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Review IJNB

Biosynthesis of Silver Nanoparticles Using Zingiber Officinalae and Evaluation of Cervical Cancer Activity

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Abstract

This research focused on evaluating the potential anti-cervical cancer properties of silver nanoparticles (AgNPs) that were synthesized using an aqueous extract derived from the rhizome of Zingiber officinale (ginger). The synthesized AgNPs were thoroughly characterized using a range of analytical techniques, including Ultraviolet-Visible (UV-Vis) spectroscopy, Fourier transform infrared spectroscopy(FTIR), and field emission scanning electron microscopy (FESEM). Characterization studies revealed that the synthesized AgNPs exhibited an average size range of 85–104 nm, with predominantly round and linear morphologies, dispersed in a small, scattered arrangement. Furthermore, the presence of antioxidant compounds was found using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (2,2-diphenyl-1-picrylhydrazyl) and the total antioxidant test. The DPPH result was found to be 74.89% and the total antioxidant test gives 38.2 mg/g, which provides the substantial anticancer activity of the plant extract. The cytotoxic potential of the synthesized AgNPs was evaluated against cervical cancer (HeLa) cell line using MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay] shows more apoptosis at high concentration. The biosynthesized AgNPs demonstrated cytotoxic effects in HeLa cells, indicating the anticancer potential of ZO-AgNPs. These findings suggest their possible application in the treatment of HeLa.

Keywords: Silver nanoparticles, Zingiber officinale, biosynthesis, cervical cancer, apoptosis

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INTRODUCTION

Noble metal nanoparticles have garnered significant scientific interest due to their diverse applications across biology, material science, and medicine [1]. Among them, silver nanoparticles (AgNPs) are particularly notable for their exceptional physicochemical properties – such as chemical stability and electrical conductivity – and their broad spectrum of biological activities, including antibacterial, antifungal, anti-inflammatory, antiviral, antiangiogenic, anticancer, and antiplatelet effects [2–5]. Beyond biomedical applications, AgNPs are utilized in consumer products, such as textiles [6], room sprays, detergents, wall paint formulations [7, 8], sunscreens, and cosmetics [9]. Remarkably, AgNPs have demonstrated inhibitory activity against the binding of HIV-1 to host cells under *in vitro* conditions [10]. Although traditional nanoparticle synthesis methods – such as UV irradiation, laser ablation, lithography, aerosol techniques, and photochemical reduction – are

well-established [11–13], recent research has shifted toward more environmentally friendly alternatives. Green synthesis methods using biological systems, such as bacteria [14], yeast [15], fungi [16], and plants [17] are gaining increased attention due to their nontoxic, cost-effective, and sustainable characteristics. Of these, plant-based synthesis is particularly noteworthy for its scalability, low biohazard risk, and the absence of the complex maintenance requirements typically associated with microbial culture systems [18]. Cancer remains a leading cause of death worldwide [19], and according to WHO projections, annual cases are expected to rise from 14 million in 2012 to 22 million within two decades. This trend underscores the urgency of developing effective antineoplastic agents. Natural products remain a valuable and diverse source for novel drug discovery, consistently providing promising lead compounds for a wide range of therapeutic applications [20]. Zingiber officinale (ginger), a flowering plant native to the Indo-Malayan region, is well-documented for its antimicrobial, anticancer, and antioxidant properties. In this study, we investigated the anticancer potential of AgNPs biosynthesized using Zingiber officinale extract against human cervical cancer (HeLa) cells.

MATERIALS AND METHODS

Materials, Reagents, and Instrumentation

Zingiber officinale (ginger) rhizomes were collected from the Coimbatore district of Tamil Nadu, India, and authenticated prior to use. All reagents and chemicals employed in the study were of analytical grade, sourced from Sigma (USA) and S.D. Fine Chemicals (Chennai, India). Key equipment included an LT Labtronics microprocessor-based spectrophotometer, a Shimadzu FTIR spectrometer (Model 100l), and a Robonik ELISA reader. HeLa cell lines were provided by the National Centre for Cell Science, Pune, India, and maintained at the Centre for Bioscience and Nanoscience Research Laboratory, Eachanari, Coimbatore, Tamil Nadu.

