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Phytosynthesis Of Silver Nanoparticles From *Dimocarpus Longan* Lour. Fruit Pulp Extract And Study Of Their Biomedical Potentials: Antibacterial, Antioxidant And Anticancer

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Abstract

The synthesis of silver nanoparticles (AgNPs) from *D. longan* Lour. fruit pulp extract was successfully achieved, as confirmed by UV-visible spectroscopy (max absorbance at 289 nm), DLS (size of < 100 nm and about 250 nm), and Zeta potential (highly stable with -11.8 mV) analysis. The ABTS, DPPH, and metal chelating analyses demonstrated that PhS-AgNPs, in their native state, exhibit substantial antioxidant activities. Thus, PhS-AgNPs can be utilized as antioxidants to alleviate oxidative stress. PhS-AgNPs exhibited substantial antibacterial efficacy against bacterial pathogens (superior active against Gram-ve related to Gram+ve bacteria), rendering them very advantageous in the treatment of bacterial-mediated illnesses and valuable in biological domains. The results of the MTT and morphological investigation indicate that PhS-AgNPs exhibit potent anticancer capabilities on human breast cancer cells (MDA-MB-231) and have significant potential as a cancer therapy in the field of biomedicine. However, it is crucial to do a comprehensive analysis of the anticancer mechanism and any safety risks before concluding that PhS-AgNPs can be used as an effective drug for cancer treatment.

Key words: *Dimocarpus longan* Lour. fruit, Silver nanoparticles, Antioxidant activity, Antibacterial activity, MTT assay, Anticancer activity.

1. Introduction

Nanotechnology, a field of study involving materials with dimensions ranging from 1 to 100 nm, has gained significant interest in various fields such as science, electronics, and environmental cleaning. Physicist Richard Feynman introduced the term "nanotechnology" in 1959. Nanoparticles of important metals, including palladium, mercury, nickel, copper, silver, platinum, gold, and cobalt have significant and potential roles in multiple fields of physics, chemistry, and biology. Nanoparticles have numerous applications in cancer therapy, cell labeling, drug delivery, wastewater remediation, antimicrobial agents, diagnostics, cosmetics, biomarkers, cell biology, ointments, chemical sensing, food industry, antioxidants, imaging, catalysis, anti-inflammatory agents, and wound healing (Silva, 2004).

Silver nanoparticles (AgNPs) are versatile nanoparticles with enhanced biological, physical, and chemical properties, making them widely used in medical fields and healthcare. They have greater biocompatibility compared to traditional pharmaceuticals, enhancing targeted distribution and effectiveness. AgNPs also possess antibacterial, antiviral, cancer prevention, thrombolytic, and anticoagulation activities, potentially showing anti-diabetic properties (Shaikh et al., 2021).

Nanoparticles can be synthesized using natural and chemical/physical resources. Their biocompatibility makes them ideal for biomedical applications. Especially, biogenic nanoparticles are free from hazardous by-products and offer numerous benefits, including efficient, environmentally friendly manufacturing techniques, economic feasibility, and compatibility with live organisms. In this scenario, plants' extracts/components serve as capping and stabilizing agents and are highly biocompatible (Chopra et al., 2022).

The present study explores the role of *Dimocarpus longan* Lour. fruit pulp extract in stabilizing and reducing the formation of phytosynthesis silver nanoparticles (PhS-AgNPs), revealing their antioxidant, antibacterial, and anticancer properties.

2. Materials and methods

2.1 Chemicals and reagents

The chemicals used in the study were sourced from HiMedia, and Sigma-Aldrich, Bengaluru, India.

2.2 Collection of *Dimocarpus longan* Lour. fruits

The *Dimocarpus longan* Lour. fruits were collected from the Western Ghats, Tirupathi, India. The pulp was cleaned, dried, pulverized, and macerated (Dharajiya et al., 2017). The extract was passed through filter paper and preserved in vacuum-sealed bags. The fruit pulp extract was then used for the phytosynthesis of silver nanoparticles (PhS-AgNPs).

