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Original Article

Green Synthesis of Silver Nano Particles Structural Characterization and their Antioxidant and Anticancer Potential Using Adenocarcinoma (A549) Cell Line

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ABSTRACT

Background: Over the past two decades, Silver Nanoparticles (AgNPs) have demonstrated a wide range of antioxidant and anticancer properties. Vinca alkaloid exhibits the anticancer efficacy by direct metaphase arrest of cell division. **Aim:** The present study is to develop a green synthesis method for producing silver nanoparticles using vinca, the antioxidant and anticancer potential was assessed using A549 cells. **Materials and Methods:** The synthesized AgNPs were analyzed using Fourier Transform Infrared (FTIR) analysis, UV-visible spectrophotometer (UV), Scanning Electron Microscope (SEM) and Spectra Max i3X energy-dispersive X-ray (XRD) Spectroscopy to determine their physico-chemical and morphological characteristics. **Results and Discussion:** The FTIR spectrum of vinca AgNPs exhibited absorption bands at 692 cm^{-1}, 684 cm^{-1}, 611 cm^{-1}, 592 cm^{-1}, 578 cm^{-1}, 554 cm^{-1}, 548 cm^{-1}, 539 cm^{-1} and 526 cm^{-1}, indicating the presence of silver ion bounded nanoparticles derived from Vinca leaf. These findings suggest that vinca-coated AgNPs possess multiple functions that contribute to their stability. XRD data analysis revealed Bragg's reflections in the XRD pattern (2θ) at 24.75, 31.59, 37.56, 53.01, 64.93 and 76.27, confirming the crystalline nature of the green synthesized AgNPs. Elemental analysis was conducted to determine the elemental composition of the sample, which indicated that approximately 60% of the prepared nanoparticles were bound with silver ions, supporting the formulation. Antioxidant studies were performed using the DPPH assay at different concentrations of AgNPs, while cell-based cytotoxic assays were conducted using different concentrations of $AgNO_3$. **Conclusion:** The results demonstrated that the nanoparticles inhibited the proliferation of A549 cells and reduced cellular motility, indicating their promising anticancer efficacy.

Keywords: Silver Nanoparticle, Vinca, Anticancer, *Catharanthus roseus*, Antiproliferative.

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INTRODUCTION

Silver Nanoparticles

Nanotechnology is one of the rapidly expanding areas of study in technology, engineering and science that deals with nanoscale material manipulation. In the fields of biochemistry and commercial goods, nanomaterials are widely used in coatings, material packaging, cosmetics, targeted medicine delivery and drug carriers.[1,2] AgNPs, or silver nano particles, are inert inorganic metal particles with a size range of 1-100 nm.[3] These minuscule particles of metallic silver exhibit distinct characteristics such as stability, antibacterial activity, conductivity and catalysis.[4] They are therefore frequently used as antioxidant, antibacterial, antimicrobial and anti-inflammatory drugs.[5] As an alternative, plant extracts and microorganisms can be used to physiologically synthesise AgNPs.[6] It has been discovered that the synthesis rate of plant extract is higher than that of chemical processes and green synthesis produced by microorganisms.[7,8] It is well recognised that plant extracts contain in wide range of phytochemicals with distinct structures and bioactivities, including flavonoids, alkaloids, ketones, carboxylic acids and aldehydes. "The silver nanoparticles demonstrate superior antibacterial,[9] antifungal and antiviral properties compared to metallic silver and silver compounds.[10,11]" Utilizing biological processes to create nano particles is biocompatible because plants, in particular, release functional biomolecules that actively decreased metal ions.[12] Both ''Top-down and ''Bottom -up" methods can be used to create NPs. An assortment of techniques, including pulse laser ablation, evaporation-condensation and ball milling, are employed to break down a suitable bulk material into minuscule particles through size reduction in a top-down approach. Conversely, the bottom-up approach enables atoms to self- assembles into new

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nuclei which subsequently develop into nano scale particles, there by facilitating the chemical and biological creations of Nano Particles (NPs).[13]

In comparison to physical and chemical processes, green synthesis is more environmentally benign, economically advantageous and readily scaled up for the production of large quantities of Nano Particles (NPs).

Vinca Alkaloids

Vinca (*Catharanthus roseus)* is a tropical perennial herbaceous plant of the Apocynaceae family. Eight species make up this group; seven are unique to Madagascar (*C. coriaceus, C. lanceus, C. longifolius, C. alis, Cov. roseus, C. scitulus* and *C. trichophyllus*) and one is native to India (*C. pusillus*).[14] Vinca alkaloids are a class of medications derived from the stem of the *Catharanthus roseus* G. Don Periwinkle plant found in Madagascar. Natural extracts of these alkaloids with cytotoxic and hypoglycaemic properties are made from the pink periwinkle plant. Their famed capacity to combat cancer is another attribute. Vibesine (VDS), Vinblastine (VBL), Vinorelbine (VRL) and Vincristine (VCR) are the four main vinca alkaloids used in clinical practice. The US has authorised the use of VCR, VBL and VRL. One alkaloid that was isolated from *Vinca rosea* Linn. is vinblastine sulphate. When used *in vitro*, this compound is a stat kinetic oncolytic drug that stops developing cells in metaphase. *Catharanthus roseus*, also known as Madagascar periwinkle or leurocristine, or simply "VCR," is a kind of vinca alkaloid. It functions as a mitotic inhibitor and is frequently applied in chemotherapy for cancer.[15]

Nanoparticle

Nanoparticles are defined by the International Organization for Standardization (ISO) as Nano-objects whose exterior dimensions are all within the Nano scale range. In this situation, there shouldn't be any appreciable variations in the Nano object's longest and shortest axes' lengths. However, if there is a notable difference in dimensions (usually exceeding three times), alternative terms like Nano fibers or Nano plates may be more appropriate than the term NPs.[16]

MATERIALS AND METHODS

Preparation of Leaf Extracts

The leaves of *Catharanthus roseus*, also known as Nithya Kalyani, were gathered, carefully cleaned in distilled water and then dried at 37°C. Afterwards, the dried leaves were pulverised with a mortar and pestle to a fine powder, which was then utilised as stock for the subsequent extraction procedure.

Extraction of Plant Leaf

Using a liquid to extract a desired ingredient from a solid material is known as soxhlet extraction. An organic solvent, such as ethanol, methanol, acetone, hexane, or ethyl acetate, can be the liquid, or solvent. The solute is dissolved by the solvent, but the other solid matrix elements remain intact. To extract vinca leaves using Soxhlet extraction process the following steps includes as

Use 20 g of *vinca roseus* plant sample with 70 mL of ethanol (B.P 60-65°C) as a solvent.

Reflux for 6 hr at constant temperature 60°C.

Soak materials with 90% ethanol and leave the mixture overnight.

Microwave assisted extraction of leaves of*C. roseus*.

Phytochemical Analysis

Vinca rosea, a highly valuable and extensively utilized medicinal plant, underwent an initial investigation to determine its phytochemical composition. In this study, the secondary metabolites in the methanol, petroleum ether, ethanol and aqueous extracts made from *Vinca rosea* Linn. leaves were evaluated both qualitatively and quantitatively. Numerous phytochemical components, such as alkaloids, flavonoids, sterols, phenols, tannins and glycosides, were found, according to the analysis as shown in Table 1.

