RESEARCH PAPER

Green synthesis of Silver Nanoparticles from Annona muricata L. Leaf Extract: Characterization and Assessment of Antioxidant and Anti-Cancer Potential

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ABSTRACT
Silver nanoparticles (AgNPs) have been extensively explored due to their varied biological properties. Annona muricata. L leaf extract-derived silver nanoparticles (AM-AgNPs) were synthesized and characterized using UV–Visible spectroscopy, ATR-FTIR spectroscopy, and Scanning Electron Microscopy (SEM). A characteristic surface plasmon resonance peak at 440 nm was detected in the UV–Visible absorption spectrum, confirming AgNPs formation. SEM analysis revealed particle sizes ranging from 350 to 650 nm, while ATR-FTIR identified functional groups involved in the reduction and capping of silver ions, providing insights into surface morphology and stabilization. Phytochemical profiling by LC–MS and DPPH study revealed a 25% enhancement in antioxidant activity in the AM-AgNPs compared to A. muricata leaf (AML) extract alone. In addition, a sustained release of 77.3% over 24 hr. established effective entrapment and prolonged delivery potential. The entrapment efficiency of phytochemicals within AM-AgNPs was found to be in the range of 74.23–81.09%, representing strong phytochemical association with the nanoparticles. Cytotoxicity evaluated by MTT assay using MCF-7 breast cancer cell lines showed concentration-dependent growth inhibition, with an IC_{50} value of 74.51 µg/mL.

Keywords: Antioxidant, Anti Proliferative, Silver nanoparticles, MCF-7, MTT Assay
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INTRODUCTION

Nanotechnology has emerged as a rapidly evolving field with significant potential in health and pharmaceutical research, predominantly through the expansion of nanoparticle-based therapeutic systems[1]. Among various metallic nanoparticles, silver nanoparticles (AgNPs) have gained considerable attention due to their exclusive physicochemical properties, including high surface-to-volume ratio, surface plasmon resonance behaviour, and prominent biological activities. These characteristics make AgNPs promising candidate for applications in drug delivery, antimicrobial therapy, antioxidant systems, and cancer treatment [2].

Conventional physical and chemical methods for synthesizing silver nanoparticles often involve toxic reagents, high energy consumption, and environmental concerns, limiting their biomedical applicability[3]. In contrast, green synthesis using plant-based extracts has gained increasing interest as an eco-friendly, cost-effective, and sustainable alternative[4]. Plant metabolites such as polyphenols, flavonoids, alkaloids, and proteins act as reducing, capping, and stabilizing agents during nanoparticle synthesis, resulting in biocompatible and stable nanoparticles suitable for therapeutic use[5]. The widespread use of traditional synthetic drugs has led to the emergence of microbial resistance, significantly reducing their long-term efficacy. Moreover, many synthetic drugs are allied with adverse side effects, encouraging researchers to explore alternative therapeutic approaches with improved safety profiles. Consequently, growing attention has been directed toward natural medicines due to their potential reduced toxicity.

Globally, oncologists employ various conventional cancer treatment modalities, including chemotherapy, radiation therapy, and surgical intervention [6]. However, many chemotherapeutic agents are associated with significant

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side effects, as their non-target-specific action often results in damage to both cancerous and healthy cells[7]. In parallel, cancer treatment strategies are evolving from conventional chemotherapeutic approaches toward targeted, green-synthesized nanomedicines that offer enhanced efficacy with minimized side effects.

Annona muricata L., commonly known as soursop or graviola, is a tropical medicinal plant belonging to the family *Annonaceae*. The plant is widely distributed in tropical and subtropical regions of the world, including Central and South America, the Caribbean, Africa, Southeast Asia, and the Indian subcontinent [8]. In India, *Annona muricata* L. is commonly cultivated in southern and coastal regions, where it is traditionally used for medicinal and nutritional purposes, and its leaves—rich in polyphenols, flavonoids, and other bioactive compounds—represent a rich source for the green synthesis of nanoparticles[9]. Green synthesized silver nanoparticles have demonstrated antioxidant activity comparable to natural compounds, making them a promising alternative to synthetic antioxidants and cytotoxic reagent [10].

This study aimed to synthesize and characterize silver nanoparticles using *Annona muricata* L. leaf extract and to evaluate their antioxidant and anticancer activities, with a focus on their potential to enhance selective cytotoxicity against breast cancer cells. The uniqueness of this work portrays in employing *Annona muricata* leaf extract as a sustainable biogenic route for nanoparticle synthesis, coupled with the dual evaluation of their antioxidant and anticancer efficacy, thereby bridging green nanotechnology with targeted cancer therapies.

