

Green synthesis of galangin mediated silver nanoparticles from *Alpinia officinarum* for antioxidant, antibacterial and antibreast cancer applications and computational studies

Amrita Yadav

MES Abasaheb Garware College

Swati C. Jagdale

Dr. Vishwanath Karad MIT World Peace University

Varsha S. Honmore

vhonmore@gmail.com

MES Abasaheb Garware College

Research Article

Keywords: Green synthesis, *Alpinia officinarum*, Galangin-silver nanoparticles, Anti-breast cancer, DFT, Molecular docking

Posted Date: September 2nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10583730/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Green synthesis of galangin mediated silver nanoparticles from *Alpinia officinarum* for antioxidant, antibacterial and antibreast cancer applications and computational studies

Amrita Yadav^{a,b}, Swati C. Jagdale^c, Varsha S. Honmore^{a*}

^a Postgraduate and Research Center, Department of Chemistry, MES Abasaheb Garware College, Pune, Maharashtra, India, 411007

^b Interdisciplinary School of Science, Savitribai Phule Pune University, Pune, Maharashtra, India 411007

^c Department of Pharmaceutical Sciences, School of Health Sciences and Technology, Dr. Vishwanath Karad MIT World Peace University, Pune, Maharashtra, India 411007

*Corresponding Author

Dr. Varsha S. Honmore

Postgraduate & Research Centre, Department of Chemistry,
MES Abasaheb Garware College, Pune, India 411007

Tel.: 91-20-41038263; fax: 91-20-25438165

E-mail address: vshonmore@yahoo.com / vshonmore@gmail.com

Abstract

Nowadays study of silver nanoparticles is becoming increasingly fascinating for the study of biomedical applications. Green methods produce eco-friendly substances and byproducts, utilize minimum energy, and do away with the need for expensive chemicals. This study is focused on the green synthesis of galangin-silver nanoparticles (G-AgNPs) from *Alpinia officinarum*, followed by their characterization and possible application for antioxidant, antibacterial and anti-breast cancer activities, DFT studies and molecular docking.

Synthesized G-AgNPs were characterized by UV-visible spectroscopy, FTIR, XRD, Raman, FESEM-EDS and dynamic light scattering techniques. Formation of G-AgNPs was indicated by a colour shift from whitish to a dark brown solution and further it was confirmed by presence of a surface plasmon resonance at 365nm in UV-visible spectra. The G-AgNPs were evaluated for antioxidant, antibacterial and anti-breast cancer activities. Significant antioxidant potential of G-AgNPs was demonstrated with IC₅₀ value of 5.67 µg/ml in DPPH, 6.09 µg/ml in ABTS and 6.83 µg/ml in H₂O₂ assay. The cytotoxic potential evaluated by MTT assay revealed the promising antiproliferative effects against anti-breast cancer cell lines, with an IC₅₀ value of 12.4 µg/ml. The theoretical results derived from DFT calculations enrich experimental data, helping the structured design of G-AgNPs with customized functionality for advanced technological applications. Molecular docking studies conducted on 4LMN and 8AI0 protein receptors showed strong binding of galangin, suggesting its anti-breast cancer potential via MAPK and ERα pathways. Overall, the synthesized G-AgNPs represent a promising frontier in developing advanced therapeutics for combating the breast cancer, infectious diseases and oxidative damage.

Keywords: Green synthesis, *Alpinia officinarum*, Galangin-silver nanoparticles, Anti-breast cancer, DFT, Molecular docking

1. Introduction

The plant *Alpinia officinarum*, also known as lesser galangal, possesses multiple therapeutic qualities and is used in numerous medications. Consumers growing interest in natural products and the health-promoting bioactive components derived from *Alpinia officinarum* prompted the creation of novel methods for producing those components on a large scale. *Alpinia officinarum* is a medicinal plant recognized for its dried rhizome, which has been employed for centuries to ease symptoms such as stomach aches, colds, ulcers and diarrhoea [1,2]. This plant is a member of the Zingiberaceae family. Various research have demonstrated that plants in this family possess multiple biological activities, including antioxidant, anti-inflammatory, and antitubercular properties [3]. *Alpinia officinarum* is cultivated in regions such as Japan, Thailand, and Vietnam, with its roots in China. The plant grows best between 19°C-27°C under high humidity and phytoconstituents in the plant are maximum at four to five years of age [4].

Galangin, a bioactive compound classified as 3,5,7-trihydroxyflavonoid, is considered a key constituent of lesser galangal and has been explored for various uses like anti-inflammatory, anti-oxidative, anti-microbial and anticancer properties [5,6]. Despite of having great potential for its application as therapeutic agent, poor aqueous solubility, low bioavailability, and rapid metabolism limits the therapeutic uses of galangin [7]. Nanoparticles prepared of metals and ligand from natural resources improves the solubility, stability and effectiveness of such crude products [8,9]. The unique physicochemical properties and biological activities of these nanoparticles arise from their small size and high surface-to-volume ratio [10]. These attributes render them highly valuable for various applications across industries, medicine, and technology.

Synthesis of silver nanoparticles (Ag-NPs) using green chemistry approach utilizes the natural product extracts to reduce silver ions. The flavonoids like galangin act as a reducing agent which produces highly stable, biocompatible, and eco-friendly nanoparticles [11]. This sustainable method eliminates the need for toxic and hazardous chemicals typically required in conventional synthetic processes [12,13].

Metal nanoparticles with a wide variety of morphologies have been created using living things and their extracts, including bacteria, fungi, algae and plants. For example- *Momordica cymbalaria* leaf extract-mediated bio-fabricated Ag-NPs exhibited notable cytotoxic effects against cancer cell lines [14]. In another research, iron nanoparticles synthesized from *Azadirachta indica* and *Eucalyptus gunni* demonstrated effectiveness degradation of dicamba via Fenton oxidation [15]. Likewise, *Canthium Parviflorum* leaf extract was utilized in the biosynthesis of gold NPs, showing promising potential in removing dyes and nitrophenol from industrial wastewater [16]. The approach based on green chemistry has significantly increased in popularity owing to its straightforwardness, affordability, eco-friendliness, and potential for scalability.

In this study, we have utilized isolated galangin from *Alpinia officinarum* followed by green synthesis of galangin-silver nanoparticles (G-AgNPs) and an in-depth characterization to evaluate the structure and morphology, computational studies for 4LMN and 8AI0 protein receptor binding and evaluation for their application in the biomedical field.

2. Materials and methods

2.1. Chemicals and Reagents

Alpinia officinarum rhizome was purchased from Naturalife Alagapuram, Salem, Tamil-Nadu India. Silver nitrate (99.9% pure), and ethanol were purchased from Fisher Scientific, Pune, Maharashtra, India. The DPPH (1,1- diphenyl-1-picrylhydrazyl) was procured from Tokyo Chemical Industry India Pvt. Ltd and ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was purchased from BLD Pharm, Hyderabad, India. L-ascorbic acid and potassium persulfate were purchased from Advent, Navi

Mumbai, Maharashtra, India. MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide), Hydrogen peroxide (30% w/v) and ciprofloxacin was procured from Sigma-Aldrich, Bangalore, Karnataka, India. All the chemicals used in the experimental study were AR grade with more than 97% purity and all the solutions were prepared in deionized water.

