Optical and biomedical applications of eco-friendly biosynthesized silver nano spheres using zingiber officinale root extract

E Ramya 1,4 *, L Jyothi 2,4 *, P Vivek Vardhan 3, N Sri Ram Gopal 1 and Narayana Rao Desai 2

1 Department of Science and Humanities, MLR Institute of Technology, Dundigal, Telangana-500043, India
2 School of Physics, University of Hyderabad, Telangana–500046, India
3 Department of Biotechnology, School of Life Sciences, Pondicherry University, Puducherry–605014, India
4 Authors to whom any correspondence should be addressed.

E-mail: eramya@uohyd.ac.in and jyothik6@yahoo.co.in

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Abstract

Eco-friendly bio-compatible silver nanoparticles (Ag NPs) were successfully synthesized using Zingiber officinale extract in a simple green route at room temperature. The phytoconstituents present in Zingiber officinale (Z. officinale) extract act as reducing and stabilizing agents. The size and crystallinity of spherical Ag NPs were confirmed through transmission electron microscopy (TEM) and X-ray diffraction (XRD) studies. The formation of silver nanoparticles was also confirmed from the UV–vis and FTIR spectra. Luminescence properties of europium (Eu) and samarium (Sm) complexes in the presence of silver were studied. The intensity of luminescence from Eu and Sm complexes was found to get enhanced or quenched with their concentrations in Ag NPs. Interesting nonlinear optical properties exhibited by Ag NPs were observed in the Z-scan experiment suggesting that they can be used as optical limiters for the picosecond (ps) time scale green laser. Silver nanoparticles were tested against colon cancer (HCT116) cells in vitro by MTT assay and they exhibited smaller IC50 values with better inhibition efficiency. Ag NPs induced apoptosis through the activation of Reactive oxygen species (ROS) and Caspase-3 pathways. Antibacterial activity of Ag NPs was analysed against Acinetobacter baumannii (A. baumannii) and Staphylococcus aureus (S. aureus) and they were found to be efficient in inhibiting the bacteria. The results indicate that the biosynthesized eco-friendly nanoparticles having high stability can lead to many applications such as good luminescence enhancement, optical limiting characteristics, anticancerous and antibacterial properties in optics and biomedicine.

1. Introduction

Nanotechnology has gained attention and shows rapid growth due to its capability to tune the properties of materials [1]. Nanoparticles (NPs) exhibit exclusive electronic, magnetic, chemical, mechanical, optical, medicinal and catalytic properties in comparison with bulk materials because of the quantum size effect and large surface to volume ratio [2]. Among all the metals, silver is fascinating owing to its ease of preparation, chemical and physical properties, disinfecting nature and medicinal value particularly acting as effective anticancer agent. Its various applications extend to biosensors, ultrasensitive detection, bio-imaging, oxidative catalysis, nano-electronics, surface enhanced Raman scattering, and antimicrobial activity [3–5]. A number of techniques are feasible for the preparation of Ag NPs, such as chemical, electrochemical, photochemical, irradiation methods, Langmuir-Blodgett and biological techniques [6]. Many of these techniques are hazardous, complicated and costly. They have drawbacks due to toxic solvents and by-products with high energy consumption. Consequently, there is a demand to establish green synthesis of NPs using micro-organisms and plant extract to make it environmental friendly and cost-effective. Organic synthesis of NPs by microorganisms, enzymes is complex and requires elaborate process by cell culture and environmental issues. Thus plant extracts are promoted for preparation of nanoparticles [4]. Several reports have appeared on synthesis of silver NPs by
plant extracts. Some of them are using: Radish [7], Pinus desiflora (red pine) [8], ginko, persimmon, mokryeon, oriental plane, Cycas leaf [9], drumstick leaf [10], Shorea tumbugaia stem bark [11], Cinnamon [12], papaya [13], bishkaphra roots [14], Jatropha curcas latex [15], jack fruit leaf [16], big-sage [17], coriander leaf [18] and wild croton leaf [19] etc.

Ginger root belongs to the family Zingiberaceae, which has been consumed as a spice and also as medicine. 3% of essential oil contained in ginger causes fragrance of spice. Sesquiterpenoids with zingiberene are major constituents of ginger. Compounds like farnesene and β-sesquiphellandrene bisabolene belong to sesquiterpenoids (citral, cineol and β-sesquiphellandrene). Gingerol-related components possess high antifungal, antimicrobial, anticancerous, antioxidant and anti-inflammatory pharmaceutical properties [20–22]. Hence ginger has been serving as an oriental traditional medicine used in treating many diseases like cold, cough, nausea, rheumatism, cardiac disorders, inflammation and tumors.

Luminophors (III) draw good attention because of their potential applications in fluoroimmunoassay, light emitting diodes (LED) and optical signal amplification. Mostly, recent work is concentrated on synergy of luminophores with metallic nanostructures, quantum dots, organic molecules, dyes or polymers to achieve higher luminescence. An increase in the radiative decay rate of luminophores can be achieved by introducing a resonant Plasmon mode of a metal nanoparticle. The ability of the photoluminescence (PL) enhancement is based on: (i) the distance between metal nanoparticle and rare-earth ion, (ii) coordination around rare-earth ions and (iii) the excitation wavelength. This demonstrates that the enhancement owes to coupling of the dipoles of lanthanide ion transitions with Plasmon modes of metallic nanostructures, resulting in enhancement several orders of magnitude in radiative decay rates when the separation them is below 20 nm [23–26]. Other reason for the enhancement of luminescence from emitters is due to the surface-enhanced fluorescence (SEF), where the separation between metal surface and emitter is a crucial factor [27].