Characterization and Biosynthesis of Silver Nanoparticles

The 40 mL of extract and 154 mL of deionized water were combined with 6.0 mL of $AgNO_3$ (20 mm) to initiate the biogenesis of the nanoparticles. Next, colour changes resulting from the reaction of $AgNO_3$ with the extract concentration at 80°C were looked for in the reaction mixture. Following the reaction, the material took on a reddish-brown hue. It was then centrifuged for 10 minutes at 12,000 rpm. The resulting pellet was cleaned using acetone and

Table 1: Methodology of Phytochemical Analysis.

Phytochemical	Methodology
Carbohydrates	After treating 2 mL of extract with 1 mL of Molisch's reagent, a few drops of concentrated H_2SO_4 were added.
Tannins	The mixture of 1 mL extract and 2 mL 5% $FeCl_3$ was added.
Saponins	In a graduated cylinder, 2 mL of extract and distilled water were added and the mixture was agitated for 15 min.
Terpenoids	Chloroform (2 mL) and concentrated H_2SO_4 were applied to 0.5 mL of the extract.
Anthraquinones	1 mL of the extract received a few drops of 10% NH_4OH added.
Steroids	A small amount of concentrated H_2SO_4 and an equivalent volume of chloroform were added to 1 mL of extract.
Proteins	A few drops of $CuSO_4$ and 1 mL of 10% NaOH were added to 1 mL of extract.

methanol after three rounds of deionized water washing. The pellet was preserved for additional characterizations once it had dried. Using an Ultraviolet-Visible (UV-vis) spectrophotometer (i3X) with a wavelength range of 230-600 nm, the synthesis of silver nanoparticles was examined.

UV-visible Spectrophotometer (UV) Analysis

The Vinca solutions underwent scanning within the wavelength range of 200-600 nm in comparison to the corresponding buffer solution (blank). A Shimadzu 1900i double beam UV spectrophotometer (Japan) was used to measure the absorbance of the Vinca solution and determine the wavelength at which maximum absorbance happens.

Fourier Transforms Infrared (FTIR) Analysis

On a Bruker ATR alpha device, attenuated total reflection-fourier transform infrared spectroscopy, or ATR-FTIR, was used to collect the samples' infrared spectra. The instrument was maintained at an ambient temperature of 25.0±0.50C. The analytical procedure was straightforward and did not require any special preparation of the samples. To secure the sample, a little portion was placed on the zinc solenoid crystal plate and the anvil was turned. After that, the samples were scanned in the 4000-400 cm^{-1} region to record the spectra. This made it possible to identify different functional groups using the German-made Bruker II Alpha apparatus and FTIR/ATR.

XRD Analysis (Rigaku Smartlab-Japan)

In materials research, X-ray diffraction analysis, also referred to as is a technique used to determine a substance's crystallographic configuration. XRD makes it possible to identify the crystalline phases that are present in a material by first irradiating it with X-rays and then evaluating the intensities variable scatter angles of the X-rays that are released. In turn, this offers helpful information on the material's chemical makeup. The phases that are present are then ascertained by comparing the collected data with reference databases. A concentrated X-ray beam is aimed at the sample at a particular angle of incidence during an X-ray reflectometry investigation. This causes the X-rays to diffract in various ways based on the sample's crystal structure, particularly the inter atomic distances. The locations of the diffracted X-rays that arise are measured.

SEM Imaging (HITACHI S 3400-JAPAN)

This investigation was carried out to look in the silver nanoparticles' structure and determine their size and shape. The AgNPs had a uniform size distribution and a spherical shape, as shown by the SEM (Scanning Electron Microscopy) pictures. The AgNPs were about 50 nm in size on average. Those vinca-coated AgNPs were likewise shown to be evenly distributed and clear of clumping or agglomeration by the SEM pictures. The surface of the AgNPs appeared smooth and free of any deformities or irregularities. Overall, the SEM analysis confirmed that the AgNPs produced in the current study were of high quality and had the desired morphological characteristics.

Destructive Size Display

Through dynamic light scattering, the size of the nanoparticles was ascertained using the Microtrac Equipment (Nanotrac, USA) size of nanoparticle analyzer. This is a common method used to determine the pattern of distribution of particle sizes and the average hydrodynamic diameter. The temperature at which dynamic light scattering experiments were made was 250°C.

Antioxidant Assay

DPPH Radical Scavenging Assay

Introduction

When the biological system's antioxidant capability is exceeded by the generation of Reactive Oxygen Compounds (ROS), oxidative stress results. Numerous human disorders, such as heart disease, high blood pressure, diabetes mellitus, inflammation, cancer and AIDS, have been connected to this imbalance.[17] Conversely, in low concentrations compared to oxidizable substrates, antioxidants are compounds that can prevent or postpone oxidative reactions. They achieve this by mechanisms such as electron donation, metal ion chelation and sparing of antioxidants. By reducing the burden of free radicals, antioxidants can stabilize them and prevent further damage. The test for DPPH scavenging is an economical and simple way to evaluate a compound's ability to scavenge free radicals.[18,19]

Principle

Also referred to as 1,1-diphenyl-2-picryl hydrazyl, or DPPH, is a purple-colored, stable free radical. 1,1-diphenyl-2-picryl hydrazine, a colourless substance whose absorbance at 517 nm can be used to determine its concentration.

Methodology Used

Reagents

It is recommended to keep the DPPH component (the Eastern European Community Nr. 217-591-8, Sigma, USA) at or below freezing temperatures.

Ensure to use HPLC grade methanol (Ranbaxy Chemicals) for your experiments.

Inhibitor (reference standard): Ascorbic acid (1 mg/mL).

Preparation of working solutions

DPPH: 1 mg was dissolved in 6 mL of methanol of the HPLC (high-performance liquid chromatography) grade.

Ascorbic acid: 1 mg was dissolved in 1 mL of methanol.

Procedure

The assay for DPPH is carried out in accordance with Rajakumar *et al.'s* approach. In conclusion, various levels of the test solution are mixed with 80 µL of DPPH solution and HPLC grade methanol is used to adjust the volume to 240 µL. The test samples are examined at concentrations in the range of 320 to 10 µg/mL and the control standard is assessed at ranges between 30 and 1.25 µg/mL. After the reaction mixture has been well combined, it is incubated for 15 min at 25°C. A semi-autoanalyzer is then used to test the mixture's absorbance at a wavelength of 517 nm. Additionally, a control reaction is carried out devoid of the test sample.

