *Escherichia coli*, *Salmonella enterica, and Pseudomonas aerogens* were determined. Antifungal activity was carried out against *Aspergillus flavus* and *Aspergillus niger.* Anticancer activity was studied in A549 cancer cells using Tryphan blue dye using fusing haemocytometer.

Keywords: UV visible spectra - Scanning electron microscopy - Fourier transform infrared spectroscopy

Introduction

Nanoparticles are the sub-nanosized colloidal structures composed of natural and synthetic polymers and size range from 10nm to 1000nm (Gupta *et al.,* 2011). Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. The magnetic, chemical, mechanical, optical and electrical properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the material surface becomes significant. Recent developments in nanotechnology allow us to produce, characterize and change functional properties of nanoparticles for use in catalysts (Cornell and Schwertman, 1996), gas sensors (Liu *et al.,* 1999), optical magnetic recording (Awschalom and Divincenzo, 1995) and various biomedical applications including magnetic resonance imaging (MRI), hyperthermic treatment for malignant cells, targeted drug and gene delivery, and magnetic cell separation (Gupta and Gupta, 2005). With the availability of these new applications, new techniques should be developed to scale the processes to commercially accepted levels at the same time develop lower cost approaches to nanoparticle synthesis.

Biological synthesis of nanoparticles is mostly preferred because they are regarded as safe, cost-effective, sustainable and environment friendly processes for the synthesis of silver nanoparticles (Amar Ratan *et al.,* 2013). Silver nanoparticles have been successfully synthesized using bacteria (Ratan Das *et al.,* 2011), fungi (Ahmad *et al.,*2003), actinomycetes (Prakasam *et al.,*2012) and plant extracts (Gardea-Torresdey *et al.,*2002). Silver nanoparticles have been successfully employed in catalysis, pharmaceutical nano engineering, drug delivery, sensor development, electronics-DSSC and allied sectors (Zhao *et al.,*1998). The Objective of the present study is to biologically synthesize and characterize silver and Zinc nanoparticles using *Bacillus subtilis* and *Streptomyces* PDS1 and to determine their Antimicrobial and Anti-cancer activity.

Materials and Methods

Culture maintenance for synthesis of silver and zinc nanoparticles

The bacterial cultures of *Bacillus subtilis* and *Streptomyces PDS1* were obtained from Centre for

Bioscience and Nanoscience Research, Coimbatore. The cultures were maintained at Nutrient agar plates and Actinomycin isolation agar respectively at 4°C. The bacterial cultures were inoculated on to the sterile nutrient broth and allowed to grow for 12 hours at 37° C under the pH 7.0.

Synthesis of silver and Zinc nanoparticles

The cell free filtrate was obtained by centrifugation of *Bacillus subtilis* and *Streptomyces PDS1* at 10,000 rpm for 10 minutes and temperature was maintained at 4°C. For the synthesis of silver nanoparticles 10 ml of the cell free filtrate was brought in contact with 1 mM of silver nitrate final concentration in 100 ml Erlen Meyer flask and agitated at 37°C in dark conditions under neutral pH 7. Simultaneously, control without Silver nitrate solution was incubated under same conditions. Similarly, *Streptomyces PDS1* cell filtrate along with Zinc sulphate and Zinc nitrate of exactly 3 grams each were added to the supernatant, followed by continuous magnetic stirring for 5 hours. Then, Phosphate buffer solution was added to collect the pellet through centrifugation at 10,000 rpm for 10 minutes. The zinc nanoparticles were synthesized. Simultaneously, control without Zinc sulphate and Zinc nitrate was incubated under same conditions.

UV-VIS studies of silver and zinc nanoparticles

When the Reaction solution was recorded at an interval between 0 – 24 hrs the Silver nanoparticle and zinc nanoparticles depicted a series of typical UV- VIS spectra. Under normal pH 7.0 the change in light absorption and change in intensity of the light yellow color during 24 hours incubation, a peak was observed in the visible region of 360nm.– (Elico, UV-VIS SL 159).

SEM studies for silver and zinc nanoparticles

Scanning Electron Microscopic (SEM) analysis was done in Cochin University, Kerala. Thin films of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes for emitting characteristic X-rays. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample for silver and zinc nanoparticles.

