Effect of biofilm formation and cytotoxicity of biogenic silver nanoparticles (AgNPs) synthesized from a medicinal plant *Sterculia foetida* L.

N. Vasanth[a,c], T. Dons[b], L. J. Michaelraj[a], P. Ganesan[c,*], S. H. Salmen[d], S. A. Alharbi[d], S. Mutheeswaran[c], M. Anthonysamy[a], S. Ignacimuthu[c,] A. Stalin[e]

[a]*Department of Botany, St. Xavier's College, (Affiliated to Manonmaniam Sundaranar University), Palayamkottai, Tirunelveli, Tamilnadu, India*

[b]*Department of Botany, Sri Saradha College for Women, Perambalur, -621113,Tamil Nadu, India*

[c]*Interdisciplinary Research Centre in Biology, Xavier Research Foundation, St. Xavier's College (ManonmaniamSundaranar University), Palayamkottai, Tirunelveli, Tamilnadu, India and The Anna and Donald Waite Chair, Creighton University, Omaha, USA*

[d]*Department of Botany and Microbiology, College of Science, King Saud University, PO Box -2455, Riyadh -11451, Saudi Arabia*

[e]*State Key Laboratory of Subtropical Silviculture, Department of Traditional Chinese Medicine, Zhejiang A&F University, Hangzhou, China*

The biogenic synthesis of silver nanoparticles mediated by medicinal plant *Sterculia foetida* was the prime experimental analysis of present study. The most important aim and focus of this study was to synthesis the AgNPs via biological method and the same had been evaluated against bio-film formation and dose dependent cyto-toxicity against cancer cells. This *in vitro* comprehensive analysis show that Ag had more advantage than other metals, the NPs was mediated by plant residue. And the NPs were further characterized by UV showing the sharp absorption peak at 455 nm; the identification of corresponding functional section proved by the parameter like FTIR, where conversion of Ag ions and capping agent is determined, the analysis on X- ray diffraction demonstrate the AgNPs found to be crystalline nature and face-centered like cubic structure. FESEM cum EDAX has showed the surface morphology with 40-50nm. After the physiochemical characterization, the AgNPs were evaluated with biofilm formation and cancer cells. In cyto-toxic study, two cell lines such as MCF 7 lung cancer cell line and A549 Breast cancer cell line were experimented and the values are AgNPs (IC50 =11.50± 0.05 µg and IC50 = 5.5± 0.05 µg/mL); $AgNO_3$- (IC50 = 5.8± 0.05 µg and IC50 = 6.5± 0.05µg/mL) and *Sterculia foetida* (IC50 > 5000 µg/mL and IC50 < 5000 µg/mL). Another application of this present study is anti-biofilm assay. The selected bacterial strains are methicillin-resistant *Staphylococcus aureus;* PA 14-*Pseudomonas aeruginosa and Vibrio cholerae.* Hence, the findings recommend that silver nanoparticles from medicinal plant *Sterculia foetida is* effective and can be used against bacteria and more precisely for cancer cell study.

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Key words: Biofilm, Crystalline, Cancer cells, Cyto-toxicity, Nanoparticles, Diffraction

1. Introduction

The Nanotechnology sector has gained its attention in *de novo* due to their broad utilization and being forefront in the fields like catalysis, sensing, electronics, photonics and medicine [1, 2]. However the bio-synthesis of metallic nanoparticles have attained much attention in recent times, in particular plant mediated methods to obtain MNPs have much advantages than synthetic methods. Since the former is very conducive in nature, it is highly utilized method for preparation of NPs [3]. The major perspective in bio-synthesis method is fabrication of sustainable

* Corresponding author: sundarganeshps@gmail.com
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materials; it is also a major tool in the field of medicine, in particular cancer biology [4]. Now as far as nanobiotechnology is concerned this particular science extensively studied and exploited for tumor treatment via drug delivery system [4]. New drug finding is one of the challenging elements for cancer treatment. Though number of methods is there to treat the cancer, but chemotherapy is widely used one. As a result, the chemo method causes side effects including bone marrow suppression [5, 6]. Moreover the increasing the number of cancer metastasis and multi drug resistant aspects seems to challenging task [7]. Hence utilization of green method to synthesis NPs and advances in NPs research for innovative therapeutics has made this science to foremost level [8]. The novel modalities with therapeutic purpose loaded with nanomaterials have the scope in cancer diagnosis. The so-called nondrug materials are biocompatiable, non-toxic and biodegradable [9, 10]. Scientifically AgNPs seems to effective tool against microbial community in the open environment. The synergistic antimicrobial and biofilm inhibition is found with AgNPs[11]. Since Silver is attaining its momentum [12], it is quite useful to combine the plant material with metallic compound. The plant material *Sterculia foetida*, which is also medicinal, is a large, straight, deciduous tree growing upto40 m. Therefore AgNPs is a promising tool as it develops the penetration of drugs to cancer cells [13]. Hence this experimental work reports the synthesis, characterization and comprehensive biological evaluation of ANPs that are functionalized the *S. foetida* a medicinal plant, used for diverse purposes by human community.

