Green Synthesized silver nanoparticles utilizing Gmelina arborea Leaves and assessed its Antibacterial, Antifungal, Antioxidant and Anticancer Potential

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Abstract

An eco-friendly approach was employed to synthesize silver nanoparticles (L-AgNPs) using *Gmelina arborea* leaf extract as a reducing and capping agent. UV-Visible spectroscopy exhibited the molecular interaction resonance peak at 402 nm and a discernible colour change from pale yellow to dark brown confirmed the synthesis of L-AgNPs. Fourier transform infrared spectroscopy (FTIR) identified the families of compounds from the leaf extracts that acted as electron donors and reducing Ag^+ ion into metallic silver and capping the nanoparticles. X-ray Diffraction (XRD) confirmed the Face-centered Cubic crystalline structured of synthesized L-AgNPs, and Scanning Electron Microscopy (SEM) revealed predominantly spherical particles, while the existence of silver was confirmed by EDAX analysis. The size of the L-AgNPs was determined using the Transmission Electron Microscopy (TEM) analysis. Antibacterial activity was assessed against two Gram-positive and Gram-negative bacteria. Assessed the antifungal efficiency at a concentration of 10 µL/mL, against *Candida tropicalis* and *Aspergillus fumigatus*, L Ag NPs exhibited greater antifungal activity against *Candida tropicalis* than *Aspergillus fumigatus*. L Ag NPs exhibited a zone of inhibition of 8 mm while the positive control Fluconazole showed 9 mm. L Ag NPs has 73.63% antioxidant activity at concentration of 100 µg/mL, whereas ascorbic acid exhibited 82.45 % at the same concentration. The half-maximal inhibitory concentration (IC_{50}) for DPPH radical scavenging activity was 31.95 µg/mL. The IC_{50} value of L-AgNPs against PA1 Ovarian cancer cell lines was 5.69 µg/mL. The study highlights the potential of biosynthesized L Ag NPs as biocompatible and eco safe or environmentally safe antimicrobial and anticancer agent.

Keywords: *Gmelina arborea* leaf extract; Silver nanoparticles; Antibacterial; Antifungal; Antioxidant; Anticancer activity.

How to cite this article: Dayana BM, Prakash JTJ, Sowmiya K, Meena S. Green Synthesized Silver Nanoparticles Utilizing *Gmelina arborea* Leaves and Assessed its Antibacterial, Antifungal, Antioxidant and Anticancer Potential. Int J Drug Deliv Technol. 2026;16(69s):1579-1589. DOI: 10.25258/ijddt.16.69s.153

1. Introduction

Nanotechnology is a multidisciplinary field which includes colloidal science, chemistry, physics, and biology focused on phenomena at the nanoscale [1]. Matter at its nanoscale shows distinct structural, biological and optical properties which make it applicable to play a master role in various approaches like drug delivery, biosensors, controlling the rate of abnormal cell proliferation, inhibiting the growth of infectious agents, and regulating the abnormal molecular mechanism in the body [2, 3]. Synthesis of nanoparticles by physical and chemical methods produces harmful byproducts because toxic chemicals are used in the preparation and this causes severe reaction conditions leads to toxicological concerns [4, 5].

One of the alternative method to the traditional method for producing nanoparticles is green synthesis which is ecologically responsible. They address many of the toxicological and ecological issues related with these methods [6]. Organic and botanical materials such as plants, leaves, root, bark, bacteria and some other tools are natural resources, that are used in these environmentally benign methods to break down metal ions into nanoparticles. Plant-based synthesis is an efficient method for the production of nanomaterials such as silver, gold, platinum, and palladium nanoparticles because it is simple, cheap,

and safe for living things [7].Among all these nanomaterials silver nanoparticles (AgNPs) have shown more antibacterial, antioxidant, and catalytic characteristics. Silver compounds in low amounts are reasonably safe for human body, in addition to being able to do many different things [8,9]. Because of this reason silver is considered as a major choice for biological uses including medication delivery, wound healing, and antibacterial coatings [10]. It also shows how important green synthesis is for making nanotechnology safer and more eco friendly [11-13].