Ag nanoparticles also exhibit good third order nonlinear optical (NLO) properties and showed their application as optical limiter [28]. Bio synthesized Ag NPs show better optical limiting properties even though the bio molecules cap the Ag NPs [29–32].

Cancer has been a major uncontrolled degenerative health problem. Identification with least side effects and locating the new anti-cancer drug has become crucial aspect of current research in cancer therapy. With extraordinary antimicrobial and anticancerous activities, application of Ag NPs have become more extensive in medicine as they are playing an important role in medicinal devices, dressings, nano-lotions, gels etc [33]. They exhibit antibacterial activities against gram-positive and gram-negative bacteria. Majority of bacteria have grown their own resistance to antibiotic drugs, thus forcing us to develop a replacement for antibiotics in future. Ag NPs are more attractive due to their non-toxic and antibacterial behaviour in broad range with no side effects to human body. Various studies reported that Ag NPs remarkably diminish the function of mitochondria with induced cell apoptosis or necrosis. Au NPs are simply bound with thiol and amine groups, which modifies the surfaces with DNA and amino acids which have good biocompatibility in clinical applications. The cytotoxicity of these NPs depends on the size, shape and surface modification [34–38].

The goal of the study is to improve the role of green chemistry both in optics and in biomedicine. Current work demonstrates the luminescence efficiency of eco-friendly Zingiber officinale based biosynthesized Ag NPs on lanthanide complexes and also optical limiting efficiency. The study validates the possible in vitro anti-proliferative effects of bio synthesized silver nanoparticles against the HCT116 cell lines. The report presents the antibacterial activity of the NPs against (A. baumannii) and Staphylococcus aureus (S. aureus). Moreover, the report elucidates the efficiency of NPs in luminescence enhancement, optical limiting, anticancerous and antibacterial activities.

2. Materials and methods

2.1. Reagents

Silver nitrate (AgNO₃) was obtained by Sigma Aldrich. Zingiber officinale roots were purchased from a local market. Human colon (HCT116) cell lines were provided by National Centre for Cell Science (NCCS), Pune, India.

2.2. Preparation of extract from Zingiber officinale root extract

First the fresh roots were thoroughly rinsed in distilled water many times and 10 grams of it were cut and added to 100 ml of distilled water and heated for 5 min. The mixture was cooled to room temperature and was filtered with a filter paper (Whatman no. 1). The solution was again filtered with 1 mm filter paper to avoid granules and stored in a refrigerator.
2.3. Synthesis of Silver nanoparticles

Briefly, AgNO₃ (1 mM) aqueous solution was prepared and 3 ml of root extract was added to 30 ml of 1 mM silver nitrate solution. Initially the solution was transparent. After few minutes the solution turns to dark ash colour indicating the formation of Ag NPs. The experiment was repeated thrice to confirm the reproducibility.

3. Experimental details

Silver solutions were coated on a silica substrate and dried at room temperature to record the XRD spectrum by Cu–Kα, X-radiation (λ = 1.5406 Å) of Bruker D8 diffractometer operated at 30 mA and 40 kV power with scan rate of 0.05°/min over the 2θ range 30–80°. The FTIR spectra of Ag NPs solution was recorded using Thermo-Nicolet 6700 in the 400–4000 cm⁻¹ region. The reduction of silver ions to silver NPs was confirmed by recording the absorption spectrum on UV-visible spectrometer JASCO in the 300–800 nm range with 1 nm resolution. TEM images and Energy dispersive X-ray analysis (EDX) were recorded on TECHNAI G2 S-Twin at 20 kV voltage to investigate the morphology, size and crystallinity of Ag NPs. Field emission scanning electron microscopy (FESEM) studies were accomplished on Carl ZEISS-Ultra 55 FE-SEM model. Photoluminescence (PL) emission spectra of liquid Ag NPs with rare-earth ions were measured on HORIBA YVON spectrometer over the range of 570–670 nm by exciting at 350 nm. Third order nonlinear optical studies were performed by Z-scan using a ps Nd:YAG laser (EKSPRA-2143A) operating at 10 Hz pulses. Ag NPs dispersed in water were used in 1 mm quartz cuvette for recording the Z-scan data. The output light is received with photodiode and a reference photodiode monitoring the laser fluctuations.