2. Materials and methods

2.1. Plant identification followed by Leaf Extract

The medicinal plant material was ***Sterculia foetida*** were collected from the district Tiruchirappalli, Tamilnadu, followed by identity of the taxonomic position of the plant material was done at BSI, India. One gram of plant powder taken and the same was boiled in 100 ml of DW for about five minutes and allowed to settle at normal temp. The filtrate process was done using No.1 filter paper (0.45 μm).

2.2. Synthesis process of AgNPs from *S. foetida*

The waterlogged solution of 1mM $AgNO_3$ stock was made ready. From the stock solution, proper aliquots were taken and same was allowed for reaction process with plant residue for bio-synthesis of AgNPs. Still to make it be more prolific incubation period is set about 1 hour to 24 hrs for the formation of AgNPs. Finally, plant residue operates as capping agent for the developing AgNPs**.**

2.3. Analytical characterization of AgNPs (14,15)

The overall characterization of synthesized AgNPs was documented to envisage shape and size of the NPs. The reduction of Ag is done using double beam UV-vis spectrophotometer at 200-700 nm. The functional group identification and capping agent in formation of NPs were identified through FTIR. Further to find the crystal nature of NPs, the XRD equipped with Cu-Kα radition; and using monochromatic wavelength in a 2θ range from 20º-80º (XRD-Model-D8 advance, BRUKER Germany). FESEM was utilized along EDAX in order to find the size and shape via elements present in the synthesized solution.

2.4. Anti-Biofilm experiment

2.4.1 Effect of AgNPs on Bacterial biofilms

The 24-well microtiter plate was utilized to foresee the possibility in inhibition of biofilm formation by AgNPs.A 1% of selected bacterial strains such as *Staphylococcus aureus; Pseudomonas aeruginosa; Vibrio cholera* (107CFU ml^{-1}) was used as an inoculums for LB medium devoid of and with AgNPs(100 mg ml^{-1}). Subsequently, the wells were cleaned with DW. Further all well were stained at 500μl of 0.5% crystal violet solution (w/v). Then the process of destining was done with 1ml of ethanol solvent for 15 min and measurement made at 560nm. The inhibition pattern of AgNPs in 100mg/ml-^{1} was experimented against biofilm formation.

2.4.2. Effect of AgNPs on cell lines

The cyto-toxic activities of were experimented with the cancer cell lines by means of MTT assay. The two cell lines, MCF-7 human breast cancer cells and A549 human lung cancer cells were procured from NCCS-national center for cell science, India. Further, the obtained cancer cells were supplemented at DMEM high glucose medium and the same was added with 15% fetel bovine serum and 20ml of antibiotics such as penicillin with 37^0C in a humidified atmosphere of 5% CO_2.

Cell viability assay: the biogenic AgNPs, AgNO3 and Plant residue were together suspended in DMSO. The solution was diluted with a media to get various concentrations. 100 µl of samples aliquot were added to the wells 5 X 10^3 oftwo cancer cell lines per well. The DMSO was utilized as control. The 20µl of MTT residue was added in each well and incubated at 37ºC. The data were collected at 3 replicates each and the same was used to calculate the mean. Acridine Orange (AO) and Ethidium Bromide (EB) staining process: The Apoptotic proceeding was done by Ao/EB staining. The cells were treated with IC50 for 12h. Further, at incubation, the cells were collected with cold PBS and number of cells for each were keep count for viable and Apoptotic or necrotic with staining methods.

Statistical analysis: The obtained results and data were measured as Mean±SD from the least three independent experimentations. The various groups were compared using two-way ANOVA.

