Section A-Research paper
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ANTIBACTERIAL AND ANTICANCER ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES USING METHANOL EXTRACT OF *CALOTROPIS GIGANTEA* FLOWERS

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ABSTRACT

Silver nanoparticles (Ag-NPs) reflects high potential as an effective antimicrobial, cancer therapy with minimal side effects. Ag-NPs preparation techniques is designed as an ecofriendly manner. Using the flower extract of *Calotropis gigantea* plant, a powerful medicinal plant used to cure a variety of ailments, we biologically synthesized Ag-NPs using methanol in this study. The phytochemicals found in medicinal plants, such as polyphenols, flavonoids, and tannins, serve as reducing and stabilizing agents. As a result, they can be used in biological synthesis processes. The work suggests a novel strategy that biosynthesized Ag-NPs. Utilising a variety of techniques, the biosynthesized Ag-NPs were characterized, and the MCF-7 cell line was used to test their cytotoxicity. Surface plasmon resonance bands for Ag-NPs was discovered using ultraviolet-visible spectroscopy at 425 nm. Antibacterial activity of Silver nanoparticles of *Calotropis gigantea* flower extract was carried out against bacterial pathogens like Escherichia coli and *Salmonella typhi* by disc diffusion method. Maximum zone of inhibition were noticed in the *Salmonella typhi* followed by *Escherichia coli*. All the values showed $p<0.001$ level of significance

Keywords: Silver nanoparticles, FT-IR, SEM, UV-Vis Spectrophotometer, Bio reduction, Antibacterial activity, Bacterial pathogens, *Escherichia coli* and *Salmonella typhi*.

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INTRODUCTION

Despite optimistic developments in pharmaceutical biotechnology and the creation of new medications, cancer and infectious diseases continue to rank second to cardiovascular diseases as the leading causes of mortality and morbidity worldwide [1]. In addition, because of their rapid genetic evolution and genetic systems of adaptability, the majority of microbes are naturally equipped to resist the effects of therapeutic medicines. Have lost their interest in developing new antibiotic

compounds due to their narrow profit margin [2]. Consequently, it is crucial to develop efficient and low-cost processes for the production of therapeutic agents to combat the mentioned health problems. Significant advancements in nanoscience and nanotechnology, especially in the areas of diagnosis and treatment, are encouraging for addressing the problem of the emergence of antimicrobial drug resistance and enhancing life quality. Physiochemical designed biocompatible nanomaterials can offer efficient treatments for infectious and cancerous diseases [3].

It has been suggested that various physical and chemical processes can be used to create biocompatible nanoparticles with improved therapeutic qualities. The production of nanoparticles on a wide scale frequently necessitates high temperatures and/or pressures, which may not be economically feasible. Nevertheless, some chemical processes can result in nanoparticles with useful properties. One drawback of chemical procedures, meanwhile, is the use of poisonous and dangerous solvents.

Green synthesis has just been introduced as a straightforward, practical, and eco-friendly alternative method for creating nanoparticles. Biological substances (such plant extracts), microbes, or even eukaryotic cells function as reducing and stabilising agents in a conventional green synthesis, producing desired nanoparticles with predetermined properties [4, 5].

In this study, we looked at the viability of synthesizing Ag-NPs utilising the methanol solution derived from flower extract of *Calotropis gigantea*. The herbal substance was extracted using a very gentle, soxhlet extraction method at a low temperature, using methanol as solvent. We suggest that this bio-safe extract functions both as a stabilising and a reducing agent. The cytotoxicity of the synthesized nanoparticles was evaluated, and their anticancer, and antibacterial characteristics were described.

MATERIALS AND METHODS

Calotropis gigantea flowers extract

The freshly obtained flowers were dried for 15-20 days and then the flower grinded using mixer grinder to convert in to a powder form. The collected powdered form of flowers 1 kg was soaked with 9 liters methanol to obtain 39.59 gm of extracted compound by using Soxhlet apparatus. The Soxhlet apparatus is used to boil the solvent (in round bottom flask) so that it circulates during the process. A siphon mechanism is used up, which periodically empties and pours the solvent back into the round bottom flask of Soxhlet. Initially, the solvent methanol is heated to 30-40 degree temperature. The solvent vapor goes up the refining, and due to presence of cold water flowing through a condenser, it condenses the solvent. A portion of the coveted compound breaks up in the warm solvent. At the point when the Soxhlet chamber is full, the chamber is purged by the siphon. The methanol along with the extracted compound comes back to the round bottom flask. Now, the round bottom flask contains methanol and some extracted compound from *Calotropis gigantea* flowers. The solvent is heated again, and the process explained above is repeated several times, preferably 72 hrs. After numerous cycles, the coveted compound is

amassed in the bottom flask and the preserved methanol extract was used for additional examination [11].