MATERIALS AND METHOD

Analytical grade methanol was purchased from, Merck. Silver Nitrate ($AgNO_3$, ≥99.9%), procured from Sigma-Aldrich. Dialysis bags were obtained from **Sigma-Aldrich (Merck Millipore).** LC–MS grade Acetonitrile and Formic Acid used for chromatographic analysis were purchased from SD Fine Chemicals, India. Water used for LC–MS analysis and sample preparation was generated in-house using Merck **Milli-Q system.** 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and MTT reagents were purchased from Sigma-Aldrich. All the chemicals utilized were of analytical grade.

Sample Collection and Preparation of *Annona muricata* Leaf Extract (AML)

Fresh and healthy *Annona muricata* leaves were collected during the monsoon season (June 2025) from Marahalli village, Doddaballapur taluk, Bengaluru Rural district, Karnataka, India (77.595684° E, 13.367644° N) as shown in the Figure 1. The collected leaves were thoroughly washed with distilled water and shade-dried at 37 °C for 48 hr. The dried leaves were pulverized to a fine powder, and 15 g of the powder was extracted with 150 mL of methanol by incubation at 70 °C for 2 hr. The resulting methanolic extract was concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use [11].

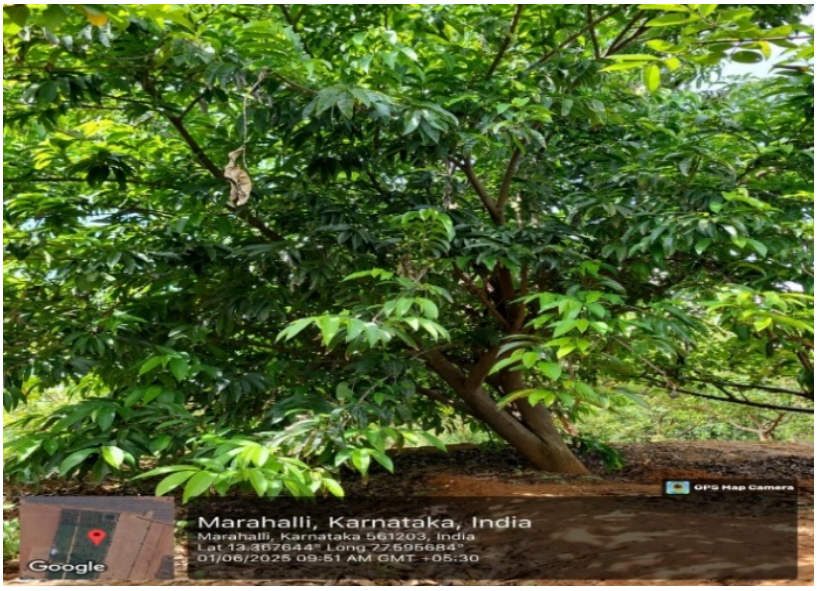

Figure 1. Sample Collection Place

Synthesis of Silver Nanoparticles (AM-AgNPs)

Silver nanoparticles were synthesized using *Annona muricata* leaf (AML) extract as a reducing and stabilizing agent. An aqueous solution of silver nitrate was prepared and maintained under continuous magnetic stirring. The plant extract was slowly introduced into the silver nitrate solution in a controlled manner to initiate the reduction process, and the reaction mixture was stirred at 500 rpm to ensure uniform dispersion. After initial mixing, the reaction was allowed to proceed for an additional 30 min under constant stirring. The resulting mixture was then subjected to high-speed homogenization at 4000 rpm for 4 hr to promote efficient nucleation and growth of nanoparticles. Following homogenization, the mixture was concentrated using a rotary evaporator maintained at 40–60 °C for 24 hr to remove excess solvent, yielding the biosynthesized silver nanoparticles [12, 13].

Entrapment Efficiency

UV–Visible spectroscopy was used to estimate the entrapment efficiency of *Annona muricata* phytochemicals with silver nanoparticles; the AM-AgNPs suspension was centrifuged (12,000 rpm, 20 min), the supernatant absorbance (440nm) was compared with the initial extract, and efficiency calculated using the given equation [12].

Where: -

A_0: represents the absorbance of the plant extract before nanoparticle synthesis

A_s: denotes the absorbance of the supernatant after centrifugation.

Scanning Electron Microscopy

The surface morphology and topography of the synthesized AM-AgNPs were analyzed using a scanning electron microscope (SEM; Hitachi SU3500). Images were recorded in secondary electron mode to obtain detailed surface morphological features [14].

ATR-FTIR Spectroscopic Analysis

Attenuated Total Reflection–Fourier Transform Infrared (ATR-FTIR) spectroscopy (Bruker Alpha II FTIR) was employed to identify the functional groups present on the surface of the synthesized AM-AgNPs. The obtained spectra were analyzed to identify characteristic vibrational bands corresponding to functional groups associated with biomolecules involved in the reduction and stabilization of the nanoparticles [15].