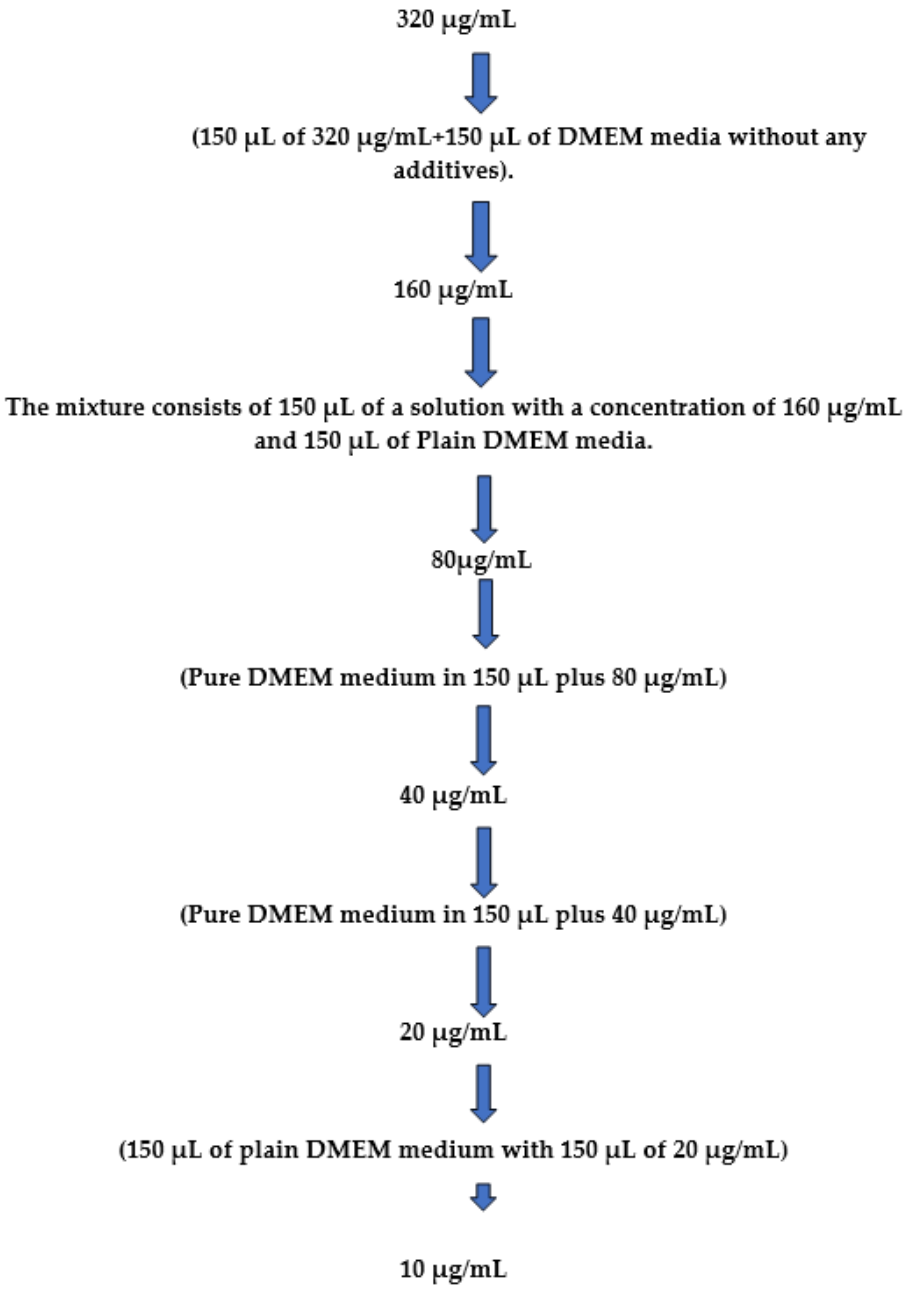
Statistical evaluation

Experiments are conducted with the Graph Pad prism to determine the Half Maximal Inhibiting Concentration (IC_{50}). This phrase refers to the amount of a material needed to 50% impede a process that is biologic, such as that of an enzymes cell, cell receptors, or microbe.

Calculations

Calculating percentage growth inhibition:

$$\%\ Inhibition = \frac{(OD\ of\ Control - OD\ of\ Sample)}{OD\ of\ Control} \times 100$$



Cytotoxicity Studies for A549 Cell Line

Introduction

Previously, the evaluation of harmful impacts induced by unknown substances in a controlled laboratory environment encompassed the enumeration of viable cells following the application of a dye. Other ways included measuring DNA synthesis by adding radioisotopes, using automated cellular counters and using other methods that depended on dyes plus cellular activity.

Goal

This Standardised Operating Procedure (SOP) aims to provide clear and concise instructions to perform the MTT method cytotoxicity assay.

Materials and Methods

1. After being filtered using using 0.2 µ m filter, the Multipurpose Treatment Powder is stored at temperatures between 2 and 8°C. This ensures its availability for regular usage or allows for long-term storage by freezing.
2. DMSO
3. SpectraMax i3X.

Test sample preparation

DMSO was used to create 32 mg/mL solutions for cytotoxicity investigations. Then, using DMEM plain media, successive two-fold dilutions were made with a range of 320 µg/mL to 10 µg/mL for treatment purposes.

Culture Medium And Cell Lines

The ATCC provided the Mino cell line. The starting cells were grown in DMEM mixed with 10% Foetal Bovine Serum (FBS), 100 µg/mL of streptomycin and 100 IU/mL of penicillin in a controlled atmosphere at 37°C with 5% CO_2. The culture was continued until the cells reached complete coverage. A solution comprising 0.2% of the total trypsin, 0.02% EDTA, as well as 0.05% insulin in PBS was used to separate the cells. After the vitality of the cells was assessed, they were centrifuged. Subsequently, 50,000 cells were inserted into each well of a 96-well plate and cultured in a CO_2 incubator with 5% CO_2 for a whole day at 37°C.

Reagents' source

We purchased DMEM, RPMI, Trypsin, Pen Strep and FBS from Invitrogen.

Procedure

Trypsinization was applied to the monolayer cell culture and 5.0 x 105 cells/mL was the final cell count achieved using the appropriate medium containing 10% FBS. Afterwards, 100 microtiter plates with a diluted cell solution (50,000 cells per well) had been placed to each well. Once a partial surface had developed after 24 hr, the supernatant that had formed was removed and the monolayer that was formed was given one medium wash. Subsequently, 100 µL of various test drug doses were put onto the microtiter plate partial monolayer. The plates had been incubated in a mixture containing 5% CO_2 for 24 hr at 37°C. After the incubation period, each well received 100 µL to MTT (5 mg/10 mL up MTT in PBS) and the test solutions were disposed of.

Calculating Inhibition

$$\%\ Inhibition = \frac{(OD\ of\ Control - OD\ of\ Sample)}{OD\ of\ Control} \times 100$$

Statistical Evaluation

IC_{50} Value

The half-maximal inhibitory concentration, or IC_{50}, measures how well a substance inhibits biological or metabolic activity.

RESULTS

Phytochemical Analysis: Results shown in Table 2.

Table 2: Phytochemical Analysis.

Phytochemical	Methodology	Observation
Carbohydrates	1 mL of Molisch's reagent was added to the extract, along with a tiny quantity of concentrated H_2SO_4.	The creation of a ring with a purple or reddish ring.
Tannins	1 mL of this extract was combined with 2 mL of 5% $FeCl_3$.	production of a dark blue or greenish-black ring
Saponins	Distilled water and 2 mL of extract were put into a graduated cylinder. and the mixture was agitated for 15 min.	A sheet of foam one centimetre thick forming
Terpenoids	A mixture of concentrated H_2SO_4 and 2 mL of chloroform was put to 0.5 mL of the extract.	The boundary becoming a reddish-brown colour.
Anthraquinones	A few drips of 10% NH_4OH that were added to a single millilitre of extract.	Formation of a pinkish precipitate
Steroids	Chloroform, a millilitre of extract and a small amount of concentrated H_2SO_4 were combined.	Formation of a brown colour ring
Proteins	Add a few drops liquid $CuSO_4$ and 1 mL of 10% NaOH to 1 mL of extract.	Creation of a mauve or purple blue.