Phytochemical analysis

Using recognized biochemical techniques, the phytochemicals coumarine, anthocynine, quinones, carbohydrates, alkaloids, saponins, starch, steroids, C. glycosids, proteins, acids, phenol, terpenes, and tannins were screened for in the methanol extract of *Calotropis gigantea* flowers [34,35].

Synthesis of silver nanoparticles using methanol extract

All of the chemicals were purchased from Mumbai, India's Himedia Laboratories Pvt. Ltd. *Calotropis gigantea* powder weighing 3 grams was heated in 100 ml of methanol before being filtered through whatmann no1. 95 ml of a 1 mm $AgNO_3$ solution were added along with 5 ml of methanol from this. The flasks were incubated for 24 hours at room temperature. The sample's biosynthesis of Ag-NPs is indicated by the colour change from yellowish to dark (Kasthuri *et al.,* 2009).

The formation of the Ag-NPs

The creation of Ag-NPs throughout the reduction process is evidenced by a visible shift in the color of the reaction solution from colorless to dark brown (Fig. 2)

Analysis of UV-Vis Spectrum

UV-Vis spectroscopy may easily detect the synthesis of silver nanoparticles by lowering the corresponding metal ion solution with *Calotropis gigantea* flowers extract. A Perkin-ElmerLamda-45 spectrophotometer was used to determine the absorption spectra of flower extract quantities and metal content in the 500-800 nm region.

Fourier Transform Infrared Spectroscopy (FT-IR)

The sample was measured using Shimadzu 8400 sand, which has a spectral range of 4000-400 cm^{-1} and a resolution of $4cm^{-1}$. Powder samples for FT-IR measurements were created in the same way that powder diffraction samples were. The FT-IR spectra of silver nanoparticles after synthesis were studied to determine the various functional groups for the formation of silver nanoparticles.

SEM analysis

In an ion coater, the powdered Ag-NPs were uniformly spread and sputter coated with platinum for 120 seconds before being observed by SEM (JEOL-JSM 6360 MODEL, JAPAN).

Antibacterial activity

Silver nanoparticles of methanol extract were evaluated for their antibacterial activity using the disc diffusion method developed by [16]. Sterile blank discs were submerged in the sample in a Petri dish for around two hours. The discs could now be used. Discs were placed in MHA agar plates that had already been colonized. Following an hour of diffusion, the plates were incubated at 37°C for 24 hours. The zone of inhibition was measured using a conventional ruler (ZOI). Measurements of the ZOI (Zone of Inhibition) were made in millimeters. Antibiotic ampicillin (10 µg/ml) was used as the positive control.

Anticancer activity

In 24-well plates with MEM containing 10% FBS, MCF-7 (1104 cells/ml) were seeded, and the plates were incubated for 24 hours at 37°C with 5% CO_2 and 95% O2. Fresh serum-free media was introduced after the medium had been withdrawn, rinsed with PBS, and maintained in the incubator for an hour. After starvation, methanol extract was applied to the cells, and Ag-NPs were produced at eight different concentrations, including (1000, 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 µg/ml), and the cells were then cultured for two and four hours. Supernatant was removed after incubation and 100 µl of Dimethyl Sulfoxide (DMSO) was added to dissolve the crystals. Using DMSO as the blank, the absorbance at 570 nm was determined using a spectrophotometer. Following is how the cell viability % was determined:

$$\text{Cell viability \%} = \frac{\text{OD of Experimental Sample}}{\text{OD of Control Sample}}$$

Based on the above calculation, the IC50 concentration against MCF-7 cell line was determined.

Analytical Statistics

The six trials' findings were presented as Mean SEM. Using the software version of Pad Prism V.5.0, a one-way ANOVA was conducted. To determine the level of significance at $p<0.001$, $p<0.01$, and $p<0.05$, all parameters, including antibacterial, anticancer were statistically analyzed.

