Research Article

Green Synthesized *Annona muricata* Seed-Mediated Silver Nanoparticles for Oral Cancer Therapy

***Pranjal Mutkekar[1], Sanket Patil[1], Sarvadnya Bedare[2], Pratiksha Desai[2], Dr. Prasanna Sutar[3]**
1. M.Pharm Department of Pharmaceutics, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, India.
2. B.Pharm, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, India.
3. Professor, Department of Pharmacognosy, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, India.
***Corresponding author's E-mail:** pranjalmutkekar@gmail.com

Received: 10-05-2026; **Revised:** 23-07-2026; **Accepted:** 29-07-2026; **Published online:** 20-08-2026.

ABSTRACT

Oral squamous cell carcinoma (OSCC) continues to be a significant cause of cancer-related mortality with a need for safer and more effective therapies. The purpose of this study was to synthesize, characterize silver nanoparticles (AgNPs) by using the seed extract of *Annona muricata* (AM) and to assess their in vitro anticancer activity on the oral squamous carcinoma cell line (OECM1). The green synthesis was used in which the seed extract was used as both reducing and stabilizing agent. Characterization was done by using UV-Visible spectroscopy, dynamic light scattering (DLS), zeta potential analysis, SEM, TEM, EDX, FTIR, and entrapment efficiency analysis. The formation of AgNPs was confirmed by a characteristic SPR peak at 452 nm. The average size of the nanoparticles was 138.2 nm as measured by hydrodynamic diameter and 68 nm as determined by TEM, with the majority of particles spherical in shape. The medium stability of the colloidal suspension was reflected by the zeta potential of −17.11 mV. The morphology and elemental composition of the nanoparticles, as well as the participation of hydroxyl, carbonyl and phenolic groups during their synthesis was confirmed by SEM, TEM, EDX and FTIR. The entrapment efficiency was 73.13%. MTT assay showed that it exhibited dose-dependent cytotoxicity against OECM1 cells with IC_{50} of 67.85 µg/mL. The results indicated that A. muricata-mediated AgNPs have favorable physicochemical properties and promising anticancer action against OSCC, which further requires the mechanistic and in vivo studies.

Keywords: *Annona muricata*, green synthesis of silver nanoparticles, oral squamous cell carcinoma, OECM1 and anticancer activity.

INTRODUCTION

Oral cancer (mouth, tongue and lip cancers) is currently the sixth most common cancer worldwide.[1] With India accounting for about one-third of the global burden, this disease is a big global health problem and an estimated 77,000 new cases and 52,000 deaths are reported annually in India.[1] Tobacco use, betel-quid chewing, high alcohol consumption, and chronic viral infections (like the human papillomavirus, or HPV) are the leading risk factors for oral cancer.[1,2] One major issue in oral cancer management is that most cases, especially in developing countries, are diagnosed at a very advanced stage (III-IV), with only 20- to 50% 5-year survival rate.[1,3]

The usual treatment options for oral cancer include surgery, radiotherapy and chemotherapy.[1,2] These interventions, however, frequently have high morbidity and socioeconomic impacts and cause severe disfigurement.[3] Moreover, numerous drugs made chemically have a way of causing side effects, from gastrointestinal troubles to more serious complications such as heart failure and stroke.[4] Therefore, new non-invasive and cost-effective diagnostic and therapeutic approaches which can specifically target malignant cells while sparing cells from systemic toxicity are urgently needed.[1,5]

With this background, the use of medicinal plants as alternatives to synthetic drugs has been accepted worldwide because of its high content of secondary metabolites with relatively low side effects.[4] The Annonaceae family includes *Annona muricata*, commonly referred to as Soursop or Graviola, which has been widely studied for its numerous healing properties, especially its strong effects against cancer and various types of oxidative stress.[5,6] The leaves of *A. muricata* have been the subject of numerous studies, however, the seeds of *A. muricata* are also rich in bioactive compounds, including alkaloids, flavonoids and acetogenins that are unique to this family and are potent as antitumour agents.[6,7,8] The seeds can be easily collected without killing the plant and also have high radical scavenging activity, indicating its potential for pharmaceutical uses.[9]

Another field of revolution in cancer treatment is the field of nanotechnology, which has allowed us to create the development of Nanoparticles (NPs), enhancing the pharmacokinetic and pharmacodynamic properties of drug delivery.[5] The Silver nanoparticles (AgNPs) are the most frequently used nanoparticles because of their unique optical and electrical properties and their well-documented inhibitory and bactericidal effects.[10,11] The synthesis of AgNPs with plant extracts is considered to be a cost-effective and environmentally friendly process as it excludes the use of toxic reducing agents.[11,12,13] In this biogenic method the phytochemicals in the plant extract act as both reducing agents and capping agents and frequently intensify the medicinal properties of the nanoparticles formed by the process, in the process of which they produce synergistic effects.[13]

The selective cytotoxicity of the *Annonaceous acetogenins* and the special characteristics of silver nanoparticles make this research promising for the development of a new therapeutic agent capable of attacking oral cancer.[2,5] This

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

could be a more patient-tailored and safer treatment option for people who have this disfiguring disease.[13]

MATERIALS AND METHODS

All the solvents utilized are of analytical grade. They were procured from Shri Venkatesh Enterprises Dharwad. Soxhlet apparatus for extraction. Seeds were collected from Absons House, Malappuram, and Kerala India. They were authenticated by Dr. Harsha Hegade, Scientist-E ICMR, Belgaum. Oral cancer cell lines (OECM 1) from Infinite Biotech Institute of Research and Analytics Maharashtra, India.

