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Green Synthesis, Characterization, And Assessment Of Cytotoxic Activity Of Silver Nanoparticles Synthesized Using Calotropis Gigantea Leaf Extract Against THP-1 And PC-3 Cancer Cell Lines

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Abstract:
Nanotechnology has emerged as a pivotal field with diverse applications, particularly in biomedicine, due to the unique properties of nanoparticles such as high surface area to volume ratios and distinctive optical, magnetic, and chemical characteristics. Silver nanoparticles (AgNPs) have garnered significant attention for their potential in cancer therapy, drug delivery, and antimicrobial applications. Traditional methods of nanoparticle synthesis often involve toxic chemicals, prompting the need for eco-friendly alternatives. This study explores the green synthesis of AgNPs using Calotropis gigantea leaf extract, a plant known for its medicinal properties, including antioxidant, anti-inflammatory, and cytotoxic activities. The synthesized AgNPs were characterized using UV-Visible spectroscopy, XRD, FESEM, EDX, FT-IR, DLS, and Zeta potential analysis, confirming their crystalline structure, morphology, and stability. The cytotoxic activity of the biosynthesized AgNPs was evaluated against THP-1 (human leukaemia) and PC-3 (prostate cancer) cell lines using the MTT assay. Results demonstrated significant anti-proliferative effects, with IC50 values of 17.22±0.1007 µl/ml for THP-1 and 26.18±0.047 µl/ml for PC-3 cells, indicating potent cytotoxic activity. The findings highlight the potential of C. gigantea-mediated AgNPs as a promising anticancer agent, offering a cost-effective and eco-friendly alternative to conventional cancer therapies. This research underscores the importance of green synthesis in developing nanomaterials with significant biomedical applications, particularly in oncology.
Key Words: *Anti-cancer activity, THP-1, PC-3, Calotropis gigantea, Silver nanoparticles*

INTRODUCTION:

Nanotechnology has gained significant attention in recent years as one of the lucrative fields of study and research, offering distinctive character and a plethora of applications in varieties of sectors (Erci F., 2018). Due to their well-documented small size, high surface area to volume ratios, and unique characteristics in optical, magnetic, chemical, and mechanical properties, nanoparticles have emerged as promising biomedical applicants as antioxidants, antibiotics, anti-cancerous agents, and drug delivery agents (Tanase C., 2019). Noble metal nanoparticles, which include silver, platinum, titanium, gold, Zinc, and copper, have gained substantial interest for application in biomedical fields and multi-utility due to therapeutic and diagnostic characteristics (Behboodi *et al.*, 2019). The conventional approach to the synthesis of nanoparticles includes chemical and physical methods that have pronounced toxicity levels and are also hazardous in nature. Plant-mediated green synthesis of nanoparticles is cost-effective, environmentally friendly, less hazardous, and also requires a short time in synthesis (Gengan *et al.*, 2013). Usually, in the plant-based technique of synthesis of silver nanoparticles, the extracts of different parts of plants, such as leaves, stems, latex, fruits, peels, etc., are being used as the source of stabilizing and capping agents for the biosynthesized metal nanoparticles (Olga *et al.*, 2022). Nanomaterials synthesised from various natural organic agents have pivotal applications in diagnostic procedures, advanced medical device development, and drug delivery, which makes the way for

the progression of techniques for intensification and improvement of human health (Kumar *et al.*, 2024). Plants contain a plethora of bioactive metabolites such as polyphenols, flavonoids, tannins, polysaccharides, terpenoids, alkaloids, amines, and aldehydes, which act as capping agent, stabilizing agents, and reducing agents which play essential role in the alteration and reduction of metallic ions to metallic nanostructures, leading to the formation of desired nanoparticles with defined features (Rajan *et al.*, 2015). Among all metallic nanoparticles, the silver nanoparticles (AgNPs) are the best contender due to their exclusive biological, chemical, and physical properties (Ahn *et al.*, 2019). The green technique offers a wide range of advantages in the synthesis of silver nanoparticles over the other processes because the green technique is extracellular and uses extracts from leaves or plant parts rather than the entire plants. This is a cheaper alternative, and turns out to be eco-friendly as well (Shankar *et al.*, 2004).

Calotropis gigantea, commonly called the "crown flower' plant, is a large shrub belonging to the family Apocynaceae, widely distributed in the Indian subcontinent, and can be easily identified by the presence of soft tomentum on the abaxial side of leaves and stem (Rehman *et al.*, 1991). The Indian Ayurvedic system and the traditional medicine include this plantforits medicinal value, which finds its use in the treatment of muscular spasm, rheumatoid arthritis, asthma, and also in cough syrup and purgative. The leaves of the Calotropisplant contain a plethora of known and documented compounds, which include coroglaucigenin, 16a-hydroxycalotropin, calotoxin, dienoic acid, 12β-hydroxycoroglaucigenin, frugoside, mevalonolactone, calotropagenin, desglucouzarin, and calactinic acid, along with bioactive compounds like 15b-hydroxy cardenolides (1,2) and a 16a-hydroxycalactinic acid methyl ester (3) (Singh *et al.*, 2011).The leaf extract of this plant has already been evaluated for its different pharmacological efficacies and medicinal uses, such as antioxidant, antimicrobial, hypoglycemic, anti-inflammatory, anti-malarial, in paralysis, and snake bite, among other important medicinal potentialities (Sharma *et al.*, 2022; Sreewardhini et al., 2023). Further, the crude extract of *C. gigantea* leaves have been reported to possess significant cytotoxic activity against human colon cancer cells (WiDr cells) (Mutiah *et al.*, 2017).

