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Antioxidant, Antimicrobial and Anticancer Properties of Phyto-Synthesized Silver Nanoparticles Using Aqueous Tuber Extract of *Ceropegia spiralis* Wt.

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Abstract
Biosynthesis of silver nanoparticles is under exploration due to wide biomedical applications and research interest in present advancement of nanotechnology. *Ceropegia spiralis* aqueous tuber extract is mixed with $AgNO_3$, for the formation of Silver nanoparticles initially confirmed by visual observation of color change and further confirmed by using UV–Vis spectroscopy. The nanoparticles were characterized by TEM equipped with EDAX and DLS. UV–Vis result shows that maximum Surface Plasmon Resonance (SPR) band for synthesized nanoparticles at 449 nm. Transmission electron microscope result reveals spherical in shape with 10.41-23.72nm and the weight percentage of AgNPs in the sample were measured with EDAX found 72.17%. Dynamic Light Scattering data gives the size of Nanoparticles in the liquid sample found 5.0 nm along with the negative value -17 mV of Zeta potential indicates the high stability of Nanoparticles. Antimicrobial studies of AgNPs showed highest Zone of Inhibition against *Staphylococcus aureus* (21.67mm) and *Candida tropicalis* (18mm) among bacterial and fungal strains. Anticancer activity of AgNPs exhibited potential anticancer activity towards HeLa (Human Cervix Adenocarcinoma) cancer cell lines, shows 74.39% cell death with cell viability 25.61%. Overall, *C. spiralis* is efficient in the synthesis of nanoparticles paves a way for better antimicrobial and anticancer therapeutic drug potentials to be design by pharmaceuticals.
Keywords
Silver Nanoparticles, Biosynthesis, Surface Plasmon Resonance (SPR), UV–Vis spectroscopy, Transmission Electron Microscope (TEM), Hela Cell lines
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INTRODUCTION:
Nanotechnology is emerging field with its application in science and technology for the purpose of manufacturing new materials at the nano scale level [1]. Silver nanoparticles (AgNPs) showed many important properties such as antimicrobial, anti-inflammatory, wounds healing and application in antibiotic, diagnostic, therapeutic fields. AgNPs are non-toxic so that used against diseases caused by bacterial contamination. Silver nanoparticles showed significant antibacterial property by attaching with highly reactive faces of bacterial cell wall inhibiting

their metabolism and other major functions [2-5]. Plant extract contains biomolecules such as vitamins, polysaccharides, proteins, amino acids, enzymes, and organic acids can act as both reducing as well as capping agents in the bio synthesis of silver nanoparticles [6].

C. spiralis Wight (Apocynaceae) is a slender, erect herb with depressed tubers, opposite leaves, sessile 10-20cm long narrowly linear, base and apex often curved and twisted at the tip. Flowers 3-5 cm long. greenish-purple, cymes, mostly solitary. Fruit of two slender follicular mericarp (Fig 1) [7]. Flowers peculiar with ornamental potential. it is endemic to Peninsular India [8].

The tuberous roots are edible [9], which contain starch, sugar, gum, albuminoids, fats, and crude fibers are valuable constituents in many traditional medicinal systems of India. *Ceropegia* species are storehouse of various valuable phytoconstituents that are routinely used in traditional Indian ayurvedic drugs for the treatment of gastric disorders, diarrhoea, dysentery, urinary tract ailments, etc [10]. Pharmacological importance of the genus *Ceropegia* is mainly due to the presence of pyridine alkaloid "cerpegin", which is potentially antipyretic, analgesic, local anesthetic, antiulcer, mast cell stabilizing, hepato-protective, tranquilizing, and hypotensive [11].

Poor seed setting, low seed germination, scarcity of pollinators and indiscriminate exploitation of edible tubers of *Ceropegia spiralis* seems to be the main hindrance for its natural regeneration to maintain the wild population. The genus, *Ceropegia* is under threat owing to either destructive collection or habitat degradation. Fifty species are present in India [12]. Out of which 28 species are endemic to Peninsular India [13,14].

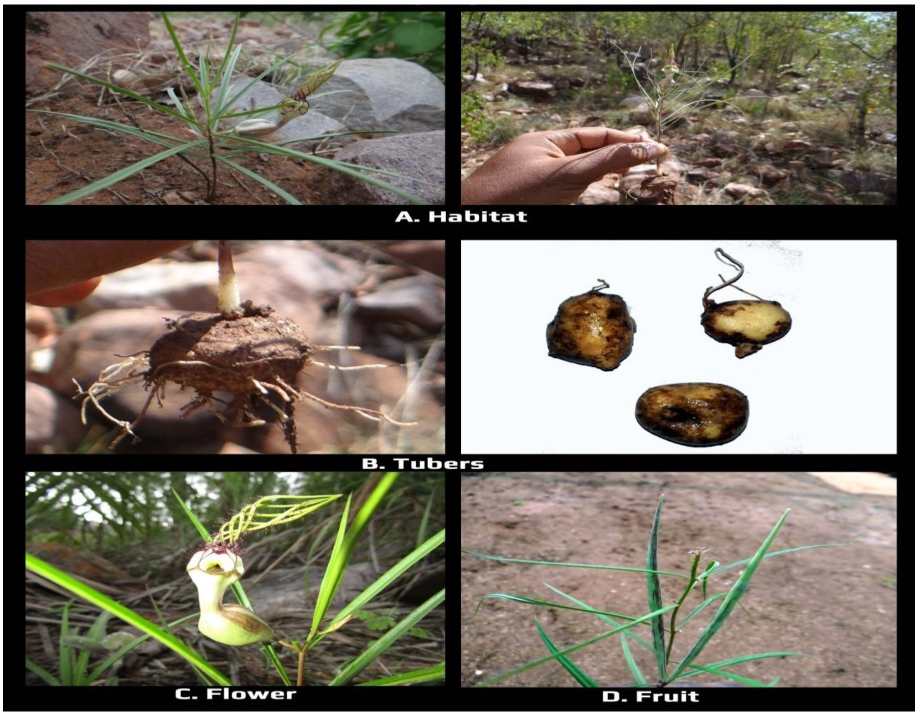

Fig 1: *Ceropegia spiralis*

MATERIALS AND METHODS

Plant collections, identification, extract preparation and Synthesis of AgNPs:

The ***C. spiralis*** tubers were collected from Jaapali area of Tirumala hills, Chittoor District of Andhra Pradesh, India and are identified by the Flora of the Presidency of Madras [15] and cross checked by herbarium (voucher no.2205) deposited in the Department of Botany, Sri Venkateswara University, Tirupati [16].

The tubers were washed several times with tap water to remove the dust particles and shade dried to evaporate the residual moisture. Then dried tubers made into fine powder with the help of electric blender. The tuber extract was prepared, by adding 5g of fine powder with 100 ml of Milli Q ultra-pure distilled water in a 500 ml Erlenmeyer flask the mixture was heated at 70^{0}C for 30 min and then filtered through sterile muslin cloth followed by whatmann No.1 filter paper. This filtrate was used for the synthesis of silver nanoparticles and was used in subsequent biological activities.