Fourier transform infrared spectroscopy (FTIR) Analysis for silver and zinc nanoparticles

The chemical bonds present in the silver and zinc nanoparticles were analyzed using FTIR spectrum. The *Bacillus subtilis* and *Streptomyces PDS1* filtrate containing the extracellular proteins secreted by the bacteria in the presence of silver and zinc followed by centrifugation at 10,000 rpm for 10 minutes. The readings were recorded in FTIR spectrometer**.**

Antimicrobial activities

Bacterial susceptibility for silver and zinc nanoparticles

The antibacterial susceptibility test were carried out by using *Bacillus subtilis* silver nanoparticles and *Streptomyces PDS1* zinc nanoparticles against the microorganisms like *Escherichia coli*, *Salmonella enterica , Pseudomonas aerogens* using well diffusion method. By using Muller hinton agar, the cultures were swabbed and well were made using sterile cork-borer; the wells were loaded with the 10µl of nanoparticles and 10µl of antibiotic (Vancomycin 30mcg) which was used as control. The zone of inhibition was observed and recorded.

Fungal susceptibility for silver and zinc nanoparticles

The antifungal susceptibility test were carried out by using *Bacillus subtilis* silver nanoparticles and *Streptomyces PDS1* zinc nanoparticles against the fungi like *Aspergillus flavus* and *Aspergillus niger* using well-diffusion method. Potato dextrose agar plates were prepared, the cultures were swabbed onto the sterile agar medium and the well was created using cork-borer, the wells were filled with 10µl nanoparticles and 10µl glycerol were used as control. The zone of inhibition was observed and recorded.

Anti-cancer activity of silver and zinc nanoparticles

Cell viability and cytotoxicity assays were used for drug screening and cytotoxicity tests of chemicals. They are based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Trypan Blue is a widely used assay for staining dead cells. A549 cancer cells were sub cultured in DMEM (Dulbecco's Modified Eagle's Medium)media supplemented with 2 mM L-glutamine adjusted with 1.5 g/L sodium bicarbonate and 90% fetal calf serum incubated at 37 °C in 5% CO_2 incubator.10µl of synthesised silver nanoparticles and zinc nanoparticles were added to the A549 cancer cells separately seeded in 96-well microtitre & incubated at 37 °C for 24 hours. At the end of the treatment 20µl of tryphan blue was added. After 30 minutes, the viability was measured using haemocytometer and calculated by the formula; Percentage Of anticancer activity **=** Control-Treated/control*100.

Results and Discussion

Synthesis of Silver and Zinc Nanoparticles

In the present study the silver and zinc nanoparticles were synthesized using *Bacillus subtilis* and *Streptomyces PDS1.* After incubation the color change occurred in the cell free extract of *Bacillus subtilis* when challenged with 1 mM Silver nitrate changed color from yellow color to light yellow color and *Streptomyces PDS1* cell free extract with Zinc sulphate and Zinc nitrate changed colour from white to pale yellow colour in 12 hours and attained maximum intensity after 24 hours with intensity increasing during the period of incubation indicated the formation of silver nanoparticles. Control sample showed no change in color of the cell filtrates when incubated under same conditions. pH of the synthesized silver nanoparticles and zinc ions were 7.0.

UV – VIS spectral studies for silver and zinc nanoparticles

The bacterial cell growth were observed using turbidity. The optical density was measured at 360nm with control (without nanoparticles) and synthesized samples (silver and Zinc Nanoparticles) ***(Graph 1 & 2)***.similar results were recorded by Suneetha *et al.,* 2004.

SEM analysis for silver and zinc nanoparticles

The plate of SEM images with 40,000 magnification shows that the silver nanoparticles are aggregated. In the micrographs it has been observed that the nanoparticles were in the size ranging from 80 ± 120 nm with a variety of morphology in structure ***(Figure 1).***The plate of SEM images with 10,000 magnification shows that the zinc nanoparticles are aggregated. In the micrographs it has been observed that the nanoparticles were in the size range from 70 ± 110 nm with a variety of morphology in structure ***(Figure 2).***Similar method was followed for SEM analysis of Zinc oxide nanoparticles by Vani *et al.,* 2011.

