Biogenic Mediated Green Synthesis Of Silver Nanoparticles: Assessment Of Antimicrobial And Cytotoxic Potential

A.Baskaran[1]*, S. Anbazhakan[2], S. Sivaranjani[3], K.Subasankari[4] and R. Ramya[5]

[1] Assistant Professor of Botany, Thanthai Hans Roever College (Autonomous), Perambalur, Tamil Nadu.
[2] Associate Professor of Botany, Alagappa Government Arts College, (Affiliated by Alagappa University), Karaikudi.
[3] Assistant Professor of Biotechnology, Bon secours college for women (Autonomous) Thanjavur.
[4] Assistant Professor of Biotechnology, School of Engineering and Technology, Dhanalakshmi Srinivasan University Samayapuram.
[5] PG & Research Department of Biotechnology, Thanthai Hans Roever College, Perambalur, Tamil Nadu.
*Corresponding Author: A.Baskaran

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Abstract - **The current study aimed to investigate the green synthesis of silver nanoparticles (AgNPs) using *Barleria longiflora* stems extracted with an aqueous solution. The synthesized AgNPs were characterized and evaluated for their antimicrobial activity and toxicity. *Barleria longiflora* stems were extracted with an aqueous solution to synthesize silver nanoparticles (AgNPs). The synthesis was confirmed by a distinct color shift from pale yellow to reddish-brown. The AgNPs were characterized using UV spectrophotometry, FTIR analysis, SEM analysis, and EDAX analysis. Antimicrobial activity of the ethanol extract of AgNPs was carried out by the Resazurin method against *Escherichia coli, Staphylococcus aureus, Candida albicans*, and *Aspergillus fumigates*.**

Keywords - ***Barleria longiflora,* AgNPs, FTIR, SEM and EDAX.**

1. INTRODUCTION

Nanotechnology is a magical concoction that affects every aspect of life, the economy, and science. Nanotechnology encompasses the engineering of materials at the molecular level, yielding innovative solutions in fields such as biomedical research, healthcare, and more [1]. Recently, nanotechnology has garnered significant attention due to the distinct characteristics of nanoparticles, which exhibit remarkable properties at the 1-100 nanometer scale [2]. To improve the use of nanoparticles in biomedicine, a great deal of work is being done to develop safe, non-toxic, reliable, and environmentally friendly techniques for producing and assembling nanoparticles with the right sizes and shapes [3]. A variety of nanomaterials are available, such as alginate, copper, zinc, silver, titanium, gold, and magnesium [4]. In contrast to other precious metal nanoparticles, silver nanoparticles (AgNPs) are the subject of much research on account of optical, antibacterial, and anticancer properties [5]., antioxidant, antidiabetic [6] and larvicidal properties, in addition to their affordability [5]. Although there are several ways to create nanoparticles, chemical methods are the most often used. However, the synthesis process of these methods usually calls for hazardous chemicals, potentially threatening human well-being and environmental safety [7]. These techniques are also associated with excessive energy usage and costly post-processing [8]. With a focus on protecting the environment and ecosystem, the green synthesis of nanoparticles is considered a more sustainable and cost-effective approach. An emerging field is the biosynthesis of nanoparticles tapping into nature's resources such bacteria, fungi, enzymes, algae, and plant extracts [9]. Plant-based nanoparticle extraction naturally streamlines the procedure, making it extremely effective and environmentally friendly [10]. Green chemistry-based nanomaterials made from medicinal plants have recently become a viable method of treating a number of illnesses [11]. According to the majority of research, the acanthaceae family contains unique phytochemical substances with important medical applications [12]. There are at least 4,000 species in the vast, primarily tropical family Acanthaceae [13]. With numerous species, *Barleria* is the third-biggest genus in the Acanthaceae family [14]. The little blooming shrub Barleria longiflora, also called Kattukanakambaram, grows to a height of 1-2 meters and is used extensively as a vital medicinal herb in traditional medicine [15]. Organic fabrication of silver nanoparticles utilizing stem extract from *Barleria longiflora*, thorough characterisation using a variety of methods, and evaluation of their antibacterial and anticancer qualities were the main objectives of this study.