5ml of aqueous tuber extract, 50ml of 1 mM Ag $(NO_3)^2$ was added and the sample was left at room temperature, until the gray color of solution changed to light brown. The synthesis of AgNPs was confirmed by the brown color. So, in the current study the biosynthesis of silver nanoparticles (AgNPs) with ***C. spiralis*** tuber extract was prepared without any toxic chemicals which is popularly known as "Green synthesis" or "Green Method"

Characterization of silver nanoparticles

The bio-reduction of pure Ag+ ions was carried out with the aqueous tuber extract of ***C. spiralis*** was monitored periodically by sampling of the 1µl and the optical absorbance of silver nanoparticles suspended in distilled water was recorded on UV–Vis Spectrophotometer (Nanodrop 8000 UV Vis spectrometer) at 220–750 nm wavelength range. The reaction solutions were carried out at room temperature on spectrophotometer at a resolution of 1 nm. Particle size and zeta potential measurement experiments were carried out by using a Horiba Nanopartica SZ-100 instrument. Fourier-Transform Infra-Red (FT-IR) spectra of synthesized SNPs were analyzed between the range 4,000 to 500 cm-1 with an IRAFFINITY-1, IR by ATR method. Crystalline nature of metallic silver nanoparticles was examined using an X-ray diffractometer (XRD) from Bruker, D8 advance, Germany. XRD-6000 equipped with Cu, Ka radiation source using Ni as filter at a setting of 40 kV/30 mA. Transmission electron microscopy (TEM) technique was used to visualize the morphology of the AgNps. At the 200 kV ultra-high-resolution transmission electron microscope (FEI-TECNAI G2 20 TWIN).TEM Grid was prepared by placing 5 µL AgNp Solution on Carbon- Coated Copper grids drying under lamp [17 – 23].

Antioxidant Activity [DPPH]:

DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging method involves the stock solution prepared by dissolving 4 mg of DPPH in 100 ml of methanol and stored at 20°C. 2 ml of this solution was added to 1 ml of *C. spiralis* tuber aqueous extract and *C. spiralis* AgNPs at different concentrations (25-100µg/ml). Ascorbic acid was used as a standard. Where RSA is Radical scavenging activity (RSA), *Ac* is the absorbance of the control, and *as* is the absorbance of the sample or standard [24]. Using the fallowing formulae

$$\textbf{Radical Scavenging Activity} = \frac{(Ac-As)}{(Ac)} \textbf{X100} \rightarrow \textbf{(1)}$$

Antimicrobial studies of SNPs

Biosynthesized silver nanoparticles were analyzed for antimicrobial activity against three Gram-positive bacterial strains like *Staphylococcus aureus* MTCC-3160, *Enterococcus faecalis* MTCC-2729, Streptococcus *pyogenes* ATCC 19615 and two Gram-negative bacterial strains like *Enterobacter aerogenes* MTCC-2822, and *Salmonella typhimurium* MTCC-3231. Antifungal studies were carried out in three fungal strains like, *Aspergillus niger* MTCC 281, *Candida albicans* MTCC-183 *C. tropicalis* MTCC-184, procured from Department of Botany, Bharathidasan University, Tiruchirappalli, Tamil Nadu. Disc diffusion assay method was carried out using standard protocol [25]. Different concentrations (10,20,30,40, µg/ml of Plant extract, SNPs and Kanamycin/Fluconazole was applied on separate filter paper discs (Whatman No. 1) filter paper with 6 mm diameter), and allowed to dry before being placed on the agar medium. The *C. spiralis* tuber extract was used as positive controls, AgNO3 is negative control respectively. Kanamycin and Fluconazole (5mcg/disc) were used as standard controls for bacterial and fungal strains, respectively.

Anticancer activity:

AgNPs of *C. spiralis* was subjected to MTT 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye by(MTT) to purple formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces by MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportionate to the number of viable cells and can be measured spectrophotometrically at 570nm [26,27]. HeLa cell line (Human Cervix Adenocarcinoma) is procured from National Centre for Cell Sciences (NCCS), Pune, India. The Dulbecco's Modified Eagle's Medium with high glucose is used to growing up 2×10^4 cells per well in 96-well plates and incubated in 5% CO_2 atmosphere at 37^0C for 24 h supplemented with 2 mM/L glutamine, 10% Foetal Bovine Serum (FBS) with 10 µg/ml of *Ciprofloxacin* [28].

Afterwards medium was expelled and treated with different concentrations (12.5, 25, 50, 100 and 200µl/ml) AgNPs of *C. spiralis* incubated for 24hrs. Further, remove spent media and add 100 µl of MTT reagent with the 0.5mg/ml concentration and incubate the plate for 2.5hrs for the reaction. Later, remove MTT reagent completely and add 100ul of 100% Dimethyl sulfoxide (DMSO) to solubilize the formazone crystals completely and measure the absorbance at 570 nm using 96 well Plate reader. (The 0.1% of DMSO used to dissolve the nanoparticles and set as negative control and 15 µM *Camptothecin* treated cell lines were set as positive control. The initial experiment was maintained for 0 to 24 h of timeline period with 12 h of time gap period to check probability of cell toxicity. It provides specific time course period to allow functional cell mortality to understand the experiment in a flexible and adaptable way. According to the results, significant cytotoxicity was observed at 24-hrs at 37^0c incubation period. The percentage of cell viability was calculated by the following formula [29]

$$\textbf{Percentage of Cell viability} = \frac{\textbf{OD value of treated cell lines}}{\textbf{OD value of control}}\ \textbf{X 100} \quad \rightarrow (2)$$

RESULTS

Ultraviolet–visible spectroscopy of *C. spiralis*-AgNPs

UV-Visible spectroscopy is a straightforward technique for the detection and for confirming the formation of nanoparticles. The bio-reduction of Ag+ ions by using the tuber aqueous extract of *C. spiralis was* monitored from time to time by sampling of the 1 µl aliquots and the optical absorbance was recorded on the Nano drop 8000 UV-Vis spectrophotometer between 220 – 750 nm wavelength range. The UV-visible spectra (Fig. 2) of the bioreduced Cs-AgNPs solution showed an absorbance peak at 449 nm which is a characteristic surface plasmon resonance (SPR) peak of silver nanoparticles, confirms their biosynthesis. The size and shape of the Cs-AgNPs reflects on the absorbance peak [30]. It is well known that the SPR range of silver nanoparticles is between 390 nm to70 nm, earlier reports on biosynthesized AgNPs also reveals the similar results that as that of the SPR AgNPs is between 400nm to 450 nm range, it also reveals that the AgNPs are small and spherical in shape, and the size range between 20nm to 100nm [31]. In the present study the SPR of Cs-AgNPs is 449nm, indicates that the nanoparticles are small and spherical in shape further confirmed by TEM analysis and Particle size analysis.

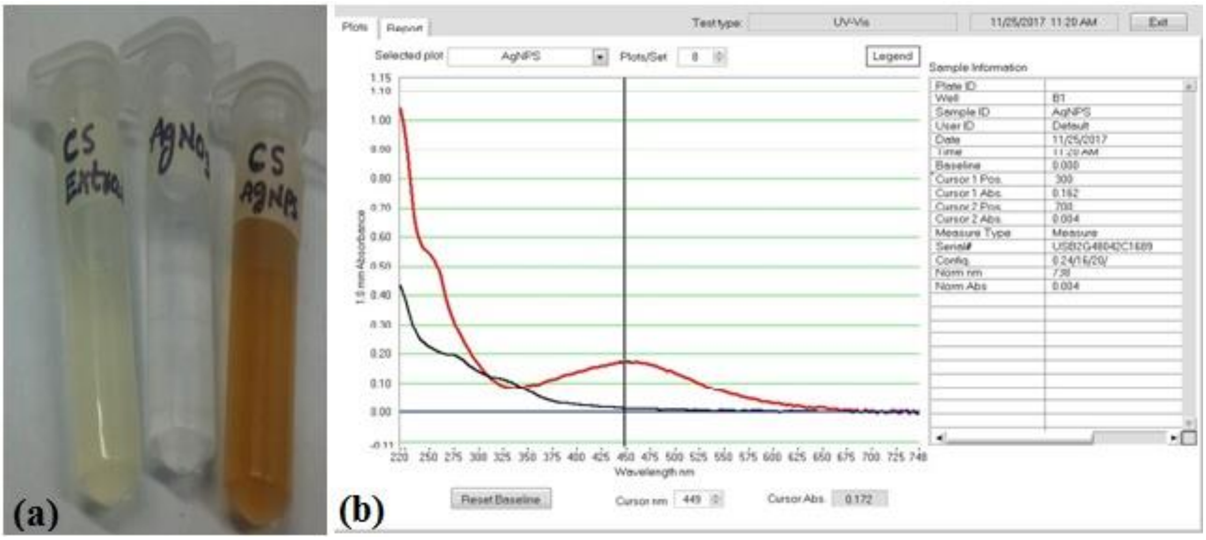

Fig 2: (a) Synthesized silver NPs of *C. spiralis* mixture Colour change from grey to brown. **(b)** UV- VIS analysis of synthesized NPs shows peak at 449 nm.