2.3 Phytosynthesis and characterization of silver nanoparticles

The process of reducing silver ions into AgNPs using *D. longan* Lour fruit pulp extract was carried out in an aqueous solution as per the methodology of Ramesh et al., (2015). The formation of PhS-AgNPs is monitored for color changes and analyzed using a UV-Vis spectrophotometer. Following, PhS-AgNPs are recovered using centrifugation. The obtained pellet is washed with deionized water, freeze-dried, and powdered. The as-synthesized PhS-AgNPs were characterized by UV-vis spectroscopy, dynamic light scattering, zeta potential, and scanning electron microscope analysis.

2.4 Antioxidant potential of phytosynthesized silver nanoparticles

The antioxidant potential of PhS-AgNPs was assessed by ABTS and DPPH assay as per the methodology of Das et al., (2023) and Thanh et al., (2022).

2.5 Antimicrobial activity of phytosynthesized silver nanoparticles

The agar disk diffusion experiment demonstrated the antibacterial activity of PhS-AgNPs, indicating their effectiveness against bacteria, which is carried out as per the methodology of Ydollahi et al., (2016). The broth-dilution approach was used to demonstrate the precise antibacterial activity of PhS-AgNPs as per the methodology of Espinel-Ingroff et al., (2009).

2.6 Anticancer activity of phytosynthesized silver nanoparticles

The anticancer effect of PhS-AgNPs on human breast cancer (MDA-MB-231 cells) was carried out by MTT and morphological observation as per the methodology of Tomankova et al., (2014) and Dessard et al., (2024).

2.7 Statistical analysis

Three replicates of each experiment were conducted (n = 3). The experimental data was represented using the mean ± standard deviation (SD). The data were analyzed for statistical significance using Analysis of Variance. The findings were deemed statistically significant when the p-value was less than or equal to 0.05.

3. Results and discussion

3.1 Phytosynthesis and characterization of silver nanoparticles

In our study, the synthesis of AgNPs was monitored using color change and UV-Vis spectroscopy. The color of the reaction mixture changed within 12 minutes, turning yellowish brown, and then reddish brown after 50 minutes, due to the reduction of silver metal ions (Ag^{+}) into AgNPs (Ag°) by the active molecules present in the extract of *D. longan* Lour. fruit pulp extract. The color is believed to be caused by SPR excitation. After that phytosynthesized AgNPs (PhS-AgNPs) were subjected to centrifugation at 14,500 rpm for a duration of 45 minutes, followed by three rinses with deionized water. The phytosynthesized AgNPs (PhS-AgNPs) pellet was freeze-dried using a lyophilizer. The PhS-AgNPs were subsequently desiccated and pulverized into a fine powder using a mortar and pestle.

In the present study, the initial validation of PhS-AgNPs synthesis was established using UV-visible spectroscopy, which utilized a theory of plasmon resonance. An absorption peak at 289 nm was observed (**Fig. 1**). In support of our result, the AgNPs produced from the aqueous leaf extract of *Azadirachta indica* exhibited a UV-Vis peak within the wavelength range of 436-446 nm (Ahmed et al., 2016). The UV–vis spectra of produced AgNPs from carob leaf extract showed SPR peak at 420 nm (Awwad et al., 2013).

