Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Characterisation and Cytotoxicity Analysis of Green Synthesised Silver Nanoparticles Using Fruit Peel Extract of *Citrus pennivesiculata* (Lush.) Tanaka

Akhila George*, Sheba P. T., Elsam Joseph

Department of Botany and Centre for Research, St. Teresa's College (Autonomous), Ernakulam, Kerala, India.

ARTICLE INFO

Article history:

Received: 26 September, 2023

Revised: 01 December, 2023

Accepted: 04 December, 2023

Published: 30 January, 2024

Keywords:

Silver nanoparticles, *Citrus pennivesiculata*, Cytotoxicity analysis, Transmission electron microscope, Anticancer activity

DOI:

10.25004/IJPSDR.2024.160105

ABSTRACT

Silver nanoparticles were green synthesized using the aqueous extract of *Citrus pennivesiculata* (Lush.) Tanaka, J. fruit peel. The metallic silver was reduced to silver nanoparticles by the action of secondary metabolites in the fruit peel. The characterization of silver nanoparticles was done by UV-visible spectrophotometry, transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). UV-vis spectrophotometry of the silver nanoparticles showed an absorption peak at 435 nm. The TEM analysis showed that the spherical diameter of the particle ranged between 2 to 34 nm. The XRD analysis proved the crystalline nature of the synthesized silver nanoparticles. The FTIR analysis of the synthesized nanoparticles showed the presence of alcohols, phenols, aromatic esters, monosubstituted alkynes, disubstituted alkenes, sulfoxide, amino, and other functional groups. Cytotoxicity and anticancer activity of the green synthesized silver nanoparticles were determined using the mouse fibroblast cell line (L929) and human breast cancer cell line (MCF-7), respectively. The lethal concentration (LC_{50}) of silver nanoparticles on the L929 cell line was found to be 48.521 µg/mL, and that of the MCF-7 cell line was 21.625816 µg/mL. The synthesized silver nanoparticles revealed cytotoxic activity in a dose-dependent manner. The conclusions drawn from this research could be beneficial for nanotechnology-based biomedical applications.

Introduction

Nanotechnology is an innovative multidisciplinary area of science that offers nanoscale materials with applications in the medical, electrical, mechanical, molecular, and structural material fields.[1] The study and application of nanotechnology have been expanding day by day.[2] The application of nanotechnology and novel techniques for creating and utilizing nanomaterials have seen significant developments in the fields of food and agriculture,[3] cancer therapy and imaging,[4,5] and biocompatible nanomaterials have also been utilized as drug, vaccine, and gene vehicles for therapy.[6] However, the contemporary methods for chemically synthesizing nanoparticles have raised concerns about environmental pollution and its potentially dangerous side effects. As a result, the use of specific green chemistry-based methods for the synthesis of nanoparticles is essential because they are non-toxic and environment-friendly.[7] In contrast to chemical processes, biosynthesized nanoparticles are environmentally safe, less expensive, long-lasting, and capable of producing a wide range of shapes (spheres, prisms, or plates) with sizes ranging from 1 to 100 nm.[8] Metallic nanoparticles have drawn much interest from the biomedical industry.[9] Plant-based metallic nanoparticles have also exhibited excellent non-cytotoxic potential against normal human cells, antioxidant activity against free radicals like DPPH, antibacterial activities against gram-positive and gram-negative bacteria, and antifungal activities against *Candida* species.[10]

***Corresponding Author:** Ms. Akhila George

Address: Department of Botany and Centre for Research, St. Teresa's College (Autonomous), Ernakulam, Kerala, India.

Email ✉: georgeakhila16@gmail.com

Tel.: +91-9747280451

Relevant conflicts of interest/financial disclosures: Financial support was received from Mahatma Gandhi University, Kottayam, Kerala, India, as a University Junior Research Fellowship. The authors have no relevant financial or non-financial interests to disclose.

Much attention has been paid to the creation of bio-nanoparticles, particularly silver nanoparticles, because of their potential utility in medical diagnostics,[11,12] therapeutics, antioxidants,[13] anti-bacterial[14] and cytotoxic applications.[15] Drug resistance is unlikely to occur in silver nanoparticles, which implies that the use of silver nanoparticles may be a potential remedy for the issue of drug resistance.[16] Silver nanoparticles in textiles, coatings, food packaging, catheters, oral care, therapeutics, and diagnostics are becoming more prevalent.[17] Reports also suggest that silver and silver-based products are the most widely used and efficient antimicrobial agents to eradicate pathogenic microorganisms.[1] Additionally, the silver nanoparticles also exhibit anti-cancer properties against various cancer cell lines such as MCF-7 breast cancer cells,[18] HCT116 colon cancer cells,[19] prostate cancer cells,[20] HeLa cells[21] and lung carcinoma A549 cells.[22] The green synthesized silver nanoparticles from leaf extracts of *Vitex negundo* L. are of proven efficacy as an anticancer agent against human colon cancer cell line HCT15.[23] The ethanolic extract of *Puncia granatum* L. var. *spinosa* also inhibits human prostate cell proliferation *in-vitro.*[24] To synthesize AgNPs, a wide range of organisms, including bacteria, algae, fungi, and plants, have been investigated.[25] The laborious procedure of maintaining cell culture becomes unnecessary when plants are used to synthesize nanoparticles. A simple mix of an aqueous plant extract and an aqueous silver nitrate solution is enough for the process. The plant extract also acts as a reducing and stabilising agent for synthesising nanoparticles in solution.[26]