Among the various biomedical applications of the silver nanoparticle synthesized using the green plant extract, one of the prominent applications of these tiny particles is observed in their role as anti-cancerous agents. Cancer is among the most deadly and globally distributed ailments, which is distinguished by uncontrolled division of the mutant cells. The pathophysiology of cancer is extremely intricate and is frequently brought on by genetic mutation or deregulation brought on by the exposure of normal cells to xenobiotics or environmental contaminants or the potent carcinogens (Gali-Muhtasib *et al.*, 2020). Radiation therapy, chemotherapy, and surgery are listed as the conventional methods of cancer treatment (Damyanov *et al.*, 2018). The nanosized materials have distinctive chemical and physical properties that make them a potent contender for the biomedical application in the field of treatment of cancerous growth. In the attempt to mitigate some of the adverse consequences of traditional cancer treatment, the emerging nanotechnology has gained substantial interest (Liang *et al.*, 2016).

Previous studies have established that the plant extract-based silver nanoparticles can enter the cancer cells through endocytosis and followed by their penetration into the mitochondria, where they can affect the cellular respiration and produce highly reactive free radicals like reactive oxygen species (ROS) (Hsin *et al.*, 2008). The formation of free radicals inside the cancerous cells can lead to the impairment of some important biomolecules which includes DNA and proteins. The green-synthesised silver nanoparticles have also been established to encourage the process of apoptosis i.e. programmed cell death in the cancerous cells by modulating the process of autophagy, which occurs as a result of a series of steps of molecular events in the mitochondria of cancerous cells (Sanpui *et al.*, 2011; Peynshaert *et al.*, 2014). The impairment of lysosomal functions and resulting autophagy in the THP cancer cell line has already been reported to be induced by AgNPs (Xu *et al.*, 2015). According to a few other studies, the green-synthesised AgNPs may also have an impact on the vascular endothelial growth factor, which is important for angiogenesis in tumors (Kalishwaralal *et al.*, 2009). The scientific studies to date have established that the green-synthesized AgNPs

have marked cytotoxic activity against different cancer cell lines through ROS production, modulation of apoptosis and autophagy, and other molecular mechanisms (Jabir *et al.*, 2021; Alvarez-Cirerol *et al.*, 2024). The silver nanoparticles synthesized from the latex of *C. gigantea* have been reported to have marked cytotoxic activity against the MCF-7 cancer cell line (Immaculate *et al.*, 2020) and HeLa cells (Chandrasekaran *et al.*, 2015). However, to date the AgNPs synthesized from leaf extract of *C. gigantea* has been assessed for their antioxidant and antimicrobial properties (Rengarajan *et al.*, 2024) among other significant biomedical efficacies. Therefore, the present study has been undertaken for the synthesis of silver nanoparticles using leaf extract of *C. gigantea*, their characterization, and to evaluate the leaf extract-based AgNPs for their anti-cancerous properties by determining their cytotoxic activity against selected cancer cell lines, i.e., PC-3 and THP-1.

MATERIALS AND METHODS:

Collection of sample and preparation of extract:

Fresh leaves of the plant *C. gigantea* were collected from East Singhbhum, Jharkhand, India. The extraction is done by following previously established standard methods. The leaves were washed and dried under shade for 7-8 days and then powdered. 25 gm of fine powder was dissolved in 500 ml of double-distilled water and then boiled for 10 minutes at 80°C, and then the solution was allowed to cool down to room temperature. Now the solution was filtered through Whatman filter paper. The filtrate was collected and stored at 4°C for further use (S. Revathi *et al.*, 2024).

Synthesis of silver nanoparticles:

A 1 mM solution of silver nitrate ($AgNO_3$) was prepared by dissolving 0.169 g of $AgNO_3$in 1000 ml of deionised water, and the solution was kept covered with foil to prevent its oxidation by direct exposure to sunlight. Now, 10 ml of extract was added to 90 ml of $AgNO_3$solution, followed by continuous stirring. The solution was then incubated at 80°C for 4 hrs. The change in colour of the solution preliminarily infers the synthesis of silver nanoparticles, which was then confirmed by various analyses for characterization of nanoparticles.

Characterization of Silver Nanoparticles:

The reduction of silver ions and thereby the synthesis of extract-mediated silver nanoparticles was preliminarily shown by the colour change of the solution after incubation. Various analytical techniques were used for the characterisation of biosynthesised Cg-AgNPs, like UV-visible spectroscopy, XRD (X-ray diffraction), FESEM (field emission scanning electron microscopy), EDX (energy dispersive X-ray) analysis, FT-IR (Fourier transmission infrared) spectroscopy, DLS (dynamic light scattering), and zeta potential analysis.

The absorption spectra of the sample were recorded with a UV-visible spectrophotometer (Perkin Elmer, USA; Lambda-25)between the wavelengths of 200 and 800 nm using deionised water for adjusting the baseline reference, and the resultant peak referred to the synthesis of silver nanoparticles.The structural analysis of biosynthesized silver nanoparticles was done by using an X-ray diffractometer (Rigaku, Japan; Smart Lab 9KW) and the data was recorded for a wavelength of 0.154 nm (X-ray wavelength) in a 2θ range of 10° to 80°.The XRD analysis was done on a fine and dried powdered sample. The presence of different functional groups on the surface coating of Cg-AgNPs was determined through FTIR (Shimadzu Co., Japan; IR Prestige 21) analysis by scanning the spectrum in the range of 400 to 4000 cm^{-1}. The size, shape, topography, and morphological properties of Cg-AgNPs were analysed by FESEM-EDX (Carl Zeiss Microscope Ltd., Germany; Sigma 300). The surface charge and hydrodynamic diameter of Cg-AgNPs was analysed by DLS analysis (Malvern Inst. UK; Zeta Sizer Nano ZS).

