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Research Article

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM CATHARANTHUS ROSEUS FOR BREAST CANCER THERAPY

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ABSTRACT

Hypothesis: Green synthesis of silver nano particle is an environmentally friendly approach and it is already established that plant extract have high potential for production of silver nanoparticles with wide applications. *Catharanthus roseus* is one of the anti-cancerous plants belonging to the family Apocynaceae due to the presence of vinca alkaloids. The plant has certain chemical constituents which is known to have anti-bacterial and anticancer activity which acts as both capping as well as reducing agent, therefore the green-synthesised silver nanoparticles (AgNPs) from *Catharanthus roseus* has additive *in-vitro* cytotoxicity effect against human breast cancer (MCF-7) cells. Due to their nano size which might be contribute potent effect for breast cancer therapy.

Experiment: The current study is designed to reveal the *in-vitro* cytotoxicity effect of green-synthesised silver nanoparticles (AgNPs) against human breast cancer (MCF-7) cells AgNPs are synthesised by using *Catharanthus roseus* leaf extract as a potent reducing agent. Characterization is done by UV-Visible Spectroscopy, XRD Analysis, TEM Analysis, and FT-IR Analysis. Cytotoxicity study is done by MTT assay in MCF-7 cell-line.

Findings: The face centered cubic crystal structure crystalline and spherical silver nanoparticles of 07-33 nm in size are synthesized. The toxicity potential of the green synthesized AgNPs on human breast cancer cells has been examined using MCF-7 cell line by the3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay. Green-synthesized AgNPs induce cytotoxicity on MCF-7 cell lines was found to be higher with increased concentration of AgNPs. Complete mortality rate was observed in 250 µg/ml concentration of AgNPs. IC50 of AgNPs was found to be 113.068 µg/ml.

Keywords: Green-synthesis, *Catharanthus roseus*, Silver nanoparticles, TEM, XRD, MTT assay MCF-7 cell line.

INTRODUCTION

Green synthesis of silver nanoparticles is evolving into an essential branch of nanotechnology. The Emerging significance of noble metal nanoparticles (Gold and Silver) in the area of nanotechnology due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools not only for medical imaging also for treating diseases [1]. Nanoparticles are structures ranging from approximately 1-100 nm [2,3]. Nano size results in specific physiochemical characteristics such as high surface area to volume ratio, which potentially results in high reactivity [4]. Physical method, Chemical method and Biological method (Green synthesis) are the three major methods for synthesis of nanoparticle. Chemical approaches are toxic and expensive. Thus, there is a growing need to develop environmentally and economically friendly processes, which do not use toxic chemicals in the synthesis protocols. Thus the role of green synthetic method was emerged which utilizes Bacteria, Fungi, Algae and Plants for the synthesis of silver nanoparticles [5]. The rate of reduction of metal ions using plants has been found to be much faster as compared to micro-organisms and resulting in the formation of stable metal nanoparticles due to environment friendly approach. Green synthesis of silver nanoparticles has been reported using the extracts of plants such as Jatropa curcas [6], Boswellia ovalifoliolata [7], Coriandrum sativum [8], Calotropis gigantean [9], Bitter Apple (Citrullus colocynthis) [10], Cassia auriculata [11], Eucalyptus hybrida [12], Trianthema decandra [13], D. carota extract [14] etc.

Green synthesis of silver nanoparticles is an emerging field of research and it is already established that plant extract have high potential for production of silver nanoparticles with wide applications. Breast cancer is one of the leading types of cancer in the number of new cases diagnosed and it is the most prevalent type of cancer in women. *Catharanthus roseus* (Linn.) G. Don (Fig. 1) is one of the anti-cancerous plant belonging to the family Apocynaceae due to the presence of vinca alkaloids. The

Figure 1: *Catharanthus roseus.*

plant have certain chemical constituents which is known to have anti-diabetic, anti-bacterial, anti-ulcer, anti-helminthic, anti-diarrhoeal and hypotensive activity. The present study focused on the biosynthesis of silver nanoparticles for breast cancer therapy [15-19].

MATERIALS AND METHODS

Fresh leaves of C.roseus Linn were identified and collected from local places of Pampady, Thrissur, Kerala, India and the taxonomic identification was made by Dr. V.K. Sreenivas, Asst. Professor and Head, Department of Botany, Sri Vyasa N.S.S. College, Vyasagiri, Wadakkanchery, Thrissur and a voucher specimen was kept for further reference. Silver nitrate solution was obtained from Nice chemicals Pvt. Ltd, Kochi.