UV Spectra of $AgNO_3$ Nano Particle

The Vinca solutions underwent scanning within the wavelength range of 200-600 nm in comparison to the corresponding buffer solution (blank). A double beam UV spectrophotometer (Shimadzu 1900i, Japan) was used to measure the penetration of the Vinca solution in order to determine the wavelengths at which maximum absorbance happens as shown in Table 3 and Figure 1.

FTIR Study

The purpose of the FTIR study was to pinpoint the putative groups of function and biomolecules in charge of efficiently capping and stabilising the synthesised AgNPs. The spectrum of FTIR of the Vinca AgNPs shown in Figure 2 and 3 revealed absorption bands at 692 cm^{-1}, 684 cm^{-1}, 611 cm^{-1}, 592 cm^{-1}, 578 cm^{-1}, 554 cm^{-1}, 548 cm^{-1}, 539 cm^{-1} and 526 cm^{-1}, indicating the presence of silver ion bounded nanoparticles derived from Vinca leaf.

XRD analysis (rigaku smartlab-JAPAN)

The XRD pattern of the synthesized AgNPs shown in Table 4 and Figure 4 revealed the presence of Bragg reflections at 2θ angles of 24.75, 31.59, 37.56, 53.01, 64.93 and 76.27. These reflections clearly indicate the existence of different lattice planes, allowing for the indexing of the silver structure as Face-Centered Cubic (FCC). The study's findings provide clear evidence that the green synthesized AgNPs possess a crystalline nature, as demonstrated by the XRD pattern.

SEM Imaging (Hitachi S 3400-Japan)

The Scanning Electron Microscope (SEM) operated at an accelerating voltage of 10kV using Hitachi S 3400 was utilized to capture images of the powders. A small amount of powder, measuring a few µg, was affixed to a stub using a double-sided sticky carbon tape. The stub was then placed inside the SEM chamber and examined at various magnifications to enhance the clarity of the particle morphology and topology. In order to obtain clearer images with better magnification, gold sputtering was conducted to prepare the Nano particulates. Through this specific study, it was confirmed that the sample possessed a nanostructure. Furthermore, elemental analysis was done to ascertain the percentage elemental composition of the given sample. The results revealed that approximately 60% of the prepared nanoparticle was bound with silver ions, confirming the formation of the Nanoparticle which is shown in Figures 5-8.

Analysis of particle size and zeta potentialVinca's AgNPs were previously synthesised and refined via X-ray diffraction analysis, SEM+Edax, FTIR spectroscopy and UV-vis spectroscopy. Subsequently, the sample underwent analysis by Zetasizer Nano ZS (Make: Microtrac, Model: Nanotra-USA). To prepare the stock solution of AgNPs, a concentration of 10 mg/mL in Nano water was utilized, followed by ultrasonication using an ultrasonic bath

Table 3: UV Spectra of $AgNO_3$ Nano Particle.

nm	$AgNO_3$	nm	$AgNO_3$
230	1.2087	420	1.1999
240	1.1923	430	1.2222
250	1.1759	440	1.2139
260	1.1595	450	1.1774
270	1.1431	460	1.1166
280	1.1267	470	1.054
290	1.1103	480	0.9835
300	1.0939	490	0.9066
310	1.046	500	0.8346
320	1.0903	510	0.7636
330	1.0967	520	0.6969
340	1.0989	530	0.6341
350	1.0351	540	0.5766
360	1.031	550	0.5285
370	1.0425	560	0.482
380	1.0696	570	0.4415
390	1.096	580	0.4073
400	1.123	590	0.3743
410	1.1606	600	0.3357

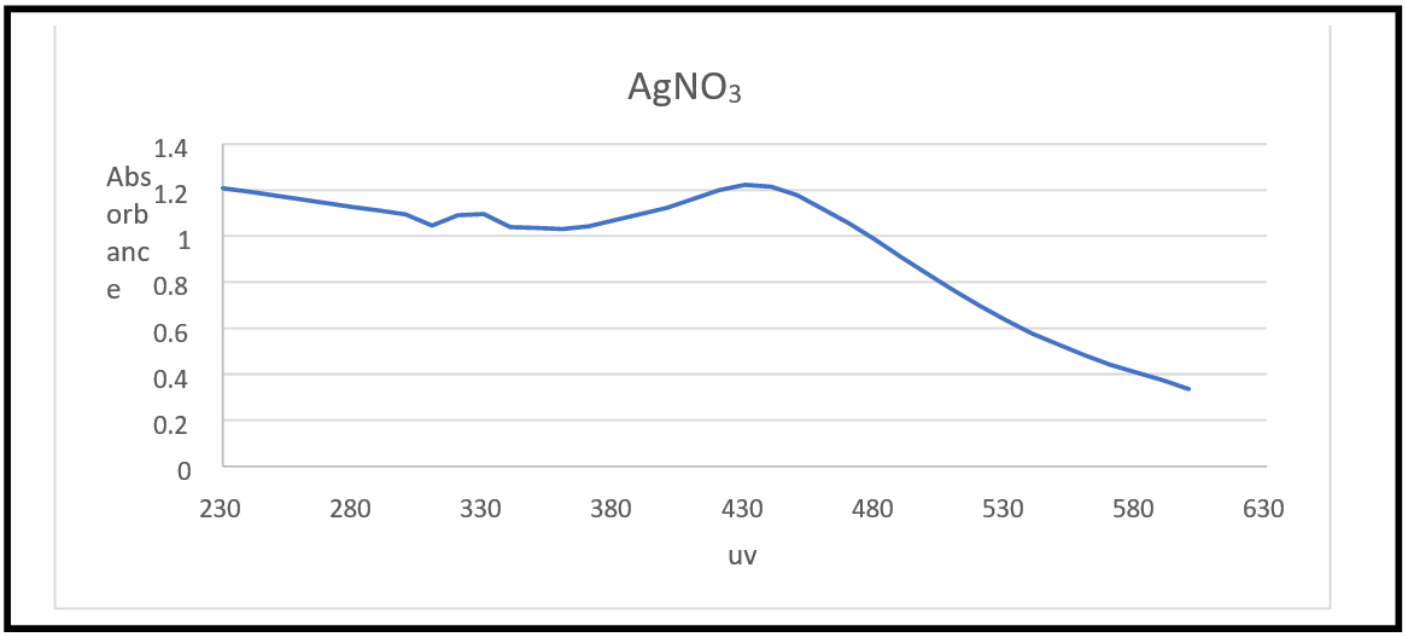


Figure 1: UV Spectra of $AgNO_3$ Nano Particle.

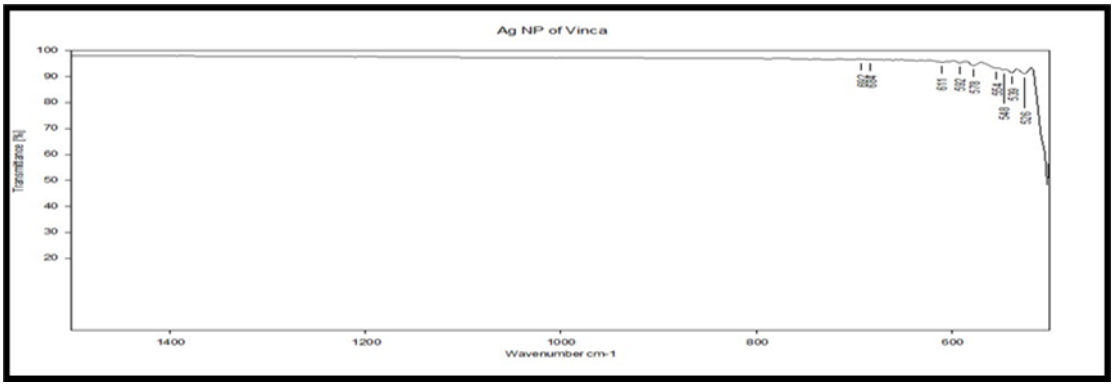


Figure 2: FTIR AgNP of vinca.

