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Biosynthesis of Silver Nanoparticles Using *Carica Papaya* Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity Against Cancer Cell Lines

Inchara D[1], Sibi Sanjith[2] , Buvaneshwari S[3]

Department of life science, Garden City University

Incharad234@gmail.com

Assistant Professor, Department of life science, Garden City University

Buvaneshwari.s@gcu.edu.in

Abstract

This study explores a green, eco-friendly approach for synthesizing silver nanoparticles (AgNPs) using aqueous leaf extract of *Carica papaya*. The plant extract acts as both a reducing and stabilizing agent, leading to the formation of biologically active nanoparticles. Characterization of the synthesized nanoparticles was performed using UV–Visible spectroscopy, FTIR, XRD, and SEM, confirming the presence of stable, spherical silver nanoparticles with a crystalline face-centered cubic structure averaging around 200 nm in size. Phytochemical analysis revealed the presence of bioactive compounds—such as flavonoids, phenols, and alkaloids—that contribute to the reduction and stabilization processes. The Cp-AgNPs Ag-NPs demonstrated notable antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with stronger effects observed against Gram-negative strains. Furthermore, the antiproliferative potential of Cp-AgNPs was assessed using the MTT assay on various human cancer cell lines (MCF-7, A549, HCT-116, and Caco-2), showing a dose-dependent decrease in cell viability. These findings highlight the potential of *Carica papaya*-mediated silver nanoparticles as promising candidates for antimicrobial and anticancer applications, aligning with sustainable practices in nanomedicine.

Key words: Ag-NPs (Silver nanoparticles), *Carica* papaya, MTT Assay , MCF-7, A549, HCT-116, Caco-2

1. INTRODUCTION

1.1 Introduction on nanoparticles

Biosynthesized nanoparticles are gaining popularity for their eco-friendly production and broad range of biological uses. This study presents a simple, affordable, and environmentally safe method for creating silver nanoparticles using *Carica papaya* leaf extract, which acts as both a natural reducing and stabilizing agent. The resulting silver nanoparticles (Cp-AgNPs) were analyzed using techniques like UV–Vis, FTIR, XRD, SEM, and DLS to confirm their structure and properties. Phytochemical screening showed that the extract contains active plant compounds that help in nanoparticle formation. When tested, the Cp-AgNPs displayed strong, dose-dependent antibacterial effects—especially against *Staphylococcus aureus*—and showed promising anticancer activity in MTT assays across selected cancer cell lines.

Nanotechnology has opened new doors in medicine, agriculture, and food science due to the unique properties of nanoparticles—such as their tiny size, large surface area, and enhanced chemical behavior. While traditional chemical methods for making nanoparticles can be harmful, plant-based synthesis offers a safer and more sustainable option. Plants naturally provide bioactive molecules like flavonoids, alkaloids, and polyphenols that assist in the creation of stable, functional nanoparticles. Silver nanoparticles, in particular, stand out for their antimicrobial and anticancer potential, especially due to their ability to slowly release silver ions in a controlled way. By merging plant-based methods with nanotechnology, this study highlights a greener path toward future medical and therapeutic innovations.

1.2Introduction on *Carica papaya*

Carica papaya, more commonly known as papaya or pawpaw, is a fast-growing tropical species that originated in southern Mexico and Central America. Although it resembles a tree in appearance, it is actually an herbaceous plant with a hollow stem and a crown of large, deeply lobed, umbrella-like leaves. The plant thrives in warm, moist environments and is now cultivated widely across tropical and subtropical regions, with India being the world's leading producer.

Papaya plants may occur as either dioecious, bearing male and female flowers on separate plants, or hermaphroditic, where both reproductive structures occur together. A notable feature of this species is cauliflory—flowers and fruits often sprout directly from the trunk. The fruits are generally oval to pear-shaped, with a sweet orange or reddish interior, and a central cavity packed with black, peppery-tasting seeds.

Nutritionally, papaya is valued as an excellent source of vitamin C, beta-carotene, folate, and potassium. It also contains two key enzymes, papain and chymopapain, which support digestion and help reduce inflammation. Beyond being consumed as fruit, many parts of the plant are used in traditional medicine. For example, papaya leaves are employed in remedies for dengue and digestive disorders, the latex is harvested for papain used in various industries, and the seeds are recognized for their antimicrobial activity.

Because of its fast growth, multiple health applications, and economic importance, Carica papaya remains a significant agricultural crop and a widely enjoyed dietary fruit across tropical regions.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh leaves of *Carica papaya* were carefully gathered for this study. To ensure they were free from dust or surface impurities, the leaves were thoroughly washed with distilled water. After cleaning, they were air-dried in the shade—a step that helps retain the natural phytochemicals within the leaves. Once completely dry, the leaves were ground into a fine powder using either a blender or a mortar and pestle, depending on what was available.