Synthesis of silver nanoparticles

According to a procedure already published (M. Bhanu Prakash et al.) briefly, 10 g of *Catharanthus roseus* plant leaves were taken and cut down into small pieces, and then they were surface cleaned by running tap water, followed by distilled water and boiled in 100 ml of distilled water at 60°C for 20 mins. After that the aqueous leaf extract was filtered through the whatsmann. No. 1 filter paper, and the filtrate was stored at 4°C and could be used within one week. 2 ml of aqueous leaf extract was added to the Erlenmeyer flask containing 98 ml of $AgNO_3$ (10-3 M) and the mixture was incubated at room temperature [17].

Study center

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CHARACTERIZATION OF SILVER NANOPARTICLES

UV-VIS spectral analysis

Synthesis of silver nanoparticles can be observed by UV-VIS spectroscopy. The reduction of the Ag^+ ions was monitored by periodic sampling of the small aliquot of the sample into distilled water and the UV-VIS spectra were recorded by Shimadzu UV-1800 Spectrophotometer from 200-800 nm.

Transmission Electron Microscopy

TEM technique was employed to envisage the size and shape of Ag nanoparticles. HR-TEM measurements were made using a 200 kV Ultra High Resolution Transmission Electron Microscope (Joel/JEM 2100). TEM grids were prepared by placing a drop of particle solution on a carbon-coated copper grid and drying under lamp.

XRD analysis

XRD analysis of the sample of Ag nanoparticles was prepared using a Bruker AXS D8 Advance diffractometer, using Cu-Kα X-rays of wavelength (λ) = 1.54056 Å as source and operated at a voltage of 40 kV and a current of 35 mA. The sample was scanned in 2θ ranging from 10° to 80° with a step size 0.02° and step time 32.8 s. XRD patterns were analyzed to determine peak intensity, position and width. Fullwidth at half-maximum (FWHM) data was used with the scherrer's formula to determine mean particle size. Scherrer's equation is given by

$$D = 0.9\lambda\beta\cos\theta$$

Where d is the mean diameter of the nanoparticles, λ is wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction angle θ.

Breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune, India. MCF-7 cells were trypsinized and grown as a monolayerin a DMEM medium containing 10% FBS and incubated at 37°C for 3 days in 5% CO atmosphere. Cell viability was measured using MTT assay. The cultured MCF 7 cells were placed on a 96 well microtitre plate (10,000 cells/plate) and the cells were subjected to different concentrations of AgNPs (31.25-250 µg/ml) and incubated for 24 hours at 37°C in 5% CO_2 atmosphere. After incubation, 20 l of MTT (2 mg/ml) in MEM-PR was added to each well and further incubated for 3 hours at 37°C in 5% CO atmosphere. The supernatant was removed and iso-propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The cytomorphological changes were observed under an inverted phase-contrast microscope (Table 1).

FT-IR Analysis

The prepared silver nanoparticles from the leaf extract were centrifuged at 10,000 rpm for 20 min and then the pellet obtained is washed thrice with distilled water. Then it is dried in an oven at 60°C in an oven for 24 h. This powdered sample of the formed silver nanoparticles from the extract was subjected to FT-IR analysis using Bruker AlphaFT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany.

***In-vitro* cytotoxicity study**

MTT assay

The *in-vitro* anticancer studies of synthesized silver nanoparticles were determined by MTT assay using MCF-7 breast cancer cell line. The increase in concentration of AgNPs results in increased cytotoxicity in MCF-7 cells. The IC50 value of AgNPs was found to be 113.068 μg/ml. It was shown that the morphological variations were observed such as loss of membrane integrity, inhibition of cell growth, cytoplasmic condensation and cell clumping results indicate that the AgNPs treated MCF-7 cells undergone cell death, whereas the non-treated cells were active. In recent times, biosynthesis of nanomaterials is exposed as a viable and facile alternative strategy, mainly because of its green chemistry principles. Apart from the eco-friendly scheme, interaction of biocompounds with noble metals will be helpful for the development different tools and devices for a wide range of applications in biology and medicine.

Biogenic AgNPs induce cytotoxicity on MCF-7 cell lines was found to be higher with increased concentration of AgNPs. There was a change in the percentage of cell viability in control and AgNPs (0, 31.25, 62.5, 125 and 250 μg/ml) treated with MCF-7 cells. Complete mortality rate was observed in 250 μg/ml concentration of AgNPs. IC50 of AgNPs was found to be 113.068 μg/ml. The Diverse morphological alteration was observed in AgNPs treated MCF-7 cells, however no such effects were seen in untreated cells. It was shown that the morphological variations were observed such as loss of membrane integrity, inhibition of cell growth, cytoplasmic condensation and cell clumping results indicate that the AgNPs treated MCF-7cells undergone cell death, whereas the non-treated cells were active.