2. MATERIALS AND METHODS

2.1. *Collection And Cold Extraction Of Plant Materials*

Barleria longiflora L.f, an Acanthaceae family member, was obtained from a collection site in Tiruchirappalli District, Tamil Nadu. A 10g stem sample was measured and immersed in 100ml of sterile distilled water. After a 24-hour incubation period, the extract was filtered using sterile filter paper. The filtrate was procured and employed for synthesis of silver nanoparticles.

2.2. *Synthesis Of AGNPS*

Silver are synthesized by adding 10 ml of aqueous extract to 90 ml of 3mM aqueous AgNO3 solution at normal temperature. A colorimetric indication of the reduction reaction was observed, with the solution changing from pale yellow to reddish brown, signifying the formation of Ag0 from Ag+ ions. The reddish-brown coloration of the solution indicated the preliminary formation of silver nanoparticles via plant-extract-mediated synthesis.

2.3. *UV–Visible Spectrophotometer*

UV-Visible spectroscopy was employed to monitor the spectral response of the synthesized AgNPs, with absorbance measurements taken on a Thermo Scientific Evolution 201 spectrophotometer across the 200-800 nm wavelength range. This method has diverse applications, including semiconductor manufacturing, materials science, energy resource analysis, and forensic science, where it is used to examine trace evidence and authenticate documents.

2.4. *Fourier Transform Infrared Spectroscopy (FTIR) Analysis*

The FTIR spectroscopy technique is based on the infrared region of the electromagnetic spectrum, where wavelengths are longer and frequencies are lower than those of visible light. This field comprises various techniques, mainly focused on absorption spectroscopy. The FTIR spectrum revealed the presence of biomolecules that played a crucial role in the synthesis and stabilization of nanoparticles. Finally, the sample was ground into a fine powder using potassium bromide (KBr) salt. The powder mixture was then compressed into a translucent pellet using a mechanical press, enabling the passage of light for spectral analysis. FTIR spectra were recorded on a Jasco 6300 spectrometer in ATR mode, scanning the range of 400-4000 cm−1.

2.5. *Scanning Electron Microscopy (SEM-EDAX) Analysis*

SEM was employed to scrutinize the surface morphology and shape of the fabricated AgNPs nanoparticles. These nanoparticles size and morphology were assessed operating scanning electron microscopy (SEM; TESCAN VEGA3 SBU).

2.6. *Antimicrobial Activity*

2.6.1. *Minimum Inhibitory Concentration (MIC) Using Resazurin Microtitre Assay:*

A 270 mg sample of resazurin was dissolved in 40 ml of sterile distilled water to prepare the resazurin solution. The solution was thoroughly mixed using a vortex mixer to guarantee complete dissolution and homogeneity. The assay was performed in a 96-well plate format, maintaining strict aseptic conditions throughout. A sterile 96 well plate was labeled. 100 µl of sample was carefully pipetted into the first well of the 96-well plate. Subsequent wells received 50 µl of nutrient broth and potato dextrose broth, followed by serial dilution. 10 µl volume of resazurin indicator solution was added to each well. Each well was then inoculated with 10 µl of bacterial suspension and 10 µl of fungal suspension. To prevent dehydration of microorganisms, each plate was loosely wrapped with cling film. Following incubation at 37 °C for 18-24 hours, the plate was visually examined for color changes. Any color change from purple to pink or colorless was considered a positive result. The minimum inhibitory concentration (MIC) value was determined as the lowest concentration at which this color change occurred.

2.7. *Invitro Cytotoxicity Activity (VERO - NORMAL CELL LINE)*

2.7.1. *MTT assay*

The cytotoxic activity of the samples against VERO (normal) cells was evaluated using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [16]. Cells were seeded at a density of 1 × 105/well in 96-well plates, with each well containing 0.2 ml of medium. The plates were then incubated in a 5% CO2 humidified incubator at 37°C for 72 hours. Following the 72-hour incubation, various concentrations of the test samples dissolved in 0.1% DMSO were added to the wells, and the plates were further incubated for 24 hours in a 5% CO2 humidified incubator. The cell morphology was observed under an inverted microscope at 40X magnification, and photomicrographs were captured. Following removal of the sample solution, 20 µl of MTT reagent was added to each well. The plates were then incubated at room temperature for 2-4 hours. Subsequent to the incubation, 1ml of DMSO was added. The number of viable cells was determined by spectrophotometric analysis at an absorbance wavelength of 540nm. 50% inhibition of cell viability (IC50) value was ascertained illustratively. The influence of the samples on the augmentation of VERO cells was stated as the % cell viability, using the following formula:

Calculation

% cell viability = A540 of treated cells / A540 of control cells × 100%

3. RESULT AND DISCUSSION

3.1. *Synthesis Of AGNPS*

The present study aimed to investigate the eco-friendly synthesis of silver nanoparticles (AgNPs) using aqueous extracts of *Barleria longiflora* stem powder.