3.1. Cytotoxicity (MTT assay)

The cytotoxic assay of prepared Ag NPs on HCT116 cancer cells was obtained by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cell lines were grown in Dulbecco’s modified Eagle’s medium (DMEM), with 10% fetal bovine serum (FBS), penicillin (100 μg ml⁻¹), streptomycin (100 U ml⁻¹) at 37 °C atmosphere of 5% CO₂. The cell lines were seeded for 24 h before exposure to Ag NPs. The actively grown HCT116 cells were seeded in a 96-well plate at a density of 1 × 10⁴ per well, incubated in DMEM/1% FBS. After that, media was replaced with Ag NPs of various concentrations at 25, 50, 75, 100 and 125 μM. The cells were incubated at 37 °C for 1 h with 20 μl MTT solution. The MTT solution was removed and replaced by DMSO. Optical density (OD) was obtained at 550 nm by scanning ELISA plate reader. Cell culture medium with cells act as negative control. Cell viability measurements were calculated from the ratio of mean optical density to the negative control. The cytotoxicity measurements of Ag NPs are compared with Oxaliplatin (OXP) drug as positive control.

3.2. Apoptosis assay using ANNEXIN V

Combining of Annexin V-FITC/PI to cancer cells was obtained using Annexin V-FITC Apoptosis detection Kit I (BD Biosciences, New Jersey, and USA). 2 ml of HCT116 cells (3 × 10⁶/2 ml) were treated with Ag-NPs and camptothecin (15 μM) for 24 h and cleaned with PBS twice. These were suspended in buffer (100 μl) and changed to culture tube (5 ml). Then, 5 μl of FITC Annexin V combined with cells incubated at 25 °C in dark for 15 min. The binding buffer (400 μl) with PI (5 μl) added to every tube. Then the cells were studied on flow cytometer (Becton Dickinson, FACS Calibur, USA).

3.3. Caspase-3

In vitro caspase activity in Ag-NPs, both camptothecin (15 μM) treated and untreated cells were studied using FITC rabbit anti-active caspase-3 antibody (BD Biosciences, New Jersey, USA). Cancer cells were cleaned in PBS and permeabilized using BD Cytotox/Cytoperm Kit at RT for 20 min, then pelleted and cleaned with BD buffer. Cancer cells were subsequently stained with FITC rabbit anti-active caspase-3 antibody (BD Biosciences, clone C92-605). Then, cells were cleaned and suspended in BD buffer to study by flow cytometry (Becton Dickinson, FACs Calibur, New Jersey, USA).

3.4. Reactive oxygen species (ROS) generation

Intracellular ROS levels in Ag-NPs, both camptothecin (15 μM) treated and untreated cells, were studied using 2',7'-dichlorofluorescin diacetate (H₂DCFDA) (Life Technologies, Invitrogen, India). Cells were cleaned in PBS twice and suspended (1 × 10⁶ cells ml⁻¹) in H₂DCFDA working solution (10 μM) and incubated for 30 min at 37 °C in dark. Then cells were re-suspended in 400 μl of pre-warmed DPBS (CELLclone, India) and analyzed by flow cytometry (Becton Dickinson, FACS Calibur, New Jersey, USA).
3.5. Antibacterial activity
Antibacterial activity of Ag-NPs was obtained by well diffusion assay against the Gram-negative bacteria \textit{[Acinetobacter boumannii (ATCC-17978)]} and Gram-positive bacteria \textit{[Staphylococcus aureus (ATCC 33591)]}. Bacterial cultures were prepared to 0.5 McFarland standards prior to the assay. Pure bacterial cultures were subcultured on Luria Bertani (LB) agar medium and uniformly swabbed on individual plates. 10, 20, 30 $\mu$l of Ag NPs at concentrations was poured into wells, allowed to diffuse and incubated at 37°C for 24 h. Antibacterial activities were studied by the diameter of zone of inhibition.

4. Results and discussions
4.1. X-ray diffraction (XRD)
X-ray diffraction spectrum of silver nanospheres synthesized by \textit{Zingiber officinale} extract is seen in figure 1. XRD pattern shows four distinct reflections for Ag NPs at scattering angles of 38.1°, 44.5°, 64.5°, and 77.4° corresponding to the planes \{111\}, \{200\}, \{220\}, and \{311\} respectively. The reflections display the cubic face centred (FCC) crystalline structure of silver. Observed data was compared with JCPDS No. 04-0783 patterns. The broad width of the reflections indicated that the particles are nano-sized and size of silver nanoparticles was approximated by Debye–Scherrer equation

$$D = \frac{K\lambda}{\beta \cos \theta}$$

Here, D is mean size of particles, K is shape factor, $\lambda$ is wavelength, $\beta$ is full width half maximum of the peak, $\theta$ is angle of diffraction from the XRD spectrum.

From above equation, the diameter of nanoparticles is estimated as $\sim$6.1 nm (calculated from \{111\} plane diffraction peak). This is comparable with the TEM data.

The diffraction peaks at 27.1°, 32.3°, and 46.3° are due to constituents present in \textit{Zingiber officinale}, which act as capping and stabilizing agents. Hence, these results affirmed that silver nanoparticles were formed by reduction of silver ions by \textit{Zingiber officinale} extract \[3, 39\].