Different plant extracts were used in studies on green-synthesized silver nanoparticles (AgNPs), which have revealed the photocatalytic, anti-cancer, anti-bacterial, anti-oxidant and other biological activities of the green synthesized AgNPs.[27] *Citrus* fruits of the Rutaceae family are one of the main tree crops grown worldwide. There are several species in this genus. *Citrus limon* peels, particularly its flavonoid compounds, have been found to contain valuable agents in cancer treatment.[28] The varied applications of citrus fruits in the food industry are related to their exclusive chemical compositions. The phytochemicals derived from citrus fruits have several health benefits, including anti-oxidant,[29] anti-inflammatory,[30] anti-cancer,[31] and anti-microbial[32] properties. They also have beneficial effects on the heart, brain, and liver and in the management of obesity.[33] The secondary metabolites of citrus fruits are linked to a reduced risk of cancer, including gastric cancer, breast cancer, lung tumorigenesis, hematopoietic malignancies, etc.[34] The *Citrus* flavonoids are also linked to an arrest in the advancement of hyperglycaemia by being bound to starch and decreased hepatic gluconeogenesis.[35]

Silver nanoparticles were synthesized from various *Citrus* species. The AgNPs synthesized from *Citrus limon* fruit peel have shown antibacterial activity against many human pathogenic bacteria.[28] The AgNPs from *Citrus sinensis* fruit peel demonstrated good antibacterial activity against gram-positive and Gram-negative bacteria.[36] AgNPs containing *Citrus* limon leaves have also been effective medications for treating human cutaneous wounds.[37] Considerable cytotoxicity was demonstrated by AgNPs synthesized from citrus flavonoids hesperidin, naringin, and diosmin.[38] The green-synthesized AgNPs from citrus juice can be used efficiently against cancer cell lines in combination with other anti-cancer medications.[39]

Citrus peels and their specific secondary metabolites have been identified as anti-cancer agents. These secondary metabolites or phytochemicals have been shown to reduce silver atoms to silver nanoparticles. Each *Citrus* species has a unique phytochemical composition, which implies that their ability to synthesize AgNPs varies from species to species.[40] This work attempts to evaluate the anti-cancer potential of the peels of *Citrus pennivesiculata* (Lush.) Tanaka. *C. pennivesiculata* (Lush.) Tanaka is a medium-sized tree that is commonly found in tropical Asia. An in-depth literature study revealed that only a few research or analytical investigations were done on *C. pennivesiculata* (Lush.) Tanaka. The current research is focused on the green synthesis of silver nanoparticles using the aqueous fruit peel extract of *C. pennivesiculata* (Lush.) Tanaka. The green synthesised nanoparticles have been studied for structural and morphological characteristics and as an anticancer agent. This study can help evaluate the anti-cancer and cytotoxic properties of the plant and its potential therapeutic applications in cancer treatment in the future.

Materials and Methods

Collection of *C. pennivesiculata* (Lush.) Tanaka Fruit Peel and Preparation of Extract

The fruits of *C. pennivesiculata* (Lush.) Tanaka was collected from Chalakkudy, Thrissur, Kerala, India and the plant was authenticated through Kerala Forest Research Institute, Thrissur, Kerala. The specimens were accessed to the KFRI herbarium (KFRI Herbarium Accession Number 18375). The fruit peels were thoroughly cleaned, sliced into tiny pieces, and dried for a week in the shade. Then, the dried samples were coarsely powdered and preserved in a container for further use. Eight grams of the powdered peels were mixed with 80 mL distilled water and left to boil for five minutes in a boiling water bath. The extract obtained was filtered through Whatman No. 1 filter paper and then stored at 4°C for further use.[41]

Green Synthesis of Silver Nanoparticles

The fruit peel extract of *C. pennivesiculata* (Lush.) Tanaka was used for the green synthesis of silver nanoparticles. In 100 mL of I-mM of the aqueous solution of silver nitrate

($AgNO_3$) was prepared in a 250 mL Erlenmeyer flask, and 40 mL of the fruit peel extract was added into the flask for the bio-reduction of the $AgNO_3$ into silver (Ag^+) ions. The mixture was incubated for 24 hours at 37°C.[42] The gradual change in color of the reaction mixture from pale yellow to dark brown was observed after 24 hours of incubation. The reaction mixture of biosynthesized AgNPs was centrifuged at 6000 rpm for about 15 minutes. The resulting AgNPs were washed with double distilled water, followed by washing with distilled ethanol, dried and ground at room temperature for further use.[43]

Characterization of Silver Nanoparticles

UV-visible spectroscopy analysis

The AgNPs were characterized using a UV-visible spectrophotometer (Thermo Scientific Evolution 201/220, USA) to confirm the reduction of $AgNO_3$ to Ag^+. The UV-vis spectrum was recorded in the 200 to 900 nm range with a UV-vis spectrophotometer. The typical optical spectrum of AgNPs ranges between 350 to 550 nm).[44]

Transmission electron microscopy analysis

The morphology of the green synthesized AgNPs was examined using transmission electron microscopy (TEM). A drop of AgNPs solution was loaded on a carbon-coated copper grid, and the solvent evaporated.[28] The high-resolution images were captured using a TEM (Jeol/Jem 2100 Japan).

