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ORIGINAL ARTICLE

Cytotoxic properties (MDA-MB-231- an epithelial breast cancer cell line) and bactericidal activity of Silver nanoparticles mediated by *Strobilanthes ciliata nees*

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Abstract

The sciences that intrigue about nanotechnology are increased ever before and improved permeability in its applications at various arenas. Precisely, the Silver nanoparticles (AgNPs) implication is deep-time perspective to understand that fascinating nanomaterials are involved in different biomedical applications. Since NPs application derivates are physical and chemical based but biological methods is still holds a profound impact. Hence, the medicinal plant *Strobilanthes ciliata* was utilized to synthesis AgNPs and the same was subjected to UV irradiation. And subsequent application of the crude biosynthesis of AgNPs was experimented with various physico-chemical parameters. Biosynthesized particle characterization was carried out by color pattern and UV-visible spectroscopy and followed by FTIR, SEM and finally experimenting with cancer cells as cyto-toxic study. The characterization result of particle size of synthesized AgNPs is 77 nm. The highest peak obtained in FTIR is at 3439.48cm−1 (OH) and the UV range is 477 nm. As far as the cancer cells are concerned, the cell survival rate at 1 mcg is 62% and at 1.5 mcg is 30% which is optimal. The lattice planes of XRD are face-centered-cubic (FCC) structure. The Particle size of S.*ciliata* mediated AgNPs is 77.7 nm. Biological methods attain much concern and forethought because bio-resources are used as precursors in synthesis of NPs. Therefore, herein we exclusively have done the plant-mediated metallic NPs synthesis, characterization and applications in detailed summary. The present research observation will hold enrich understanding about AgNPs interaction and the mode of synthesis with various expertise and subject.

Keywords: *Cancer Cells; Characterization; Green Chemistry; Nanomaterials; Strobilanthes Ciliata.*

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INTRODUCTION

The dread disease known as breast cancer is foremost causes of demise in among women around the world in recent times [1]. This particular disease is said to be the 5th most common cancer related with death [2]. It is estimated that each day people lose their lives due to cancer disease that includes 81% of lung cancer mortality were recorded in the year 2022 [3]. Since materials with nanometer dimensions and their derived applications are in tune to treat health connected issues, it has expanded much awareness in scientific sector, precisely towards the inhibition of cancer metastasis and related process is gaining momentum [4]. In general, Apoptosis is a usual cell death process in natural system. It depends upon certain death receptors (DR) involving Bcl-2,

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CASP8, CAPS10, Bik with signalling bio-molecules [5]. But the so-called phyto-constituents from medicinal plants that posse's flavonoid, polyphenols and few secondary metabolites hold the anti-neoplastic properties. Due to ineffective and intake process the utilization of the same become complicatedness. The above elements can be overcome by the green nanotechnology [6]. In recent times, plant residue mediates the synthesis of NPs due improved stability cum biocompatibility. The eco environmental approach have emerged and gained importance due to lesser toxicity when compared to chemical hazards to synthesis NPs [7]. Numerous studies and experimentation process vouch for this synthesis cum characterization of AgNPs and have been testimonial for various findings [8-10] but only few reports are found on cyto-toxic efficacy of NPS. Hence this research experiment includes a detailed account on biosynthesis of AgNPs from *S. ciliata* extracts. This approach is uncomplicated, non-toxic, eco-friendly [8] hence sustainable; consequently the intent of this research experiments is to have a better comprehend and encapsulate the means and methods of NPs invading the proliferation of malignant cells. Therefore, this particular research experiments will analysis the AgNPs cytotoxic effects on breast neoplasia and bring about certain mechanism towards the elucidation of the same.

MATERIALS AND METHODS

Plant specimen collection

The medicinal plant material was procured from MS Swaminathan Research Foundation, Wayanad, and Kerala. The collected plant materials were thoroughly washed and shade dried. The obtained plant is then weighed, powdered and stored.

Chemicals and Glasswares

Silver nitrate is most important chemical required for synthesis of nano particles. Glasswares like petri dish, beakers, conical flask, funnel, Whatman filter paper, pipette and etc. Nutritive agar and nutritive broth were used for antibacterial assay.