Fourier transform infrared spectroscopy (FTIR) for silver and zinc nanoparticles

Bacillus subtilis Extract depicts a series of FTIR spectra of the reaction solution recorded at intervals of 24 hrs. A highest peak at 1083.04cm-1 cm-1 was found to have been appeared. These FTIR results are found to line with the finding of UV-VIS spectroscopic studies. ***(Graph 3).****Streptomyces PDS1* Extract depicts a series of FTIR spectra of the reaction solution recorded at intervals of 24 hrs. A highest peak at 1071.88cm-1 cm-1 was found to have been appeared. These FTIR results are found to line with the finding of UV-VIS spectroscopic studies. ***(Graph 4)***

Antimicrobial activity

Bacterial susceptibility for silver and zinc nanoparticles

The zone of inhibition of silver and zinc nanoparticles against the microorganism *Escherichia coli*, *Salmonella enterica , Pseudomonas aerogens* were measured.The ***Figure 3(A) and table 1*** clearly indicates that *Bacillus subtilis* silver nanoparticles has the good antibacterial activity against the *Escherichia coli*. The ***Figure 3(B) and table 2*** clearly indicates that *Streptomyces PDS1* zinc nanoparticle *has* good antibacterial activity against the *Pseudomonas aerogens*.Similar results were recorded for Antibacterial activity of silver nanoparticles against *Escherichia coli* (Zhao *et al.,* 1998).

Fungal susceptibility for silver and zinc nanoparticles

The zone of inhibition of silver and zinc nanoparticles against the fungi *Aspergillus flavus* and *Aspergillus niger* were measured. The present study clearly indicates that silver nanoparticles from *Bacillus subtilis* has the good antifungal activity against the fungi *Aspergillus niger* ***(Figure 4A and table 3)*** and zinc nanoparticles showed minimal activity than silver nanoparticle ***(Figure 4B and table 4)***.

Anti-cancer activity for silver and zinc nanoparticles

In this method, cell viability was determined by counting the unstained cells with haemocytometer. However, Tryphan Blue staining cannot be used to distinguish between the healthy cells and the cells that are alive. In the Colony Formation method, the numbers of cell colonies are counted using a Haemocytometer as a cell viability indicator. Dead cell are stained as blue ***(Figure 5)***. Percentage Of anticancer activity of Silver nanoparticle and Zinc nanoparticle were found to be 46.66% and 37.14% respectively. Similar study was done to determine Anti-cancer activity of Zinc oxide nanoparticles against A549 cancer cells at 10 concentrations between 60-80 % (Suneetha *et al.,* 2004).

Table 1: Antibacterial activity of biologically synthesized Silver nanoparticles

Organisms	*Bacillus subtilis* (culture filtrate)	Silver Nanoparticles	1 Mm$AgNo_3$	Vancomycin(30 mcg)
Escherichia coli	0.5mm	0.6mm	0.4mm	0.8mm
Salmonella enterica	0.4mm	0.2mm	0.5mm	Nil
Pseudomonas aerogens	0.3mm	Nil	0.3mm	Nil

Table 2: Antibacterial activity of biologically synthesized Zinc nanoparticles

Organisms	*Streptomyces PDS1* (culture filtrate)	Zinc nanoparticles	$ZnSo_4$ + $ZnNo_3$	Vancomycin (30 mcg)
Escherichia coli	0.4mm	Nil	0.4mm	0.8mm
Salmonella enterica	Nil	Nil	0.6mm	Nil
Pseudomonas aerogens	Nil	0.2mm	0.3mm	Nil

Table 3: Antifungal activity of biologically synthesized Silver nanoparticles

Organisms	*Bacillus subtilis* (Culture Filtrate)	Sliver nanoparticles	$AgNo_3$	Glycerol
Aspergillus flavus	0.2 mm	0.3 mm	0.2 mm	0.4 mm
Aspergillus niger	0.2 mm	0.4 mm	0.3 mm	0.2 mm

Table 4: Antifungal activity of biologically synthesized zinc nanoparticles

Organisms	*Streptomyces PDS1* (culture filtrate)	Zinc nanoparticle	Glycerol
Aspergillus flavus	0.2 mm	0.1 mm	0.2 mm
Aspergillus niger	0.3 mm	0.2 mm	0.2 mm