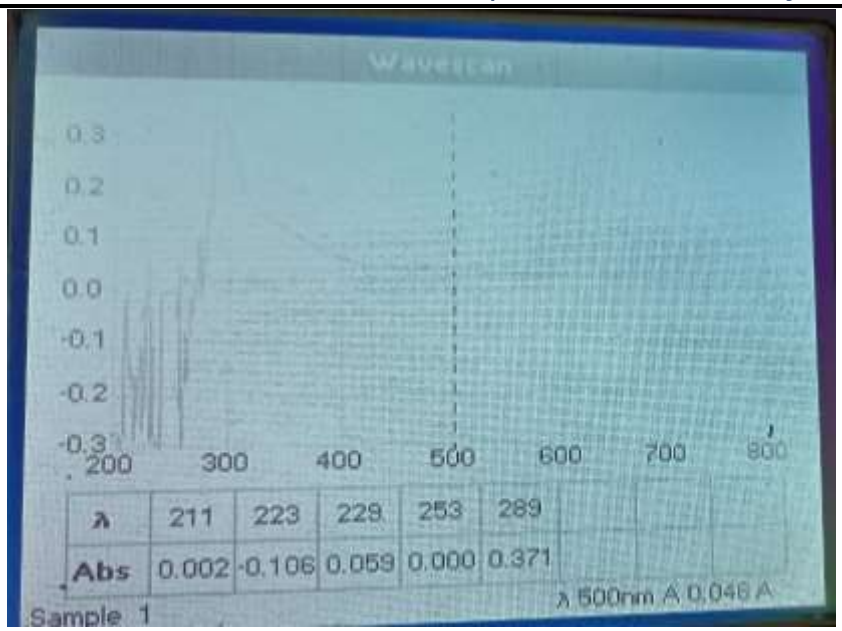

Figure 1: UV-visible spectrum of PhS-AgNPs

The particle sizes of the produced PhS-AgNPs were verified using DLS. The synthesized PhS-AgNPs were found to be in the nanoscale range, with an average size of < 100 nm and about 250 nm (**Fig. 2**).

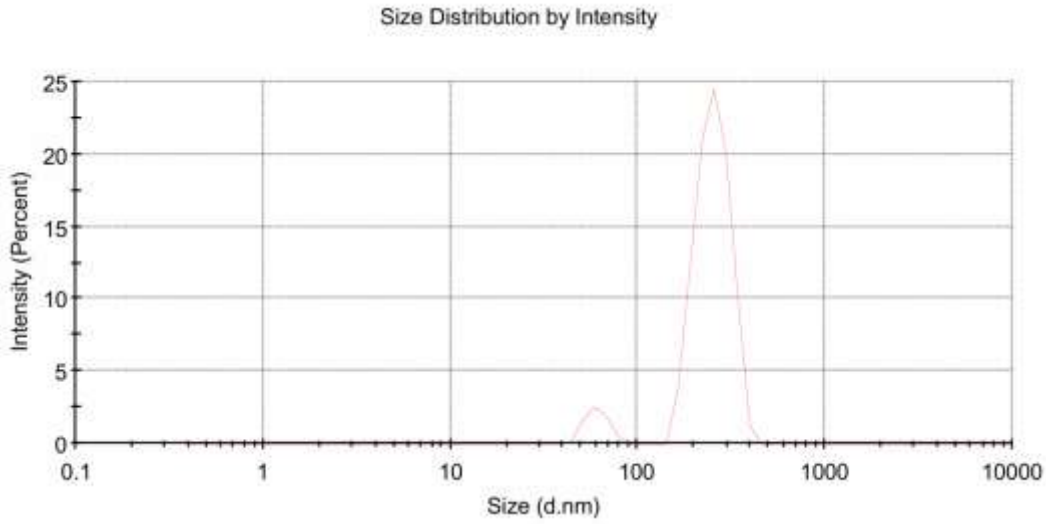

Figure 2: DLS spectrum of PhS-AgNPs

PhS-AgNPs possess a negative charge and displayed a zeta potential of -11.8 mV, as shown in **Figure 3**. The presence of polyphenols in the VC fruit extract is likely responsible for the negative charge observed on PhS-AgNPs. The dispersion form of PhS-AgNPs is facilitated by the presence of negative electrostatic forces. This negative charge could be due to plant molecules presence around PhS-AgNPs. In support of our study, negative value suggested that the nanoparticles were appropriate and prevented aggregation (Ashraf et al., 2019). The AgNPs synthesized using *Eucalyptus citriodora* and *Melaleuca cajuputi* possessed zeta potentials of -36.49 and -31.16 mV, respectively.

