Research article

Synthesis, characterization and biomedical applications of silver nanoparticles

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(Received: May 2021 Revised: June 2021 Accepted: June 2021)

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ABSTRACT

Introduction and Aim: Silver nanoparticles (AgNPs) have been extensively useful in biomedical applications. This study aims to synthesize silver nanoparticles by lasers ablation and to use them as an anti-bacterial and anti-cancer agent.

Materials and Methods: According to the current study, Ag-nanoparticles can be synthesized easily using pulsed laser ablation on a 99.81% pure silver target immersed in deionized water. The prepared nanoparticles were characterized by UV-vis spectroscopy and X-ray diffraction (XRD), transmission electron microscopy (TEM), and atomic force microscopy (AFM) technique. Using a well-diffusion process, the anti-bacterial action of the synthesized Ag nanoparticles was tested versus two Gram-positive bacteria species (S. aureus and S. pyogenes) and two species of Gram-negative bacteria (E. coli and P. aeruginosa). The anticancer activity of the silver nanoparticles was evaluated by the MTT assay.

Results: The generated AgNps had a maximum absorbance peak of 400 nm. The XRD analysis verified that the synthesized silver nanoparticles had been nanocrystalline. The AgNPs did not affect any of the blood parameters. Gram-negative bacteria are more affected by silver than Gram-positive bacteria. The Ag nanoparticles had been shown a maximum anti-bacterial action at a concentration of 80 µg/ml and had a lower effect with 20 µg/ml concentration while their efficacy at 40 and 60 µg/ml concentrations appeared to be variable against all bacterial species. The findings show that AgNPs have a cytotoxic influence on cancer cells in 80 µg/ml concentration.

Conclusion: In comparison to Gram-positive bacteria, silver nanoparticles show high antibacterial activity against Gram-negative bacteria. The prepared nanoparticles have a potent effect on cancer cells and restricted harmful effects on RBCs.

Keywords: Nanobiotechnology; antimicrobial; toxicity activity; tumor therapy.

INTRODUCTION

Nanoscience is an increasingly growing field that has led to the development of a broad range of synthesized metal nanoparticles at the nanoscale level (1). Nanoparticles are nanoscale particles of sizes varying from 1 to 100 nanometers. Silver nanoparticles (AgNPs) have become increasingly common among metallic nanoparticles due to their unique chemical, physical, and biological properties (2). Nanoparticles are commonly used for several applications of their size, morphological properties, distribution, and structure (3). Due to their exceptional physicochemical properties, the specific surface atom and surface area of metal nanoparticles are both high. Metal nanoparticle synthesis is world famous because of its important applications in medicine, electronics, energy, and chemistry (4). Metal nanoparticles have been prepared using a range of techniques, including physical, chemical, and mechanical methods. The synthesis of metal nanoparticles has many applications in many fields such as biomedical, cosmetic, pharmaceutical, environmental bioremediation, bioimaging, and drug delivery (5). AgNPs have been widely applied in wound dressings, surgical instruments, bone prostheses, cancer therapies, water treatments, bactericidal, biosensing, and electronics (6). Ag-NPs are used in biomedicine for in vitro and in vivo studies of anti-inflammatory drug catalysts, cytotoxicity, and treatment of colon, breast, and gastric cancer. It has been revealed to be active against a variety of bacteria and in the treatment of diabetes (3). Besides that, when used in relatively low quantities, Ag-NPs are considered less harmful to mammalian cells or nontoxic and ecologically friendly. Previous investigations evidenced the significant antibacterial effect of AgNPs on both gram-negative and gram-positive bacteria (7). Ag nanoparticles are effective against over 650 pathogens and having a wide range of activity. As a result, nano silver is now regarded as one of the most promising antibiotic alternatives because it appears to have a high possibility to resolve the problem of multidrug resistance, which is frequently observed in some bacterial strains (8). In different cancer cells, silver nanoparticles play an important role. AgNPs have been proposed to be promising tools for developing more efficient and robust active anticancer therapies (9).

This research aims to synthesize and characterize colloidal silver nanoparticles using the method of laser ablation in liquid to assess the silver nanoparticles...
influence on the activity of bacteria, toxicity on cells of human blood, and cancer cell.