Phytochemical Profiling of AM-AgNPs by Using LC-MS

LC–MS analysis was performed using an Agilent HPLC system coupled to a Waters Micro mass Quattro Micro API triple quadrupole mass spectrometer operated in full-scan MS mode with electrospray ionization (ESI) in positive ion mode. Chromatographic separation was achieved on a C18 column (50 × 4.6 mm, 3.5 µm) using a binary mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (acetonitrile, MS grade) at a flow rate of 1.2 mL/min under gradient conditions (85% A/15% B at 0.01 min, 25% A/75% B at 6 min, and 85% A/15% B at 11 min), with a total run time of 15.01 min. The optimized source parameters included a capillary voltage of 3.45 kV, cone voltage of 33 V, extractor voltage of 3.0 V, source temperature of 110 °C, and desolvation temperature of 350 °C, with nitrogen used as the desolvation (750 L/h) and cone (50 L/h) gas. Data were acquired with a scan time of 0.2 s per scan and processed using MassLynx software (version 4.1), with analytes identified and quantified based on their retention times and m/z values using calibration standards [16].

***In vitro* Release Assay**

The release profiles of AML and AM-AgNPs were evaluated using the dialysis bag diffusion method. Equivalent amounts of each sample were dispersed in PBS (Phosphate Buffer Saline at pH 7.4) and loaded into pre-soaked dialysis membranes. The bags were immersed in 50 mL PBS at 37 ± 0.5 °C under continuous stirring at 100 rpm for 24 hr [17]. The collected samples were filtered through a 0.1 µm membrane filter and analyzed using LC–MS to quantify the released plant extract. The cumulative percentage release was calculated and plotted against time to compare the release profiles of AML and AM–AgNPs.

DPPH scavenging effect

The antioxidant activity of plant extract–mediated silver nanoparticles was determined using the DPPH radical scavenging assay following the method of Mensor et al. (2001) [18, 19]. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{[100 - \{\text{Absorbance (sample)} - \text{Absorbance(blank)}\}}{\text{Absorbance (blank)}] \text{ x } 100}$$

Preparation of cells

The MCF-7 human breast adenocarcinoma cell line (NCCS, Pune, India) was cultured in high-glucose DMEM supplemented with 10% FBS and 1% antibiotic–antimycotic at 37 °C in a humidified 5% CO_2 incubator. At 85–90% confluence, cells were trypsinized (0.25% trypsin-EDTA) and seeded into 96-well plates at 2 × 10^4 cells/well, followed by 24 hr incubation prior to treatment [20].

MTT Cytotoxicity Assay

The anticancer activity of the samples on MCF-7 breast cancer cell lines was determined by the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay to evaluate the cytotoxicity activity as prescribed by Horiuchi et al. (1988) [21]. Cell viability (%) and IC_{50} values were calculated from concentration–response curves by linear regression analysis [22].

RESULTS AND DISCUSSION

Green Synthesis and UV-Vis Characterization of AM-AgNPs

The synthesis of *Annona muricata*–mediated silver nanoparticles (AM-AgNPs) were initially evidenced by a visible color change of the reaction mixture from pale green to reddish-brown, which gradually intensified to dark brown or black upon prolonged incubation, indicating successful nanoparticle formation due to surface plasmon resonance (SPR) excitation of silver nanoparticles [23,24]. This colour transition is a well-recognized optical signature arising from the collective oscillation of conduction electrons on the nanoparticle surface [25]. Elevated temperature and extended incubation time facilitated efficient reduction of Ag^+ ions by phytochemicals present in the *A. muricata* leaf extract, thereby enhancing nanoparticle yield and stability [26]. The subsequent darkening of the reaction mixture at later

stages may be attributed to nanoparticle growth, segregation, and partial aggregation governed by Brownian motion and nucleation–growth dynamics consistent with the LaMer mechanism [27]. The formation of AM-AgNPs was further confirmed by UV–Visible spectroscopy, which exhibited a characteristic SPR absorption band in the range of 320–520 nm, with a distinct peak centered at 440 nm as shown in the Figure 2, a feature typically associated with spherical silver nanoparticles synthesized via green routes [28].

