

Synthesis of silver nanoparticles from extracts of *Scytonema geitleri* HKAR-12 and their *in vitro* antibacterial and antitumor potentials

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ABSTRACT

In the present study silver nanoparticles (AgNPs) have been synthesized through the cell-free extracts of the rooftop dwelling cyanobacterium *Scytonema geitleri* HKAR-12. UV-VIS spectroscopy, FTIR, X-ray diffraction, SEM and TEM were used for the determination of morphological, structural and optical properties of synthesized AgNPs. Extracts of *Scytonema geitleri* HKAR-12 have the ability to reduce AgNO₃ to Ag⁰. Sharp peak at 422 nm indicated the rapid synthesis of AgNPs. FTIR results showed the presence of different groups responsible for the reduction of AgNO₃ to AgNPs. XRD pattern confirmed the crystalline nature of AgNPs. SEM showed the bead shape structure of AgNPs. TEM confirmed the actual size of AgNPs to be ranging between 9-17 nm. AgNPs showed antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli* strain1 and *E. coli* strain 2 and 11 µg/mL of AgNPs effectively inhibited the growth of MCF-7 cells. Hence, *Scytonema geitleri* HKAR-12, isolated from the rooftop could serve as a desirable biological candidate for convenient and cheap production of AgNPs having antimicrobial and anti-cancerous properties.

Keywords: Silver nanoparticles, Cyanobacteria, Green synthesis, Antibacterial activity, MCF-7 cells.

Abbreviations: Nanoparticles: NPs, Silver nanoparticles: AgNPs; double-distilled water: DDW.

1. INTRODUCTION

Metallic nanoparticles (NPs) have numerous applications in various fields of life, such as food processing, cosmetics, therapeutics and clinical diagnostics, electronics, agriculture and environmental remediation, wastewater treatment, packaging and textiles [1-14]. In spite of having several unique and novel properties, metallic NPs also causes several health hazards to living organisms including human which are related to its production. Hence, synthesis of NPs have raised severe concerns in the scientific community and this has led to the exploration of non-toxic and environmentally benign methods of its biosynthesis utilizing renewable sources of energy, are operable at low temperature and consume less energy [14-15].

Cyanobacteria, the ecologically and economically important autotrophic prokaryotes appeared on the Earth ~2.5 billion years ago and are very successful oxygenic photosynthesizers [16]. They are important primary producers of the Earth as they significantly contribute to the fixed carbon budget in terrestrial and aquatic ecosystems as well. Cyanobacteria produce several value-added products of commercial as well as of ecological importance [17-20]. Several metabolites both extracellular, as well as intracellular, are produced by several strains of cyanobacteria having antifungal, antibacterial, antiviral and antialgal potentials [19, 21].

Cyanobacteria have been utilized in the biological synthesis of metallic NPs [22-28] as they are a potential source of pharmaceuticals, biofuels, colored pigments and other important biomolecules [19-21]. They are important microorganisms which have the capability to fix atmospheric N₂. The enzyme "nitrogenase" reduces atmospheric dinitrogen gas (N₂) to

ammonia. Cyanobacteria are a better biological candidate for NPs synthesis because of having a high growth rate and biomass productivity [14].

AgNPs have plasmonics property in the visible region and exhibit broad-spectrum biocidal activity. In comparison to other metals, silver is not very toxic for animals including humans. Several studies reported synthesis of AgNPs using fungi, bacteria, and algae, but these studies have utilized whole cell masses in synthesis of AgNPs [24-30]. However, few workers have employed cell extract for the synthesis of metal NPs [24, 31]. Cell extracts of the cyanobacterium *Anabaena doliolum* were utilized for synthesis of AgNPs [32]. Husain et al. [33] investigated biosynthesis of AgNPs from 30 different cyanobacterial strains. Filamentous cyanobacterium *Westiellopsis* sp. (A15) was used for synthesis of AgNPs by Lakshmi et al. [34]. Cyanobacterial culture filtrate, when exposed to aqueous Ag ions, were reduced into AgNPs. FTIR analysis revealed that protein was responsible for reduction of silver ions. Presence of several functional groups was revealed by FTIR characterization and studies also suggested the role of proteins or enzymes in the bio-reduction of Ag ions into AgNPs [35]. Sonker et al. [31] synthesized AgNPs utilizing the cell extract of the cyanobacterium *Nostoc* sp. strain HKAR-2 isolated from hot springs. Use of cyanobacterial extracts for the NPs biosynthesis is being widely practiced these days as extraction of NPs formed inside the cell is a very cost-ineffective and complex process.

