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Anticancer Potential of Green Synthesized Silver Nanoparticles Using Flower extract of *C. guianensis* against Human breast adenocarcinoma (MCF7) Cell line

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Abstract:
The objective of this study was to examine the anti-cancer properties of silver nanoparticles (AgNPs) that were produced through the use of plant flower extract derived from the *C. guianensis*. The green synthesis of silver nanoparticles (AgNPs) was achieved using a plant flower extract of *C. guianensis*. The characterization of silver nanoparticles (AgNPs) was conducted by UV-visible spectroscopy, The MTT assay was employed to evaluate the efficacy of silver nanoparticles (AgNPs) in inhibiting the growth of human breast adenocarcinoma (MCF7) cell line, with a specific focus on their anti-cancer properties. The investigation using UV-Visible spectroscopy revealed the presence of a distinct peak in the range of 300-600 nm wavelength, which is indicative of the occurrence of surface plasmon resonance (SPR) and the formation of silver nanoparticles (AgNPs). The findings of the anti-cancer investigation indicate that AgNPs had cytotoxic effects on MCF7cell line, at concentrations, 31.25µg/ml, 62.5µg/ml, and 26.29µg/ml the cell viability was 85.30%, 72.30%, and 62.97%, respectively. Whereas, at the highest test concentration (i.e., 250 µg/ml) around 50.76% cell viability was observed. Indicating ~50% cell mortality. The present study provides evidence supporting the potential anti-cancer activity of silver nanoparticles (AgNPs) synthesized using the plant flower extract of *C. guianensis*.

Key Words: AgNPs, Flower extract, Uv-vis, Cytotoxicity.

Introduction:

The scientific community has shown significant interest in Nobel metal nanoparticles due to their wide range of applications in several fields such as biology, material science, and medicine [1]. Silver nanoparticles have garnered significant interest because of their unique physiochemical properties, including chemical stability and electrical conductivity, as well as their many biological activities such as antibacterial, antifungal, anti-inflammatory, antiviral, antiangiogenesis, anticancer, and antiplatelet activity. Moreover, the utilisation of silver nanoparticles has been observed in many applications such as clothes [6], room spray, laundry detergent, wall paint composition [7, 8], sunscreens, and cosmetics [9]. In vitro experiments have demonstrated that silver nanoparticles possess the ability to hinder the binding of the HIV-1 virus to host cells [10]. While there are numerous techniques for preparing metal nanoparticles, including UV radiation, laser ablation, lithography, aerosol technologies, and photochemical reduction [11–13], there is a growing emphasis on environmentally friendly synthesis methods involving bacteria [14], yeast [15], fungi [16], and plants [17].The process of green synthesis of nanoparticles is characterised by its cleanliness, lack of toxicity, cost-effectiveness, and environmental friendliness. The utilisation of microbe-mediated synthesis, despite its potential, has been constrained in industrial applications due to the necessity of maintaining sterile conditions. In contrast, the use of plant extracts for the production of nanoparticles holds significant value owing to its potential for easy scalability, reduced biohazardous properties, and avoidance of the cumbersome procedures associated with maintaining cell lines. Nanotechnology is now being employed in the field of medicine to facilitate the creation of novel antibacterial and anti-cancer medicines, with the ultimate objective of formulating enhanced therapeutic

interventions and approaches for addressing cancer. Cancer is a significant global health concern and is classified as a Non-Communicable Disease in the Global Burden of Diseases report. Cardiovascular disease is the primary cause of mortality, followed by the condition under discussion, which resulted in the deaths of around 8.8 million individuals globally in 2015 [1].Extensive research has been conducted since the early 2000s to investigate the possible efficacy of nanoparticles as a technique for addressing cancer. In recent times, scholars have been engaged in the advancement of metallic nanoparticles as potential anti-cancer therapies.

Based on our current understanding and comprehensive review of the literature, it appears that the use of *C. guianensis* for the manufacture of silver nanoparticles has not been documented. This study aimed to investigate the green synthesis of silver nanoparticles (AgNPs) using *C. Guianensis* and afterwards characterise the synthesised nanoparticles. Similarly, the aforementioned method was employed to evaluate their impact on biological systems.