Preparation of Nanoparticles

For the preparation of *S. ciliata* AgNPs 95ml of 1N of $AgNO_3$ and 5 ml of plant phytochemcial residue is mixed and kept in sunlight for about 2 hours in the morning at 30°C. After that 100 ml the sample is taken for consecutive characterization (Particle size, UV, FTIR, XRD and SEM) prior to centrifugation at 5000 rpm.

Characterisation of AgNPs

UV

The quantitative technique such as UV- visible spectrum is a systematic method that determines the amount of discrete wavelengths [11] or visible light which are wrapped up by or transmitted through a test sample was done via (Shimadzu UV-1800). **FTIR:** Fourier transform infrared spectroscopy (FTIR) [12] is perhaps to find chemical bonds (functional groups) present in compounds. The instrument is equipped with JASCO IRT-7000 and mode of operating at a resolution of 4cm-1. **SEM:** Scanning electron microscopy (SEM) is a modern method to acquire higher resolution images mainly on surface fractions. A powerful investigative tool for morphological image is (ZEISS EVO-MA 10). **XRD:** X-ray diffraction is an effective tool to study the crystalline of the nanostructures [13]. It can be estimated using the Debye-Scherrer equation. The formula is constant (k) =0.94 and wavelength=1.5406. It provides data on configuration, phases, preferred crystal orientations (texture), and different structural parameters, together with common grain size, crystallinity. **Particle size:** Particle size analysis is one of the important tests, which proves whether the prepared sample is nano particle or not. An object with spherical size can be quantitatively and unambiguously defined through its diameter. Sample is used for analyzing the particle size of prepared *S.ciliata* AgNPs.

Anticancer activity

Breast malignancy cell lines (MDA-MB-231) was obtained NCCS, Pune, India. The Dulbecco modified eagle medium (DMEM) was used for culturing along with Glucose medium (Sigma-Aldrich, U.S.A.) along with Fetal Bovine Serum (20%) and together with antibiotics such as Penicillin or Streptomycin incubated in 96 well plate at 37°C with a well humidified environment of 3% CO_2(Thermo scientific, USA). Each section of the experiments was performed by utilizing the cells from passage 10 or less.

Cell viability Assay

S.ciliata mediated silver nanoparticles were suspended in Dimethyl sulfoxide (DMSO) to formulate initial volume. 200 µL of these samples

have been added to wells holding 3×10^3 MDA-MB-231 cancerous cells per well. The DMSO was considered as controlling agent. 20 µL of MTT solution containing (5 mg/mL in PBS) was poured and same was kept for incubation for 4 h at 37 °C. The formazan artificial dye was dissolved through addition of 100 µL of DMSO in each well. Absorbance was recored at 570 nm (dimension) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, iMark, USA). Information has been gathered to calculate for particular mean, the % of inhibition was estimated from this information with the following formula:

$$\frac{\text{Mean of absorbance of untreated cells (Control) - Mean of absorbance of treated cells}}{\text{Mean of absorbance of untreated cells (Control)}} \text{ X } 100$$

The Acridine Orange (AO) and Ethidium Bromide (EB) staining procedure

In order to analysis the apoptotic morphology, the AO/EB staining method was experimented. The procured cells were kept at the concentration IC50 for about 24h. The cell harvesting was done followed by washing of the same with cold PBS. Then, it is further diluted and immersed with PBS at a concentration of 5×10^5 cell/mL and mixed with 25 µL AO/EB solution (3.8 µL of AO and 2.5 µL of EB in PBS) on glass microscopic slide and analyzed under fluorescent microscope. 300 cells each for single experiment were taken and sample was measured for viable, apoptotic or necrotic by staining. The membrane integrity and followed by apoptotic and necrotic cells calculated.

Anti-bacterial activity

The synthesized *S.ciliata* AgNPs were used to analyze the antibacterial activity against the bacterial pathogens by disc diffusion method. Bacterial cultures such as (MTCC 443) *Escherichia coli* and (MTTC 121) *Bacillus subtilis* were obtained. The fresh inoculum was taken in the experiment along with nutrient broth. The incubation maintained at 37 °C for 24 hours and used after standardization.