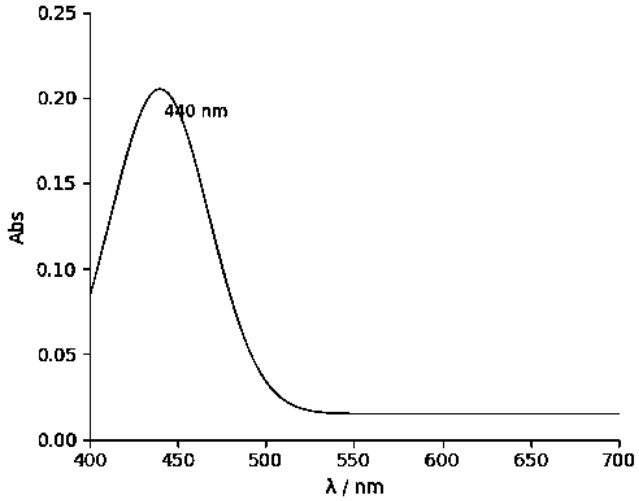

Figure 2: UV-Visible Spectrum of AM-AgNPs showing SPR peak at 440nm

Entrapment Efficiency

Entrapment efficiency refers to the proportion of phytochemicals successfully entrapped or associated with nanoparticles relative to the total phytochemicals present in the extract. The entrapment efficiency of AM-AgNPs was found in the range of 74.23–81.09%, highlighting the strong phytochemical association and stabilization capacity of the extract in nanoparticle formation. Recent studies confirm that such high efficiency reflects the rich presence of bioactive compounds in *A. muricata* leaves, which act as reducing and capping agents during nanoparticles biosynthesis [29].

Characterization of AM-AgNPs by SEM

The morphology of the AM-AgNPs was examined using SEM as demonstrarted in the Figure 3. The SEM images revealed that the particles appeared irregular and polydisperse, which is typical in green synthesis due to the diverse phytochemicals acting as reducing and stabilizing agents. Particle size estimation from SEM images indicated sizes ranging approximately from 350nm to 650 nm. The observed agglomeration is characteristic of green-synthesized nanoparticles and is attributed to organic capping by plant biomolecules [30, 31].

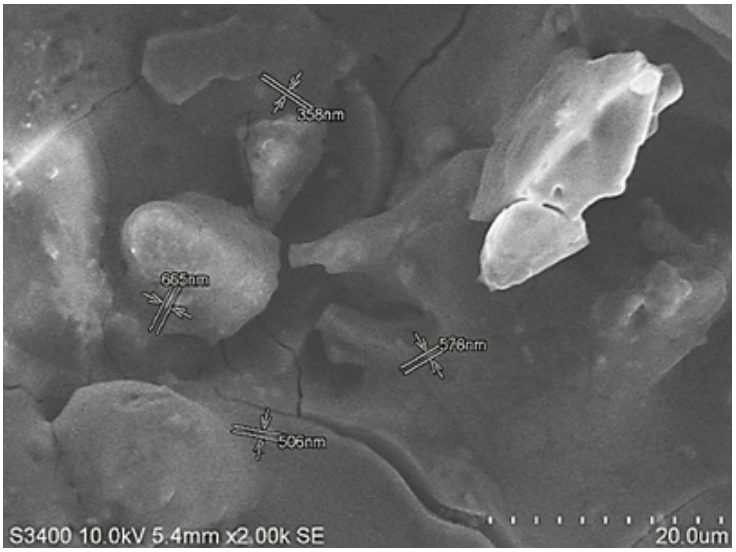

Figure 3: SEM image of *Annona muricata*- leaf extract mediated silver nanoparticles.

Characterization of AM-AgNPs by ATR-FTIR

FTIR spectra of the synthesized silver nanoparticles (AM-AgNPs) confirmed their formation and stabilization by plant-derived biomolecules. A broad band at ~3400 cm^{-1} indicated O–H stretching vibrations of phenols/alcohols, suggesting their role in reduction and capping of Ag^{+} ions. The peak at ~2920 cm^{-1} corresponded to C–H stretching of aliphatic chains, while a strong absorption near 1650 cm^{-1}

was attributed to C=O stretching of amide I or ketone groups, consistent with proteins and polyphenolic compounds involved in nanoparticle synthesis. Additional peaks at ~1380 cm^{-1} and ~1100 cm^{-1} represented C–N and C–O/C–O–C vibrations, indicating contributions from amino compounds, polysaccharides, and flavonoids in stabilization as shown in the Figure 4. Weak absorptions in the 600–800 cm^{-1} region suggested Ag–O interactions, confirming silver–biomolecule binding. Collectively, these spectral features demonstrate that phytochemicals acted as reducing and capping agents, forming a stable organic coating that prevented agglomeration and ensured colloidal stability, thereby validating the green synthesis of AgNPs [32, 33].