Preparation of Plant Extract

Fresh rhizomes of Zingiber officinale were collected and thoroughly washed to remove impurities. A sample of 25 g was weighed, crushed, and mixed with 50 mL of distilled water (refer to Figure 1). The mixture was incubated at 40 °C for 24 hours in a shaking incubator operating at 60–70 rpm. Following incubation, the extract was filtered using Whatman No. 1 filter paper. The resulting filtrate was covered with aluminum foil to shield it from sunlight and environmental contaminants and stored at room temperature for subsequent analysis.

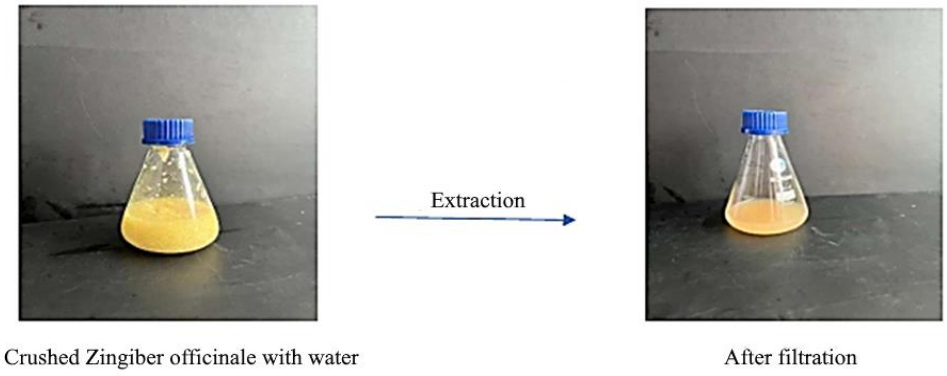


Figure 1. Preparation of plant extract.

Phytochemical Analysis of Plant Extract Alkaloids

- *Alkaloids:* Few milliliters of the plant extract were treated with Wagner's reagent was gently introduced along the inner walls of the test tube, and the formation of a reddish-brown precipitate confirmed the presence of alkaloids.
- *Flavonoids:* A 0.5 g sample of ginger was combined with water in a test tube and thoroughly shaken. Upon the addition of sodium hydroxide, an intense yellow coloration appeared, which

turned colorless with the subsequent addition of diluted hydrochloric acid, indicating the presence of flavonoids.

- *Tannins*: An amount of 0.5 g of ginger powder was combined with 20 mL of water and heated. After filtration, the mixture was treated with a 0.1% ferric chloride solution. The formation of a brownish-green coloration indicated the presence of tannins.
- *Saponins:* To test for saponins, 0.5 g of ginger were combined with water and gently heated. A few drops of olive oil were then added to the mixture, which was shaken vigorously. The appearance of a stable emulsion confirmed the presence of saponins.
- *Glycosides:* A 100 mg sample of the extract was dissolved in 1 mL of glacial acetic acid with a drop of ferric chloride. This mixture was then gently layered beneath with 1 mL of concentrated sulfuric acid. The appearance of a distinct brown ring at the junction of the two layers signified the presence of deoxy sugars, which are indicative of cardenolides.
- *Steroids:* To test steroidal compounds, 0.5 g of the sample was dissolved in 2 mL of chloroform and carefully treated with concentrated sulfuric acid. The emergence of a reddish-brown color at the interface between the two layers confirmed the presence of steroidal constituents.
- *Phenols:* To identify phenolic compounds, 50 mg of the extract was dissolved in 5 mL of distilled water. Then, 2 mL of a 1% gelatin solution containing 10% sodium chloride was added. The appearance of a white precipitate confirmed the presence of phenolic constituents in the sample.
- *Oxalates:* A 5 mL portion of the extract was mixed with 1 mL of concentrated sulfuric acid and left to stand undisturbed for 1 hour. Subsequently, two drops of potassium permanganate were introduced into the mixture. The formation of a persistent red color confirmed the presence of oxalates.

ANTIOXIDANT ANALYSIS

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

A 0.004% (w/v) DPPH solution was prepared in methanol. Sample and standard solutions were also prepared using methanol, with concentrations of 1 mg/mL for the test sample and 0.05 g/mL for ascorbic acid (standard). For the assay, 500 µL of the sample was mixed with 1 mL of the DPPH solution and 0.4 mL of 50 mM Tris-HCl buffer. The sample was kept in the absence of light for 30 minutes to prevent photodegradation during incubation. The absorbance was measured at 517 nm using a spectrophotometer, with methanol used as the blank for calibration. Antioxidant activity was expressed in mg/g, using ascorbic acid as the standard reference compound.