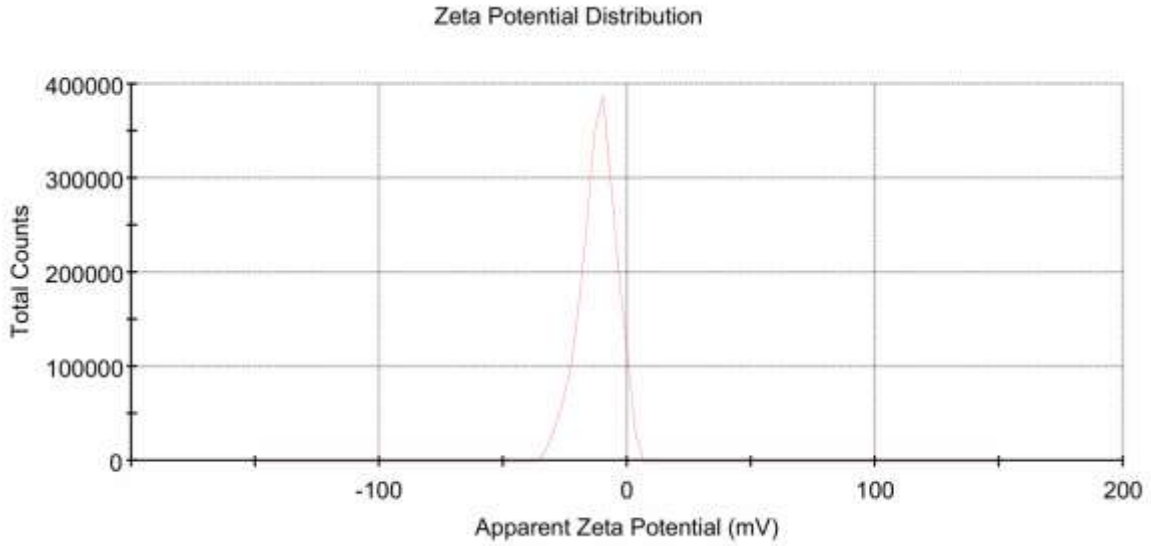

Figure 3: Zeta potential of PhS-AgNPs

3.2 Antioxidant potential of phytosynthesized silver nanoparticles

In the ABTS assay, the antioxidant potential of PhS-AgNPs was found to be potential and have demonstrated a capacity to scavenge ABTS free radicals that is dependent on the dosage, indicating their antioxidant potential (**Fig. 4**). The EC_{50} values, which represent the concentration needed to block 50% of free radicals, were determined to be 71.04 ± 2.34 µg/mL.

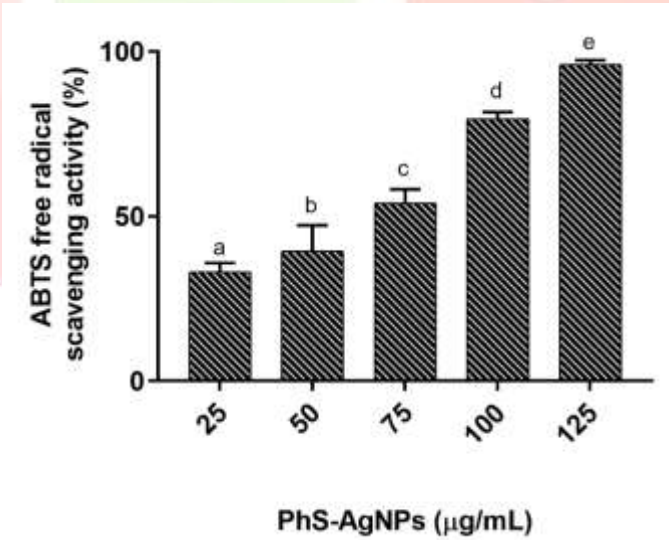

Figure 4: Antioxidant potential of PhS-AgNPs was assessed using ABTS free radical scavenging assay in a dose-dependent way. The analysis was conducted in triplicate (n = 3), and the results were reported as the mean value plus or minus the standard deviation. The statistical significance between the test samples was assessed using Tukey's test, and a *p*-value of ≤ 0.05 was deemed to be statistically significant. The bars

in the specific study group, labeled with distinct alphabets, represent statistically significant results (*p*-value ≤ 0.05).