Fourier transform infrared spectroscopy analysis

The synthesized AgNPs were subjected to Fourier transform infrared spectroscopy (FTIR) (Thermo Nicolet IS50, USA). The analysis was carried out with KBr pellets, and all the measurements were recorded at a wave range of 400 to 4000 cm^{-1}.

X-ray diffraction analysis

The green synthesized silver nanoparticles were coated on a glass substrate and read under an X-ray diffractometer (XRD) (Bruker D8, Germany). The scanning was carried out in the 2θ region from 0 to 90°. The typical crystallite size (L) was calculated using the Scherer equation.

$$L = \frac{K\lambda}{\beta \cos\theta}$$

Where λ used is the wavelength (1.5418 Å) of X-rays, θ represents the Bragg's angle, β is the full-width half maximum (FWHM) of the diffraction profile at 2θ scale, and k is constant, which is equal to unity and related to both to the crystal shape and to how θ is defined.[45]

Cytotoxicity assay

Cytotoxic assays were performed using a mouse fibroblast cell line (L929) and human breast cancer cell line (MCF-7) procured from the National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified Eagle's medium, DMEM (Sigma-Aldrich, USA). The cell line was cultured in a 25 cm^2 tissue culture flask with DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium bicarbonate (Merck, Germany), and an antibiotic solution containing penicillin (100 IU/mL), Streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The MTT assay method was used to determine the viability of cells after direct inspection of the cells using an inverted phase contrast microscope.[21,46]

The percentage of growth inhibition was calculated using the formula;

$$\% \text{ of viability} = \frac{\text{Mean OD of Sample X 100}}{\text{Mean OD of Control}}$$

Cytotoxicity assays were carried out in triplicates, and the results were represented as mean ± standard deviation.

Results

Characterization of Biosynthesized AgNPs

In this study, an aqueous fruit peel extract of *C. pennivesiculata* (Lush.) Tanaka was used for the biosynthesis of AgNPs (Fig. 1). Following the addition of silver nitrate to the aqueous fruit peel extract, a change in color from pale yellow (Fig. 2a) to dark brown was observed (Fig. 2b). This change in color indicated the excitation of surface plasmon resonance by AgNPs.[47] After 24 hours of incubation, the reduction of AgNPs in the reaction mixture was confirmed by UV-vis spectrophotometry. The absorption spectrum of the nanoparticles showed a maximum peak at 435 nm (Fig. 2c).

Microscopic attributes of the AgNPs, including morphology and particle size, were determined through TEM analysis. Fig. 3 shows the TEM images of AgNPs synthesized by the

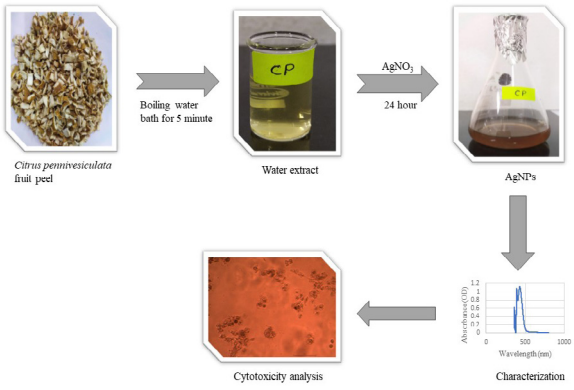

Fig. 1: Flowchart of green synthesis of AgNPs from *C. pennivesiculata* (Lush.) Tanaka fruit peel extract (CP) and its cytotoxicity analysis

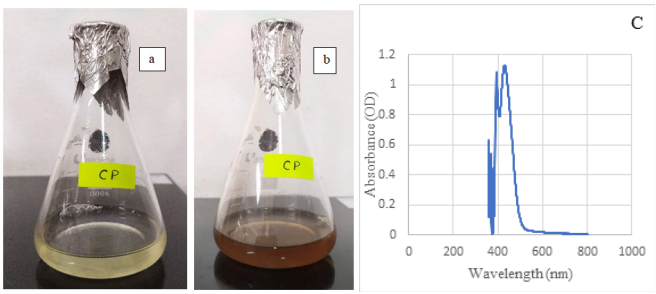

Fig. 2: Synthesis of silver nanoparticles from *C. pennivesiculata* extract (CP extract) (**a-**Initial reaction mixture of CP **b-**Reaction mixture after 24 hours, **c-**UV- visible spectrum of green synthesised AgNPs)

fruit peel extract of *C. pennivesiculata.* As demonstrated by the TEM image, the particles were mainly spherical, with a diameter ranging from 2 to 34 nm.

The XRD pattern of the AgNPs synthesized by *C. pennivesiculata* fruit peels is shown in Fig. 4. The characteristic diffraction peaks at 2θ values 38.115, 44.207, 64.477 and 77.443 represent the 111, 200, 220 and 311 sets of lattice planes, respectively. This agrees with metallic silver's face-centered cubic (FCC) structure (JCPDS File No: 00-004-0783). The average size of synthesized AgNPs calculated from the Scherrer equation was 3 nm.

The FTIR spectroscopy analysis (Fig. 5) was carried out to propose the possible biomolecules responsible for the synthesis of AgNPs. The spectrum showed significant absorption bands corresponding to different chemical groups in the extract containing the synthesized AgNPs.

