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Research Article

Safed Musli (*Chlorophytum borivilianum* L.) Callus-Mediated Biosynthesis of Silver Nanoparticles and Evaluation of their Antimicrobial Activity and Cytotoxicity against Human Colon Cancer Cells

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With the advancement of nanobiotechnology, eco-friendly approaches of plant-mediated silver nanomaterial (AgNP) biosynthesis have become more attractive for biomedical applications. The present study is a report of biosynthesizing AgNPs using *Chlorophytum borivilianum* L. (Safed musli) callus extract as a novel source of reducing agent. $AgNO_3$ solution challenged with the methanolic callus extract displayed a change in color from yellow to brown owing to the bioreduction reaction. Further, AgNPs were characterized by using UV–visible spectrophotometry, X-ray Diffraction (XRD), Atomic Force Microscopy (AFM), and Fourier Transform Infrared Spectroscopy (FTIR). UV–vis spectrum revealed the surface plasmon resonance property of AgNPs at around 450 nm. XRD pattern with typical peaks indicated the face-centered cubic nature of silver. AFM analysis confirmed the existence of spherical-shaped and well-dispersed AgNPs having an average size of 52.0 nm. Further, FTIR analysis confirmed the involvement of different phytoconstituents of the callus extract role in the process of bioreduction to form nanoparticles. The AgNPs were more efficient in inhibiting the tested pathogenic microbes, namely, *Pseudomonas aeruginosa*, *Bacillus subtilis*, Methicillin-resistant *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* compared to callus extract. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay confirmed the cytotoxic property of AgNPs against human colon adenocarcinoma cell line (HT-29) in a dose-dependent manner. At higher concentrations of 500 μg/mL AgNPs, the cell viability was observed to be only 7% after 24 hours with IC_{50} value of 254 μg/mL. Therefore, these AgNPs clearly endorse the manifold potential to be used in various biomedical applications in the near future.

1. Introduction

As an emerging field of science in the modern world, nanotechnology has greatly benefited humans. Nanotechnology is aimed at producing and utilizing nanosized materials measuring between 1 and 100 nm [1]. The unique features of nanosized materials make them more attractive for application in various fields, especially for delivering drug molecules, image analysis, as a biomarker, biodetection of macromolecules or pathogens, etc. [2]. Several types of metals are being

used for the synthesis of nanomaterials for specific biomedical applications. They include silver (Ag), gold (Au), titanium dioxide (TiO_2), zinc oxide (ZnO), copper oxide (CuO), magnesium oxide (MgO), calcium oxide (CaO), and silica (Si). These nanostructures exhibit unique physicochemical and biological properties, including strength, plasticity, durability, and functions. Thus, they are widely applied in different areas, including electronics, biomedicine, and bioengineering [3]. As silver possess antimicrobial activity, it is being widely used in the preparation of various antimicrobial agents for the past few years [4, 5]. Today, silver is used to synthesize silver nanoparticles (AgNPs) for different applications in the fields of medicine, food, health care, etc. This is due to the fact that AgNPs with a larger surface area-to-volume ratio possess unique biological, electrical, thermal, and optical properties [6–8].

There are several approaches to synthesize AgNPs, including chemical, physical, and biological methods [3, 9–11]. However, the preferred method is by using the biological route that involves plant compounds or plant extracts, microbes, or their products. This is mainly because of safety, cost-effectiveness, and environment-friendly aspects. On the other hand, chemical and physical methods involve toxic chemicals, a lot of energy, great pressure, and high temperature [6, 9, 12]. AgNPs were successively produced using different plant extracts, such as *Leptadenia reticulata* [9], *Cassia didymobotrya* [10], *Andrographis paniculata* [12], *Prunus japonica* [13], *Talinum triangulare* [14], *Euphorbia antiquorum* [15], *Thymbra spicata* [16], and *Cleome viscosa* [17]. Recently, AgNPs are being synthesized from the plant callus as a novel source. For instance, the callus induced from *Catharanthus roseus*, *Sesuvium portulacastrum*, *Taxus yunnanensis*, *Centella asiatica*, *Cucurbita maxima*, etc., are used for the biosynthesis of AgNPs [4, 18–21]. Advantageously, callus cultures mitigate the problems of wild plant source scarcity. In addition, callus extracts are more efficient in producing more distinct and scattered AgNPs compared to those biosynthesized using leaf extracts with higher bioactivities [4, 21].