4.2. FTIR
The FT-IR spectrum of \textit{Zingiber officinale} extract produced silver nanoparticles is seen in figure 2. Major bands noticed at 3317, 1638, 1381, 1082, 815, 721 and 611 cm$^{-1}$ are interpreted below. The characteristic peak 3317 cm$^{-1}$ is the stretching mode of N–H or C=O. 1638 cm$^{-1}$ is attributed to N–H bonding vibration of amides indicating their existence in extract during the reduction of nanoparticles. The band at 1381 cm$^{-1}$ is ascribed to C=C alkane stretching vibration. The band at 1082 cm$^{-1}$ is attributed to the stretching vibration of C=O. The bands at 815, 721 and 611 cm$^{-1}$ are the strong signals of heterocyclic compounds, the active components of \textit{Z. officinale} such as alkaloids and flavonoids, which act as capping agents. These peaks reveal the presence of heterocyclic compounds in extract which are responsible for reduction of the silver ions \[40, 41\].

4.3. TEM images
Figure 3 shows the TEM images and elemental compositions of the synthesized \textit{Zingiber officinale} extract capped Ag NPs. TEM analysis (figure 3a) clearly revealed the uniform and well dispersed formation of spherical nanoparticles. From figures 3(b) and (c), HRTEM and SAED patterns show the crystalline nature of the Ag NPs.
and its corresponding planes are observed. These have been supported by the XRD. Spherical particles’ mean diameter is around 16.8 nm with a range from 12 to 24 nm. Figure 3(e) shows the EDX pattern indicating the presence of chemical composition of Ag. The percentage of elements found to be Silver (Ag) 13%, Iron (Fe) 24%, and Copper (Cu) 77%. The other elements like Fe and Cu served as capping agents bound to the surface of the Ag NPs. Table 1 compares the d-spacing values obtained from TEM and XRD where both of them match closely [42].

4.4. FESEM
Bio synthesized silver nanoparticles are confirmed by the structural analysis with FESEM and the results are shown in figure 4. FESEM analysis revealed that the formation of Ag NPs capped with biomolecules had uniform distribution with spherical shape. The size of the nanoparticles varied from 10 to 20 nm in diameter.

4.5. UV–vis absorption
When Z. officinale root extract was added to aqueous AgNO₃, the solution turned from transparent to dark ash colour owing to excitations of surface Plasmon of Ag nanoparticles. Appearance of surface Plasmon resonance (SPR) peak in UV–vis absorption spectra which is around 435 nm. Figure 5 (inset 5(ii)) clearly indicates the formation of silver NPs. Shape of the Ag NPs determines the number of SPR bands appearing in the absorption spectra, as predicted by the Mie theory [26]. Based on this, absorption spectra of spherical NPs have only one SPR band while the other shapes lead to two or more Plasmon bands. Single SPR band shown in inset (ii) indicates that the biosynthesized Ag NPs are uniformly distributed as nanospheres, which also matches with the XRD and TEM analysis.

Figure 5 shows the absorption spectra at different concentrations ((a) 5, (b) 10, (c) 20, (d) 50, (e) 70, (f) 100 and (g) 200 μl) of Ag NPs with 15 μl concentration of lanthanide complex like europium tetrafluoroacetionate, (Eu(TTFA))₃ complex. The absorption spectra of mixture solution shows peaks at 262 nm, 342 nm and 410 nm and a peak at around 300–400 nm is red-shifted moderately owing to the interaction of Ag NPs and europium molecules. This indicates the influence of plasmonic field of silver on the complex ions. With increase in the concentration of Ag NPs, the Plasmon band at 410 nm is same as pure silver solution as shown in inset 5(ii). Inset figure 5(i) shows the linear increase in absorption with increase of silver concentration in europium (20 μl) demonstrating that NPs are not getting agglomerated [7].

4.6. Photoluminescence
The effect of the presence of silver on the luminescence emission from Eu (TTFA)₃ and Sm (TTFA)₃ are investigated with various Ag NPs concentration (λ_exc = 350 nm) and the results are shown in figures 6(a) and (b)). Three luminescence emission peaks centred at 614, 577 and 590 nm are assigned to ⁷D₀ → ⁷F₂ (electric dipole), ⁵D₀ → ⁴F₀ and ⁵D₀ → ⁴F₅ (magnetic dipole) transitions for Eu(TTFA)₃ respectively (figure 6(a)). Similarly, figure 6(b), shows the luminescence spectra of Sm (TTFA)₃ with different concentrations of silver. The three bands at (⁶G₅/₂ → ⁶H₇/₂) 566 nm, (⁶G₅/₂ → ⁶H₇/₂) 602 nm (magnetic dipole) and (⁶G₅/₂ → ⁶H₉/₂) 645 nm (electric dipole) appear. Inset figures show the dependence of luminescence intensity on the concentration of Eu complex (20, 30, 40 μl) and Sm complex (200, 220, 240 μl) with varying Ag concentrations from 5 to 300 μl. In the case of Eu³⁺ ions, enhancement factors (EF) for electric-dipole transition (⁷D₀ → ⁷F₂) is 8.7. For magnetic dipole transitions (⁷D₀ → ⁷F₀ and ⁵D₀ → ⁷F₁) EFs are 3.81 and 5.44 in the presence of Ag NPs. In case of Sm³⁺ ions, EF’s of electric dipole transition (⁶G₅/₂ → ⁶H₉/₂) is 3.6 and magnetic dipole transitions (⁶G₅/₂ → ⁶H₇/₂ and ⁶G₅/₂ → ⁶H₇/₂) are 1.81 and 3.04 respectively.