Total Antioxidant Activity

A 0.5 mL aliquot of the sample was combined with 0.5 mL of a reagent mixture composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The solution was incubated at 50°C for 90 minutes alongside a blank. The samples were cooled to room temperature, and the absorbance was measured using a spectrophotometer at 695 nm. Antioxidant capacity was expressed in mg/g, with ascorbic acid as the reference standard.

Preparation of AgNP

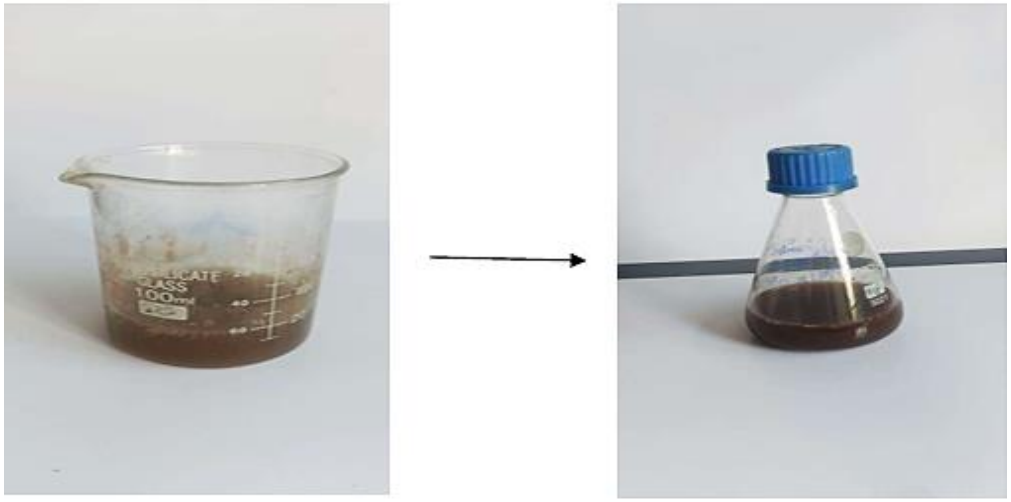
Add 1.698 g of silver nitrate ($AgNO_3$) to 20 mL of distilled water and continuously stirred with a mechanical stirrer. The plant extract was divided into control (8 mL) and sample (20 mL), and then 20 mL of the sample was added slowly to $AgNO_3$ and kept in a dark place for 48 hrs. The green color solution (Ag+) was turned into reddish brown color (AgO), which indicates the presence of nanoparticle. Then subjected to ultraviolet (UV)-Visible spectrophotometric analysis. The prepared AgNPs were analyzed by FTIR and SEM methods. The preparation of nanoparticles was shown in Figure 2.

Characterization of AgNP

- *UV-Vis Spectroscopy Analysis:* The 1 mM AgNPs solutions the color changes of filtrate after adding precursor ($AgNO_3$) was monitored by visual observation, and the absorbance at

wavelength 200–600 nm to detect the maximum surface plasmon resonance (SPR). When AgNPs are exposed to light, the electrons on their surface collectively oscillate, leading to the absorption of light at specific wavelengths, which is the SPR band. The presence and intensity of this peak are strong indicators of the successful formation of AgNPs. The wavelength and shape of the SPR band provide important information about the size and structural features of the nanoparticles.

- *Fourier Transform Infrared Spectroscopy (FTIR):* FTIR spectroscopy was used for the characterization studies of synthesized AgNPs at the range of 400–4000 cm^{-1}. It helps identify functional groups on the surface of AgNPs. Studying the interactions between silica dioxide and zinc oxide nanoparticles with surfactants. Characterizing the toxicity of different nanoparticles on microorganisms and studying the physisorption of molecules at the surface of nanomaterials.
- *Scanning Electron Microscopy (SEM):* Field Emission Scanning Electron Microscopy (FESEM) was employed to examine the shape and surface morphology of the synthesized AgNPs. Under ambient conditions, the sample was mounted on a double sided conductive affixed to a sample holder. The samples were then analyzed using the MIRA 3 TESCAN scanning electron microscope, operated at an accelerating voltage of 15.0 kV.