2.2. Synthesis of G-AgNPs through green synthesis method

Green synthetic approach was used for the synthesis of G-AgNPs. Before the synthesis of galangin-silver nanoparticles, isolation of galangin was performed through ethanolic extract [17]. For the synthesis of G-AgNPs, an individual mixture of 20 ml of isolated galangin (1×10^{-8} M) was combined with 60 ml of a (1×10^{-8} M) silver nitrate solution. This mixture was continuously stirred using a magnetic stirrer at a temperature range of 45-50°C for a duration of approximately 1.5 hours. The colour of the solution transitioned from a whitish to a dark brown shade, which is an indication of the G-AgNPs formation (**Fig. S1**) and this phenomenon was due to surface plasmon resonance. Afterward, the solution was allowed to cool and was centrifuged at 8000 rpm for 20 min. The resulting paste was collected and permitted to dry in a hot air oven at 80°C for approximately 24 hours, ultimately yielding a black-coloured nanoparticles.

2.3. Characterization of G-AgNPs

The formation of G-AgNPs was verified by using a double-beam UV-Vis spectrophotometer (Shimadzu UV-1900i, U.S.A.). Each sample of G-AgNPs was diluted in deionized water and a 50µL aliquot of each suspension was introduced into a cuvette for the purpose of UV-Visible absorption spectrum data acquisition.

The presence of functional groups and formation of G-AgNPs was examined by FTIR spectroscopy studies using ATR module of the ALPHA II FT-IR spectrometer from Bruker Corporation U.S.A. An extensive analysis of the topography, size distribution, and aggregation of G-AgNPs was carried out through the use of field emission scanning electron microscope (FESEM, Nova Nanosem NPEP303) and elemental composition assessment was performed via energy-dispersive X-ray spectroscopy (EDS). Single-crystal X-ray diffraction data of the synthesized nanoparticles were acquired at low temperature by Bruker D8 Venture diffractometer equipped with an Oxford Cryostream 700 Plus cooling system and a Kryoflex II device. Phase composition data was recorded by Raman spectroscopy on FT-Raman Bruker Equinox 55 spectrometer equipped with liquid-nitrogen cooled germanium detector. Size distribution, hydrodynamic radius and zeta potential was analysed by diffraction light scattering (DLS) technique using Malvern PANalytical Aeris benchtop X-Ray Diffraction machine. Density Functional Theory (DFT) calculation was performed to examine the theoretical structure of G-AgNPs using BURAI 1.3, a graphical user interface (GUI) of Quantum ESPRESSO software. The in-silico binding prediction of galangin with 4LMN and 8AI0 protein receptors was assessed by Gein Dock Suite version 2.5.7.0 software.

2.4. In-vitro activities

The G-AgNPs were tested for antioxidant, antibacterial and anti-breast cancer activities. Antioxidant activities were evaluated through ABTS, DPPH and H₂O₂ assays. Antibacterial activities were evaluated for gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria. Cytotoxic potential was assessed on MCF-7 breast cancer cell line.

2.4.1. Antioxidant Activity

2.4.1.1. DPPH Scavenging Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was determined using the Blois method with slight modifications. The antioxidant activity of silver nanoparticles and ascorbic acid (standard) were assessed and compared based on their ability to neutralize the DPPH free radicals. Each test sample or standard (10-100 μ L) was added to 2 mL of 0.33% DPPH solution in methanol in individual test tubes and incubated for 30 minutes at 37 °C for 30 minutes in the dark. After the incubation, the absorbance of each solution was measured at 517 nm using UV- Vis spectrophotometer against blank solution. The percentage of DPPH radical scavenging activity was calculated using the standard formula (supplementary data). All the experiments were conducted in triplicate, and the results are expressed as mean $\pm$ standard deviation (SD).

2.4.1.2. ABTS Scavenging Assay

The ABTS scavenging assay was performed to measure the antioxidant capacity of the samples by an end-point approach via neutralization of ABTS $^{\bullet+}$ radical cations. A reaction mixture of 7mM ABTS and 2.5mM potassium persulfate solution at a 2:1 (v/v) ratio was incubated in dark for 16h to generate ABTS $^{\bullet+}$ radicals. Resulting solution was diluted with ethanol to adjust absorbance at 734nm. The microplate assay was conducted by adding 10 μ L of G-AgNPs or ascorbic acid (standard) solution into the specific wells containing 200 μ L of the diluted ABTS solution in each well. The mixture was incubated in dark for 30 minutes and the absorbance was recorded at 734nm.

2.4.1.3. Hydrogen Peroxide (H_2O_2) Scavenging Assay

The H_2O_2 scavenging activity was determined by aliquoting 600 μ L of H_2O_2 solution in test tubes for blank, sample and standard followed by addition of 100 μ L of G-AgNPs and ascorbic acid (standard) solution in their respective tubes. The volume was made up to 4ml with phosphate buffer in all test tube. An identical reaction mixture without sample served as a negative control. All the test tubes were incubated for 10 minutes at ambient temperature. The absorbance of the remaining H_2O_2 was recorded at 230nm against the phosphate buffer blank.

2.4.2. Antibacterial Activity

Anti-bacterial potential of the G-AgNPs was evaluated in standard petri dishes using agar diffusion method. Before the experiment, Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial cultures were first grown in nutrient broth at 37°C and continuous shaking at 160 rpm for 24 h to achieve active growth. The turbidity of the cultures was standardized using a colorimeter at 540 nm, adjusting the optical density to 0.05 to obtain a uniform bacterial inoculum. Mueller-Hinton agar plates were prepared and aseptically inoculated with 100 μ L of each standardized bacterial suspension using a sterile spreader to form a consistent culture. The inoculum was allowed to absorb on to the plate for 10 min. Wells were carefully punched into the agar plates for test and reference standard application. An aliquot of 50 μ L of G-AgNPs (100 μ g/mL) and ciprofloxacin standard (10 μ g/mL) solution was added to their respective wells [18]. The plates were then incubated at 37 °C for 24 hr followed by zones of inhibition measurement surrounding each well. All antibacterial experiments were performed in triplicate, and the inhibition zones were expressed as mean $\pm$ standard deviation (SD).

2.4.3. In-Vitro anti-breast cancer activity

The MCF-7 human breast carcinoma cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. The confluent single layered cells were wash with 1X phosphate buffered saline (PBS; pH 7.4, Invitrogen, Cat. No. AM9625) and detached using 0.25% Trypsin-EDTA with phenol red (Gibco, Cat. No. 25200072). After cell detachment, the complete growth medium was added to

neutralize the reaction. This cell suspension was seeded into 96-well plate at a count of 0.01 million cells/well in minimum essential medium eagle-alpha (MEM α ; Gibco, Cat. No. 12561056) with 10% fetal bovine serum (FBS; Gibco, Cat. No. A5256701) and 1X antibiotic-antimycotic solution (Gibco, Cat. No. 15240096). The cells were treated with standard (positive control) and G-AgNPs solution in triplicate at concentrations ranging from 1.0 to 200 $\mu\text{g/mL}$.