The green synthesized AgNPs from *C. pennivesiculata* fruit peel were studied for their cytotoxic activity against the L929 cell line (Fig. 6) and MCF-7 cell line (Fig. 7) *in-vitro* by MTT assay at different concentrations. As shown in Tables 1 and 2, the cell viability decreased by increasing the concentration of the AgNPs.

Discussion

Synthesis of silver nanoparticles from aqueous fruit peel extract of *C. pennivesiculata* (Lush.) Tanaka was done in a cost-effective and eco-friendly manner. The presence of AgNPs in the reaction mixture was confirmed by the change in color of the solution from pale yellow to dark brown.[48,49] The UV-visible spectroscopy analysis further verified the formation of AgNPs, which showed an absorption peak at 435 nm. The absorption spectrum of AgNPs typically ranges between 350 to 550 nm.[50] The TEM analysis revealed that the nanoparticles were spherical in shape with the diameter varying from 2 to 34 nm. The varied size of the nanoparticles was due to the presence of different phytochemicals in the plant extract.[51] The shape of the nanoparticles was found to be hexagonal, spherical or triangular, depending upon the phytochemical composition and concentration of the plant extract from which they were generated.[52]

The crystalline characteristics of AgNPs were demonstrated by XRD analysis. The XRD pattern clearly

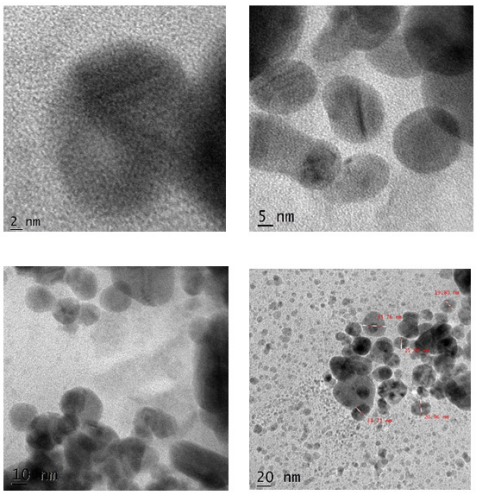

Fig. 3: TEM micrographs of green synthesized AgNPs from *C. pennivesiculata* (Lush.) Tanaka fruit peel

Table 1: Cytotoxic effects of AgNPs synthesized from *C. pennivesiculata* fruit peel on L929 cell line

Sample concentration (µg/mL)	*Viability % (mean ± SD)*	*Inhibitory %*
0 (Control)	100 ± 0.00	0
6.25	85.49 ± 0.19	14.51
12.5	75.21 ± 0.39	24.79
25	68.19 ± 0.32	31.81
50	48.81 ± 0.44	51.19
100	40.25 ± 0.26	59.75

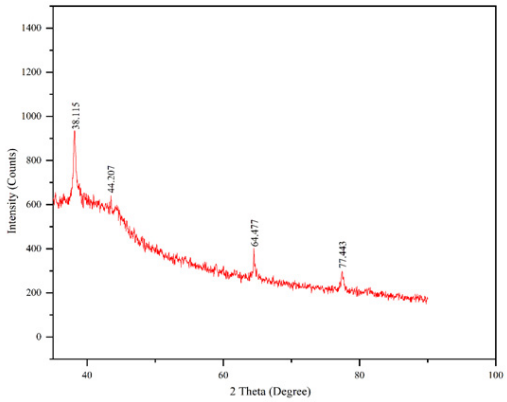

Fig. 4: XRD pattern of green synthesized AgNPs from *C. pennivesiculata* (Lush.) Tanaka fruit peel

showed the prominent peaks at 2θ values 38.115, 44.207, 64.477 and 77.443 related to the reflections from the 111, 200, 220, and 311 lattice planes. These specific XRD peaks are due to the presence of a face-centered cubic (FCC) structure of the crystalline AgNPs.[53] Plane 111 was more prominent compared to that of the other planes, which could be attributed to the overriding orientation of the 111 plane.[1] The average crystallite size of AgNPs calculated using the Scherrer equation was 3 nm, which aligned with the particle size obtained from the TEM image.

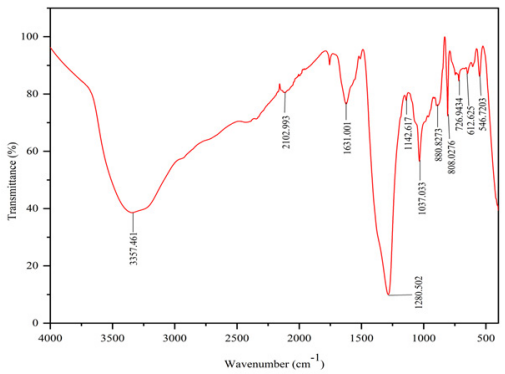

Fig. 5: FTIR spectrum of green synthesized AgNPs from *C. pennivesiculata* (Lush.) Tanaka fruit peel