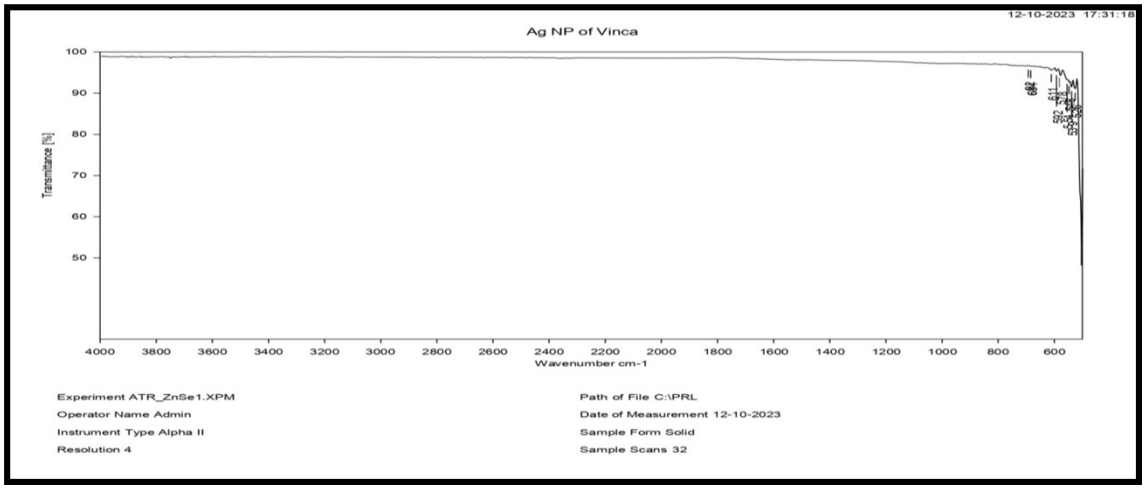


Figure 3: FTIR AgNP of vinca.

for 5 min. The solution was then transferred into the liquid cell for measurement of particle size and zeta potential. The stability of the AgNPs was assessed through zeta potential analysis. According to the data presented in the Figure 4, With a typical particle size of 465 nm, the AgNPs' zeta potential measurement showed a value of -4.53 mV, showing that they are stable which is shown in Table 5.

Assay for scavenging DPPH radicals (Table 6 and Figures 9 and 10)

Calculating percentage growth inhibition

$$\% \, Inhibition = \frac{(OD \, of \, Control - OD \, of \, Sample)}{OD \, of \, Control} \times 100$$

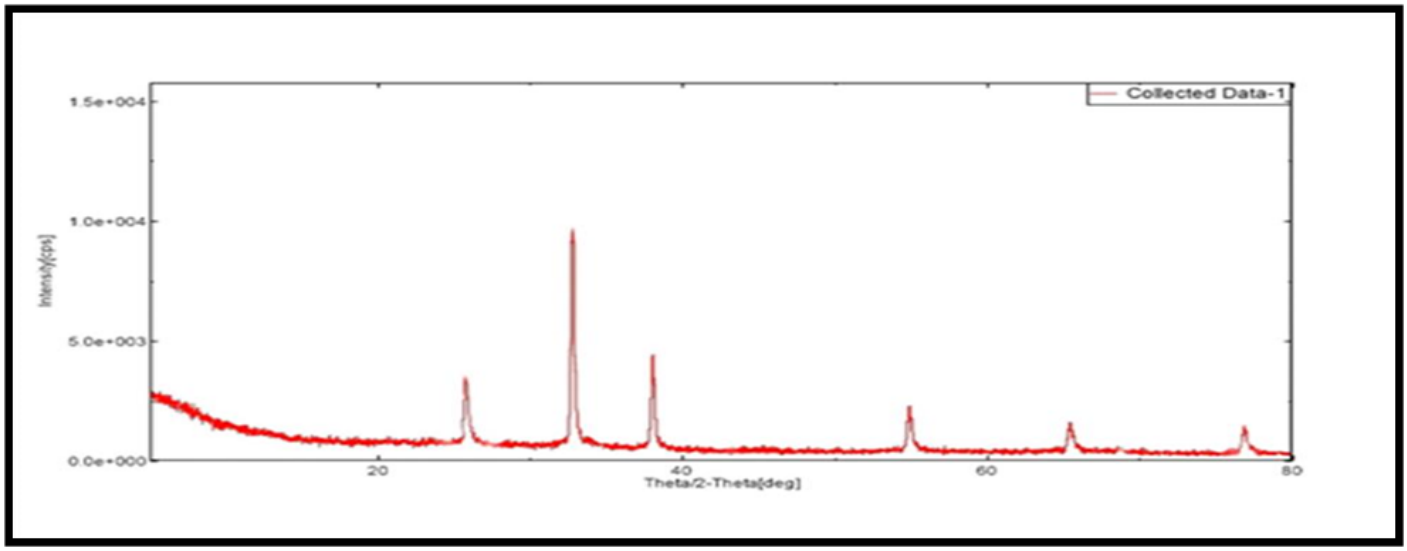


Figure 4: XRD of $AgNO_3$ Nano Particle.

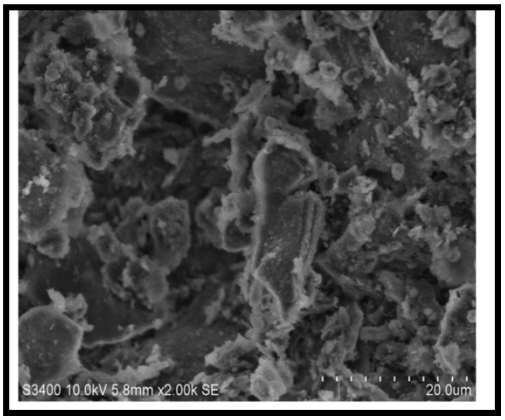


Figure 5: SEM Imaging.

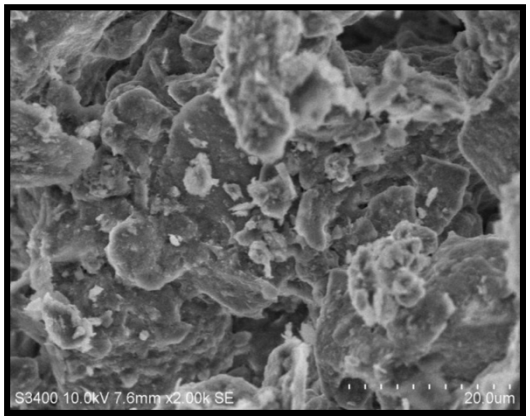


Figure 6: SEM Imaging.

Table 4: XRD of $AgNO_3$ Nano Particle.