Figure 2. FTIR spectra of Zingiber officinale extract used for biosynthesized Ag NPs.
Figure 3. (a) TEM images, (b) High resolution (HRTEM) image, (c) SAED rings pattern, (d) size distribution and e) EDAX pattern of bio reduced Ag NPs.

Table 1. d-spacing values of Ag NPs from XRD and TEM analysis.

| Planes (indexed in XRD) | XRD d-spacing (Å) | TEM d-spacing (Å) |
|-------------------------|-------------------|-------------------|
| 111                     | 2.354             | 2.358             |
| 200                     | 2.033             | 2.037             |
| 220                     | 1.442             | 1.451             |
| 311                     | 1.235             | 1.236             |
Figure 4. FESEM pictures of silver nanoparticles.

Figure 5. UV–vis absorbance spectrum of different concentrations of silver nanoparticles with Eu 20 μl complex. Inset figure (i) Linear dependence of silver concentration on absorbance and (ii) Pure Ag NPs absorption spectrum.

Figure 6. (a) Emission spectra (λ<sub>exc</sub> = 350 nm) of 15 μl Eu(TTFA), with various concentrations of silver nanoparticles: (a) 5, (b) 10, (c) 30, (d) 50, (e) 70, (f) 100, (g) 200 μl (h) 300 μl. Inset Figure shows the silver concentration versus luminescence intensity. Different europium concentrations are (a) 5 μl (b) 15 μl and (c) 25 μl. Figure 6(b) Emission spectra of 220 μl Sm(TTFA), (λ<sub>exc</sub> = 350 nm) with various silver: (a) 5, (b) 10, (c) 30, (d) 50, (e) 70, (f) 100, (g) 200 μl (h) 300 μl. Inset figure shows silver concentration versus PL intensity. Various samarium concentrations are (a) 200 μl (b) 220 μl and (c) 240 μl.
Table 2: Ag concentration and ratio between two transitions.

| Ag concentration (μl) | \( \frac{I(7F_2)}{I(7F_1)} \) | \( \frac{I(5D_0)}{I(4G_5)} \) |
|------------------------|-------------------------------|-----------------------------|
| 5                      | 2.93                          | 1.2                         |
| 10                     | 3.07                          | 1.46                        |
| 30                     | 3.42                          | 1.45                        |
| 50                     | 6.92                          | 1.4                         |
| 70                     | 4.53                          | 1.36                        |
| 100                    | 7.66                          | 1.37                        |
| 200                    | 4.37                          | 1.36                        |
| 300                    | 2.37                          | 1.47                        |

The normalised emission intensity of electric dipole transition increases up to a certain concentration of Ag and then decreases with further increase of Ag, which could be due to re-absorption of Plasmon resonance (SPR). From inset of figure 6(ii), the absorption of Ag NPs over the wavelength range ~300–600 nm practically covers the emission of the rare-earth complex (figure 6). From Forster mechanism [19, 33], the absorption is a competition between SPR band of Ag NPs and rare-earth complex. However, the energy transfer from lanthanum ions to silver NPs i.e., the emitted energy from complex is absorbed by Ag NPs. Therefore the enhancement is due to the balance between SPR and SEF (surface enhanced fluorescence) and also it depends on size, concentration and the medium around of Ag NPs.

The electric dipole transitions of \( ^7D_0 \rightarrow ^7F_2 \) (Eu) and \( ^4G_{5/2} \rightarrow ^6H_{9/2} \) (Sm) are very sensitive to the local field while the magnetic dipole transitions \( ^7D_0 \rightarrow ^7F_1 \) and \( ^4G_{5/2} \rightarrow ^6H_{7/2} \) in Eu and Sm complexes respectively are less sensitive. The intensity ratio between electric and magnetic dipole transitions of \( ^7D_0 \rightarrow ^7F_2 \rightarrow ^5D_0 \rightarrow ^7F_2 \) and \( ^4G_{5/2} \rightarrow ^6H_{9/2} \rightarrow ^4G_{5/2} \rightarrow ^6H_{7/2} \) of rare-earth complexes show the degree of asymmetry around rare-earth ions are shown in table 2. In case of Eu and Sm complexes, the intensity of \( ^7D_0 \rightarrow ^7F_2 \rightarrow ^5D_0 \rightarrow ^7F_2 \) and \( ^4G_{5/2} \rightarrow ^6H_{9/2} \rightarrow ^4G_{5/2} \rightarrow ^6H_{7/2} \) emissions increase relatively with Ag concentration and the increase is by a factor of about 2.61 and 1.3 respectively [20, 21, 43].

The intensity ratio, shown in table 2, between electric and magnetic dipole transitions of rare-earth complexes indicate the symmetry around rare-earth ions. In case of Eu complex, at maximum emission enhancement, intensity emission ratio first decreases and then increases. It means that (i) there is an energy transfer between lanthanum ions and silver NPs, (ii) induced surface Plasmon resonance of silver NPs influence the field around rare-earth ions. The emission from rare-earth ions depends on the size of Ag NPs as the SPR peak changes with size. In case of Sm complex, the intensity ratio did not alter significantly, which indicates that Ag NPs’ SPR does not have resonant interaction with the lanthanum ions.