Chlorophytum borivilianum L. (Safed musli) is a valued medicinal plant, having copious bioactive components, such as phenols, saponins, flavonoids, alkaloids, tannins, steroids, triterpenoids, and vitamins. The plant is effective in curing chronic leucorrhoea, diabetes, arthritis, high blood pressure, and delayed menopause [22, 23]. To overcome the problems of Safed musli cultivation in the field, plant tissue culture approaches have been adopted to obtain its bioactive compounds. Safed musli callus culture as a reliable source for plant secondary metabolites has been proven previously by Charl et al. [24]. Further, they have also reported the antimicrobial and antioxidant activities of Safed musli callus extract. However, to date there is no report on the biosynthesis of AgNP using Safed musli plant or its callus. Therefore, the present study reports a biological method of synthesizing AgNPs using Safed musli callus extracts to evaluate their biological properties.

2. Materials and Methods

2.1. Preparation of Callus Extracts of Safed Musli. To initiate the callus cultures of Safed musli, the method explained by Nakasha et al. [23] was followed. Briefly, shoot buds of Safed musli were inoculated on solid Murashige and Skoog medium containing 5 mg/L 2,4-dichlorophenoxy acetic acid and cultured for 4 weeks and then harvested. To prepare the callus extract, 20 g fresh weight callus was grinded along with 100 mL of methanol and boiled for about 5 min. By using the Whatman no. 1 filter paper, the extract was filtered and kept at 4°C. The extract was used for AgNP preparation within 1 week.

2.2. Biosynthesis of AgNPs. About 10 mL of callus extracts was challenged with 90 mL of 1 mM $AgNO_3$ (silver nitrate) solution contained in an Erlenmeyer flask (250 mL). The reaction mixture was kept at room temperature on a shaker (150 rpm) without light. The change in color was recorded periodically up to 5 hours, and the AgNPs were stored at room temperature for 3 months to check the stability. The reaction mixture was centrifuged at 20,000 rpm for 15 min to concentrate the biogenically synthesized AgNPs for further characterization.

2.3. Characterization of AgNPs

2.3.1. UV-Visible Spectral Analysis. The change in color formation in the reaction mixture was visually monitored. About 2 mL of the solution was periodically collected after 1, 3, and 5 hours of incubation, and the reduction of silver ions was measured at 300–600 nm UV-visible spectrum using a spectrophotometer (ELICO, India).

2.3.2. X-Ray Diffraction (XRD) Analysis. On the glass slide, a single drop of AgNP solution was added and coated. It was later analyzed to record the crystalline nature of the biosynthesized nanoparticles by using an X-ray diffractometer (XRD), model XRD-6000, Shimadzu, Japan, with 40 kV and 30 mA with Cu ka radiation at 2θ angel.

2.3.3. Atomic Force Microscopy (AFM). Using AFM (A.P.E. Research A100, Italy), AgNPs were characterized to observe their morphological features. At first, the solution containing AgNPs was sonicated at room temperature for 15 min by using an ultrasonicator. Later, the AgNP solution was dried to form a thin layer on a mica-based glass slide, and this was used for observing under AFM.

2.3.4. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. FTIR analysis of biogenically synthesized AgNPs was performed by using a Perkin Elmer FTIR spectrum-using KBr pellet using a Shimazdu IR Prestige-21 FTIR instrument with a diffuse reflecting mode (DRS-8000). All the measurements were carried out in the range of 400–4000 cm^{-1}.

2.4. Antibacterial Activity Evaluation. The biosynthesized AgNPs were assessed for their antimicrobial activity using a disc diffusion method against common human pathogenic Gram-positive bacterial strains, *Bacillus subtilis* B29 (ATCC 29737), Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC700698) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 15442), and *Escherichia coli* E266 (Gram-negative), and one fungal species, *Candida albicans*

90028. All microbial strains were procured from the Laboratory of Molecular Biomedicine, Institute of Bioscience, UPM, Serdang, Malaysia. All bacterial strains were maintained on the Mueller-Hinton Agar (MHA) media, while *C. albicans* 90028 was cultured on the potato dextrose agar (PDA) medium. To evaluate antibacterial activities, the disc diffusion assay method was employed with little modifications [24]. Briefly, the pure culture of each microbe was evenly swabbed onto the separate petri plates using sterile cotton swabs. The culture medium was placed with sterile discs (6 mm in diameter) precoated with different concentrations (100, 200, and 300 μg/mL) of AgNPs and the methanolic leaf extract. Dimethyl sulfoxide (DMSO) (10 μg/μL) and gentamycin (10 μg/disc) were used as negative and positive controls, respectively, against all the tested microbes. Each treatment was replicated 5 times, and the experiment was repeated twice. All plates were incubated at 37°C for 24 hours, and the appearance of the zone of inhibition (mm) was recorded with the help of a ruler.