Sl. No.	2-theta (deg)	d(ang.)	FWHM (deg)	Int. (counts deg)	Int. W(deg)	Asym. factor	Size (ang.)	Rel.int.I	Rel. height
1	24.75	2.0348	0.983	46.2	1.42	2.43	91.46	37.01	22.36
2	31.59	2.63181	0.126	256.38	0.2263	0.65	75.73	100	100
3	37.56	2.26567	0.23	119	0.324	1.401	388.85	42.92	27.33
4	53.01	1.57242	0.2381	62.1	0.388	1.416	439.8	21.69	13.28
5	64.93	1.32585	0.2843	44.16	0.402	1.301	315.02	15.95	7.73
6	76.27	1.1411	0.6499	51.31	0.971	0.332	154.42	21.67	35.92

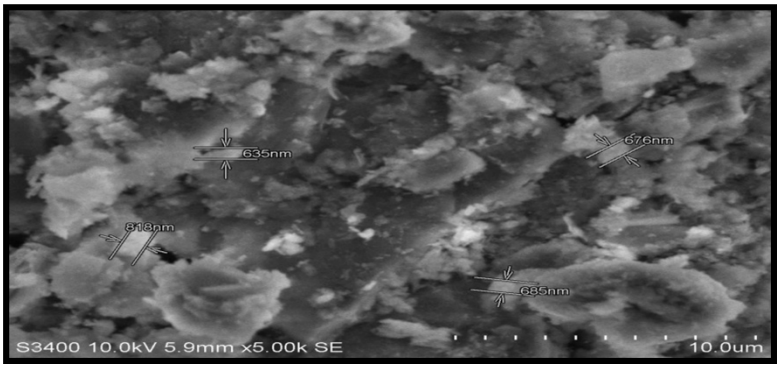


Figure 7: SEM Imaging .

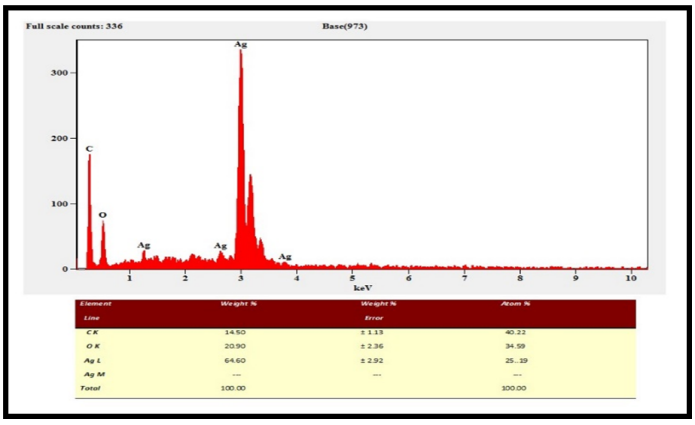


Figure 8: SEM Imaging.

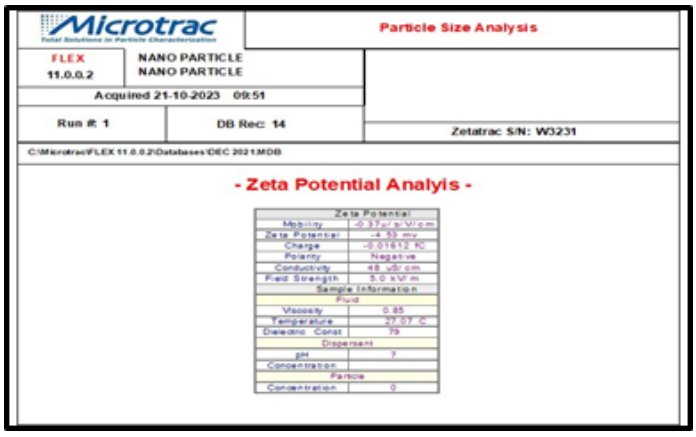


Table 5: Particle Size Analysis.

Table 6: DPPH Assay.

	Conc µg/mL	OD@517	%INHIBITION	IC_{50}
Ascorbic acid	0	0.716	0.00	15.19
	1.125	0.628	12.31	
	3.25	0.542	24.35	
	7.5	0.435	39.22	
	15	0.314	56.14	
	30	0.18902	73.62	
Vinca $AgNO_3$	0	0.736	0.00	184
	10	0.617	16.17	
	20	0.583	20.77	
	40	0.518	29.62	
	80	0.436	40.76	
	160	0.329	55.29	
	320	0.200	72.83	

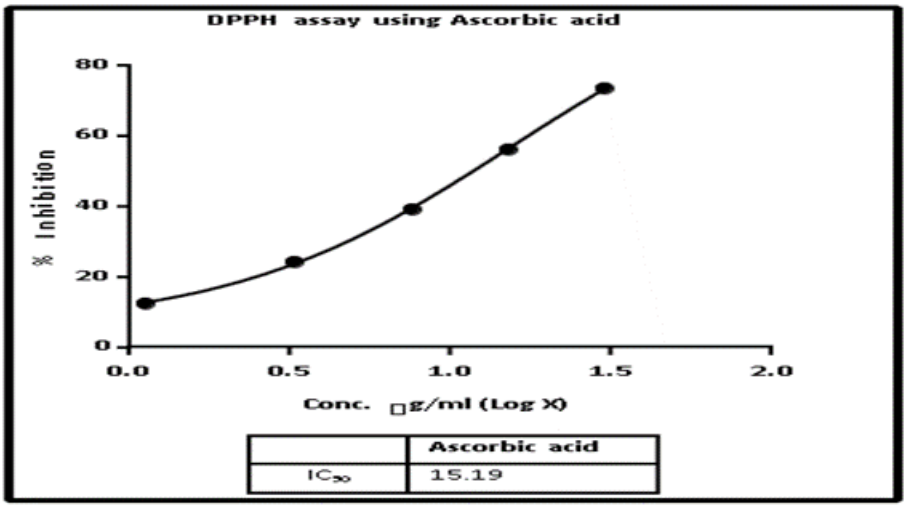


Figure 9: DPPH assay using ascorbic acid.

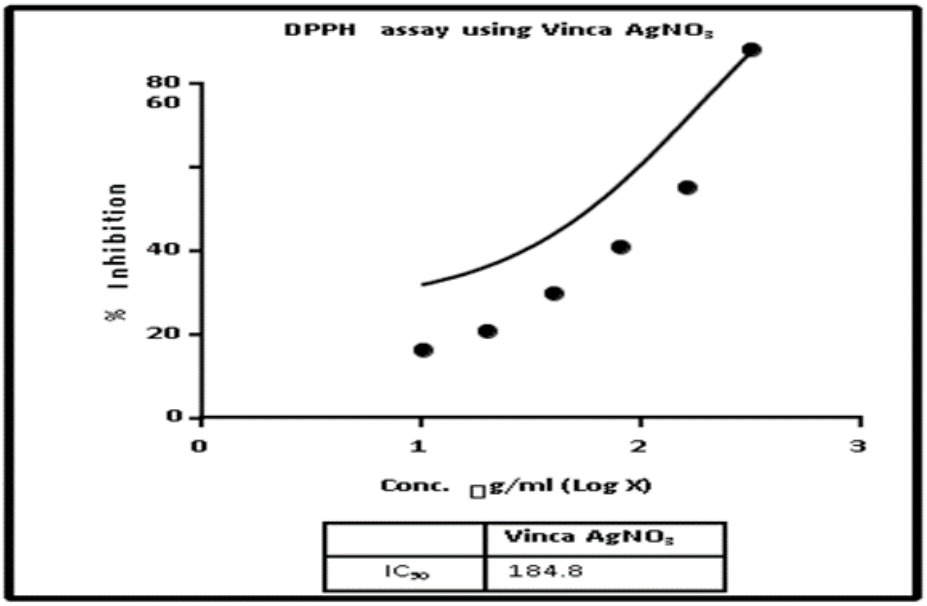


Figure 10: DPPH assay using vinca $AgNO_3$.