RESULTS AND DISCUSSION

***Calotropis gigantea* a green synthesis of Ag-NPs and UV-Vis spectroscopy**

Figure: 1 Color changes

Ag+ was only ignited at the nanoscale by reductions of metal ions in plant flowers (the creation of nanoparticles is confirmed by a colour change from yellow to yellowish brown – Figure 1). The synthesis of white $AgNO_3$ and green compounds is superior to that of physical and chemical methods because it is less expensive, more environmentally friendly, and simpler to scale up for large-scale synthesis. High pressure, energy, temperature, and harmful chemicals are not necessary [33].

UV – VISIBLE SPECTRUM

Ag-NPs made from the methanol extract of *Calotropis gigantea* flower was observed using UV-visible spectroscopy, and it was discovered that the absorbance was greatest between 560 nm. Under UV-visible spectroscopy, when methanol extract and silver nitrate solution were incubated for longer periods of time, more Ag-NPs were produced (Fig. 2). The secondary metabolites in the flower extract function as a reducing and a capping agent for the synthesis of Ag-NPs, and together they drastically inhibited the formation of silver nitrate into silver ions. Previous studies [8, 14, 18, 22] that used a greener method to create silver nanoparticles with less harmful effects on people confirm our work [27,31].

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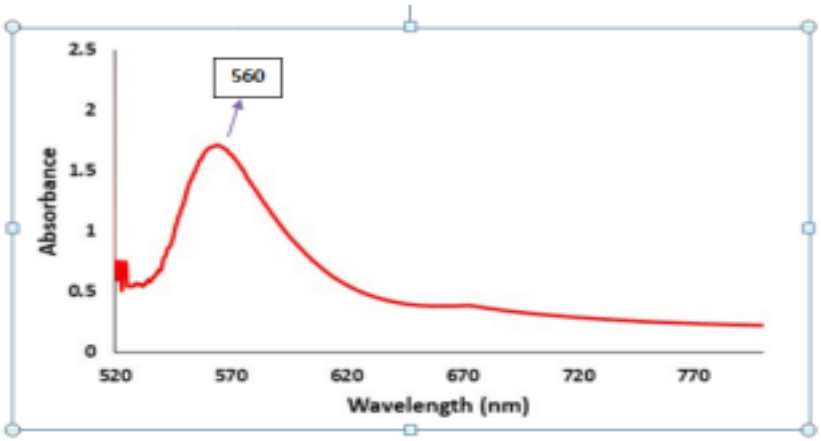


Figure 2: Uv Vis spectrum of synthesized silver nanoparticles of methanol extract of *Calotropis gigantea* flowers

FT-IR SPECTRUM

The presence of active functional groups in the chosen medicinal plant, which also functions as a reducing and capping agent, may be the cause of the creation or synthesis of silver nanoparticles, as shown by FT-IR spectra (Figs. 3). The FT-IR spectrum (Fig. 3b) of silver nanoparticles showed peaks at 3439(Alkenes C=C-H), 1625(Nitro Groups N=O Stretch), 1362 (AmidesN-HStretch) and 530(alkyl halides) cm^{-1}. This represents the different functional groups of biomolecules on the surface of nanoparticles. The very accurate analytical technique known as FT-IR allows for the detection and visualization of molecules' components, chemical structure, chemical bonds, functional groups, and bonding configurations [6]. To identify the compounds that serve as coating and stabilising agents as well as to spot the reduction of silver ions, AgNPs are characterised using FT-IR [7].