2.2 Preparation of Leaf Extract

About 100 grams of the powdered leaf material was accurately weighed and mixed with 250 mL of distilled water in a conical flask. This mixture was then either gently heated at 80°C for 30 minutes or placed on a mechanical shaker at room temperature for 24 hours to extract the plant compounds. The solution was first filtered using muslin cloth and then passed through Whatman No.1 filter paper to remove any remaining solids, resulting in a clear extract. This extract was stored at 4°C until it was needed for nanoparticle synthesis.

2.3 Preparation of Silver Nitrate Solution

To prepare the silver nitrate solution, 0.1 grams of $AgNO_3$ was dissolved in 100 mL of distilled water to make a 1 mM solution. To prevent the silver from breaking down when exposed to light, the solution was kept in an amber-colored bottle.

2.4 Synthesis of Silver Nanoparticles

For the synthesis, between 5 to 25 mL of the papaya leaf extract was added to 100 mL of the prepared silver nitrate solution. This mixture was stirred constantly at room temperature or gently warmed to 40°C. Over the course of 1 to 24 hours, a visible color shift from pale yellow to reddish-brown indicated the formation of silver nanoparticles. To isolate the nanoparticles, the solution was centrifuged at 10,000 rpm for 1 minute.

2.5 Confirmation of Nanoparticle Formation

The formation of silver nanoparticles was confirmed using **UV-Visible spectroscopy**, which detects a specific peak—typically around 435 nm—that indicates surface plasmon resonance, a hallmark of silver nanoparticles. Additional tests were conducted for detailed analysis:

- **FTIR (Fourier Transform Infrared Spectroscopy)** was used to identify functional groups involved in stabilizing the nanoparticles.
- **XRD (X-ray Diffraction)** helped confirm the crystalline nature of the particles.
- **SEM/TEM (Scanning or Transmission Electron Microscopy)** provided detailed images to examine the size, shape, and surface features of the nanoparticles.

METHODS

To confirm the formation and quality of the synthesized silver nanoparticles (Cp-AgNPs), several characterization techniques were used. **UV–Visible spectroscopy** helped detect the signature absorbance peak of silver nanoparticles, while **SEM** provided detailed images of their shape and surface. **XRD analysis** was used to confirm their crystalline structure and size using the Debye–Scherrer equation.

For antibacterial testing, both *E. coli* and *S. aureus* were cultured. Growth inhibition was monitored over time, and the **well diffusion method** was used to assess how effectively different concentrations of Cp-AgNPs could prevent bacterial growth. The **Minimum Inhibitory Concentration (MIC)** was determined by measuring bacterial survival at various nanoparticle concentrations.

Phytochemical screening of the *C. papaya* leaf extract identified the natural compounds responsible for nanoparticle formation, such as flavonoids and tannins.

To evaluate anticancer activity, four human cancer cell lines (MCF-7, A549, HCT-116, and Caco-2) were grown in culture. Cells were treated with various concentrations of Cp-AgNPs, and the **MTT assay** was used to measure how effectively the nanoparticles reduced cell viability. A visible drop in cell survival with increasing nanoparticle concentration indicated strong antiproliferative potential.

3. RESULT AND DISCUSSION

3.1 Phytochemical Analysis

Screening the *Carica papaya* leaf extract revealed a rich mix of bioactive compounds known for their medicinal properties. The aqueous extract contained key phytochemicals like alkaloids, flavonoids, tannins, saponins, phenolic compounds, glycosides, terpenoids, and steroids.

Each of these plays a unique role in promoting health:

- **Alkaloids** (such as carpaine) have strong antimicrobial effects and can support heart health.
- **Flavonoids** and **phenolic compounds** are well-known antioxidants that also help reduce inflammation.
- **Tannins** fight off infections, while **saponins** boost the immune system.
- **Glycosides** assist in managing inflammation and offer antibacterial support.
- **Terpenoids** and **steroids** are valued for their antiviral and anti-inflammatory activities.

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These compounds were identified using standard lab tests—such as Dragendorff's, Mayer's, and Wagner's reagents for alkaloids, and the foam test for saponins. Interestingly, compounds like cardiac glycosides and anthraquinones, which are sometimes present in other medicinal plants, were not found in the papaya leaf extract.