Gamhar, or *Gmelina arborea*, is a fast-growing deciduous tree that belongs to the *Lamiaceae* family. This plant is well known in the countries of South and Southeast Asia and used for many therapeutic characteristics and also used in traditional herbal treatments [14]. This plant possess many bioactive phytochemicals especially the leaves, bark and roots that contain flavonoids, alkaloids, and tannins. These bioactive elements shows more potential to destroy bacteria, reduce inflammation, and protect cells from damage. Cancer is a disease that causes death because of the abnormal cells in the body to proliferate and divide without control [15]. It is still one of the biggest health problems in the world, killing around seventy lakh people every year, even in very developed, industrialized countries. Recent estimates say that in 2017, cancer killed more than 9.5 million people around the world, making it the second most common cause of death behind heart disease [16, 17]. Various kinds of cancer exist, and each one affects the body in a different way. Among them. Lung carcinoma is the most widespread and dangerous types.

The 2020 Global Cancer Report says that lung cancer affects about 18 out of every 100,000 people and is the second largest cause of cancer fatalities. Breast cancer is another common and dangerous type of cancer [18]. Researchers have looked into a lot of different things, including plants, in their pursuit for effective cancer treatments. Herbal plants have been utilized for millennia to cure a wide range of ailments, and they are still important in modern medicine. Roots, leaves, bark, and seeds from medicinal plants can all be taken out and processed to make pharmaceutical chemicals that could be used to treat diseases [19-21]. It is interesting to note that around 60% of the anticancer medications now on the market come from plants.

The green synthesis method is an eco-conscious framework to make nanoparticles than conventional chemical and physical procedures, which often use toxic chemicals and utilizes lot of energy. After the silver nanoparticles were successfully made using biosynthesis, the study will also look at how well they fight cancer in vitro [22]. This study aims to find out if *Gmelina arborea*-mediated L-AgNPs could be a new and effective cancer treatment by looking at how hazardous they are to certain cancer cell lines. The results of this study may help the domain of nanomedicine thrive and encourage the nanotherapeutics derived from plant-based that have milder effects and are more compatible with living things. Use of G. arborea leaves provides a sustainable, low-cost, and non-toxic alternative to traditional chemical/physical methods. Also emphasize the dose dependent responses which indicates superior potency compared to other plant-based AgNPs.

2. Experimental section

2.1. Materials

Gmelina arborea plant's young and healthy leaves of Gmelina arborea were collected from areas nearby areas of Trichy. The leaves were thoroughly washed with distilled water to clean contaminants and the leaves were then shade-dried and left to sit at room temperature for approximately two weeks while avoiding direct sunlight to preserve phytochemicals. The following chemicals were used in this study. Silver nitrate ($AgNO_3$), Sodium hydroxide (NaOH), and ethanol (CH_3CH_2OH) which were obtained from Sigma-Aldrich, India. All chemicals were collected used as received without further purification.

2.2. Preparation of *Gmelina arborea* extract

Leaf sample was prepared by mixing 2 g of powdered dried leaves with 100mL of distilled water and heated using a heating mantle at 60°C for 10 minutes . After cooling, the extract was filtered through Whatman No.1 filter paper. The filtrate was stored at 4°C for further use. The prepared *Gmelina arborea* leaf extract was used for the synthesi L-AgNPs.

2.3. Preparation of silver nanoparticles from *Gmelina arborea* extract

To synthesize silver nanoparticles (L-AgNPs), we employed *Gmelina arborea* leaf extract as a reducing and stabilizing agent. To perform the synthesis, 80 mL of a 1 mM silver nitrate ($AgNO_3$) solution was carefully mixed with 20 mL of a freshly prepared aqueous filtrate of *Gmelina arborea* leaves in a sterile reaction vessel at room temperature. The reaction mixture was gently stirred at room temperature to facilitate the bio reduction process. The progressive colour change from pale yellow to dark brown provided visual evidence of silver nanoparticle formation. The reaction mixture was incubated for 24h to ensure the complete reduction of Ag^+ ions. The formation of L-AgNPs results surface plasmon resonance (SPR) which causes the color change, which is the first obvious indication that nanoparticles have been formed.