Figure.1**:** SEM image of silver nanoparticles

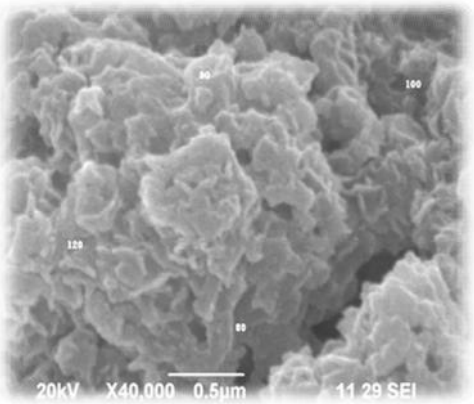


***Figure.2*: SEM image of zinc nanoparticles**

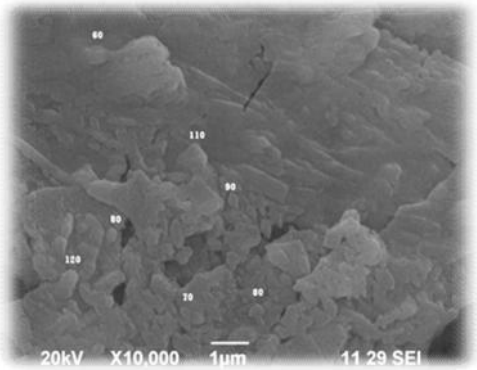
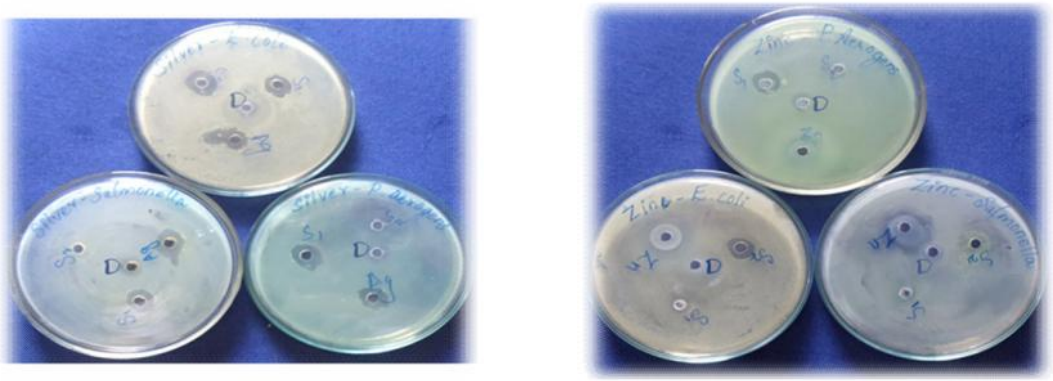


Figure 3: Antibacterial activity of biologically synthesized Silver (A) and Zinc (B) nanoparticles

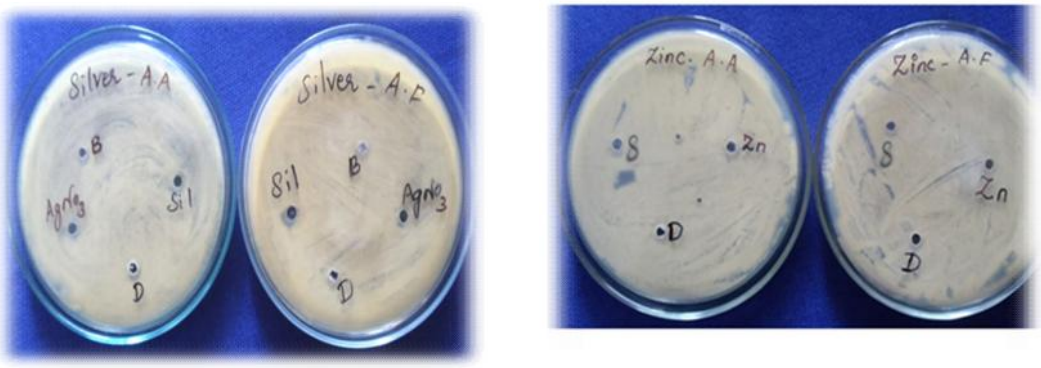


(A) **(B)**

(A): Ag -1mM silver nitrate, S1- *Bacillus subtilis* (Culture Filtrate), S2 - Silver nanoparticle D - Vancomycin Antibiotic (30 mcg).

(B): Zn - Zinc sulphate and Zinc nitrate, S1 - Zinc nanoparticles, S2 - *Streptomyces PDS1* (Culture Filtrate), D – Vancomycin antibiotic (30 mcg)