Before adding ($AgNO_3$) After adding ($AgNO_3$)

Figure 2. Preparation of silver nanoparticles.

Cytotoxicity by MTT Assay

The HeLa cell line was subcultured in RPMI medium, supplemented with sodium carbonate, glucose, and 10% bovine serum albumin (BSA) to support cell viability. Following the addition of all components into T-flasks, cells were incubated in a CO_2 incubator set to a temperature of 37°C, pH 7.0–7.5, and relative humidity of 70–80% for 24–72 hours. Cell growth was confirmed by observation under an inverted microscope, and the cultured cells were used for further experimentation.

For the MTT assay, cells were seeded into 96-well plates and allowed to adhere for 24 hours at 37°C in a 5% CO_2 environment with 70–80% humidity. Cells were then treated with varying concentrations of the test sample, alongside a blank control (DMSO), an untreated control (cell line alone), and standard reference drug (Doxorubicin at 12.5 µg). After 24 hours of incubation, wells were rinsed with DMSO, followed by trypsin treatment. Following this, 20 µL of MTT reagent was dispensed into each well, and the plates were gently agitated before being incubated for an additional 24 hours at 37°C in a CO_2-controlled environment. Post-incubation, the reaction mixture was carefully removed, and formazan crystals formed by viable cells were solubilized by adding 100 µL of DMSO to each well and mixing thoroughly. After ensuring complete dissolution, the absorbance was recorded at 570 nm using a 96-well ELISA plate reader. The cell viability percentage was then determined using the following formula:

Cell Viability (%) = (Absorbance of Sample / Absorbance of Control) × 100

RESULTS & DISCUSSION

Phytochemical Analysis

The phytochemical analysis of Zingiber officinalae shows Alkaloids, flavonoids, Tannins, Saponins, Glycosides, Steroids, Phenols, and Oxalate with a strong positive result. This is shown in Table 1 and Figure 3.

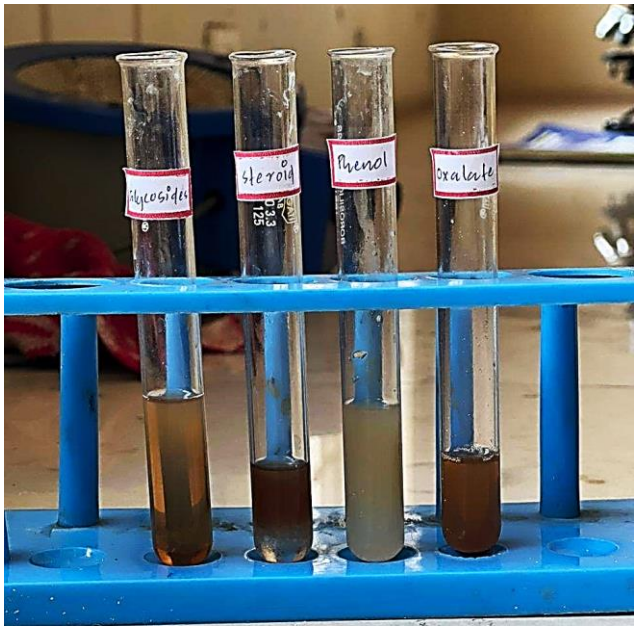


Figure3. Picturization for phytochemical analysis.

Table 1. Phytochemical analysis of Zingiber Officinale rhizome extract.

S.N.	Test	Observation	Result
1	Alkaloids	Formation of reddish-brown precipitate.	+
2	Flavonoids	Formation of colorless solution.	+
3	Tannins	Formation of brownish- green coloration.	+
4	Saponins	Formation of soluble emulsion.	+
5	Glycosides	Formation of brown ring at interface	+
6	Steroids	Formation of reddish- brown color at the interface.	+
7	Phenols	Formation of white precipitate	+
8	Oxalate	Formation of steady red color.	+

DPPH (2,2-diphenyl-1-picrylhydrazyl)

The antioxidant activity of Zingiber officinalae was found to be 74.89%. This is shown in Figure 4.

Total Antioxidant Test

The total antioxidant was found to be = 382 mg/g. This is shown in Figure 5.