2.4. Characterization

The synthesized L-AgNPs were studied by UV-Vis spectroscopy was scanned by PerkinElmer Lambda 365 precisely. The elemental make up of the synthesized L-AgNPs was studied using Perkin

Elmer, spectrum TWO at the range of 4000 to 400 cm^{-1}. The electrostatic surface charges was analyzed using the Zetasizer Nano ZS instrument. The synthesized L-AgNPs were subjected to DLS measurement techniques were done by micromeritics, Nano Plus. XRD measurement was carried out by Panalytical/x'pert3. SEM combined with EDAX were analyzed using Tescan Oxford, Vega3 Inca. TEM helped to identify the physical arrangement of the L-AgNPs using a JEOL 2100 electron microscope. The antimicrobial activities of the synthesized L-AgNPs were assessed by disc diffusion method. All the microbial species were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Sabouraud's dextrose agar/broth(Hi media Pvt, Bombay, India) were served as a culture media for the antifungal assays. Antimicrobial assessment was repeated thrice. The free radical scavenging capacity of the prepared L-AgNPs was tested using DPPH assay and it performed thrice. Cancerous strains were maintained in DEME supplemented with heat-inactivated 10% FBS and 1% antibiotics, and the morphology of cancer cells after the treatment of synthesized L-AgNPs was studied under an inverted microscope.

3. Results and Discussion

3.1. UV-Visible spectroscopy

The absorption peak in the UV-Vis spectroscopy provides information about the particle size and dielectric property of the surrounding medium of the nanoparticles. The synthesized L-AgNPs showed an absorption peak at 402 nm in the UV-Vis region [Fig. 1(a)]. This peak is attributed to surface plasmon resonance. The phytochemicals present in the leaves of *Gmelina arborea* were used as a reducing and stabilizing agents, and the intensity of the SPR peak indicated their effectiveness [23]. The absorption of visible light occurs through the excitation of localized surface plasmon resonance, where conduction electrons in the metal nanoparticles oscillate collectively against the positively charged ionic lattice. Dipole oscillation is induced when the incident light matches the resonance frequency of the nanoparticles [24].

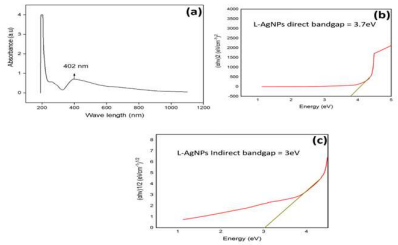

Fig. 1 (a) UV-Vis spectra of synthesized L-AgNPs, (b) The direct band gap of synthesized L-AgNPs, (c) The indirect band gap of synthesized L-AgNPs

The electrons in the nanoparticle resonate causing the absorption of electromagnetic radiation. The L-AgNPs exhibited a characteristic absorption peak at approximately 402 nm. The absorption peak at 402 nm corresponds to the transverse surface plasmon resonance in the silver nanoparticles [25]. Fig. 1(b, c) shows the direct and indirect band gaps of synthesized L-AgNPs. The indirect band gap of 3.0 eV corresponds to a wavelength which is close to the observed absorption peak. This facilitates ROS-mediated disruption of bacterial cell membranes. The direct band gap of 3.7 eV further indicates the quantum-confined nature of the nanostructure and high electron availability at the nanoparticle surface which shows prolonged antimicrobial and anticancer actions.

3.2. FTIR spectroscopy

To identify the functional groups present in the synthesized L-AgNPs that acted as capping and reducing agents, FTIR spectroscopy was carried out. Fig. 2 and 3 represents FTIR spectroscopy of *Gmelina arborea* leaf extracts and L-AgNPs which represents the vibrational modes of the chemical bonds in the molecule. Table 1 summarizes the FTIR peak positions and the corresponding functional groups of *Gmelina arborea* leaf extract and L-AgNPs. The observed absorption peaks were located at 3436, 2926, 2842, 2416, 1634, 1389, 1250, 1068, 838, 670, 587, 461 cm^{-1}. FTIR spectrum of L-AgNPs revealed strong IR bands at 3436 and 1634 cm^{-1}. The strong broad band at 3436 cm^{-1} was assigned to N-H stretching mode in the linkage of proteins. The bands associated with the vibration of the functional groups such as alcohols, phenols and carboxylic acids are likely responsible for the reduction of the Ag^{+} ion and the stabilization of the synthesized L-AgNPs [26-28]. The peak at 1634 cm^{-1} correspond to the C=O stretching mode of the amine I group which is characteristics of proteins. The peak at 670 cm^{-1} was assigned to the C-H bending vibration of aromatic compounds [29].