4.7. Life time decay curves

Figure 7 shows the fluorescence decay curves of \( ^7D_0 \) and \( ^4G_{5/2} \) levels of Eu\(^{3+}\) and Sm\(^{3+}\) ions with and without 70 μl of Ag NPs at 613 nm and 645 nm under 350 nm excitation respectively. The transitions from \( ^5D_0 \rightarrow ^7F_{0.1,2} \) show longer lifetimes. The curves were fitted with single exponential decay. The lifetime of Eu complex is 97 μs and in the presence of silver NPs, the life time increases to 216 μs. We observe only a small change from 6.3 μs to 10.2 μs in Sm complex owing to the presence of silver NPs. At the off resonant excitation of Ag NPs with 350 nm excitation, there is an appreciable energy transfer from the Ag NPs to the lanthanum ions (both Eu and Sm). This energy transfer keeps increasing as we increase the concentration of the Ag NPs. However, at higher concentration, the Ag NPs are likely to cluster and their resultant absorption shifts towards the red region. At such high concentrations, we would expect a reverse energy transfer, that is, from the Eu and Sm to the Ag NPs, thereby reducing the luminescence from the rare-earth complexes. This means that a small variation in luminescence lifetime of rare earth ion affects the luminescence yield. This enhancement increases the decay rates, thus increase in quantum yields [21, 44]. The population of \( ^7D_0 \) or \( ^4G_{5/2} \) level therefore depends on the concentration of rare-earth ions [23], or the emission intensity ratio is proportional to rare-earth concentration. The quantum efficiencies are unaltered which means lifetime and intensity ratios are invariant as shown in table 2.

4.8. Optical limiting studies

The nonlinear optical absorption measurements of Zingiber officinale synthesized silver NPs were carried through open aperture Z-Scan technique (figure 8) with Nd:YAG laser (EKSPLA-2143A) at 532 nm, 30 ps and 10 Hz. In short, in normal Z-scan experimental setup, a Gaussian profile beam is focused by a lens. 1 mm thick sample cuvette is moved along the focused beam. At focus, the sample sees a maximum intensity and it
continuously reduces from focus in two directions. An f/40 configuration was used here. The width of the sample should be less than the Rayleigh range, which is \( \sim 3 \) \( \text{mm} \) for the lens used in the present set up. Neutral density filters and apertures are used to alter the intensity of laser and for beam shaping, respectively. By moving the sample across the focus, the experimental data is measured through boxcar averager (model SRS 250) with the use of analog-to-digital (ADC) card to obtain a good averaging of the pulses and the output is given to a computer. The nonlinear absorption coefficient \((\alpha_2)\) was measured by fitting the transmittance equation \([28, 39, 45]\)

**Figure 7.** Fluorescence decay curves of europium and samarium complexes with and without Ag NPs at 613 nm and at 645 nm respectively with excitation at 350 nm.

**Figure 8.** (a) Open aperture Z-scan curves, (b) Optical limiting curves of Ag NPs with 532 nm, 30 ps pulse width and 10 Hz rep rate.
Here, \( z_0 = \frac{\pi \omega_0^2}{\lambda} \) - Rayleigh range, \( \omega_0 \) - beam waist at \( Z = 0 \), \( I_0 \) - intensity on sample at focus, \( \lambda \) - laser excitation wavelength, \( a_2 \) - nonlinear absorption coefficient, and effective path length \( L_{\text{eff}} = \frac{1 - e^{-a_2 z_0}}{a_0} \), sample of length \( L \), \( a_0 \) is linear absorption coefficient.

Figure 8 depicts the \( Z \)-scan of open aperture data with different intensities of green synthesized Ag NPs. Symbols represent the experimental data and solid lines are theoretical fits using equation (1). The experimental data shows a reverse saturable absorption (RSA) behaviour due to the excitations of SPR band to free carrier band of nanoparticles and also a two-photon absorption (TPA) from ground state. The theoretical fits obtained lead to the absorption coefficient \( \alpha_2 = 10.2 \times 10^{-9} - 15.2 \times 10^{-9} \text{cm}^2 \text{W}^{-1} \) at various intensities in the range of 0.78 GW cm\(^{-2}\) to 3.9 GW cm\(^{-2}\) [18]. Figure 8(b) displays the optical threshold limiting data of Ag NPs at 532 nm with ps laser. The measured optical limiting threshold value is 54 mJ cm\(^{-2}\).