MATERIALS AND METHODS

Production of Ag nanoparticles

Silver metal powder with high purity in de-ionized water was used as the target. Their purity was measured by (Skyray EDX P730, USA) device. These powders were compressed under 10 tons to form a pellet with 1.5 cm in diameter, 3mm in thickness. A pulsed Nd: YAG laser system at 1064 nm wavelength with maximum energy 350 mJ per pulse was used for target ablation. The output pulse duration is 9 ns and the repetition rate 1 Hz. The beam diameter of 2.4 mm was used for laser ablation. The laser was applied with a convex lens with a 120 mm focal length to achieve high laser fluence as shown in figure 1. The setup had been used with an ablation time of 30 mins for the perpetration of AgNPs.

Characterization of synthesized AgNPs

UV-visible (Optima SP-3000DB, Japan) spectrophotometer had been used to confirm the formation of Ag-nanoparticles. The colloidal sample's absorbance spectrum was measured in the 350-600 nm range.

X-ray diffraction (XRD) analysis had been conducted by a Japanese Shimadzu XRD 6000 using CuKα radiation with 1.54060 Å wavelengths operated at 40 kV and 30 mA at a 20 angle pattern. Scanning was carried out in the region of 20°- 80°. The obtained images were matched to the Joint Committee on Powder Diffraction Standards (JCPDS) library to account for the crystalline structure.

Transmission electron microscopy (TEM) (Philips, Germany) testing was achieved to obtained images for the nanoparticles at 0.3 nm resolution with operating voltage of 200 KeV. The test samples for TEM were prepared by spreading a drop of nanoparticles solution onto standard silver (200 meshes) in order to get the morphology properties (shape and size).

Atomic force microscopy (AFM) technique was used to pattern the morphology and surface roughness of the silver nanoparticles by a direct surface image of sample.

Effect of AgNPs on human blood components

The blood samples were collected from twelve healthy volunteers in EDTA tubes to prevent clotting. The procedure was performed by applying AgNPs at different concentrations of 20, 40, 60, and 80 µg/ml to blood samples for one hour at 37°C and compared with untreated blood samples using a complete blood count (CBC) test to assess the possible toxic effect. The CBC was achieved by a programmed completely digital hematology analyzer (Mindary, USA).

Assay for antibacterial activity

A well diffusion method had been employed to inspect the antibacterial action of the prepared AgNPs contra some pathogenic bacteria including Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes and Staphylococcus aureus. On Mueller Hinton agar (Oxoid, England) plates, an inoculum with turbidity equal to that of 0.5 McFarland tube containing 1x10⁸ cfu per ml of bacterial culture to be examined was spread with a sterile swab moistened with bacterial suspension, the agar medium was subsequently punched using an 8 mm cork borer. 0.2 ml of Ag nanoparticles were poured into the wells with diverse concentrations (20, 40, 60, 80) µg/ml, the negative control wells were filled with deionized water. The inoculated plates were then incubated at 37°C overnight. The plates were recorded for zones of inhibition, after the incubation period. Three replicates were conducted for the experiment (10).

Cell cytotoxicity assay

Lung cancer cells (A549) were obtained from the biotech Cell Bank Unit in Iraq. The A549 cell line was assessed.
cultured in Dulbecco's Modified Eagle's Medium (Fisher, USA), enhanced using 10 percent of fetal bovine serum, 1% penicillin/streptomycin, and L-glutamine (Sigma, USA). In a humidified incubator, the cells were cultured at 5% CO2 and 37°C (11).

Anticancer activity of the prepared Ag-nanoparticles on A549 cells was determined by the MTT assay which was used to estimate the cell cytotoxicity. The MTT assay was performed according to a standard protocol. At a density of 1×10^3 cells per ml, cells were cultured in a 96-well plate. After 24 hours, Ag-nanoparticles were prepared in culture media at different concentrations ranging from 0 to 80 µg/ml, and each concentration was tested on cells in triplicate wells for 72 hours. After 72 hours, plates were screened for background Ag-NP absorbance in each well; 10 µl of MTT solution was added to each well and permitted to crystallize at 37°C for 4 hours; and 100 µl of acidified isopropanol was added to each well. To dissolve the formazan crystals, the solution was thoroughly blended. ELIZA reader used to test absorbance at 570nm (12).