3. Results

3.1. Characterization studies

3.1.1. UV–Visible absorption Analysis of AgNPs

The AgNPs show signs of yellowish to brown coloration due to the excitation process of surface Plasmon vibrations takes place in AgNPs. However the UV spectroscopy also show the single SPR band formation at 300 nm indicating the small sized particles , while the longer wavelength indicate the presence of anisotropic NPs. Hence in this present study UV spectrum show 455nm (Fig. 1), indicating the presence of various bioactive compounds like polyphenols which an ultimate source of reduction of Ag ions.

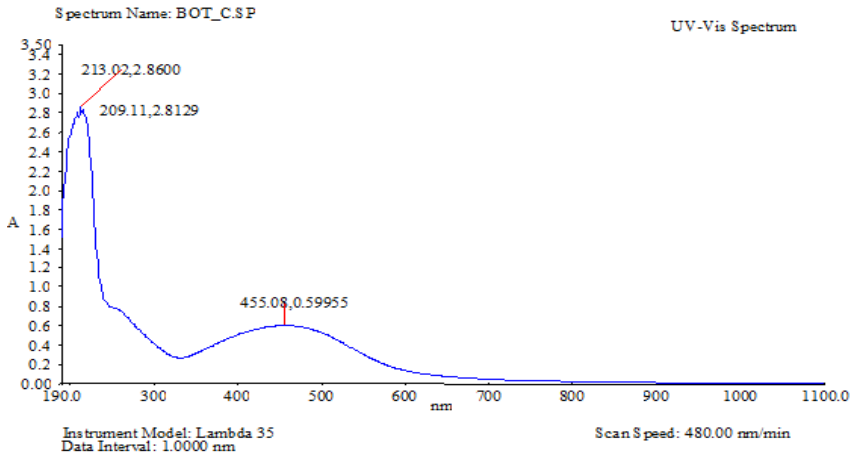


Fig. 1.UV Spectrum Peak value of AgNPs of Sterculia foetidaL.

3.1.2. Fourier Transform Infrared Spectroscopy (FTIR) studies of AgNPs

The dual process played by plant residue as a reducing and capping agents become notified by FTIR analysis showing various functional groups. The obtained absorbance bands of 2919.83 assigned to CH3,CH2 and CH stretch and the functional groups is Alkanes. And the 2396.15 cm-1peak show the Phosphine. The assimilation of peaks at 2518 and 1763 cm-1

corresponded to O-H bend and C-O stretch at primary alcohol. Hence FTIR results (Fig-2) indicates that the plant extract might have been engrossed in the construction of AgNPs as the reductant and capping agent.

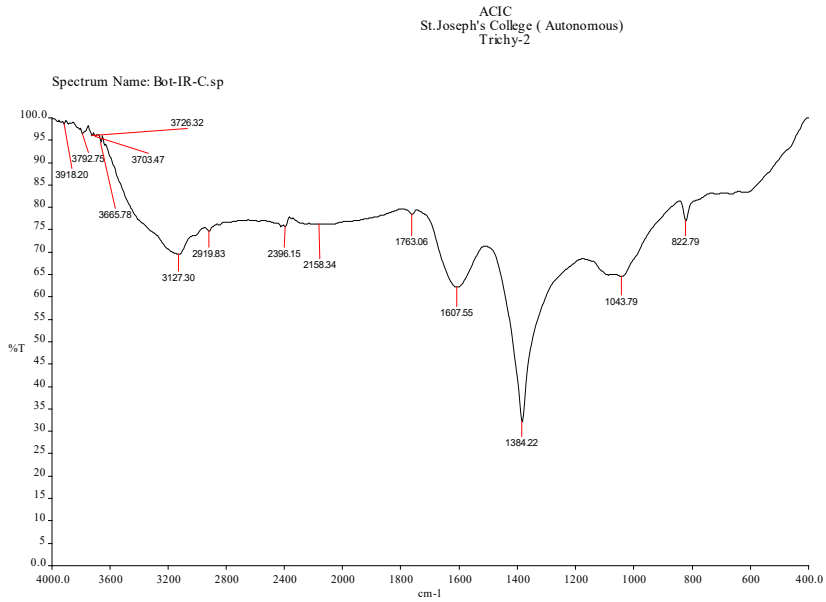


Fig. 2.FT-IR Peak value and its functional groups of AgNPs of Sterculia foetida L.