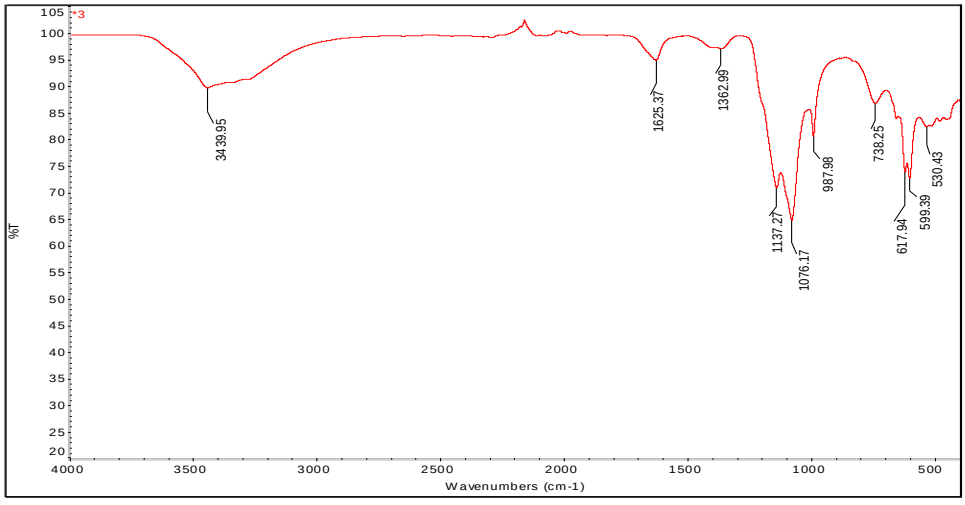


Figure 3: FT-IR Image Of Synthesized Ag-NPs of *Calotropis gigantea*

SEM IMAGES

SEM was used to look at the morphology of the silver nanoparticles (Fig. 4). In some areas, agglomerations of Ag-NPs were visible in the obtained images as well-defined spherical shapes. The average particle size in the SEM images' chosen region was estimated to be 50-105 nm. [13,15,19] Observed the same trend of outcomes with silver nanoparticles mediated by *C. pareira* L.

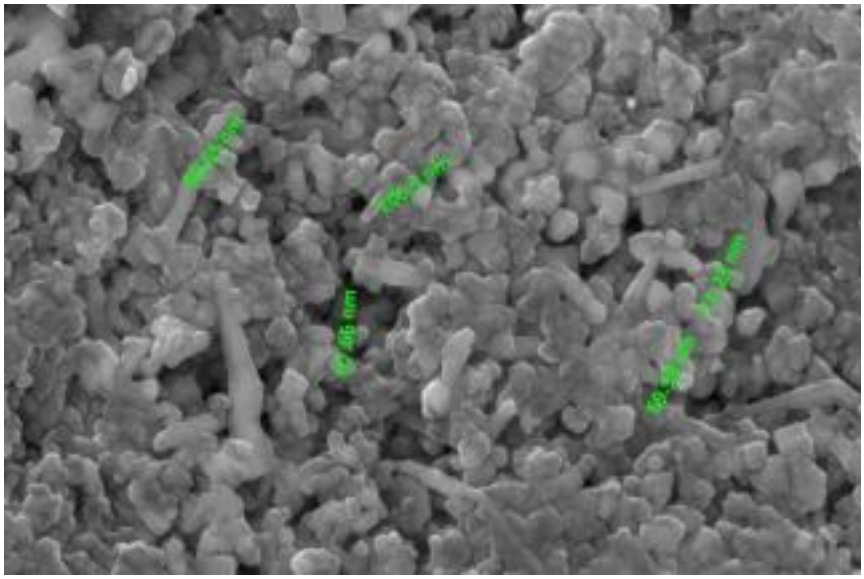


Figure 4: SEM Image of Synthesized Ag-NPS

Antibacterial activity

The antibacterial qualities of the synthesized Ag-NPs have been examined using a variety of methods, including disc diffusion, the conventional broth dilution method, and an assessment of the turbidity of culture media. These nanoparticles exhibit substantial antibacterial behaviour against the studied Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria, according to the data shown in Table 1 and Figure 5. Ag-NPs are widely employed in medicine, textile coatings, food preservation, dye reduction, wound treatment, antiseptic creams, and a variety of environmental applications because of their antibacterial characteristics. Both Gram-positive and Gram-negative bacteria were susceptible to Ag-NPs' antibacterial effects, which were dose dependent. The concentration of the Ag-NPs determined how effective they were against bacteria; more Ag-NPs had a greater inhibitory effect on microbial development [9]. The binding of the microorganism's membrane through electrostatic violation, the rupture of the cell wall, and the impact on internal activities like DNA, RNA, and protein synthesis have been reported as the mechanisms of Ag-NPs' bacterial activity [12-13, 16, 17, 26].