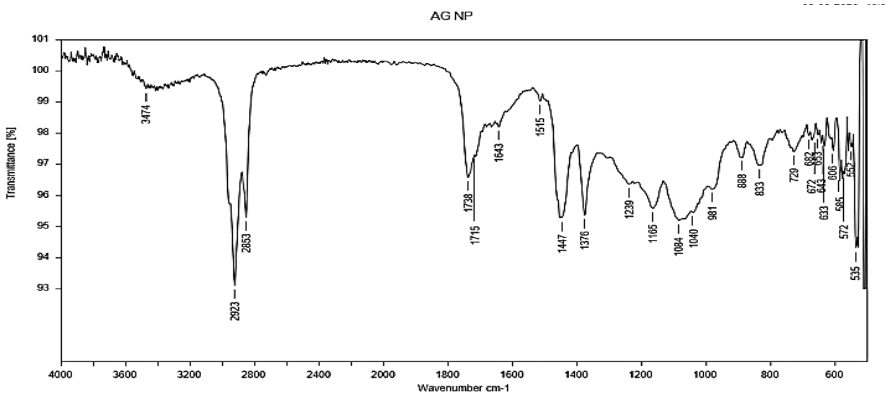

Figure 4: FTIR spectrum of *Annona muricata* leaf extract- mediated silver nanoparticles

LC–MS Analysis of Phytochemicals Associated with AM-AgNPs

LC–MS analysis was performed to qualitatively investigate the phytochemical constituents associated with *Annona muricata* leaf extract–mediated silver nanoparticles (AM-AgNPs). The total ion chromatogram (TIC) of AM-AgNPs as shown in the Figure 5 exhibited multiple resolved peaks, indicating the presence of a heterogeneous population of plant-derived biomolecules associated with the nanoparticle surface. Among these, three dominant peaks were observed at retention times of approximately 0.384, 0.542, and 1.438 min, suggesting the prevalence of relatively polar phytochemical constituents. The early elution behavior on the C18 column is characteristic of low- to moderately polar compounds, which are commonly reported in *Annona muricata* leaf extracts and are known to participate in the reduction and stabilization of silver nanoparticles during green synthesis [34].

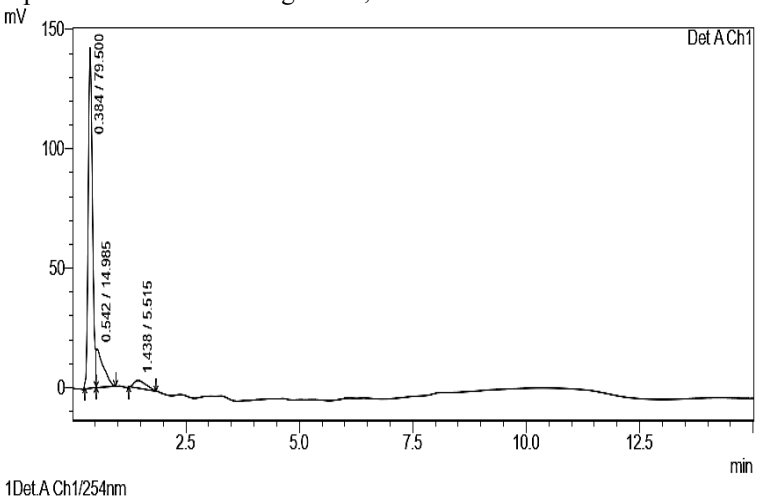

Figure 5: LC-MS chromatogram of AM-AgNPs.

The mass spectrum acquired in positive electrospray ionization mode (ESI^+) as shown in the Figure 6 revealed multiple protonated molecular ions and adduct peaks, indicating the presence of nitrogen-containing and semi-polar compounds such as alkaloids, certain terpenoids, and acetogenin-related structures. Positive ion mode is particularly effective for detecting basic and neutral phytochemicals, which are frequently implicated in nanoparticle surface functionalization through coordination and electrostatic interactions. The diversity of ion signals observed in ESI^+ mode supports the adsorption of multiple bioactive phytochemical classes onto the AgNPs surface[35].

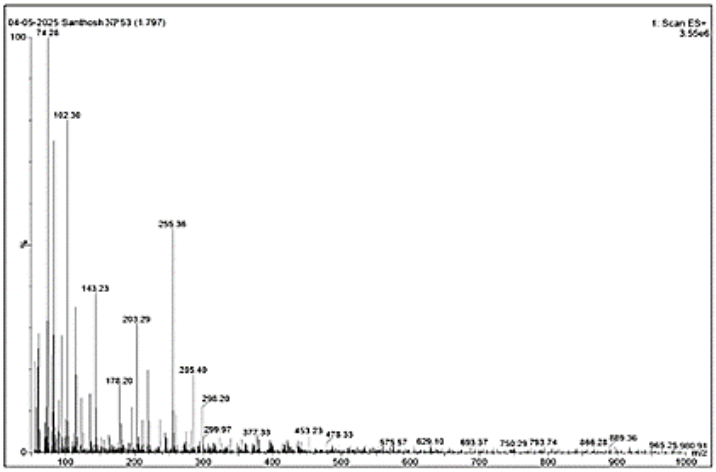

Figure 6: Mass spectrum of AM-AgNPs in positive ionization mode (ESI+)

