Probe Sonication-Based Green Synthesis AgNPs Using *P. Guajava* Leaves Extract and Anticancer Behavior MCF-7 Cell Lines

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ABSTRACT

Purpose: The present work is devoted to the green and probe sonication-based fabrication of AgNPs using the leaves extract of P. guajava. **Materials and Methods:** Various analytical techniques have been used to characterize the synthesized AgNPs, which examine significant chemical and physical characteristics of AgNPs. **Results:** The green synthesized AgNPs were used against a breast cancer cell line (MCF-7). The FTIR study indicates the presence of O-H, C=O, C=C, and C-Ag bonds on the surface of AgNPs. The XRD peaks of AgNPs were found to be 27.8º, 32.2º, 38.1º, 44.4º, 45.6º, 57.1º, 64.5º, and 77.9º respectively. The MTT assay of AgNPs showed increased cytotoxicity against MCF-7 cell lines with the half maximal inhibitory concentration of 24.44 μg/mL. **Conclusion:** The findings suggest that AgNPs synthesized from *P. guajava* leaves exhibit significant anticancer activity against MCF-7 cell lines, highlighting their potential for therapeutic applications.

Keywords: AgNPs, Anticancer Activity, Green and probe ultrasonic-based synthesis, Leaves Extract, *P. guajava*.

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INTRODUCTION

Nanotechnology, a fast-growing field, will be one of the main forces behind the upcoming technological revolution of the 21st century. NPs which may be of any shape, have at least one 100 nm or less dimension.[1-3] Nanoscale structures in gene sequencing, medication delivery, and diagnostics provide the nanotechnology interface between biomedical engineering and biotechnology.[4,5] Because of their unique physicochemical properties' NPs have received much attention in biological applications. Destructive or constructive methods can be used to create metal-based NPs. Metal-based NPs such as Aluminum (Al), Cobalt (Co), Cadmium (Cd), Gold (Au), Copper (Cu), Iron (Fe), Lead (Pb), Zinc (Zn), and Silver (Ag) are frequently used in NPs synthesis.[3] The use of silver nanoparticles, or AgNPs, is increasing throughout a range of industries, such as food, cosmetics, medicine, the environment, and healthcare, owing to their distinct physical and chemical properties.[4] Green synthesis offers sustainable, affordable, eco-friendly, and non-toxic methods for synthesizing Nanoparticles (NPs). It involves extracts from plant parts and biometabolites from organisms, making it a simple, sustainable alternative to conventional processes.[5] In green synthesis, the biologically active components present in the extract behaves as capping and reducing agents during the synthesis of NPs.[6,7] AgNPs have been synthesized in several ways, including reducing silver compounds in solution, to meet demand in bioscience, biotechnology, and other fields.[6] Ag is considered to have anticancer effects. Natural reagents, including sugars, biodegradable films, and leaf extracts, were used to fabricate NPs, making them crucial in nanotechnology research.[7] Cancer is the world's deadliest disease. New anticancer medications are expected to be used to treat the cancer.[8] MNPs are an option for chemotherapeutic drugs and are on the rise in disease research. MNPs have the potential for use in many applications.[9] AgNPs show essential anticancer properties. These NPs show anticancer activity in different ways. One of them is to start the release of important cell components, causing damage and finally dead cells.[10]

The guava plant, or *Psidium guajava* (*P. guajava*), is a small, evergreen shrub member of the Myrtaceae family. Antioxidants,