The cell viability was measured after 24 h of treatment using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. To prevent the turbidity and measurement interference from drug residue in media, the used media was aspirated and the wells were rinsed with $1\times$ PBS. A fresh medium containing 0.5 mg/mL MTT reagent was added to each well and incubated at 37°C for 4 h in the dark. After incubation, the plate was centrifuged at $200\times g$ for 5 min to secure the cells and crystals. 50 μL of the supernatant was carefully pipetted out without disturbing the cells and crystals and 100 μL of sterile DMSO was added to solubilize the purple formazan crystals. After a 30-min incubation at room temperature, the absorbance was measured at 540 nm using a microplate reader. The background absorbance of the blank was subtracted from all readings. Untreated cells served as a negative control and blank wells (medium only) were used to subtract the background absorbance. The trypan blue exclusion method was used to determine the percent viability in the wells [19].

3. Results and discussion

3.1. Characterization of G-AgNPs

3.1.1. UV-Visible analysis

UV-Visible spectra of the synthesized G-AgNPs were recorded to verify the product formation. The colour transition of the reaction mixture from whitish to dark brown, revealed the reduction of silver ions (Ag^+) and the formation of G-AgNPs. The resulting dark-brownish coloration of the solution is due to surface plasmon resonance (SPR) phenomena caused by oscillation of electrons and subsequently resonance under light waves [20]. UV-Visible absorption peak at 365 nm confirmed the successful formation of G-AgNPs (**Fig. 1**).

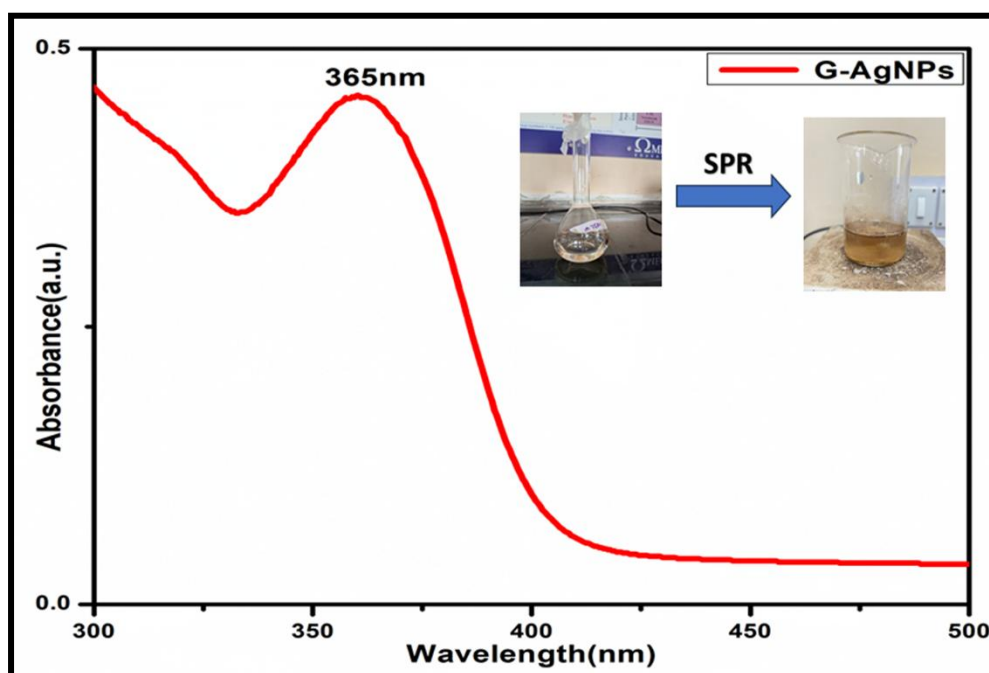


Fig. 1 UV-Visible spectra of G-AgNPs

3.1.2. FTIR spectroscopy studies

FTIR analysis was used to confirm the presence of characteristic functional groups in synthesized nanoparticles. The recorded FTIR spectra (**Fig. 2**) of the G-AgNPs indicated the functional groups present in galangin and silver-oxygen bond. The spectra show a ketone group (C=O) stretching vibration at wavenumber 1733 cm^{-1} and an alcohol (OH) group stretching vibration at wavenumber 3307 cm^{-1} . A peak at wavenumber 535 cm^{-1} corresponds to Ag-O-Ag bonds [21]. Likewise, the characteristic peak for stretching vibration of C=C and C-O bond can be seen in the spectra at 1596 cm^{-1} and 1260 cm^{-1} respectively.

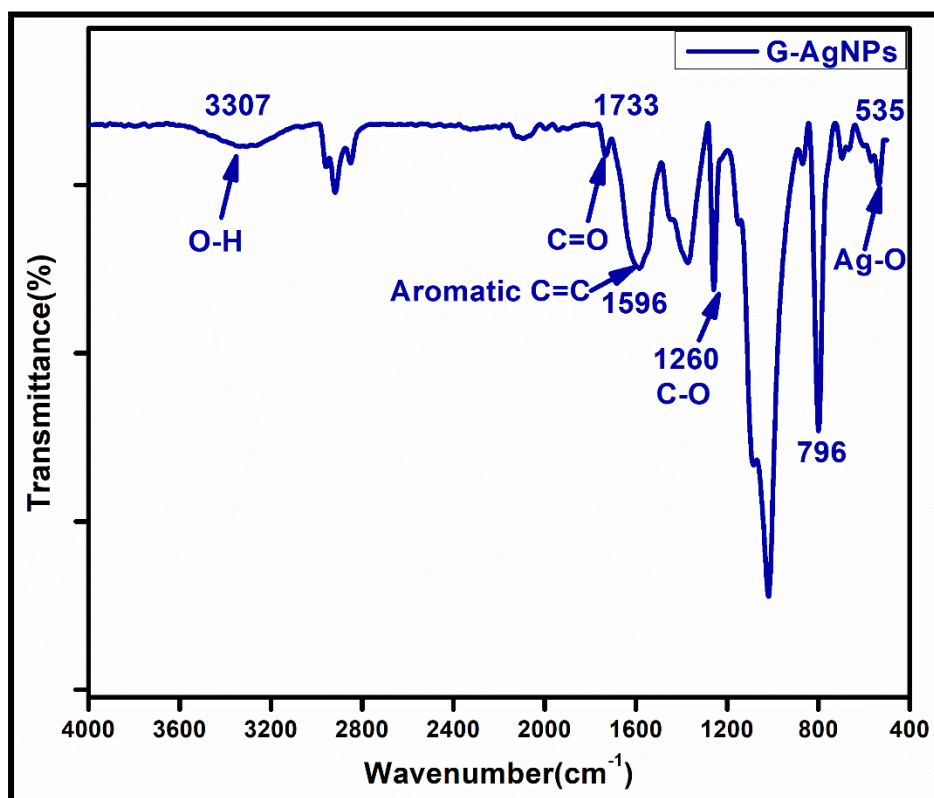


Fig. 2 FTIR spectra of G-AgNPs

3.1.3. XRD analysis

The XRD diffraction pattern recorded for the synthesised G-AgNPs shows (**Fig. 3a**) 2θ values of 27.80° , 32.23° , 38.17° , 46.24° , 54.85° , 57.58° , 67.49° and 74.52° . The characteristics peaks for crystalline particles are equivalent to crystallographic peaks. The diffraction peaks were found to be in good agreement with the cubic crystal structure of silver-nanoparticles as indicated by JCPDS data 96-900-7041 and shown in the inset **Fig. 3a**. The X-Ray diffraction pattern was analysed by Rietveld refinement. The refinement yielded a good fit between the observed and calculated profile as indicated by the low values of reliability factors and a satisfactory chi square (χ^2) value nearly 1. The retrieved refinement data characterized by $a = 8.490\text{ \AA}$, $b = 8.440\text{ \AA}$, $c = 7.915\text{ \AA}$, $\alpha = \beta = \gamma = 90^\circ$, and unit cell volume = $570.5\text{ \AA}^3$, conclusively places the sample in the tetragonal crystal system. The inexplicable peaks in the figure (**Fig. 3a**) might have been caused by the crystalline and amorphous organic phases that are present when the G-AgNPs crystallise. The Debye-Scherrer equation was applied to determine