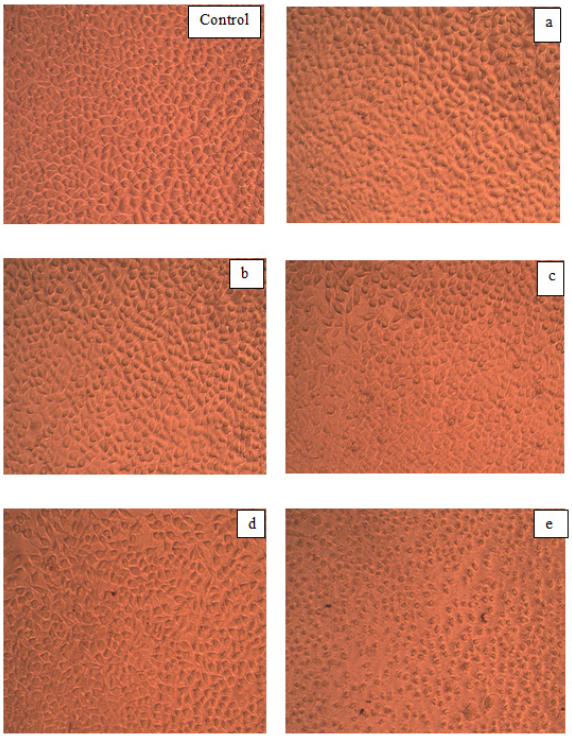

Fig. 6: Cytotoxic effect of AgNPs on mouse fibroblast cell line (L929) at different concentrations. (a) 6.25 µg/mL (b) 12.5 µg/mL (c) 25 µg/mL (d) 50 µg/mL e. 100 µg/mL

FTIR helped to identify the functional groups involved in AgNP synthesis. The broad band at 3357.461 cm^{-1} was due to the O-H stretching of alcohol or phenol groups,[54] whereas the sharp band at 1280.502 cm^{-1} corresponded to the C-O stretching of an aromatic ester. The 2102.993 and 2102.993 cm^{-1} peaks represented the C≡C stretching of monosubstituted alkyne and C=C stretching of disubstituted alkene, respectively. The band at 1142.617 cm^{-1} indicated the C-N stretching vibrations of amino groups, which are commonly present in proteins and act as ligands for AgNPs and increase the stability of AgNPs.[55] The bands at 1037.033 and 546.7203 cm^{-1}

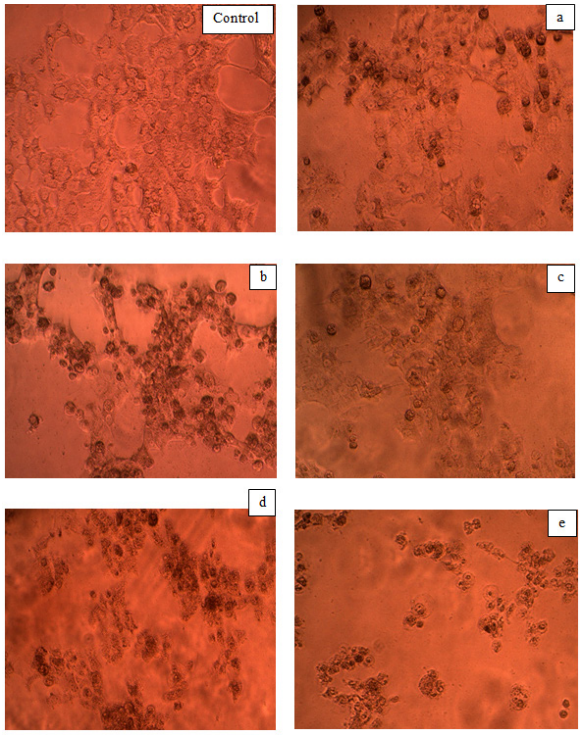

Fig. 7: Cytotoxic effect of AgNPs on human breast cancer cell line (MCF-7) at different concentrations (a) 6.25 µg/mL (b) 12.5 µg/mL (c) 25 µg/mL (d) 50 µg/mL (e) 100 µg/mL

Table 2: Cytotoxic effects of AgNPs synthesized from *C. pennivesiculata* fruit peel on MCF-7 cell line

Sample concentration (µg/mL)	*Viability % (mean ± SD)*	*Inhibitory %*
0 (Control)	100 ± 0.00	0
6.25	72.76 ±0.66	27.24
12.5	66.98 ± 0.64	33.02
25	44.65 ± 0.88	55.35
50	38.16 ± 0.25	61.84
100	25.74 ± 0.59	74.26

are attributed to the S=O stretching of sulfoxide and C-Br stretching of the halo compound, respectively. The bands at 880.8273 and 808.0276 represented the C-H bending. The peaks at 726.9434 cm^{-1} and 612.625 revealed the out-of-plane bending vibrations of the O-H and C-H groups, respectively.[56]

The cytotoxicity of AgNPs on normal cells (L929: mouse fibroblast cells) and cancer cells (MCF-7: human breast cancer cells) were analyzed using the MTT assay method. The lethal concentration (LC_{50}) of AgNPs on L929 was found to be 48.521 µg/mL, and that of MCF-7 was 21.625816 µg/mL. The cytotoxic effect of AgNPs was due to their ability to stimulate reactive oxygen species in the cellular components.[57] Cancerous cells uptake more AgNPs than normal cells which makes them more susceptible to damage.[58] The silver nanoparticles synthesized from *C. pennivesiculata* (Lush.) Tanaka showed promising anticancer activity against human breast cancer

cells (MCF-7) in a dose-dependent manner. It was found that the concentration at which AgNPs affect normal cells was more than twice as high as the concentration at which it affect cancerous cells.