### Table 3. MTT assay on HCT116 cells treated with various concentrations of Ag NPs±SD.

| HCT116 cell lines | Concentration (\( \mu \text{g/ml} \)) | % Cell viability | % Cell inhibition |
|-------------------|--------------------------------------|-----------------|------------------|
| Control           | 100                                  | 100             | 0                |
| Zingiber officinale | 100                                | 100             | 0                |
| Oxaliplatin       | 57.76                                | 42.24           | 57.76            |
| 25                | 56.04 ± 1.32                         | 43.96 ± 1.32    | 56.04 ± 1.32     |
| 50                | 48.85 ± 1.21                         | 51.15 ± 1.21    | 48.85 ± 1.21     |
| 75                | 45.58 ± 0.97                         | 54.42 ± 0.97    | 45.58 ± 0.97     |
| 100               | 37.91 ± 0.42                         | 62.09 ± 0.42    | 37.91 ± 0.42     |
| 125               | 24.84 ± 0.23                         | 75.16 ± 0.23    | 24.84 ± 0.23     |

\[
\Gamma_{\text{OA(2PA)}} = \frac{1}{2^{5/2}(1 + x^2/z_0^2)} \quad (1)
\]

4.9. Cytotoxic assay (MTT assay)

The *Zingiber officinale* extract mediated Ag NPs were tested for cytotoxic effect using MTT on HCT116 cell lines as shown in figure 9. Cell lines were tested after 24 h incubation at 37 °C in 5% CO\(_2\) by varying the concentration of biosynthesized Ag NPs (25, 50, 75, 100, and 125 \( \mu \text{M} \)) in *Zingiber officinale* extract shows zero inhibition, indicating that the extract does not play any role in toxicity. The synthesized Ag NPs significantly increased the cell death in the treated HCT116 cell lines as shown in figure 9 and table 3 compares with commercially available drug Oxaliplatin (OXP). A significant cytotoxic effect of half maximal inhibitory concentration value (IC\(_{50}\)) of Ag NPs is 49.97 \( \mu \text{g ml}^{-1}\). Ag NPs inhibited the proliferation of HCT116 cells dose dependently which demonstrates that the Ag NPs prepared using *Zingiber officinale* have great promise as an anticancer agent [46].

Figure 10 shows fluorescence images (20\( \times \)) of HCT116 cells with (a) untreated cells, (b) treated cells with control (OXP), cells treated with Ag NPs with different concentrations (c) 25 \( \mu \text{M} \) (d) 50 \( \mu \text{M} \) (e) 70 \( \mu \text{M} \) and (f) 125 \( \mu \text{M} \). The morphological observation of cell death was performed by exposing HCT116 colon cancer cells to Ag NPs as shown in figure 10. Microscopic observations in the treated cancer cells show distinctive morphological shape changes when compared to the control. Nanoparticle (Ag NPs) treated cells appeared as
clusters and restricted cell spreading patterns as compared to control. This might be because of structural and functional changes in mitochondria. Bright spots on the cell surface might be due to adsorption of Ag NPs on the surface of the cells.

4.10. Apoptosis assay
Apoptosis is a programmed cell death process, in which, asymmetry of plasma membrane, blebs formation and condensation of nucleus will occur. The loss of plasma membrane asymmetry is early characteristic of apoptosis. In apoptotic bodies, phosphatidylserine (PS) is altered from inner leaflet to the outer leaflet of membrane [19]. Annexin V is a binding protein which binds to PS and propidium iodide (PI) is a dye used for labelling the early or late apoptotic bodies [20]. Live cells with undamaged membranes are not stained with PI and dead cells are stained with PI. Therefore, cells are viable means Annexin V and PI are negative (−); cells in early apoptosis means Annexin V is positive (+) and PI is negative (−); cells in late apoptosis or dead means Annexin V and PI are both positive (+).

In flow cytometry figure 11, HCT116-untreated and camptothecin-treated cells were stained with Annexin V and PI kit. In untreated, 93.3% cells were live and were non-apoptotic (Annexin V and PI are negative). In case of camptothecin-treated cells, 46.5% were live cells and increase in early-apoptotic (Annexin V (+) and PI (−)) cells were observed from 3% to 34.6% from untreated to treated cells. In case of Ag NPs treated, slight increase in late apoptotic (Annexin V (+) and PI (+)) cells is observed. Thus, Ag NPs treated cells were showing late apoptotic pathway. In FSC-H plots, the increment in the apoptotic cells is also reflected by altering the scattered light. In apoptosis, cells will shrink, which means decrement in the scattered light. As compared with untreated and camptothecin- treated cells, Ag NPs treated cells showed decrease in the scattered light. Percentage of apoptotic cells when untreated and when treated with Camptothecin and Ag NPs can be seen from the bar graph. The results indicates that Ag NPs treated cells are apoptotic bodies.

4.11. Caspase 3 expression
Caspases are important moderators of apoptosis, which normally play an important role in activating death protease and cleavage of cell proteins. Activation of caspase-3 proteolytically cleaves and activates other caspases, leading to apoptosis. Apopain, Rabbit IgG antibody can identify the active form of caspase-3. It has been used to visualize the existence of active form of caspase-3 in HCT116 cells.

From SSC-H plots in figure 12, as compared with untreated and Camptothecin–treated cells, Ag NPs treated cells showed decrease in the scattered light. The results from Counts and bar graphs also show that the untreated cells in M1 region were negative for active caspase-3, whereas 66.49% treated cells were in M2 region, i.e., positive for active caspase-3 staining. This result is in agreement with the cells treated with Camptothecin (48.26%), which is known to induce apoptosis, thus indicating Ag-NPs induced caspase-3 dependent apoptotic cell death in HCT116 cells [47].