3.1.3. Field emission scanning electron microscope (FESEM) and EDAX analysis of AgNPs

The topographical analysis with FESEM with EDAX shows the mean particle size of the AgNPs. This particular image (Fig-3) shows the surface morphology with spherical nature. In this present study too indicate the particle mean size ranges from 40-50 nm. The obtained results confirm that plant leaf extracts are involved in large production of AgNPs. The EDAX spectrum (Fig-4, 5) indicates the signal at Ag region leading the formation of AgNPs. The Ag was the major constituent element comparable with other elements. The elements includes Ag ,O,C,Cl,Ca,Na,Si,Mg,Al . The conversion pattern of AgNO3 to AgNPs was confirmed by the dence peak at Ag. The graphical image indicates the Ag as major element validating the spectrum depicting of reduction Ag to AgNPs.

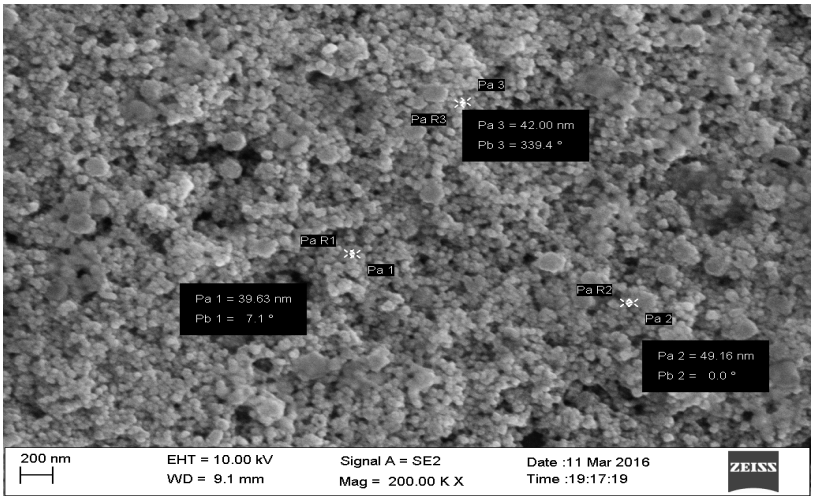


Fig. 3.FESEM image of AgNPs.

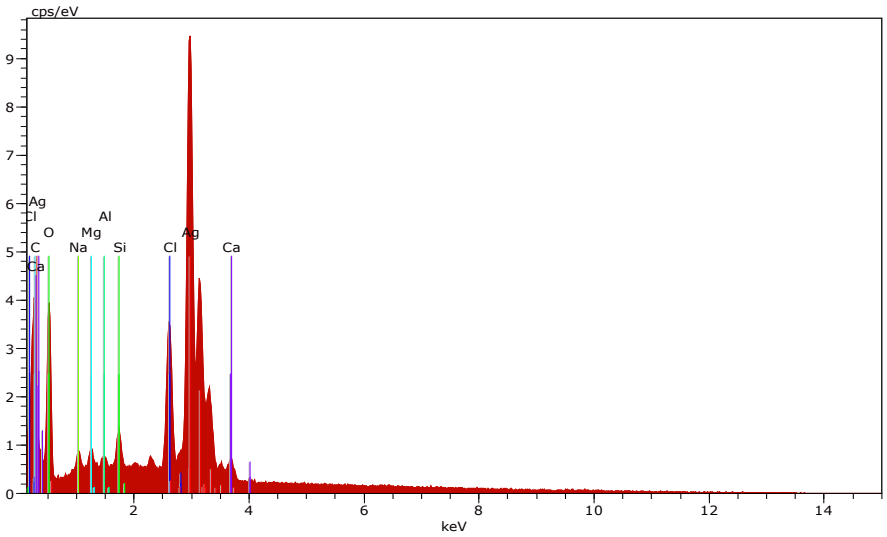


Fig. 4.Energy Dispersive X-ray Spectroscopy (EDAX) of AgNPs.

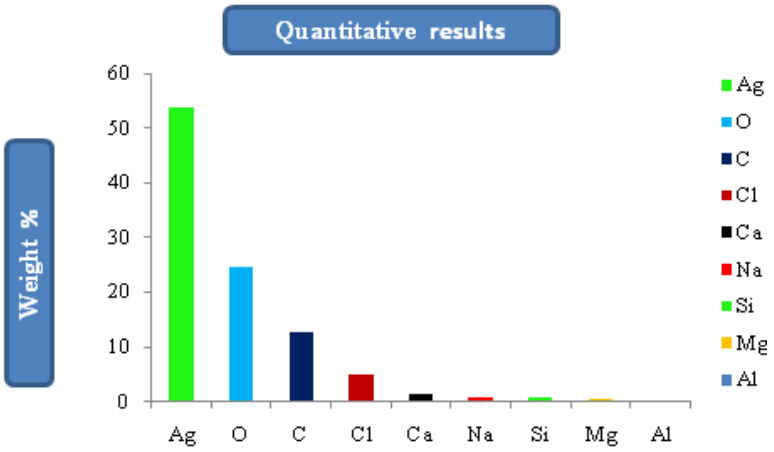


Fig. 5.Graphical representation of EDAX.