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antibacterial, anti-inflammatory, and anticancer features are some of the possible health advantages of guava that are attributed to its chemical constitution, namely the presence of vitamins, minerals, and phytochemicals.[11] Since it contains these bioactive chemicals, guava leaf extract is often employed in the green synthesis of Nanoparticles (NPs), providing capping and reducing agents that assist in the formation and stability of NPs.[12] In the present work, AgNPs have been synthesized using *P. guajava* leaf extract and used as anticancer agents in treating breast cancer MCF-7 cell lines.[6] AgNPs show potential for treating breast cancer via selective targeting, improved cytotoxicity, inhibiting cell migration and invasion, anti-metastatic features, etc. The present study demonstrated an efficient and eco-friendly synthesis of AgNPs and can be applicable for large-scale production of AgNPs. The objective of green synthesis of Ag NPs is to produce environmentally benign, sustainable nanoparticles for implementation in biotechnology, medicine, and environmental remediation. Excellent antimicrobial capabilities, use of renewable sources in the synthetic process, and a reduction of harmful chemicals are all advantages of this inexpensive and efficient method.[6-8] MCF-7 is widely used for breast cancer cell lines that have been propagated for many years by several groups. MCF-7 cells test the efficacy and toxicity of potential anti-cancer drugs, including chemotherapeutic agents and targeted therapies. MCF-7 breast cancer cells express Estrogen Receptors (ER), making them responsive to estrogen. These cells have a high proliferative rate, so they can quickly grow.[11,14]

MATERIALS AND METHODS

Materials

All chemicals used in this work were of AR grade. Guava Leaves (*P. guajava),* Double Distilled Water (DDW), Silver Nitrate ($AgNO_3$), and Dimethyl Sulfoxide (DMSO) were used in the present work.

Preparation of Leaf Extract

P. guajava leaves (Figure 1) were collected from nearby Dehradun, Uttarakhand. Plant authentication was done by the BSI (Botanical Survey of India) Dehradun, Uttarakhand with accessioned no. 1523. The fresh leaves of *P. guajava* were collected and thoroughly washed in DDW. These leaves were cut into fine pieces and then crushed with a mortar pestle. 10 g of guava leaves were weighed and dissolved in 100 mL of DDW and then sonicated for 30 min. Now, the content, and the filtrate (extract), were preserved at 4°C for further use.[9,16]

Synthesis and characterization of AgNPs

A 0.1 M $AgNO_3$ solution was used to synthesize AgNPs. Different concentrations (1, 2, 3, and 4 mL) were added separately to 10 mL of 0.1 M $AgNO_3$ solution to synthesize Ag-1, Ag-2, Ag-3, and Ag-4. The resulting samples were incubated in a dark chamber for 3 hr., to reduce photo-activation of $AgNO_3$. The reduction of Ag^+ to metallic Ag conversion took place by the color change of the solution from light yellowish to dark brown, which initially specifies the formation of AgNPs. The produced AgNPs were sonicated for 2 min in a probe sonicator and centrifuged at 2000 rpm for 30 min. The residues were washed with DDW and then kept in the oven at 60°C for 2 hr.[9,16,20] Dried powder was collected and was further evaluated for various parameters. Different analytical methods such as XRD (X-ray diffraction), FTIR (Fourier-Transform Infrared Spectroscopy), SEM (Scanning Electron Microscopy), and TEM (Transmission Electron Microscopy) were used for the characterization of AgNPs.

Figure 1: Collected leaves of *P. guajava.*

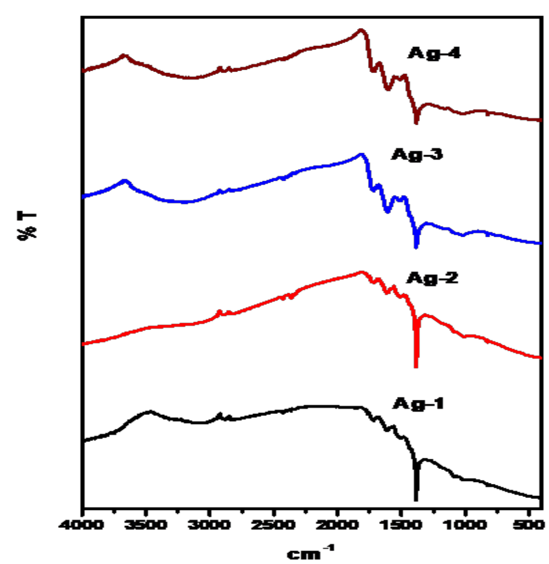

Figure 2: FTIR Spectra of AgNPs (at different concentrations).