the average crystalline size of the G-AgNPs. The average size of the formed G-AgNPs was determined to be 22.19 nm using this equation. Additional figures are provided in supplementary data (**Fig. S2** and **S3**)

$$D = \frac{k\lambda}{B \cos \theta}$$

Where D = crystal size; k = Scherrer constant; λ = wavelength of the X-ray; θ = Full-width half maximum of the diffraction peak.

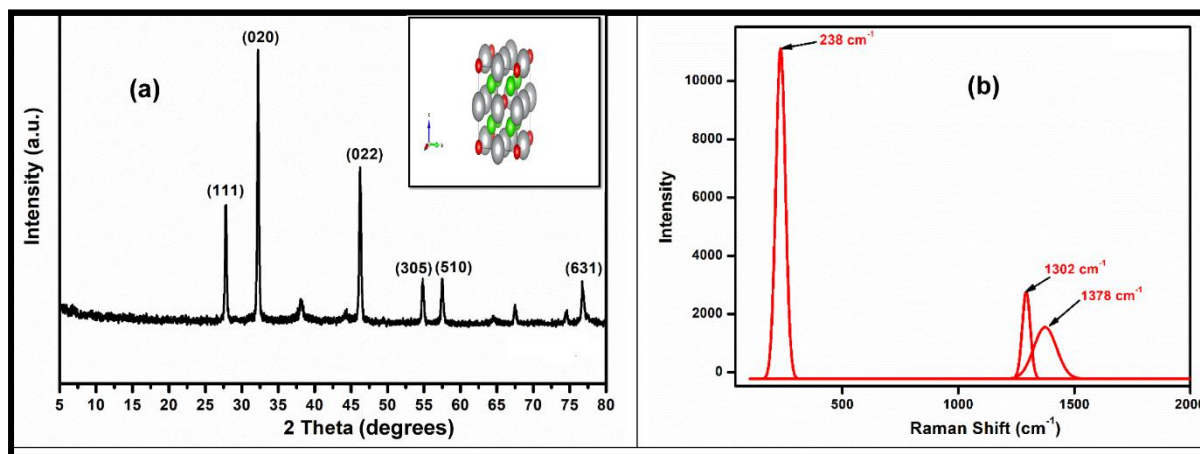


Fig. 3 (a) XRD and (b) Raman spectra of G-AgNPs

3.1.4. Raman analysis

Nanoparticles demonstrated considerable enhancement of Raman signals in Raman spectroscopy, ascribed to the surface-enhanced Raman scattering (SERS) phenomenon. The Raman spectrum (**Fig. 3b**) confirms the successful synthesis of the G-AgNPs. A very intensive peak at 238 cm^{-1} is the characteristics of Ag-O stretching vibrations [22]. The high signal amplification of this peak is due to the binding of galangin over the surface of silver nanoparticles. The D band, located at around 1302 cm^{-1} , is ascribed to disordered carbon, presumably derived from the organic molecule (galangin) in the G-AgNPs. The D band, situated at approximately 1378 cm^{-1} , can be attributed to the presence of galangin coating onto the nanoparticles.

3.1.5. FESEM-EDS analysis

The FESEM analysis was performed to determine the structure and morphology of the prepared G-AgNPs (**Fig. 4a**). This analysis revealed that the synthesized G-AgNPs are in cubic shape and in the size range of 20-100nm, which is aligned with the size obtained from XRD analysis. The elemental contents of G-AgNPs were measured by employing Energy Dispersive X-ray Spectroscopy (EDS) analysis (**Fig. 4b**). The elemental silver in the G-AgNPs was confirmed by a prominent absorption peak at 3 keV [23]. The mapping confirms the elemental composition and distribution of carbon indicated by red signal (**Fig. 4c**), silver peaks by cyan signal (**Fig. 4d**), oxygen by green colour (**Fig. 4e**) and uniform homogenous distribution of elements in the synthesized G-AgNPs, as shown in the inset figure. (**Fig. 4b** and **4f**)

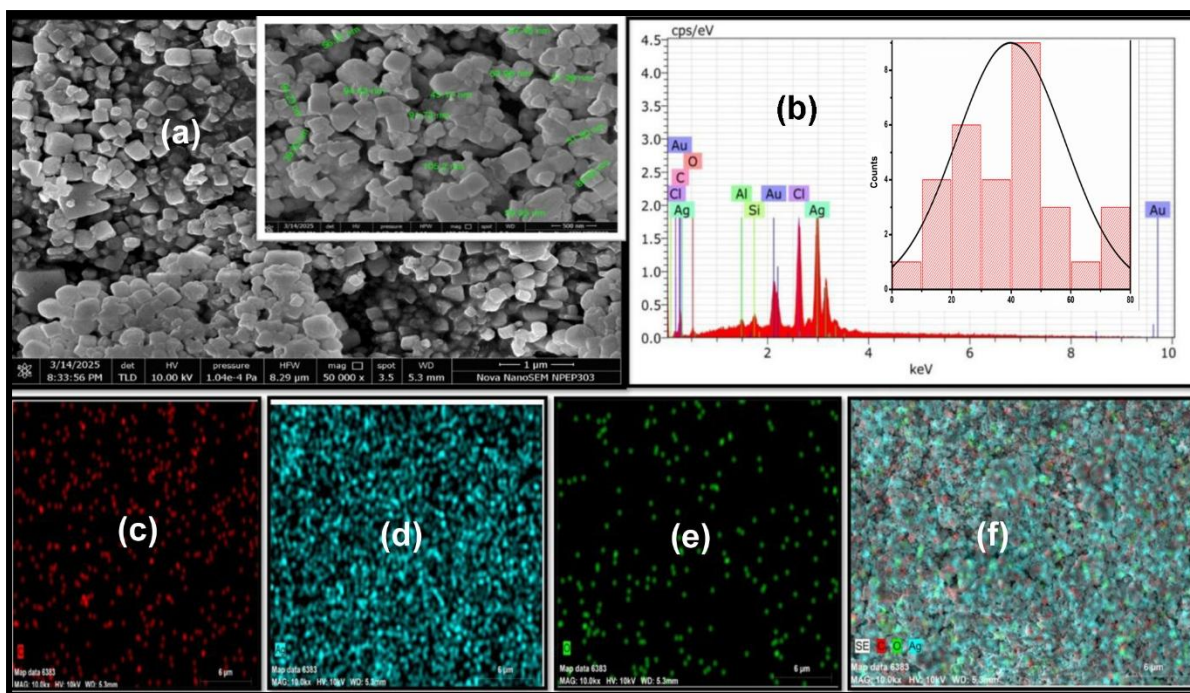


Fig. 4 FESEM-EDS image; (a) FESEM (b), Overlaid EDS spectra, elemental distribution image (c) through (f)