***Figure.*4: Antifungal activity of biologically synthesized Silver (A) and zinc (B) nanoparticles**

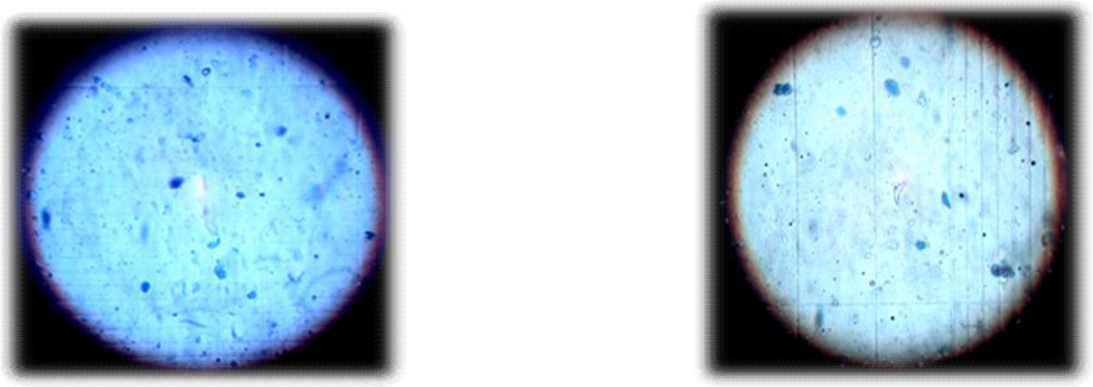


(A) **(B)**

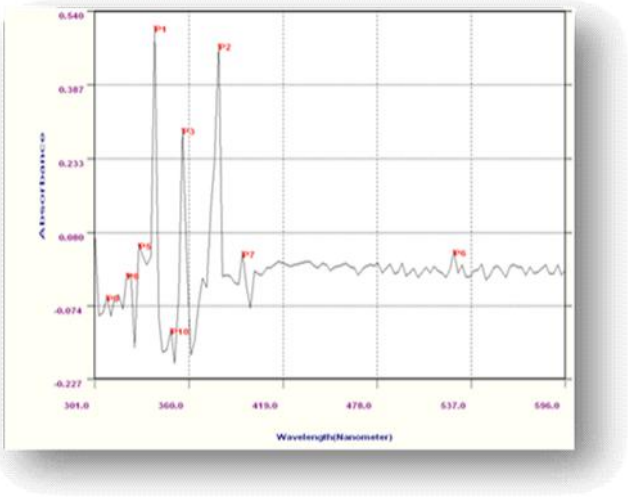
A.A- *Aspergillus niger,* A.F- *Aspergillus flavus* (A) Sil - Silver nanoparticles, B - *Bacillus subtilis* (Culture Filtrate), AgNO3 - 1 mM silver nitrate; D – Glycerol(B) S - *Streptomyces PDS1 (*Culture Filtrate), Zn - Zinc nanoparticles, D - Glycerol

***Figure 5*: Anticancer activity of biologically synthesized Silver (A) and Zinc (B) nanoparticles.**

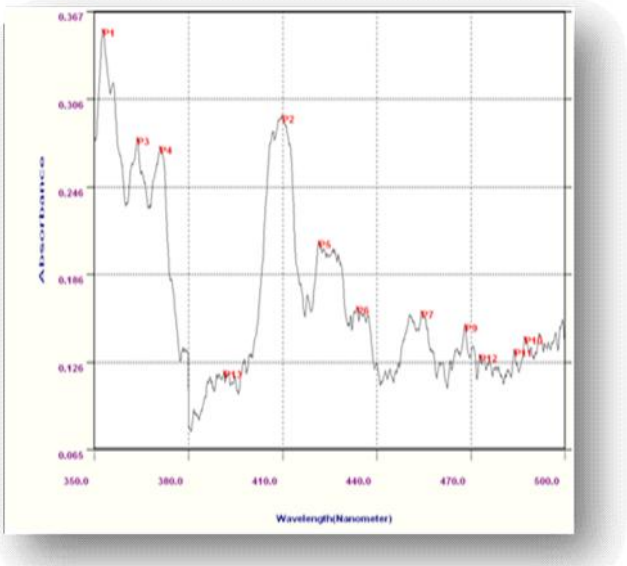
A **B**



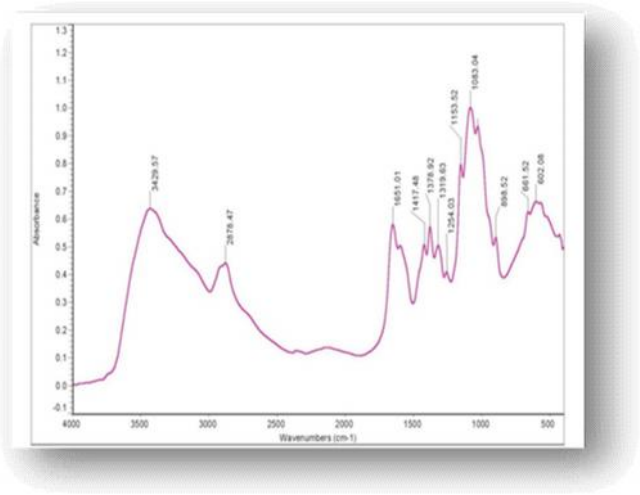
Graph.1: ***Bacillus subtilis*** **extract in UV – VIS spectroscopy**