Concentration (μg/ml)	Absorbance	% Viability
250	0.0605	26.76991
125	0.0690	30.53097
62.5	0.1940	85.84071
31.25	0.2100	92.92035

Table 1: % viability at different concentrations.

RESULTS AND DISCUSSION

The colour of the freshly prepared aqueous extract obtained from the leaf of *Catharanthus roseus* changed when silver nitrate solution is added. The reduction of pure +Agions was monitored by measuring the UV-Visible spectrum of the reaction medium at an interval of 2, 3, 4, 24 hours (Fig. 2). The appearance of reddish brown colour indicates the formation of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to the excitation of surface plasmon vibrations in silver nanoparticles [15]. A stable absorption peak at 448 nm (Fig. 3) was obtained. It was reported that the nanoparticles which were showing maximum absorbance around the 450 nm will have the spherical

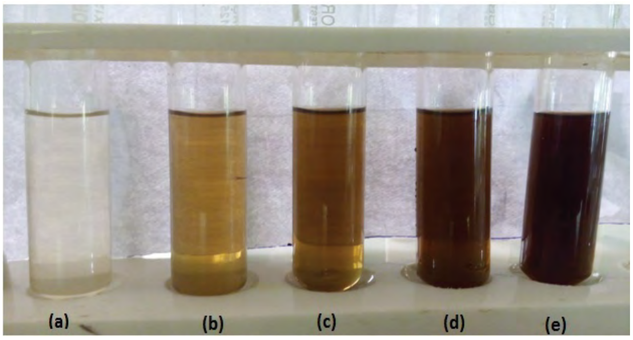

Figure 2: Digital Photographs of (a) Pure 1 mM $AgNO_3$ solution (b) 1 mM $AgNO_3$ and leaf extract solution after 2 hours of incubation at RT (c) after 3 hours (d) after 4 hours (e) after 24 hours.

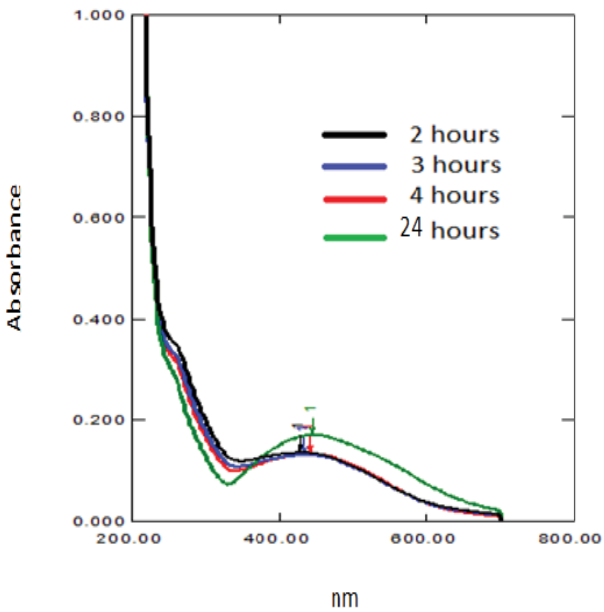

Figure 3: UV-VIS absorption spectra of aqueous silver nitrate with *Catharanthus roseus* leaf extract at different time intervals.

shape, and the silver nanoparticles that are formed may be poly-dispersed in condition because the spectra exhibiting the broadening of peak.

TEM was employed to characterize the size, shape, and morphology of formed silver nanoparticles. The TEM image (Fig. 4) of silver nanoparticles shows that most of the prepared silver nanoparticles are spherical in shape. A few agglomerated silver nanoparticles were also observed which indicates the possible sedimentation at a later time. It is reveal that there is variation in particle sizes and the particle size ranges from 7 nm to 33 nm and the average particle size was found to be 19 nm (Fig. 5). X-ray diffraction (XRD) analysis is used for determining the crystal structure and purity of a material. Fig. 6 shows the XRD pattern with the diffraction peaks at 31.077, 38.185, 44.469 and 55.164 corresponding to the (110), (111), (200) and (211) facets of the face centered cubic crystal structure. Two diffraction peaks observed at 38.185 and 44.469 in the 2θ range 10-800can be ascribed to the (111) and (200) reflection planes of face- centered cubic (fcc) structure of Ag phases. These diffraction peaks are well consistent with the standard data file JCPDS No:04-0783 [16]. In addition, residual peaks were also observed at 27.852, 32.252, 46.214, 64.501 and 73.391. These peaks are due to the constituents present in C. roseus extract. Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effect. Broader peaks signify smaller particle size and reflect the effects of experimental conditions on the nucleation and growth of the crystal nuclei. The average crystalline size calculated using Debye scherrer equation with the width of the (111) peak was found to be 25 nm.