3.1.6. *Dynamic Light Scattering (DLS) analysis*

The physicochemical properties of the G-AgNPs were evaluated by DLS to determine the hydrodynamic radius their distribution and surface charge. The average hydrodynamic diameter of G-AgNPs determined by the DLS technique was 156.6 nm with polydispersity index (PDI) value 0.261, which indicates that the synthesized NPs are in moderately uniform size and in a well-dispersed state. (**Fig. 5a**). As shown in the graph, the rapid decay of the autocorrelation function (**Fig. 5c**) confirmed the presence of small and highly mobile nanoparticles undergoing Brownian motion. Interestingly, a near-neutral zeta potential of -0.0146 mV was measured in this experiment (**Fig. 5b**). In general, a near-zero zeta potential signals in a nanoparticle system of metals like silver is seen as a lack of electrostatic repulsion that can result in rapid coagulation and instability. But, in our case the G-AgNPs maintained the colloidal stability and was free from aggregation, which was confirmed by the lower PDI value. Despite of near zero zeta potential, the stability of G-AgNPs was imparted by a potent steric hindrance mechanism wherein the bulky, non-ionic aromatic structures of the galangin formed a dense layer over the metallic silver cores. This organic layer acts as a physical spatial barrier that effectively overrides attractive Van der Waals forces preventing core aggregation and validating the successful synthesis of a stable G-AgNPs [21, 22].

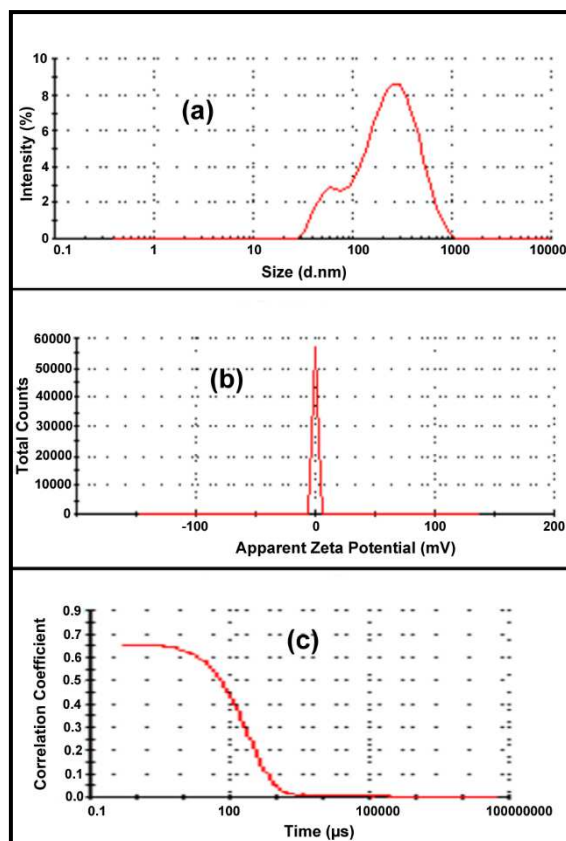


Fig. 5 DLS analysis graph; size distribution (a) Zeta potential (b) Autocorrelation graph (c)

3.2. In-vitro activities for G-AgNPs

3.2.1. Antioxidant activities

The antioxidant potential of the G-AgNPs was thoroughly evaluated using three complementary in-vitro models called DPPH, ABTS, and H_2O_2 scavenging assays. Ascorbic acid served as the standard reference compound for all three assays. Comparative scavenging profiles for all three assays across a wide concentration range from 20 to 100 $\mu\text{g/ml}$ is shown in the consolidated **Table 1** and **Fig. 6**. Both synthesized G-AgNPs and standard reference showed a linear dose-dependent performance in radical scavenging.

DPPH Radical Scavenging: Synthesized G-AgNPs showed radical clearance from 20 $\mu\text{g/mL}$ ($15.62 \pm 0.17\%$) reached a maximum scavenging activity of $50.40 \pm 0.45\%$ at the maximum concentration of 100 $\mu\text{g/mL}$. The G-AgNPs showed lower but robust scavenging capability than standard ascorbic acid which achieved almost total clearance ($99.00 \pm 0.84\%$) at the same peak concentration.

ABTS Radical Cation Scavenging: The G-AgNPs performed a steady neutralization across the given range from $11.72 \pm 0.50\%$ to $31.27 \pm 0.26\%$ but efficiency was lower than the ascorbic acid standard, which demonstrated an inhibition rate of $85.70 \pm 0.45\%$ at the maximum concentration.

Hydrogen Peroxide (H_2O_2 Decoupling): In this assay, the synthesized G-AgNPs demonstrated slightly better efficacy at the lowest concentration (20 $\mu\text{g/mL}$) and exhibited a scavenging efficacy of $15.67 \pm 0.90\%$ as compared to the ascorbic acid ($13.40 \pm 0.70\%$). But at the highest concentration (100 $\mu\text{g/mL}$), the G-AgNPs achieved a maximum scavenging effect of $40.70 \pm 0.38\%$ compared to $68.80 \pm 0.55\%$ for the reference standard.

Overall, the antioxidant potential of G-AgNPs was lower than the ascorbic acid but demonstrated a steady and predictable acceleration in efficacy up to the maximum used concentration limit. The calculated IC₅₀ values obtained for G-AgNPs in DPPH, ABTS and H₂O₂ were 5.67, 6.09 and 6.83 µg/mL while it was found to be 3.59, 3.70, and 2.85 µg/mL for Ascorbic Acid.

The radical-scavenging property of the synthesized G-AgNPs is due to the rich layer of galangin capping adsorbed onto the silver core surface during synthesis. The functional groups present in this flavonoid layer facilitate the electron transfer and stabilize the reactive radicals into non-radical derivatives [26].

Table 1: In-vitro antioxidant profile of G-AgNPs

Concentration (µg/ml)	% Scavenging activity					
	DPPH assay		ABTS assay		H ₂ O ₂ assay	
	G-AgNPs	Ascorbic acid	G-AgNPs	Ascorbic acid	G-AgNPs	Ascorbic acid
20	15.62 ±0.17	16±0.21	11.72±0.5	14.5±0.11	15.67±0.9	13.4±0.70
40	20.56 ±0.21	32.1±0.33	16.67±0.9	39±0.19	23.19±0.16	34.5±0.18
60	36.30±0.32	57.3±0.47	22.49±0.15	56.38±0.28	25.52±0.23	43.5±0.27
80	43.42±0.38	73±0.68	27.15±0.20	69.18±0.37	36.77±0.28	58.2±0.41
100	50.40±0.45	99±0.84	31.27±0.26	85.70±0.45	40.70±0.38	68.8±0.55
IC ₅₀	5.67±0.34	3.59±0.70	6.09±0.04	3.70±0.45	6.83±0.39	2.85±0.63