In the DPPH study, PhS-AgNPs showed significant antioxidant activity in scavenging DPPH free radicals, similar to the ABTS free radical scavenging assay. The PhS-AgNPs have demonstrated a capacity to scavenge DPPH free radicals, with the level of effectiveness being depending on the dosage (**Fig. 5**). The EC_{50} value, which represents the concentration needed to block 50% of DPPH free radicals, was determined to be 67.91 ± 3.56 µg/mL. PhS-AgNPs produced in their original form possess significant antioxidant properties and can be employed as antioxidants to mitigate oxidative stress (Ravindran et al., 2013).

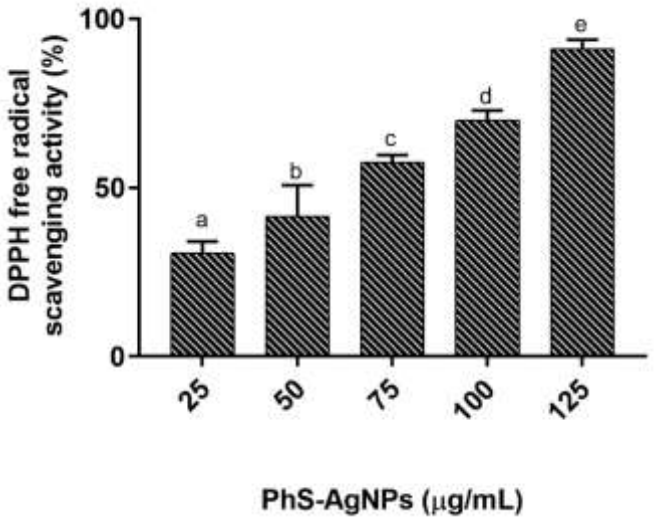

Figure 5: Antioxidant potential of PhS-AgNPs was assessed using DPPH free radical scavenging assay in a dose-dependent way. The analysis was conducted in triplicate (n = 3), and the results were reported as the mean value plus or minus the standard deviation. The statistical significance between the test samples was assessed using Tukey's test, and a *p*-value of ≤ 0.05 was deemed to be statistically significant. The bars in the specific study group, labeled with distinct alphabets, represent statistically significant results (*p*-value ≤ 0.05).

3.3 Antimicrobial potential of phytosynthesized silver nanoparticles

3.3.1 Agar disk-diffusion method

In the present study, the disk-diffusion method was employed to assess the antibacterial activity of PhS-AgNPs. The disk-diffusion method is commonly employed to evaluate the antimicrobial efficacy of nanoparticles, antibiotics, or plant extracts. The agar plate surface is inoculated using a method akin to the disk-diffusion technique, where a certain amount of the microbial inoculum is evenly spread over the entire agar surface. The disk-diffusion method is employed to evaluate the overall susceptibility of a certain concentration of an antimicrobial agent. This method relies on the principle that when an antimicrobial agent is placed on agar that has been previously inoculated with the test bacterial pathogen, the moisture is absorbed and the antimicrobial agent spreads outwardly across the agar medium, generating a gradient of antimicrobial concentration (Tendencia, 2004).

The current investigation examined the antibacterial effectiveness of produced PhS-AgNPs against a diverse array of bacterial pathogens, encompassing both Gram-negative and Gram-positive bacteria (**Table 1**). The PhS-AgNPs have demonstrated greater antibacterial efficacy against Gram-negative bacteria compared to Gram-positive bacteria. Gram-negative bacteria were treated with PhS-AgNPs at a concentration of 300 µg/disk displayed higher activity. The antimicrobial activity of the treatment was found to be highest against Gram-negative bacteria *Escherichia coli* – MTCC 1302, with a zone of inhibition measuring 21.56 ± 1.08 mm at 300 µg/disk. The lowest antimicrobial activity on Gram-negative bacteria was seen against *Pseudomonas aeruginosa* – MTCC 741, with a zone of inhibition measuring 19.34 ± 1.27 mm at 300 µg/disk.