Microscopic Study

The seeds of *Annona muricata* were soaked in warm water for 3 days to remove the seed coat and fixed in 10% formalin. Samples were dehydrated in a series of ethanol (60-100%), cleared in xylene, infiltrated with molten paraffin and embedded in paraffin blocks at 60°C. Paraffin blocks were cut with a microtome, mounted onto glass slides, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). The sections were then dehydrated, cleared, mounted and viewed with a compound light microscope for histopathological studies.[14]

Extraction of seeds

The seeds of *Annona muricata* were dried and powdered then extracted by Soxhlet apparatus. The seed powder was placed in a thimble, weighed and extracted with ethanol as the solvent. The extraction was carried on until the whole plant material was exhausted, as shown by the colourless siphon tube solvent. The extract obtained was concentrated by a water bath to remove excess solvent.[8,9]

Qualitative Phytochemical Screening

The ethanolic extract of *Annona muricata* seeds was screened for various phytochemical constituents using standard qualitative methods. Alkaloids were detected using Mayer's and Dragendorff's reagents. Phenolic compounds were identified using the ferric chloride test. Proteins were detected by the Biuret test, carbohydrates by Molisch's test, flavonoids by the alkaline reagent test, saponins by the foam test, tannins by the ferric chloride test, fats and oils by the spot test, glycosides by Keller–Killiani test, and vitamins by standard qualitative procedures. The development of characteristic colour changes or precipitates indicated the presence of the corresponding phytoconstituents.[15]

Synthesis of silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized by adding 10 mL of ethanolic extract of the seeds of *Annona muricata* dropwise to 100 mL of 1 mM solution of silver nitrate at 70°C with continuous magnetic stirring. The reaction pH was adjusted to 9 with 1% (w/v) NaOH, and incubated overnight in the dark at room temperature. The appearance of a dark brown color confirmed the formation of AgNPs. The collected nanoparticles were centrifuged at 10,000 rpm for 30 min, washed three times with distilled water to wash the residual extract and re-centrifuged.[16]

Characterization of AgNPs

UV - Visible spectroscopy analysis:

The absorbance of the samples was measured using a UV–Visible spectrophotometer (UV-1900i, Shimadzu Corporation, Kyoto, Japan). Samples were analysed in quartz cuvettes with a 1 cm path length at the predetermined wavelength of 420 nm. Distilled water was used as the blank, and all measurements were performed in triplicate.[17]

Dynamic Light Scattering (DLS) Analysis:

The synthesized silver nanoparticles were characterized by Dynamic Light Scattering (DLS) analysis on Malvern Panalytical Zetasizer. The solution of nanoparticles was dispersed in DI water and analysed at 25 °C. Measurements were made in disposable polystyrene cuvettes and intensity based scattering data provided particle size distribution. The Z-average diameter and polydispersity index (PDI) were determined by cumulants analysis method.[18]

Determination of zeta potential:

Using electrophoretic light scattering, the zeta-potential of the prepared silver nanoparticles was measured with a Zetasizer Nano ZS90 (Malvern Panalytical, UK). The nanoparticle suspension was diluted with Milli-Q water (1:10, v/v) before the analysis. The measurements were taken with a DTS1070 folded capillary cell at 25 °C in triplicates and the average value was used to determine surface charge and colloidal stability of the nanoparticles.[19]

Scanning Electron Microscopy (SEM):

The surface morphology of the synthesized silver nanoparticles was investigated by Scanning Electron Microscope (SEM, SU3800, Hitachi, Japan). Samples of nanoparticles were dried, attached to aluminum stubs and then studied in high vacuum at 30 kV accelerating voltage, 6.1 mm working distance and 15,000× magnification. The resulting micrographs were analysed to find the shape of the particles, as well as the surface morphology.[19]

Energy Dispersive X-ray Spectroscopy (EDX):

Energy Dispersive X-ray Spectroscopy (EDX) coupled with Hitachi (SU3800) Scanning Electron Microscope (SEM) was used to determine elemental composition of the synthesized silver nanoparticles. These samples of nanoparticles were then dried and placed on aluminium stubs for analysis at an accelerating voltage of 30 kV. The EDX spectra were obtained and analyzed to calculate the elemental composition, such as wt. % and at. % of the elements detected.[20]

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Transmission Electron Microscopy (TEM):

Transmission Electron Microscopy (TEM) was used to investigate the morphology and particle size of the nanoparticles synthesized. The diluted nanoparticle suspension was dropped onto a carbon-coated copper grid, air-dried at room temperature and then viewed in a TEM. The micrographs obtained were analyzed to evaluate the particle morphology, size and dispersion properties.[21]

Entrapment Efficiency:

The entrapment efficiency (EE%) of seed extract loaded silver nanoparticles of *Annona muricata* was indirectly calculated. The unentrapped extract in the supernatant was collected after centrifuging the nanoparticle suspension at 10,000 rpm for 20 min. The supernatant was diluted 10-fold with distilled water (1 mL of supernatant was transferred to 10 mL of distilled water) and the absorbance measured at 230 nm with a UV–Visible spectrophotometer, blanked with distilled water. Free extract concentration was determined from the calibration curve (Y = mx+ c) and the efficiency of entrapment was determined by the following equation:[22]

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total Extract} - \text{Free extract}}{\text{Total extract}} \times 100$$

FTIR Analysis:

Fourier Transform Infrared (FTIR) spectroscopy was used to determine the functional groups of the extract of *Annona muricata* seeds and their contribution for reduction and stabilization of the synthesized green silver nanoparticles (AgNPs). The FTIR spectra of the seed extract (Sample B) and silver nanosuspension (Sample A) were obtained using Bruker FTIR spectrometer with Attenuated Total Reflectance (ATR) accessory at room temperature ranging from 4000–400 cm^{-1}. The spectra obtained were analyzed and compared to find the characteristic functional groups and to ensure participation of phytochemicals in the synthesis and stabilization of nanoparticles.[23]

Anticancer Activity:

The synthesized silver nanoparticles were tested in vitro for their anticancer activity against OECM1 (oral squamous carcinoma cell line) by MTT assay. The OECM1 cells were maintained at 37ºC in the presence of 5% CO_2 and humidity at DMEM with FBS and antibiotic-antimycotic solution. Cells (1 × 104 cells/well) were then seeded in 96-well plates and then treated with various doses of silver nanoparticles (20–100 µg/mL) for 24 h. The negative and positive controls used were DMSO (0.2% in PBS) and 5-fluorouracil (5-FU), respectively. After the treatment MTT solution (5mg/mL) was added and the plates were incubated for 4 h. The formazan crystals formed were dissolved in DMSO and absorbance was recorded at 550nm in a microplate reader. The percentage of inhibition and cell viability were determined against the untreated control cells.[24]

RESULTS AND DISCUSSION

Microscopic Study:

The transverse section of seed (figure 1) of *Annona muricata* showed well organized seed tissue, compact parenchymatous cells and distinct cell walls. The food reserves were stored in many cells in the tissue. Large storage cavities or oil containing cells were also observed, suggesting the presence of bioactive constituents which might impart their medicinal and nutritional attributes to the seed. The structure of the seed was intact, which was confirmed by the intact cellular architecture.

Extraction of seeds:

Ethanol was used as a solvent with a Soxhlet apparatus to extract the powdered seeds of *Annona muricata*, which was successful. The extract obtained was yellow in colour and sticky, which is an indication of the presence of other phytochemicals like fats, oils, phenolics and other secondary metabolites.

a b c

Figure 1: a. Seeds of *Annona muricta*, b. T.S of *Annona muricata* seed at 45x, c. T.S of *Annona muricata* seed at 10x

Qualitative Phytochemical Screening:

Table 1: Qualitative test for different phytoconstituents.

Phytochemical Constituents	Alkaloids	Phenol	Proteins	Carbohydrates	Flavonoids	Alkaloids	Saponin	Tannins	Fats and Oils	Glycosides
Inference	+	+	-	+	+	+	+	-	+	+

(+)present, (-) absent.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Synthesis of silver nanoparticles:

Visual observation (figure 2) was initially used as a test to confirm the synthesis of silver nanoparticles using *Annona muricata* seed extract. When the seed extract was added to the silver nitrate ($AgNO_3$) solution, the reaction mixture showed an obvious change in colour from pale yellow to dark brown. This colour change is believed to be due to the excitation of surface plasmon resonance (SPR) of the silver nanoparticles and is regarded as preliminary evidence of the formation of nanoparticles. The one with the control (seed extract), however, did not exhibit any changes in colour during the entire experimental period. The colour change observed suggests that phytoconstituents of *Annona muricata* seed extract have reduced the silver ions (Ag^+) to silver nanoparticles.

Characterization of AgNps

UV - Visible spectroscopy analysis

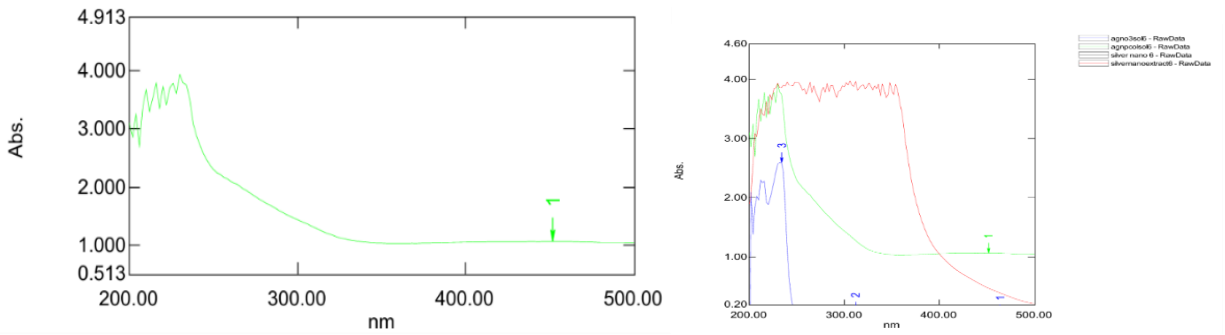
UV–Visible spectroscopy (figure 3) was performed to confirm the biosynthesis of silver nanoparticles using *Annona muricata* seed extract. A characteristic surface plasmon resonance (SPR) absorption peak was observed at 452 nm, confirming the reduction of Ag^+ ions to metallic silver nanoparticles by the phytoconstituents present in the seed extract. The absence of a corresponding SPR peak in the silver nitrate solution and seed extract alone further verified nanoparticle formation. The broad absorption band suggests the formation of moderately polydisperse and stable silver nanoparticles.