Material and Methods:

C. guianensis were collected from September to December from the surroundings of University campus, (Lat long), and comparing the specimens to the herbarium collection of departmental herbarium museum in place, confirmed the specimens' authenticity. The cell culture medium, antibiotics-antimycotic solution, trypsin, and fetal bovine serum (FBS) were purchased from Invitrogen, a reputable American supplier. The plastic goods and other commodities were acquired from local vendor. These chemicals and reagents were purchased from manufacturer Sigma.

Preparation of Flower Extract

The aerial component of *C. Guianensis* was obtained and subjected to multiple washes with distilled water in order to eliminate any dust particles. Subsequently, it was dried in a shaded environment. The plant flower that had been dried by exposure to air was fragmented into smaller segments, subjected to maceration in distilled water, underwent filtration using gravitational force, and had its solvent removed through evaporation under reduced pressure using a rotary evaporator. The desiccated extract was stored at a temperature of 4°C.

Synthesis of Silver Nanoparticles

$AgNO_3$ is the most commonly used metal precursor in the synthesis of AgNPs. PFE (Plant Flower Extract)solution was mixed with $AgNO_3$ solution of 1 mM (100 ml), which pre-conditioned to the set parameters. Immediate color change from light green to brown color was observed, indicating the ultra-fast synthesis of AgNPs in the reaction mixture.

Ultraviolet-visible Spectra Analysis

The UV-visible spectra of nanoparticles that were synthesized through biological processes were acquired using a double-beam UV-visible spectrophotometer with a resolution of 1 nm. The test solution was placed in a quartz cuvette, and its optical density was examined at wavelengths ranging from 300 to 600 nm. The graph was generated by plotting the independent variable of wavelength on the X-axis and the dependent variable of absorbance on the Y-axis.

Cell culture

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The MCF-7 cell lines derived from human breast cancer were acquired from the National Center for Cell Science (NCCS) located in Pune, India. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Hi Media Laboratories, India).

Effect of AgNPs on cytotoxicity

The impact of silver nanoparticles (AgNPs) on cellular proliferation was assessed through the utilization of the MTT (dimethyl thiozolyl diphenyl-tetrazolium bromide) test. In brief, the cells were suspended at a concentration of 3×10^5 cells per milliliter. The cells were carefully arranged in 96-well microtiter plates, with each well containing 200 µl of solution. They were then subjected to a controlled environment with a temperature of 37 °C and a humidity level of 95% air and 5% CO_2 for duration of 24 hours. The MCF-7 cancer cells were subjected to various concentrations of silver nanoparticles produced from flowers. The control cultures underwent treatment with Dimethyl sulfoxide (DMSO). Cell viability was assessed using the MTT test after a period of 5 days, and subsequently, the cytotoxic concentration (C50) was determined.

Results and Discussion

Silver nanoparticles (AgNPs) have shown great potential in the field of nanotechnology due to their unique ability to combat several detrimental processes in the field of bioscience. Various methodologies have been employed in order to obtain an improved alternative for the synthesis of silver nanoparticles (AgNPs), encompassing physical, chemical, and biological techniques. In contemporary times, the utilization of aqueous extracts from green leaves for the synthesis of AgNPs has gained popularity due to its ability to circumvent laborious and costly procedures [20]. In recent years, numerous researchers have explored the utilization of plants as viable and accessible sources for the synthesis of biocompatible nanoparticles. This approach has several notable advantages, including biocompatibility, cost-effectiveness, absence of toxicity, and the ability to produce nanoparticles of high purity [21].Previous research has demonstrated the significant contribution of many plant constituents, including chemicals, secondary metabolites, and biomolecules such as carbohydrates, lipids, phenols, flavonoids, tannins, acids, resins, and terpenes, in the mitigation of nanoparticle ions. [22]
In the present study, biosynthesis of AgNPs with C. guianensis flower extract was achieved to evaluate thecytotoxic effects of AgNPs on the MCF-7 cell line.

The silver nitrate solution, which lacked colour, underwent a transformation to a dark brown colour, suggesting the presence of silver nanoparticles (AgNPs). The presence of a brown colour can be ascribed to the presence of surface plasmons [23], which arise from the collective oscillations of valence electrons inside the electromagnetic field of the incident radiation.