In the present investigation, we used the cell extract of the rooftop dwelling cyanobacterium *Scytonema geitleri* HKAR-12 for the

synthesis of AgNPs. We also attempted to screen the *in vitro* anticancerous and antibacterial activity of the biosynthesized

2. EXPERIMENTAL SECTION

Preparation of Cell Extract for Green Synthesis of AgNPs.

Culture of rooftop dwelling cyanobacterium *Scytonema geitleri* HKAR-12 was grown at $28 \pm 2^\circ\text{C}$ in a culture room under axenic conditions in autoclaved BG-11 (without nitrogen) medium under continuous fluorescent white light of 12 Wm^{-2} with a 14/10 light/dark cycle. To prepare the cell extract, pellet of *Scytonema geitleri* HKAR-12 (10.0 g wet weight) was suspended in 100 mL of sterile double-distilled water (DDW) and sonicated in a Sonicator (Sonics Vibra™) for 5 min at maximum output and duty cycle. The cell-free extract was centrifuged at $10,000 \times g$ for 15 min and filtered through Whatman No. 1 filter paper.

For the synthesis of AgNPs, the cell extract (pH 7.0) was distributed equally (50 mL each) into two flasks. To one flask, AgNO_3 was added to attain a final concentration of 1 mM. The second flask contained only cell extract and served as the positive control. Both the flasks were kept in light and incubated at 25°C for 102 h. The formation of AgNPs was monitored visually at an interval of 2 h by observing the change in color of the reaction mixture. The bioreduction of silver ions was monitored by the spectroscopic analysis of the reaction mixture in the range of 200-800 nm in a UV-VIS spectrophotometer at known time intervals. For the quantitative estimation, the AgNPs formed in the cell extract from known amount of culture (wet weight) were centrifuged at $10,000 \times g$ at 4°C for 15 min and the weight of the dried pellet was determined [32, 36].

Biosynthesis of AgNPs.

For synthesizing AgNPs, equal volume (5 mL) of cyanobacterial cell-free extract and AgNO_3 solution was mixed, and incubated in light at 25°C for 120 h. The synthesis of AgNPs was observed by recording the change in color of the reaction mixture. Cell extract served as a positive control and 1 mM AgNO_3 solution as the negative control. Conditions such as AgNO_3 concentration, different environmental conditions (dark, light), pH, the volume of cyanobacterial extract, temperature, and time were optimized for optimum AgNPs synthesis.

Characterization of Nanoparticles.

UV-VIS Spectroscopy. UV-VIS spectrophotometer was used for evaluating the synthesis of AgNPs. Absorption was taken at different time interval by taking 2 mL mixture which contained AgNO_3 and cyanobacterial extract of *Scytonema geitleri* HKAR-12. Absorption spectra were taken between 200-800 nm by using Hitachi-UV-1800 spectrophotometer.

FTIR Observations. FTIR analysis of the centrifuged and lyophilized sample was performed for identifying possible functional groups present on AgNPs surfaces (By using Varian 3100 FTIR spectrophotometer). For FTIR, at room temperature, a small amount of dried AgNPs was grinded along with KBr pellet having a resolution of 4 cm^{-1} and range of $400\text{-}4000 \text{ cm}^{-1}$. Recording of the spectra of the extract of *Scytonema geitleri* HKAR-12 was done prior and after the biosynthesis of AgNPs.

Scanning Electron Microscopy (SEM). Morphological characterization of the AgNPs was done by using SEM (Quanta-

AgNPs.

200 FEI, Netherland). For SEM, on a copper grid (carbon coated), thin film of the sample was formed by dropping of a small amount of the sample on the grid, and with the help of blotting paper extra solution was removed followed by drying the film on the SEM grid by putting it under a mercury lamp for 5 min.

Transmission Electron Microscopy (TEM). TECNAI G2-TWIN-FEI TEM (Transmission electron microscopy) was used to determine the size of biologically synthesized AgNPs. Biosynthesized AgNPs were sonicated for preparing the sample. Then the sample (a drop) was kept on a copper grid (carbon and formvar coated) followed by drying under infrared lamp prior to the experiment. TEM was carried at an accelerating voltage of 200 Kv.