Disc diffusion method

The disc diffusion method was experimented to find out inhibition zone. The fresh and overnight grown cultures of inoculum were spread on to agar plates. The sterile paper discs made with 5mm diameter contain 1N, 2N, 3N, $AgNO_3$, Dil.H_2O, and antibiotic. After 24 hours the zone of inhibition was measured.

RESULTS

Visual observation

The first observation is the colour change obtained when the prepared *S.ciliata* AgNPs is kept in sunlight. It turns from transparent to brown colour after few hours of sunlight exposure.

UV

were characterized by UV–Vis spectroscopy, a prominence technique for the structural categorization NPs

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S.ciliata AgNPs were determined by UV-Vis spectroscopic analysis, a analytical technique for the absorption categorization of NPs. The obtained wavelength holding range of 200–700 nm is normally used to characterize the metal based nanoparticles. In this present study, the peak of UV is 477 nm observed (Fig. 1). Moreover, in certain points AgNPs of *Coccinia grandis* and *Phyllanthus emblica* had showed similar surface Plasmon resonance at 442 nm and at 443 nm too (Table 1).

FTIR

The FTIR spectrum of the *S. Ciliate* mediated AgNPs show in the range of 4000–400 cm^{-1}. However, certain peaks are blunt are with various intensities to show absorption of each functional group. The peak at 3439.48 cm^{-1} is assigned to –OH group. Alkynes group could be determined by peak 2145.77 cm^{-1}. Since most of the peaks are downwards the peak 2093.24 cm^{-1} are aromatic group. Alkenes group are found in peak 1635.82 cm^{-1}. NO_2 group are found in peak 1303.57 cm^{-1}. The range from 1250 cm^{-1} downwards is the specific and important range for different characteristics of each compound e.g. 1250.13

cm^{-1} refers to C-O-C groups, 1164.65 cm^{-1} refers to C-F and 700.00 cm^{-1} refers to C-Br. The highest peak is obtained at 3439.48 cm^{-1} and 700.00 cm^{-1} (Fig. 2) (Table 2). Thus various functional groups present in the plant had become capping agents to covert silver ions into AgNPs.

SEM

The *S.ciliata* mediated AgNPs exhibits a solid morphology and topography results in SEM. Thus, the agglomerated *S.ciliata* AgNPs show a distinct spherical shape. The aggregation and dispersal of AgNPs is found often. *S.ciliata* AgNPs showed 92.06 µm, 105 µm and 79.76 µm respectively. The SEM images of *S.ciliata* AgNPs exemplify that most of the particles are moderately dispersed and barely agglomerated in nature. Consequently, AgNPs that are agglomerated show spherical shape (Figs. 3a, 3b).

XRD

The biosynthesized AgNPs using *S.ciliata* plant extract showed a confirmed attribute peaks examined in the XRD images. *S.ciliata* AgNPs includes Braggs reflection clearly showing the presence of (0010), (400), (103), (103), and (004)

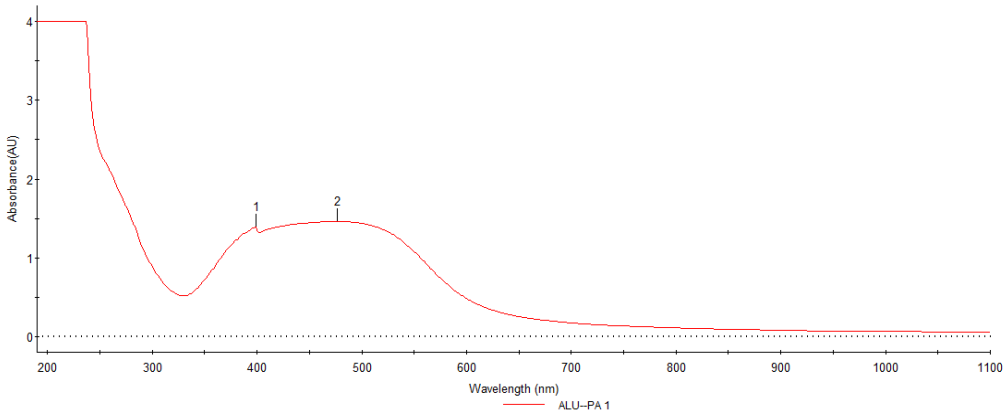


Fig. 1. UV spectroscopic analysis of silver nanoparticles mediated by *S. ciliate*.