**Statistical method**

All the assessments were carried out three times. The data were presented as a mean value, with graphs created in the Microsoft Excel software. Statistical significance was assessed using GraphPad Prism 7 Software.

**RESULTS**

**Synthesis and characterization of Ag nanoparticles**

The current study has revealed that Ag-nanoparticles can be quickly synthesize using pulsed laser ablation on a 99.81% purity silver target that is immersed in deionized water. Rising the number of pulses would contribute to greater output of particle concentrations. The size of the particles reduces as laser pulse repetitions raise. The results of UV-vis spectroscopy analysis showed that the maximum absorbance peak of produced AgNps was 400 nm (Fig. 2).

The XRD analysis of the silver nanoparticles produced using the laser ablation technique is shown in figure (3). It proved that the synthesized silver nanoparticles were nanocrystalline. Once the X-ray light reflects on silver crystal, it results in the creation of many diffraction patterns which reflect the physicochemical characteristics of the crystal structures. With face-centered cubic (FCC) symmetry, all the peaks match pure silver metal. The XRD analysis reveals that the formed Ag-nanoparticles are crystalline. According to the Debye–Scherrer formula, the size of synthesized Ag nanoparticles was 5.2 nm. Four peaks at 20 degrees of (111), (200), (220), and (311) planes matching to 38.2328, 44.5945, 64.5913, and 77.6305 values of Ag are detected and contrasted with the silver file No. 04–0783 in the standard powder diffraction card of JCPDS. The resulting particles are FCC Ag-nanoparticles, according to the XRD report.
TEM image of nanoparticles has exhibited the spherical shape of AgNPs and contained a diameter within the size range of 5-40 nm and the average size nano-particles in sample is about 20 nm diameter. We have utilized the TEM analysis for the purpose of distinguishing the synthesized AgNPs based on morphology, size, and composition (Fig. 4).

Atomic Force Microscopy (AFM) illustrated the spherical shape of AgNPs that had been systematically distributed on the silicon surface. The surface roughness could be seen in 3D (Fig. 5).

The Ag-NPs effects on components of human blood

Some hematological parameters had been determined to study the effect of Ag-nanoparticles on blood composition, including, packed-cell volume (PCV), platelet (PLT), hemoglobin (HGB), white blood cell (WBC), and red blood cell (RBC). The influences of AgNPs on some hematological parameters are presented in table (1), the findings clarified that AgNPs had no significant influence on all blood parameters.

Table 1: Effect of AgNPs on blood parameters

| Blood parameters     | The mean value of AgNPs treated blood sample | Value of untreated blood sample |
|----------------------|---------------------------------------------|---------------------------------|
| Packed-cell volume   | 44%                                         | 43%                             |
| Platelet             | 281000/mm³                                   | 282000/mm³                     |
| Hemoglobin           | 15 g/dL                                      | 14 g/dL                         |
| White blood cell     | 8400/mm³                                     | 8500/mm³                       |
| Red blood cell       | 5.5 million/mm³                              | 5.4 million/mm³                |
Antibacterial activity of AgNPs

The results of this study appeared that Ag nanoparticles have a higher effect against all bacterial isolates at 80 μg/ml concentration, and lower effect at 20 μg/ml concentration while appeared variable results in their effectiveness at 40 and 60 μg/ml concentration (Table 2 and Fig. 6).

Table 2: Antibacterial activity of different concentrations for Ag NPs

| Concentration (µg/ml) | S. aureus | S. pyogenes | E. coli | P. aeruginosa |
|-----------------------|-----------|-------------|---------|--------------|
| 20                    | 8.56      | 9.33        | 14.56   | 16.66        |
| 40                    | 11.55     | 12.27       | 16.33   | 19.16        |
| 60                    | 14.56     | 15.17       | 18.33   | 21.50        |
| 80                    | 19.56     | 20.83       | 23.55   | 26.16        |
| Control (Distilled water) | -         | -           | -       | -            |

Fig. 6: Antibacterial activity of AgNPs at varied concentrations (C= control)

A Study of Anticancer Activity of AgNPs

The anticancer action of AgNPs was examined by the MTT assay. The results indicated a good level of cytotoxic action in 80 μg/ml concentration (Fig. 7).