UV-Visible Spectroscopy Analysis

λ max of Zingiber officinale extract scanned at a range of 200–600 nm and absorbance at 446 nm. This is shown in Figure 6.

Fourier Transform Infrared Spectroscopy (FTIR)

The functional group present in nanoparticles of Zingiber officinalae are carbon bromide, carbon chloride, amide, nitrogenoxide, aromatic, alkene, and carboxylic acid with strong variable and weak intensity. This is shown in Table 2 and Figure 7.

Scanning Electron Microscopy (SEM)

The SEM micrograph of synthesized AgNPs was magnified in 20 um and 16.5 mm wide at an accelerated voltage of 10kV and the particles were round and linear-shaped with an average diameter of 85–104 nm. The SEM image shows a scattered structure, which is shown in Figure 8.

Anticancer Activity Against HeLa

In control, there is a presence of more cell viability. In low concentration (2 ug), the cell viability has been reduced (low apoptosis occurs). In high concentration (10 ug), the apoptosis occurs than low concentration. Based on comparing the absorbance values of treated samples to control samples, in high concentration (10 ug) occurs more than apoptosis than low concentration. This is shown in Table 3 and Figures 9 and 10.

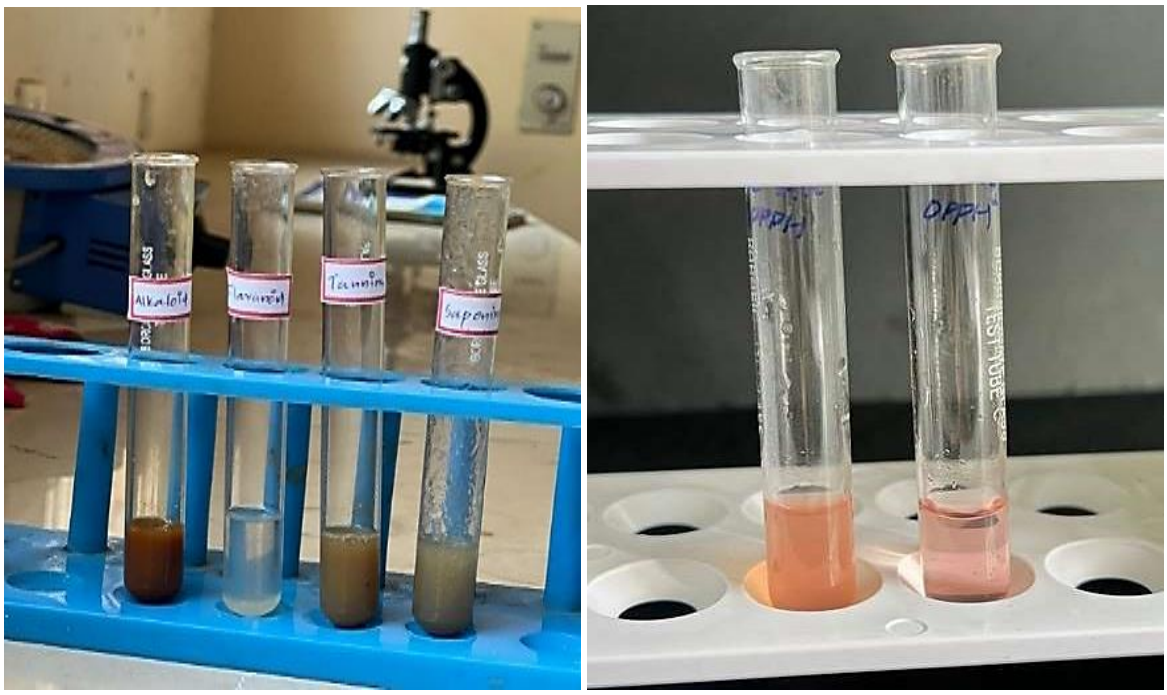


Figure 4. DPPH assay for antioxidant test.