Anticancer activity (MTT-ASSAY)

The silver nanoparticles' cytotoxicity property was intended to be achieved using an MTT assay method. The cytotoxicity of the sample was conducted at MCF-7 (NCCS Pune) cell was analyzed with MTT assay 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.[13] In Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% FBS (Fetal bovine serum), and 1% antibiotic solution with 5% CO_2, the 96 well plates technique was used over the cells (1000 cells/well). Ensuingly, the cells were conducted from the formulations (varying concentrations were formulated in a complete medium). Untreated, cells were considered as control, and cells without MTT were referred to as blank. The final concentration containing the 250 µg/mL of MTT solution was treated to the cell culture for incubation of 24 hr followed by 2 more hours. After incubation, the supernatant solution was removed, and the obtained cell layer matrix was dissolved in DMSO (100 mL) and analyzed with an Elisa Plate reader (iMark, Biorad, USA) at the wavelength of 540 nm. GraphPad Prism 6 software were used to determine the IC_{50} of the sample. Images were taken with a camera.[14,15]

RESULTS

Characterization of AgNPs

The FTIR spectra of AgNPs (prepared at different concentrations) are presented in Figure 2.[16,17] The characteristics peaks of Ag-1 were assigned at 3314, 2881, 2827, 1762, 1684, 1569, and 823 cm^{-1}. For Ag-2, the FTIR peaks have been assigned at 2882, 1717, 1615, 1539, and 840 cm^{-1}. The FTIR peaks of 3195, 1718, 1609, 1523, and 960 cm^{-1} were assigned for Ag-3. In the case of Ag-4, the FTIR peaks were found to be 3132, 2882, 1715, 1606, 1528, and 945 cm^{-1}. The XRD patterns of AgNPs at different concentrations are presented in Figure 3. These patterns indicate the semicrystalline

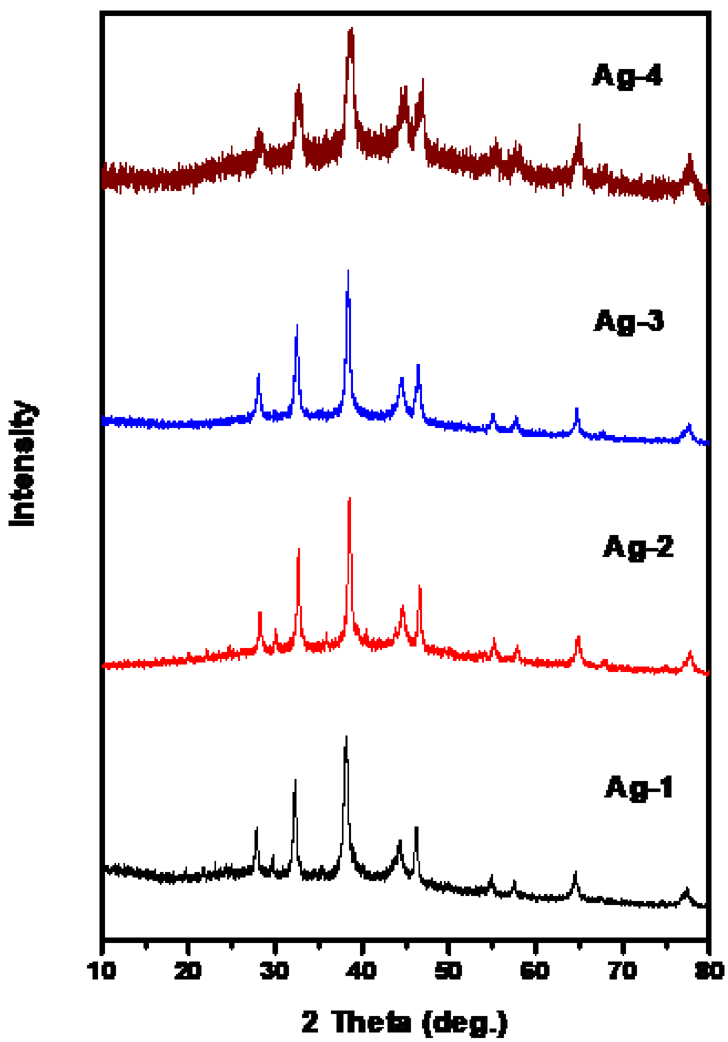

Figure 3: XRD pattern of AgNPs (at different concentrations).