Fourier transform infrared (FTIR) spectra analysis of *C. spiralis*-AgNPs

The functional groups of tuber extract and synthesized SNPs were identified using FT–IR spectroscopy between the scan ranges of 4000–500 cm^{-1}. The FT–IR spectrum (Fig. 3) obtained for tuber extract and AgNPs displayed a number of absorption peaks like 3328.09 cm^{-1} assigned for O-H (Stretch) bond of Phenols, 1649.12 $cm^{-1}$for C=C bond (Stretch) of alkanes, 1546.47 cm^{-1} for N–H (Bend) bond of amines, 1298.83 cm^{-1} for C–O (Stretch) bond of aliphatic amines, 1102.36 $cm^{-1}$for C–N (Stretch) bond of alcohols, 739.31 $cm^{-1}$for C-Cl (Stretch) bond of alkyl halides, and 3347.87 cm^{-1} for O–H (stretch) bond of alcohols/phenols, 1646.67 cm^{-1} for C=C (Stretch) bond of alkenes and 1082.57cm^{-1} for C–N (Stretch) bond of aliphatic amines. Most of the peaks appeared in the tuber extract, disappeared after synthesis of SNPs. Based on FT–IR analysis it is confirmed that the broad peaks of phenols (3328.09 cm^{-1}) and primary amines of proteins (1546.47 cm^{-1}) are interacting with biosynthesized SNPs and acting as a reducing agents [32].

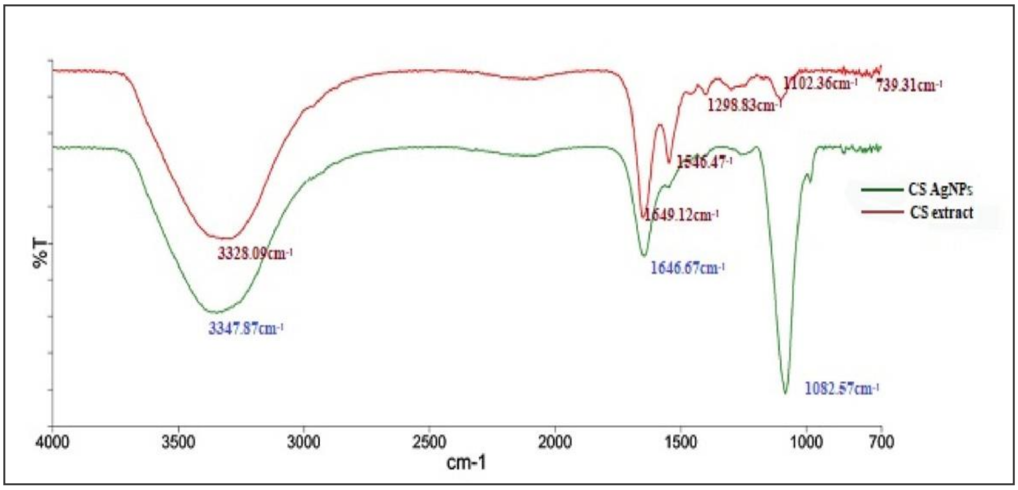

Fig 3: FTIR spectra of green synthesized AgNPs from aqueous tuber extract of ***Ceropegia spiralis***.

Particle size and Zeta potential analysis of *C. spiralis*- AgNPs:

The particle size of the biosynthesized *C. spiralis*-AgNPs are detected by the intensity and laser diffraction method using the biosynthesized colloidal solution in which the *C. spiralis*-AgNPs are polydispersed in mixture solution. The distribution of *C. spiralis*-AgNPs size range of 2 nm to 8 nm at an average size found to be 5.0 nm (Fig. 4a) with and PI value of 0.282 (poly disperse index). Further the zeta potential analysis of Cs-AgNPs was detected to be -17.1 mV, due to its high negative zeta potential it prevent the *C. spiralis*- AgNPs from agglomeration in the medium, leading to long term stability, because of the electrostatic repulsive force between the *C. spiralis*-AgNPs. The zeta potential l(Fig. 4b) of AgNPs of ***Ceropegia spiralis*** tuber extract was found to be -17.1mV [33,34].

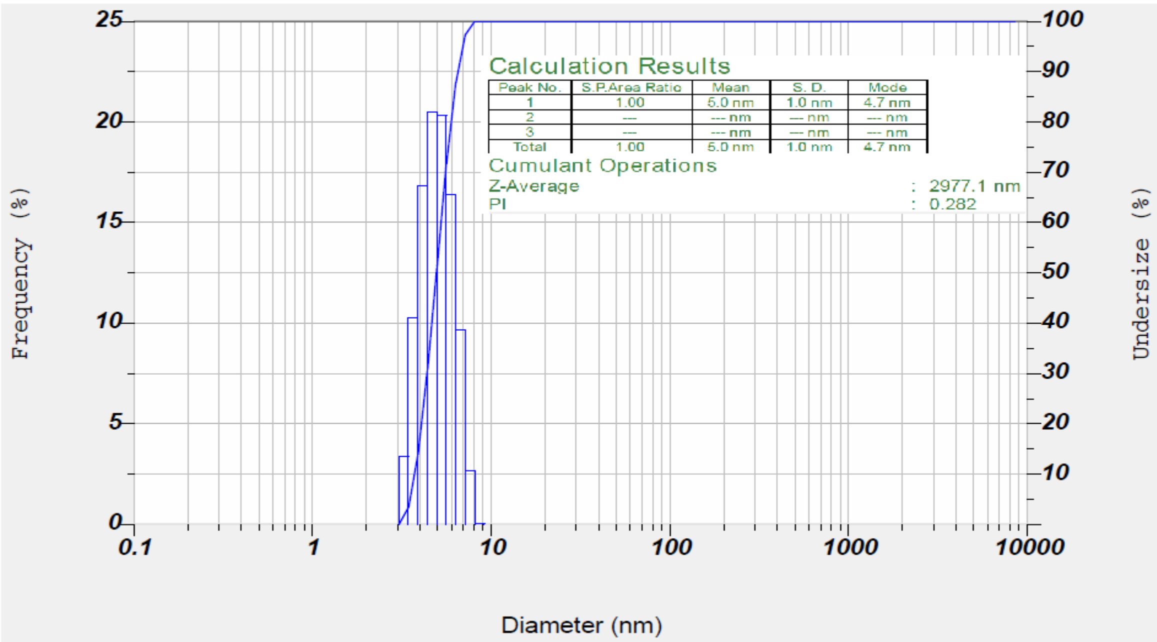

Fig 4a: Particles size distribution curve for –AgNPs of ***C. spiralis***

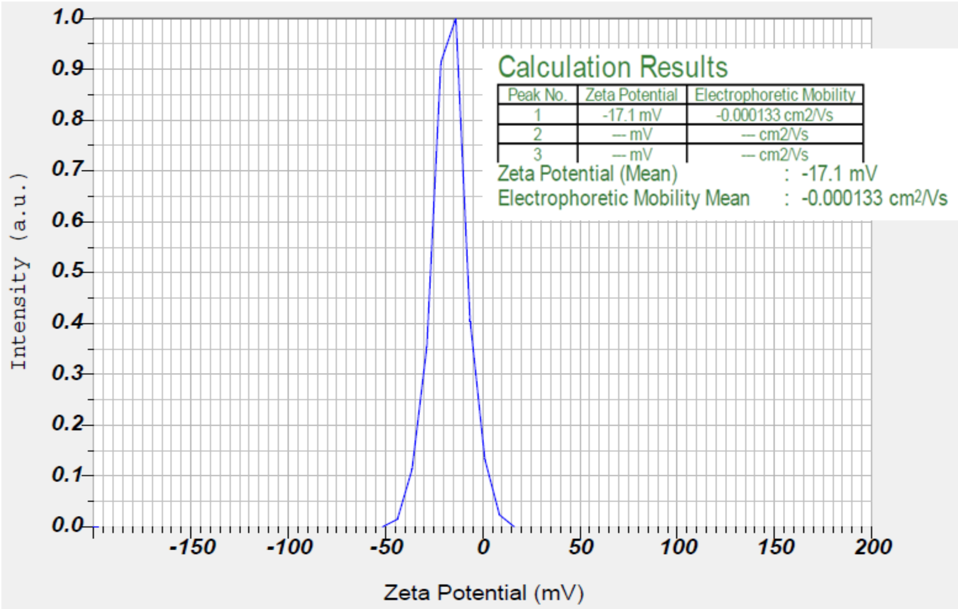

Fig 4b: Zeta potential of green synthesized AgNPs from tuber aqueous tuber extract of ***C. spiralis***