XRD Analysis. The XRD analysis (PAN analytical X pert PRO Model) was done to determine the dimension of biologically synthesized AgNPs with h, k, l value. The aqueous solution of AgNPs (45 mL) synthesized from cell extract of *Scytonema geitleri* HKAR-12 was centrifuged for 30 min at 10,000 rpm and pellet was dissolved in DDW (5 mL) followed by lyophilizing the sample (Christ Alpha 1-2 LD plus) to get the NPs in powder form. The diffraction pattern was obtained with conditions at 40 kV and 30 mA in Cu, K-alpha radiation. Debye-Scherrer's equation was utilized for determining the particles size (L) of the AgNPs.

$$L = 0.9\lambda / \beta \cos\theta$$

Where, X donates the wavelength of the X-ray, β is full width and half maximum and θ is the Bragg's angle.

Antibacterial Activity. The AgNPs synthesized from the cyanobacterial extract of *Scytonema geitleri* HKAR-12 were tested for their antimicrobial activity by disc diffusion method against bacteria like *Pseudomonas aeruginosa*, *Escherichia coli* strain 1 and *E. coli* strain 2 following standard method [37]. The pure cultures of organism were subcultured on nutrient agar medium at 35°C on rotary shaker at 200 rpm. Using sterile glass spreader, bacterial strains were swabbed uniformly on the plates. Antibiotic of disc size 3 mm had been kept on nutrient agar plates. $10 \mu\text{g/mL}$ of AgNPs solution were soaked into separate discs on the plates. Streptomycin was tested for the sensitivity towards the three bacterial strains. For this study, Streptomycin subcultured as positive control and DDW taken as negative control. The plates with NPs treatment and control were incubated at 35°C for 18 h, followed by the measurement of zone of inhibition.

Antitumor Activity. MTT (3-[4, 5-dimethylthiazol-2-yl] 2, 5-diphenyltetrazolium bromide) (Sigma-Aldrich Company, St. Louis, Mo) assay was utilized for assessment of *in vitro* cytotoxicity and survival of cells on AgNPs toxicity. In 24 well plates (with a density of 40,000 cells/well/2 mL media), MCF-7 cell line (exponentially growing) was seeded followed by incubation at 37°C . The media was replaced with fresh complete DMEM media (2 mL) after 18 h of incubation. Sterilized phosphate buffer saline (PBS) (20 μL) having NPs (of varying concentration) was added in each well and incubated at 37°C .

Experimental setup with AgNP-free phosphate-buffer saline was taken as control. Cells were washed thoroughly with sterile PBS after 48 h of incubation with NPs for proper removal of NPs from the cells for preventing any interference with MTT reagent. To each well, fresh complete DMEM media (500 μ L) having 0.4 μ g of MTT reagent was added followed by incubating the plate at 37°C for 5 h. After that remaining MTT solution in medium was aspirated off. DMSO (500 μ L) was added for solubilizing the formed formazan crystals. The microtiter plate was shaken (10 min) and color of formazan crystals (purple) was measured by recording the optical density (OD) at 570 and 630 nm using a microplate reader (Spectra Max M2, MTX Lab System). For background correction, measured OD was calculated by subtracting the OD at 630 nm from that of at 570 nm. The number

of viable cells reflected the anticancerous activity as it is indirectly proportional to the number of cells which are viable which in turn, is proportional directly to OD. For control, OD of cells without any drug treatment was taken.

Percent inhibition = $(\text{Observed OD value} / \text{Control OD value}) \times 100$
 IC_{50} (50% inhibitory concentration) was used for expressing the anti-proliferative activity of biosynthesized AgNPs. IC_{50} denotes the concentration of the AgNPs which results in a 50% decrease in the control level of proliferation.

Statistical Analysis. All the experiments were performed in triplicate and results have been presented as mean values of three replicates. One-way analysis of variance was performed for statistical analyses.

3. RESULTS SECTION

Confirmation of biosynthesized AgNPs by UV-VIS Spectroscopy.