Assessment of anti-cancerous activity (MTT assay) against Human Leukemia (THP-1) cell line:

The cytotoxicity of the provided samples on the THP-1 cell line (procured from NCCS Pune-Human leukaemia monocytic cell) was determined by MTT assay (Tihauan *et al.*, 2020; Van Meerloo *et al.*, 2011; Fotakis and Timbrell, 2006). The cells (10000 cells/well) were cultured in a 96-well plate for 24 h in RPMI

1640 medium (RPMI 1640-AT060-1L) supplemented with 10% FBS (fetal bovine serum, HIMEDIA-RM 10432) and 1% antibiotic solution at 37°C with 5% CO_2. The next day, cells were treated with different concentrations of the Cg-AgNPs solution. After incubation for 24 hours, MTT Solution was added to cell culture and further incubated for 2 h. Cells without treatment were considered as Co-controls, cells without MTT were considered as blank. At the end of the experiment, culture supernatant was removed, and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO -SRL-atno.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm and 66nm. M.*IC50 was* calculated by using the software Graph Pad Prism -6. Images were captured under an inverted microscope (Olympus eK2) using a camera (AmScope digital camera 10 MP Aptima CMOS).

Calculation:-

$$\% \boldsymbol{Viable\ Cells} = \boldsymbol{A(test)/A(control)} \times \mathbf{100}$$

(A_{test} = Absorbance of test sample)
($A_{Control}$ = Absorbance of Control)

Assessment of anti-cancerous activity (MTT assay) against Prostate cancer (PC-3) cell line:

The cytotoxicity of the extract-based silver nanoparticle solution on the PC-3 (procured from NCCS Pune) cell line was determined by MTT assay. The cells (10000 cells/well) were cultured in a 96-well plate for 24 h in Ham's F12K medium supplemented with 10% FBS (fetal bovine serum, HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-streptomycin-Sigma-Aldrich P0781) at 37 °C with 5% CO_2. The next day, cells were treated with different concentrations of the Cg-AgNPs. A stock solution of Cg-AgNPs was prepared in DMSO and further diluted to get different concentrations in incomplete cell culture medium (without FBS). After incubation for 24 hours, MTT Solution (5 mg/ml) was added to cell culture and further incubated (in an air-banned CO_2 incubator with a head force of HF90) for 2 h. Cells without treatment were considered controls, and cells without MTT were considered blank.

RESULTS AND DISCUSSION:

(A) **(B)**

Fig 1: The colour change after incubation preliminarily confirms the synthesis of AgNPs, and (B) Dried Cg-AgNPs

The fundamental chemical reaction that results in the formation of silver nanoparticles with various phytochemical constituent compounds acting as capping and stabilising agents is the reduction of silver ions present in the $AgNO_3$ solution by the phytochemical constituent compounds present in the leaf extract of *C. gigantea*, as shown in Figure 1.

XRD Analysis

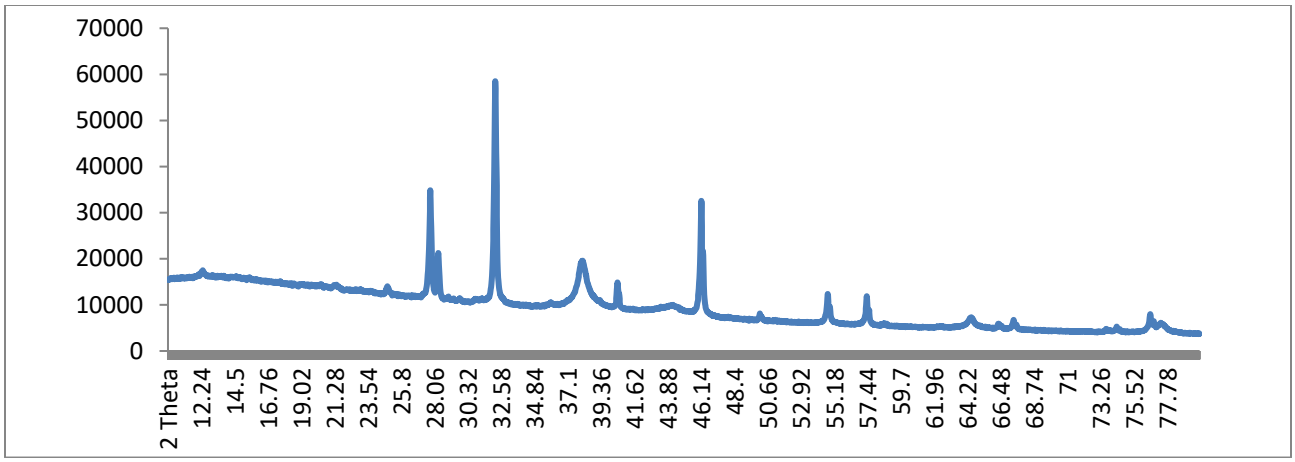


Figure 2: XRD analysis of Cg-AgNPs showing major peaks of intensities with respect to 2 Theta values