Determine the Size and Zeta Potential of particles.

Dynamic Light Scattering (DLS) and zeta potential analysis were conducted (figure 4) to assess the particle size distribution and colloidal stability of the silver nanoparticles biosynthesized. The DLS analysis showed that in the process formed nanoparticles within the nanometric range in which the mean hydrodynamic particle size is 138.2 nm. The narrow size distribution observed for the particles suggested a relatively uniform formation of nanoparticles. The increased hydrodynamic size relative to the electron microscopy particle size, could be explained by the presence of the phytoconstituents from *Annona muricata* seed extract onto the surface of the nanoparticle, which creates a hydration layer around the particles. The zeta potential analysis revealed that the surface of nanoparticles was negative and that they had moderate colloidal stability with a value of −17.11 mV. It is believed that the negative charge of the surface is caused by the presence of biomolecules from plants which are adsorbed on the surface and give electrostatic stabilization and reduce the aggregation of the particles during storage.

a b c

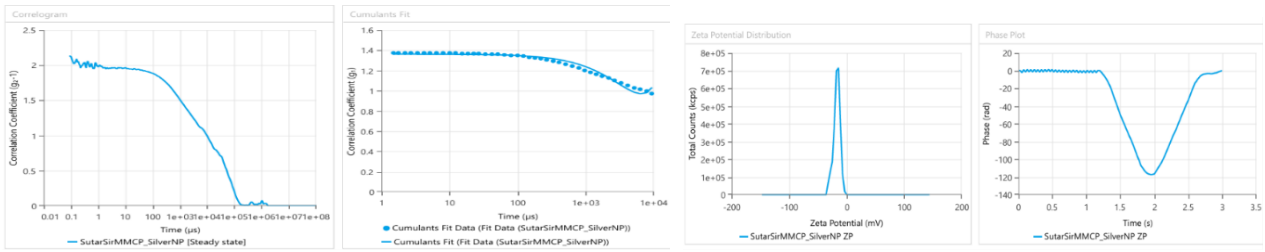
Figure 2: a. *Annona muricata* seeds extract, b. silver nitrate solution, c. Synthesized AgNps



a b

Figure 3: a) UV–Visible absorption spectrum of silver nanoparticles synthesized using *Annona muricata* seed extract. b) Comparative UV–Visible spectral overlay of $AgNO_3$ solution, *Annona muricata* seed extract, and silver nanoparticle suspension. The appearance of a distinct SPR peak around 452 nm in the nanoparticle.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.



a b

Figure 4: a. Correlogram and cumulants fit obtained from dynamic light scattering (DLS) measurements of synthesized silver nanoparticles. b. Zeta potential distribution and phase plot of synthesized silver nanoparticles.

Scanning electron microscopy (SEM)

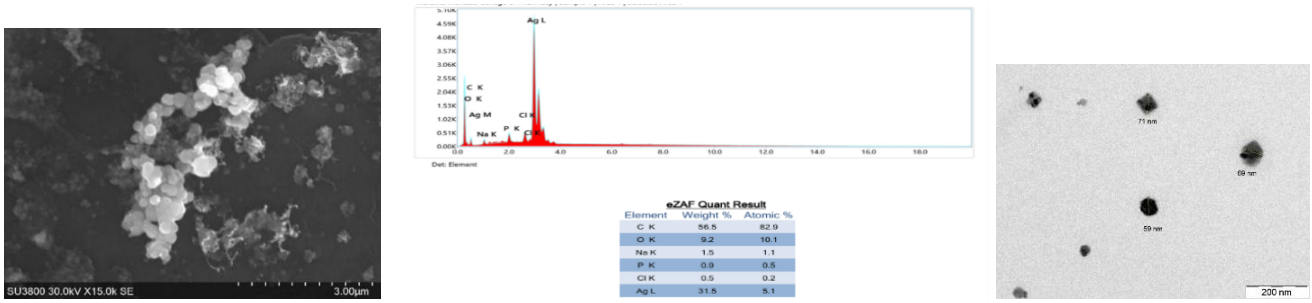
The morphology of the synthesized AgNPs was studied by scanning electron microscopy (SEM). SEM image showed that the nanoparticles were mostly spherical in shape and had an aggregating or clustering property. This observed aggregation could be due to interparticle attractive forces and high surface energy of nanoparticles while drying and preparing the sample. In spite of the aggregation, the particles showed a fairly uniform morphology, which is proof of successful synthesis of silver nanoparticles. The size of the particles in SEM image was in nanometre range and matched with the hydrodynamic size from DLS analysis.