Fig. 7: Cytotoxic action of AgNPs in lung cancer (cell line A549)

Fig. 8: Cytotoxicity of Ag-nanoparticles compared with control on A549 lung cancer cell line (MTT assay). Statistical significance was assessed using GraphPad Prism 7 Software.
DISCUSSION

The size of the nanoparticles can be affected by various synthesis conditions, such as pulse wavelength, laser fluences, and solvent kind. Nanoparticle behavior, efficacy, safety, and biodistribution are all influenced by their physicochemical properties. Therefore, AgNPs characterization is beneficial for evaluating the essential features of the synthesized particles. UV-vis spectroscopy, XRD, TEM and AFM are among the analytical techniques used to characterize the samples. The most effective and simplest technique for confirming nanoparticle formation is UV-vis spectrophotometry analysis (13). The study by Leersnyder et al., (14) had been observed that small Ag-nanospheres (10–50 nm) have a small absorbance peak close to 400 nm. UV-vis spectroscopy is just a very convenient and dependable technique for the prime characterization of synthesized nanoparticles. It is also simple, easy, sensitive, fast, and requiring only a short measurement time. AgNP has matchless optical properties that allow them to interact strongly with specific light wavelengths. AgNP absorption is affected by dielectric medium, particle size, and chemical environment (15).

XRD was used to analyse the structural properties of the Ag NPs and to identify the phase and crystallinity of AgNPs. The diffraction peaks corresponding to synthesized Ag NPs match well with the pure silver metal (Fig. 3). No extra diffraction peaks corresponding to impurities were detected, indicating that relatively pure AgNPs was obtained and crystalline in nature. FCC symmetry of AgNPs compared with JCPDS number 04-0783 confirms the XRD peaks obtained in the current study.

The AgNPs morphology was analysed by TEM, which provides a clear understanding of the size, size distribution and morphological characters of nanoparticles. Figure 4 shows the Ag NPs are spherical in shape and in the range of 5–40nm. The average size of Ag NPs is 20nm. The particle size determined from the TEM analysis is in good agreement with that of the XRD analysis. These results were consistent with the previously reported study (16).

We have observed the diameter of Se-NPs through the obtained Atomic Force Microscopy (AFM). Figure 5 illustrates the spherical shape of AgNPs that had been well dispersed throughout the silicon surface. The obtained results have been consistent with the gathered data from the TEM analysis, while the achieved outcomes of AFM analysis have been in correspondence with the report of Kazemi et al., (17).

Ag nanoparticles exhibited more efficiency against Gram-negative bacteria contrast with Gram-positive bacteria, the variation in the response of both G-ve and G+ve bacteria to the same prepared Ag nanoparticles arises from the variation between bacteria in the cell wall structure. Since silver ions operate on several targets, it's difficult, if not impossible, to pinpoint the exact cause of death in a silver-treated bacterium (18). The antimicrobial activity of silver nanoparticles is related to four distinct mechanisms: 1- Intracellular structures (ribosomes, mitochondria, vacuoles) and biomolecules (DNA, lipids, protein) are destroyed by penetration of AgNPs into the cell. 2- By generating reactive oxygen species (ROS) and free radicals, AgNPs caused cellular toxicity and oxidative stress, 3- AgNP adhesion to the cell wall and membrane surfaces, and 4- Altered signal transduction processes (19). The antibacterial activity of silver nanoparticles is thought to be due to their smaller particle size, which has a better ability to penetrate bacteria, particularly Gram-negative bacteria (20). Against certain bacteria, AgNPs with a diameter of 5–10 nm has both bacteriostatic and bactericidal properties (21). Domínguez et al., (22) reported that colloidal silver generated ROS in Gram-negative bacteria at lower and higher concentrations but not in Gram-positive bacteria, this could demonstrate why colloidal silver has a lower bactericidal action against Gram-positive bacteria. Similar findings by Kim et al., (23) revealed that the differences in composition, thickness, and structure of cells between Gram-positive and Gram-negative may clarify why E. coli display significant inhibition by Ag nanoparticles, while S. aureus is less inhibited. Silver ions antimicrobial potency is affected by the composition and thickness of the microorganism’s cell walls, as well as variances in the peptidoglycan layer organization (19). Gram-positive bacteria have a thicker cell wall and a negative charge on the peptidoglycan layer permits silver ions to bind to the bacteria and prevents them from functioning, making them more resistant to silver (24).