Figure 2 shows the X-ray diffraction pattern of silver nanoparticles synthesized from aqueous leaf extract of *C. gigantea*. The graph shows diffraction peaks at or around 2θ = 27.8, 32.2, 38.14, 46.18, and 54.76. When this result is compared with the standard, the diffraction pattern shows that the synthesized silver nanoparticles are crystalline in structure. The different peaks correspond to the planes (100), (111), (200), and (220) of silver crystals, respectively (correlated to JCPDS/ICDD card number 04-0783) (Ali *et al.*, 2023).The average particle size is calculated by using the Debye-Scherrer formula:

$$D = \frac{0.9\lambda}{\beta Cos\theta}$$

Along with the Bragg peaks that are indicative of silver nanocrystals, other unidentified peaks are also seen, which indicates that the crystallisation of the bioorganic phase takes place on the surface of the silver nanoparticles. The diffraction pattern also shows unassigned peaks, noise, and peak broadening, which might be due to the biochemical constituent molecules present in the extract or the crystallisation of the bioorganic phase on the surface of nanoparticles (Velavan and Amargeetha, 2018).

UV-Visible spectroscopy:

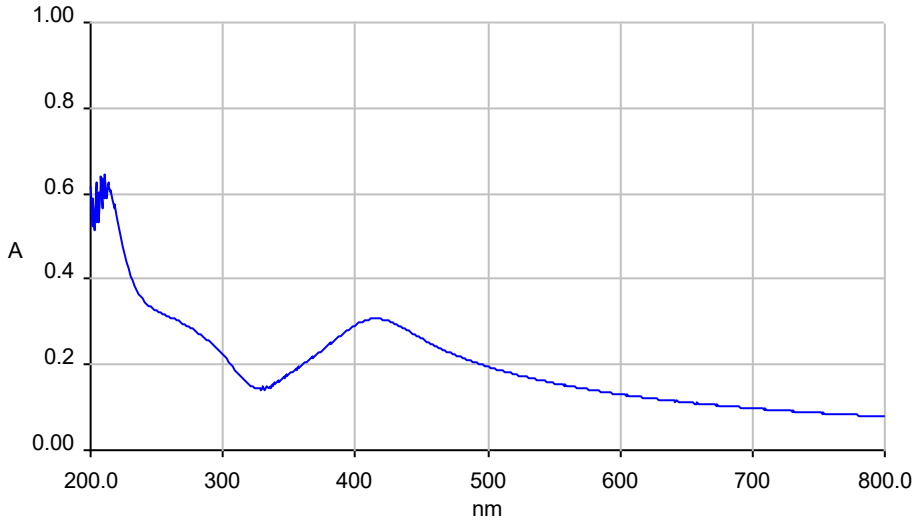


Fig. 3: UV-Visible spectrum of biosynthesized Cg-AgNPs

One of the main analytical methods for characterizing silver nanoparticles is UV-visible spectroscopy since the particles exhibit their maximum absorbance in the UV range. The *C. gigantea* leaf extract-based silver

nanoparticles (Cg-AgNPs) show a typical absorption peak at and around 420 nm due to the phenomenon of surface plasmon resonance. The band of UV-visible spectrum ranges approximately between 400 and 450 nm when the nanoparticles are smaller, whereas it may be between 450 and 495 nm when the nanoparticles are larger (R. Anith Jose *et al.*, 2022). The UV-Visible spectrum of the present analysis shows an intense surface plasmon resonance peak at around 410-420 nm, which confirms the presence of silver nanoparticles.

DLS and Zeta Potential:

The DLS analysis measures the dynamic change in the intensity of light scattered due to Brownian motion of synthesized nanoparticles (Ghani *et al.*, 2022). The Zeta potential of the synthesised Cg-AgNPs was evaluated to be -13.3 mV, which indicates that the nanoparticles have good structural stability, as the negative surface charge repels the particles in the solution, which results in the formation of stable colloids with good dispersion (Tavan *et al.*, 2023; Elamawi *et al.*, 2018). The Z-average of the biosynthesized Cg-AgNPs was found to be 183.1 nm with a polydispersity index (PdI) value of 0.243, which infers the possibility of formation of the aggregates (Tavan *et al.*, 2023).Cg-AgNPs was found to have a larger hydrodynamic diameter when compared to FESEM and XRD examinations because of the thickness of the hydration shell formed in the aqueous environment and the capping of phytochemical constituent compounds over the NPs surface (Hanachi *et al.*, 2022).

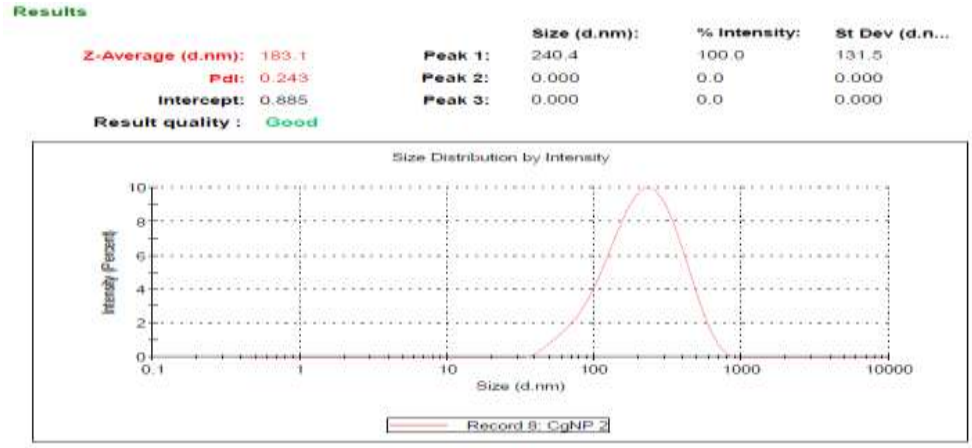


Fig. 4: Showing the Z-average peak (size distribution) of biosynthesized Cg-AgNPs