DPPH Assay Results

Cytotoxicity studies for A549 cell line (Tables 7, 8 and Figure 11,12)

Calculating percentage growth inhibition:

$$\%\ Inhibition = \frac{(OD\ of\ Control - OD\ of\ Sample)}{OD\ of\ Control} \times 100$$

DISCUSSION

Silver nanoparticles are produced from the aqueous leaf extract of *Vinca rosea*. These nano particles possess anti-plasmodial activity. The leaves of *Catharanthus roseus* have various properties, such as antimicrobial, antifungal, antioxidant, antibiotic, cancer-fighting, wound healing and antiviral effects. The green synthesis process used for the preparation of these nano particles is both time-saving and cost-effective, as well as environmentally friendly. The silver nanoparticles synthesized using silver nitrate and the leaves of *Catharanthus roseus* have demonstrated a high level of efficacy against specific strains of bacteria. Furthermore, these synthesized Silver Nanoparticles (AgNPs) exhibit a greater cytotoxic activity against A549 lung cancer cells.

During the studies, the vinca solutions were analyzed using a double beam UV-spectrophotometer (Shimadzu 1900i-Japan) within the range of 300-600 nm against the respective buffer solution. The FTIR spectrum of vinca AgNPs revealed absorption bands at 692 cm^{-1}, 684 cm^{-1}, 611 cm^{-1}, 592 cm^{-1}, 578 cm^{-1}, 554 cm^{-1}, 548 cm^{-1}, 539 cm^{-1} and 526 cm^{-1}, indicating the formation

MTT Assay Results

Table 7: Standard drug.

Doxorubicin	Conc (µg/mL)	OD@540	%Inhibition	IC_{50}
	0	0.732	0.00	2.72
	0.6	0.614	16.12	
	1.2	0.541	26.09	
	2.5	0.384	47.54	
	5	0.215	70.63	
	10	0.142	80.60	

Table 8: Vinca Nanoparticles.

Vinca $AgNO_3$	Conc (µg/mL)	OD@540	%Inhibition	IC_{50}
	10	0.671	10.41	208.5
	20	0.6249	16.57	
	40	0.555	25.90	
	80	0.4383	41.48	
	160	0.409	45.39	
	320	0.205	72.63	

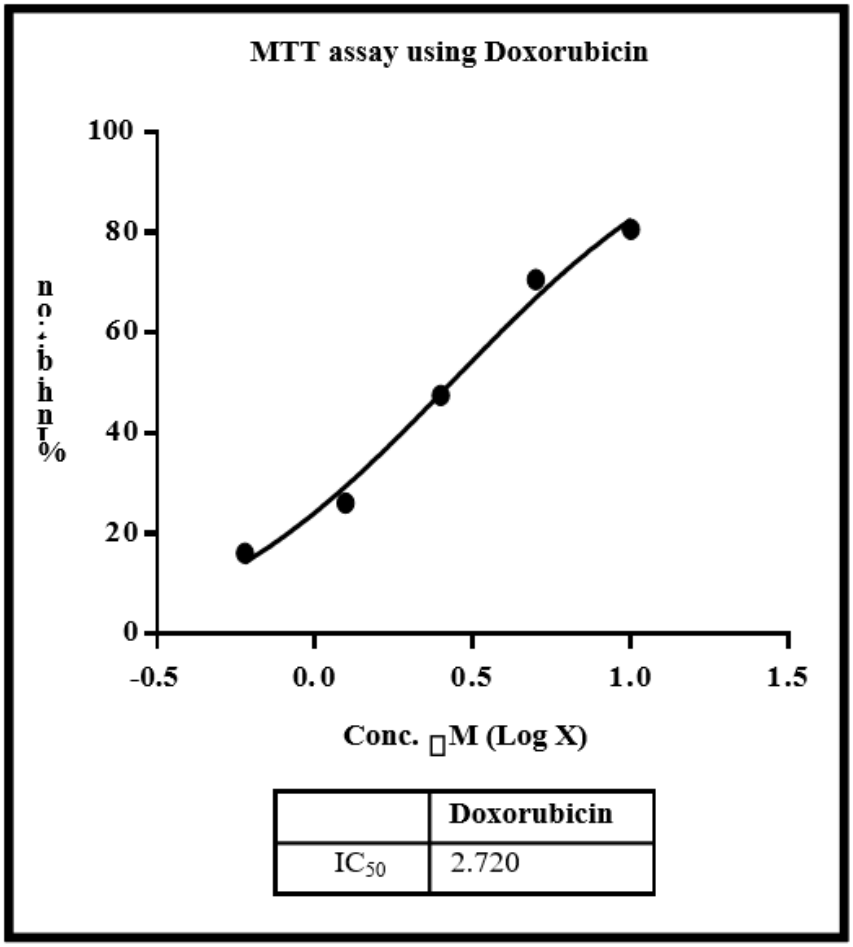
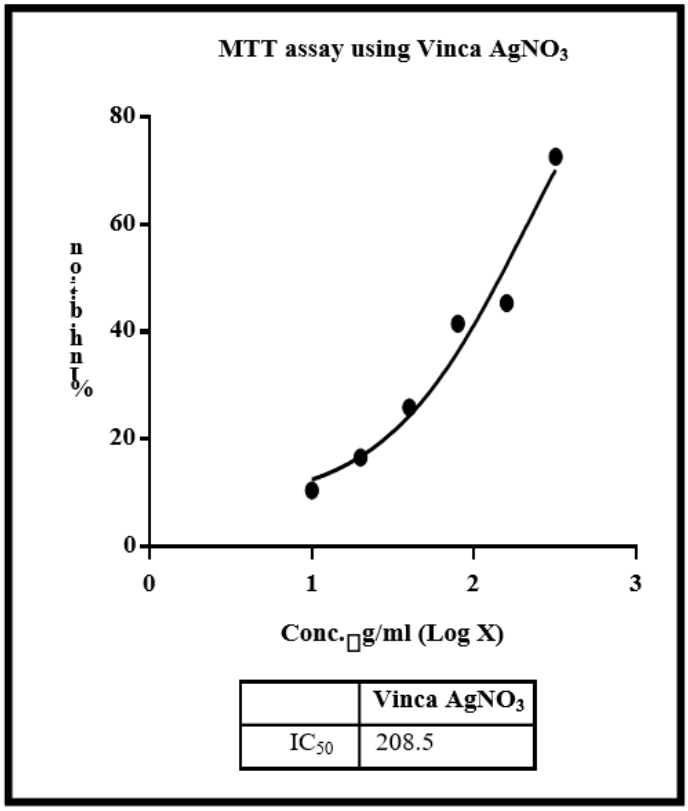


Figure 11: MTT assay using Doxorubicin.