3.1.4. X-Ray Diffraction (XRD) investigation of AgNPs

The analysis of crystalline character of AgNPs was authticated by X-ray diffraction (XRD) pattern. The diverse diffraction, peaks at 32.24°,38.05°, 44.41°, 77.47°, corresponding to the planes (111),(200). Hence XRD pattern clearly show (Fig-6) that the AgNPs produced in this current study are crystalline in nature. It also confirms the existence of Ag colloids in the sample with Braggs reflection. These reflections clearly indicate the present of planes.

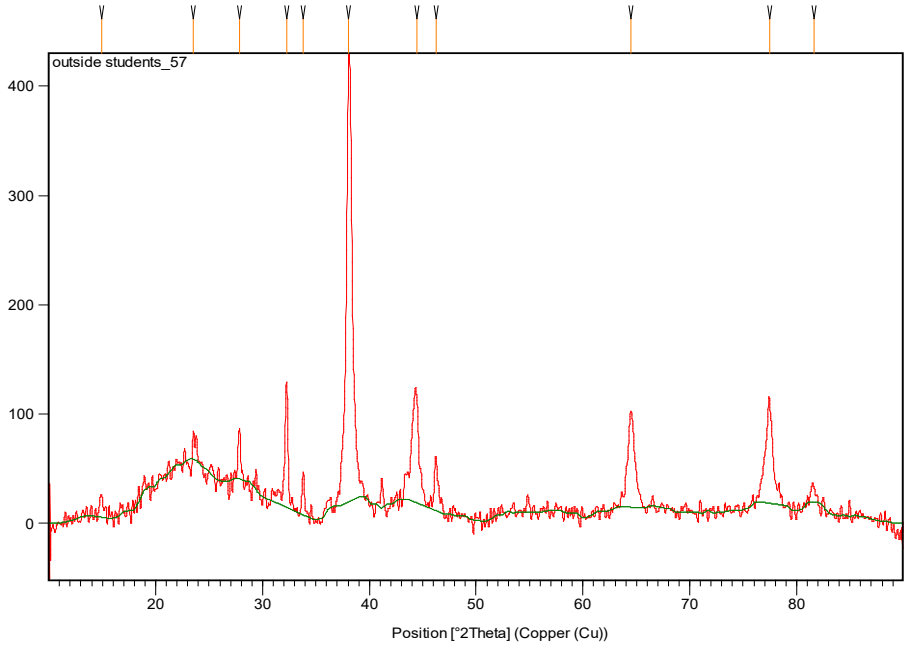
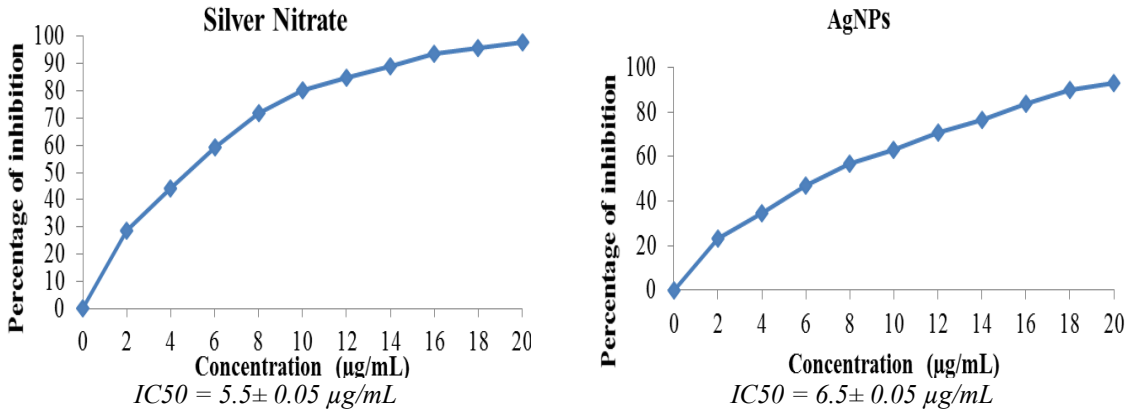


Fig. 6.X-ray Powder Diffraction (XRD).