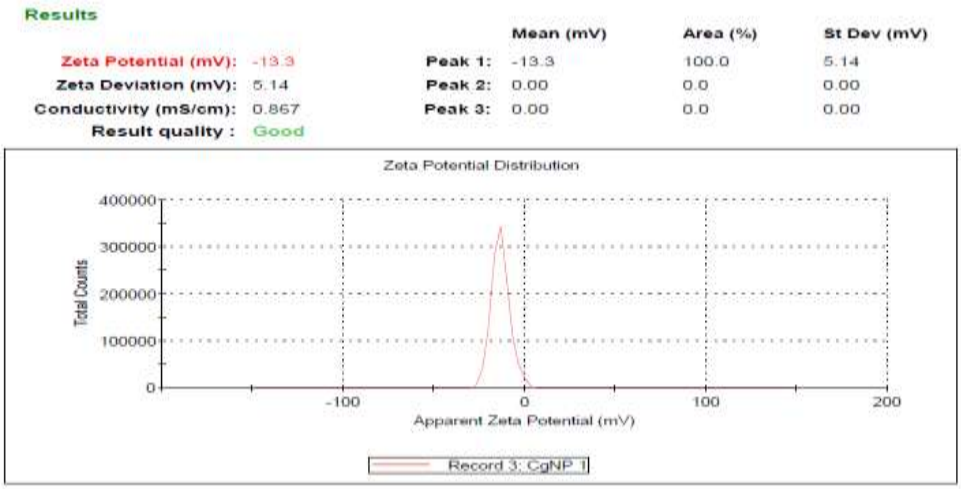
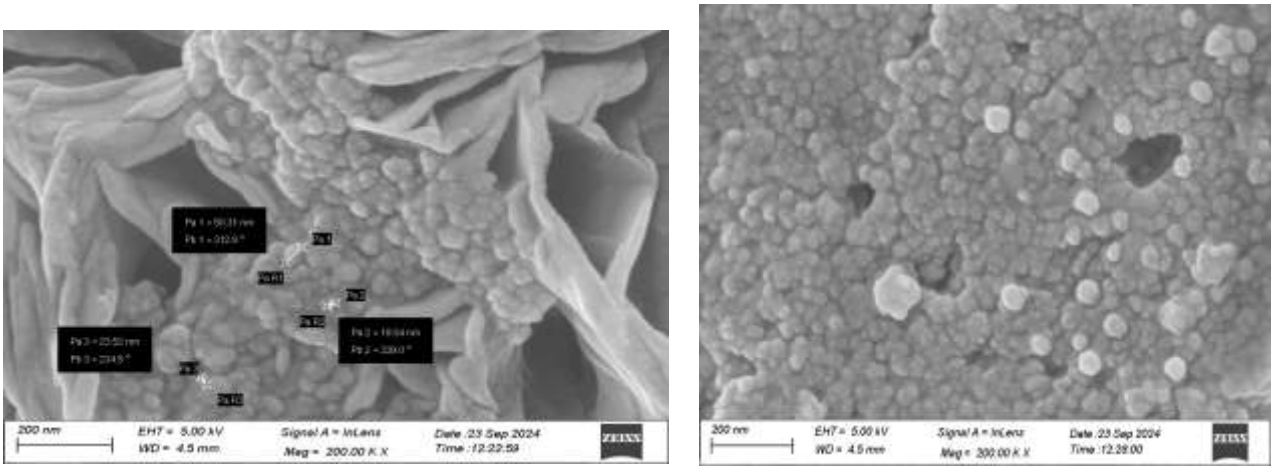


Fig. 5: Showing Zeta potential spectrum of biosynthesized Cg-AgNPs

FESEM and EDX analysis:

The FESEM was used to assess the surface morphology, topography, and size of the nanoparticles. The FESEM images depicts that the Cg-AgNPs are quasi-spherical, or ovoid in shape with particle size ranging mostly <51 nm. Some larger-sized particles can also be observed, which are due to the natural tendency of aggregation of the Cg-AgNPs or may be due to the evaporation of solvent during the preparation of the sample (Tavan *et al.*, 2023). The aggregation of two or more reducing moieties adhered to the surface of performed nuclei might be responsible for the agglomeration of Cg-AgNPs. Therefore, it could be inferred that the biochemical constituent compounds present in the C. *gigantea* extract have served as the reducing and capping agents during the synthesis of silver nanoparticles (Mohammad and Hasan, 2024). The EDX analysis of the present sample shows the highest peak at 3 keV, which infers the presence of metallic silver nanocrystals (Al-Dbass *et al.*, 2022). The EDX spectrum also shows additional peaks referring to the presence of other elements, including platinum, oxygen, chlorine, iron, and potassium, which might serve as capping agents, bound to the surface of the silver nanoparticles and may be due to the adsorption of other biomolecules at the surface of AgNPs (Salar RK *et al.*, 2016). As a strong Ag peak was observed at 3 keV, as per the sources, it confirms the presence of Ag in the sample analyzed (Fazli Khuda *et al.*, 2023).