The shape and size of AgNPs influence their cytotoxicity, for example, it was reported that silver nanoparticles with spherical shapes (30 nm), lengths of 1.5–25 µm, and diameters of 100–160 nm exhibited possible cytotoxic actions on human lung epithelial A549 cells. The most potential explanation is that AgNPs in this shape and size range can directly adhere to cell surfaces and start causing cytotoxicity (25). By increasing ROS formation, DNA destroys, and damaging cellular ultra-structures, AgNPs may stop the growth of tumor cells. Furthermore, AgNPs can trigger tumor cell apoptosis by controlling signaling pathways and inactivating proteins, as well as preventing tumor cell metastasis by blocking angiogenesis within the lesion (13). Silver nanoparticles alter the expression of several signaling molecules complicated in cell proliferation, cell viability, cell survival, apoptosis, and cytotoxicity (26). The cytotoxic activity may be caused by the Ag-nanoparticles interfering with the appropriate functioning of the protein resulting in an alteration of
the cellular chemistry. Some investigations indicate that after silver nanoparticles enter cells, they can cause incomplete unfolding and protein assembly, as well as interact with thiol-rich enzymes. Ag-NPs have been stimulated cytotoxicity due to aggregation in the liver which causes oxidative cell injury (27).

CONCLUSION

Based on the observations, it can be deduced that pulsed laser ablation strong be used to synthesize silver nanoparticles. The findings of the current study indicate that silver nanoparticles display high Gram-negative antibacterial activity compared to Gram-positive bacteria and have a significant in vitro cytotoxic effect against cancer cells, also with limited harmful impacts on RBCs components. Thus further, in the area of biomedical applications, they may be used.

CONFLICT OF INTEREST

Author declares that there is no conflict of interest for this study.

REFERENCES

1. Hamouda, R. A., Hussein, M. H., Abo-elmagd, R. A., Bawazir, S. S. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium Oscillatoria limnetica. Scientific Reports. 2019; 9: 13071.

2. Ndikou, M., Noah, N. M., Andala, D. M., Masika, E. Green synthesis and characterization of silver nanoparticles using Citrulus lanatus fruit rind extract. International Journal of Analytical Chemistry. 2017; 2017: 8108504.

3. Espinosa, J. C. M., Cerriots, R. C., Morales, A. M. R., Guerrero, K. P. S., Contreras, R. A. S., Macías, J. H. Characterization of silver nanoparticles obtained by a green route and their evaluation in the bacterium of Pseudomonas aeruginosa. Crystals. 2020; 10: 395.

4. Anandalakshmi, K., Venugopal, J., Ramasamy, V. Characterization of silver nanoparticles by green synthesis method using Pedadium murex leaf extract and their antibacterial activity. Applied Nanoscience. 2016; 6: 399-408.

5. Gamboa, S. M., Rojas, E. R., Martínez, V. V., Vega-Baudrit, J. Synthesis and characterization of silver nanoparticles and their activity as an antibacterial agent. International Journal of Biosensors & Bioelectronics. 2019; 5(5): 166-173.

6. Liao, C., Li, Y., Tong, S. C. Bactericidal and cytotoxic properties of silver nanoparticles. International Journal of Molecular Sciences. 2019; 20: 449.

7. Dong, Y., Zhu, H., Shen, Y., Zhang, W., Zhang, L. Antibacterial activity of silver nanoparticles of different particle size against Vibrio parahaemolyticus. PLoS ONE. 2019; 14(9): e0223232.

8. Salomoni, R., léo, P., Montemor, A.F., Rinaldi, B. G., Rodrigues, M. F. A. Antibacterial effect of silver nanoparticles in Pseudomonas aeruginosa. Nanotechnology, Science and Applications. 2017; 10: 115-121.

9. Raja, G., Jiang, Y., Suh, J., Kim, H., Ahn, S.H., Kim, T. Micronellular environmental regulation of silver nanoparticles in cancer therapy: a critical review. Cancers. 2020; 12: 664.