3.2. Cytotoxic study

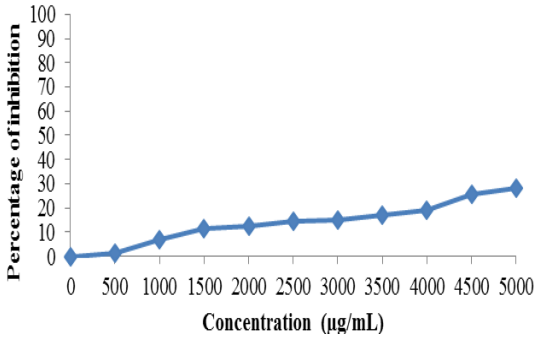
Cell viability experiments via Acridine orange (AO) and ethidium bromide (EB) stain. The effectiveness of AgNPs with cancer lines were analyzed by cell viability assay and (AO) and EB-Ethidium bromide stain assay. The different concentrations of AgNO3, AgNPs and plant extract were used. The significant inhibition was observed during the assay. In particular the AgNPs drastically repressed the development of cancer cell in a dose –dependent manner (Fig-7 &8). Several reports on AgNPs induced cell toxicity by invading the cytoplasm and mitochondria. It is also said to be chromosomal mutations and Genotoxic damages between 24 to 72 h after the treatment with AgNPs. Assumingly AgNPs induces the so-called oxidative stress that can eventually the ground reason for Genotoxicity by DNA adducts and DNA breaks. The biogenic AgNPs induces apoptosis reaction on MCF-7 cell line that is substantiated using AO/EB staining. Thus the present results indicate that there is lesser effect on cell viability and proliferation corresponding to plant extract and AgNO3. At a culmination, MCF 7 lung cancer cells and A549 breast cancer cell lines were experimented (Table:1&2), and show the values for AgNPs (IC50 =11.50± 0.05 µg and IC50 = 5.5± 0.05 µg/mL); Silver Nitrate- (IC50 = 5.8± 0.05 µg and IC50 = 6.5± 0.05 µg/mL) and *Sterculia foetida*(IC50 > 5000 µg/mL and IC50 < 5000 µg/mL). Hence the medicinal plant mediated biosynthesized AgNPs (*Sterculia foetida)*can be of potential therapeutic agent in the treatment of various cancer cell lines.



IC50 = 5.5± 0.05 µg/mL

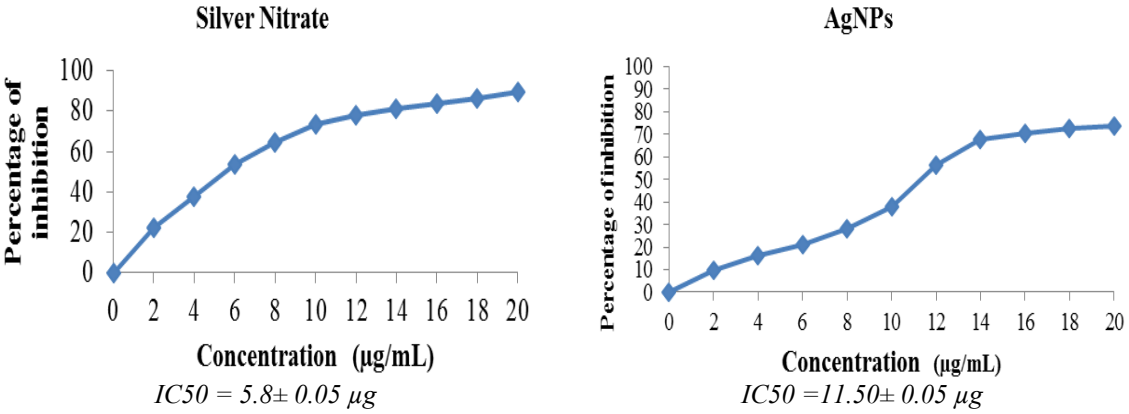
IC50 = 6.5± 0.05 µg/mL

***Sterculia foetida* L.**



IC50 < 5000 µg/mL

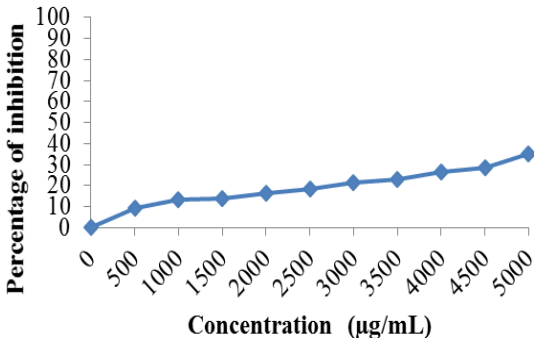
Fig. 7.Cyto-toxic assay of AgNPs against A549 cancer cell lines.