(A) **(B)**

Figure 6 (A) and (B): FESEM images of nanoparticles (at a resolution of 200 nm) showing nanoparticles with sizes 50.31 nm, 23.52 nm and 19.04 nm

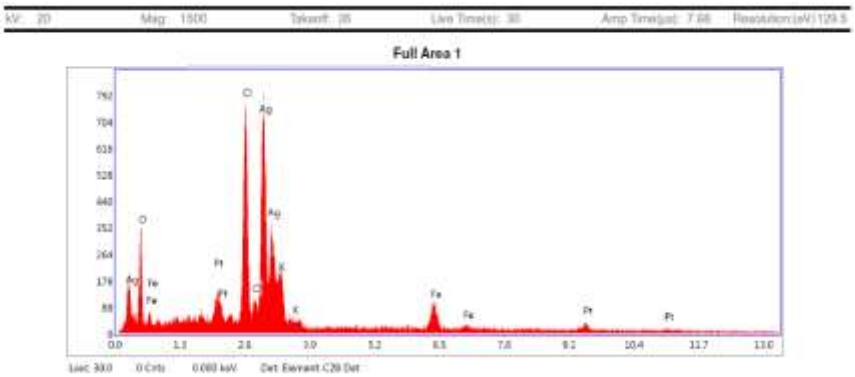


Fig. 7: EDX spectra of biosynthesized Cg-AgNPs

FT-IR analysis:

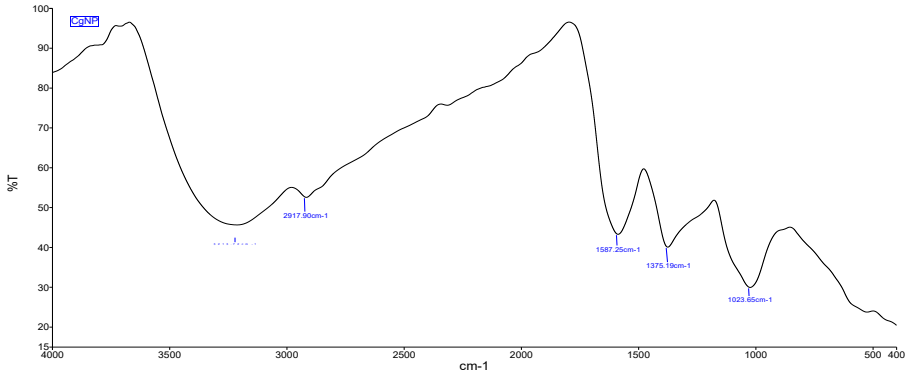


Fig. 8: FT-IR spectrum of biosynthesized Cg-AgNPs

S. No.	Wave No. (cm^{-1})	Corresponding functional Group
1	2917.90	-C-H, -C-N stretching (Methyl group, methoxy group or nitrate bonds, Aromatic group)
2	1587.25	-C-N, -C-C (aromatic ring, presence of proteins)
3	1375.19	-C-O stretching/ O-H deformation
4	1023.65	-C-O stretching/ O-H deformation
5	3400-3200 (Broad)	Normal polymeric –OH stretch

Table 1: Showing major peaks of FT-IR spectrum and their corresponding functional groups

The FTIR spectrum of the Cg-AgNPs was used to identify the functional groups of the biochemical constituent compounds, which serve as reducing and capping agents during the synthesis of nanoparticles (Mohammad and Hassan, 2024). The FT-IR spectrum was recorded for the mid-IR range of wavelengths from 400 to 4000cm^{-1}. The wave numbers in the FT-IR spectrum of Cg-AgNPs and their corresponding functional groups are listed in Table 1 which reveals the presence of biochemical compounds with functional groups like alcohols, phenols, amines, hydroxyl, amides, carbonyls etc. (Coates, 2000; Nandiyanto *et al.*, 2019), acting as capping and stabilizing agents.

Assessment of anti-cancerous activity (MTT assay) against Prostate cancer (PC-3) cell line and Leukemia (THP-1) cell line:

Based on the results obtained from the MTT assaythe IC_{50}value (concentration of an inhibitor/sample/formulation at which the viable cells are reduced by half) of Cg-AgNPs solution was found to be 26.18±0.047 µl/mland 17.22±0.1007µl/ml against the PC-3 cancer cell line(Fig. 8), and the THP-1 cancer line (Fig. 9) respectively, which marks that the biosynthesized Cg-AgNPs has significant anti-proliferative activity or cytotoxic activity against the tested cancer cell lines. The morphology of the cancer cell lines at their respective IC_{50} concentrations of Cg-AgNPs is shown in figure 10.Several previous works have shown that the silver nanoparticles synthesized from plant extracts possess marked cytotoxic activity against different cancer cell lines (Amar Kumar *et al.*, 2024; Simon *et al.*, 2022). Firdhous and Lalitha (2015) have reported that the silver nanoparticles synthesized from *Alternanthera sessilis* extract had an IC_{50} concentration of 6.85µg/ml against the PC-3 cancer cell line. In another work carried out by Muhammad *et al.* (2024), the cytotoxic activity (IC_{50}) of silver nanoparticles synthesized from *Cephalaria tchihatchewii extract against the PC-3 cell line was found to be 7.84 ± 1.21µg/ml.*Khedr *et al.* (2022) assessed the cytotoxic activities of silver nanoparticles synthesized from extracts of different parts of the plant *Cynara scolymus* L. and reported that the silver nanoparticles synthesized from flower extract, stem extract, and bract extract were found to have IC_{50} values of 2.47±0.24, 83.4±2.19, and 14.29±1.23 µg/ml, respectively, against the PC-3 cancer cell line, and the cytotoxic activity of biosynthesized silver nanoparticles may be attributed to the induction of