Aqueous extract was used to extract the dried stem powder of *Barleria longiflora.* The color shift from pale yellow to reddish-brown signifies the successful reduction of silver ions (Ag+) to metallic silver (Ag0). This confirms the formation of synthesized silver nanoparticles (Figure 1 A and B). This color change can be attributed to the quantum confinement effect, a size-dependent phenomenon that influences the optical properties of nanoparticles [17].

FIGURE 1: SYNTHESISED SILVER NANOPARTICLES

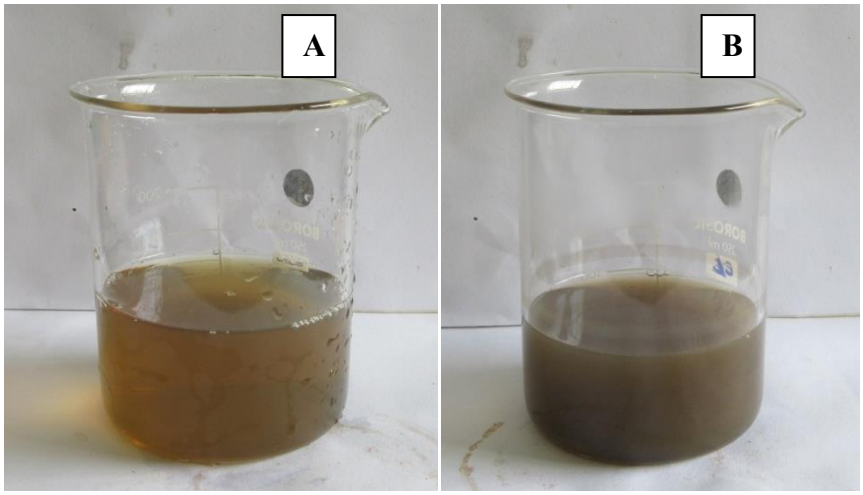


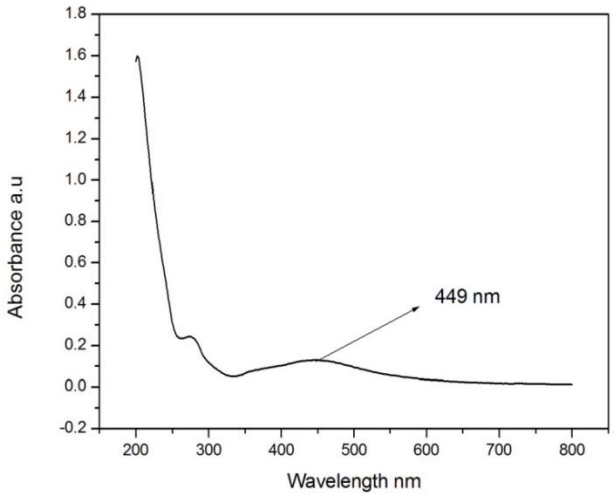
TABLE 1: ANTIMICROBIAL ACTIVITY OF AGNPS AGAINST BACTERIAL AND FUNGAL PATHOGENS

S.No	Microorganisms	Growth of Inhibition										
		1000µl	500µl	250µl	125µl	62.5µl	31.2 µl	15.6 µl	7.8 µl	STD 10 µl	DMSO	Culture
1	*S. aureus*	-	-	+	+	+	+	+	+	-	+	+
2	*E. coli*	-	-	-	-	+	+	+	+	-	+	+
3	*C. albicans*	-	-	-	-	+	+	+	+	-	+	+
4	*A. niger*	-	-	-	+	+	+	+	+	-	+	+

3.2. *UV–Visible Spectrophotometer*

UV-visible investigation and band energy parameters for the synthesized nanoparticles are portrayed in Figure 2. Silver nanoparticles exhibit a characteristic UV absorption peak within the wavelength range of 400-450 nm [17]. UV-Visible spectroscopy analysis revealed a distinct absorbance peak at 499 nm, corresponding to the silver nanoparticles biosynthesized using *Barleria longiflora* stem extract (Figure 2).