Figure 10. Fluorescence images (20×) of HCT116 cells treated with (a) untreated cells; (b) cells treated with control (OXP); cells treated with Ag NPs (c) 25 μM (d) 50 μM (e) 70 μM and (f) 125 μM.
4.12. Intracellular ROS assay by H₂DCFDA

During apoptosis process, there is alteration of mitochondrial function, which results in overproduction of (reactive oxygen species) ROS. ROS contain hydroxyl radicals or peroxides with unpaired electrons. Excess ROS can damage the DNA, proteins, and lipids leading to cell death. H₂DCFDA (2′, 7′-dichlorodihydrofluorescein diacetate) is a ROS indicator and non-fluorescent fluorescein, which is oxidized and converted into fluorescent 2′, 7′- dichlorofluorescein (DCF) by intracellular ROS. To investigate whether Ag-NPs induced ROS generation in HCT116 cells, we measured cellular ROS by H₂DCFDA (Life Technologies, Invitrogen, India) from figure 13. We found that there was an increase in the percentage of ROS (43.15%) compared to the untreated control. This result is comparable with the cells treated with the Camptothecin (38.7%), which is known to induce ROS generation by apoptosis. The results indicate that the Ag-NPs induced excessive generation of ROS in HCT116 cells [48].

4.13. Antibacterial activity

The antibacterial studies of bio-synthesized silver nanoparticles were obtained against Acinetobacter baumannii (A. baumannii) and Staphylococcus aureus (S. aureus) (figure 14). The antibacterial activities (inhibition zones) of

![Figure 11. (Apoptosis): The dot plots and histograms representing HCT116, untreated, treated with 15 μM camptothecin and treated with Ag NPs. These cells were co stained with annexin V-FITC and PI. Bar graphs showing percentage of apoptotic cells.](image-url)
Figure 12. (Caspase-3): The dot (SSC-H) plots representing HCT116, untreated and treated with Camptothecin and with Ag NPs. These cells were stained with FITC rabbit anti-active caspase-3 antibody and analysed by flow cytometry. Graphs and histograms show the counts and percentage of cells with active caspase-3.

Figure 13. (ROS): The dot plots and histograms representing HCT116, untreated, treated with Camptothecin and with Ag NPs. Graphs and histograms show the percentage of cells with ROS.
various concentrations of silver nanoparticles (10, 20 and 30 μl) against A. baumannii are 1.6, 1.7 and 1.8 cm and for S. aureus, they are 1.7, 2 and 2 cm (table 4).

The Zingiber officinale synthesized silver nanoparticles against bacteria displayed unique zones of inhibitions in well diffusion method. In literature, it is reported that silver nanoparticles exhibited antibacterial activities against different bacteria, although the mechanism of silver nanoparticles affecting the system is not well understood. The bactericidal activity depends on the size, shape and synthesis methods of silver nanoparticles [49]. But among all the shapes and methods, spherical nanoparticles with smaller sizes (large surface area) and biologically synthesized have excellent antibacterial activities [50–53]. The results show that spherical biosynthesized silver nanoparticles are more effective against A. baumannii than S. aureus bacteria with increase in concentration.

5. Conclusions

Simple green synthesis technique is used to prepare eco-friendly biocompatible Ag NPs with no surfactants and chemicals, as the Zingiber officinale extract itself acted as reducing and stabilizing agent with its natural bioreduction potential. The synthesised nanoparticles were spherical in shape having small size around 20 nm and were highly stable for six months at ambient conditions indicating that the adapted synthesis technique is straightforward, economical, non-toxic and biodegradable. The presence of Ag NPs at particular concentrations exhibited appreciable luminescence enhancements with Eu$^{3+}$ and Sm$^{3+}$ ion complexes. The enhancement was found to be higher for europium complex due to an efficient energy transfer from NPs. This was also confirmed from the lifetime studies of Eu$^{3+}$ and Sm$^{3+}$ ion complexes, where Eu$^{3+}$ ion has shown a significant increase in the emitter life time. Ag NPs also exhibited enhanced optical limiting behaviour in ps regime, which is due to two photon absorption. In vitro cytotoxicity studies revealed that the Ag NPs exhibited excellent toxic effect against HCT116, colon cancer cell lines. Ag NPs exhibit greater abilities to inhibit HCT116 cells proliferation and enhanced cellular uptake. The studies showed that apoptosis was the dominant mode of cell death and Ag NPs promote Caspase-3 apoptotic pathway through ROS generation. Moreover, the NPs also
exhibited greater antibacterial activity against (A. baumannii) and Staphylococcus aureus (S. aureus) at lower concentrations. Hence, green synthesized Ag NPs have efficient optical, biomedical properties and can be used as luminescence enhancers, optical limiters, fluorophores in various applications like bio imaging and drug delivery.

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Conflicts of interest

There are no conflicts of interests.

ORCID iDs

E Ramya @ https://orcid.org/0000-0002-8497-7315
L Jyothi @ https://orcid.org/0000-0001-5884-2401
N Sri Ram Gopal @ https://orcid.org/0000-0002-6407-2017

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