Table 1. UV-VIS spectrum of AgNPs.

NO	Peak (nm)	Peak (Au)
1.	398.75	1.395
2.	477.00	1.461

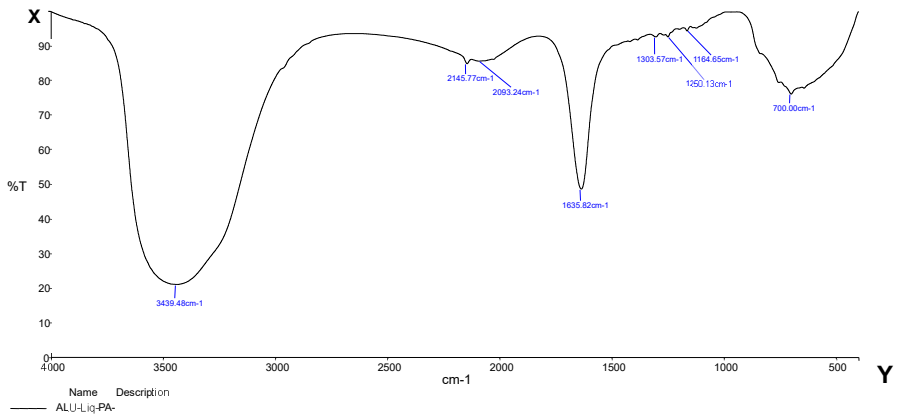
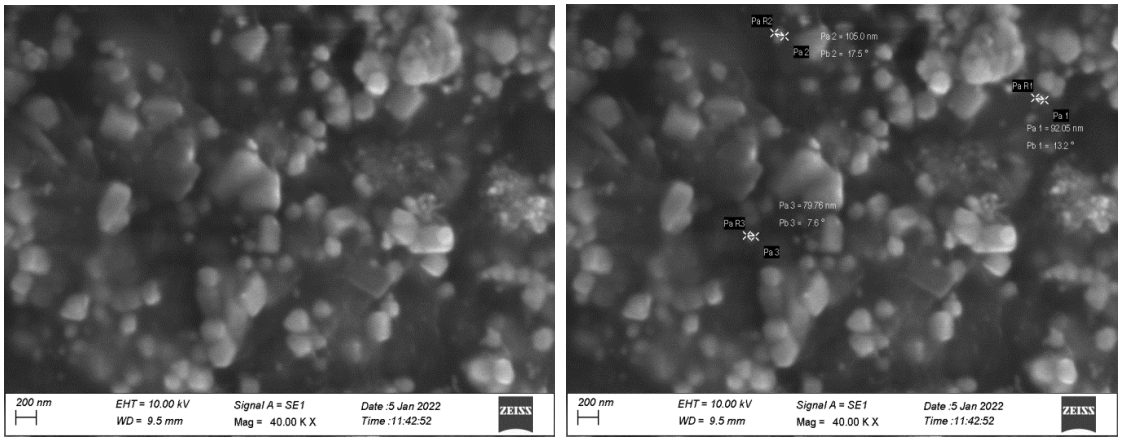


Fig 2. FT-IR peak value AgNPs mediated by *S. ciliate*.

Table 2. FTIR analysis of AgNPs.

VALUES	FUNCTIONAL GROUP	SYMBOLS
3439.48cm^{-1}	Alcohol	OH
2145.77cm^{-1}	Alkenes	c=c
2093.24cm^{-1}	Aromatics	
1635.82cm^{-1}	Alkenes	c=c
1303.57cm^{-1}	Nitrogen dioxide	NO2
1250.13cm^{-1}	Carbon monoxide	Co-C
1164.65cm^{-1}	Carbon floride	C – F



a b

Fig. 3(a). SEM image indicating shape of AgNPs, and b) SEM image indicating the size of AgNPs.

set of lattice planes and they can be indexed as hexagonal structure of AgNPs. The XRD pattern therefore in reality suit U.S.A. that AgNPs are crystalline in nature. The middling grain size of NPs outlined is established using Scherr's formula $d = (0.9\lambda/\beta s \cos(\theta\pi))$. The XRD pattern is thus display a crystalline in nature (Fig. 4).