2.5. Evaluation of Cytotoxicity against Colon Cancer Cell Line HT-29. We evaluated the cytotoxic effect of mycogenic AgNPs on colon cancer cell line HT-29 as reported earlier [9]. In brief, the cells were grown on the Dulbecco's Modified Eagle's Medium (DMEM) contained with penicillin (100 U/mL), streptomycin (100 g/mL), L-glutamine (2 mM), and fetal bovine serum (10%). Approximately 5×10^4 cells were used for the inoculation in a well of 96-well plates. A CO_2 incubator adjusted to 37°C was used to incubate the cells for 48 hours. To study the cytotoxicity, the cells were treated with biosynthesized AgNPs (10, 20, 40, 80, 120, and 160 μg/mL) and incubated for 48 hours to evaluate cell survivability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. Firstly, fresh MTT solution (5 mg/mL) was prepared and about 10 mL of it was dispensed to each well. Further, it was kept for the incubation up to 4 hours under the same conditions. Using a multiwell ELISA plate reader, the absorbance was documented at 570 nm. The obtained absorbance was transformed into cell viability percentage using the given formula below:

$$\%\ \text{cell viability} = \frac{\text{Values of optical density in the experimental samples}}{\text{Values of optical density in the control samples}} \times 100. \quad (1)$$

2.6. Statistical Analysis. All the experiments were replicated three times and repeated thrice. The data obtained from each experiment was represented as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Callus Formation and Synthesis of AgNPs. Synthesizing AgNPs through the biological route has gained more importance in recent times because of the fact that the biological method yields stable and uniform AgNPs with superior pharmacological significance [8, 10]. The present study involved the use of Safed musli callus extract as a substrate to synthesize AgNPs at room temperature. In this study, yellow-colored friable calli formed after 2 months were harvested (Figure 1).

Apparently, Safed musli calli at this stage are considered as matured and well developed to secrete plant secondary metabolites. Hence, calli harvested after 2 months were utilized in the process of synthesizing AgNPs [23, 24]. In general, the production and features of nanoparticles vary depending on the bioactive compounds occurring in solvent extracts of a plant species [9]. When the $AgNO_3$ solution was challenged with the methanolic callus extract of Safed musli, there was a change in the color from yellow to light brown due to bioreduction reaction (Figure 2). This clearly suggests the biosynthesis of AgNPs, which is correlated to the excitation of surface plasmon resonance vibrations in AgNPs [9, 18, 25]. The color change was immediately observed within an hour, and the intensity of the color increased with the incubation time up to 5 hours. However, more than 5 hours of incubation showed no observable change in the color. The color intensity increased gradually with an increase of incubation time and remained the highest after 5 hours of incubation. Till now, the exact mechanisms involved in the biosynthesis of AgNPs from plant extracts are not clearly understood. However, some possible mechanisms which might be involved in the biosynthesis are being proposed. Accordingly, the cellular enzymes along with the occurrence of diverse classes of phytocompounds, such as phenolics, flavonoids, phytosterols, terpenoids, organic acids, alkaloids, and alcohols, occurring in plant extracts might efficiently reduce forming AgNPs from silver ions [26, 27]. Previously, researchers have reported that the incubation duration for completing the bioreduction of silver ions to form AgNPs varies from one plant species to another due to differences in the occurrence of phytoconstituents in the plant extracts [9, 10].