nature of Ag-1, Ag-2, and Ag-3 and the amorphous nature of Ag-4. The common characteristic peaks of AgNPs were found to be 27.8°, 32.2°, 38.1°, 44.4°, 45.6°, 57.1°, 64.5°, and 77.9°. The surface morphology of *P. guajava* leaves extracts mediated with AgNPs was examined by SEM and illustrated in Figure 4. The TEM images of highly efficient AgNPs (Ag-3) are presented in Figure 5.

Anticancer activity

The anticancer behavior of synthesized AgNPs was investigated via the MCF-7 cell line. A remarkable inhibitory effect of AgNPs was observed after the incubation period. Figure 6 presents the anticancer assay of AgNPs. The study was conducted at different concentrations of Ag NPs such as 1, 10, 50, 100, 250, 500, and 1000 µg/mL (Figure 7). With this figure it can be observed that maximum cell viability for cancer cells was obtained for the control sample. With the treatment, a considerable decrease in cell viability was determined. When cells cultured were untreated with AgNPs, 100% cell viability was observed. MCF-7 cell lines were treated with different concentrations of *P. guajava* leaves extract-assisted AgNPs (Ag-1, Ag-2, Ag-3, Ag-4). The IC_{50} values of all the AgNPs against MCF-7 cell lines were 34.99 µg/mL, 32.7 µg/mL, 24.44 µg/mL, and 54.83 µg/mL respectively.[18] According to the results, the Ag-3 formulation emerged as the best formulation.

DISCUSSION

FTIR spectroscopy measures absorption wavelengths in samples to identify molecular structure, used in fields like food science, forensic medicine, clinical medicine, environmental analysis, and analytical chemistry. The FTIR technique has been used to detect the presence of different bonds or functional groups on the surface of AgNPs.[16-18] The assigned peaks of Ag-1 reveal the presence of O-H (str.; water absorbed), C-H (str.; hydrocarbon chains); C=O (str.), C=C (str.), O-H (def.), C-Ag, etc. bonds.[11,12] The peaks of Ag-2 are related to C-H (str.), C=O, C=C, O-H, and C-Ag bonds.[16-18] The FTIR peaks of Ag-3 indicate the presence of O-H, C-H, C=O, C=C, C-Ag, etc. bonds. On the other hand, the FTIR peaks of Ag-4 reveal the presence of O-H, C=O, C=C, C-Ag, etc. bonds.[12,19] X-ray Diffraction (XRD) is a non-destructive technique used in fields like physical, chemical, pharmaceuticals, and forensics to analyze materials' structure, composition, and properties using the Bragg Law. The obtained XRD peaks are related to the 220, 111, 200, 124, 240, 220, and 31 planes, respectively.[20-24] Using electron beams to study a sample's surface, a Scanning Electron Microscope (SEM) concentrates