The biosynthesis of AgNPs from *C. pennivesiculata* (Lush.) Tanaka was found to be a suitable method for synthesizing nanoparticles in a large scale. The synthesised AgNPs showed good cytotoxic activity against Human breast cancer cell lines which may suggest its potential use as an anticancer agent. Additional *in-vivo* studies can elucidate their mechanism of action at the molecular level in the human body. If these medical applications of AgNPs are further substantiated by future studies, this may pave the way for their routine use in the treatment of the general population.

Acknowledgement

The authors thank Dr V B Sreekumar, KFRI, Thrissur, Kerala, India, for authentication of the plant sample. We want to extend our sincere thanks to the Sophisticated Test and Instrumentation Centre (STIC), Kochi, Kerala, India, for sample analysis using FTIR, XRD and TEM.

References

1. Kanmani P, Lim ST. Synthesis and structural characterization of silver nanoparticles using bacterial exopolysaccharide and its antimicrobial activity against food and multidrug resistant pathogens. Process Biochemistry. 2013;48(7):1099–1106.
2. Oves M, Ahmar Rauf M, Aslam M, Qari HA, Sonbol H, Ahmad I, *et al*. Green synthesis of silver nanoparticles by *Conocarpus lancifolius* plant extract and their antimicrobial and anticancer activities. Saudi Journal of Biological Science. 2022;29(1):460–471.
3. He X, Deng H, Hwang H min. The current application of nanotechnology in food and agriculture. Journal of Food and Drug Analysis. 2019;27(1):1–21.
4. Misra R, Acharya S, Sahoo SK. Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discovery Today.2010;15(19):842–50.
5. Wang X, Yang L, Chen Z (Georgia), Shin DM. Application of Nanotechnology in Cancer Therapy and Imaging. CA: Cancer Journal of Clinicians. 2008;58(2):97–110.
6. Taran M, Karimi N, Almasi A. Benefits and Application of Nanotechnology in Environmental Science: an Overview. Biointerface Research in Applied Chemistry. 2020;11(1):7860–70.
7. Nagati V, Koyyati R, Donda M, Alwala J, Kudle K rao. Green Synthesis and characterization of Silver nanoparticles from *Cajanus cajan* leaf extract and its antibacterial activity. International Journal of Nanomaterials and Biostructures.2012;2:39–43.
8. Basavegowda N, Rok Lee Y. Synthesis of silver nanoparticles using Satsuma mandarin (*Citrus unshiu*) peel extract: A novel approach towards waste utilization. Materials Letters. 2013;109:31–33.
9. Nadagouda MN, Varma RS. Green and controlled synthesis of gold and platinum nanomaterials using vitamin B2: density-assisted self-assembly of nanospheres, wires and rods. Green Chemistry. 2006;8(6):516–518.
10. Zangeneh MM. Green synthesis and chemical characterization of silver nanoparticles from aqueous extract of *Falcaria vulgaris* leaves and assessment of their cytotoxicity and antioxidant, antibacterial, antifungal and cutaneous wound healing properties. Applied Organometallic Chemistry. 2019;33(9):1–16.
11. El-Sayed IH, Huang X, El-Sayed MA. Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. Nano Letters. 2005;5(5):829–834.
12. Alsamhary KI. Eco-friendly synthesis of silver nanoparticles by *Bacillus subtilis* and their antibacterial activity. Saudi Journal of Biological Science. 2020;27(8):2185–2191.
13. Lim YY, Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. LWT - Food Science and Technology. 2007;40(9):1664–1669.
14. Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S, Yun YS. *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids and Surfaces B: Biointerfaces. 2009;73(2):332–338.
15. Safaepour M, Shahverdi AR, Shahverdi HR, Khorramizadeh MR, Gohari AR. Green Synthesis of Small Silver Nanoparticles Using Geraniol and Its Cytotoxicity against Fibrosarcoma-Wehi 164. Avicenna Journal of Medical Biotechnology. 2009;1(2):111–115.
16. Lim HK, Asharani PV, Hande MP. Enhanced Genotoxicity of Silver Nanoparticles in DNA Repair Deficient Mammalian Cells. Frontiers Genetics. 2012;3:104-108.
17. Stevanović MM, Škapin SD, Bračko I, Milenković M, Petković J, Filipič M, *et al*. Poly(lactide-co-glycolide)/silver nanoparticles: Synthesis, characterization, antimicrobial activity, cytotoxicity assessment and ROS-inducing potential. Polymer. 2012;53(14):2818–2828.
18. Gurunathan S, Han JW, Dayem AA, Eppakayala V, Park JH, Cho SG, *et al*. Green synthesis of anisotropic silver nanoparticles and its potential cytotoxicity in human breast cancer cells (MCF-7). Journal of Industrial and Engineering Chemistry. 2013;19(5):1600–1605.
19. Gurunathan S, Qasim M, Park C, Yoo H, Kim JH, Hong K. Cytotoxic Potential and Molecular Pathway Analysis of Silver Nanoparticles in Human Colon Cancer Cells HCT116. International Journal of Molecular Sciences. 2018;19(8):2269-2272.
20. Firdhouse MJ, Lalitha P. Biosynthesis of silver nanoparticles using the extract of *Alternanthera sessilis*-antiproliferative effect against prostate cancer cells. Cancer Nanotechnology. 2013;4(6):137–143.
21. RamKumar Pandian S, Anjanei D, Raja L, Sundar K. PEGylated silver nanoparticles from *Sesbania aegyptiaca* exhibit immunomodulatory and anti-cancer activity. Materials Research Express. 2018;6:1–15.
22. Gurunathan S, Jeong JK, Han JW, Zhang XF, Park JH, Kim JH. Multidimensional effects of biologically synthesized silver nanoparticles in *Helicobacter pylori, Helicobacter felis*, and human lung (L132) and lung carcinoma A549 cells. Nanoscale Research Letters. 2015;10:35.
23. Prabhu D, Arulvasu C, Babu G, Manikandan R, Srinivasan P. Biologically synthesized green silver nanoparticles from leaf extract of *Vitex negundo* L. induce growth-inhibitory effect on human colon cancer cell line HCT15. Process Biochemistry. 2013;48(2):317–324.
24. Sineh Sepehr K, Baradaran B, Mazandarani M, Khori V, Shahneh FZ. Studies on the Cytotoxic Activities of *Punica granatum* L. var. *spinosa* (Apple Punice) Extract on Prostate Cell Line by Induction of Apoptosis. International Scholarly Research Notices. 2012;2012:1–6.
25. Patil MP, Palma J, Simeon NC, Jin X, Liu X, Ngabire D, *et al*. Sasa borealis leaf extract-mediated green synthesis of silver–silver chloride nanoparticles and their antibacterial and anticancer activities. New Journal of Chemistry. 2017;41(3):1363–1371.
26. Kumar V, Yadav SC, Yadav SK. *Syzygium cumini* leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization. Journal of Chemical Technology and Biotechnology. 2010;85(10):1301–1309.
27. Patra JK, Baek KH. Green synthesis of silver chloride nanoparticles using *Prunus persica* L. outer peel extract and investigation of antibacterial, anticandidal, antioxidant potential. Green Chemistry Letters Reviews. 2016;9(2):132–142.
28. Alkhulaifi MM, Alshehri JH, Alwehaibi MA, Awad MA, Al-Enazi NM, Aldosari NS, *et al*. Green synthesis of silver nanoparticles using Citrus limon peels and evaluation of their antibacterial and cytotoxic properties. Saudi Journal of Biological Sciences. 2020;27(12):3434–41.
29. Singh J, Sood S, Muthuraman A. *In-vitro* evaluation of bioactive