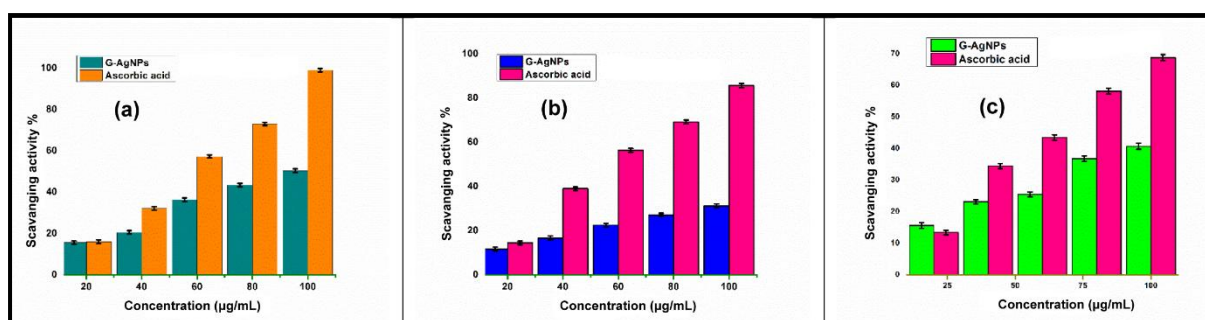


Fig. 6 Antioxidant assay (a) DPPH, (b) ABTS, (c) H₂O₂

3.2.2. Antibacterial Assay

Antibacterial capability of the green synthesized G-AgNPs was quantitatively evaluated against two Gram-positive strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative strains (*Pseudomonas aeruginosa* and *Escherichia coli*) using agar well diffusion method. The measured zones of inhibition (ZOI) after a 24-hour incubation period are shown in **Table 2** and **Fig. 7**.

Table 2: Antibacterial zone of inhibition of G-AgNPs and reference standard

Bacterial stain	Zone of Inhibition (mm±SD)	
	G-AgNPs (100µg/ml)	Standard (10µg/ml)
<i>Bacillus subtilis</i>	2.44±0.16	3.46±0.15
<i>Staphylococcus aureus</i>	2.04±0.11	2.87±0.09
<i>Pseudomonas aeruginosa</i>	1.88±0.03	2.95±0.19
<i>Escherichia coli</i>	1.87±0.04	3.51±0.10

The synthesized G-AgNPs demonstrated measurable antibacterial activity against all four tested bacterial strains. The highest susceptibility to G-AgNPs was observed in the Gram-positive bacterium *B. subtilis*, with a zone of inhibition (2.44 ± 0.16) followed by *S. aureus* (2.04 ± 0.11). Comparable susceptibility was observed in both the gram-negative bacterial strains with zone of inhibition 1.88 ± 0.03 and 1.87 ± 0.04 for *P. aeruginosa* and *E. coli* respectively. The bactericidal action of G-AgNPs could be due to strong adhesion of G-AgNPs to bacterial cell membrane and controlled release of silver ions which eventually causes the cell wall damage and cell death [27].

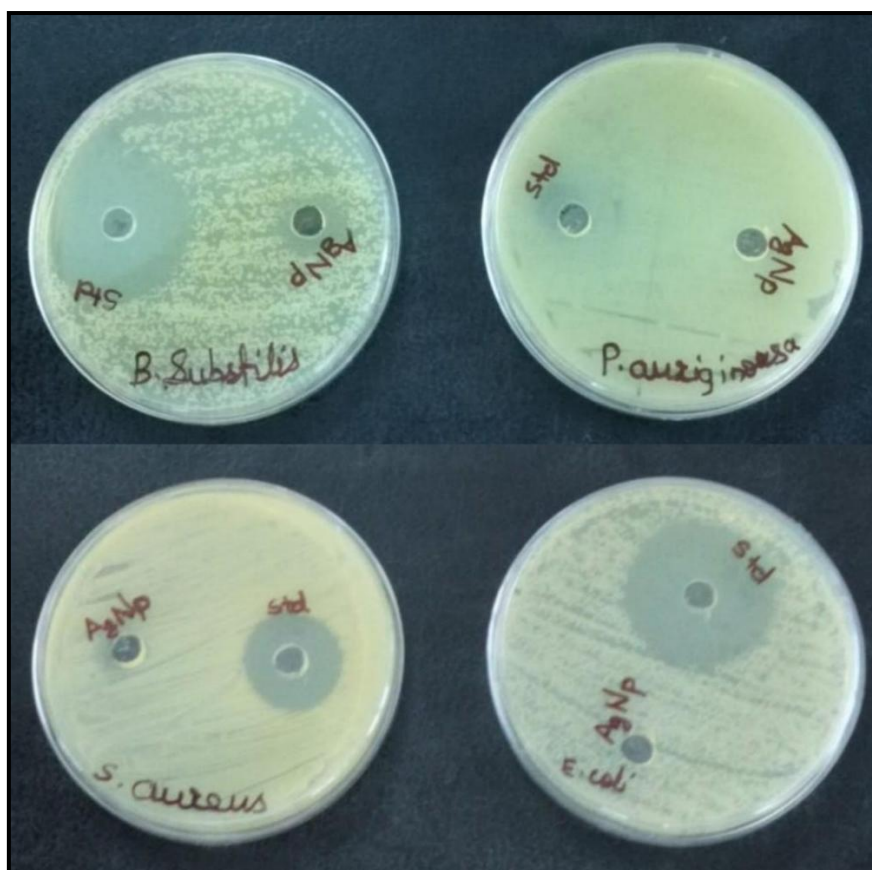


Fig. 7 Antibacterial activity of G-AgNPs against Gram-positive and Gram-negative bacteria

3.2.3. *In-vitro* cytotoxicity and anti-breast cancer potential

The synthesized G-AgNPs were evaluated for in-vitro antiproliferative activity against the human breast cancer cell line (MCF-7) in the concentration range from 1 to 200 $\mu\text{g/ml}$ using cisplatin as the reference standard. Cell viability determination was based on the trypan blue exclusion method.

G-AgNPs and Cisplatin showed a dose-dependent reduction in MCF-7 cellular viability (**Fig. 8** and supplementary **Fig. S4**). At the lowest tested dose (1.00 $\mu\text{g/mL}$) G-AgNPs preserved a high cell viability of approximately 93.9% and progressively declined with the increasing concentration and reached a baseline of approximately 6.40% viability at the peak concentration of 200 $\mu\text{g/mL}$. The G-AgNPs exhibited significantly superior therapeutic potency with IC_{50} value of 12.4 $\mu\text{g/mL}$ as compared to cisplatin, IC_{50} value 20.2 $\mu\text{g/mL}$.

The augmented cytotoxicity of G-AgNPs highlights its potential as a novel anti-breast cancer biomaterial.

The mechanism behind this enhanced cell death is due to synergism between the silver core and the galangin layer around AgNPs. The outer layer of the G-AgNPs seems to increase surface affinity for cell membranes and facilitates faster cellular uptake and more severe intracellular stress than traditional chemical formulations like cisplatin causing the biogenic nanoparticles to kill the cancer cells effectively at relatively lower doses. Once the G-AgNPs are inside the cells via endocytosis, they release silver ions which stimulate the intracellular accumulation of reactive oxygen species (ROS) and this pathway eventually leads to apoptosis [26, 27].