Change in color of the solution was observed from transparent solution to dark red after the addition of cyanobacterial extract to 1 mM $AgNO_3$ after 120 h of incubation (Fig. 1) due to the formation of AgNPs. With time, the intensity of color enhanced, and maximum change in color (dark brown) was recorded after 120 h (Fig. 1). On the other hand, the colour of $AgNO_3$ solution or cyanobacterial cell extract (light blue) did not changed even after 120 h of incubation. The absorbance of the sample was recorded between 200-800 nm by using Hitachi-2000 UV-spectrophotometer. The solution gave an absorbance peak, which was centered at 420 nm (Fig. 2). This peak was due to the phenomenon of surface plasmon resonance (SPR) exhibited by synthesized AgNPs. At different time intervals (0, 24, 48, 60, 84, 96 and 120 h) the absorption peak was recorded (Fig. 3). The SPR increased at 420 nm with increasing time interval, which indicated the enhanced synthesis of AgNPs. Different parameters were used for the effective synthesis of AgNPs, but it was observed that the best condition for the maximum synthesis of AgNPs was at room temperature on pH 7 with 25 mL of *Scytonema geitleri* HKAR-12 cell extract mixed with 20 mL of 1mM $AgNO_3$ solution in continuous light conditions. It was observed from the spectra that as time progressed the, rapid synthesis of AgNPs resulted in sharper and narrower peak at 420 nm. The initiation of reduction of $AgNO_3$ solution into AgNPs started after 12 h of the addition of $AgNO_3$ solution into the cell extract and reduction of $AgNO_3$ solution completed after 120 h of incubation.

FTIR analysis of biosynthesized AgNPs.

FTIR analysis of the AgNPs and cell extract indicated different peaks which represent different functional groups (Fig. 4). The interaction of NPs with biomolecules present in cell extract of *Scytonema geitleri* HKAR-12 showed intensive peak at 3405.89 cm^{-1} (O-H), 2925.74 cm^{-1} (C-N), 1633.75 cm^{-1} (C=O) and other peaks (Fig. 4 A), similarly, the stretching frequencies in formed AgNPs had the peaks at 3403 cm^{-1} (O-H), 2926 cm^{-1} (C-N) cm^{-1} (C-N), 2851 cm^{-1} (CH_3) and 1633 cm^{-1} (C=O) along with several new peaks (Fig. 4B).

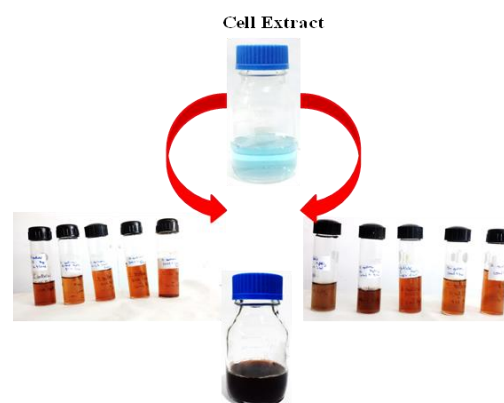


Figure 1. Change in color of solutions after 120 h of incubation. Cell extract of *Scytonema geitleri* HKAR-12 (light-blue) and reaction mixture containing *Scytonema geitleri* HKAR-12 cell extract and $AgNO_3$ (1 mM) (dark brown). The development of brown color was due to the bio-reduction of $AgNO_3$ to Ag^0 .

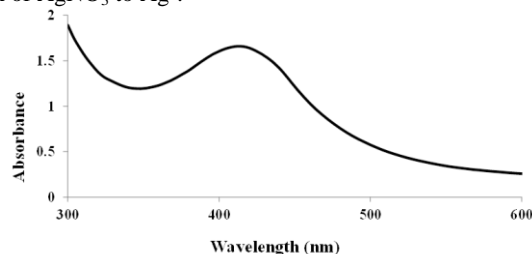


Figure 2. UV-VIS absorption spectrum of AgNPs synthesized from *Scytonema geitleri* HKAR-12 which was centred at 420 nm after 120 h of incubation.

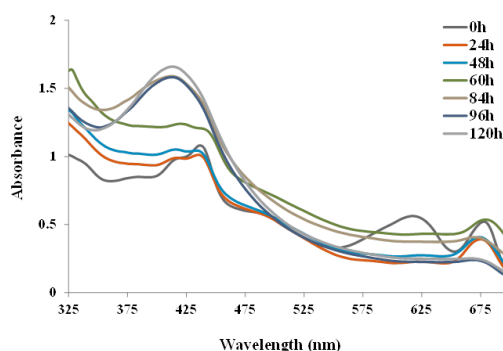


Figure 3. Absorption spectra at different time intervals from the aqueous solution of $AgNO_3$ solution having cell extract of *Scytonema geitleri* HKAR-12.