Complementary analysis in negative electrospray ionization mode (ESI^-) as Figure 7 showed prominent deprotonated molecular ions, consistent with the presence of acidic and phenolic constituents, including flavonoids, phenolic acids, and ascorbic acid-related compounds. Negative ion mode is well suited for detecting hydroxyl-rich and redox-active phytochemicals, which are widely reported as key contributors to antioxidant activity and metal ion reduction. The combined use of ESI^+ and ESI^- modes therefore provide a broader qualitative fingerprint of the phytochemical corona associated with AM-AgNPs [36].

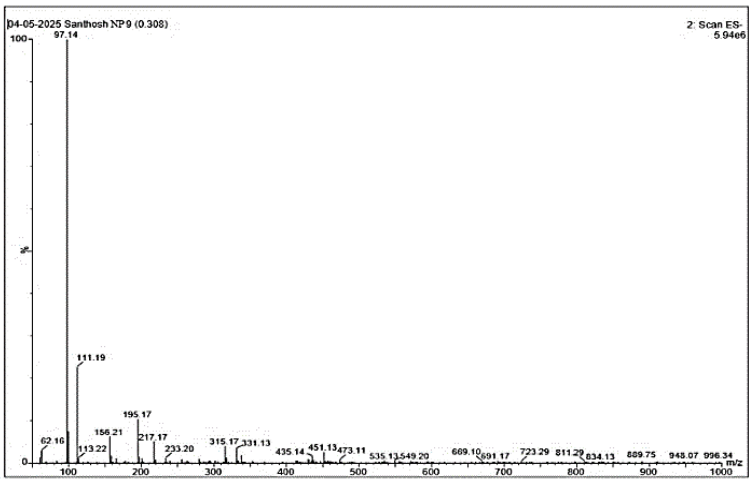

Figure 7: Mass spectrum of AM-AgNPs in negative ionization mode (ESI-).

Although individual compounds were not confirmed using authentic standards or MS/MS fragmentation, the chromatographic profiles and ionization patterns observed in Figures 5, 6 and 7 are consistent with previously reported LC–MS profiles of *Annona muricata* leaf extract, where acetogenins, alkaloids, flavonoids, terpenoids, and phenolic compounds were identified as dominant constituents [37]. These phytochemical classes are well documented for their roles as reducing and capping agents in green nanoparticle synthesis and for their contribution to biological activities.

***In vitro* Release Assay**

The *in-vitro* release study showed distinct differences between free AML extract and AM-AgNPs. Free extract displayed rapid diffusion with nearly complete release within 6–8 hr, whereas AM-AgNPs revealed a sustained release profile, reaching 77.3% cumulative release at 24 hr. The slower release from AM-AgNPs is likely due to the interaction of phytochemicals with the nanoparticle surface, forming a diffusion-limiting barrier. Similar sustained release behavior has been reported for other plant extract–mediated AgNPs, supporting the role of nanoparticle association in lengthening release as shown in the Figure 8 [38].

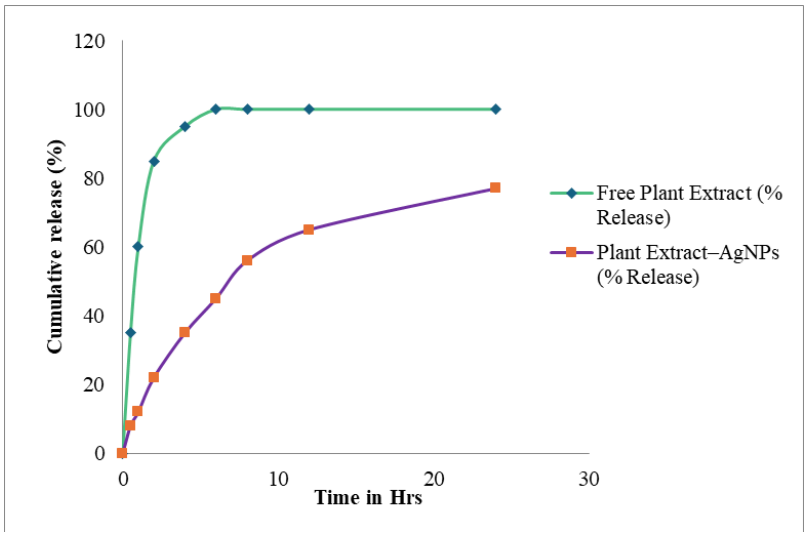

Figure 8: *In Vitro* Release assay with *Annona muricata* leaf extract and AM-AgNPs