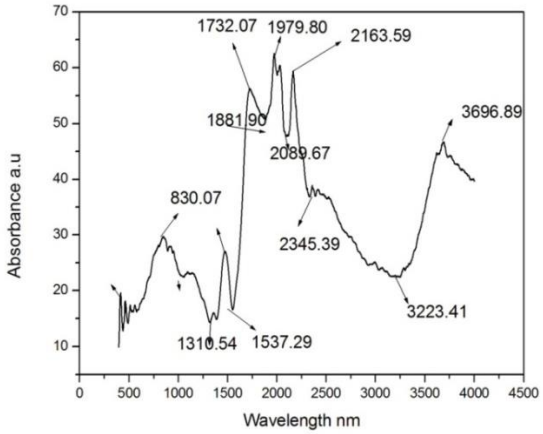
FIGURE 2: UV ANALYSIS OF SILVER NANOPARTICLES



3.3. *Fourier Transform Infrared Spectroscopy (FTIR) Analysis*

The functional groups present in the fabricated nanoparticles were analyzed using FTIR (Figure 3). FTIR interpretation of silver nanoparticles synthesized found peaks at 3223.41, 2345.39, 2163.59, 1732.07, 1537.29, 1218.54 and 830.07 cm^{-1} which correspond to functional groups O–H stretch, H–bonded (alcohols, phenols),–C≡ C– stretch (alkynes), C=O stretch (aldehydes, saturated aliphatic), N–O asymmetric stretch (nitro compounds), C–N stretch (aliphatic amines) and C–Cl stretch (alkyl halides).

FIGURE 3: UV ANALYSIS OF SILVER NANOPARTICLES



3.4. *Scanning Electron Microscopy (SEM-EDAX) Analysis*

Scanning Electron Microscopy (SEM) analysis was performed to assess the morphological characteristics and particle size distribution of the synthesized nanoparticles [18]. Scanning Electron Microscopy (SEM) is a ubiquitous technique employed for characterizing nanoparticles, providing invaluable insights into their physical morphology and surface characteristics. The capping of nanoparticles served as a protective mechanism, preventing their agglomeration and ensuring stability within the medium. The AgNPs exhibited a polydispersed spherical morphology, with particle sizes ranging from 15.7 nm to 28.3 nm, indicating a relatively narrow size distribution (Figure 4).

The elemental composition of the AgNPs was identified and quantified using Energy-Dispersive X-ray Analysis (EDAX) [19]. EDAX of synthesized silver nanoparticles is portrayed in (Figure 5). The EDAX spectrum revealed a prominent peak at approximately 3 keV, confirming the presence of elemental silver. SEM analysis revealed that the nanoparticles exhibited a spherical morphology, and EDAX spectroscopy confirmed their composition, displaying a dominant peak corresponding to silver.

