International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps

Research Article **Open Access**

Synthesis and Characterization of Silver Nanoparticles Using *Basella Rubra* Linn and their *In-vitro* Cytotoxic Studies

Rajathi.K[1], S.Suja[2] & N.Kannikaparameswari[3]

[1]*Assistant professor, PG and Research Dept. of Biochemistry, Dr. N.G.P Arts and Science College, Kalapatti road, Coimbatore-48.*
[2]*Assistant Professor, PG and Research Department of Biochemistry, P.S.G College of Arts and Science, Coimbatore*
[3]*Associate Professor, PG and Research Department of Biochemistry, Dr. N.G.P Arts and Science College, Kalapatti road, Coimbatore-48.*

A B S T R A C T

To synthesise the silver nanoparticles using the extracts of *Basella rubra Linn* and to investigate the antibacterial activity against human pathogens and invitro cytotoxic activity o human breast cancer cell line (MCF-7).In the present study silver nanoparticles were synthesized using the methanolic extract of *Basella rubra* and were characterized using UV-Visible spectroscopy and SEM analysis. Further these synthesized silver nanoparticles were evaluated for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* by disc diffusion method. The silver nanoparticles were assessed for its cytotoxic activity on human breast cancer cell line (MCF-7).SEM showed the formation of silver nanoparticles with an average size of 20-25nm.Biosynthesised silver nanoparticles showed potent antibactericidal activity against human pathogenic bacteria. The invitro screening of the biosynthesized silver nanoparticles showed potent cytotoxic activity against the human breast cancer cell line.

Keywords: *Basella rubra*, Silver nanoparticles, human pathogens

A R T I C L E I N F O

CONTENTS

Article History: Received 10 February 2015, **Accepted** 15 March 2015, **Available Online** 27 April 2015

***Corresponding Author**
Rajathi.K
Assistant professor,
PG and Research Department of Biochemistry, Dr. N.G.P Arts and Science College, Kalapatti road, Coimbatore-48.
Manuscript ID: IJCPS2506

PAPER-QR CODE

Citation: Rajathi.K, et al. Synthesis and Characterization of Silver Nanoparticles Using *Basella Rubra* Linn and their *In-vitro* Cytotoxic Studies. *Int. J. Chem, Pharm, Sci.*, 2015, 3(4): 1633-1636.

1. Introduction

Nanotechnology offers possibilities of great advancement in variety of industries by manipulating materials on the atomic or molecular level and thus obtains novel characteristics and function of smaller constructed materials; these smaller materials are referred to as nanomaterials and defined as a particles less than 100 nm in at least one direction [1]. Nanotechnology is presently applied in electrical engineering, chemistry, material science and cosmetic/and sunscreens. Medicinal sciences are investigating the use of nanotechnology to improve medical diagnosis and treatments [2].

Due to the outbreak of the infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance the pharmaceutical companies and the researchers are searching for new antibacterial agents. In the present scenario nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and its unique chemical and physical properties. The most effectively studied nanoparticles today are those made from noble metals in particular Ag, Pt, Au and Pd. Metal nanoparticles have tremendous application in the area of catalysis, optoelectronics, diagnostic biological probes and display devices. Among the above four, silver nanoparticles play a significant role in the field of biological system, living organisms and medicine [3-5]. Silver nanoparticles have attracted intensive research interest because of their important application as antimicrobial, catalytic, and surface-enhanced Raman scattering effect[6].Silver has been used as an antimicrobial agent for centuries; the recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics. It is generally recognized that silver nanoparticles may attach to the cell wall, thus disturbing cell –wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulphur- containing compounds such as DNA and protein [7].

In the present study silver nanoparticles were synthesized using the methanolic extract of *Basella rubra* and were characterized using UV-Visible spectroscopy and SEM analysis. Further these synthesized silver nanoparticles were evaluated for their antibacterial activity against *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus* and *Klebsiella pneumonia* by disc diffusion method. The silver nanoparticles were assessed for its cytotoxic activity on human breast cancer cell line (MCF-7).

2. Matirials and Methods

The present investigation on "Synthesis and characterization of Silver nanoparticles using Basella *rubra Linn* and their In vitro cytotoxic studies" was carried out in research laboratory of Dr .N.G.P Arts and Science College Coimbatore. The experimental design of the present study is given below:

Collection of Plant Sample:

The *Basella rubra Linn* plant were collected from the Ukkadam market, Coimbatore, Tamilnadu.