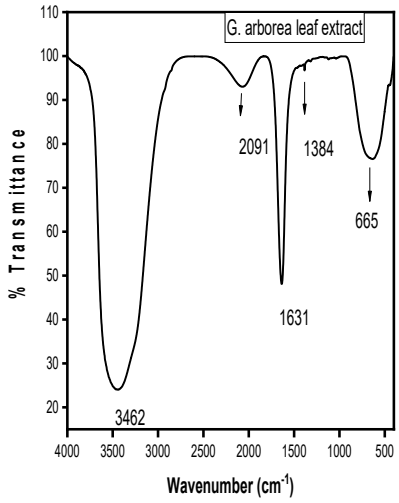

Fig. 2: FTIR analysis of *Gmelina arborea plant extract*

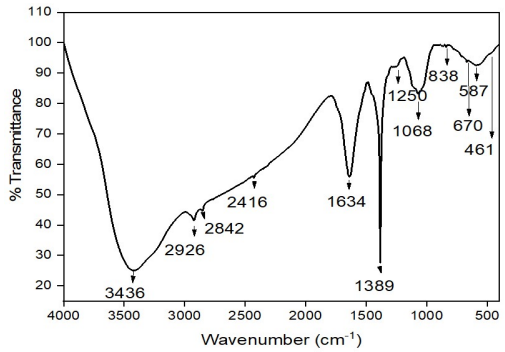

Fig. 3: FTIR analysis of synthesized L-AgNPs

Table 1 Peak values, chemical class of *Gmelina arborea plant extract and* L-AgNPs

Extract	Peak value Wave number (cm^{-1})	Functional group
G. arborea Leaf extract	3462	N-H stretching mode in linkage protein
	1631	C= O stretching mode in amine I group
	1384	CH_3 bend
	665	C-H bending mode of aromatic compounds
L Ag NPs	3436	N-H stretching mode in linkage protein
	2926	= C-H stretch
	2842	-C-H stretch
	1634	C= O stretching mode in amine I group
	1389	CH_3 bend
	1068	C-F
	670	C-H bending mode of aromatic compounds

3.3. Dynamic light scattering (DLS) and zeta potential

Fig. 4 (A) represented the Z- average diameter obtained from the DLS analysis for L-AgNPs. It was 98.80 nm with a PDI value of 0.400 . The standard deviation for DLS was 801.3 nm**.** DLS measures the hydrodynamic diameter, which includes both the metallic core of the L-AgNPs and any surface adsorbed bio-moieties that act as used as stabilizers [30]. The electric potential difference between the flowing liquid and the stationary layer that adheres to the dispersed nanoparticles is known as the zeta potential [31]. Results shown in Fig. 4 (B) demonstrated that the produced nanoparticles exhibited a zeta potential of -24 mV and were negatively charged and standard deviation is 5.52 mV. The outcome reveals that the negatively charged surface of synthesized nanoparticles exhibited a good colloidal nature with good stability and were well spread throughout the medium. Because of the strong particles repulsion, the particles remained uniformly dispersed and they did not aggregate [32].

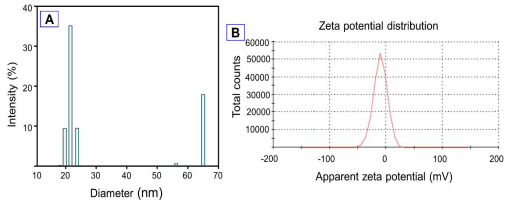

Fig. 4: (A) DLS studies and (B) Zeta potential of synthesized L-AgNPs

3.4. XRD analysis

The crystalline phases of the green synthesized L-AgNPs were identified from the XRD patterns shown in Fig.5. Bragg diffraction peaks were obtained at 2θ values of 32° and 64°. The lattice planes for silver which corresponding to the (111) and (220) crystallographic planes [33]. Through the utilization of Scherrer's formula $D = 0.9\lambda / \beta \cos\theta$, the average crystallite size of the nanoparticles synthesized through bio reductionprocess was measured [34]. The obtained results closely match the reference data from JCPDS file no. 04-0783. The average crystallite size was found to be 28.43 nm.