FIGURE 4: SEM ANALYSIS OF AGNPS

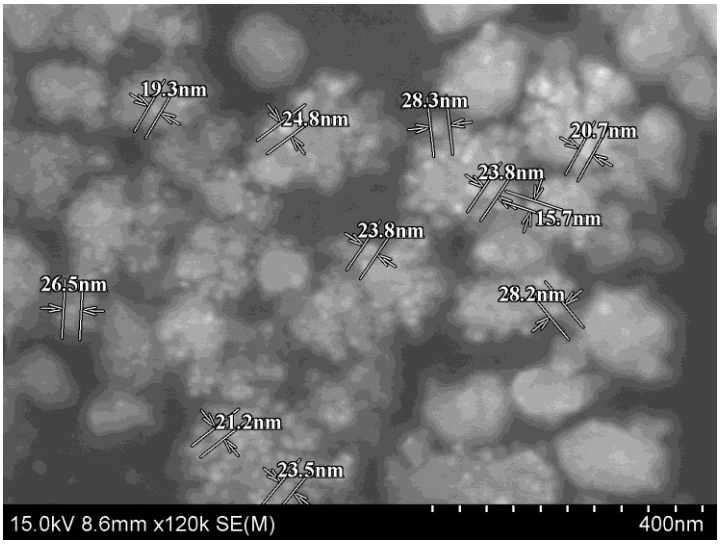
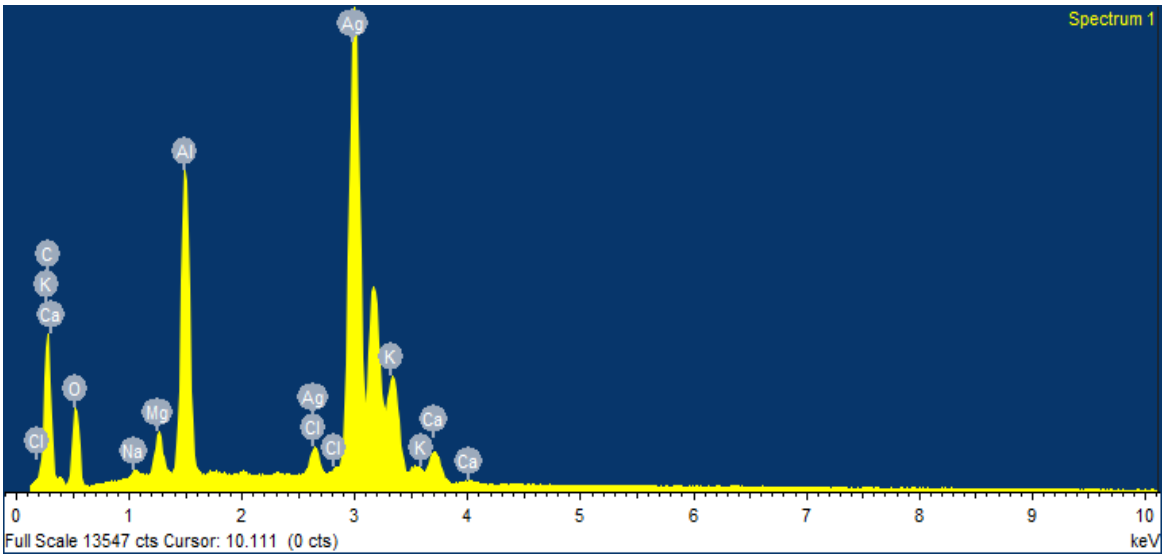


FIGURE 5: EDAX ANALYSIS OF AGNPS



3.5. *Antimicrobial Activity*

3.5.1. *Minimum Inhibitory Concentration (MIC) Using Resazurin Microtitre Assay*

The Resazurin assay was employed to assess the antimicrobial efficacy of the ethanol extract of silver nanoparticles (AgNPs). The ethanol extract of AgNPs exhibited robust antibacterial properties, effectively inhibiting the growth of both Gram-positive and Gram-negative bacteria associated with food borne illnesses, highlighting its broad-spectrum antimicrobial potential. Dimethyl sulfoxide (DMSO) was used as the negative control failed to show any activity. The antibacterial activity showed highly activity against *Escherichia coli* (MIC value 125 µg) and less activity against *Staphylococcus aureus* (MIC value 500µg) (Figure 6 and Table 1). Antifungal activity showed effective activity against *Candida albicans* (MIC value 250) and less activity against *Aspergillus fumigates* (MIC value 500). The result revealed that

the all microbes showed best antimicrobial activity. *Escherichia coli* and *Candida albicans* showed best antimicrobial activity when compared with other microbes (Table 2).

