Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Supple 1, 2015

Original Article

A GREEN NANO-BIOTECHNOLOGICAL APPROACH FOR THE SYNTHESIS OF SILVER NANOPARTICLES USING THE SEED COAT OF *TAMARINDUS INDICA*, STUDY OF ITS ANTIBACTERIAL AND ANTICANCER ACTIVITY

J. CHRISTY, D. DHARANEYA, VINMATHI. V, JUSTIN PACKIA JACOB. S*

Department of Biotechnology, St. Joseph's college of Engineering, OMR, Chennai 119
Email: drjpjacob@gmail.com

Received: 08 Aug 2015 Revised and Accepted: 24 Sep 2015

ABSTRACT

Objective: The present study was designed to investigate the antibacterial and anti cancer effect of silver nanoparticle synthesized using the seed coat extract of *Tamarindus indica.*

Methods: In this study, aqueous extract of *Tamarindus indica* seed coat is used for synthesizing silver nanoparticles (AgNPs). Plant metabolites in the aqueous extract act as both reducing and capping agents resulting in the formation of stable and shape-controlled AgNPs. The AgNPs thus synthesized were characterized using UV-Vis absorption spectroscopy, FTIR and SEM analysis. The nanoparticles were tested for their antibacterial effect against human pathogenic bacteria and anticancer effect against HeLa cell line.

Results: Our results suggested that the aromatic compounds like flavanoids in the plant extract are responsible for the reduction of AgNP's. SEM analysis revealed fairly well dimensioned square shaped nanocrystals. The results were positive proving that the synthesized AgNPs possess excellent antibacterial and anticancer properties.

Conclusion: The synthesized AgNPs showed maximum anti-bacterial and anti-cancer effect.

Keywords: Silver nanoparticle, UV–Vis absorption spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM).

INTRODUCTION

Nanoparticles are normally considered as particles with a size of 1-100 nm. They display completely novel and improved properties, when compared with larger particles such as catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties [1]. Among various nanoparticles, silver nanoparticles (AgNPs) have gained a lot of attention because of their potential applications in varied fields such as oxidative catalysis, biosensors, conductive coatings, nanoelectronics (single-electron transistors, electrical connects),antibacterial activity, etc [2]. Due to the antibacterial property of the AgNPs, in medicine, they are used in skin ointments and creams containing silver to prevent infection of burns and open wounds, medical devices and implants prepared with silver-impregnated polymers [1].

There are several methods available for the synthesis of silver nanoparticles for example, electrochemical, thermal decomposition of silver compounds, radiation assisted, sonochemical, microwave assisted process and reduction in solutions. But these chemical methods have the disadvantage of some toxic chemicals used in the process to be absorbed on the surface that may have an adverse effect in the medical applications [3]. Therefore, an alternate safe, eco-friendly method is much desired. Hence green route of synthesis using plant extracts has made an attractive platform for nanoparticle synthesis.

Plant metabolites present in the plant extract such as terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins reduce metal ions and result in the formation of nanoparticles. These metabolites coat the nanoparticles in order to stabilize them [4]. Thus plant metabolites present in the extract act as reducing, capping and stabilizing agent. In the past various medicinal plants have been used to synthesise nanoparticles thus incorporating the medicinal property of the plant into the biosynthesised NPs. Plants such as *Piper longum* [5], *Plumeria rubra* [6] *Crataeva nurvala* [7], *Nigella sativa* [8] has been used in the past to synthesise AgNPs.

In this study, for the first time we report the synthesis of silver nanoparticles by the aqueous extract of seed coat of *Tamarindus indica* (Tamarind). The biosynthesized AgNPs were characterized and tested for their antibacterial and anticancer activity.

MATERIALS AND METHODS

Plant material and preparation of the extract

The seed coat of *T. indica* was separated from seeds by soaking the seeds in distilled water for 2-3 d. The extract was prepared by boiling 5g of seed coat in 200 ml of sterile distilled water for 10 min and filtered using Whatman filtrate paper. The filtrate was refrigerated for further use.