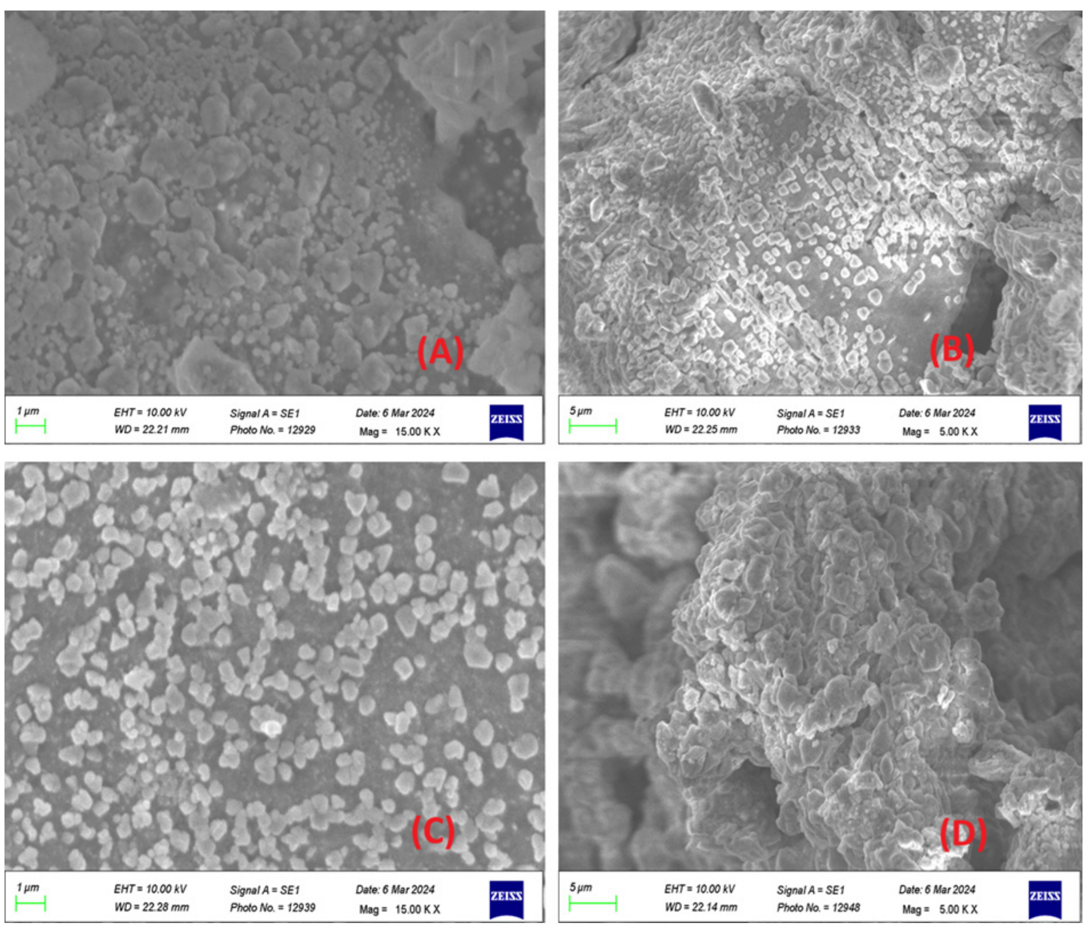

Figure 4: SEM images of AgNPs (at different concentrations).