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Activity

The DPPH assay was showed to measure the free radical scavenging activity of AM-AgNPs compared to leaf extract alone. This method assesses the electron-donating ability of antioxidants, which counterbalances the DPPH radical by forming stable molecules with an absorption maximum of 517 nm. The reaction involves a color change from purple/violet to colorless by the pairing of the odd electron on the nitrogen atom. The degree of decolorization specifies the extent of the reduction. Markedly, AM-AgNPs demonstrated higher free radical scavenging activity compared to the leaf extracts alone as shown in the Figure 9. These results are consistent with previous findings which generally correlates with free radical scavenging activity[39].

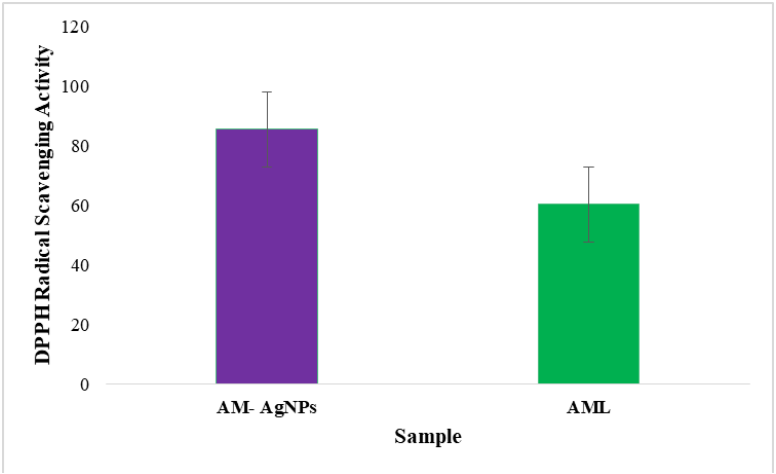

Figure 9: DPPH activity of AM-AgNPs and *Annona muricata* leaf extract alone.

Anticancer Activity of AM-AgNPs

In vitro cytotoxicity studies were conducted using AM-AgNPs dissolved in DMSO against MCF-7 breast cancer cell lines at concentrations of 5, 25, 50, 100, and 150 µg/mL, with Doxorubicin as the standard reference drug. At 5 µg/mL, AM-AgNPs displayed minimal cytotoxicity, showing 94.7% cell viability. Still, viability gradually declined with increasing concentrations, reaching 28% at 150 µg/mL. The overall cytotoxic response of AM-AgNPs against MCF-7 cells yielded an IC_{50} value of 74.51 µg/mL, representing the concentration essential to inhibit 50% of cell growth. The IC_{50} thus reflects the potency of the compound in suppressing cancer cell proliferation[40].

The assessment of MCF-7 cells showed concentration-dependent cytotoxic effects following treatment with AM-AgNPs as shown in the Figure 10. Cells treated with 5 µg/mL AM-AgNPs mainly sustained normal morphology with minimal structural changes, consistent with high cell viability. At 25 µg/mL, slight morphological changes and partial loss of cell adhesion were observed, indicating the beginning of cytotoxicity. Further increase to 50 µg/mL caused in a noticeable reduction in cell density and increased cellular deformation, viewing moderate cytotoxic effects. Marked morphological impairment, including general cell rounding, shrinkage, and detachment, was evident at 100 µg/mL. At the highest concentration of 150 µg/mL, severe cytotoxicity was observed, characterized by extensive cell death, cellular debris, and a noticeable reduction in viable MCF-7 cells,

verifying the observed decrease in cell viability and the IC_{50} value.

The enhanced anticancer activity of AM-AgNPs, relative to the AML extract, advocates a synergistic interaction between silver nanoparticles and plant-derived bioactive compounds—particularly annonaceous acetogenins, which are well-known for their pro-apoptotic and antiproliferative effects against breast cancer cell lines [41, 42].