Bio-synthesis of silver nanoparticles

40 ml of 1 mM silver nitrate solution was added to different volumes (1 ml, 3 ml, 5 ml) of an aqueous seed coat extract. The above three different solutions were subjected to different conditions like sunlight irradiation, incubation in dark at room temperature and UV exposure. The solutions were maintained in the above condition until a color change was observed and it was centrifuged at high rpm in order to separate the synthesized nanoparticles.

UV-Vis spectra analysis

Bioreduction of silver nanoparticles is responsible for the colour change and it was monitored using UV-Vis Double beam Spectrophotometer Systronics 220 by scanning the sample solution between the wavelength 300-800 nm. It gives the maximum absorbance value to prove the presence of silver nanoparticles.

SEM analysis

Scanning Electron Microscopic analysis is used to detect the size and shape of the synthesized nanoparticles. It was carried out at IIT, Chennai using FEI Quanta 200 SEM machine. A thin film of the sample was obtained by loading a small amount of the dried sample on a circular metallic sample holder and the sample was covered with a thin layer of gold by sputter coating. Then the film was allowed to dry and the images of nanoparticles were taken.

FTIR analysis

FTIR analysis also was carried out at IIT, Chennai, using Perkin Elmer Spectrum1 FTIR instrument to determine the functional groups of compounds that helped in the bioreduction of silver ions to AgNPs.

Antibacterial assay

The AgNPs were tested for antibacterial activity by an agar well diffusion method against four human pathogenic bacteria *Escherichia coli, Klebsiella pneumonia Staphylococcus aureus*, *and Pseudomonas aeruginosa.* Each bacterial culture was grown in nutrient broth for 24 h and the grown culture (100 µl) was spread onto a sterilized plate containing nutrient agar by spread plate method. Wells was made on the nutrient agar plates using a micropipette. The AgNPs were diluted using serial two-fold dilutions in concentrations ranging from 100 to 5µg/ml. 20 µl of the diluted AgNP solution was added to the wells and incubated for 24 h at 37˚c and the zone of clearance was observed on the next day.

Anticancer assay

The *HeLa* cell line obtained from obtained from National Centre for Cell Sciences; Pune (NCCS) was used for the present study.

Cells (1 × 10^5/well) were plated in 24-well plates and incubated in 37 °c with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum100 µl/well (5 mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 h. After incubation, 1 ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ cell viability} = (\text{Test OD}/\text{Control OD}) \times 100$$

The graph was plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

Primary detection of the formation of AgNPs was done by visual observation. Colour change of the mixture solution exposed to different conditions from light yellow to dark brown indicated the presence of AgNPs in the solution. This colour change is due to the optical property of nanoparticles called surface plasmon resonance [9]. Among the various conditions and methods used, incubation in the dark at room temperature method was very effective and 3 ml of seed coat extract showed the maximum yield of nanoparticles. Colour formed when kept in dark remained unchanged for several days indicating the stability of the nanoparticles formed.

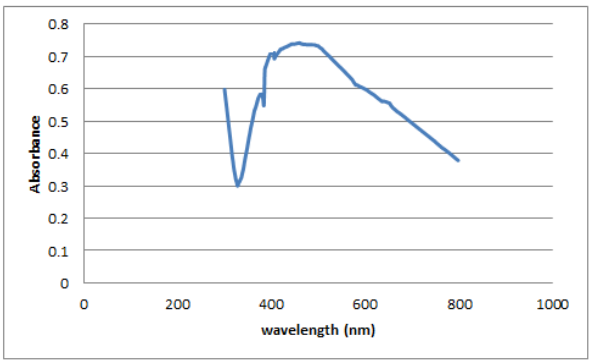

Fig. 1: UV-Vis absorption spectra of AgNPs by the extract of *Tamarindus indicus*

UV-Vis spectra analysis

Preliminary confirmation of AgNPs in the solution was done by UV-vis absorption spectroscopy. Fig.1 shows the maximum absorption value at 454 nm. The SPR bands are influenced by the size, shape, morphology, composition and dielectric environment of the prepared nanoparticles [10]. Reports suggested that, the absorption peaks in the range 425-475 nm correspond to AgNPs of fine nature [11].

FTIR analysis

FTIR studies on the samples were carried out using Perkin Elmer Spectrum1 FTIR spectroscopy, scanned at a range 450-4000 cm^{-1} of resolution to ensure the formation of silver nanoparticles.