FIGURE 6: ANTIMICROBIAL ACTIVITY OF AGNPS AGAINST BACTERIAL AND FUNGAL PATHOGENS

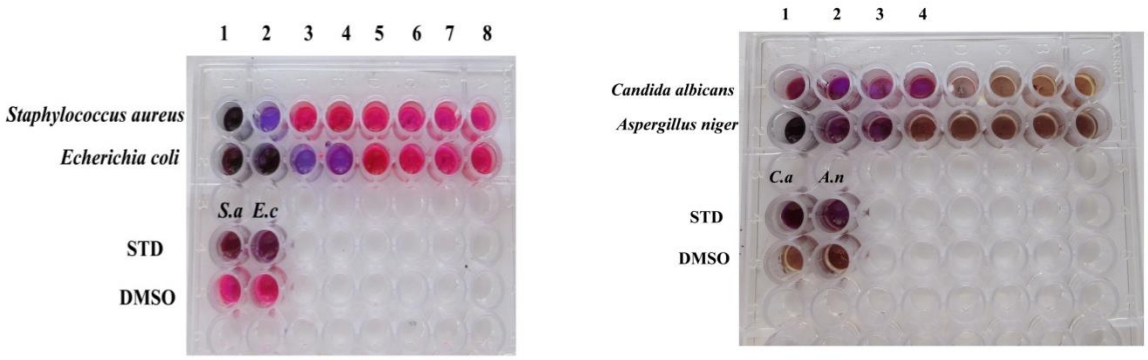


TABLE 2: MIC VALUES FOR ANTIMICROBIAL ACTIVITY OF AGNPS AGAINST BACTERIAL & FUNGAL CULTURES

S.No	Microorganisms	MIC Value (µg)
1	*S. aureus*	500
2	*E. coli*	125
3	*C. albicans*	125
4	*A. niger*	250

4. *MTT assay*

The various doses of AgNPs 1000, 500, 250, 125, 62.5 and 31.2 µg/ml and vehicle control/Negative control (DMSO) control (without samples) were validated for cytotoxicity activity against normal (VERO) cell line. A dose-dependent decrease in cell count was observed in the cell lines with increasing concentrations of the samples. The toxicity result was found at 50 % of cell viability at 68.75 µg concentration of the samples in normal VERO cell line (Table 3, Figure 7 and Figure 8). The result shows less toxicity property for AgNPs.

TABLE 3: *CYTOTOXICITY EFFICACY OF AGNPS* AGAINST NORMAL *CELL LINE (VERO)*

S.No	Concentration µg/ml	Absorbance 540nm	% cell Viability
1	1000	0.02	1.6
2	500	0.05	4.1
3	250	0.15	12.5
4	125	0.37	30.8
5	62.5	0.62	51.6
6	31.2	0.87	72.5
7	15.6	1.01	84.1
8	DMSO	1.20	100
9	Control Cells	1.20	100

FIGURE 7: CYTOTOXICITY EFFICACY OF *AGNPS* AGAINST NORMAL CELL LINE (VERO)