Figure 1 Shows the UV-Vis spectra of the produced silver nanoparticles (AgNPs), indicating the occurrence of plasmon resonance at a wavelength of 400 nm. The wavelength at which AgNPs exhibit maximum absorbance (λ max) often falls within the range of 400–500 nm [24]. The absorption of surface plasmons is influenced by various factors, including the morphology and dimensions of the particles, the distance between them, and the dielectric constant of the surrounding medium [25,26]. Previous studies have documented comparable findings [27,28].

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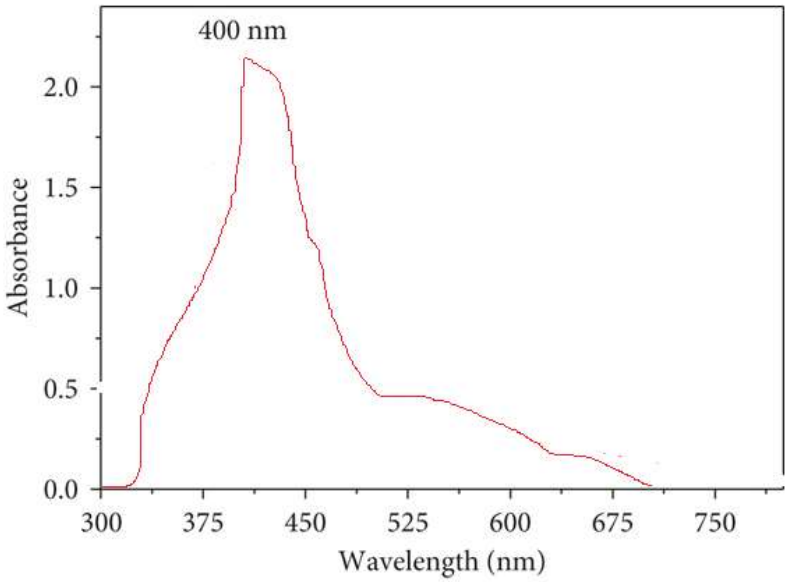

Figure: 1 Ultraviolet-visible absorption spectra of synthesized silver nanoparticles (AgNPs).

MCF7 cancer cell line is a human breast adenocarcinoma cells. The cytotoxicity of the silver nanoparticles (AgNPs) was evaluated in vitro against MCF-7 cells, a human breast cancer cell line, at various concentrations. The vitality of the tumor cells was determined using the MTT assay. The produced silver nanoparticles (AgNPs) demonstrate significant cytotoxicity when tested against MCF-7 cell lines.At the test concentration, i.e., 181.1 µM, cisplatin (Positive control) showed 49.85% cell viability. Approximately 50% reduction in the cell viability. On the other hand, in the presence of AgNPs (Test sample) showed very good cell viability (i.e., 96.46%) even at low concentration (i.e., 15.62 µg/ml). The cell viability gradually decreased as the concentration of the AgNPs increased. Indicating the AgNPs activity in concentration-dependent manner. At concentrations, 31.25µg/ml, 62.5µg/ml, and 26.29µg/ml the cell viability was 85.30%, 72.30%, and 62.97%, respectively. Whereas, at the highest test concentration (i.e., 250 µg/ml) around 50.76% cell viability was observed. Indicating ~50% cell mortality (Figure2). Indicating that the biosynthesized AgNPs were not particularly toxic to A549 cancer cell line. IC_{50} value was found to be 262.3µg/ml (Figure3).The deadly effects of silver (Ag) are attributed to the active physicochemical interaction between Ag atoms and the functional groups present in intracellular proteins, as well as the nitrogen bases and phosphate groups found in DNA.[30]. Inverted microscope images show the morphological changes observed in presence of AgNPs and standard. In comparison with untreated (Negative control) cells, treated (Standard and AgNPs) show characteristic morphological changes in MCF7 cell line (Figure 4). These characteristic changes were observed during the apoptosis stages of the cells. Figure5represent the microscopic images of MCF7 cell line in presence of standard and AgNPs (at different test concentrations).The hallmark of apoptosis such as cell shrinkage, blebbing, shape distortion, and decreased cell growth was observed in MCF7 cell line [31].