FTIR spectra of AgNP's produced by *Tamarindus indicus* seed coat extract are shown in the fig. (Fig.2), bands are observed at 3727, 3406, 2925, 2633, 2351, 1623, 1384, 1084 and 633 cm^{-1}. The above bands were compared with the FTIR spectra of $AgNO_3$ and extract alone. The bands at 3406 and 2925 cm^{-1} are broadened in the extract alone, but the narrow band in the AgNP's showed that reduction of silver ions in NP's. The band at 1623 cm^{-1}represent the involvement of C=N in plane vibrations of aminoacides indicated the involvement of aminoacids from the plant extract in the reduction of AgNP's.

The peak at 1384 cm^{-1} after the bio reduction of AgNP's indicated the C=C stretching mode in the aromatic compounds which confirmed the aromatic compounds like flavanoids in the plant extract are responsible for the reduction of AgNP's. In the earlier study [12] reported the presence of alkaloids, anthraquinone and cyanogenic glycosides in the seed coat extract, this may be responsible for the reduction of silver nanoparticles.

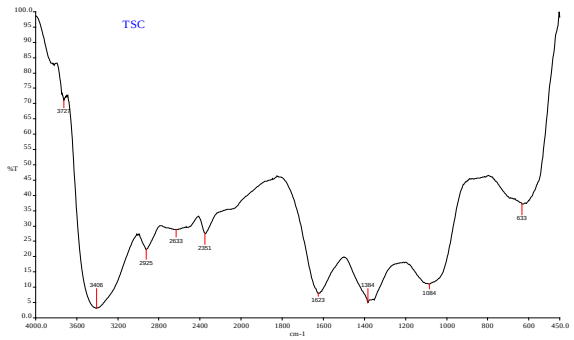

Fig. 2: FTIR spectra of AgNPs synthesized using seed coat extract *of Tamarindus indicus*

SEM analysis

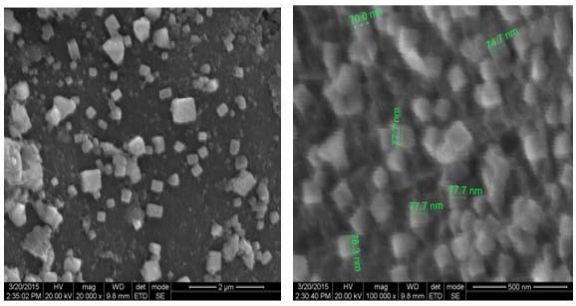

Fig.3a, b SEM image of AgNPs

The size and topology of the AgNPs were studied using SEM analysis. SEM analysis revealed fairly well dimensioned square shaped nanocrystals of size ranging between 70–78.3 nm dia.

Antibacterial assay

Zone of clearance observed in the plates indicated the bactericidal effect of the AgNPs synthesized. The clear zone was measured and given in the Table.1. Gram positive bacteria, *Staphylococcus aureus* had the smallest zone of clearance while the Gram negative bacteria, *Pseudomonas aeruginosa* had the largest zone of clearance. This is because the nanoparticles could not easily penetrate the thick

peptidoglycan layer of Gram positive bacteria but the peptidoglycan layer of Gram negative bacteria is thin which allowed easy penetration and destruction of bacterial cells [13] indicates that AgNPs showed antibacterial effect against *Klebsiella pneumonia* up to a concentration of 10µg/ml, *Escherichia coli* and *Pseudomonas aeruginosa* against and up to a concentration of 100µg/ml of AgNPs.

Table 1: Antimicrobial effects of AgNPs

S. No.	Microorganism	Zone of inhibition at dilution 1:9 (mm)	Zone of inhibition at dilution 1:49 (mm)	Zone of inhibition at dilution 1:99 (mm)
1	*Escherichia coli*	14	11	9
2	*Klebsiella pneumonia*	14	10	-
3	*Staphylococcus aureus*	15	-	-
4	*Pseudomonas aeruginosa*	15	13	7