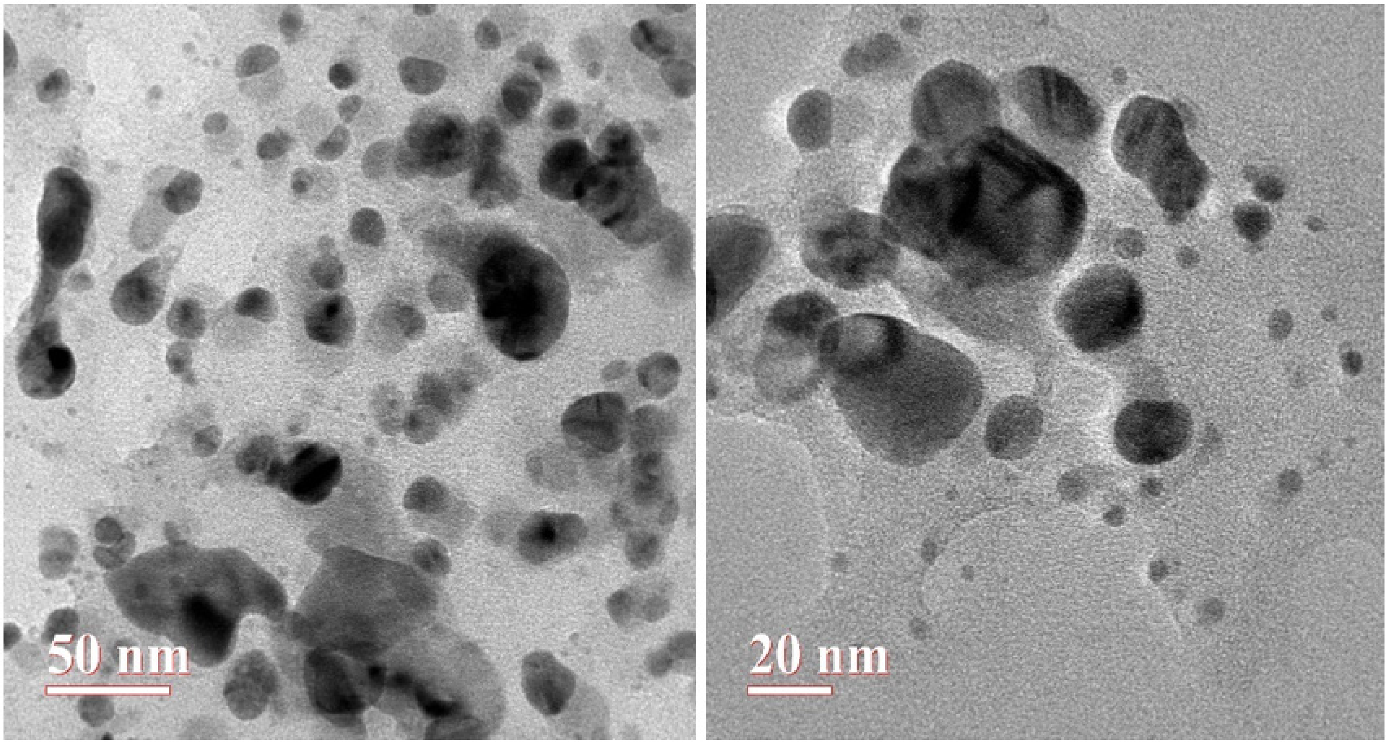

Figure 5: TEM images of Ag-3.