Preparation of the extract

10g of plant powder was weighed and it is mixed with 100 ml of methanol. The extraction was carried out in a shaker for 48 hrs. The solution was filtered through Whatmann.No.1 filter paper. The filtered sample was collected in conical flasks. The obtained extract was used for the synthesis of silver nanoparticles.

Preparation of Silver nitrate solution

1mM silver nitrate solution was prepared by the concentration of 0.017g in 100 ml double distilled water and stored.

Metal –plant extract interaction

90 ml of the silver nitrate solution was taken in conical flask. To this add 10 ml of the leaf extract. The colour change of the silver nitrate solution was found from colourless to dark brown Incubate the conical flask at room light for 72 hours

MTT cell proliferation assay

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0 C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT Assay

The MTT (4, 5-dimethylthiazolyl, 2,5-diphenyltetrazolium bromide) cell proliferation assay measures the cell proliferation rate and conversely, the reduction in cell viability when metabolic events lead to apoptosis or

necrosis. The yellow compound MTT (Sigma) is reduced by mitochondrial dehydrogenases to the water insoluble blue formazan compound, depending on the viability of the cells. Cells (3104 cells/ml) were grown on microtiter plates (200 µl of cell suspension/well) in 96 well microplates with serial dilutions of extract. 72 h later, 20 µl of a MTT solution (5 mg/ml in PBS) were added in each well. The plate was incubated for 4 h at 37°C in a CO2 incubator. After incubation, 180 µl of medium were removed from each well and 180 µl of DMSO/methanol (50:50) were added to each sample. The preparations were mixed thoroughly on a plate shaker with the cells containing formazan crystals. When all the crystals were dissolved, absorbance was measured at 570 nm with a microplate reader (Elx 800 microplate reader).

SEM Analysis of Silver Nanoparticle

Scanning electron microscope (SEM) analysis was done by using Hitachi S-4500 SEM machine. Thin films of the sample were 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 were allowed to dry by putting it under a mercury lamp for 5 minutes.

Antibacterial Activity: The antibacterial effects of silver nanoparticles were assessed against a variety of microorganisms including *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, and Pseudomonas aeroginosa* with disc diffusion method. In brief, 100 micro litre of overnight grown bacterial cultures were plated on nutrient agar plates. Wells were cut and 50 microlitre AgNP solutions was dispensed in each well. An antibiotic disc tetracycline was used as control. The plates were incubated at 37 ^{o}C for 24 hours and the zone of inhibition were observed only in presence of AgNP and documented.

3. Result and Discussion

Conformation of Silver Nanoparticle Synthesis

The conformation of synthesis of silver nanoparticles by plant extract can be done by periodically monitoring the change in colour, change in pH and change in absorbance value.

Change in Colour

The periodical colour change in reaction mixture containing Silver nitrate and *Basella rubra linn* extract was monitored for 28 hours. From the results, the synthesis of silver nanoparticles by the plant extract was confirmed by the change of colour from pale yellow to dark brown during various time intervals.

Change in pH

The periodical change in pH of reaction mixture was monitored for 72 hours and the results were presented in table-1.

Table 1: Periodical change in pH during the Biosynthesis of silver nanoparticles

Time	0 min	1 hr	2 hr	4 hr	8 hr	16hr	24hr	28hr	72hr
pH	7.03	7.12	7.18	7.27	7.34	7.46	7.51	7.65	7.8

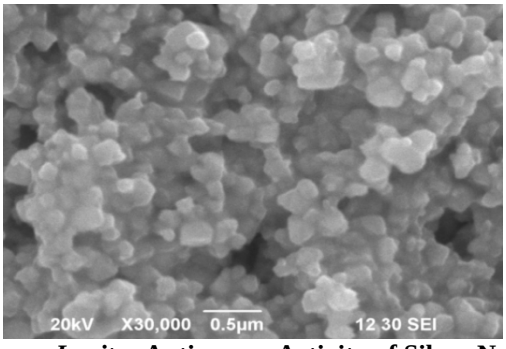
From the results, the change in pH due to the Synthesis of silver nanoparticles was conformed.

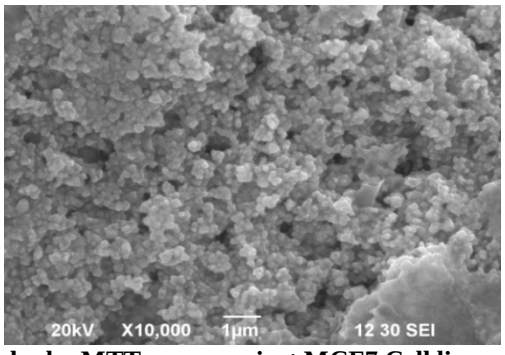
Optical Density Measurement:

UV-VIS spectroscopy can be used to examine the formation of silver nanoparticles in aqueous suspensions and the observations were presented in Table 2. The increase in absorbance at 420 nm confirmed the synthesis of silver nanoparticles by *Basella rubra linn* formed in the reaction mixture (Jain *et al*., 2009).