Synthesis of silver nanoparticles from extracts of *Scytonema geitleri* HKAR-12 and their *in vitro* antibacterial and antitumor potentials

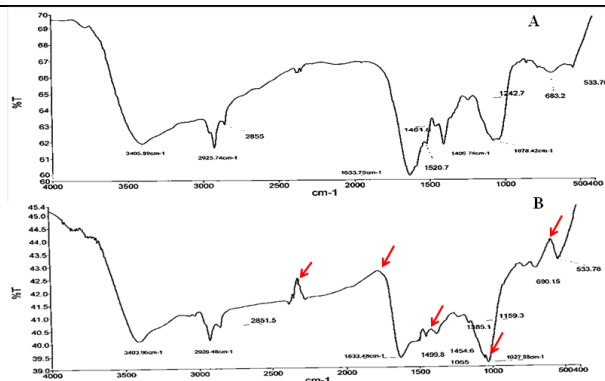


Figure 4. FTIR spectra of freeze-dried samples of AgNPs. (A) Cellular extract of *Scytonema geitleri* HKAR-12 (Control) and (B) Biosynthesized AgNPs from cell free extract of *Scytonema geitleri* HKAR-12 and AgNO₃ (1 mM) solution after 124 h of incubation. Arrows indicate new peaks appearing in the spectrum of synthesized AgNPs.

XRD pattern of AgNPs.

The nature of the AgNPs formed from the cell-free extract of *Scytonema geitleri* HKAR-12 was detected by using XRD (Fig. 5). Four intense peaks from 20° to 80° at 2θ were observed in the XRD pattern. When this whole spectrum was compared with standard, it was found that the AgNPs formed were crystalline in nature. The peaks at 2θ values were 32.58°, 46.56°, 55.12°, and 76.96° which corresponded with (111), (200), (220) and (311) plane for silver. The unassigned peaks might be because of the bioorganic phase crystallization which are usually present on the NPs surface.

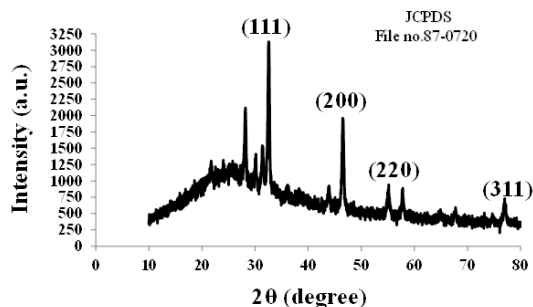


Figure 5. XRD pattern showing the facets of crystalline AgNPs after bioreduction. The peak at 2θ values 32.58°, 46.56°, 55.12°, and 76.96° corresponded to (111), (200), (220) and (311) plane for silver.

SEM images of biosynthesized AgNPs.

External morphology of biosynthesized AgNPs was demonstrated by using SEM.

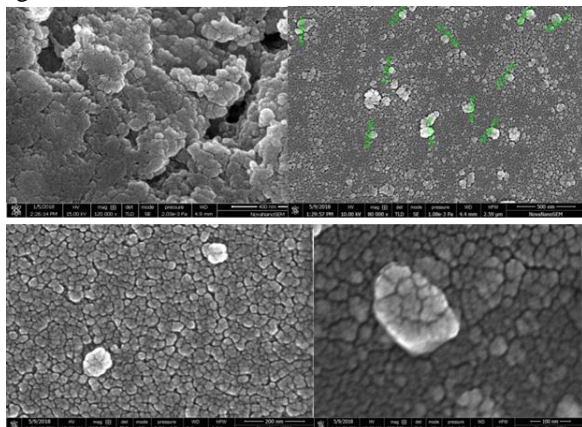


Figure 6. SEM micrograph images of biosynthesized AgNPs at different magnification showing the spherical-shaped structures.

SEM images (Fig. 6) confirmed that the metal particle was present in nano-sized. It was found from the SEM images that the AgNPs were spherical/bead-shaped in structure.

TEM images of biosynthesized AgNPs.

Size analysis of biosynthesized NPs is usually determined with the help of TEM. TEM images showed the size of NPs in the range of 9-17 nm (Fig. 7), which were spherical and well dispersed in nature.