Energy Dispersive X-ray Spectroscopy (EDX)

Energy-dispersive X-ray spectroscopy (EDS) was used to investigate the elemental composition of the synthesized silver nanoparticles. A strong response was seen at the EDS spectrum for the presence of silver (Ag), indicating the formation of silver nanoparticles. The silver content in the selected areas was around 23.3-31.5 wt.% while the rest were mainly carbon and oxygen content. Tiny levels of sodium, phosphorus and chlorine were also found. High levels of carbon and oxygen might be linked to phytochemicals or other organic biomolecules that could have been adsorbed on the surface of the nanoparticles, that presumably served as reducing and capping agents during the synthesis of the nanoparticles. The elemental presence of Ag confirmed by the EDS results was in accordance with the successful biosynthesis of AgNPs.

Transmission Electron Microscopy (TEM)

Silver nanoparticles were characterized for morphology and particle size by Transmission electron microscopy (TEM). TEM micrographs showed that the primary shape of the nanoparticles was predominantly spherical to quasi-spherical with sizes of 56–80 nm (Figure 11a) and 59–71 nm (Figure 11b). The nanoparticles were well dispersed with minimal aggregation, suggesting the biomolecules used in the biosynthesis process provided effective stabilization. The morphology observed confirms the successful formation of silver nanoparticles with nanoscale dimensions. The TEM image particle size estimation was about 68 nm. The particle size variations can be explained by the differences in nucleation and growth of nanoparticles. The TEM image also confirms the SEM observations and shows that the synthesized AgNPs are indeed on a nanometre scale.



a b c

Figure 5: Characterization of synthesized silver nanoparticles (AgNPs): (a) Scanning Electron Microscopy (SEM) image showing surface morphology of AgNPs, (b) Energy Dispersive X-ray (EDX) spectrum confirming the elemental composition and presence of silver, and (c) Transmission Electron Microscopy (TEM) image showing the morphology and nanoscale particle size of AgNPs.

Entrapment Efficiency

The entrapment efficiency of *Annona muricata* seed extract-loaded silver nanoparticles was determined by measuring the concentration of free (unentrapped) extract in the supernatant using UV–Visible spectrophotometry at 230 nm. Based on the calibration curve (Y = 0.261384X + 0.0398589), the diluted supernatant (absorbance = 1.093) corresponded to a free extract concentration of 4.03 mg/L, which was 40.3 mg/L (0.0403 mg/mL) after applying the dilution factor. Considering the initial extract content of 0.15 mg in the nanoparticle formulation, the entrapment efficiency was calculated to be 73.13%. This result indicates

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

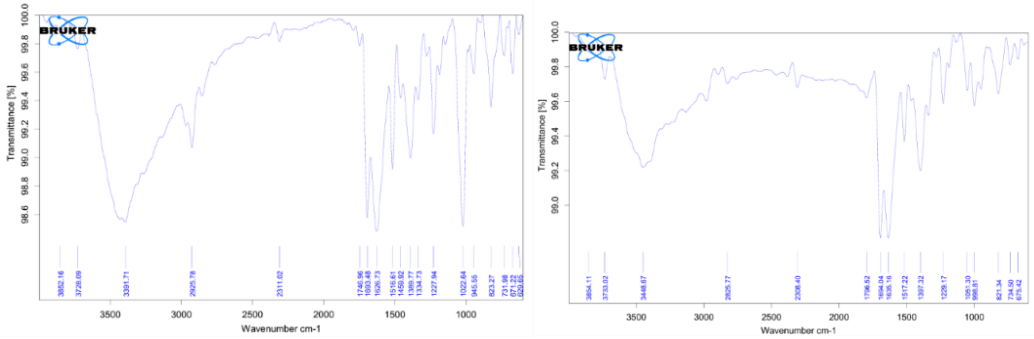
efficient loading of the phytoconstituents into the silver nanoparticles, while 26.87% remained unentrapped in the supernatant. The high entrapment efficiency may be attributed to the interaction of phenolic compounds, flavonoids, alkaloids, and other phytochemicals with silver ions during nanoparticle formation, contributing to stable nanoparticle synthesis.

FTIR Analysis

FTIR analysis (figure 6) was performed to identify the functional groups involved in the reduction and stabilization of silver nanoparticles synthesized using *Annona muricata* seed extract. The FTIR spectrum of the seed extract showed characteristic absorption bands corresponding to hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), aromatic C=C, amine (N–H/C–N), and C–O functional groups, indicating the presence of phenolics, flavonoids, proteins, and other phytoconstituents. The FTIR spectrum of the synthesized silver nanosuspension exhibited similar bands with slight shifts in the O–H (3448.67 to 3391.71 cm^{-1}), aromatic C=C (1635.16 to 1626.73 cm^{-1}), and C–O (1051.30 to 1022.64 cm^{-1}) stretching vibrations. These spectral shifts indicate the interaction of phytochemicals with silver ions during nanoparticle formation, confirming their role as reducing and capping agents. The retention of the major functional groups along with the observed peak shifts confirms the successful synthesis and stabilization of *Annona muricata*-mediated silver nanoparticles.