Particle size

The machinery of configuration NPs was determined on the basis of uniformity of particle size as well as magnitude level of defined dimensions. Hence, the wide range of particle distribution and condensed particles are corresponding to uniform in size. Therefore, the particle size of *S.ciliata* AgNPs is 77.7 nm (Fig. 5).

Cyto-toxic study

As the result of feeding the sample to breast cancer cell clearly shows that the drug concentration at 1 and 1.5 mcg concentration is active and optimal compared to all other concentration of drugs. And on further study the result shows that it also causes nuclear damage to the cells. The cell survival rate at 1 mcg is 62.043458 and at 1.5 mcg is 30.929265 which is optimal. The result summarize that *S.ciliata* AgNPs are cytotoxic. In some of these studies, plant crude extracts were used against cancer cells' proliferation and cell viability. It can be explained that the mechanism of AgNPs inhibition in cancer cells [14] the compounds such as phenolic compounds play a vital role in it [15-16]. Diverse prospect exist for nanobiology application and this results assures that the alternative for chemotherapy [17]. NPs exhibits the advantage of more effective and targeted usage in a eco-friendly greener manner [18] have great possibility to use in biomedical applications too [19-21] (Figs. 6a, 6b).

Anti-bacterial assay

This study has been conducted to evaluate the antimicrobial activity *S. Ciliate* mediated AgNPs against human pathogens including two reference strain such as *Bacillus subtilis* and *Escherichia*

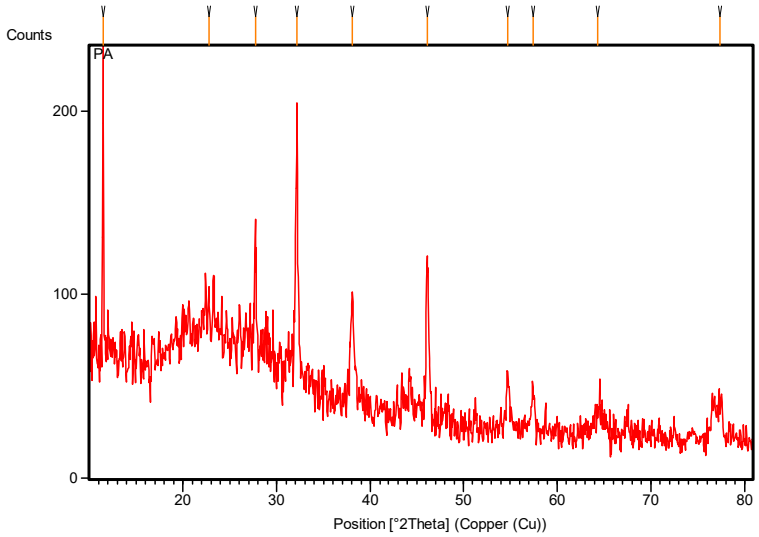


Fig. 4. X-ray diffraction (XRD) analysis of AgNPs.

NanoPlus
Common

Intensity Distribution S/N : 411615

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Date	: 05-Jan-22	File Name	: PA		
Time	: 10:36:57	Sample Information	:		
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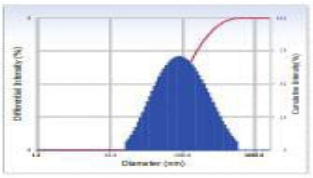
Version 5.22 / 3.00

Intensity Distribution



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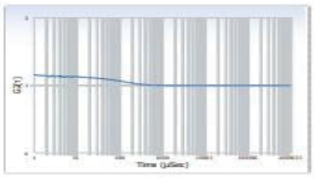


Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	126.3	100.8
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	126.3	100.8

Residual : 3.088e-003 (O.K)