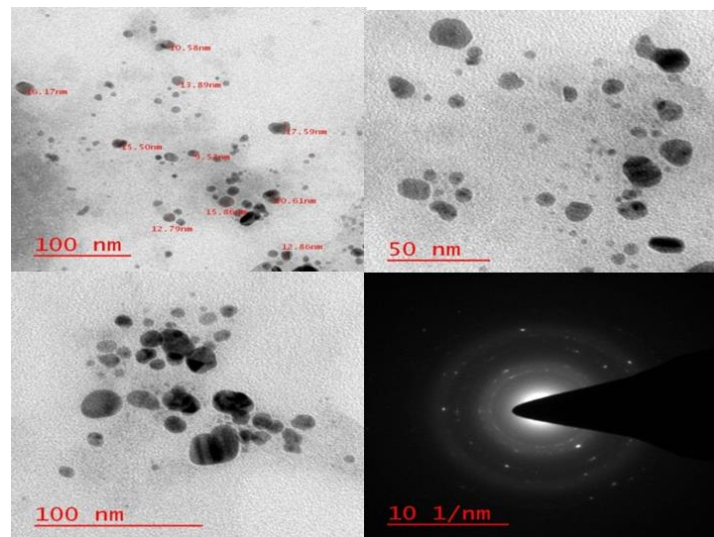


Figure 7. TEM images of biosynthesized AgNPs recorded on carbon coated copper grid.

Anticancerous activity of biosynthesized AgNPs.

MTT assay was used to analyse the cytotoxic effect of the synthesized AgNPs on the survival of MCF-7 cell line. AgNPs treated MCF-7 cells showed the cytotoxicity in dose dependent manner. The IC₅₀ was calculated with the concentrations of 50, 20, 10, 5, 2 and 0 μg/mL. Negative control for the experiment was AgNPs dissolved in the buffer. However, at lower concentration, the biosynthesized AgNPs did not show significant cytotoxicity, but their cytotoxicity enhanced with increasing concentration of AgNPs from 0 μL/mL to 50 μL/mL and minimum inhibitory concentration was found to be at 11 μg/mL (Fig. 8).

Antibacterial activity of biosynthesized AgNPs.

The antibacterial potential of synthesized AgNPs was investigated against three bacterial strains i.e., *Pseudomonas aeruginosa*, *Escherichia coli* strain 1, and *E. coli* strain 2 by using disc diffusion method (Fig. 9).

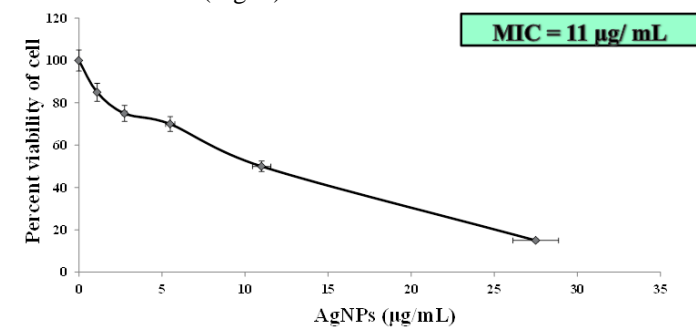


Figure 8. Determination of *in vitro* cytotoxicity of AgNPs against MCF-7 cells by MTT assay. Data are expressed as mean ± SD of three replicates. Cytotoxicity percentage is expressed relative to untreated controls.

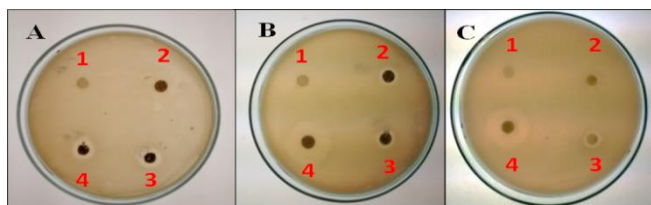


Figure 9. Zone of inhibition on three bacteria formed around the discs (A) *Pseudomonas aeruginosa* (B) *E. coli* strain 1 (C) *E. coli* strain 2. DDW served as negative control and Streptomycin served as the positive control. (Disc 1: DDW; 2: Cyanobacterial extract; 3: AgNPs; 4: Streptomycin).