X-Ray diffraction analysis (XRD) of *C. spiralis* - AgNPs:

XRD is an advanced spectroscopic technique used to evaluate the crystalline nature of biosynthesized *Cs*-AgNPs. The XRD pattern obtained revealed that Cs-AgNPs are end centered monoclinic structures in nature. (Fig. 5) CS-AgNPs analysis of synthesized nanoparticles showed 9 Bragg reflections at 27.97, 32.53, 38.32, 44.37, 46.39, 55.01, 57.55, 64.68, 77.46 corresponds to 110, 120, 111, 200, 132, 221, 220, 220, 311 integer 'hkl' planes, respectively. This indicates end-centered monoclinic crystalline nature of nanoparticles. This result was affirmed by cross checking the obtained data with JCPDS Card no. 89-3722, 84-1261 [35,36].

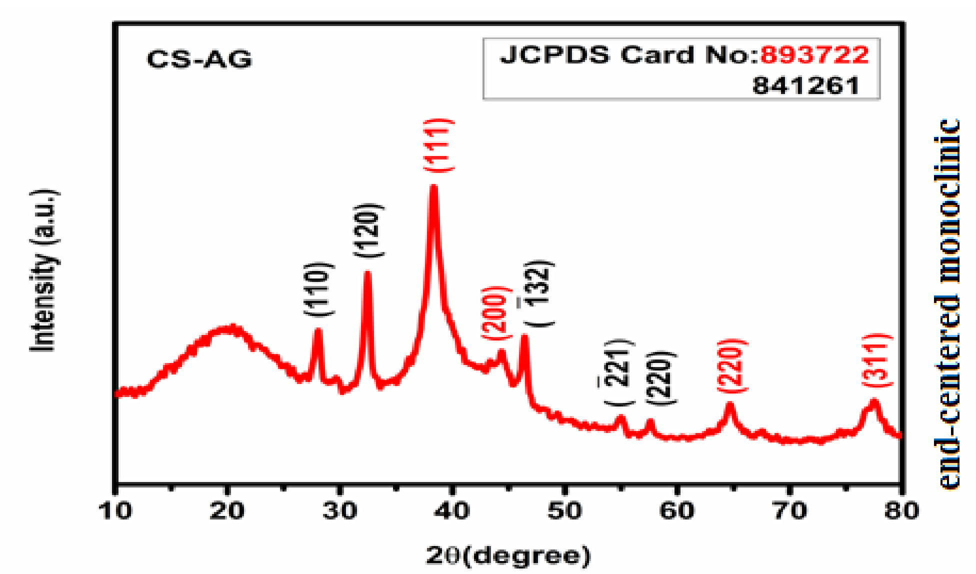

Fig 5: XRD pattern of green synthesized AgNPs from aqueous extract of ***C. spiralis***.

Transmission electron microscopy (TEM) analysis of *C. spiralis*-AgNPs

TEM analysis was performed to determine the shape and size of the biosynthesized Cs-AgNPs, using 200 kV FEITecnai G@ 20 S-TWIN High resolution TEM (VIT, Vellore). The TEM image analysis revealed that the Cs-AgNPs are roughly spherical in shape, size in-between 10.41-23.72 nm (Fig 6a). The SAED pattern analysis also reveals that the particles have different planes of end-centered monoclinic lattice which already confirmed by XRD analysis.

Particle analysis:

The synthesized nanoparticles were analyzed by different image processing software tools like ImageJ. ImageJ (Image processing and analysis in Java) Micrographs from TEM analysis showed **10.89nm** average sizes of the particles with 0.72 adjacent 'R' square values calculated by using the following Gaussian fitting formula (**Fig. 6b**):

$$y = y_0 + \left(A / \left(w \times \mathrm{sqrt}\,(PI/2)\right)\right) \times \exp\left(-2 \times \left((x - xc) / w\right)^2\right) \rightarrow (3)$$

The average size of the nanoparticle was calculated as 10.79 nm. The size measured by DLS is slightly greater than HR-TEM because the DLS measured the hydrodynamic diameter [37]. The result analysis confirmed that both HR-TEM and XRD analysis showed a similar average size of nanoparticles. The slight differences in the average size of nanoparticles are due to the preparation of sample time and variable instrumental conditions. However apart minute differences, the size measured by DLS and HR-TEM analysis showed nearly similar results (**Table 1**).