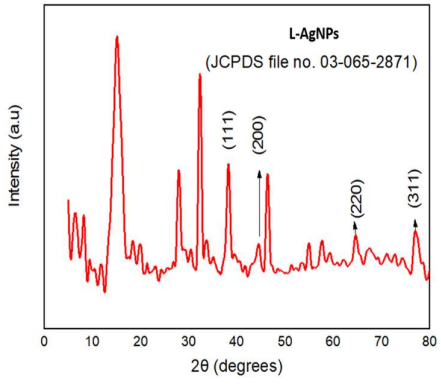

Fig. 5 XRD pattern of synthesized L-AgNPs

3.5. SEM and EDAX analysis

The SEM image of synthesized L-AgNPs is shown in Fig. 6 (A). The biosynthesized L-AgNPs exhibited predominantly spherical particles. The agglomeration of silver nanoparticles is generated attributed to the hydroxyl groups present in the aqueous leaf extract [35]. The particles with the size ranging from 6.2 to 8.2 nm can be injected into the human blood serum or human platelets easily. Therefore the green synthesized nanoparticles with low dimensions could be incorporated in biomedical applications in curing infectious diseases [36].

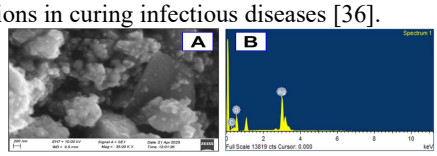

Fig. 6 (A) SEM image of synthesized L-AgNPs, and (B) EDAX of L-AgNPs

EDX analysis was performed to explore the elemental composition of the biosynthesized L-AgNPs, as shown in Fig. 6(B). The EDX spectrum reveals a strong signal at 3 keV, which is characteristic of elemental silver nanocrystals due to surface plasmon resonance. The presence of Carbon, potassium in the sample is also detected [37].

3.6. TEM analysis

Fig. 7(A) shows the morphology and surface structure of the synthesized nanoparticles which were predominantly spherical. The edges of the particles appeared lighter than the centers, indicating the presence of biomolecules, such as proteins, capping the L-AgNPs. TEM analysis showed that the average particle size was approximately 20 nm. Fig. 7 (B) shows the histogram of the particle size distribution for the L-AgNPs determined from the TEM image. The crystalline nature of the synthesized L-AgNps was identified from selected area electron diffraction pattern (SAED) as shown in Fig. 7 (C) [38].

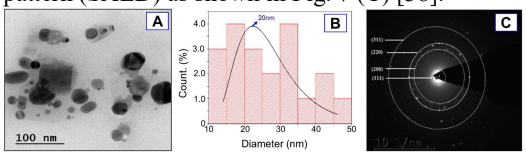

Fig. 7 (A) HR-TEM image, (B) Histogram, and (C) SAED pattern of synthesized L-AgNPs

3.7. Biological studies

3.7.1 Antibacterial capacity of L-AgNPs

The agar disc diffusion test was employed to measure the bactericidal effect of L-AgNPs. 60 mm petri dishes were prepared with Muller-Hinton Agar and inoculated with target test organisms.

Sterile 6mm filter paper discs were loaded with 10 µL of Amoxicillin, silver nitrate solution, plant extract and L-AgNPs, respectively. Amoxicillin served as a positive control.

The discs were positioned on the agar surface enabling the compound to diffuse. The plates were then incubated for 24 h at 37ºC. The diameter of the growth inhibition zones were recorded in millimeters. All experimental runs were conducted in triplicate.

Fig. 8 (A-D) shows the antibacterial activity against *Staphylococcus epidermidis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*. Table 2 shows the zone of inhibition of L-AgNPs for different bacterial strains [39]. The Gram-positive bacterial cell wall's thick peptidoglycan and teichoic acids may be the cause of this. Our bacterial study findings showed that *Staphylococcus epidermidis* and *Streptococcus pyogenes* exhibited the strongest inhibitory activity [40]. In this study, L Ag NPs displayed evident antibacterial activity with significant impact ($P < 0.05$) on *Staphylococcus epidermidis and Pseudomonas aeruginosa than Bacillus cereus and Streptococcus pyogenes* [41].