The diameter of inhibition zones (cm) around each disc is shown in (Table 1) The zone of inhibition formed by AgNPs against *Pseudomonas aeruginosa*, *E. coli* strain 1, and *E. coli* strain 2 was found to be 0.8 ± 0.01 , 0.6 ± 0.01 and 1.3 ± 0.04 cm respectively at $10 \mu\text{g/mL}$ concentration of AgNPs respectively. DDW taken as a negative control and it did not showed any zone of inhibition whereas antibiotic streptomycin was used as positive control and showed a good zone of inhibition (2 cm) (Table 1).

Discussion.

Several workers have attempted synthesis of NPs from cyanobacterial extracts (extracellular) as well as from intact cellular mass (intracellular) in the last decade [14-15, 22-24, 33, 38-52]. Complete cells of *Plectonema boryanum* and *Oscillatoria willei* (non-nitrogen-fixing cyanobacteria) were utilized for synthesis of AgNPs (22-24). *Anabaena* sp. *Calothrix* sp. and *Leptolyngbya* sp. were utilized for intracellular biosynthesis of Au, Ag, Pd and PtNPs [35, 41]. Extracellular AgNPs were synthesized using *Arthrospira platensis* and size, number and shape of the AgNPs were found to be dependent on the duration of exposure and concentrations of the Ag ions [53]. Mahdieha et al. [42] attempted biosynthesis of crystallized AgNPs with *Spirulina platensis* in aqueous environment. Cyanobacteria such as *Aphanothece* sp., *Microcoleus* sp., *Oscillatoria* sp., *Phormidium* sp., *Aphanocapsa* sp., *Gleocapsa* sp., *Synechococcus* sp., *Lyngbya* sp. and *Spirulina* sp. were screened for NPs synthesis [43]. Rejeeth et al. [54] employed biological approach for synthesis of stable AgNPs which were screened for their anticancerous activity against MCF-7 cells.

In presence of nitrate ions, NADH and NADH-dependent nitrate reductase utilizes electron shuttle enzymatic metal reduction process for reducing Ag^+ to Ag^0 . It might be possible that same mechanism might be employed by *Westiellopsis* sp. (A15) for the bio-reduction of Ag^+ to Ag^0 and subsequently for the biosynthesis of AgNPs as NADH and NADH-dependent nitrate reductase are also secreted by the cyanobacterium. This cofactor and enzyme mediated bio-reduction of silver into AgNPs have also been demonstrated *in vitro* by Kumar et al. [55]. AgNPs and AuNPs were prepared by Kratošová et al. [46] using cells of *Dolichospermum*. Recently, biosynthesis and characterization of spherical-thermostable AgNPs from *Spirulina platensis* was reported by Kaliampurthi et al. [56]. Vanillin, tannins, coumarins, glycogen and amide may act as stabilizing agents for bioreduction of silver for AgNPs formation as indicated by FTIR spectra of AgNPs. Recently, Keskin et al. [48] showed biosynthesis of AgNPs as well as its photocatalytic activity for light-dependent

degradation of organic dye along with their antimicrobial potentials. Attenuated total reflection FTIR (ATR-FTIR) analysis showed the role of proteins in the bio-reduction of silver as reducing agents.