Table 1: Average size measured by DLS, HR-TEM,

DLS analysis	HR-TEM
5.0 nm	10.89 nm

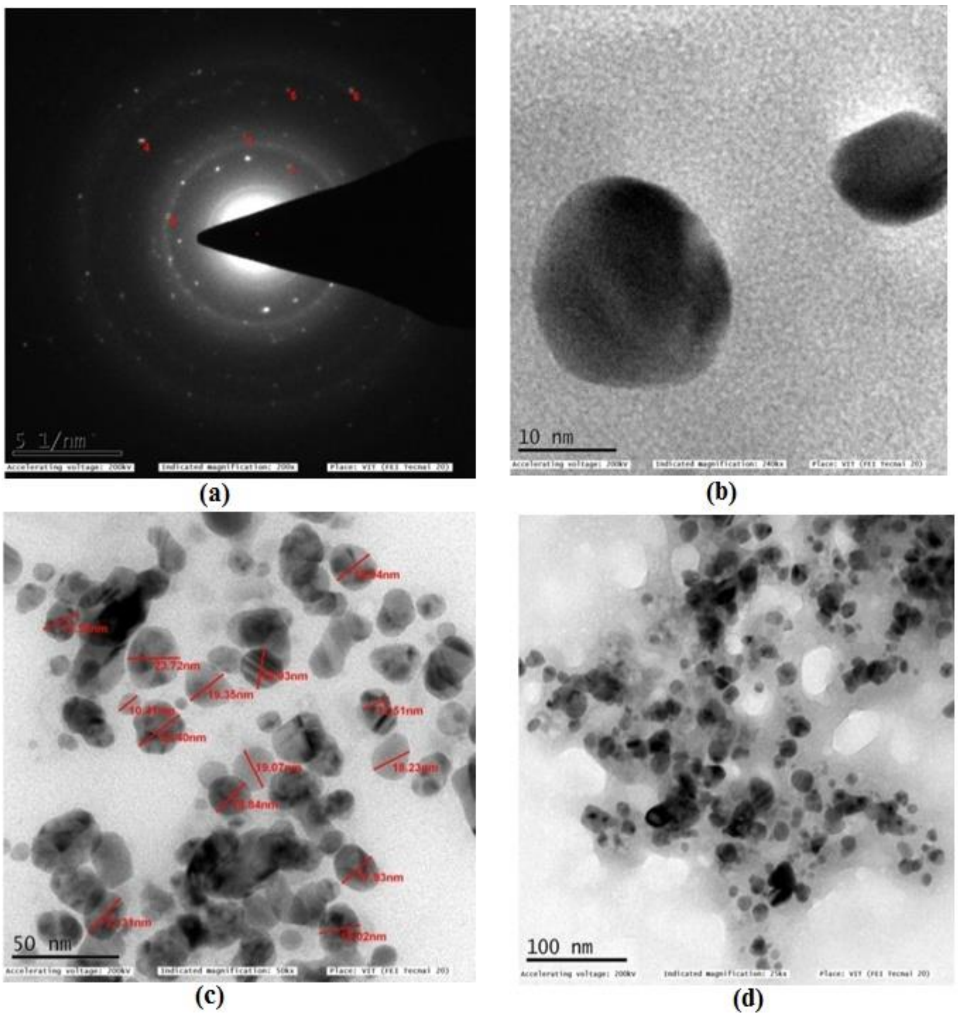

Fig 6a: TEM micrographs of biosynthesized AgNPs. **(a)** Selected area electron diffraction (SAED) of green synthesized AgNPs, **(b)** 20 nm resolution studies of green synthesized AgNPs. **(c)** 50 nm resolution studies of green synthesized AgNPs nanoparticles with 10.41-23.72 nm size. **(d)** 100 nm resolution studies of green synthesized AgNPs shows mostly spherical shaped nanoparticles.

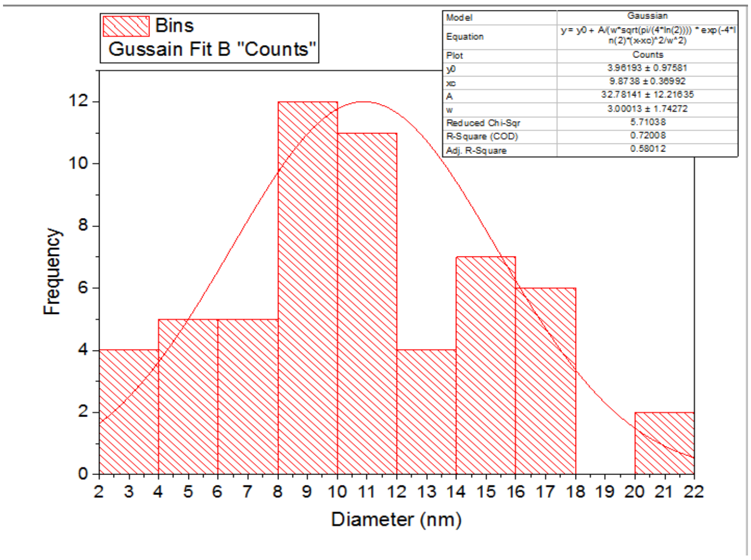

Fig 6b: Particle size analysis with Gaussian fitting formula represents average size of the particles.

Energy Dispersive X-Ray Spectroscopy (EDX) of *C. spiralis*- AgNPs

EDX analysis was performed to know the percentage of Ag present in the sample. The EDX spectra shows strong silver **72.17 %** absorption peak along with different elements with their weight percentage like Oxygen 0.95 %, Chlorine 3.48% and Copper 23.39% (Table 2) and the results indicated that the reaction product has high purity of SNPs .The EDX data revealed that, very strong signal to silver and weak signals to other elements indicates the complete reduction of silver ions to elemental silver that is Cs-AgNPs (Fig. 7).

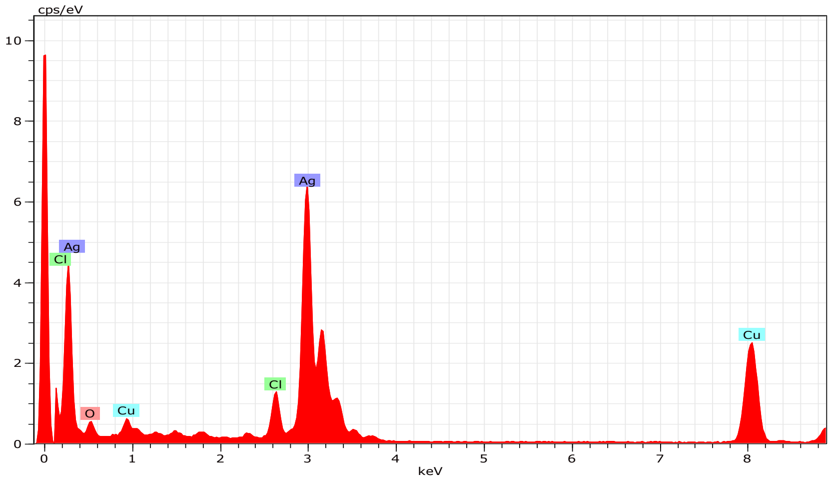

Fig 7: EDX analyses of green Synthesized AgNPs of ***C. spiralis.***

Table 2: EDX analyses of green Synthesized AgNPs of ***C. spiralis*** shows 72.17 weight percentage.

Elements	Series	Net	unn. C [wt. %]	corm. C [wt. %]	Atom. C [wt. %]	Error (3 sigma) [wt. %]
Oxygen	K – series	1155	0.95	0.95	4.99	0.20
Chlorine	K - series	7422	3.48	3.48	8.22	0.41
Silver	K - series	8320	**72.17**	**72.17**	55.99	7.07
Copper	K - series	31728	23.39	23.39	30.8	2.22
		Total	100.00	100.00	100.00	

DPPH Method of Antioxidant Analysis: *C. spiralis* - AgNPs

The synthesized AgNPs of ***C. spiralis*** showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. The antioxidant activity was increased in dose-dependent manner. The highest percentage activity was exhibited at 100 µg/ml concentration ***C. spiralis*** aqueous extract (56.32) < ***C. spiralis*** AgNPs (68.12) < Ascorbic acid (74.44) (**Table 3**and **Fig. 8**) From the results, it is concluded that Silver nanoparticles of ***C. spiralis*** possess good radical scavenging activity when compared to that of ***C. spiralis*** aqueous extract. The antioxidant activity of AgNPs by the DPPH method shows a strong absorption band at 517 nm.

Table 3: Antioxidant activity of Phyto-Synthesized AgNPs of ***C. spiralis***

Concentration	Aqueous Extracts (%)	AgNPs (%)	Ascorbic Acid (%)
25 µg/ml	14.02 ± 0.08	17.24 ± 0.36	47.45 ± 0.16
50 µg/ml	24.24 ± 0.3	27.31 ± 0.25	56.26 ± 0.42
75 µg/ml	36.14 ± 0.18	32.54 ± 0.08	66.25 ± 0.45
100 µg/ml	56.32 ± 0.2	**68.12 ± 0.41**	74.44 ± 0.42

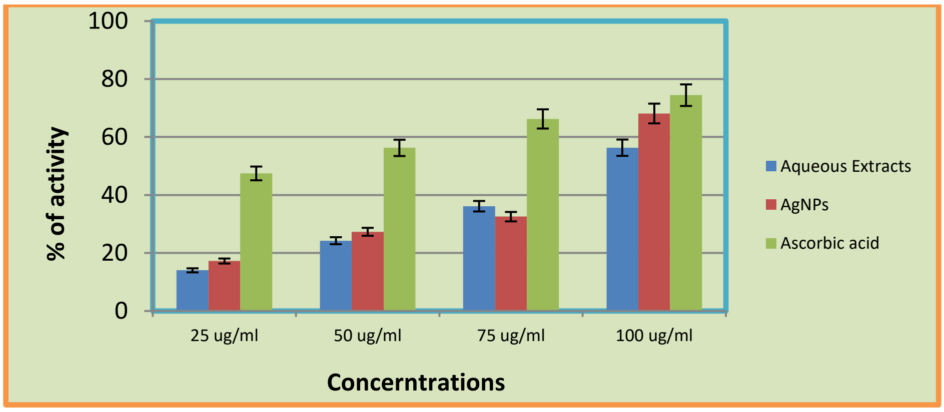

Fig 8: Antioxidant activity of Phytosynthesized AgNPs of ***C. spiralis***

Antimicrobial activity of *C. spiralis* – AgNPs:

Antimicrobial study of green synthesized AgNPs has shown noteworthy growth inhibitory results on different microorganisms. A highest zone of inhibition was observed against *Staphylococcus aureus* followed by *Streptococcus pyogenes* the diameter Zone of Inhibition (DZI) **21.67 mm, 21.33 mm**. respective against gram positive bacterial strains, Whereas against fungal strains the highest zone of inhibition was observed on *C. tropicalis* diameter Zone of Inhibition (DZI) **18mm** (Figs. 9 (a & b), 10, 11 & Table 4 & 5). In this study, antifungal activity was less when compared with anti-bacterial activity. Among the bacteria the zone of inhibition was less on gram negative bacteria when compared with gram positive bacterial strains.