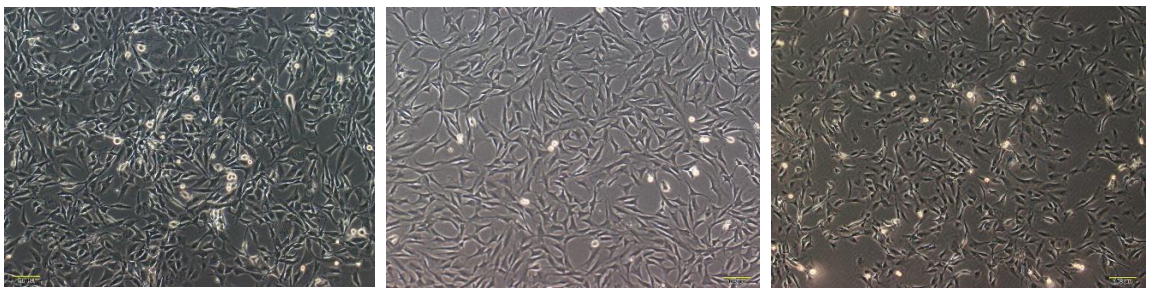
Anticancer activity

The anticancer activity of the synthesized silver nanoparticles was evaluated against the OECM1 oral squamous carcinoma cell line using the MTT assay. The nanoparticles exhibited concentration-dependent cytotoxic effects, with the percentage inhibition increasing as the concentration increased from 20 to 100 µg/ml.At concentrations of 20, 40, 60, 80, and 100 µg/mL, the nanoparticles produced 20.59%, 23.27%, 44.26%, 66.16%, and 68.77% growth inhibition, respectively. Correspondingly, cell viability decreased from 79.41% at 20 µg/mL to 31.23% at 100 µg/ml. The IC_{50} value of the sample was determined to be 67.85 µg/mL, indicating significant cytotoxic activity against OECM1 cells.The standard anticancer drug 5-fluorouracil (5-FU) exhibited stronger activity with an IC_{50} value of 38.15 µg/ml. However, the synthesized silver nanoparticles demonstrated notable anticancer potential, suggesting their suitability as a promising candidate for further investigation against oral cancer cells.



a b

Figure 6: FTIR spectra of (a) green synthesized silver nanosuspension and (b) *Annona muricata* seed extract. The spectra show characteristic functional groups involved in the reduction and stabilization of silver nanoparticles, with slight shifts in peak positions after nanoparticle synthesis, confirming successful green synthesis.



b c d

Figure 7: Treatment to cancer cells and evaluation of the cell's morphology under an inverted microscope. (a) EffectsofcompoundagainstOECM1Celllines.(b)Normal morphology of the untreated control cells and high cell density. (c) Treated cells in standard conditions showing decreased cell density, cell size shrinkage, and morphological alteration as signs of cytotoxicity. (d) *Annona muricata* mediated silver nanoparticles treated cells indicated remarkable morphological alterations like cell rounding, shrinkage, and reduced confluency, demonstrating anticancer activity. All the images were taken at the same magnification (scale bar is 100 µm).

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Sr. No	Sample Code	Conc. µg/ml	OD			Mean	% of Inhibition	%of Viability	IC50 µg/ml
1	Control		1.532						-
2	Standard (5-Furouracil)	20	0.977	0.975	0.979	0.977	36.31%	63.69%	38.15
		40	0.746	0.744	0.748	0.746	51.36%	48.64%	
		60	0.519	0.521	0.518	0.519	66.16%	33.84%	
		80	0.389	0.389	0.387	0.388	74.70%	25.3%	
		100	0.246	0.248	0.244	0.246	83.96%	16.04%	
3	Sample A	20	1.218	1.220	1.216	1.218	20.59%	79.41%	67.85
		40	1.178	1.176	1.179	1.177	23.27%	76.73%	
		60	0.855	0.857	0.853	0.855	44.26%	55.74%	
		80	0.519	0.521	0.517	0.519	66.16%	33.84%	
		100	0.479	0.481	0.477	0.479	68.77%	31.23%	

DISCUSSION

The present study demonstrated the successful green synthesis of silver nanoparticles (AgNPs) using the ethanolic seed extract of *Annona muricata* and evaluated their anticancer potential against OECM1 oral squamous carcinoma cells. Green synthesis offers an eco-friendly and cost-effective alternative to conventional chemical methods by utilizing plant phytochemicals as reducing and stabilizing agents. The qualitative phytochemical screening of the seed extract revealed the presence of alkaloids, phenolic compounds, flavonoids, saponins, carbohydrates, glycosides, and fats/oils, which may have played an important role in the reduction of silver ions and stabilization of the synthesized nanoparticles. These phytoconstituents are also known to possess antioxidant and anticancer properties, potentially contributing to the biological activity of the nanoparticles.