Since many of these phytochemicals are water-soluble, using water as the extraction medium proved both effective and environmentally friendly. Altogether, this diverse chemical profile not only supports the traditional use of papaya leaves in treating ailments like dengue and digestive issues but also explains their effectiveness in the eco-friendly synthesis of silver nanoparticles.

Figure 1. Steps involved in extraction of silver nanoparticles from *C. papaya* leaf extract.

The UV–Visible spectrum of the synthesized silver nanoparticles showed a strong, sharp peak around 400 nm—typical of surface plasmon resonance (SPR), which is unique to silver nanoparticles. This confirms their successful formation using Carica papaya leaf extract. The peak's intensity and clarity suggest the particles are present in high concentration and are mostly uniform in size, indicating a stable, well-dispersed nanoparticle solution. These results, combined with other techniques like XRD and FTIR, help paint a complete picture of the nanoparticles' structure and composition.

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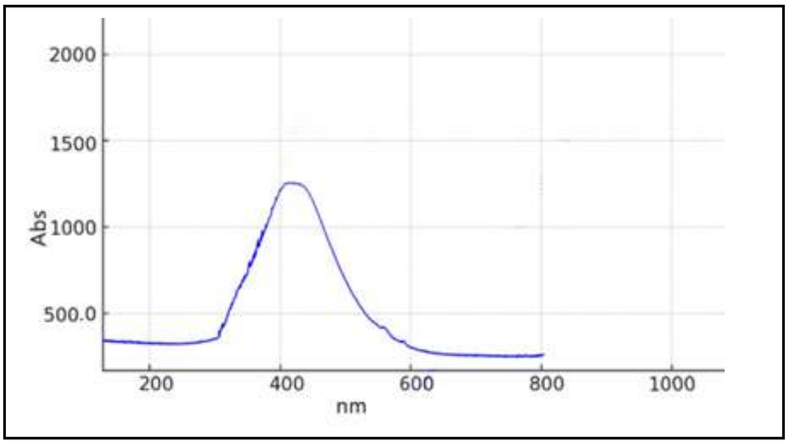

Figure 2. UV Spectra of the aqueous leaf extract Cp-AgNPs synthesized from the *C. papaya* leaf extract.

The FTIR spectrum of the silver nanoparticles synthesized with *Carica papaya* leaf extract shows key functional groups like hydroxyl, amine, carbonyl, and aliphatic chains—indicating the presence of natural compounds such as phenols, proteins, and flavonoids. These groups play a crucial role in reducing silver ions and stabilizing the nanoparticles. Overall, the spectrum confirms that papaya leaf phytochemicals actively drive the green, eco-friendly synthesis of silver nanoparticles.

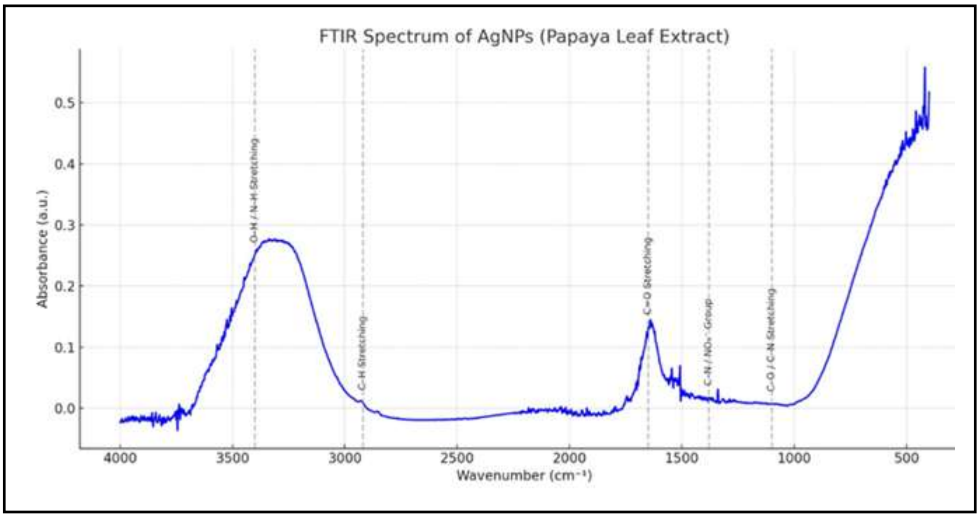

Figure 3. FTIR spectra of the *C. papaya* leaf extract (Cp-AgNPs).

The XRD pattern of the silver nanoparticles showed distinct peaks at 38°, 44°, 64°, and 77°, matching the (111), (200), (220), and (311) planes of a face-centered cubic structure—typical of pure silver. The strong (111) peak suggests good stability and high surface reactivity. These results, along with FTIR and UV–Vis data, confirm that *Carica papaya* leaf extract effectively aids in forming well-structured silver nanoparticles.