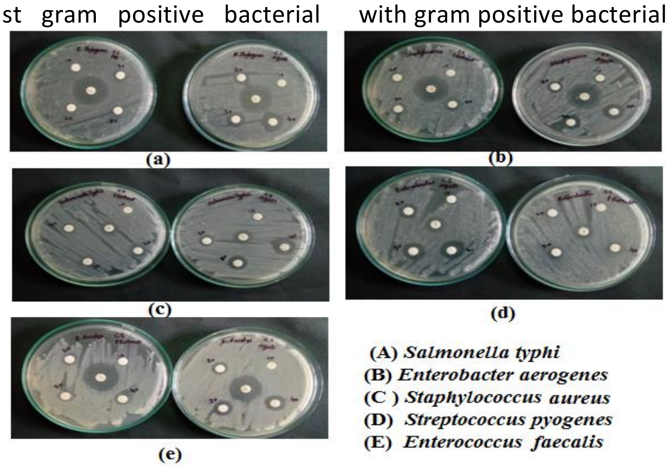

Fig 9a: Antimicrobial activity of *C. spiralis* – AgNPs**.**

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Table 4: Effect of different extracts and Phyto synthesized *Silver* nanoparticles of *C. spiralis* on clinically isolated bacterial Strains.

Concentration (µg/ml)	Streptococcus pyogenes		*Enterococcus faecalis*		Staphylococcus aureus		Salmonella typhi		Enterobacter aerogenes	
	Zone of Inhibition (mm)									
	PE	AgNPs	PE	AgNPs	PE	AgNPs	PE	AgNPs	PE	AgNPs
10 (µg/ml)	6.67 ± 0.33***	10.33 ± 0.33 ***	7.33 ± 0.33 ***	7.67 ± 0.33 ***	7.33 ± 0.33 ***	9.33 ± 0.33 ***	7.67 ± 0.33 ***	9.67 ± 0.33	7 ± 0 ***	7.33. ± 0.33 ***
20 (µg/ml)	11.33 ± 0.67 ***	12.67 ± 0.88 ***	11.67 ± 0.33 ***	12.67 ± 0.33 ***	8.33 ± 0.33 ***	14.67 ± 0.67 ***	12.67 ± 0.33	12.00 ± 0.58	10 ± 0.58 **	12.33 ± 0.33
30 (µg/ml)	11.67 ± 0.67 ***	16.67 ± 0.88 ***	12.33 ± 0.33 ***	16.33 ± 0.33 ***	9.67 ± 0.33 ***	17.67 ± 0.88 ***	13.67 ± 0.33	14.67 ± 0.67 *	12.33 ± 0.33	14.33 ± 0.33
40 (µg/ml)	13.33 ± 0.33 ***	21.33 ± 0.67 *	14.67 ± 0.67 ***	19.33 ± 0.33 ***	12.33 ± 0.33 ***	21.67 ± 0.33 **	14.33 ± 0.33 **	20.33 ± 0.88 ***	13.33 ± 0.33	18.00 ± 0.58 ***
Kanamycin	23.33 ± 0.67	24.67 ± 0.67	22.67 ± 0.33	27.67 ± 0.33	23.67 ± 0.33	25.67 ± 0.33	12.33 ± 0.33	11.67 ± 0.33	12.67 ± 0.33	13.33 ± 0.33

All the data are expressed as mean ± SEM: *** p<0.01, ** p<0.02, * p<0.03 as compared to Control group, n=4; (One – way ANOVA followed by Dunnett's test).

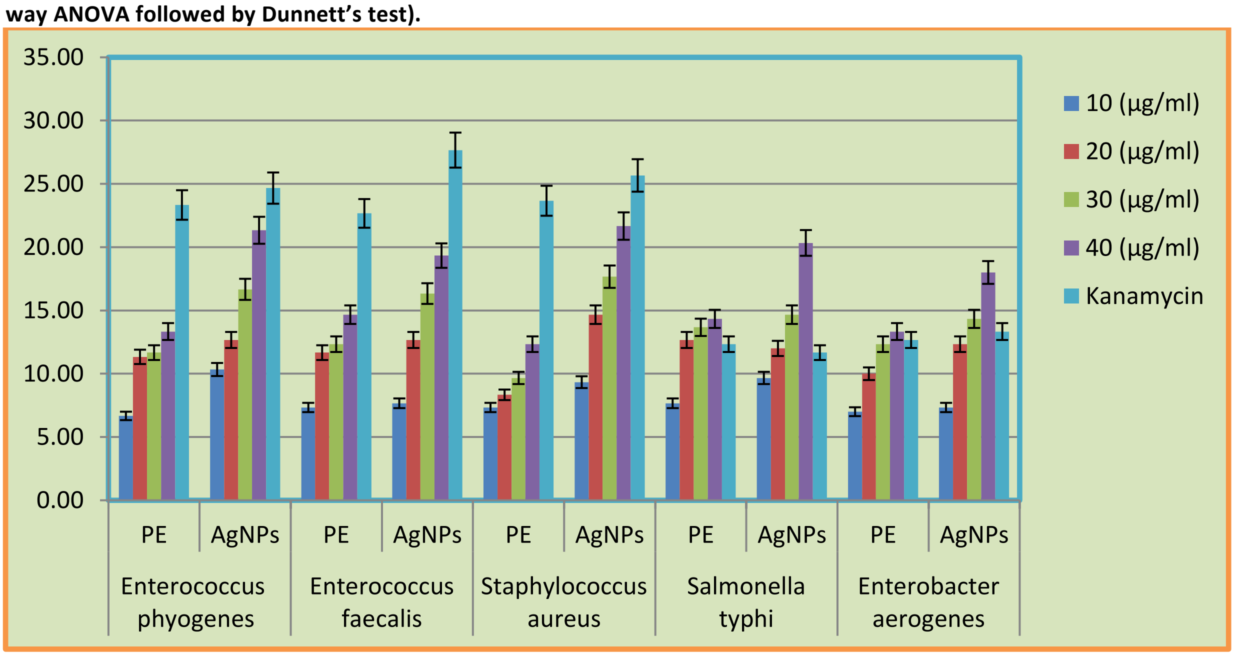

Fig 9b: Zone of inhibition of different extracts of *C. spiralis* on clinically isolated bacterial strains