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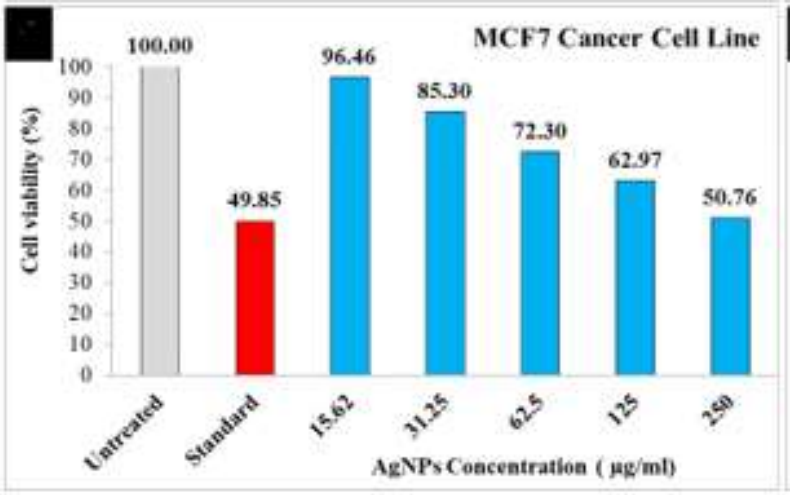

Figure: 2 MTT assay to determine the anticancer potential of the AgNPs in comparison with cisplatin (Positive control; commercial standard) against MCF7 cell line.

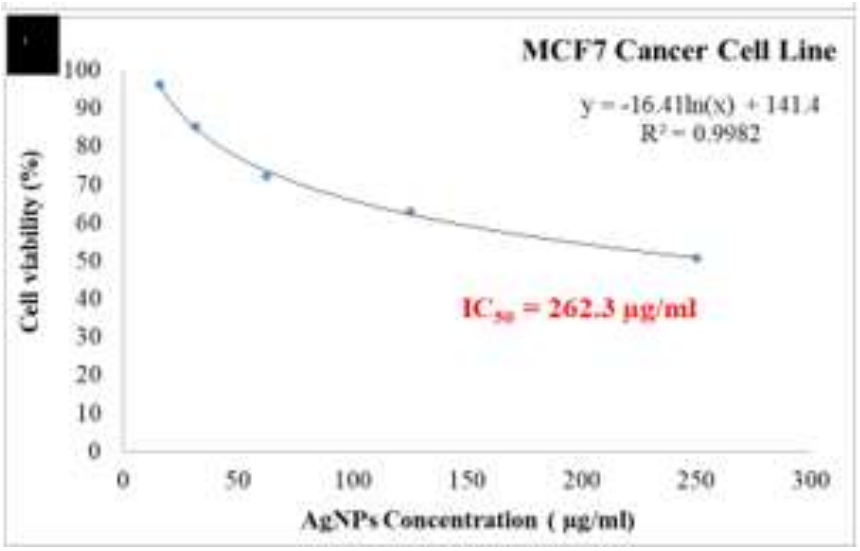

Figure: 3 Represents the plot between cell viability (in %) against different concentrations of AgNPs to determine IC_{50} value