In Gram-positive bacteria analysis, PhS-AgNPs have shown antimicrobial activity. *Staphylococcus aureus* - MTCC 740 when treated with PhS-AgNPs at a concentration of 300 µg/well, the highest zone of inhibition observed as 17.25 ± 1.34 mm. On the other hand, the lowest antimicrobial activity observed against Gram-positive bacteria was *Listeria monocytogenes* – MTCC 657, with a zone of inhibition measuring 14.80 ± 1.27 mm. The disk-diffusion test indicated that PhS-AgNPs exhibit strong antibacterial efficacy against a broad spectrum of bacterial infections. Succeeding, the micro-well dilution approach was used to quantify the exact dosage of PhS-AgNPs needed to limit the growth and eliminate the bacterial pathogens.

Table 1: The antibacterial efficacy of phytosynthesized silver nanoparticles against different bacteria was assessed using the disk-diffusion technique.

Bacterial pathogen	**Zone of inhibition (mm)**		
	100 µg of PhS-AgNPs	200 µg of PhS-AgNPs	300 µg of PhS-AgNPs
Gram-negative bacteria			
E. coli	14.29 ± 1.21	17.88 ± 1.59	21.56 ± 1.08
P. aeruginosa	12.79 ± 1.38	16.21 ± 1.81	19.34 ± 1.27
S. typhimurium	14.01 ± 1.07	16.93 ± 1.42	20.57 ± 1.28
Gram-positive bacteria			
S. aureus	12.58 ± 1.03	14.69 ± 0.92	17.25 ± 1.34
B. subtilis	11.88 ± 1.24	12.94 ± 1.16	15.94 ± 1.08
L. monocytogenes	10.32 ± 1.58	12.36 ± 1.74	14.80 ± 1.27

3.3.2 Broth-dilution method

In a broth-dilution assay, PhS-AgNPs have demonstrated greater antibacterial efficacy against Gram-negative bacteria than against Gram-positive bacteria (**Table 2**). The results of the agar-well diffusion assay and the micro-well dilution assay were determined to be similar.

Gram-negative bacteria have shown that PhS-AgNPs possess the highest level of antimicrobial activity. Bgn-SeNPs have demonstrated best MIC and MBC values against *Escherichia coli* – MTCC 1302 with 31.56 ± 4.81 and 48.73 ± 3.14 µg/mL, respectively. The antimicrobial activity of PhS-AgNPs against Gram-negative bacteria *Pseudomonas aeruginosa* – MTCC 741 was found to be the lowest. The MIC and MBC values for PhS-AgNPs against *Pseudomonas aeruginosa* – MTCC 741 were determined to be 37.92 ± 2.68 and 56.31 ± 2.85 µg/mL, respectively.

In Gram-positive bacteria, PhS-AgNPs had the highest level of antibiotic activity against *Bacillus subtilis* - MTCC 1133. The MIC and MBC values of PhS-AgNPs were measured at 39.63 ± 3.70 and 65.46 ± 5.71 µg/mL, respectively. The PhS-AgNPs exhibited the least antimicrobial efficacy against *Listeria monocytogenes* – MTCC 657, with measured MIC and MBC values of 43.30 ± 3.54 and 71.18 ± 4.09 µg/mL, respectively.

Table 2: The antibacterial efficacy of phytosynthesized silver nanoparticles against different bacteria was assessed using the broth-dilution technique.