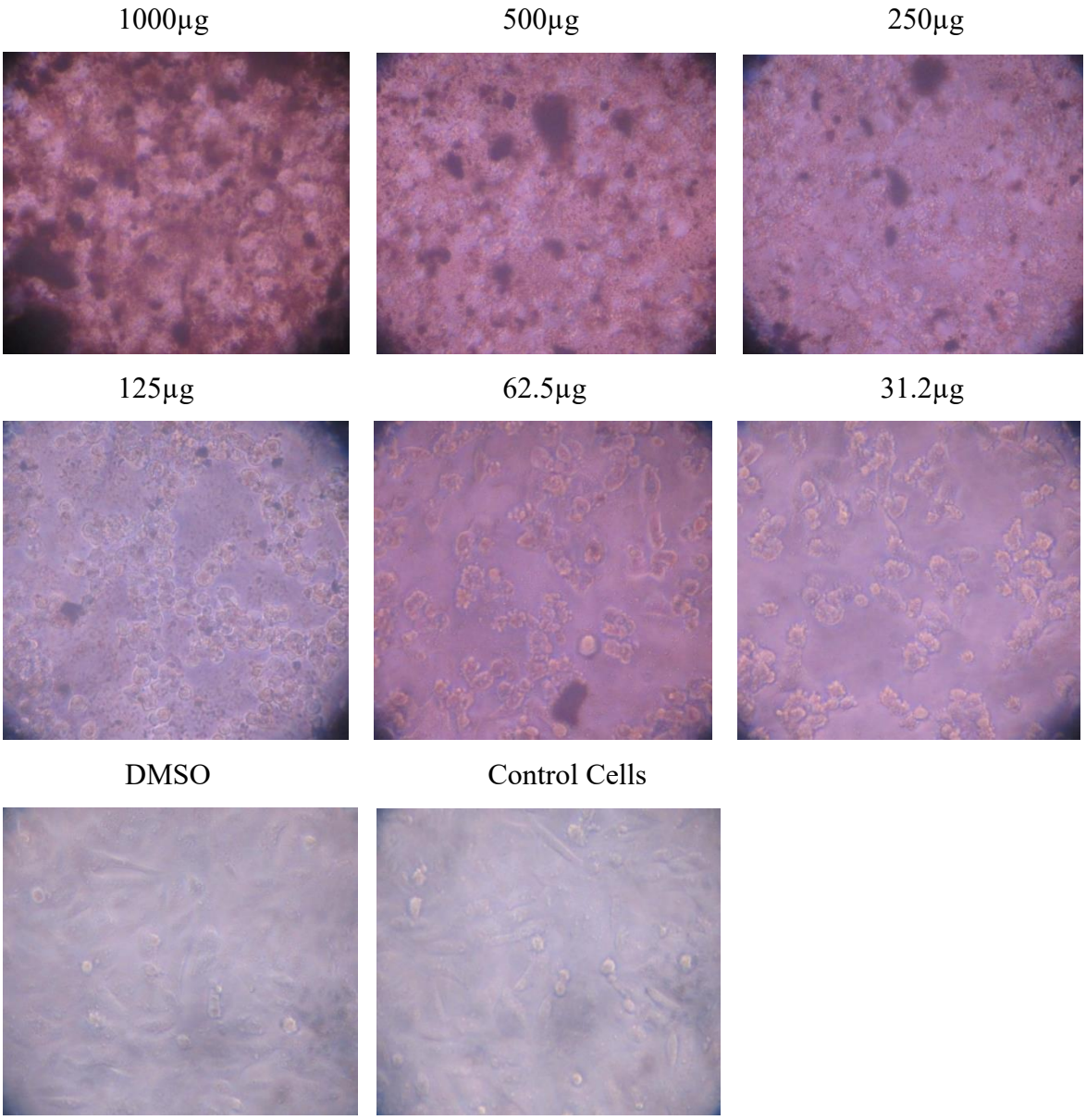
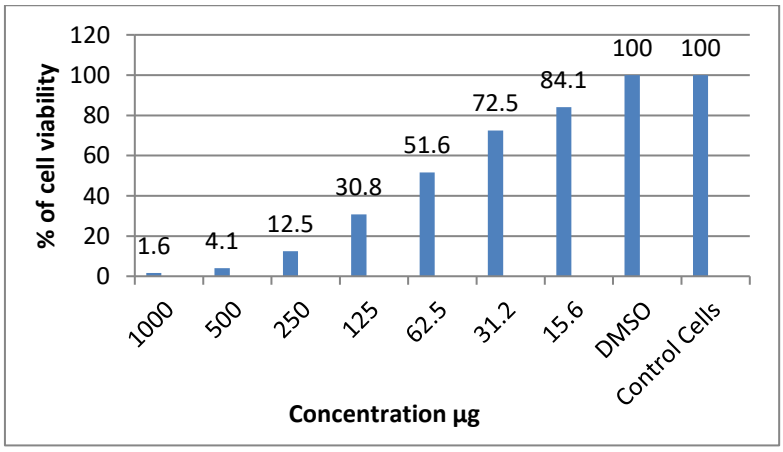


FIGURE 6: CYTOTOXICITY EFFICACY OF *AGNPS* AGAINST NORMAL CELL LINE (VERO)



5. CONCLUSION

Using extracts from *B. longiflora* stems, an efficient, quick, and environmentally friendly way to create AgNPs was created, offering a viable substitute for chemical synthesis, particularly for pharmaceutical and medicinal applications. AgNPs have a promising future in biological applications, as evidenced by their desirable shape and tiny size. The formulation and alibration of silver nanoparticles (AgNPs) made from the stem of *B. longiflora* have never been studied before. The FTIR spectra revealed that functional groups governing AgNPs' bio-reduction and stability absorb light. The antibacterial properties of the produced AgNPs were impressive, demonstrating effectiveness against fungal and bacterial pathogens, including both Gram-positive and Gram-negative food-borne bacteria AgNPs' potential as chemotherapeutic agents was indicated by their in vitro cytotoxicity, which showed that they could specifically target cancer cell lines. This investigation sets the stage for future research into the therapeutic potential of AgNPs produced from *B. longiflora*. This study serves as a vital starting point, but further investigation and testing are necessary to fully elucidate the effects of these nanoparticles in antimicrobial and anticancer contexts.

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7. CONFLICTS OF INTEREST

All the authors declare that they have no known conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

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