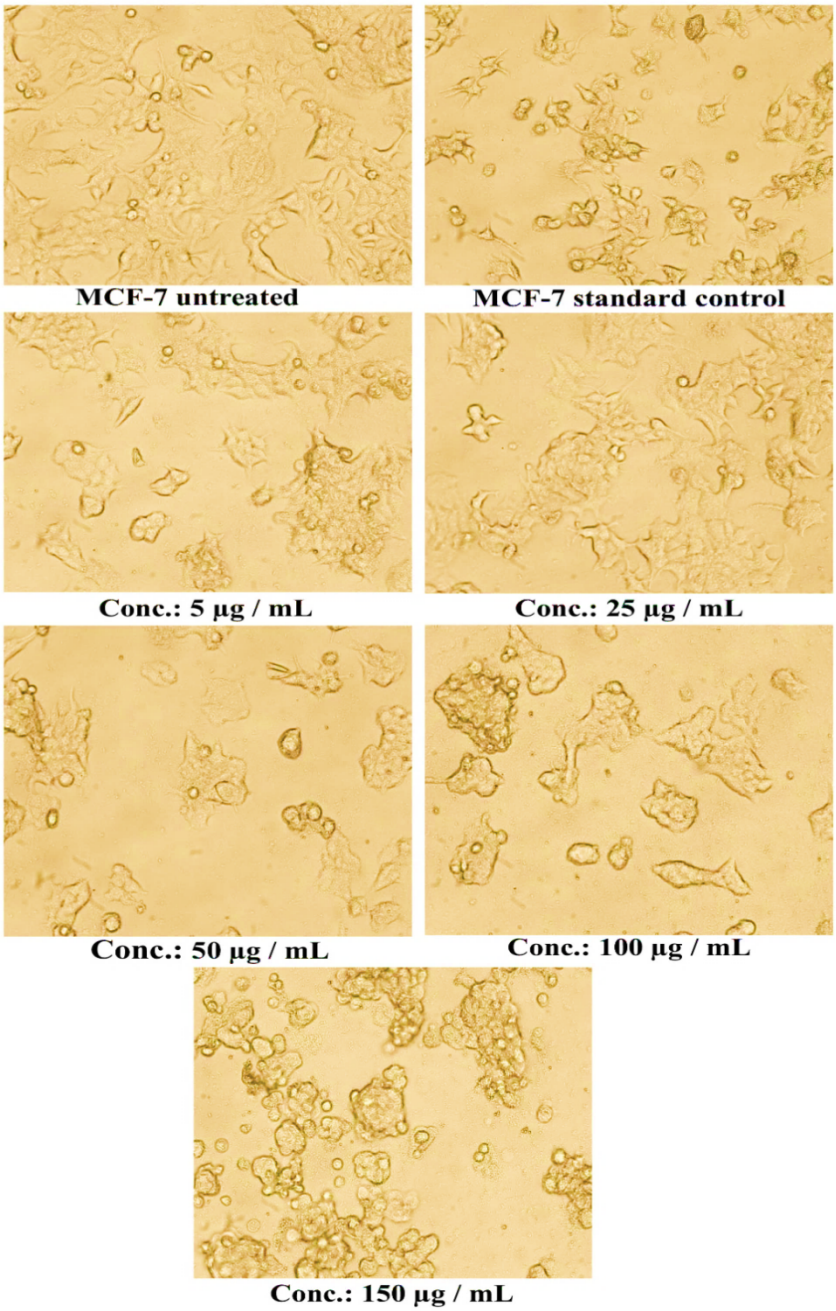

Figure 10: Observations confirmed cytotoxicity through cell morphological variations at a) Untreated, b) Doxorubicin treated, c) 5 µg/mL, d) 25 µg/mL, e) 50 µg/mL, f) 100 µg/mL, g) 150 µg/mL AM-AgNPs doses.

CONCLUSION

Green synthesis of silver nanoparticles using *Annona muricata* L. leaf extract was successfully achieved through an eco-friendly and sustainable approach. The formation of AM-AgNPs was confirmed by a characteristic surface Plasmon resonance peak at 440 nm in UV–Visible spectroscopy, while SEM analysis revealed irregular and polydisperse particle morphology. ATR-FTIR spectra demonstrated the involvement of plant-derived functional groups in the reduction and stabilization of silver ions, indicating effective phytochemical capping. LC–MS profiling supported the association of bioactive phytochemicals with the nanoparticle surface. The high entrapment efficiency and sustained release behaviour observed over 24 h highlight the suitability of AM-AgNPs

as a delivery system capable of enhancing phytochemical stability and prolonged release.

Biological evaluation showed that AM-AgNPs exhibited improved antioxidant activity and concentration-dependent cytotoxic effects against MCF-7 breast cancer cells, with an IC_{50} value of 74.51 µg/mL. The enhanced biological performance of AM-AgNPs compared to the leaf extract suggests that nanoparticles significantly improve therapeutic efficacy. The synergistic interaction between plant-derived bioactive compounds and silver nanoparticles creates an attractive therapeutic platform that amplifies anticancer activity, offering new opportunities for the development of effective and green nanotechnology-based cancer therapies. These findings indicate that plant-mediated silver nanoparticles represent a promising platform for eco-friendly antioxidant and anticancer applications, deserving further mechanistic and *in-vivo* investigations.

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Ethical Issues

Not applicable.

Conflict Of Interest

The authors declare no conflict of interest.

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