3.2. Characterization of AgNPs

3.2.1. UV-Visible Spectroscopy Analysis. The use of UV-visible spectroscopy, XRD, AFM, and FTIR analysis has provided the information related to the size, shape, dispersion, and surface area of callus extract-mediated AgNPs. The UV spectrum showed the presence of a sharp absorbance peak at around 450 nm, suggesting the occurrence of AgNPs (Figure 3). According to previous reports, the UV-visible absorption band in between 425 and 460 nm indicates surface plasmon resonance (SPR) of AgNPs [8, 18, 28]. This SPR peak along with the bioreducing agents of the callus extract may possibly be involved in capping to form and stabilize AgNPs [9]. The

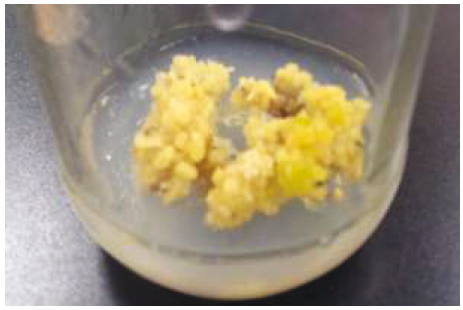

Figure 1: Showing the callus formation on MS medium supplemented with 2,4-D (5 mg/L) after 2 months.

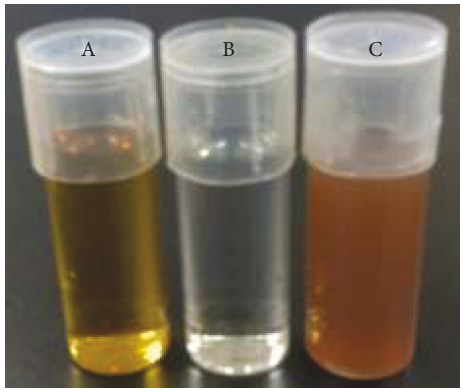

Figure 2: Yellow color of the Safed musli callus extract (A); transparent color of $AgNO_3$ solution (B), and brown color of the reaction mixture after 48 hours of exposure to $AgNO_3$ indicating the formation of AgNPs (C).

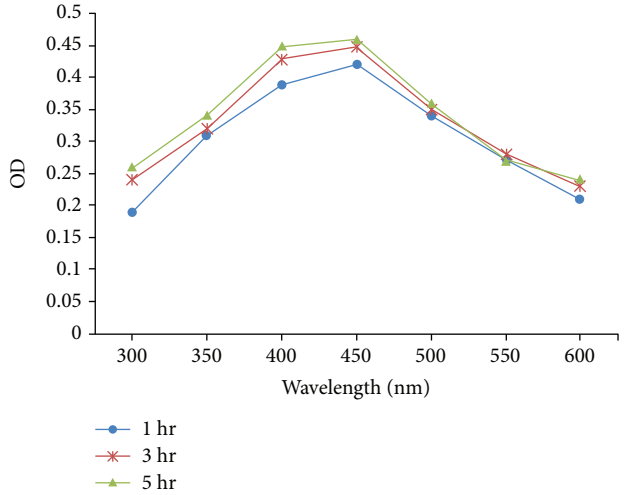

Figure 3: UV–visible absorption spectroscopy showing the characteristic SPR peak of AgNPs.

presence of a broad peak could be correlated to the polydispersal nature of AgNPs with spherical shape [29].

3.2.2. XRD Analysis. The observation of diffraction peaks of XRD analysis provides the details on crystalline nature and

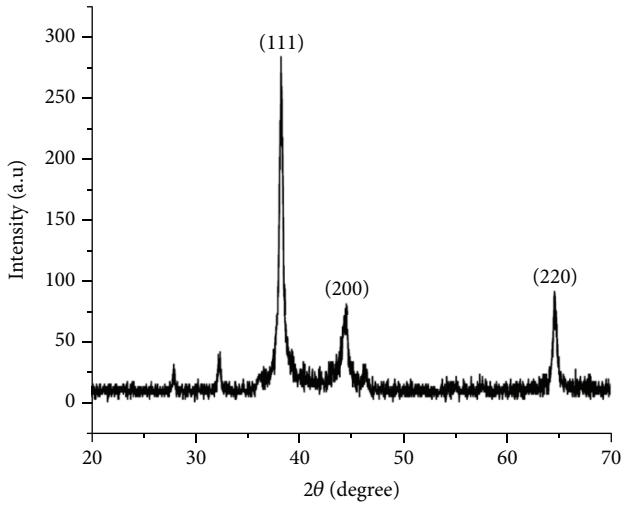

Figure 4: XRD pattern of biosynthesized AgNPs using callus extract of Safed musli.