Cumulants Results

Diameter	(d)	: 77.7	(nm)
Polydispersity Index	(P.I.)	: 0.288	
Diffusion Const.	(D)	: 6.334e-008	(cm²/sec)

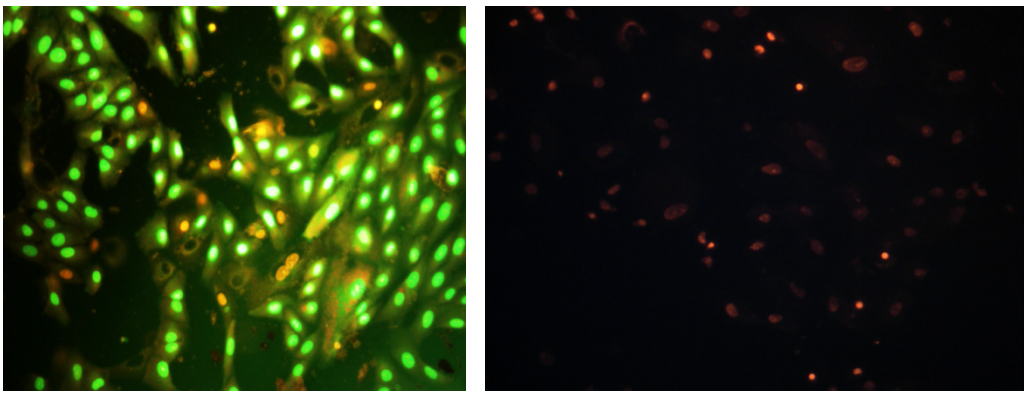
Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 28904	(cps)
Attenuator 1	: 30.38	(%)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
1.0	0.0	0.0	6.4	0.0	0.0	41.5	2.4	16.5	267.5	1.8	91.0
1.1	0.0	0.0	6.9	0.0	0.0	44.7	2.6	19.1	288.2	1.6	92.6
1.2	0.0	0.0	7.5	0.0	0.0	48.2	2.8	21.9	310.5	1.4	94.0
1.3	0.0	0.0	8.1	0.0	0.0	51.9	2.9	24.8	334.5	1.3	95.3
1.3	0.0	0.0	8.7	0.0	0.0	55.9	3.1	27.9	360.4	1.1	96.4
1.5	0.0	0.0	9.4	0.0	0.0	60.3	3.2	31.1	388.3	0.9	97.3
1.6	0.0	0.0	10.1	0.0	0.0	64.9	3.3	34.4	418.4	0.8	98.1
1.7	0.0	0.0	10.9	0.0	0.0	70.0	3.4	37.8	450.7	0.6	98.7
1.8	0.0	0.0	11.7	0.0	0.0	75.4	3.5	41.2	485.6	0.5	99.3
2.0	0.0	0.0	12.6	0.0	0.0	81.2	3.5	44.8	523.2	0.4	99.7
2.1	0.0	0.0	13.6	0.0	0.0	87.5	3.5	48.3	563.7	0.3	100.0
2.3	0.0	0.0	14.6	0.0	0.0	94.2	3.5	51.8	607.3	0.0	100.0
2.4	0.0	0.0	15.8	0.0	0.0	101.5	3.5	55.3	654.2	0.0	100.0
2.6	0.0	0.0	17.0	0.3	0.3	109.4	3.5	58.8	704.9	0.0	100.0
2.8	0.0	0.0	18.3	0.4	0.7	117.9	3.4	62.2	759.4	0.0	100.0
3.1	0.0	0.0	19.7	0.5	1.3	127.0	3.3	65.5	818.2	0.0	100.0
3.3	0.0	0.0	21.2	0.7	2.0	136.8	3.2	68.7	881.5	0.0	100.0
3.5	0.0	0.0	22.9	0.9	2.8	147.4	3.1	71.8	949.7	0.0	100.0
3.8	0.0	0.0	24.6	1.0	3.9	158.8	3.0	74.8	1023.1	0.0	100.0
4.1	0.0	0.0	26.5	1.2	5.1	171.1	2.8	77.6	1102.3	0.0	100.0
4.4	0.0	0.0	28.6	1.4	6.5	184.3	2.7	80.3	1187.6	0.0	100.0
4.8	0.0	0.0	30.8	1.6	8.1	198.6	2.5	82.8	1279.5	0.0	100.0
5.2	0.0	0.0	33.2	1.8	9.9	213.9	2.3	85.1	1378.4	0.0	100.0
5.6	0.0	0.0	35.8	2.0	11.9	230.5	2.1	87.3	1485.1	0.0	100.0

Fig. 5. DLS Particle size graph indicating the diameter of AgNPs.



a b

Fig. 6 (a). Apoptotic cells by fluorescent microscopy, and b) Apoptotic cell with stained with acridine orange.