Bacteria	**MIC (µg/mL) of PhS-AgNPs**	**MBC (µg/mL) of PhS-AgNPs**
Gram-negative bacteria		
E. coli	31.56 ± 4.81	48.73 ± 3.14
P. aeruginosa	37.92 ± 2.68	56.31 ± 2.85
S. typhimurium	36.83 ± 2.98	54.73 ± 3.06
Gram-positive bacteria		
S. aureus	41.56 ± 3.93	68.73 ± 4.96
B. subtilis	39.63 ± 3.70	65.46 ± 5.71
L. monocytogenes	43.30 ± 3.54	71.18 ± 4.09

In support of our report, AgNPs synthesized from plant extracts are widely recognized as highly effective antibacterial agents (Salayová et al., 2021). Despite great progress in understanding the antibacterial mechanism of silver nanoparticles, the specific mechanism of action remains incompletely understood. The continuous release of silver ions by silver nanoparticles can be regarded as the mechanism by which bacteria are killed. Due to electrostatic attraction and an affinity for sulfur proteins, silver ions have the ability to stick to the cell wall and cytoplasmic membrane. Attached ions can increase the permeability of the cytoplasmic membrane and cause damage to the bacterial envelope. Once free silver ions are absorbed by cells, they can inhibit respiratory enzymes, leading to the generation of reactive oxygen species and the interruption of adenosine triphosphate synthesis. Reactive oxygen species can play a significant role in causing cell membrane damage and altering the structure of DNA. Due to the significance of sulfur and phosphorus in DNA, the presence of silver ions can lead to issues in DNA replication, cell reproduction, or even the termination of microorganisms. Furthermore, silver ions have the ability to hinder the production of proteins by causing the ribosomes in the cytoplasm to undergo denaturation (More et al., 2023).

3.4 Anticancer activity

The anticancer effects of PhS-AgNPs on human breast cancer cells (MDA-MB-231) was assessed using the MTT assay and micro-morphological studies. It was found that the PhS-AgNPs impacted the viability of cancer cells. The study discovered that PhS-AgNPs had a dose-dependent growth suppression effect on human breast cancer cells (MDA-MB-231) (**Fig. 6**). The IC_{50} value, which represents the concentration needed to inhibit 50% of cell viability, was calculated for PhS-AgNPs in MDA-MB-231 cancer cells. The IC_{50} value was found to be 108.29 ± 2.78 µg/mL, respectively. The findings indicated that the PhS-AgNPs significantly inhibited the growth of cancer cells.

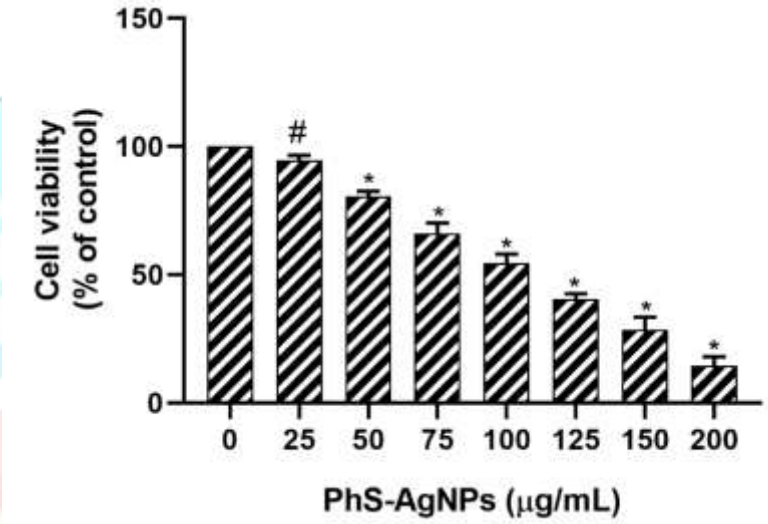

Figure 6: Dose-dependent anticancer activity of PhS-AgNPs on human breast cancer cells (MDA-MB-231). The analysis was conducted in triplicate (n = 3), and the results were reported as the mean value plus or minus the standard deviation. The statistical significance between the test samples was assessed using Dunnett's test, and a *p*-value of ≤ 0.05 was deemed to be statistically significant. The bars labeled with '*', represent statistically significant (*p*-value ≤ 0.05) related to control.