	Vinca $AgNO_3$
IC_{50}	208.5

Figure 12: MTT assay using Vinca.

of silver ion bounded nanoparticles derived from Vinca leaf. The synthetic AgNPs' XRD data analysis demonstrated the presence of Bragg's reflections in the XRD pattern (2θ) at 24.75, 31.59, 37.56, 53.01, 64.93 and 76.27.

The AgNPs powders were examined using a SEM (scanning electron microscope) equipped with a 10kV accelerating voltage utilizing the Hitachi S 3400-Japan model. For particle size and Zeta potential analysis, the sample was assessed using the Zetasizer Nano ZS (Make: Microtrac, Model: Nanotra-USA). The zeta potential measurement of AgNPs exhibited a value of -4.53 mV, indicating the stability of these AgNPs and an average particle size of 465 nm. In the DPPH antioxidant studies, the sample was analyzed at different concentrations, resulting in an IC_{50} value of 184.8 for the sample. $AgNO_3$ was tested at various doses in the cytotoxicity investigations carried out on the A549 cell line.

CONCLUSION

Silver nanoparticles (AgNPs) have been effectively stabilized by the addition of vinca, hence augmenting their antibacterial and anticancer properties. The vinca solution was scanned in the 200-600 nm wavelength range using a UV spectrophotometer to find potential functional groups and biomolecules for capping and effective stabilization. X-ray reflections clearly indicated the presence of various lattice planes, confirming the Face-Centered-Cubic (FCC) structure of silver. The XRD pattern of the green synthesized AgNPs showed a crystalline nature. SEM study confirmed the Nano-structure of the sample. Elemental analysis revealed that approximately 60% of the prepared nanoparticle was bound with silver ions, confirming the formation of nanoparticles. The stability of AgNPs was evaluated using Zeta potential analysis. The test sample was analyzed for DPPH at different concentrations and compared to the standard DOX value in cytotoxicity studies for A549 cells.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Ag: Silver; **$AgNO_3$:** Silver nitrate; **NP:** Nanoparticle; **CPE:** Cloud-point Extraction; **CMC:** Critical micelle concentration; **FFF:** Field-flow Fractionation; **HDC:** Hydrodynamic chromatography; **EM:** Electron Microscopy; **TEM:** Transmission Electron Microscopy; **SEM:** Scanning Electron Microscopy; **TIAS:** Terpenoid Indole Alkaloids; **VLB:** Vinblastine; **VRL:** Vinorelbine; **VCR:** Vincristine; **VDS:** Vindesine; **MRSA:** Methicillin-resistant *Staphylococcus aureus;* ***C. roseus*:** *Catharanthus roseus*; **DLVO:** Derjaguin-Landau-Verwey-Overbeek; **JKR:** Johnson-Kendall-Roberts; **DMT:** Derjaguin-Mullar-Toporov; **nm:** Nanometer; **MTT:** (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); **ATR:** Attenuated Total Reflection; **A549:** Adenocarcinoma cell line; **AgNPs:** Silver Nanoparticles; **XRD:** X-rays deflection; **EDXA:** Energy-dispersive X-ray analysis; **OD:** Optical Density; **FCC:** Face-centered-cubic; **DPHH:** [1,1-Diphenyl-2-picrylhydrazyl]; **IC_{50}:** Half maximal Inhibitory Concentration; **DMEM:** Dulbecco's Modified Eagle Medium; **ATCC:** American Type Culture Collection; **FBS:** Fetal Bovine Serum; **PBS:** Phosphate Buffered Saline; **°C:** Degree Celsius; **GPP:** GraphPad Prism.

REFERENCES

1. Chung IM, Park I, Seung-Hyun K, Thiruvengadam M, Rajakumar G. Plant-mediated synthesis of silver nanoparticles: their characteristics properties and therapeutic applications. Nanoscale Research Letters. 2016;11:40.
2. Kotakadi VS, Rao RY, Gaddam SA, Prasad TN, Reddy AV, Gopal DV. Simple and rapid biosynthesis of stable silver nanoparticles using dried leaves of *Catharanthus roseus*. G. Donn and its anti-microbial activity. Colloids and Surface Bio interfaces. 2013;105:194-8.
3. Calderón-Jiménez B, Monique E. Johnson ME, Montoro Bustos AR, Murphy KE, Michael R. Winchester MR, José R. Vega Baudrit JR. Silver Nanoparticles: Technological Advances, Societal Impacts and Metrological Challenges. Frontiers in Chemistry. 2017;5(6).
4. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications and Therapeutic Approaches. Int. J of Mol. Sci. 2016;17(9):1534.
5. Sangiliyandi G, Jegadeesh R, Sri Nurestri AM, Priscilla AJ, Sabaratnam V. Green synthesis of silver nanoparticles using Ganoderma neo-japonicum Imazeki: a

potential cytotoxic agent against breast cancer cells. Int. J of Nanomedicine. 2013;8:4399-413.

6. Jang SJ, Yang IJ, Tettey CO, Kim KM, Shin HM. *In vitro* anticancer activity of green synthesized silver nanoparticles on MCF-7 human breast cancer cells. Materials Science and Engineering C. 2016;68:430-5.
7. Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, *et al.* green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. Asperellum*. Nanotechnology. 2008;19(7):075103.
8. Basanagowda MP, Ashok AH. Green Synthesis of Silver Nanoparticles by *Duranta repens* leaves and their antimicrobial efficacy. Nano Trends: A Journal of Nanotechnology and Its Application. 2013;14(1):13-18.
9. Rajesh S, Raja DP, Rathi JM, Sahayaraj K. Biosynthesis of silver nanoparticles using *Ulva fasciata* (Delile) ethyl acetate extract and its activity against *Xanthomonas campestris* pv. *Malvacearum. J*. Biopest. 2012;5:119-28.
10. Verma VC, Kharwar RN, Gange AC,'Biosynthesis of antimicrobial silver nanoparticles by endophytic fungus *Aspergillus clavatus*. Biomedicine. 2010;5(1):33-40.
11. Kumar V, Yadav SK. 'Plant-mediated synthesis of silver and gold nanoparticles and their applications. J. Chem. Technol. Biotechnol. 2009;84:151-7.
12. Satyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. 'Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. Adv. Sci. Lett. 2010;3:1-6.
13. Daniel MC, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties and applications toward biology, catalysis and nanotechnology. Chem Rev. 2004;104:293-346.
14. Van der Heijden, RD I Jacobs, Snoeijer W, Hallard D, Verpoorte R. The *Catharanthus* alkaloids: Pharmacognosy and biotechnology. Curr. Med. Chem. 2004;11:1241-53.
15. Moudi M, Go R, Seok Yien CY, Nazre M. Vinca alkaloids. *Int J Prev Med*. 2013;4:1231-5.
16. Khan I, Saeed K, Khan I. Nanoparticles: properties, applications and toxicities. Arab J Chem. 2019;12(7):908-31.
17. Halliwell B, Gutteridge JMC. (1989) Free Radicals in Biology and Medicine. Clarendon Press, Oxford, 2nd edn., 416-9.
18. Arjun HB, Yasuhiro T, Ketut AI, Kiyoshi M, Katsumichi M, Dejair M, Alfredo AGH, Shigetoshi K. *J.* Ethnopharmacol. 2000:72; 239-46.
19. Rajakumar DV. Biochemical and Pharmacological studies on the antioxidant properties of Dehydrozingerone and its analogs. Pharmazie.1994; 49(7):516-19.

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