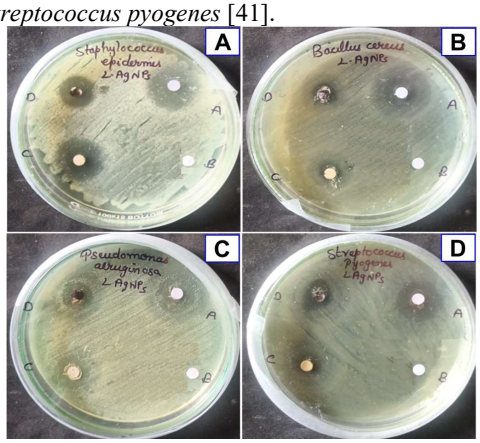

Fig. 8 Antibacterial activity of the synthesized L-AgNPs, (A) *Staphylococcus epidermis*, (B) *Bacillus cereus*, (C) *Pseudomonas aeruginosa*, and (D) *Streptococcus pyogenes*

Table 2 Antibacterial activity of L-AgNPs

Samples	**Concentrations (µL/mL)**	**Zone of Inhibition (in mm) (Diameter)**			
		Staphylococcus epidermidis	***Bacillus cereus***	***Pseudomonas aeruginosa***	***Streptococcus pyogenes***
A (Amoxicillin)	10	10.0 ± 0.1	11.1 ± 1.0	12.0 ± 0.8	9.2 ± 0.2
B (Silve	10	-	-	-	-

r)					
C (Plant extract)	10	6.1 ± 1.0	2.0 ± 1.1	9.8 ± 0.2	3.3 ± 0.7
D (Nanoparticles)	*10*	7.0 ± 0.8	4.2 ± 1.5	8.3 ± 0.3	6.0 ± 1.0

3.7.2. Antifungal activity

To evaluate the antifungal activity via the disc diffusion method, 60 mm petri dishes containing the test organisms were cultivated on Sabouraud's dextrose agar (SDA).

Sterile 6mm discs were soaked with 10 µL of Fluconazole, silver nitrate solution, plant extract and L-AgNPs.

These discs were positioned on the agar plate and allowed to pre diffuse for 30 minutes at room temperature.

Fluconazole served to validate the assay. After incubating the dishes for 24 h at 37°C, the prevention zone of bacterial growth were measured in mm.

Fig. 9 shows the antifungal activity of L Ag NPs against *Candida tropicalis*, *Aspergillus fumigatus.* The L-AgNPs were strongly inhibited by *Candida tropicalis* than by *Aspergillus fumigatus*. Silver nanoparticles prevent the growth of the fungal colony. At a concentration of 10µL/mL, L-AgNPs show a zone of inhibition of 8mm, and fluconazole showed 9 mm for *Candida tropicalis*. For *Aspergillus fumigatus,* L-AgNPs showed a zone of inhibition of 7 mm, and fluconazole showed 10 mm. The synthesized L-AgNPs showed more potential against *Candida tropicalis* than *Aspergillus fumigatus.* Table 3 shows the zone of inhibition of the sample L-AgNPs for different fungal strains [42].

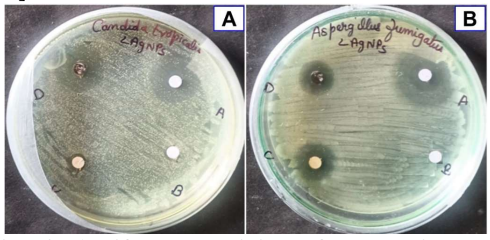

Fig. 9 Antifungal activity of synthesized L-AgNPs, (A) *Candida tropicalis*, and (B) *Aspergillus fumigatus*

Table 3 Antifungal activity of L-AgNPs

S. N	Samples	Concentrations	Zone of Inhibition (in mm)	
o		**(µL/mL)**	**(Diameter)**	
			Candida tropicalis	*Aspergillus fumigatus.*
1.	A (Amoxicillin)	10	9	10
2.	B (Silver)	10	-	-
3.	C (Plant extract)	10	2	6
4.	D (Nanoparticles)	*10*	8	7