In the morphological analysis study, when comparing untreated cells (control) to cells treated with PhS-AgNPs, the IC_{50} value affected the micromorphology of cancer cells. The control cells exhibited a vigorous morphology in cancerous cells (**Fig. 7**). The cellular micromorphology of cells treated with PhS-AgNPs at its IC_{50} value (108.29 ± 2.78 µg/mL) exhibited detrimental changes, such as the loss of cell shape and structure, the presence of cellular debris, the formation of apoptotic bodies, and so on (shown in green arrows in the figure). The MTT and morphological study's findings suggest that PhS-AgNPs possess strong

anticancer properties and have great promise as a cancer therapy in the biomedical area (Ravindran et al., 2013).

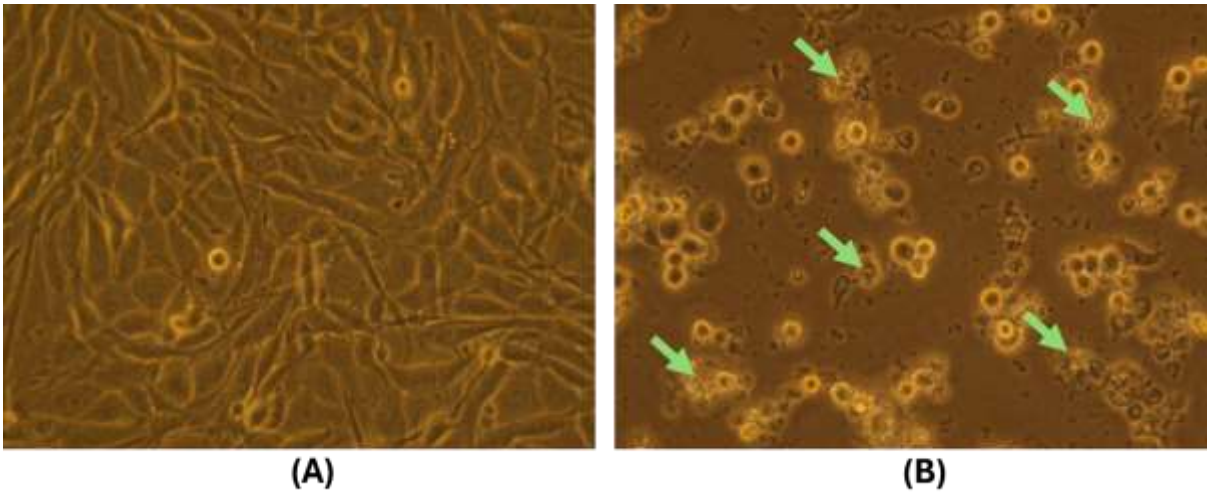

Figure 7: The morphology of human breast cancer cells (MDA-MB-231) was examined after treatment with the IC_{50} concentration of PhS-AgNPs. The photographs were recorded at a resolution of 400x. (A) Human breast cancer cells (MDA-MB-231) without treatment (control). (B) Human breast cancer cells (MDA-MB-231) were exposed to the IC_{50} of PhS-AgNPs (108.29 ± 2.78 µg/mL).

4. Conclusion

AgNPs were successfully synthesized from *D. longan* Lour. fruit pulp extract, which was confirmed by UV-visible spectroscopy, DLS, and Zeta potential analysis. ABTS, DPPH, and metal chelating analysis showed that PhS-AgNPs produced in their original form possess significant antioxidant properties and can be employed as antioxidants to mitigate oxidative stress. PhS-AgNPs demonstrated antibacterial effectiveness against various bacterial pathogens, making them highly beneficial in treating bacterial-mediated infections and beneficial in biomedical fields. The MTT and morphological study's findings suggest that PhS-AgNPs possess strong anticancer properties and have great promise as a cancer therapy in the biomedical area. Nevertheless, it is imperative to thoroughly analyze the anticancer mechanism and any safety hazards before determining PhS-AgNPs as a viable anticancer medication.

Conflict of Interest

The authors declare that there is no conflict of interest

Acknowledgment

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