Table 1: Antibacterial activity of synthesized silver nanoparticles for different pathogens

		Zone of inhibition in mm			
S.No	Organism	125µg/ml	250µg/ml	500 µg/ml	Standard
1	*Salmonella typhi*	7.13±0.25	8.66±0.80	10±0.5	9.16±0.28
2	*E.coli*	7.16±0.28	7.83±0.15	8.76±0.64	8.23±0.25

***P<0.001 Significant variation**

Mean value ± Standard Deviation (Five replicate)

E.coli

Salmonella typhi

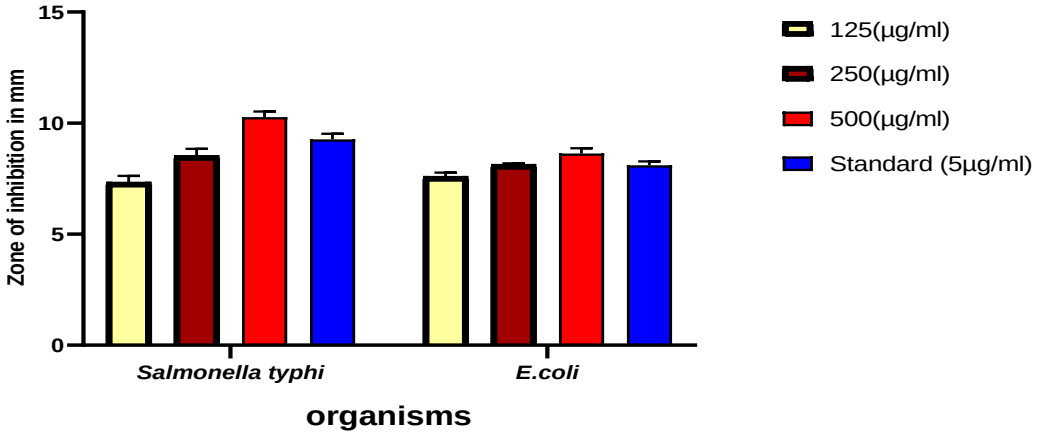


Figure 5: Antibacterial activity of synthesized silver nanoparticles for different pathogens

Anticancer activity

Human MCF-7cell lines was used to assess the anticancer efficacy of the Ag-NPs produced by *Calotropis gigantea*. The lowest IC_{50} concentrations for the green synthesized Ag-NPs was 158.62 µg/mL (Tab-2 and Fig-6). The capacity of green synthesized silver nanoparticles to prevent the growth of cancer cells in vitro may serve as a sign of potential anticancer activity [10].

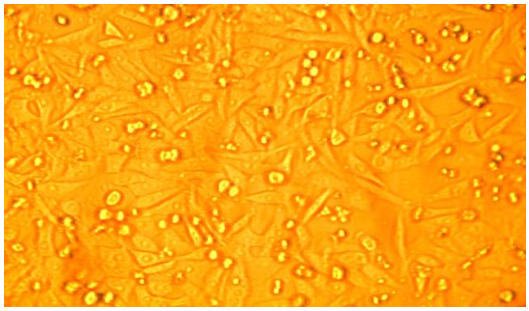
Table 2: Anticancer effect of *Calotropis gigantea* on *MCF-7 C*ell line

S.No	Concentration (µg/ml)	Absorbance (O.D)	Cell Viability (%)
1	1000	0.07	11.11
2	500	0.13	20.63
3	250	0.22	34.92
4	125	0.35	55.55
5	62.5	0.37	58.73
6	31.2	0.45	71.42
7	Cell control	0.63	100