Further the FT-IR analysis of the leaf extract mediated silver nanoparticles was performed. The FT-IR spectrum of silver nanoparticles showed principal peaks at 3650 cm^{-1} for OH stretching, 3460 cm^{-1} for N-H stretching, 2850 cm^{-1} for CH-stretching aromatic, 1643 cm^{-1} for C=O stretching, 953 cm^{-1} for CH_3- bending vibrations (Fig. 7). The above evidence proposed that the silver nanoparticles may be capped with these functional groups of vinca alkaloids and form a layer on the surface of nanoparticles could acts as reducing and stabilizing agent of the nanoparticles.

The toxicity potential of the synthesized AgNPs on human breast cancer cells has been examined using MCF-7 cell line using the MTT assay. Green-synthesized AgNPs induce cytotoxicity on MCF-7 cell lines was found to be higher with increased concentration of AgNPs. There was a change in the percentage of cell viability in control and AgNPs (0, 31.25, 62.5, 125 and 250 μg/ml) treated with MCF-7 cells. Complete mortality rate was observed in 250 μg/ml concentration of AgNPs. IC50 of AgNPs was found to be 113.068 μg/ml (Fig. 8). Particle size distribution histogram of the AgNPs determined from the TEM image as shown

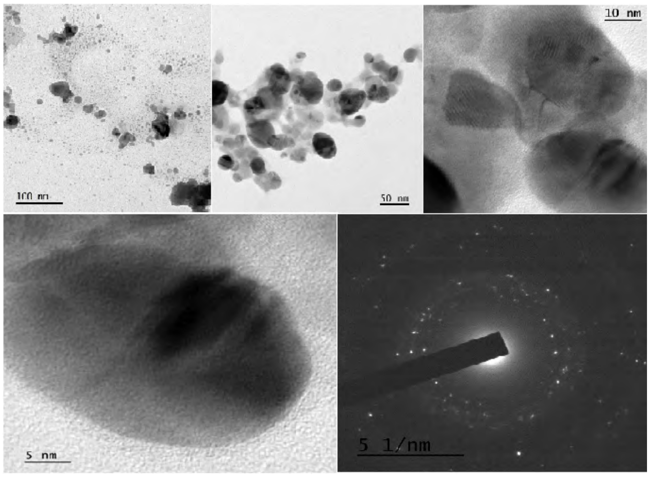

Figure 4: HRTEM micrograph of silver nanoparticles synthesised from 1 × 10^{-3} M $AgNO_3$ solution and *Catharanthus roseus* extract.

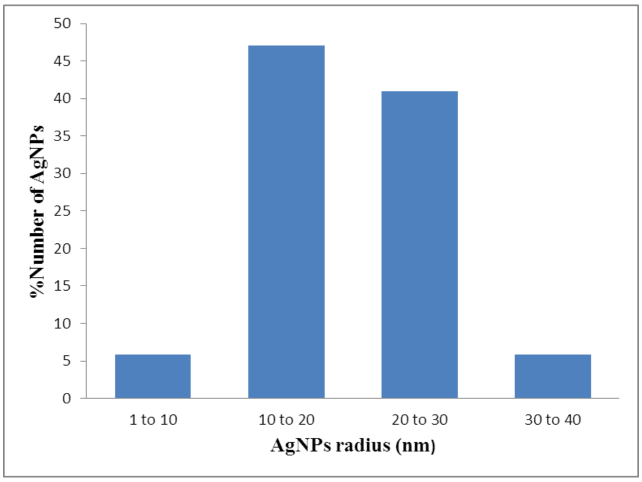

Figure 5: Particle size distribution histogram of AgNPs.

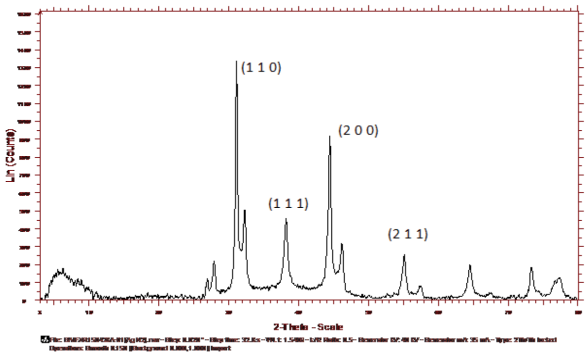

Figure 6: XRD pattern of synthesised silver nanoparticles.