compounds, anti-oxidant, lipid peroxidation and lipoxygenase inhibitory potential of *Citrus karna* L. peel extract. Journal of Food Science and Technology. 2014;51(1):67–74.
30. Yoshizaki N, Fujii T, Masaki H, Okubo T, Shimada K, Hashizume R. Orange peel extract, containing high levels of polymethoxyflavonoid, suppressed UVB-induced COX-2 expression and PGE2 production in HaCaT cells through PPAR-γ activation. Experimental Dermatology. 2014;23:18–22.
31. Chang L, Jia S, Fu Y, Zhou T, Cao J, He Q, *et al*. Ougan (*Citrus reticulata* cv. Suavissima) flavedo extract suppresses cancer motility by interfering with epithelial-to-mesenchymal transition in SKOV3 cells. Chinese Medicine. 2015;10:1–10.
32. Espina L, Somolinos M, Lorán S, Conchello P, García D, Pagán R. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. Food Control. 2011;22(6):896–902.
33. Lv X, Zhao S, Ning Z, Zeng H, Shu Y, Tao O, *et al*. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chemistry Central Journal. 2015;9:68-71.
34. Tanaka T, Tanaka T, Tanaka M, Kuno T. Cancer chemoprevention by citrus pulp and juices containing high amounts of β-cryptoxanthin and hesperidin. Journal of Biomedicineand Biotechnology. 2012;2012:1–10.
35. Shen W, Xu Y, Lu YH. Inhibitory effects of Citrus flavonoids on starch digestion and antihyperglycemic effects in HepG2 cells. Journal of Agricultural and Food Chemistry. 2012;60(38):9609–9619.
36. Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. Spectrochim Acta Part A: Molecular and Biomolecular Spectroscopy. 2011;79(3):594–8.
37. Abbasi N, Ghaneialvar H, Moradi R, Zangeneh MM, Zangeneh A. Formulation and characterization of a novel cutaneous wound healing ointment by silver nanoparticles containing *Citrus limon* leaf: A chemobiological study. Arabian Journal of Chemistry. 2021;14(7):1-5.
38. Sahu N, Soni D, Chandrashekhar B, Satpute DB, Saravanadevi S, Sarangi BK, *et al*. Synthesis of silver nanoparticles using flavonoids: hesperidin, naringin and diosmin, and their antibacterial effects and cytotoxicity. International Nano Letters. 2016;6(3):173–181.
39. Kumar A, Luhach N, Chauhan R, Badgujar H, Soni S, Chhokar V, *et al*. Synthesis and Characterization of Silver Nanoparticles Using Citrus Fruit Juice for Evaluation of Anticancer Activity Against Colo-205 Cell Lines. Journal of Nanoscience and Nanotechnology. 2021;21(6):3580–3587.
40. Ali S, Chen X, Ahmad S, Shah W, Shafique M, Chaubey P, *et al*. Advancements and challenges in phytochemical-mediated silver nanoparticles for food packaging: Recent review (2021–2023). Trends in Food Science and Technology. 2023;141:104197.
41. Vankar PS, Shukla D. Biosynthesis of silver nanoparticles using lemon leaves extract and its application for antimicrobial finish on fabric. Applied Nanoscience. 2012;2(2):163–168.
42. Adebayo-Tayo B, Akinsete T, Odeniyi O. Phytochemical Composition and Comparative Evaluation of Antimicrobial Activities of the Juice Extract of *Citrus aurantifolia* and its Silver Nanoparticles. Nigerian Journal of Pharmaceutical Research.2016;12:59–64.
43. Gul S, Ismail M, Khan MI, Khan SB, Asiri AM, Rahman IU, *et al*. Novel synthesis of silver nanoparticles using melon aqueous extract and evaluation of their feeding deterrent activity against housefly *Musca domestica*. Asian Pacific Journal of Tropical Disease. 2016;6(4):311–316.
44. Singh SS, Gunagambhire V. Biosynthesis, Characterization, and Antidermatophytic Activity of Silver Nanoparticles Using Raamphal Plant (*Annona reticulata*) Aqueous Leaves Extract. Indian Journal of Materials Science. 2014;2014:1–5.