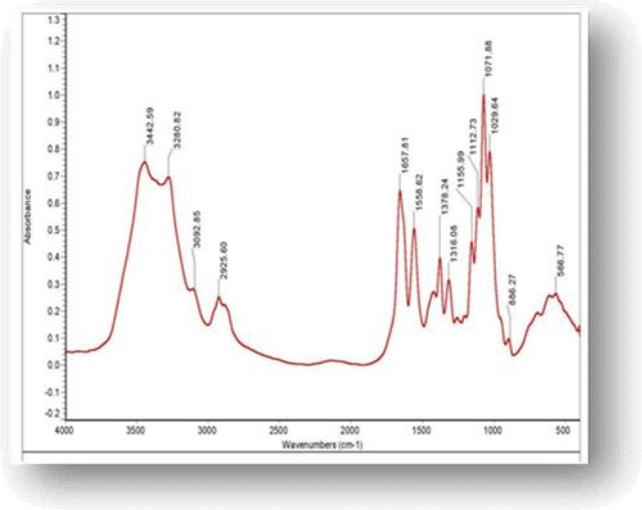
Graph.2: ***Streptomyces PDS1*** **extract in UV – VIS spectroscopy**



Graph.3: *Bacillus subtilis* Extract on a series of FTIR spectra



Graph.4: *Streptomyces PDS1* Extract on a series of FTIR spectra



Acknowledgments

The authors are thankful to the management of Sri Krishna Arts and Science College (Autonomous), Coimbatore, for providing them necessary facilities and support to carry out this work. The authors are also thankful to Dr Ragunathan, Scientist, Centre for Bioscience and Nanoscience Research, Coimbatore for support to analyze the nanoparticles.

References

Ahmad, P., Mukherjee, S., Senapati, D., Man- dal, Khan, M I., and Kumar, R. (2003). *Colloids Surface*, 28: 313

Amar Ratan., Ekta Gupta and Ragunathan R. (2013).Synthesis of Silver Nanoparticles using *Klebsiella pneumonia* and its Bio- Medical Applications, Journal enhanced research in science tech& engg, 3:1.

Awschalom D. D. and Divincenzo D. P. (1995). Complex Dynamics of Mesoscopic Magnets.*Physics Today.* **48(4)**: 43-48.

Cornell R. M. and Schwertman U. (1996).The Iron Oxides: Structure, Properties, Reactions, Occurences and Uses. New York: VCH.

Gardea-Torresdey ,J L., Parsons, J G., Gomez, E., Peralta-Videa, J., Troiani H E.,Santiago, P., and Jose Yacaman, M.(2002).Formation and Growth of Au Nanoparticles inside Live Alfalfa Plants,Nanoletters,2.

Gupta A. K. and Gupta M. (2005).Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications.*Biomaterials*. **26(18)**: 3995-4021.

Gupta Jitendraa, Prabakaran L. B., Gupta Reenaa, Govind Mohana. (2011). Nanoparticles formulation using counterion Induced gelification Technique: *invitro* Chloramphenicol release. *Int j pharm pharm sci.* **3(3)**: 6670.

Liu X. Y., Ding J. and Wang J., (1999).An alpha-Fe2O3 powder of nanosized particles via precursor dispersion.*Journal of Materials Research.* **14(8)**: 3355-3362.

Ratan Das, Sneha Gang, Siddhartha Sankar Nath.(2011).Preparation and Antibacterial Activity of Silver Nanoparticles, *Journal of Biomaterials and Nanobiotechlogy*, 2:472-475.

Suneetha, V V., Laksmi, V V. (2004). *Enzyme. Microbial Technology*, 6:31-36.

Vani, C., Sergin, G K., Annamalai.(2011).A study in the affect of zinc oxide nanoparticles in *Staphylococcus aureus*,*Internaional journal of Pharma and Bio Sciences,*2:327-329.

Zhao, G., Stevens. (1998) Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion, *Journal Se. Biometals*, 11.