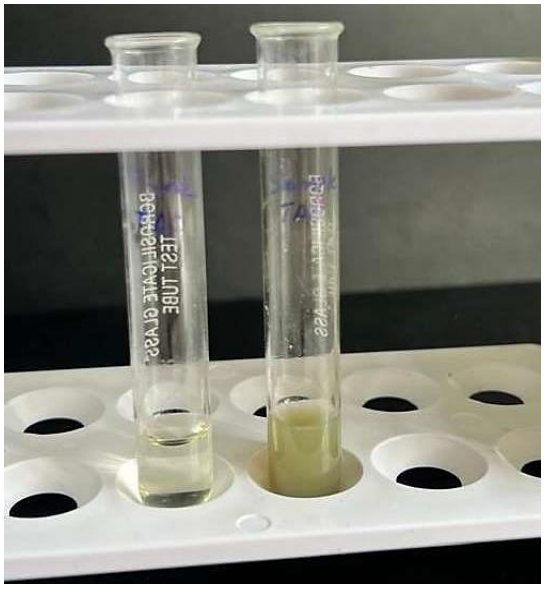


Figure 5. Total antioxidant test.

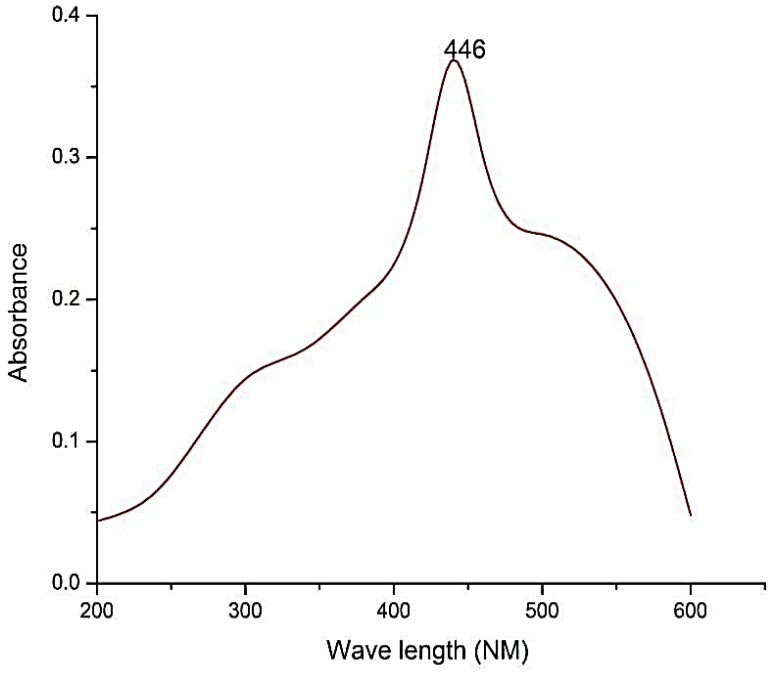


Figure 6. UV-visible spectrophotometric analysis.

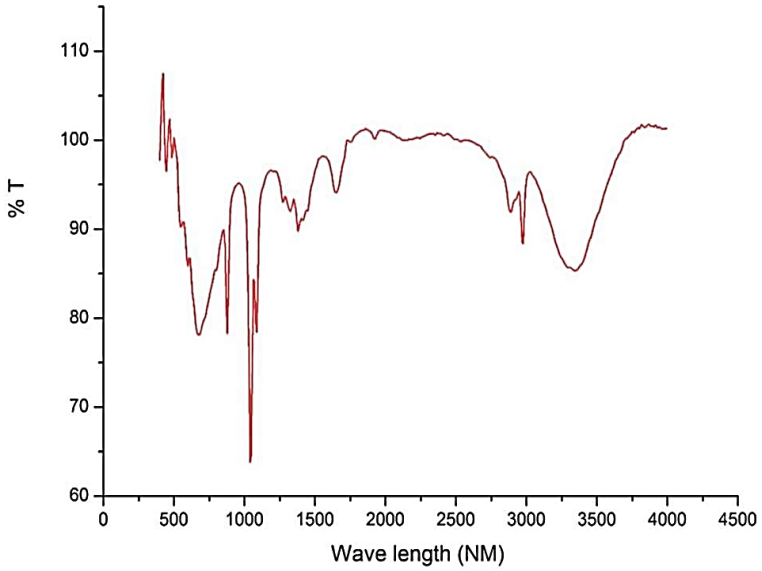


Figure 7. Fourier transform infrared spectroscopy (FTIR).

Table 2. Functional group of FTIR analysis.