chemical composition of the biosynthesized AgNPs. The result of the XRD pattern of AgNPs synthesized using Safed musli callus extract is illustrated in Figure 4. The diffracted intensities from 20° to 70° were recorded. The observed peaks at 2θ of 38.34°, 44.54°, and 64.6° correspond to (111), (200), and (220) planes, respectively, of face-centered cubic structure of silver. These results are similar to the record of the Joint Committee on Powder Diffraction Standards (JCPDS no. 04-0783). Likewise, other minor peaks observed might be correlated to the crystalline organic compounds that are adsorbed on the AgNP surface. Similar diffraction patterns were also observed by previous findings related to AgNPs synthesized from plant sources [10, 18, 27].

3.2.3. AFM Analysis. AFM analysis was carried out to record the topological features of biosynthesized AgNPs from callus extract of Safed musli. The result evidently disclosed that the existence of spherically shaped AgNPs is evenly dispersed (Figure 5). The size of the AgNPs ranged between 35.1 and 168.0 nm with an average size of 52.0 nm. The biosynthesized AgNPs were found with a roughness of 7.9 nm and root mean square roughness of 14.6 nm (Figures 5(a) and 5(b)). These observations are in confirmation with the previously reported nanoregime and spherically shaped AgNPs biosynthesized from different plant species, including *Leptadenia reticulata*, *Murraya koenigii*, *Centella asiatica*, *Cleome viscosa*, and *Coptidis rhizoma* [8, 17, 18, 27, 30].

3.2.4. FTIR Analysis. The likely interaction of biosynthesized AgNPs and different phytocompounds occurring in Safed musli callus extract was determined by FTIR analysis. These phytoconstituents are accredited to function as reducing and stabilizing agents during their AgNP biosynthesis [10, 29]. Figure 6 demonstrates the FTIR spectral data of biosynthesized AgNPs with 14 distinct peaks in the range of the 4000–500 cm^{-1} region. A broad peak at 3437.86 cm^{-1} corresponds to the stretching vibrations of –O-H and –N-H groups. Likewise, the peak at 2920.59 cm^{-1} is the result of –

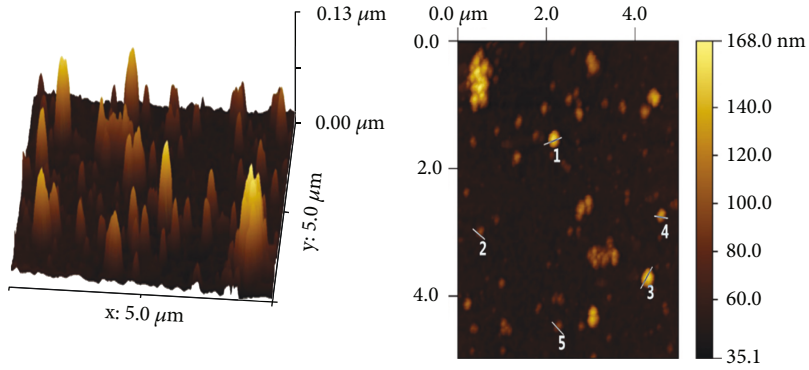

Figure 5: AFM images of AgNPs biosynthesized by callus extract of Safed musli.

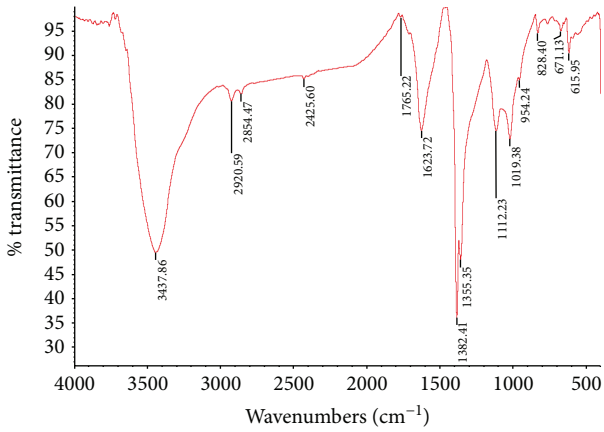

Figure 6: FTIR spectral data of AgNPs produced by callus extract of Safed musli.