Table 3. Results of antibacterial activity by AgNPs.

S. NO	Plant part Extract/ Antibiotic/Contol	Zone of Inhibition (mm) + SD	
		1.*Bacillus subtilis*	2.*Escherichia coli*
1.	Positive control (Cefotaxime)	15.3 ± 3.42	16.2 ± 5.33
2.	Negative contol ($AgNO_3$)	6 ± 2.21	7 ± 2.20
3.	AgNPs	10.3 ± 4.62	11.2 ± 2.15
4.	Plant extract	2.3 ± 1.02	4.2 ± 1.10

coli. Growth inhibition activity of AgNPs was intensified against strains like *E.coli* and gram-positive *Bacillus subtilis* using the Disc diffusion method. The standard antibiotics Cefotaxime, plant extracts, $AgNO_3$ and AgNPs were chosen. The obtained outcomes of the activity exhibited that all synthesized AgNPs had efficient inhibition capacity. The inhibition zone for AgNPs is 10.3 ± 4.62 and 11.2 ± 2.15 mm respectively (Table 3), (Figs. 7a, 7b). The plant extract and $AgNO_3$ expose less anti-bacterial activity against both strains, which can be interpreted that plant mediated AgNPs, are exclusively responsible for the antibacterial activity (zone of inhibition). Interestingly, AgNPs of plant origin exhibited effective inhibition on bacterial strains.

SUMMARY AND CONCLUSION

The development of skill based experiments has enkindled the ultimate aim in adapting the brand-new science known as (Nanotechnology), in which AgNPs biosynthesis, strategy and its relevance grow to be a part of human need. We have presented the development of *S. Ciliata* mediated AgNPs with characterization study. The study also encloses cytotoxic study and plant studies. An ecofriendly cum renewable process for the synthesis of *S. Ciliata* AgNPs was initiated. The elucidation of UV spectrum AgNPs indicates a deep absorption at 477 nm that measured as maximum and the FTIR shows 3439.48 cm^{-1} is assigned to –OH group. The regime of AgNPs is multiplication that takes place because of uncertainty of the modern-day science. The AgNPs position in the scientific fields like Biomedical and Agriculture can be focused upon, because there is no much proven report of AgNPs. The use of Ag based nonmaterial needs prior attention in order to study the equilibrium between the AgNPs exposed in open environment and it clinical perspective in terms of inhibition of cancer cells. The applications and novelty of present day nanomaterial are multifolded and it happens due to certainty present in the emerging sector of science. Due to its presence as biomaterials it has valued interest in biomedical sector too. And much needed validated literature is needed element to authenticate the new findings in the field of nanomaterial. However, continues monitoring and safer disposal of by-products from NPs is further need to be taken into account pertaining that NPs does not create any form of risk to human