Frequency (cm-1)	Functional Group	Intensity
564.2	C–Br	Strong
682.4	C–Cl	Strong
1142.46	C=O amide	Strong
1261.2	NO_2 stretch	Variable
1636.10	C≡C aromatic	Strong
2149.46	C≡O stretch	Weak
1684.31	C=C alkene	Weak
3149.94	–COOH	Strong

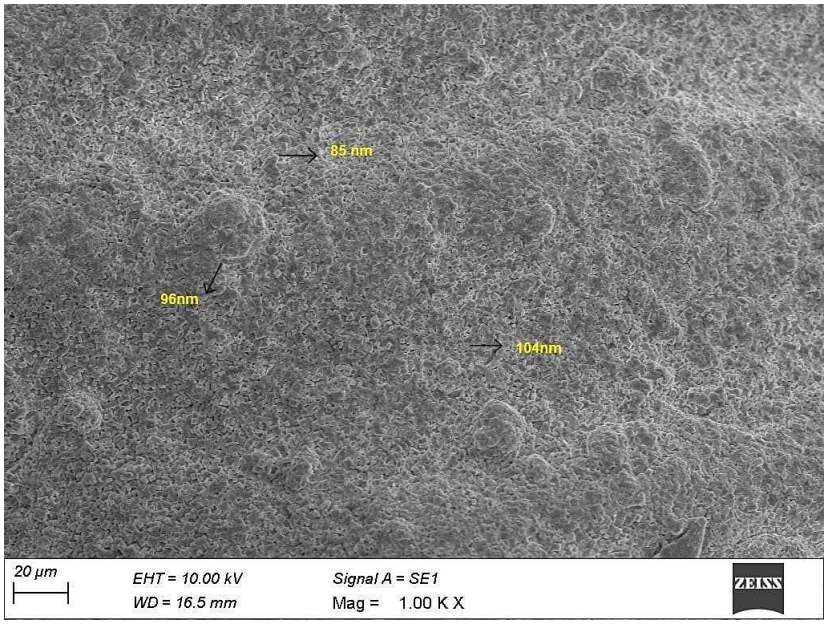


Figure 8. Scanning electron microscopy.

Table 3. Percentage of cell death.

Concentration of Sample(µg)	Of Reading	% of Cell Death
Control	0.774	0 %
2	0.639	17.44 %
4	0.586	24.28 %
6	0.507	34.49 %
8	0.461	40.43 %
10	0.380	50.90 %