In the present study, the cell-free extract of *Scytonema geitleri* HKAR-12 was capable of reducing AgNO_3 solution to form AgNPs. UV-VIS spectra are one of the most sensitive and easy ways to test the synthesis of AgNPs. As mentioned above the solution gave an absorbance peak centered at 420 nm due to SPR of AgNPs indicating its biosynthesis. A comparative analysis of the FTIR spectra revealed that similar functional groups were share the cyanobacterial cell extract and AgNPs. These functional groups reflect presence of a significant amount of phenolic compounds in the cyanobacterial extract which could reduce the silver ions into AgNPs. There was a slight change in the frequency i.e appearance of new peaks in spectrum of AgNPs such as 2250 cm^{-1} , 1700 cm^{-1} in comparison to the *Scytonema geitleri* HKAR-12 cell extract. OH group has been found to be involved in the reduction of silver ion into AgNPs. Wave band at 2925.74 cm^{-1} in cyanobacterial extract and 2926 cm^{-1} band in synthesized NPs correspond to the C-N stretching of the amine. Wave band 1633 cm^{-1} present in biosynthesized NPs might be responsible for the adsorption of biomolecule on the surface of NPs. Presence of proteins is indicated by several other peaks which are usually responsible for the stability of AgNPs. FTIR spectra of freeze-dried sample of biosynthesized NPs showed different functional groups such as carbonyl, carboxyl and hydroxyl groups of amino acids and proteins, which reduce the AgNO_3 into the silver ions and stabilized the NPs. Stretching frequencies also reflect the presence of certain aromatic amino acids like phenylalanine, tryptophan and tyrosine, which could induce the formation of AgNPs [24]. The XRD data confirmed the crystalline nature of AgNPs and this is favored by 111 facets [59]. Three other peaks may be impurities, due to addition of other organic substances to the cyanobacterial culture. The X-ray diffraction pattern peaks were broad around their bases which indicate that the AgNPs were in nano size. SEM results showed the bead shape structure of AgNPs. Further analysis with TEM also confirmed the size of AgNPs (9-17nm). The sharp peak at 422 nm indicated the rapid synthesis of AgNPs and FTIR data indicated the presence of different groups responsible for the reduction of AgNO_3 to AgNPs. XRD pattern confirmed the crystalline nature of AgNPs. Studies on the cytotoxicity of biogenic AgNPs against cancer cell lines are still in its early stage. However, several *in vitro* studies have been done on the cytotoxic effect of AgNPs on mammalian cells [58]. Toxicity of AgNPs against MCF-7 breast cancer cells was done using AgNPs synthesized from the plant *Annona squamosa* [59]. Very few reports are available on cytotoxicity of biosynthesized AgNPs synthesized from cyanobacterial cell extracts on MCF-7 breast cancer cell lines by using MTT assays. In the present study $11 \mu\text{g/mL}$ concentration of AgNPs effectively killed the MCF-7 cells. The possible cytotoxic mechanism of these biosynthesized AgNPs is still not clear but some studies state that biosynthesized AgNPs causes DNA damage and induce apoptosis in tumor cells and also affects the gene involves in cell cycle regulation [60-62].

Synthesis of silver nanoparticles from extracts of *Scytonema geitleri* HKAR-12 and their *in vitro* antibacterial and antitumor potentials

Table 1. Zone of inhibition formed by AgNPs synthesized from the cellular extract of *Scytonema geitleri* HKAR-12 against three bacterial strains.

Bacteria	Cyanobacterial extract	Antibacterial activity: Zone of inhibition (cm)		
		Zone of inhibition formed by AgNPs (10 µg/mL)	Positive Control streptomycin (25 µg/mL)	Negative Control (DDW)
<i>Pseudomonas aeruginosa</i>	-	0.8±0.01	1.0±0.03	-
<i>E. coli</i> strain 1	0.6±0.08	0.6±0.01	2.2±0.02	-
<i>E. coli</i> strain 2	0.4±0.02	1.3±0.04	2.0±0.05	-

4. CONCLUSIONS

Since long back, silver-based compounds have been used as antibacterial agent as they have strong antibacterial properties against various microorganisms [63-72]. Earlier studies have shown the antibacterial activity of biosynthesized NPs [73-75, 29]. AgNPs have small size but a larger surface area, which contribute to the antibacterial activity of AgNPs [76-77]. Size, shape, solubility, and charge on the biosynthesized NPs are crucial for their biological properties as antimicrobials [29, 78]. Large surface area of AgNPs due to their small size provide them better surface with the bacteria/microbial cell surface. Microbial cellular physiology/functioning gets disturbed by such interaction resulting in the defacing of bacterial cell membrane. Here also AgNPs showed significant antibacterial activity against *Pseudomonas aeruginosa*, *E. coli* strain 1 and *E. coli* strain 2.

The formation and stabilization of the NPs is facilitated by the vast array of active biomolecules present in the cell extract of cyanobacteria [32, 36, 48], hence cyanobacteria could serve as a potent candidate for green synthesis of NPs and their conjugates with novel secondary metabolites produced by these organisms. The mechanism underlying the antimicrobial action including as antibacterial agent of AgNPs is still not completely understood. Some reports suggest that AgNPs produce free radicals which create pores in cell wall of bacteria, hence, changing the cell membrane's permeability and finally causing cell death [79-84]. Overall, it can be concluded that the extract of *Scytonema geitleri* HKAR-12 have the ability to reduce AgNO₃ to Ag⁰ and could serve as a good candidate for NPs biosynthesis.

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