The formation of AgNPs was initially confirmed by the visual colour change from pale yellow to dark brown, which is characteristic of silver nanoparticle synthesis due to surface plasmon resonance (SPR). UV–Visible spectroscopy further confirmed nanoparticle formation by exhibiting a distinct absorption peak at 452 nm. This SPR peak falls within the characteristic range reported for silver nanoparticles synthesized using plant extracts and indicates the successful reduction of Ag^+ ions into metallic silver nanoparticles. The broadness of the absorption band suggests a polydisperse nanoparticle population, which is commonly observed in biologically synthesized nanoparticles.

Dynamic Light Scattering (DLS) analysis revealed an average hydrodynamic particle size of 138.2 nm, whereas TEM analysis showed particle sizes ranging from 56–80 nm and 59–71 nm, with an average size of approximately 68 nm. The larger size observed in DLS compared to TEM can be attributed to the hydrodynamic diameter measured in solution, which includes the adsorbed phytochemical layer surrounding the nanoparticles. In contrast, TEM measures the actual metallic core size. Similar differences between DLS and TEM measurements have been reported for plant-mediated silver nanoparticles due to capping by bioactive molecules. The TEM images further revealed predominantly spherical to quasi-spherical nanoparticles with good dispersion and minimal aggregation, indicating effective stabilization by biomolecules present in the seed extract.

The zeta potential value of −17.11 mV indicated a negatively charged nanoparticle surface and moderate colloidal stability. The negative surface charge may be attributed to the adsorption of phenolic compounds and other oxygen-containing functional groups derived from the plant extract. Such surface charges help prevent excessive aggregation through electrostatic repulsion and contribute to nanoparticle stability during storage and biological applications.

SEM analysis showed predominantly spherical nanoparticles with some degree of agglomeration. Aggregation is commonly observed in silver nanoparticles due to their high surface energy and drying effects during sample preparation. Nevertheless, the overall morphology remained relatively uniform, indicating successful nanoparticle synthesis. EDX analysis further confirmed the elemental composition of the nanoparticles by showing a strong characteristic silver signal. The presence of carbon and oxygen peaks supports the existence of plant-derived phytochemicals on the nanoparticle surface, which likely function as capping and stabilizing agents.

The synthesized AgNPs exhibited significant cytotoxic activity against OECM1 oral squamous carcinoma cells in a concentration-dependent manner. The percentage inhibition increased from 20.59% at 20 µg/mL to 68.77% at 100 µg/mL, with an IC_{50} value of 67.85 µg/ml. Although the standard drug 5-fluorouracil demonstrated higher potency (IC_{50} = 38.15 µg/mL), the AgNPs showed substantial anticancer activity, highlighting their therapeutic potential. The enhanced cytotoxic effect may result from the combined action of silver nanoparticles and bioactive phytochemicals from *Annona muricata* seeds.

The anticancer activity of AgNPs has been widely attributed to multiple mechanisms, including the generation of reactive oxygen species (ROS), induction of oxidative stress, mitochondrial dysfunction, DNA damage, and activation of apoptotic pathways. Silver nanoparticles can penetrate

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

cancer cells and disrupt cellular redox balance, leading to programmed cell death. Additionally, *Annona muricata* seeds contain annonaceous acetogenins, flavonoids, and phenolic compounds that have been reported to exhibit selective cytotoxicity against cancer cells. Therefore, the observed cytotoxic effect may arise from a synergistic interaction between the intrinsic anticancer constituents of the seed extract and the physicochemical properties of AgNPs.

Overall, the findings demonstrate that *Annona muricata*-mediated silver nanoparticles possess favourable physicochemical characteristics, including nanoscale size, spherical morphology, moderate stability, and significant anticancer activity against OECM1 oral cancer cells. These results suggest that biosynthesized AgNPs may serve as a promising nanotherapeutic candidate for oral cancer treatment. However, further investigations involving mechanistic studies, apoptosis assays, molecular pathway analysis, and in vivo evaluations are required to establish their safety, efficacy, and clinical applicability.