3.7.3. Antioxidant activity of L-AgNPs by DPPH assay method

As illustrated in fig. 10 (A), the samples display dose dependent antioxidant activity, with the highest concentration approached the efficacy of the standard ascorbic acid. The sample exhibits 73.63% antioxidant activity at a concentration of 100 µg/mL, whereas ascorbic acid achieves 82.45% at the same concentration. The proton radical scavenging action is due to antioxidants was measured[43]. Table 4 shows the L-AgNPs capacity to inhibit oxidation and neutralise harmful free radicals. Fig. 10 (B) compares the antioxidant activity of L-AgNPs with Ascorbic acid activity. The DPPH radical scavenging capacity of L-AgNPs peaked at 73.63 % at 100 µg/mL. The DPPH assay revealed that L-AgNPs are potent free radical scavengers. The antioxidant IC_{50} for L-AgNPs was identified to be 31.95 µg/mL which was compared with IC_{50} value of Ascorbic acid (20.48 µg/mL). This antioxidant capacity of L-AgNPs may result from the functional groups attached to them [44].

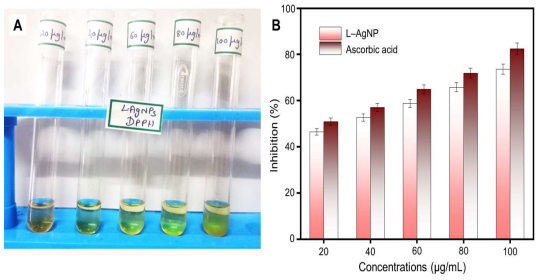

Fig. 10 (A) Antioxidant of the synthesized L-AgNPs, (B) Compared to ascorbic acid by the DPPH assay method

Table 4 Antioxidant activity of L-AgNPs

by DPPH assay method

S. No	Concentrations (µg/mL)	Antioxidant activity DPPH (%)	
		L-AgNPs	***Ascorbic acid***
1.	20	46.49	50.87
2.	40	52.63	57.01
3.	60	58.77	64.91
4.	80	65.78	71.92
5.	100	73.63	82.45
6.	IC_{50}	31.95	20.48

3.7.4. Anticancer activity

PA-1 cells were harvested , counted using a haemocytometer, and diluted in DMEM medium to a working density of 1×10^4 cells/mL.

To guarantee the correct cellular adhesion, aliquots of this cell solution were seeded into 96-well plates and cultured for 24 hours at 37°C in a humidified incubator with 5% CO2.

Control and different quantities of L-AgNPs, ranging from 2.5 to 15.0 µg/mL, were applied to the cells.

The identical settings were used to incubate the plates once more for 24 hours. The drug-containing culture medium was taken out after the 24-hour period, and new medium was used to wash the cells. Each well received 5 mg/mL of MTT dye produced in PBS, and the plates were incubated at 37°C for 4 hours. The yellow tetrazolium MTT was converted into purple formazan crystals during this incubation by metabolically active live cells.

After carefully removing the culture medium, 100 µL of concentrated DSMO was added to each well to completely dissolve the formazan precipitates.

Cell viability was quantified by measuring the absorbance at 540 nm using a multi-well plate reader [45].

The results were expressed as a percentage of stable cells with respect to the control. The half-maximal inhibitory concentration (IC_{50}) values were calculated, and the optimal doses were analyzed at various time points. The IC_{50} values were determined from the dose-responsive curve of the sample L-AgNPs, where the inhibition of 50% cytotoxicity was compared to vehicle control cells. All experiments were performed at least three times in triplicate. Fig. 11 (A-F) shows cell death of PA-1 Ovarian cancer cells for the different concentrations of synthesized L-AgNPs.