Table 2: Anticancer effects of the AgNPs on HeLa cell line

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O. D)	Cell Viability (%)
1	1000	Neat	0.03	5.55
2	500	1:1	0.06	11.11
3	250	1:2	0.08	14.81
4	125	1:4	0.12	22.22
5	62.5	1:8	0.18	33.33
6	31.2	1:16	0.23	42.59
7	15.6	1:32	0.26	48.14
8	7.8	1:64	0.34	62.96
9	Cell control	-	0.54	100

Anticancer assay

The AgNPs were tested for their cytotoxic effect using MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay on HeLa cell line. AgNPs of varying concentrations was tested after 24 h of incubation at 37 °c in 5% CO_2 using HeLa cell line. Significant cytotoxic effect was observed at IC50-15.6µg/ml of AgNPs (table 2). IC50 is the quantitative measure that indicates how much of a particular drug or other substance is needed to inhibit a given biological process by half.

CONCLUSION

Square shaped, stable silver nanoparticles with average size 74.15±4 nm were synthesized using aqueous seed coat extract of *T. indicus.* The AgNps was characterized by UV-Visible, SEM and FTIR Spectrum. Biosynthesis of AgNPs is a better alternative method over chemical synthesis since it is pollutant free and eco-friendly. The synthesized AgNPs showed maximum anti-bacterial and anti-cancer effect on HeLa cell line and hence, further studies must be conducted in an animal model in order to assess genotoxic and cytotoxic effects.

CONFLICTS OF INTERESTS

Declared None

REFERENCES

1. Kamyar Shameli, Mansor Bin Ahmad. Green biosynthesis of silver nanoparticles using *Callicarpa maingayi* stem bark extraction. Molecules 2012;17:8506.
2. Rao YN, Banerjee D, Datta A, Das SK, Guin R, Saha A. Gamma irradiation route to synthesis of highly re-dispersible natural polymer capped silver nanoparticles. Radiat Phys Chem 2010;79:1240.
3. Devendra Jain, Hemant Kumar Daima, Sumita Kachhwaha, SL Kothari. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Digest J Nanomater Biostructures 2009;4:723-37.
4. Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, Taliansky ME, *et al*. Green nanotechnologies: synthesis of metal nanoparticles using plants. Acta Nat 2014;6:20.
5. Justin Packia Jacob S, Finub S, Anand Narayanan. Synthesis of silver nanoparticles using *Piper longum* leaf extracts and its cytotoxic activity against Hep-2 cell line. Colloids Surf B 2012;91:212.
6. Hana Sarah Haseeb, Justin Packia Jacob S. Synthesis of silver nanoparticles using plumeria rubra extracts and to study its anti-bacterial properties. World J Pharm Res 2014;3:792.
7. Vinmathi V, Justin Packia Jacob S. Synthesis, Characterization, Antibacterial and cytotoxic studies of silver nanoparticles using aqueous extracts of *Crataeva nurvala* leaves-a green nano-biotechnological approach. World J Pharm Res 2014;4:953-62.
8. Mukesh Kumar P, Vinmathi V, Panchapakesan Gautam, Herald Wilson A, Justin Packia Jacob S. Green synthesis of silver nanorods using aqueous seed extract of *Nigella sativa* and study of its antidiabetic activity. Aust J Basic Appl Sci 2015;9:295-8.
9. Singh A, Jain D. Green synthesis of silver nanoparticles using *Argemone mexicana* leaf extract and evaluation of their antimicrobial activities. Digest J Nanomater Biostructures 2010;5:483.
10. Kamyar Shameli, Mansor Bin Ahmad. Green biosynthesis of silver Nanoparticles Using *Callicarpa maingayi*; 2012. p. 24.
11. Kamyar Shameli, Mansor Bin Ahmad. Green biosynthesis of silver nanoparticles using *Callicarpa maingayi* stem bark extraction. Molecules 2012;17:8506.
12. Timothy SY, Attah MO, Shamaki KB. Antiulcer and antibacterial screening of the seed coat and leaf extracts of *Tamarindus Indica* Linn. Asian J Pharm Res 2015;5:1-6.
13. Samir A Anuj, Kalpesh B Ishnava. Plant mediated synthesis of silver nanoparticles by using dried stem powder of *Tinospora cordifolia*, its antibacterial activity and comparison with antibiotics. Int J Pharma Bio Sci 2013;4:849.