45. Ismail M, Khan MI, Akhtar K, Khan MA, Asiri AM, Khan SB. Biosynthesis of silver nanoparticles: A colorimetric optical sensor for detection of hexavalent chromium and ammonia in aqueous solution. Physica E: Low-Dimensional Systems and Nanostructures. 2018;103:367–376.
46. Talarico LB, Zibetti RGM, Faria PCS, Scolaro LA, Duarte MER, Noseda MD, *et al*. Anti-herpes simplex virus activity of sulfated galactans from the red seaweeds *Gymnogongrus griffithsiae* and *Cryptonemia crenulata*. International Journal of Biological Macromolecules. 2004;34(1):63–71.
47. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and Biosystems Engineering. 2009;32(1):79–84.
48. Skiba MI, Vorobyova VI. Synthesis of Silver Nanoparticles Using Orange Peel Extract Prepared by Plasmochemical Extraction Method and Degradation of Methylene Blue under Solar Irradiation. Advances in Materials Science and Engineering. 2019;2019:1–8.
49. Khane Y, Benouis K, Albukhaty S, Sulaiman GM, Abomughaid MM, Al Ali A, *et al*. Green Synthesis of Silver Nanoparticles Using Aqueous Citrus limon Zest Extract: Characterization and Evaluation of Their Antioxidant and Antimicrobial Properties. Nanomaterials. 2022;12(12):1–20.
50. Shivakumar Singh P, Vidyasagar GM. Biosynthesis, Characterization, and Antidermatophytic Activity of Silver Nanoparticles Using Raamphal Plant (*Annona reticulata*) Aqueous Leaves Extract. Indian Journal of Material Science. 2014;2014:1–5.
51. Satpathy S, Patra A, Ahirwar B, Delwar Hussain M. Antioxidant and anticancer activities of green synthesized silver nanoparticles using aqueous extract of tubers of *Pueraria tuberosa*. Artif Cells Nanomedicine and Biotechnology. 2018;46(sup3):71–85.
52. Joy Prabu H, Johnson I. Plant-mediated biosynthesis and characterization of silver nanoparticles by leaf extracts of *Tragia involucrata, Cymbopogon citronella, Solanum verbascifolium and Tylophora ovata*. Karbala Internationa Journal of Modern Science. 2015;1(4):237–246.
53. Wypij M, Jędrzejewski T, Trzcińska-Wencel J, Ostrowski M, Rai M, Golińska P. Green Synthesized Silver Nanoparticles: Antibacterial and Anticancer Activities, Biocompatibility, and Analyses of Surface-Attached Proteins. Frontiers in Microbiology. 2021;12:1–17.
54. Kumar B, Smita K, Cumbal L, Debut A. Green synthesis of silver nanoparticles using Andean blackberry fruit extract. Saudi Journal of Biological Science. 2017;24(1):45–50.
55. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, *et al*. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Nanotechnology. 2007;18:285–290.
56. Bahrami-Teimoori B, Nikparast Y, Hojatianfar M, Akhlaghi M, Ghorbani R, Pourianfar HR. Characterisation and antifungal activity of silver nanoparticles biologically synthesised by *Amaranthus retroflexus* leaf extract. Journal of Experimental Nanoscience. 2017;12(1):129–139.
57. Venugopal K, Ahmad H, Manikandan E, Thanigai Arul K, Kavitha K, Moodley MK, *et al*. The impact of anticancer activity upon Beta vulgaris extract mediated biosynthesized silver nanoparticles (ag-NPs) against human breast (MCF-7), lung (A549) and pharynx (Hep-2) cancer cell lines. Journal of Photochemistry and Photobiology. 2017;173:99–107.
58. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nature Reviews Cancer. 2011;11(2):85–95.

HOW TO CITE THIS ARTICLE: George A, Sheba PT, Joseph E. Characterisation and Cytotoxicity Analysis of Green Synthesised Silver Nanoparticles Using Fruit Peel Extract of *Citrus pennivesiculata* (Lush.) Tanaka. Int. J. Pharm. Sci. Drug Res. 2024;16(1):29-35. **DOI:** 10.25004/IJPSDR.2024.160105