Table 2: Optical density of sample and control

Time	**O.D of sample**	**O.D of control**
0 hour	1.73	1.09
4 hour	1.88	1.09
16 hour	2.02	1.09
24 hour	2.24	1.09





***In vitro* Anticancer Activity of Silver Nanoparticles by MTT assay against MCF7 Cell lines:**

Characterization of Silver Nanoparticle

SEM Analysis

The biosynthesized silver nanostructure by employing *Basella rubra linn* extract was further characterized and size was confirmed by SEM analysis. The SEM image showing high density silver nanoparticle synthesized by the *Basella rubra* plant extract confirmed the development of silver nanostructure. The SEM analysis showed the particle size between 20-25nm.

The cytotoxicity activity of Silver nanoparticles was confirmed by MTT assay the result were presented in Table 3. The MTT assay confirmed the cytotoxic activity of Silver nanoparticles against MCF7 breast cancer cell lines. The IC 50 value was found 63.62µg/ml and there was a linear correlation (R^2 = 0.9978) between the concentration and percentage of inhibition.

Table 3: Inhibition of MCF 7 breast cancer cell by silver nanoparticles

Concentration (µg)of Silver nanoparticles	Cell Inhibition	IC 50
18.75	0.35746	63.62µg /ml
37.5	4.021448	
75	72.117	R^2=0.9978
150	94.8168	
300	99.55317	

Evaluation of Antibacterial Activity

The antibacterial activity of silver nanoparticles was investigated against various pathogenic bacteria of Gram positive (*Staphylococcus aureus*) and Gram negative strains (*Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa, Klebsiella pneumonia*) using disc diffusion technique. The diameter of inhibition zones around each well with silver nanoparticles represented in table 4.The highest antimicrobial activity was observed against *Pseudomonas aueroginosa* and the least was noticed against *Klebsiella pneumonia.*

Table 4: Zone of inhibitory activity of *Basella rubra linn* extract

Name of the organism	Zone of inhibition (mm) of various sample		
	Silver nanoparticles	Plant extract	Antibioticdisc (Gentamicin)
Staphylococcus aureus	11	8	20
Escherichia coli	12	10	18
Pseudomonas aueroginosa	20	7	26
Klebsiella pneumonia	9	6	14

4. Conclusion

Synthesized nanoparticles have shown antibacterial potential against pathogenic bacteria. Silver nanoparticle was found to inhibit bacterial growth in comparison to the plant extract and standard antibiotic disc. Inhibition of bacterial growth by silver nano particle can be attributed to damage of the bacterial cell membrane and extrusion of the cytoplasmic contents there by resulting in the death of the bacterium. In conclusion Silver nanoparticles prepared by green route found to have both antibacterial and cytotoxic activities and can very well applied in biological system.

5. References

1. Morones, Evans, Mritunjai Singh & Duangporn K, Biosynthesis of silver nanoparticles using plant extract, Journal of Biological Sciences, **2005**, 10(5): 465-467.
2. Baker C, Pradhan A, Pakstis L, Pochan D J, synthesis and antibacterial properties of silver nanoparticle, Technol, 2005, 5: 244-249.
3. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Deepak V,Pandian SRK, Muniyandi J. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf B, **2009**, 74: 328-335.
4. 4. Jain D, Daima HK, Kachhwaha S, Kothari SL, Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Dig J Nanomater Biostruct, **2009**, 4: 723-727.5. Parashar V,
5. Parashar R, Sharma B, Pandey AC, Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Dig J Nanomater Biostruct, **2009**, 4: 45-50.
6. Kamal K Panda, Mohan M Achary, Krishnaveni R, Bijaya k, Sachindra N, Surendra N, Brahma P, Invitro biosynthesis and genotoxicity bioassay of silver nanoparticles using plants, Toxicology, **2011**, 25:1097-1100.
7. Sarfaraj H, Nazeer KFH, Ravichandran V,Zaheen Hassan, Ansari, Evaluation of invitro free radical scavenging potential of different fractions of Hygrophila auriculata Heine, Asian Journal of traditional medicines, **2009**, 4(5): 179-186.