IC50 = 5.8± 0.05 µg

IC50 =11.50± 0.05 µg

***Sterculia foetida* L.**



IC50 > 5000 µg/mL

Fig. 8.Cyto-toxic assay of AgNPs against MCF 7 cancer cell lines.

Table 1. In vitro cytotoxicity assays for the complex against human lung cancer cell line (A549).

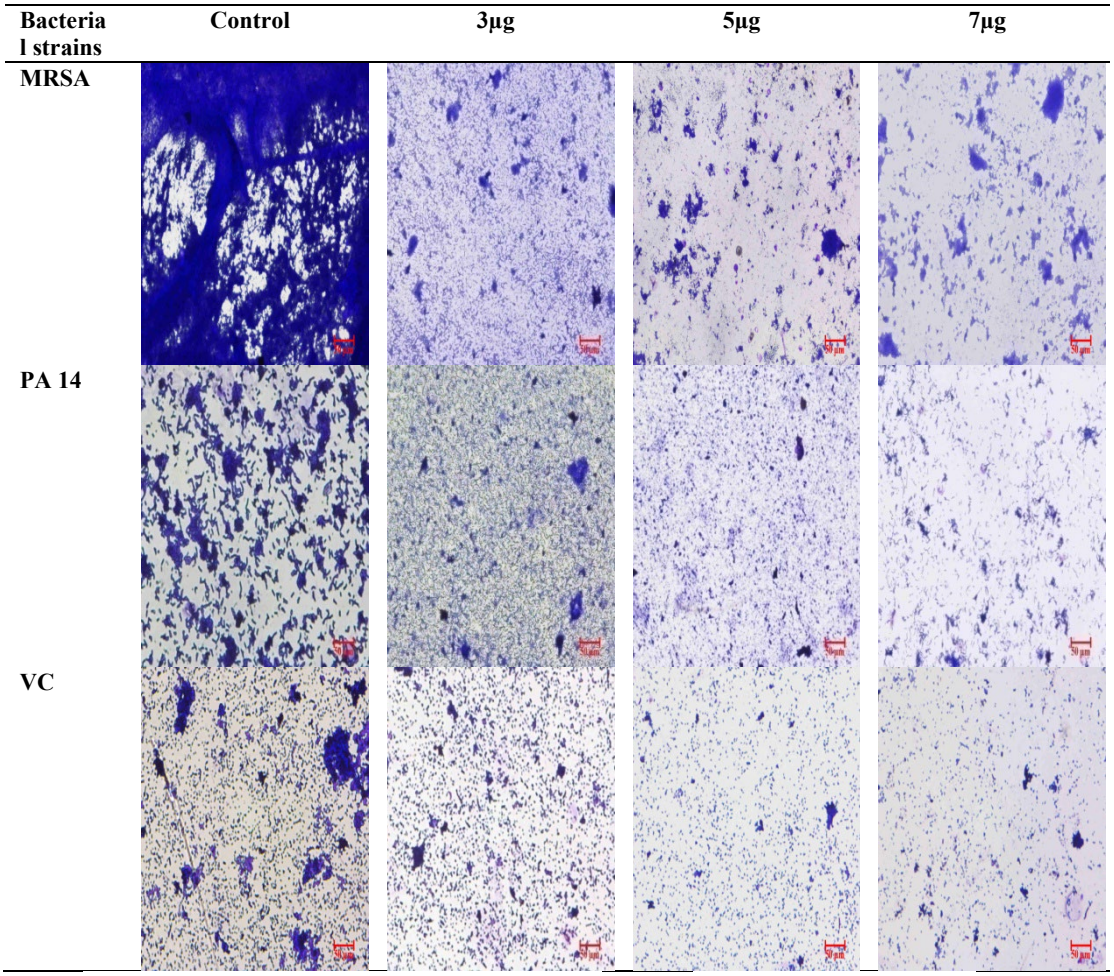
Compound	IC $_{50}$ Values (24 h)
Silver Nitrate	5.5± 0.05 µg/mL
AgNPs	6.5± 0.05 µg/mL
Sterculia foetida	> 5000 µg/mL

Table 2. In vitro cytotoxicity assays for the complex against human breast cancer cell line (MCF-7).