CONCLUSION

In the present study, silver nanoparticles (AgNPs) were successfully synthesized by the ethanolic seed extract of *Annona muricata*. All the biosynthesized AgNPs were characterized using UV-VIS spectroscopy, DLS, zeta potential analysis, SEM, EDX, TEM, FTIR, and entrapment efficiency studies, which confirmed the successful formation of biosynthesized AgNPs, nanoscale size, spherical shape, elemental composition, moderate colloidal stability, and efficient phytoconstituent loading. Further, FTIR analysis verified the participation of biomolecules extracted from plants as reducing and stabilizing agents in the process of the synthesis of nanoparticles. The synthesized AgNPs had also shown appreciable concentration-dependent cytotoxic effect against the oral squamous carcinoma cell line OECM1 with IC_{50} value as 67.85µg/mL indicating their promising anticancer potential. The combination of silver nanoparticles with the bioactive phytochemical present in A. muricata seeds may be associated with the anticancer effect. In general, the results indicate that the AgNPs produced by A. muricata serve as a green nanotherapeutic method for oral cancer treatment. Further mechanistic characterization, thorough toxicity studies and in vivo testing, are still needed before they can be proven to be safe, effective and clinically useful.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sensors International. 2020 Jan 1;1:100046.
2. Cleveland Clinic. Oral cancer: causes, symptoms and treatment [Internet]. Cleveland (OH): Cleveland Clinic; 2024 Aug 2 [cited 2026 Jun 17]. Available from: https://my.clevelandclinic.org/health/diseases/12194-cancer
3. Ziober BL, Mauk MG, Falls EM, Chen Z, Ziober AF, Bau HH. Lab-on-a-chip for oral cancer screening and diagnosis. Head & Neck: Journal for the Sciences and Specialties of the Head and Neck. 2008 Jan;30(1):111-21.
4. Liew PM, Yong YK. Stachytarpheta jamaicensis (L.) Vahl: from traditional usage to pharmacological evidence. Evidence-Based Complementary and Alternative Medicine. 2016;2016(1):7842340.
5. Mohanraj VJ, Chen YJ. Nanoparticles-a review. Tropical journal of pharmaceutical research. 2006;5(1):561-73.
6. Naik AV, Sellappan K. Physicochemical and Phytochemical Analysis of Different Plant Parts of *Annona muricata* L.(Annonaceae). Pharmaceutical methods. 2019 Jul 1;10(2).
7. Coria-Téllez AV, Montalvo-Gónzalez E, Yahia EM, Obledo-Vázquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arabian Journal of chemistry. 2018 Jul 1;11(5):662-91.
8. Tambun R, Alexander V, Ginting Y. Performance comparison of maceration method, soxhletation method, and microwave-assisted extraction in extracting active compounds from soursop leaves (*Annona muricata*): A review. InIOP Conference Series: Materials Science and Engineering 2021 Mar 1 (Vol. 1122, No. 1, p. 012095). IOP Publishing.
9. Nguyen VT, Nguyen MT, Tran QT, Thinh PV, Bui LM, Le TH, Le VM, Linh HT. Effect of extraction solvent on total polyphenol content, total flavonoid content, and antioxidant activity of soursop seeds (*Annona muricata* L.). InIOP Conference Series: Materials Science and Engineering 2020 Jan 1 (Vol. 736, No. 2, p. 022063). IOP Publishing.
10. MKNano. Nanoparticles [Internet]. Mississauga (ON): MKNano; 2026 [cited 2026 Jun 17]. Available from: https://www.mknano.com/nanoparticles/
11. Abbasi E, Milani M, Fekri Aval S, Kouhi M, Akbarzadeh A, Tayefi Nasrabadi H, Nikasa P, Joo SW, Hanifehpour Y, Nejati-Koshki K, Samiei M. Silver nanoparticles: synthesis methods, bio-applications and properties. Critical reviews in microbiology. 2016 Mar 3;42(2):173-80.
12. Parveen K, Banse V, Ledwani L. Green synthesis of nanoparticles: Their advantages and disadvantages. InAIP conference proceedings 2016 Apr 13 (Vol. 1724, No. 1, p. 020048). AIP Publishing LLC.
13. Olabemiwo OM, John G, Oyebamiji AK, Akintelu SA. Phytosynthesized bimetallic gold-copper nanoparticles using Anacardium occidentale leaf extract and its potential antidiabetic activity. Current Research in Green and Sustainable Chemistry. 2025 Oct 2:100489.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

14. Miya M, Hibara KI, Itoh JI. Preparation and Sectioning of Paraffin-Embedded Tissue for Histology and Histochemistry. Methods Mol Biol. 2025;2869:41-48. doi: 10.1007/978-1-0716-4204-7_6. PMID: 39499466.

15. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30.

16. Ezealisiji KM, Noundou XS, Ukwueze SE. Green synthesis and characterization of monodispersed silver nanoparticles using root bark aqueous extract of *Annona muricata* Linn and their antimicrobial activity. Applied Nanoscience. 2017 Nov;7(8):905-11.

17. Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. Biosynthesis of silver nanoparticles using Coriandrum sativum leaf extract and their application in nonlinear optics. Advanced science letters. 2010 Jun 1;3(2):138-43.

18. Kumavat SR, Mishra S. Green synthesis of silver nanoparticles using Borago officinalis leaves extract and screening its antimicrobial and antifungal activity. International Nano Letters. 2021 Dec;11(4):355-70.

19. Alkammash NM. Synthesis of silver nanoparticles from Artemisia Sieberi and *Calotropis procera* medical plant extracts and their characterization using SEM analysis. Biosciences Biotechnology Research Asia. 2017 Jun 28;14(2):521-6.

20. Goldstein JI, Newbury DE, Michael JR, Ritchie NW, Scott JH, Joy DC. Scanning electron microscopy and X-ray microanalysis. springer; 2017 Nov 17.

21. Murphy CJ, Sau TK, Gole AM, Orendorff CJ, Gao J, Gou L, Hunyadi SE, Li T. Anisotropic metal nanoparticles: synthesis, assembly, and optical applications. The Journal of Physical Chemistry B. 2005 Jul 28;109(29):13857-70.

22. Danaei MR, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari YM. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018 May 18;10(2):57.

23. Stuart BH. Infrared spectroscopy: fundamentals and applications. John Wiley & Sons; 2004 Aug 20.

24. Hansen MB, Nielsen SE, Berg K. 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. Journal of Immunological Methods. 119(2):203-210

For any questions related to this article, please reach us at: globalresearchonline@rediffmail.com
New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.