apoptosis or through antioxidant activation. In comparison to the previous works, the results of the present work reveal that the Cg-AgNPs are found to have a comparable cytotoxic activity against the PC-3 cancer cell line with an IC_{50} value of 26.18±0.047 µl/ml. Few studies have been conducted to date to evaluate the cytotoxicity of biosynthesised silver nanoparticles against THP-1 cancer cells. Alvarez-Cirerol *et al.* (2024) have reported that the silver nanoparticles synthesised from *Rumex hymenosepalus* root extract showed a minimal cytotoxic and apoptotic impact on the THP-1 cancer cell line at a concentration of 12.5 µg/ml. Another work done by Jabir *et al.* (2021) demonstrated that the silver nanoparticles synthesised from *Annona muricata* peel extract have marked antiproliferative activity against THP-1 cancer cells at a concentration of 17.34 µg/ml, due to the high membrane-penetrating potential of biosynthesised silver nanoparticles. In the present work the cytotoxic activity of biosynthesized silver nanoparticles against THP-1 cancer cells was found to be comparable with the results of other works 17.22±0.1007µl/ml. According to Hemlata *et al.* (2020), the biosynthesized silver nanoparticles may induce cytotoxic effects on cancer cells due to their very small size and ability to enter the cells through endocytosis along with their property to evade P-glycoprotein efflux. Several studies conducted over the past few decades have demonstrated that the use of nanoparticles has resulted in miraculous advancements in the field of medicine because of their small size, targeting ability, higher selectivity, and capacity to function as a drug delivery vehicle that can even cross the blood-brain barrier (BBB), along with increased bioavailability of drugs at the target site and reduced adverse side effects (Vinhas *et al.*, 2017; Cena and Jativa, 2018; Mostafavi *et al.*, 2022).Among all the biosynthesized metal nanoparticles, the silver nanoparticles are found to be one of the most effective anticancer agents (Khorrami *et al.*, 2019). Previous studies have reported that the biosynthesised silver nanoparticles can affect the metabolic activities of cancer cells by blocking the respiratory chain, producing free radicals like ROS (reactive oxygen species), altering DNA structure and functions, enhancing oxidative stress and mitochondrial damage, and adversely affecting cell division mechanisms along with membrane damage (Yesilot and Aydin, 2019). In the present study, the in-vitro cytotoxicity assay revealed that the Cg-AgNPs decreased the viability of leukaemia (THP-1) and prostate (PC-3) cancer cells in a dose-dependent manner. The marked cytotoxic activity of Cg-AgNPs against the tested cancer cell lines may be attributed to the physico-chemical properties of the biosynthesized silver nanoparticles along with the biochemical constituent compounds present in the *C. gigantea* leaf extract, which act as reducing as well as capping agents for the biosynthesized silver nanoparticles. Further, the results also infer that the Cg-AgNPs show more cytotoxic activity against the PC-3 cancer cell line in comparison to the THP-1 cancer cell line.

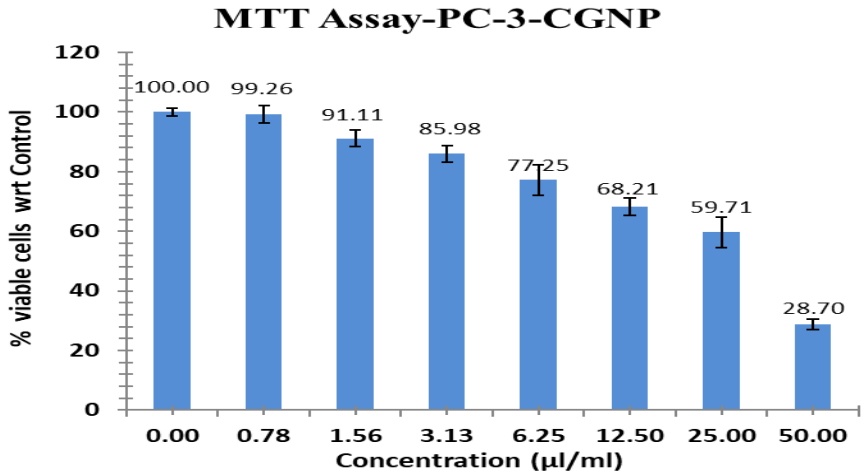


Fig. 9: Showing the cytotoxic activity of Cg-AgNPs solution at different concentrations against PC-3 cancer cell line