Compound	IC $_{50}$ Values (24 h)
Silver Nitrate	5.8± 0.05 µg/mL
AgNPs	11.50± 0.05 µg/mL
Sterculia foetida	> 5000 µg/mL

3.3. Anti-biofilm assay

The anti-biofilm assay was experimented by bio-synthesized AgNPs against biofilm forming bacteria. The multi-resistant bacterial strains are namely *Staphylococcus aureus;* PA 14-*Pseudomonas aeruginosa* and *Vibrio cholerae. The* inhibition of biofilm formation by AgNPs was assessed in a dose-dependent manner against the bacterial strains. The bacteria strains were grown-up in 19-well microtiter plates for 24 h.And 10-100µg/ml concentration of the AgNPs were supplemented at each well. The assay indicated that inhibition of biofilm construction by bacterial strains corresponds to negative control used in this experiment. Further the results show (Fig-9) AgNPs inhibits the bacterial strain at 100µg/ml. The small particles of AgNPs decrease the activity and glycocalyx matrix synthesis is arrested. However the penetration rate of biofilm also shows some difference based on the resistance. Hence this particular study would help the research world to get an idea on bacterial anti-biotic resistance and colonize abiotic with biofilms.



*** MRSA- Methicillin-resistant Staphylococcus aureus -*PA 14-*Pseudomonas aeruginosa***
VC-*Vibrio cholerae

Fig. 9.Anti-biofilm assay of AgNPs.

4. Discussion

The green method to synthesis AgNPs for anti-cancer and anti-biofilm applications is getting momentum in the recent past and also still a challenging and promising area of research interest today (18). The various bio-molecules present in the plant structuralize a stable capping layer and contributing to the stability which promotes their interaction with target cells (19). Consecutively, the AgNPs become evidence for noteworthy antimicrobial action against the bacteria like *E. coli* and *P. aeruginosa* at fewer concentrations. In contrast, the bacteria like *S. epidermidis* and *S. aureus* were also viewed to be less sensitive (20). However, the presence of thick peptidoglycan stratum consist of linear polysaccharide chains cross linked by short peptides, showing stiff structure that hold backs the penetration process of AgNPs into the bacterial membrane (21-23). Similarly, silver ions too produce ROS in cells. The elevated ROS level may have oxidative stress cum cell membrane damage, LDH release, and intracellular proteins, lipids, and DNA (24, 25). The higher the ROS level may lead to signaling cascades and results in neither apoptosis nor necrosis (26). Likewise, Ag ions discharged from AgNPs may augment their cytotoxic effect by influencing cascades that lead to intracellular toxicity denoted as the "lysosome-enhanced Trojan horse effect" (27). Furthermore, AgNPs can interrelate with the membrane proteins and develop active signaling passageways leading to the distraction of cell proliferation. Hence the full proceedings are needed to be standardised before it is fully utilised for human purpose. It has been put forwarded that AgNPs showing anti-biofilm activity and cancer cell inhibition are mediated by the development of free radicals and membrane damage

5. Conclusion and future perspectives

The conventional approach to synthesis AgNPs are expensive and have adverse effect of toxic substances; it is also alarming concern to monitor the amount of risk of contamination during the preparation of AgNPs via physical and chemical methods respectively. Hence generation biogenic NPs from plant extracts or green method seems to be important front in nanotechnology. And it is a bio-reduction of Ag nitrate solutions. The appropriate characterization using UV-Vis, FTIR, FESEM, EDAX, XRD have indicated the size, shape and prominently revealed the efficient capping and stabilizing properties of AgNPs. The size and shape determination have gained resultant cytotoxicity of plant mediated NPs. The cancer cell inhibition and anti-biofilm assay are promising bio-experiments with an acceptable therapeutic index.

Further, the plant residue is readily available to develop the eco-friendly route for large scale and synthesis well dispersed metallic NPs. In near future biochemical and enzymatic reaction in NPs for material synthesis need to be fused which consecutively will pay a way for identification and characterization of biomolecules associated with NPs. Hence we had experiment that are simple environmentally benign method of synthesis of AgNPs and found medicinal plants to be the primitive source. Production of AgNPs promisingly would bigger role in Agricultural and pharmaceutical industry and so on.

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