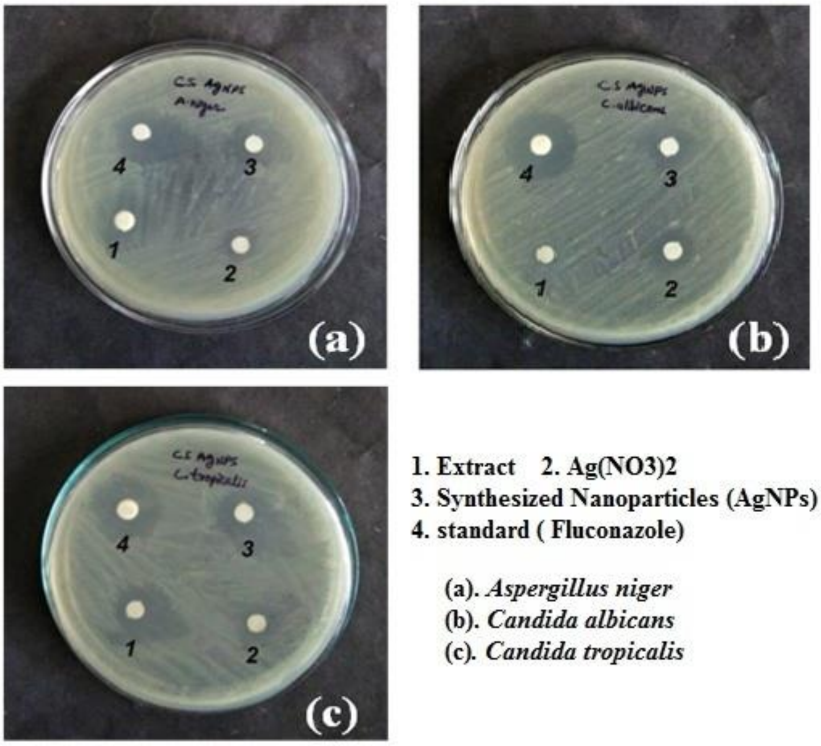

Fig 10: Antifungal activity of *C. spiralis* – AgNPs

Table 5: Effect of different extracts and phyto synthesized ***Silver*** nanoparticles of *C.spiralis* on Fungal strains

NAME OF ORGANISM	Extracts	Ag(NO3)2	AgNPs	Fluconazole
Aspergillusniger	12.75 ± 0.25 ***	11.25 ± 0.25 ***	14.5 ± 0.29 ***	20.75 ± 0.63
Candida albicans	7 ± 0 ***	9 ± 0.41 ***	14 ± 0 ***	19 ± 0
Candida tropicalis	14 ± 0.41 ***	10.75 ± 0.25 ***	18 ± 0.41	19 ± 0.41

All the data are expressed as mean ± SEM: *** p<0.01, ** p<0.02, * p<0.03 as compared to Control group, n=3; (One – way ANOVA followed by Dunnett's test).

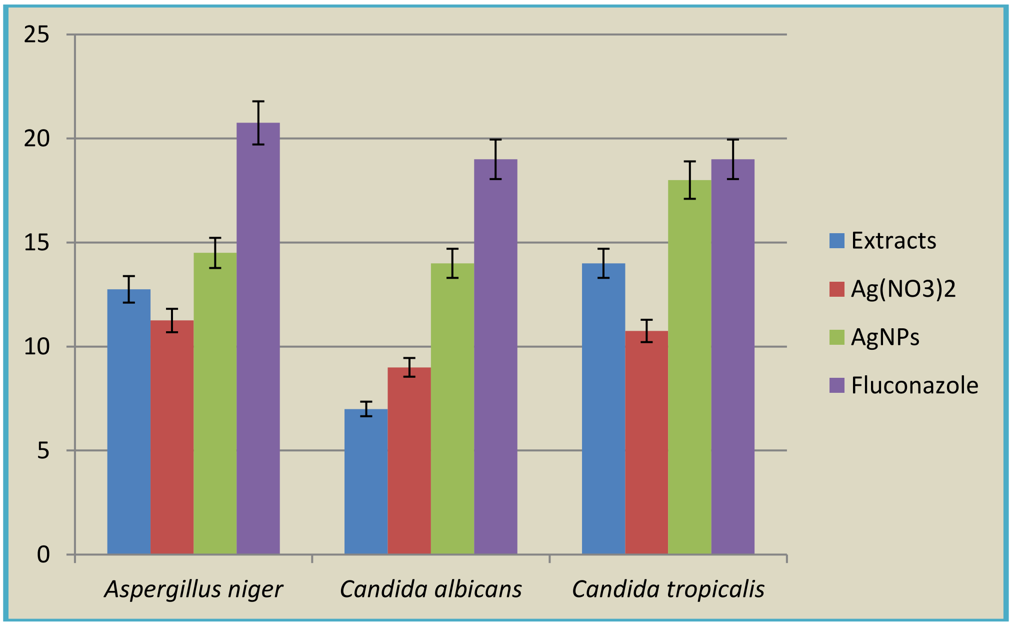

Fig 11: Zone of inhibition of different extracts of *C. spiralis* on fungal strains

Anticancer activity *C. spiralis*–AgNPs:

The HeLa cell line (Human Cervix Adenocarcinoma) was used for cytotoxicity analysis by reading formazan crystals formed by the reaction of mitochondrial dehydrogenase by MTT assay. At 48 h of time course incubation period, a significant abatement in cell viability was observed in the treated cell lines, while the concentration of AgNPs was increased from 12.5, 25, 50, 100 and 200 µg/ml. DMSO was used as a positive control to exhibit 100% of healthy proliferated cells (Figs. 12, 13& Table 6). The 50 µg/ml concentration (IC_{50}) of AgNPs may have the capability to reduce 50% of treated cell lines when compared with negative control. The cytotoxicity of nanoparticles may depend on the small size and spherical shape of the particles [38]. The Observations in Statistical data of Cytotoxicity study by ELISA Reader suggesting that against HeLa cells, Cs AgNPs showing Good cytotoxic potential properties with the IC_{50} Concentrations 59.19µg/ml But, there is no report on AgNPs synthesized from any medicinal plant to attribute anticancer activity against HeLa cell lines. From this study, the green synthesized AgNPs from tuber extract showed 10.89 nm size, spherical shaped particles, which exhibit strong cytotoxic activity against HeLa cell lines [39].

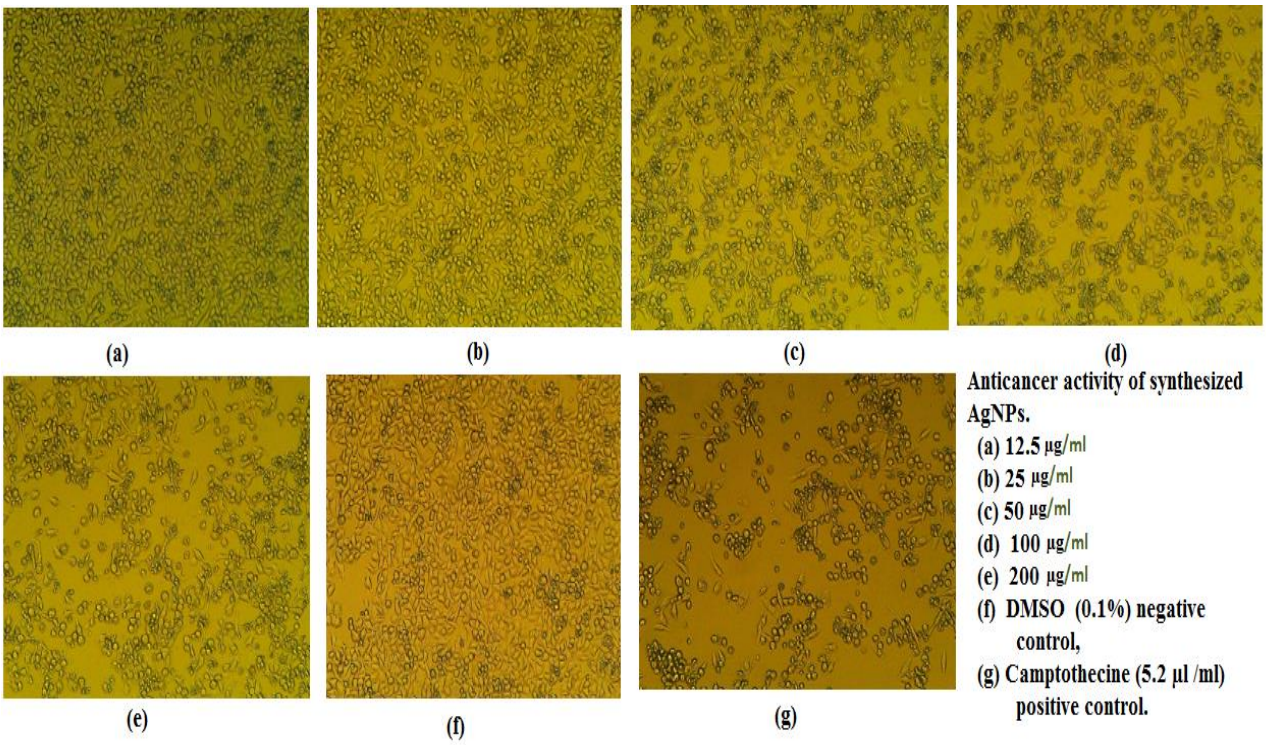

Fig 12: Anticancer activity of *C. spiralis*–AgNPs

Table 6: Anticancer effect of ***C. spiralis*** **AgNPs** on the HeLa cell lines (Human Cervix Aden carcinoma) by MTT assay.