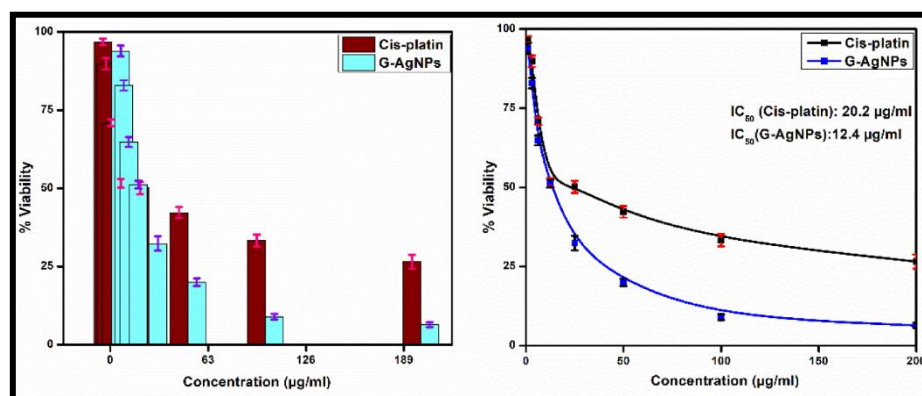


Fig. 8 Percent viability vs concentration bar graph and curve for G-AgNPs and Cisplatin

3.2.4. Density Functional Theory and Molecular Docking

Density functional theory (DFT) investigation of the G-AgNPs was performed by means of general gradient approximation (GGA) of Perdew-Burke-Ernzerhof (PBE) function in Quantum Espresso software suite [30]. The G-AgNPs models were constructed by galangin ligand on Ag₃₂ slab of triclinic primitive lattice under vacuum gap 25 Å. The kinetic energy cut-off for wave function 45.65 Ry, cut-off charge 326.261 Ry and 4x4x3 Monkhorst-pack k-point were used for geometric optimization and self-consistent field calculation (SCF) by Broyden-Fletcher-Goldfarb-Shanno algorithm (BFGS). SCF calculation iteratively the Kohn-shan equation to find the ground state electron density and total energy of a system at a fixed geometry. Simulation was performed using plane wave basis sets and ultrasoft pseudopotentials [31]. The fermi energy obtained at -3.4421 eV and total energy of SCF at -2612.32 Ry. Simulation demonstrated a significant charge localization (**Fig. 9** and supplementary **Fig. S5** through **S8**) on silver surface atoms which increases their chemical reactivity, a key factor for the interactions with cellular components and Reactive oxygen species a crucial mechanism in the apoptosis of cancer cells. The results provide atomic-scale link between practical results and theoretical exceptions, supporting the logical design of G-AgNPs as anti-breast cancer drug.

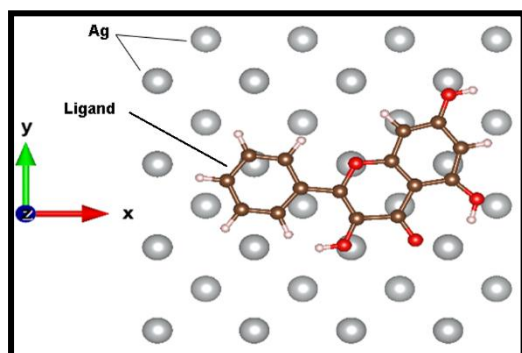


Fig. 9 DFT calculation of G-AgNPs

Molecular docking was performed to predict the galangin-receptor interaction at atomic level using Gein Dock Suite v2.5.7.0 on 4LMN and 8AI0 receptors. The molecular docking parameters were optimized and the binding parameters were evaluated using vina scoring function (supplementary table ST1 and ST2). The chemical structure of galangin was drawn in vesta software. The ligand was energy minimized using the MMFF94 force field. The protonation state was set for a physiological pH of 7.4 and partial charge were assigned using Gasteiger Kollman method as per suite protocol. The protein structure 4LMN and 8AI0 were downloaded from the protein data bank (PDB). Water molecules and co-crystallized ligand were removed using Pymol. Negative values indicate stronger binding affinity. The analysed data showed a stronger interaction with 4LMN compared to 8AI0 (**Fig. 10**).

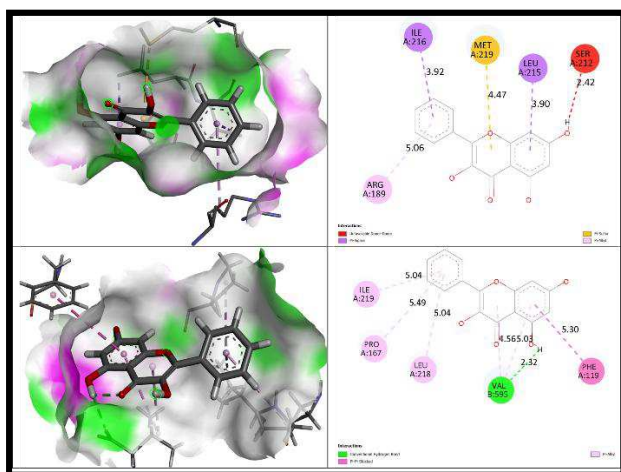


Fig. 10 2D and 3D interaction of G-AgNPs with 4LMN (top trace) and 8AI0 (bottom trace)

3.2.5. Statistical Analysis

The data related to in-vitro biological activities were analysed using one-way analysis of variance and presented as mean $\pm$ SD. ImageJ software was used to process FESEM micrograph. Origin software is used to plot graph.

4. Conclusion

In summary, this study presents a sustainable green synthesis of galangin-silver nanoparticles using *Alpinia officinarum* rhizome as a natural source. Successful synthesis of stable and uniformly distributed nanoparticles was confirmed by a comprehensive characterization. The synthesized G-AgNPs exhibited remarkable antioxidant activity, showed broad-spectrum antibacterial effectiveness against both Gram-positive and Gram-negative bacteria and demonstrated a potent cytotoxic action against MCF-7 breast cancer cells with a lower IC₅₀ value than the standard drug cisplatin.

Computational analyses of the G-AgNPs using DFT and molecular docking further elucidated the electronic structure and strong binding affinity of galangin with cancer-related protein targets 4LMN and 8AI0 that provided valuable mechanistic insights into bioactivity potential. The synergy between the silver core and galangin capping imparts enhanced biological and physicochemical properties underscoring the potential of G-AgNPs for multifunctional biomedical applications and a potential therapeutic option for combating oxidative stress, infectious diseases and cancer. The green synthesis route and demonstrated multifunctionality indicates possibilities of G-AgNPs as a strong candidate for further preclinical and clinical development. Future investigations can be explored in the in-vivo validation, biosafety assessment, and examination of additional therapeutic targets to explore their translational potential.

Authors contribution

V.H. and A.Y. conceived the original idea and designed the experiments. A.Y performed the experiments, documented results and drafted the manuscript. Molecular docking was performed by S. J. Review and final editing of the manuscript was completed by V.H.

Statements and Declarations

Competing interests: The authors declare no competing interests.

Research funding: Not applicable; this research did not receive any funding from any organization or agency.

Data availability: All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethical Approval: Not applicable.

Consent to publish: Not applicable.

Consent to participate: Not applicable.