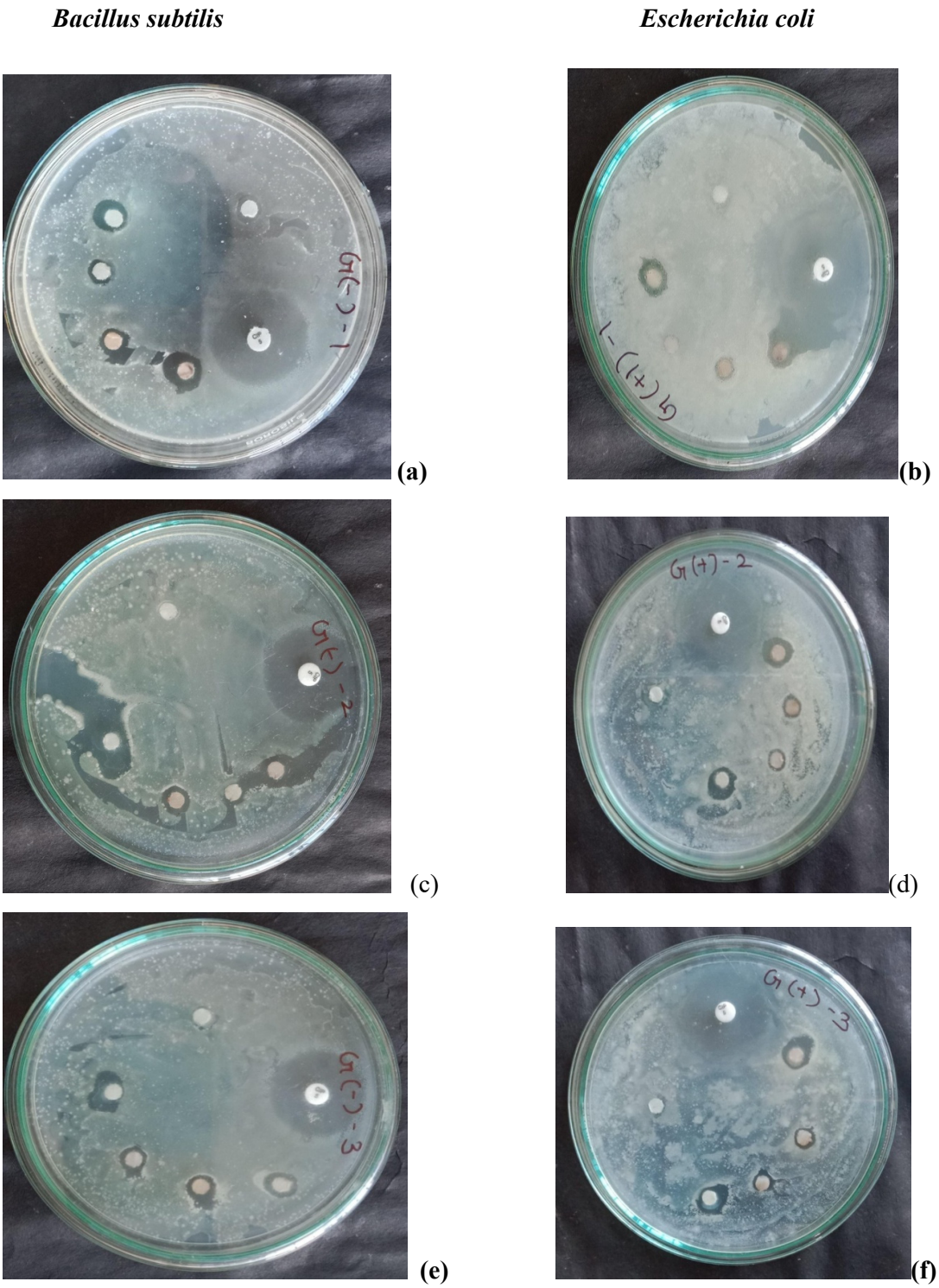


Fig. 7. Image of culture plate showing anti-microbial activity of AgNPs mediated by *S. ciliate*.

health. New avenues like agriculture and medicine need to be foucued upon in near future and plant phytochemicals such as polyphenol compounds and antioxidant properties need to be enhanced for further application in the field of nanoscience. On the other hand, some literature has assumed that there is NO considerable cyto-toxic nature against cells like macrophage therefore animal model trial is emphasized. Therefore, biological method posse's authentic factors to produce nanomaterial mediated of *S. Ciliata* and continued speculation and progress can be achieved.

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AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this article.

DECLARATIONS

The Ethics approval and consent to participate

The authors declare that they have NO conflict of interest. This research article does NOT hold any studies with human or animal subjects performed by any of the authors.

CONSENT FOR PUBLICATION

Not applicable

COMPETING INTEREST

We pronounce that whole experimental research was carried out in the absence of any commercial or financial agreement that could be construed as a potential conflict of interest.

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