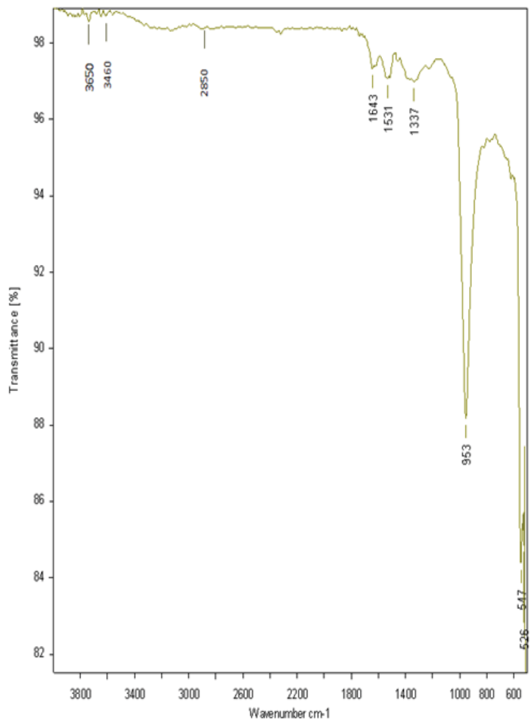

Figure 7: FT-IR spectrum of silver nanoparticles.

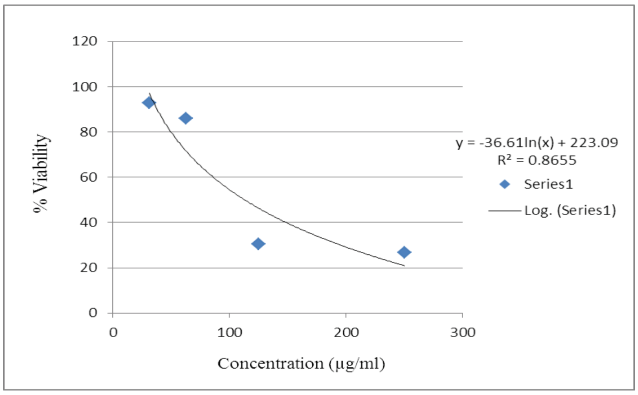

Figure 8: Graphical representation of concentration Vs % viability.

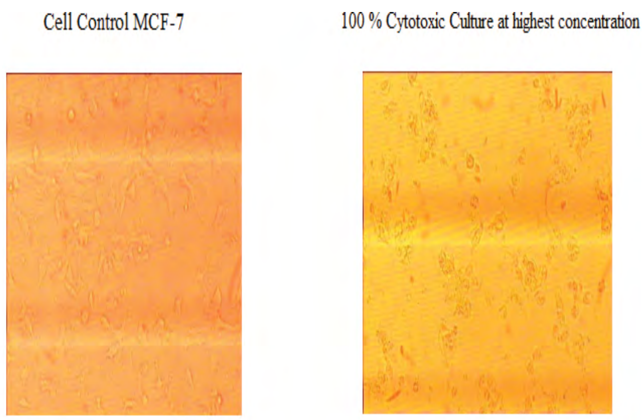

Figure 9: Phase contrast microscopic images of AgNPs gross cytomorphological changes and growth inhibition on the MCF-7 cells at 250 µg/ml and control.

in the Fig. 8. The Diverse morphological alteration was observed in AgNPs treated MCF-7 cells, however no such effects were seen in untreated cells. It was shown that the morphological variations were observed such as loss of membrane integrity, inhibition of cell growth, cytoplasmic condensation and cell clumping (Fig. 9) results indicated that the AgNPs treated MCF-7cells undergone cell death, whereas the non-treated cells were active [17,18].

CONCLUSION

In the present work, silver nanoparticles were green-synthesized using the aqueous extract of *Catharanthus roseus* leaves. The characterization was done by visual examination, UV-Visible Spectroscopy, TEM Analysis, XRD Analysis and FT-IR spectroscopy. MTT assay was used to investigate anti-cancer activity. We have developed a biosynthetic method to formulate AgNPs using aqueous leaf extract of C.roseus, which acts as a reducing as well as stabilizing agent. Particles formed are mostly polydispersed in shape. The green synthetic method is a fast, low cost and eco-friendly process in the field of nanotechnology. The study revealed that the green-synthesized silver nanoparticle provides a promising approach for the breast cancer therapy.

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