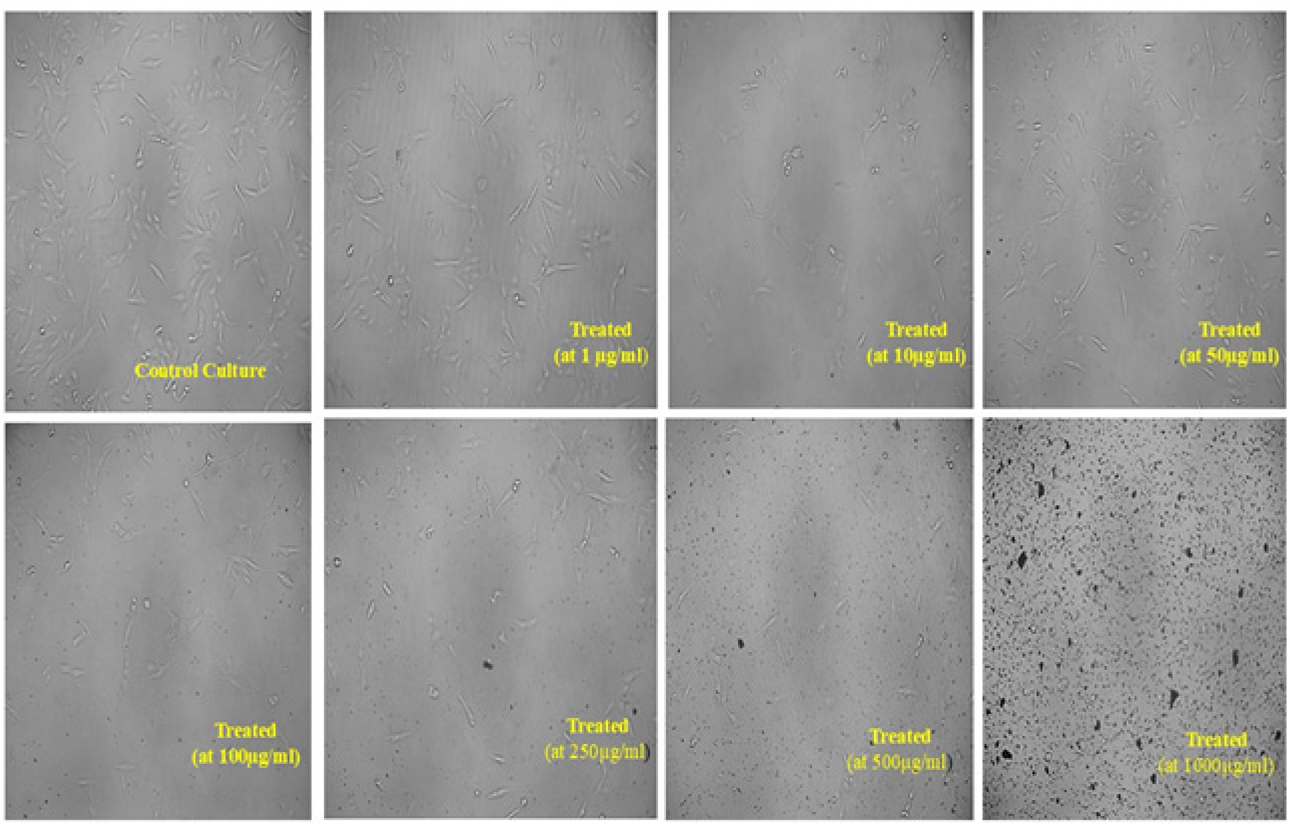

Figure 6: Effect of concentration of AgNPs on MCF-7 cells (After 24 hr).