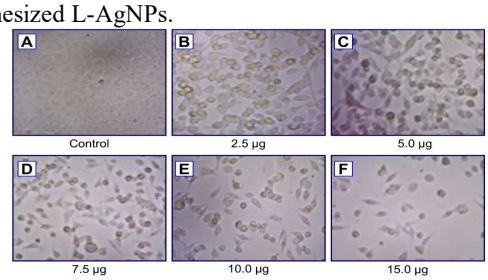

Fig. 11 Cell death of PA-1 Ovarian cancer cells for different concentration of L-AgNPs, (A) Control, (B) 2.5 µg, (C) 5 µg, (D) 7.5 µg, (E) 10µg, and (F) 15µg

Fig. 12 shows the graph of cell viability for the synthesized L-AgNPs. The IC_{50} value is 5.69 µg/mL. After incubation with L-AgNPs for 24 h, the cells exhibited 65% cell viability at a concentration of 2.5 µg/mL. When cells were exposed to higher concentrations of L-AgNPs, their vitality declined.

According to the results, biologically produced silver nanoparticles are a stable and non-toxic substance [46, 47]. These findings emphasize the significant impact of AgNPs which preserve the non toxic nature of the G. arborea [48].

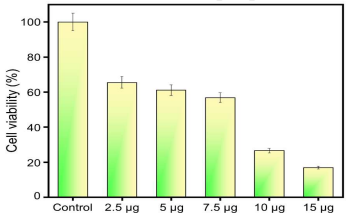

Fig. 12 Cell viability of synthesized L-AgNPs

4. Conclusion

The current study reveals that silver nanoparticles (L-AgNPs) can be synthesized in an environmentally benign manner by utilizing *Gmelina arborea* leaf extract as a natural stabilizing and reducing agent. A distinct absorption peak at 402 nm confirmed the optical characteristics of the synthesized L-AgNPs, while the accompanying colour change further indicated the successful formation of silver nanoparticles. FTIR, XRD, SEM, TEM, DLS, and zeta potential examination confirmed the existence of bioactive functional groups, a crystalline structure, a spherical shape, and good colloidal stability. The synthesized nanoparticles an average particle size of about 20 nm identified from TEM, and the zeta potential was -24 mV. The L-AgNPs produced showed considerable antibacterial activity. They worked well against both Gram-positive (*Staphylococcus epidermidis*, *Bacillus cereus*) and Gram-negative (*Pseudomonas aeruginosa*, *Streptococcus pyogenes*) bacteria, with the maximum zone observed against *Staphylococcus epidermidis*. The

antifungal activity was very strong, especially against *Candida tropicalis* and *Aspergillus fumigatus*, which showed zones of inhibition of 8 mm and 7 mm, respectively, is comparable to that of the standard drug fluconazole. The synthesized L-AgNPs exhibited potent antibacterial activity and demonstrated strong antioxidant potential in the DPPH radical scavenging assay, with a maximum scavenging effect of 73.63% at 100 µg/mL and an IC_{50} value of 31.95 µg/mL. This means that the substance may neutralize free radicals quite effectively, likely due to the plant-based capping agents. Also, the nanoparticles exhibited a strong anticancer effect on PA-1 ovarian cancer cells, with an IC_{50} value of 5.69 µg/mL. These findings indicate that the synthesized nanoparticles effectively inhibited the proliferation of cancer cells. Overall, this study demonstrates that silver nanoparticles synthesized using *Gmelina arborea* leaf extract represent a safe, biocompatible, and multifunctional agent with several potential applications in antibacterial, antifungal, antioxidant, and anticancer therapies. This eco-friendly method not only supports the goals of sustainable development but also makes a tangible difference in the field of nanomedicine. Further in vivo validation will be done in future.

Acknowledgement

The authors acknowledge St. Joseph's College Research Grant (SJCRG 2025-26) and DST-FIST, Government of India, for funding towards infrastructure and instrumentation facilities at ACIC, St. Joseph's College (Autonomous), Tiruchirappalli 620002. The authors were like to thank PG & Research Department of Physics, Government Arts College, Tiruchirappalli-620 022 to carry out this research. The authors were like to thank FIST sponsored Archbishop Casimir Instrumentation Centre, DST-FIST sponsored, St. Joseph's College (Autonomous) , Tiruchrirappalli-620 002 for providing the samples studies.

Compliance with Ethical standards

The authors declare no competing interests.

Ethical Approval

Furthermore we affirm that the research was conducted in the adherence to International, National and Institutional regulations pertaining to the production of biodiversity rights, animal experimentation and clinical research. Additionally no animals humans were injured during this investigation.

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