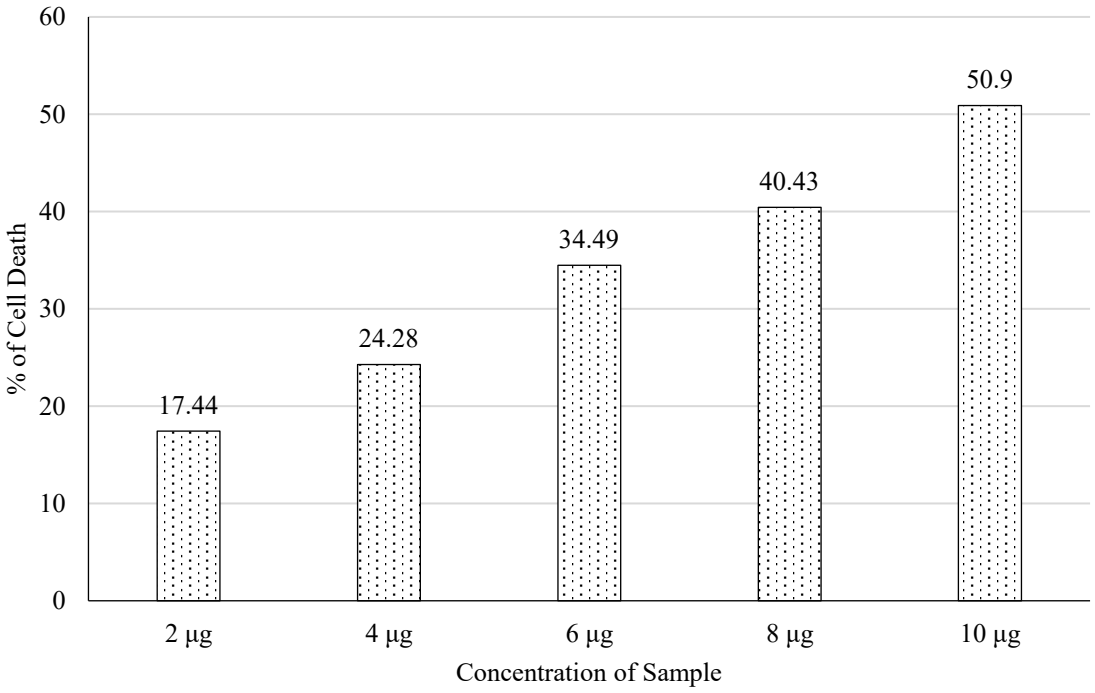


Figure 9. Anticancer activity.

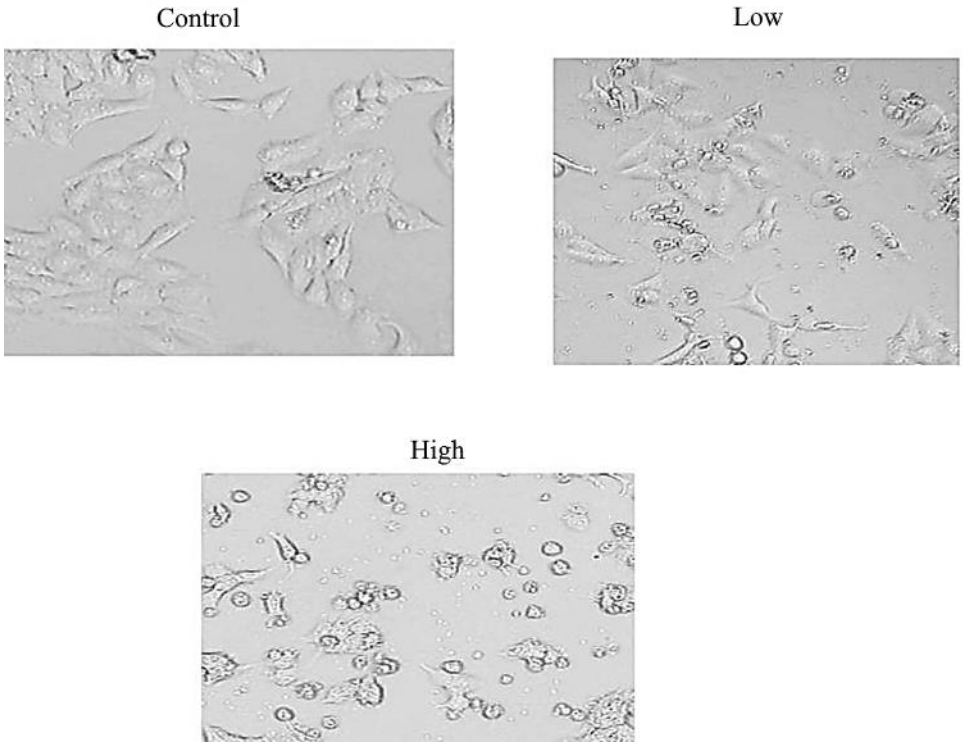


Figure 10. Cell death of HeLa cells.

CONCLUSIONS

The primary objective of this study was to assess the *in vitro* anti-HeLa potential of AgNPs synthesized using an aqueous extract of *Zingiber officinale* (ginger).

Zingiber officinale aqueous extract was found to contain alkaloids, flavonoids, phenol, saponins, tannins, steroids, glycosides, and oxalates. The presence of antioxidant compounds by using DPPH was found to be 74.89% and total antioxidant test was found to be 382 mg/g, provide the substantial anticancer activity for the plant extract, and this made the plant to be successfully used for the synthesis of AgNPs from $AgNO_3$. The color changes from green to reddish brown appeared after incubation indicates the presence of ginger AgNPs. The formation of AgNPs is confirmed by UV-Vis. UV-Visible spectroscopy for Zingiber officinale showed absorption maxima at 446 nm. AgNPs were characterized using FTIR and SEM. *In vitro* anticancer activity of Zingiber officinale extract by using Hella cell line (MTT assay) for HeLa shows good result. AgNPs synthesized via a green approach using Zingiber officinale demonstrated significant anticancer activity against HeLa cells.

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