Figure 4. XRD pattern of the synthesized Cp-AgNPs using *C. papaya* leaf extract.

The SEM image revealed mostly round silver nanoparticles averaging about 200 nm in size, evenly distributed with minimal clumping—indicating a smooth and controlled synthesis process. Their slightly larger size suggests a thin coating from papaya leaf phytochemicals, which not only helped form the particles but also stabilized them. These uniform, spherical nanoparticles are ideal for biomedical uses like drug delivery.

Antimicrobial Activity-

After 24 hours of incubation, *E. coli* showed a larger zone of inhibition than *S. aureus*, indicating that the silver nanoparticles were more effective against Gram-negative bacteria. This is likely due to their thinner, more permeable cell walls, which make them more vulnerable. The silver ions

from the nanoparticles disrupt bacterial membranes, enter the cells, and interfere with vital functions like DNA replication and protein production—ultimately causing cell death.

Antiproliferative Activity-

The Cp-AgNPs showed clear anticancer potential when tested on four cancer cell lines—A549, HCT-116, Caco-2, and MCF-7. As the nanoparticle dose increased, cell viability consistently decreased, confirming a dose-dependent effect. Some cell lines responded more sensitively than others, likely due to differences in how they interact with the nanoparticles. These findings point to strong antiproliferative activity, with IC_{50} values helping measure effectiveness, though more detailed analysis is needed to confirm the results. The IC_{50} value of Cp-AgNPs was calculated to be 88.64 µg/ml for A549, 86.11 µg/ml for HCT-116, 73.57 µg/ml for Caco-2, and 64.66 µg/ml for MCF-7 cells respectively.

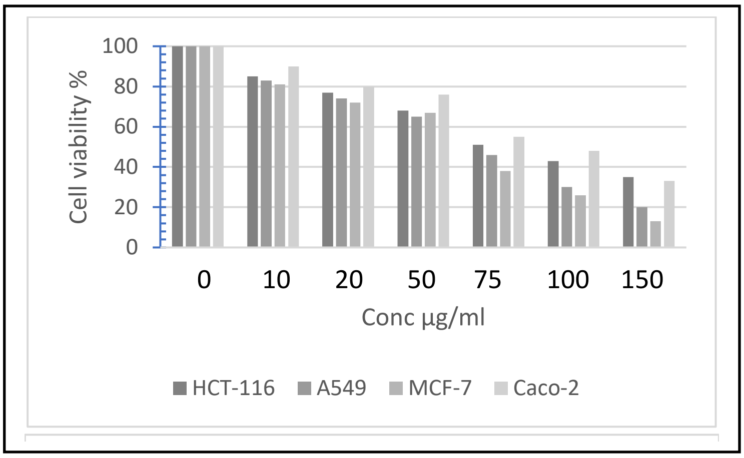

Figure 5. Effect of different concentrations of Cp-AgNPs on the viability of human cancer cell lines.

Under the microscope, each cancer cell line showed distinct shapes. MCF-7 cells were neatly spread out, A549 appeared more clustered, HCT-116 had an elongated, fibrous look, and Caco-2 formed tight, cobblestone-like clusters. These structural differences affect how each cell type interacts with nanoparticles and may explain their varied responses to treatment—highlighting the importance of cell morphology in therapeutic studies.

4. FUTURE PROSPECT

To fully understand how Cp-AgNPs fight cancer, future studies should explore their exact mechanism—like whether they cause apoptosis through oxidative stress or DNA damage. Advanced techniques (HPLC, GC-MS, LC-MS) can help identify the key bioactive compounds in *Carica papaya* extract. Broader antimicrobial testing, including against fungi and resistant bacteria, could reveal wider applications. It's also important to assess nanoparticle stability, reusability, and how they compare with those made from other plants. Finally, studying their environmental impact is crucial to ensure this remains a truly green and sustainable approach.

5.CONCLUSION

Scientists have found an innovative and eco-friendly way to create tiny silver nanoparticles using papaya leaf extract. These nanoparticles, called Cp-AgNPs, have been shown to be well-formed, stable, and uniform. In biological tests, they demonstrated strong antibacterial effects, particularly against E. coli. Furthermore, they reduced the viability of various human cancer cells in a dose-dependent manner. This dual-purpose ability to act as both an antimicrobial and an anticancer agent, combined with their green synthesis method, suggests that Cp-AgNPs have significant potential for future medical applications. Their varying impact on different cells also hints at the possibility of tailoring these nanoparticles for more targeted therapies.

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