C-H groups. The bands at 1623.72 cm^{-1} and 1376 cm^{-1} might be because of the stretching vibrations of C=C groups and the presence of C-N-like amine or C-O-like phenol groups, respectively. The wave number 1382.41 could be assigned to the $-CH_2$ group. The peak at 1019.38 is due to stretching of C=O groups. Three weak bands at 828.4, 671.13, and 615.95 cm^{-1} correspond to bending vibrations of –O-H and C-H groups. Similar observations were made by earlier researchers on other plant-based AgNPs [10, 27, 31]. Further, these absorbance peaks may be endorsed to numerous phytochemical compounds present in the callus extract of Safed musli. In support of this, a previous study by Charl et al. [24] has confirmed the occurrence of different phytoconstituents using gas chromatography–mass spectrometry analysis. Overall, FTIR data shows the multifunctionality of Safed musli callus extract in the process of bioreduction as well as to stabilize AgNPs.

3.3. Assessment of Antibacterial Activity. AgNPs exhibit a broad spectrum antimicrobial activity and, hence, are widely used in clinical applications [5, 7–9]. Nevertheless, their use as antimicrobials will be effective and can be applied only after addressing the problems of their adverse side effects [3]. Hence, we assessed antimicrobial activities of the biosynthesized AgNPs from Safed musli callus extract against human pathogens. It was observed that AgNPs efficiently inhibited all the tested bacterial strains in dose-dependent ways (Table 1). Interestingly, AgNPs exhibited a higher zone of inhibition when compared to the callus extract. The highest inhibition of AgNPs was observed against *C. albicans* (15.83 ± 1.08 mm) followed by *B. subtilis* (14.83 ± 1.60 mm) and *E. coli* (12.60 ± 0.52 mm) at 300 μg/mL concentration. However, all microbes were inhibited by AgNPs at the 300 μg/mL concentration. The maximum inhibitory activity was observed against *B. subtilis* (16.0 ± 1.7) followed by *C. albicans* (15.3 ± 0.5) and *E. coli* (14.3 ± 1.6) at 300 mg/mL concentration of AgNPs. Earlier, investigators have suggested few possible mechanisms of antimicrobial action by plant-based AgNPs. Accordingly, AgNPs denature the cell wall of microbes, destabilize the outer membrane, block cellular respiration, inhibit biosynthesis, and disrupt proton motive force. Also, higher surface area-to-volume ratio of AgNPs is responsible for the antimicrobial activity [3, 7, 8, 14]. The results of the current study clearly indicate that AgNPs synthesized from Safed musli callus extract could be used as antibacterial agents to treat many human diseases.

3.4. AgNPs against Cancer Cells. Additionally, the activity of AgNPs against cancer cell line HT-29 was carried out using the MTT assay. The results of the study are represented in Figure 7. The percentage of cell viability decreased with increased concentrations of AgNPs from 0 to 500 μg/mL. It evidently suggests that AgNPs exhibit dose-dependent cell inhibitory activities. Further, increase in the exposure time from 24 hours to 48 hours decreased the cell viability percentage. After 24 hours, the control treatments recorded 100% cell viability, while only 7% of cells survived at 500 μg/mL of AgNPs, which further decreased to 2% after 72 hours of incubation time. This signifies a high toxicity effect of AgNPs. Although biosynthesized AgNPs exhibit less toxicity at a lower dose, they induce very high lethal effect at higher doses. Similarly, earlier researchers have documented the potential cell inhibitory action of plant-based AgNPs in a dose-dependent manner [3, 8, 32]. The IC_{50} value of AgNPs was calculated to be 254, 216, and 174 μg/mL after 24 hours, 48 hours, and 72 hours, respectively, of treatment.

Table 1: Antimicrobial activities of Safed musli callus extract and its biosynthesized AgNPs against human pathogens.

Concentration (μg/mL)	Zone of inhibition (mm)				
	Bacillus subtilis B29	*Staphylococcus aureus (MRSA)*	*Pseudomonas aeruginosa ATCC 15442*	*Escherichia coli E266*	*Candida albicans 90028*
Callus extract					
100	07.33 ± 0.57	04.66 ± 2.08	04.66 ± 1.52	05.33 ± 0.57	08.00 ± 1.00
200	08.66 ± 0.57	06.00 ± 1.00	08.00 ± 1.15	06.33 ± 0.57	12.00 ± 1.73
300	10.66 ± 1.15	08.66 ± 1.15	09.65 ± 1.52	10.30 ± 1.52	12.66 ± 0.57
AgNPs					
100	09.66 ± 1.15	06.33 ± 0.57	08.16 ± 1.08	07.83 ± 0.76	12.00 ± 2.64
200	12.33 ± 1.57	09.50 ± 1.50	10.00 ± 1.00	12.30 ± 1.53	14.33 ± 1.52
300	14.83 ± 1.60	10.50 ± 1.50	12.00 ± 0.00	12.60 ± 0.52	15.83 ± 1.08