10. Jabir, M. S., Nayef, U. M., Jawad, K. H., Taqi, Z. J., Hasoon, B. A., Ahmed, N. R. Porous silicon nanoparticles prepared via an improved method: A developing strategy for a successful antimicrobial agent against Escherichia coli and Staphylococcus aureus. Materials Science and Engineering. 2018; 454: 012077.

11. Pustovalova, M., Alhaddad, L., Smetanina, N., Chigasova, A., Blokhina, T., Chuprov-Netochn, R., et al., The p53–p53BP1-related survival of A549 and H1299 human lung cancer cells after multifractionated radiotherapy demonstrated different response to additional acute X-ray exposure. International Journal of Molecular Sciences. 2020; 21: 3342.

12. Kanwal, Z., Raza, M.A., Riaz, S., Manzoor, S., Tayyeb, A., Sajid, I., et al., Synthesis and characterization of silver nanoparticle-decorated cobalt nanocomposites (Co@AgNPs) and their density-dependent antibacterial activity. Royal Society Open Science. 2019; 6: 182135.

13. Xu, L., Wang, Y., Huang, J., Chen, C., Wang, Z., Xie, H. Silver nanoparticles: synthesis, medical applications and biosafety. Theranostics. 2020; 10(20): 8996-9031.

14. Leersnyder, I.D., Rijken, H., Gelder, L.D., Driessche, I.V., Vermeir, P. High variability in silver particle characteristics, silver concentrations, and production batches of commercially available products indicates the need for a more rigorous approach. Nanomaterials. 2020; 10: 1394.

15. Zhang, X., Liu, Z., Shen, W., Gurunathan, S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International Journal of Molecular Sciences. 2016; 17: 1534.

16. Jayakar, V., Lokapur, V., Nityasree, B. R., Chalannavar, R. K., Lasrado, L. D., Shantaram, M. Optimization and green synthesis of zinc oxide nanoparticle using Garcinia cambogia leaf and evaluation of their antioxidant and anticancer property in kidney cancer (A498) cell lines. Biomedicine. 2021 Jul 7; 41(2): 206-222.

17. Kazemi, M., Akbari, A., Sabouri, Z., Soleimanpour, S., Zarrinarf, H., Khatami, M., et al., Green synthesis of colloidal selenium nanoparticles in starch solutions and investigation of their photocatalytic, antimicrobial, and cytotoxicity effects. Bioprocess and Biosystems Engineering. 2021 Jun;44(6): 1215-1225.

18. Barras, F., Assel, L., Ezraty, B. Silver and antibiotic, new facts to an old story. Antibiotics. 2018; 7: 79.

19. Dakal, T. C., Kumar, A., Majumdar, R. S., Yadav, V. Mechanistic basis of antimicrobial actions of silver nanoparticles. Frontiers in Microbiology. 2016; 7: 1831.

20. Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., et al., The bactericidal effect of silver nanoparticles. Nanotechnology. 2005; 16(10): 2346-2353.

21. Ansari, M. A., Khan, H. M., Khan, A. A., Malik, A., Sultan, A., Shahid, M., et al., Evaluation of antibacterial activity of silver nanoparticles against MSSA and MSRA on isolates from skin infections. Biology and Medicine. 2011; 5(2): 141-146.

22. Dominguez, A. V., Algaba, R. A., Canturri, A. M., Villodres, A. R., Smani, Y. Antibacterial activity of colloidal silver against Gram-negative and Gram-positive bacteria. Antibiotics. 2020; 9: 36.

23. Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., et al., Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007; 3(1): 95-101.

24. Feng, Q. L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. Journal of Biomedical Materials Research. 2000; 52(4): 662-668.

25. Ratam, Z. A., Haidere, M. F., Nurunnabi, Md., Shahriar, S. Md., Ahammad, A. J. S., Shim, Y. Y., et al., Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. Cancers. 2020; 12: 855.

26. Yuan, Y., Zhang, S., Hwang, J., Kong, I. Silver nanoparticles potentiates cytotoxicity and apoptotic potential of camptothecin in human cervical cancer cells. Oxidative Medicine and Cellular Longevity. 2018; 2018: 6121328.

27. Aziz, N., Faraz, M., Sherwani, M. A., Fatma, T., Prasad, R. Illuminating the anti-cancerous efficacy of a new fungal chassis for silver nanoparticle synthesis. Frontiers in Chemistry. 2019; 7: 65.