IC_{50} value: 158.62 µg/ml

Anticancer effect of AgNPs of *Calotropis gigantea* on *MCF-7 C*ell line

Normal MCF-7 Cell line



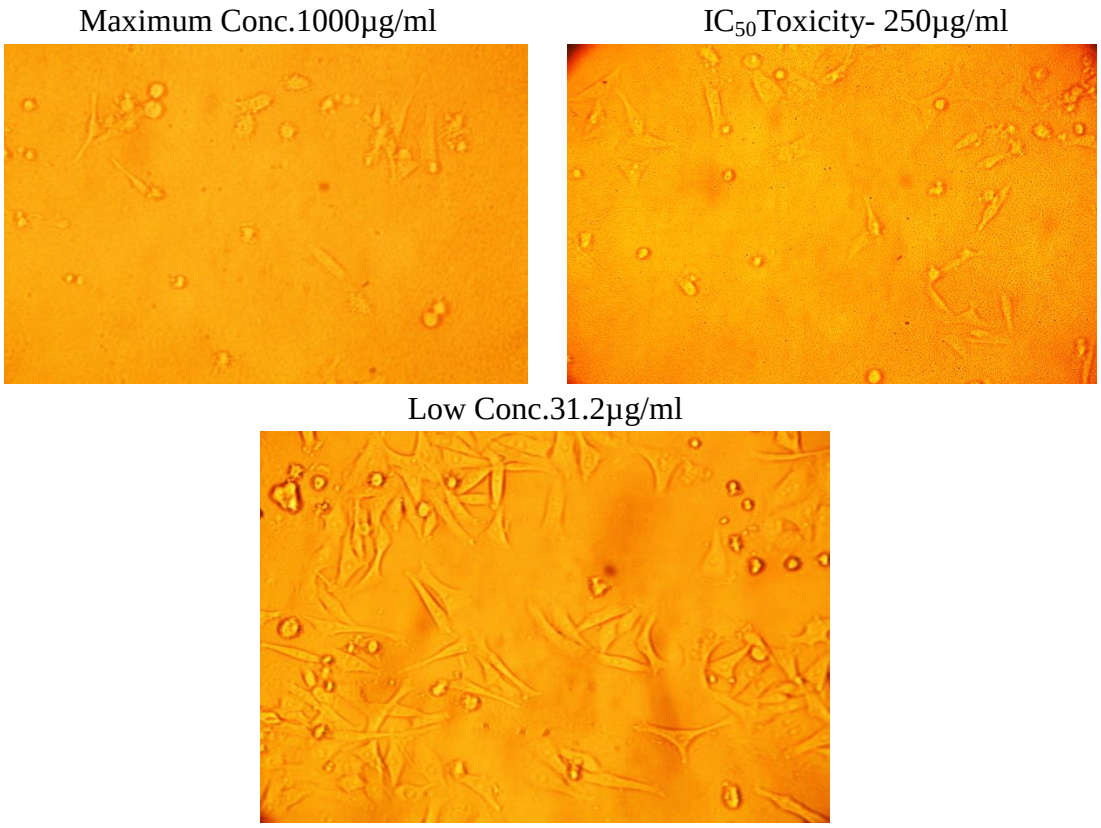


Figure 6: Anticancer effect of Ag-NPs of *Calotropis gigantea* extract on *MCF-7* Cell line

Previous studies using silver nanoparticles made by *Potentilla fulgens* against the glioblastoma and breast cancer cell lines MCF-7 and U-87 shown anticancer efficacy at concentrations of 4.91 and 8.23 mg/mL, respectively [28]. According to agents that reduce and cap silver nitrate into silver, green synthesized silver nanoparticles typically exhibit considerable anticancer action with no or no toxicity. While silver nitrate has the most potent anti-proliferative effect on cancer cells, its use is limited because of its significant toxicity towards normal cells. In this study, green-AgNPs were synthesised and demonstrated good anticancer activity against a breast cancer cell line [24, 25]. According to studies, the pericarp extract exhibited the ability to inhibit the proliferation of MCF-7 cells by triggering apoptotic cell death. By examining morphological alterations and oligonucleosomal DNA fragments, it also caused apoptosis in human breast cancer (SKBR3) cells [28-30]. Several investigations demonstrated the cytotoxic effects of mangosteen peel extract (MPE) on MCF-7 cells, which were tested using the MTT method and then analysed using the Probit method to determine the IC_{50}.

CONCLUSION

Calotropis gigantea flower extract has created silver nanoparticles by a biosynthetic method that is affordable, effective, and environmentally benign. The reduction of silver nitrate

to silver nanoparticles has been verified using the UV-Vis spectrophotometer, FT-IR, and SEM techniques. The antimicrobial sensitivity assay's well-formed zones of inhibition show that AgNPs have effective antibacterial action against the bacterial strains of *E. coli, Salmonella typhi* and anticancer (MCF-7). Silver nanoparticles created biologically have been shown to be effective antibacterial agents.

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