Generative AI and AI-assisted technologies: The authors used Quillbot for grammar and language refinement, content organization and improved readability of the manuscript. However, all the data analysis, interpretations and conclusions in the manuscript were drafted by the authors.

References

- [1] Ding P, Yang L, Feng C, et al. Research and application of *Alpinia officinarum* in medicinal field. *Chin Herb Med*. 2019;11(2):132–140.
- [2] Sharifi-Rad M, Varoni EM, Salehi B, et al. Plants of the genus *Zingiber* as a source of bioactive phytochemicals: From tradition to pharmacy. *Molecules*. 2017;22(12):2145.
- [3] Kim H-H, Bae Y, Kim S-H. Galangin attenuates mast cell-mediated allergic inflammation. *Food Chem Toxicol*. 2013;57:209–216.
- [4] Abubakar IB, Malami I, Yahaya Y, et al. A review on the ethnomedicinal uses, phytochemistry and pharmacology of *Alpinia officinarum* Hance. *J Ethnopharmacol*. 2018;224:45–62.
- [5] Honmore VS, Kandhare AD, Kadam PP, et al. Isolates of *Alpinia officinarum* Hance as COX-2 inhibitors: Evidence from anti-inflammatory, antioxidant and molecular docking studies. *Int Immunopharmacol*. 2016;33:8–17.
- [6] Zou W-W, Xu S-P. Galangin inhibits the cell progression and induces cell apoptosis through activating PTEN and Caspase-3 pathways in retinoblastoma. *Biomed Pharmacother*. 2018;97:851–863.
- [7] Ekambaram SP, Perumal SS, Paramasivam S. Galangin: advances on resources, biosynthesis pathway, bioavailability, bioactivity, and pharmacology. *Handb Diet Flavonoids*. Springer; 2023. p. 1–59.
- [8] Osman AI, Zhang Y, Farghali M, et al. Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. *Environ Chem Lett*. 2024;22(2):841–887.
- [9] Heuer-Jungemann A, Feliu N, Bakaimi I, et al. The role of ligands in the chemical synthesis and applications of inorganic nanoparticles. *Chem Rev*. 2019;119(8):4819–4880.
- [10] Yusuf A, Almotairy ARZ, Henidi H, et al. Nanoparticles as drug delivery systems: a review of the implication of nanoparticles' physicochemical properties on responses in biological systems. *Polymers*. 2023;15(7):1596.
- [11] Singh H, Desimone MF, Pandya S, et al. Revisiting the green synthesis of nanoparticles: uncovering influences of plant extracts as reducing agents for enhanced synthesis efficiency and its biomedical applications. *Int J Nanomedicine*. 2023;4727–4750.
- [12] Bhardwaj B, Singh P, Kumar A, et al. Eco-friendly greener synthesis of nanoparticles. *Adv Pharm Bull*. 2020;10(4):566.
- [13] Anwar A, Masri A, Rao K, et al. Antimicrobial activities of green synthesized gums-stabilized nanoparticles loaded with flavonoids. *Sci Rep*. 2019;9(1):3122.
- [14] Sundar M, Rajagopal G, Nivetha A, et al. Phyto-mediated green synthesis of silver nanoparticles using an aqueous leaf extract of *Momordica cymbalaria*: antioxidant, cytotoxic, antibacterial, and photocatalytic properties. *Separations*. 2024;11(2):61.
- [15] Bhaskar S, Manu B, Sreenivasa MY, et al. Synthesis of plant-based biogenic jarosite nanoparticles using *Azadirachta indica* and *Eucalyptus gunni* leaf extracts and its application in Fenton degradation of dicamba. *Water Sci Eng*. 2024;17(2):157–165.

- [16] Kumar GS, Reddy NR, Siddiqui QT, et al. A facile green synthesis of gold nanoparticles using *Canthium parviflorum* extract sustainable and energy efficient photocatalytic degradation of organic pollutants for environmental remediation. *Environ Res.* 2024;258:119471.
- [17] Honmore VS, Kandhare AD, Kadam PP, et al. Isolates of *Alpinia officinarum* Hance as COX-2 inhibitors: Evidence from anti-inflammatory, antioxidant and molecular docking studies. *Int Immunopharmacol.* 2016;33:8–17.
- [18] Skov R, Smyth R, Larsen AR, et al. Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and Etest on Mueller-Hinton agar. *J Clin Microbiol.* 2006;44(12):4395–4399.
- [19] Morten BC, Scott RJ, Avery-Kiejda KA. Comparison of three different methods for determining cell proliferation in breast cancer cell lines. *J Vis Exp JoVE.* 2016;(115):54350.
- [20] Win TT, Khan S, Fu P. Fungus-(*Alternaria* sp.) Mediated Silver Nanoparticles Synthesis, Characterization, and Screening of Antifungal Activity against Some Phytopathogens. *J Nanotechnol.* 2020;2020(1):8828878.
- [21] Ali MW, Jalil AA, Aziz FF, et al. Synergistic degradation of rhodamine B and reduction of Cr⁶⁺ over silver (II) oxide/fibrous SiO₂/ZrO₂ photocatalyst. *AIP Conf Proc.* AIP Publishing LLC; 2025. p. 020029.
- [22] Brittle SW, Foote DP, O'Neil KA, et al. A Raman-based imaging method for characterizing the molecular adsorption and spatial distribution of silver nanoparticles on hydrated mineral surfaces. *Environ Sci Technol.* 2018;52(5):2854–2862.
- [23] Ider M, Abderrafi K, Eddahbi A, et al. Silver metallic nanoparticles with surface plasmon resonance: synthesis and characterizations. *J Clust Sci.* 2017;28(3):1051–1069.
- [24] Napper DH. Polymeric stabilization of colloidal dispersions. No Title. 1983;
- [25] Javed R, Zia M, Naz S, et al. Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. *J Nanobiotechnology.* 2020;18(1):172.
- [26] Moond M, Singh S, Sangwan S, et al. Green one pot synthesis of silver nanoparticles for antioxidant and antimicrobial activities. *Discov Nano.* 2026;21(1):98.
- [27] Madhusudanan M, Mijakovic I, Singh P. Biogenic vs. Chemical AgNPs: A Comparison of Antimicrobial Potency and Stability. *Int J Mol Sci.* 2025;27(1):62.
- [28] Ullah I, Khalil AT, Ali M, et al. Green-synthesized silver nanoparticles induced apoptotic cell death in MCF-7 breast cancer cells by generating reactive oxygen species and activating caspase 3 and 9 enzyme activities. *Oxid Med Cell Longev.* 2020;2020(1):1215395.
- [29] Rai M, Ingle AP, Trzcińska-Wencel J, et al. Biogenic silver nanoparticles: what we know and what do we need to know? *Nanomaterials.* 2021;11(11):2901.
- [30] Perevalov TV, Islamov DR, Chernykh I. Atomic and electronic structures of intrinsic defects in Ta₂O₅: Ab initio simulation. *JETP Lett.* 2018;107(12):761–765.
- [31] Ivanov MV, Perevalov TV, Aliev VS, et al. Electronic structure of δ -Ta₂O₅ with oxygen vacancy: ab initio calculations and comparison with experiment. *J Appl Phys.* 2011;110(2).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplimentaryData.docx](#)
- [GRAPHICALABSTRACTNANO.tif](#)
- [DFTElectronic.mp4](#)