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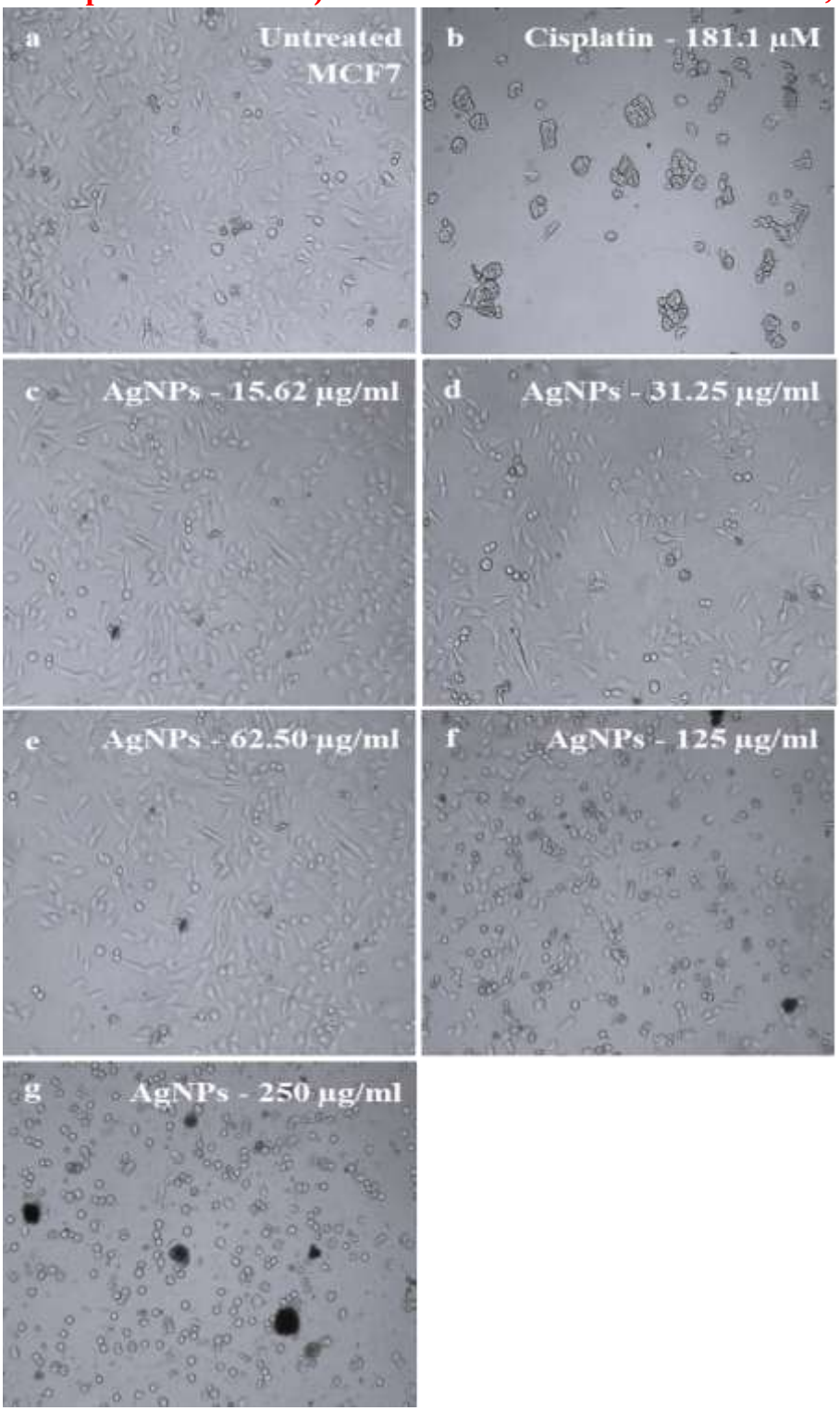

Figure 4: Inverted microscope images of MCF7 (Cancer cell line) **(a)** untreated, **(b)** cisplatin treated with 181.1 µM, used as positive control (Commercial standard) and AgNPs (Test sample) treated at different concentration i.e., **(c)** 15.62 µg/ml, **(d)** 31.25 µg/ml, **(e)** 62.5 µg/ml, **(f)** 125 µg/ml, and **(g)** 250 µg/ml.

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Conclusion:

In general, the findings of this investigation indicate that the flower extract derived from *C. guianensis* exhibits a notable capacity for synthesizing silver nanoparticles. The utilization of *C. Guianensis* flower extract for the synthesis of AgNPs offers an environmental benefit in comparison to alternative chemical synthesis approaches, as it does not generate any pollutants. Encouragingly, the produced silver nanoparticles (AgNPs) exhibited notable anti-cancer properties when tested against MCF-7 cell line. This study provides evidence that the utilization of aqueous extract of *C. guianensis* for the synthesis of AgNPs not only yields reduced environmental consequences, but also yields a product with significant potential as effective anticancer agents with life-saving properties.

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