S. No	Concentrations (µg/ml)	Absorbance (O.D)	Cell viability (%)	Cell Death (%)
1	DMSO	0.86	100	100
2	**12.5**	0.7385	85.87	14.13
3	**25**	0.5905	68.67	31.33
4	**50**	0.4335	50.41	49.59
5	**100**	0.316	36.74	63.26
6	**200**	0.16	**25.61**	**74.39**
7	**Camptothecin**	0.4425	14.58	85.42

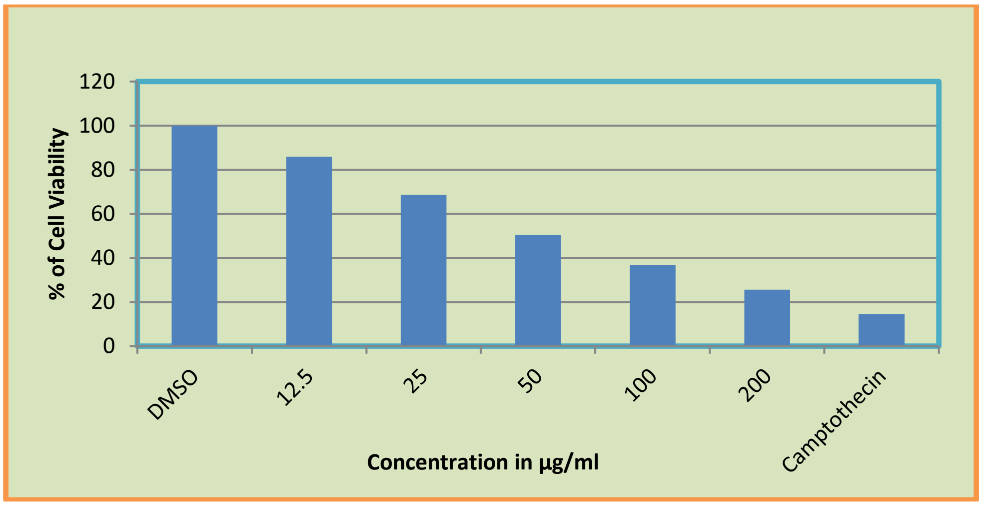

Fig 13: Anticancer activity of Phytosynthesised AgNPs from tuber extract of ***Ceropegia spiralis***.

DISCUSSION

Silver nanoparticles antifungal activity was observed less when compared to antibacterial activity may be due to fungal cell walls are made up of chitin, a fibrous substance comprising of polysaccharides having N-acetyl glucosamine and a nitrogen group, which is more firm to allow the passage of nanoparticles from the outer layer of the cell wall to the inner layer. In the case of bacteria, cell membranes are made up of peptidoglycon a polymer having sugars and amino acids, which is less firm, and passage of nanoparticles is easy when compared with fungi.

Elucidated that silver nanoparticles acts as inhibitory substances on microorganisms by the action of destroying enzymes of cell membranes. This might be possible by the small size and spherical shape of the particles have a high surface to volume ratio to interact with the cell membranes of microorganisms to exhibit the signs of growth inhibitory results [40]. Size, shape and agglomeration pattern of the nanoparticles depend on quantity-based presence of phytochemicals in the medicinal plants. This might be useful in reducing, capping and stabilization of nanoparticles to narrow size with spherical shape [41]. In this study it is found that AgNPs synthesized from tuber exhibit narrow size particles with spherical shape because *C. spiralis* is a good source for different phytochemicals especially phenols and proteins [42]

Pharmacological activities of *Ceropegia* species has been subjected to various investigations. The therapeutic importance of the genus *Ceropegia* is mainly due to the presence of '*cerpegin*', a pyridine alkaloid. DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity, ferric reducing antioxidant power (FRAP) as well as metal chelating ability of major phenolic compounds such as gallic acid, vanillin, cathechol and ferulic acids from the leaves of *Ceropegia* species such as *C. spiralis, C. panchganiensis* and *C. evansii* has been reported [43]. The leaves of *C. bulbosa* showed the highest superoxide dismutase activity [44]. *C. juncea* has possessed various potent secondary metabolites such as tannins, flavonoids and many polyphenolic compounds. Ethanolic leaf extracts of *C. juncea* showed gastroprotective and antioxidant activities in rats due to the presence of polyphenolic compounds [45]. Antibacterial activity of three *Ceropegia* species such as *C. spiralis*, *C. juncea* and *C. candelabrum* var. *candelabrum* on human pathogens *Klebsiella pnemoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* that ethanol extract showed a higher antibacterial activity as compared to chloroform and aqueous extracts [46]. Antimicrobial activity with whole plant extract of *Ceropegia pusilla* against five bacterial strains *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, *Shigella sonnei, Bacillus* sp.and four fungal strains *Candida albicans, Aspergillus* sp., *Penicillium* sp., *Mucor* sp. [47]. The crude extract of *C. deightonii* showed antimicrobial activity against *Staphylococcus aureus;* [48] *Streptococcus feacalis; Escherichia coli; Shigella dysenteriae* and *Candida albicans* Leaf extract of *C. thwaitesii* used for the plant mediated synthesis of AgNPs (silver nanoparticles) showed antimicrobial activity against *S. typhia* and *B. subtilis* [49]. Comparative studies on *in vivo* and *in vitro* tuber extracts of *C. pusilla* confirmed Antiproliferative property against HeLa cancer cell line [50]. The three *Ceropegia* species C. *spiralis*, *C. juncea* and *C. candelabrum*, screened for anti-cancer activity and confirmed the potent anticancer effect of ethyl acetate fraction of *C. spiralis* against cell lines namely HCT-118 (Colon cancer cell) [51].

CONCLUSION:

Hence the present study, reported as cost effective, eco-friendly, green approach method for production of AgNPs from the tuber extract of *C. spiralis.* The colour change pattern and surface Plasmon resonance spectra of UV–VIS data 449 nm confirms the formation of SNPs. Phenols and Proteins are mainly responsible for reduction and stabilization of these SNPs revealed by FTIR. Zeta potential analysis revealed that - 17 mV of negative value indicates greater stability of particles. XRD analysis revealed that end-centered orthorhombic crystalline nature of nanoparticles. Microscopic analysis with TEM showed spherical shaped particles with a size range from **10.41** to **23.72 nm**. These particles were mostly settled in non-agglomerated and poly-dispersed condition.

The expository synergistic efficiency of AgNPs showed growth inhibitory activity against microorganisms as well as HeLa (Human Cervix Adenocarcinoma) Cancer cell lines. Further studies need to be performed to evaluate the molecular mechanism behind the anticancer potential of the AgNPs against the Human Cervix cancer cells. The presence of higher concentrations of phenols and proteins in the tuber may be the reason behind the formation of narrow sized particles, bestowed to antimicrobial and anticancer activity. This is the first report on phyto-synthesized AgNPs from the tuber extract of *C. spiralis.* This study may pave a way for the studies of AgNPs further pharmacognostic studies against anticancer, antibacterial, and antifungal drug designing.

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CONFLICTS OF INTEREST:

There is no conflict of interest related to this work by any of the authors.

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