The experiment included DMSO (20 μL) as the negative control, while streptomycin (100 mg/mL) for bacteria and nystatin (100 mg/mL) for yeast served as the positive control. Each value represents the mean ± standard deviation (SD) of 3 replicates per treatment in 3 repeated experiments. Note: "-" represents no activity observed, while "MR" represents Methicillin-resistant.

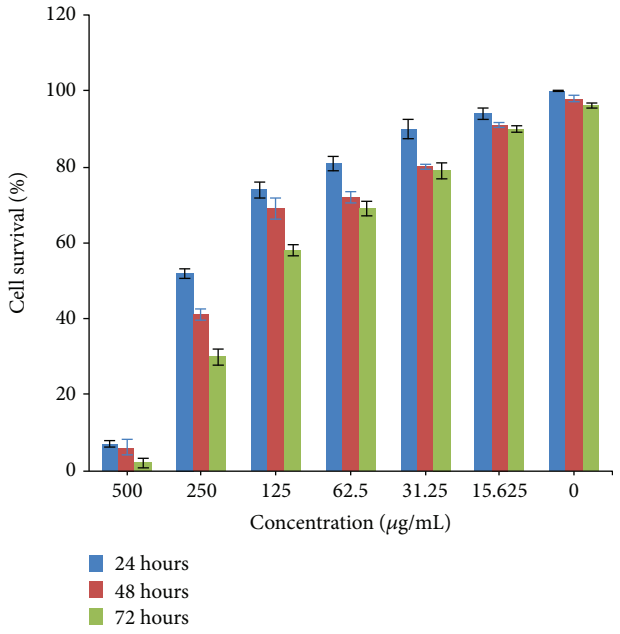

Figure 7: Cytotoxicity results of biosynthesized AgNPs using callus extracts of Safed musli.

In a previous report, it is stated that the Safed musli callus extract possesses various classes of phytochemicals [24]. Thus, phytocompound reactive functional groups, such as hydroxyl, carboxyl, and amino groups, couple with silver ions to exhibit a higher cytotoxicity. Likewise, it is proven that silver ions along with reactive functional groups interact vigorously with the cellular architecture to cause cellular damage [3, 9, 32].

In addition, silver ions possess strong affinity towards sulfhydryl groups of essential enzymes and phosphorus-comprising bases. Hence, AgNPs interact effectively with nucleic acids and cause DNA damage via disrupting the mitochondrial respiratory chain, encouraging reactive oxygen species formation, inhibiting DNA replication and cell division, promoting apoptosis, etc. [3, 32, 33]. Moreover, other characteristics of AgNPs, such as nanoregime nature, spherical shape, and particle surface, also contribute to anticancer properties. Similarly, it has been reported that nanomaterials prepared by using diverse bulk materials have elucidated their cell inhibitory activities against colon cancer cells. Specifically, the anticancer activity was mainly attributed to the chemical composition of the plant extracts and characteristics of nanoparticles, including size and morphological features of AgNPs [9, 33, 34].

4. Conclusion

In conclusion, this study describes an efficient, cost-effective, and environment-friendly approach for biosynthesizing AgNPs using the Safed musli callus extract. The biofabricated AgNPs possess spherical shape with a particle size ranging between 35.1 and 168.0 nm. The XRD pattern established that AgNPs occur in the form of nanocrystals, while AFM observation confirmed the spherical shapes of AgNPs. The FTIR spectrum revealed the occurrence of phytochemicals in the callus extracts and are attributed in the biosynthesis and stabilization of AgNPs. Further, the exhibition of antimicrobial and anticancer activity by the biosynthesized AgNPs suggests that they could be utilized in the fabrication of nanodrugs for therapeutical applications, such as antimicrobial agents, and for the treatment of colon cancers. In total, these findings clearly endorse the manifold potential of these phytofabricated AgNPs.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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