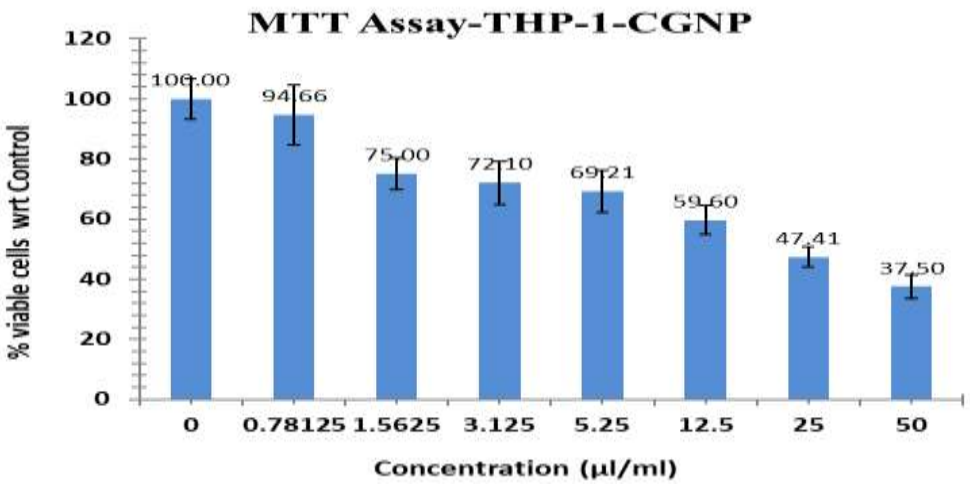
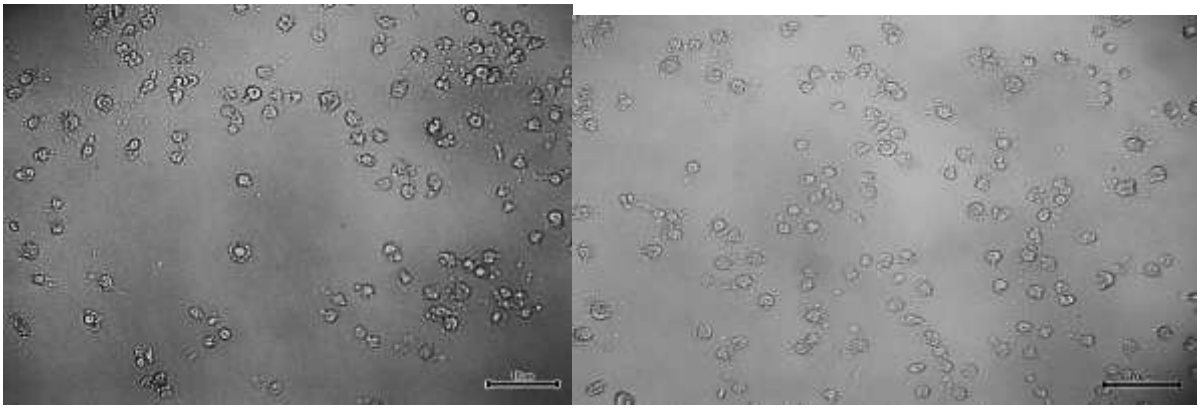
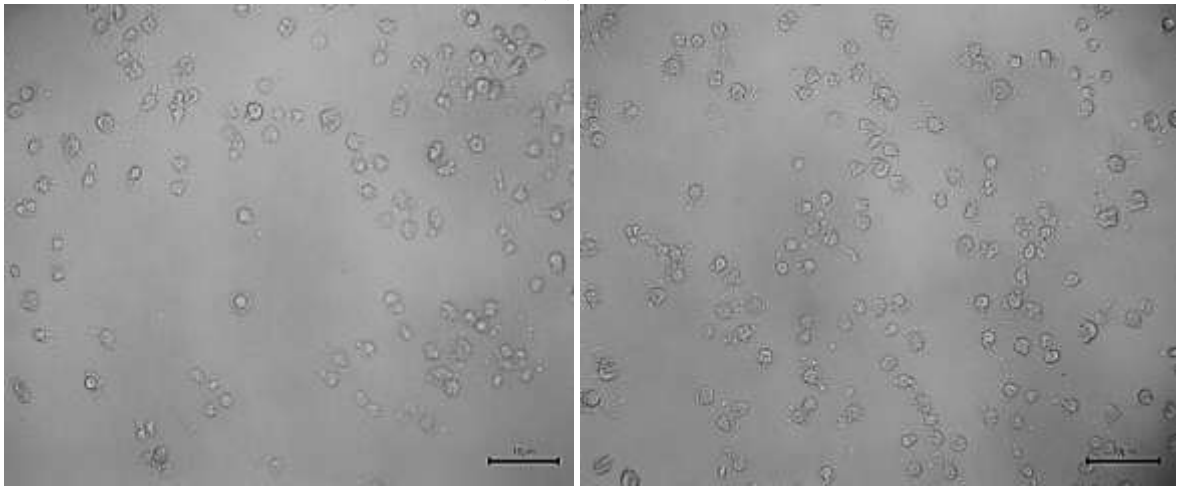


Fig. 10: Showing the cytotoxic activity of Cg-AgNPs solution at different concentrations against THP-1 cancer cell line



(A)



(B)

Fig. 11(A) and (B): Showing Morphology of PC-3 cancer cell line (A) treated with 26.18 µl/ml concentration of Cg-AgNPs and THP-1 cancer cell line (B) treated with 17.22 µl/ml concentration of Cg-AgNPs

CONCLUSION AND FUTURE PROSPECTIVES:

The present study reveals that silver nanoparticles synthesised from *Calotropis gigantea* leaf extract exhibit a strong cytotoxic effect against the selected cancer cell lines PC-3 and THP-1. The Cg-AgNPs are quasi-spherical or ovoid with particle sizes that are primarily less than 51 nm. In addition to other specified features, various characterisation approaches have revealed the presence of various functional groups of the phytochemicals constituents of *C. gigantea* leaves among the other physical and chemical characteristics of the biosynthesized Cg-AgNPs. The production of free radicals and oxidative stress, as well as other potential biochemical pathways, may be the principle by which Cg-AgNPs exhibit cytotoxic activity against the selected cancer cell lines. The current study opens up new avenues for investigation to determine the accurate molecular mechanism underlying Cg-AgNPs' cytotoxic effect, which could lead to the formation of novel anticancer medications.

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