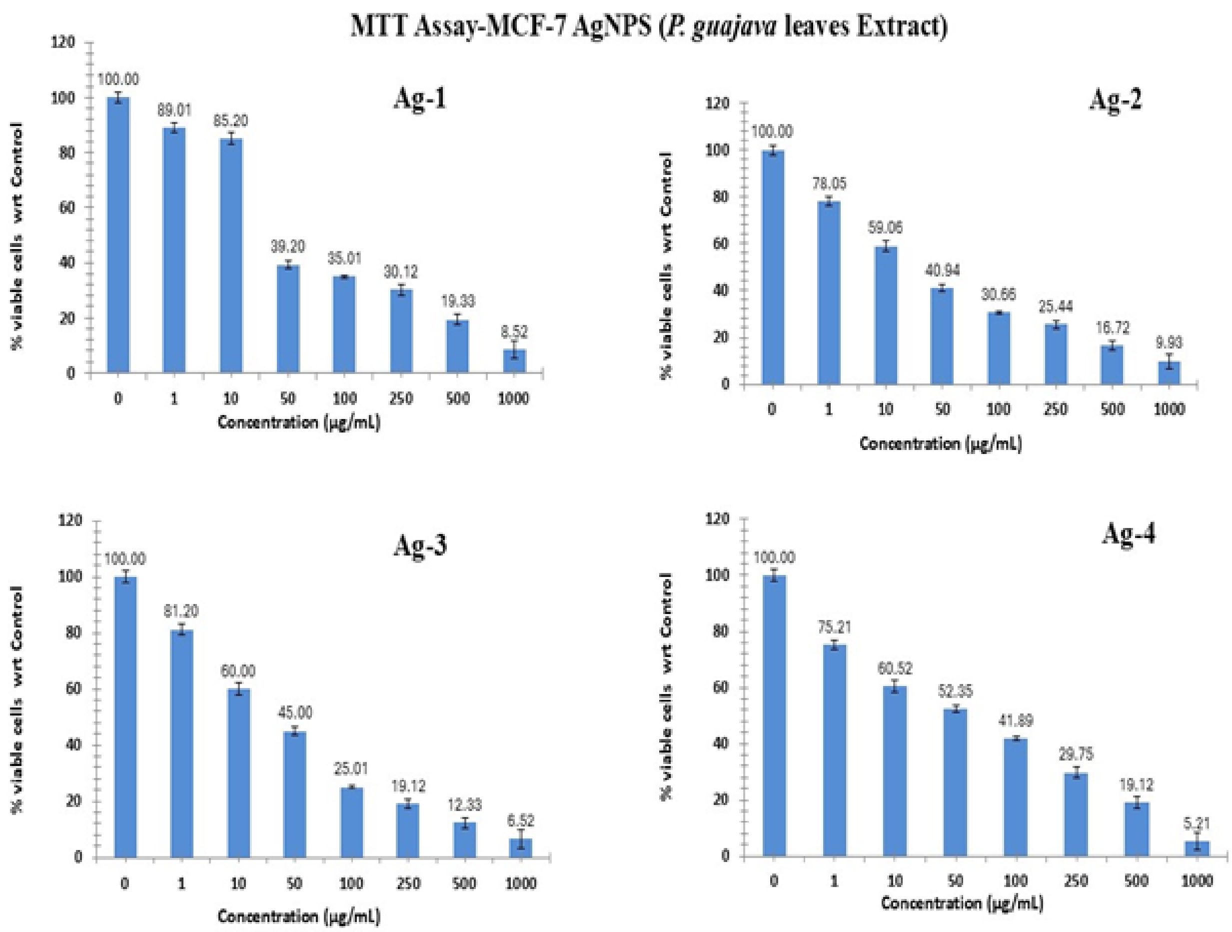

Figure 7: MTT assay of AgNPs (different concentrations) against MCF-7 cell line against *P. guajava* leaves extracts. All values are represented at ±SEM, *n*=4.

on the atoms and generates signals. Nanometre-scale sample imaging, solid-state analysis, purity, composition, and surface topography are all possible with SEM. The SEM images of Ag-1 show spherical morphology that transforms to semi-spherical (Ag-2 and Ag-3). The Ag-4 shows irregular shape morphology with agglomeration. The TEM images of highly efficient AgNPs (Ag-3) also reveal semi-spherical morphology. Figure 7 represents anticancer activity via MTT assay. Results obtained for 1ug/ml concentration revealed that the number of cancer cells decreased with the treatment. Further, a remarkable decrease in cell viability was found for samples containing 1000μg/ml AgNPs. Thus, the study showed that the concentration of AgNPs shared a proportional relationship with cell viability. Cell viability was studied at different concentrations for the *P. guajava* leaves extracts in MCF-7 cell lines and at the concentration of 1000μg/mL maximum viable cells were inhibited. The *P. guajava* leaves extract has confirmed its role as an anticancer drug.[10,20]

CONCLUSION

The synthetic approach of AgNPs based on green and probe sonication demonstrated it was easy to use, efficient, and sustainable. Characterization techniques (FTIR, SEM, XRD, and TEM) show considerable physical and chemical properties of AgNPs. High cytotoxicity efficiency and anticancer activity of AgNPs against MCF-7 breast cancer cells showed favorable results. These NPs may reduce adverse effects during the rigorous course of therapy with enhanced effectiveness. The study found a significant decrease in cancer cell viability with concentrations of AgNPs, indicating a proportional relationship between the concentration and cell viability.

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ABBREVIATIONS

NPs: Nanoparticles; **MNPs:** Metal nanoparticles; **AgNPs:** Silver Nanoparticles; **MCF-7:** Michighian Cancer Foundation; **μg/mL:** Microgram per Milliliter; **IC_{50}:** Half maximal inhibitory concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

In this research, we have performed the green synthesis of Ag NPs using leaves extract of *P. guajava*. These NPs were successfully characterized using different analytical methods and used anticancer agents against the MCF-7 cell line. AgNPS exhibited improved cytotoxicity against MCF-7 cell lines in the MTT experiment.

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