Green Synthesis, Characterization, Antimicrobial Potential and Cytotoxic Evaluation of Silver Nanoparticles derived from Marine Seaweed *Valonia Utricularis*

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Abstract

Few studies have been conducted on the production of silver nanoparticles from marine seaweeds using environmentally friendly green approach. In the current investigation, aqueous extract of Valonia utricularis (green marine algae) was used to produce silver nanoparticles and to assess their antimicrobial activity against clinically important pathogens and in vitro cytotoxic effect against HCT116 and HeLa cell lines. The effective synthesis and functionalization of the produced AgNPs were verified by characterization using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), SEM and TEM.

The biosynthesized AgNPs exhibited most potent antimicrobial protection against clinically important Gram-positive, Gram-negative and fungal pathogens and remarkable dose dependent cytotoxic effect against HCT116 and HeLa cell lines. In the future, the above environment friendly AgNP synthesis process might be readily scaled up to overcome the global challenge of multi drug resistance and treatment of cancer to meet the industrial demand.

Keywords: Green synthesis, *Valonia utricularis*, Silver nanoparticles, Antimicrobial activity, Cytotoxicity.

Introduction

Nanotechnology is a relatively young field of study that focuses on the synthesis and production of various nanoparticles with a variety of biological and pharmaceutical uses[5,33]. Since the last decade, the highest focus has been given to the physical and chemical processes for the creation of various types of nanoparticles[19,21]. The physical and chemical processes show that the metallic nanoparticles are toxic and not safe for the environment[1,6,15,22].

Recently to overcome this challenge, need of less toxic, cheap and environment friendly biological protocols to produce the metallic nanoparticles[2,5] is very necessary. Only a few publications exist regarding the production of nanoparticles utilizing marine algae, but the green synthesis of AgNPs is limited to plants, fungi, yeast, bacteria and algae[11,12,32].

The marine algae are available throughout the year and conveniently accessible to harvest in Gulf of Mannar's which is one of the richest coastal regions in the South East Asia for marine biological resources[4]. Among the biological materials, marine algae are called as bio-nano-factories because polysaccharides, proteins and other bioactive substances present in cell membranes have the ability to function as capping and reducing agents[9,10,20]. Over the period of time, silver has been gaining significant attention due to best antimicrobial and cytotoxic potential against approximately 650 types of infectious pathogens and various human cancer cell lines increasing the therapeutic out come at low concentratio[7,23].

Marine algae extract synthesis of AgNPs has attracted the attention of researchers because it is more suitable for large scale production of nanoparticles due to high metal uptake capacity, low cost and nontoxicity compared to other physical and chemical approaches[27]. The recent challenge is emergence of multi drug resistance due to excessive use of antibiotics. Incomplete treatment reduces the efficacy of antibiotics[13].To resolve the current issue, the large surface area to volume ratio of AgNPs shows broad spectrum of antimicrobial and anticancer potential against pathogenic microbes and human cancer cell lines[14,28,29].

The current study focuses on biosynthesis of AgNPs using macro algae *Valonia utricularis* (Green marine algae) collected from Mandapam, Gulf of Mannar's coastal region of Tamil Nadu to characterize the physiochemical properties for evaluation of antibacterial, antifungal and *in vitro* cytotoxic activities, controlling microbial infections and cancer.

Material and Methods

Collection and Preparation of Seaweeds: The *Valonia utricularis* seaweed sample was collected at the low tidal coastal region of Mandapam, Gulf of Mannar[4]. After collection, the sample was cleaned with seawater to remove any obvious or undesired contaminants. This was followed by a rinse with regular tap water and a final wash with

distilled water. The specimen was air dried under shadow for two weeks at room temperature, grinded to powder and kept in polythene bag and stored at room temperature[18].

Preparation of Plant Extract: After adding five grams of powdered *Valonia utricularis* seaweed to 100 milliliters of Milli-Q water, the mixture was boiled for 15±1 minutes. After the extract came to room temperature, Whatmann no. 1 filter paper was used for the filtering process and finally filtered extract was stored at 4 °C for potential use.

Biosynthesis of Silver Nanoparticles: Fresh seaweed extract was mixed separately with an aqueous solution of silver nitrate (1 mM) (Loba chemie, India) to achieve a 9:1 ratio. For three hours, this solution was kept on a shaker that rotated continuously at a temperature of 30 ± 2°C. The observed shift in color within the mixture, from a light green appearance to a darker shade, served as evidence of the reduction of Ag ions, showing that AgNPs are forming[34].

Characterization of Synthesized Silver Nanoparticles: The precursor silver nitrate salt solution (Loba chemie, India) was used to create AgNPs with *Valonia utricularis* seaweed extract using UV-Vis spectroscopy. Employing distilled water as a reference, UV-Vis spectrometer (Shimadzu-UV 1800) was utilized to evaluate the sample absorbance spectrum, across the range of 200–800 nm[25]. Using an ALPHA II small FTIR spectrometer (Bruker) in the 4000–400 cm^{-1} wavelength range, the functional groups within *Valonia utricularis* seaweed extract, playing a key in stabilizing AgNPs and reducing the metal, were determined by an examination of FTIR spectroscopy[8]. An examination of XRD was conducted using a 2θ angle pattern with monochromatic Cu Kα radiation (k = 1.5406 Å) at 40 kV and 30 mA using XPERT-PRO to examine AgNPs' crystalline structure. The scanning range encompassed 208 to 808. The acquired patterns were compared with the Joint Committee on Powder Diffraction Standards (JCPDS) database using cross-referencing to ascertain the crystalline structure of the nanomaterial[16].

To study the form and structure of the AgNPs, an FEI Quanta 200 FEG MKII scanning electron microscope (SEM) from FEI, USA was employed. This instrument functions as an environmental microscope (ESEM) and is characterized by its high resolution and high vacuum capabilities. Operating as a high-output thermal field emission microscope, it delivers a beam current exceeding 100nA and offers a resolution of 1.5 nm. Additionally, it is equipped with an 18 mm high-sensitivity backscatter (BSE) detector for enhanced atomic number contrast.

A TEM study was performed to ascertain the AgNPs shape, size and arrangement. The TEM assessments were executed at 200 kV utilizing the HITACHI H-800 instrument. On a copper grid coated with carbon, a tiny amount of the diluted bio-reduced solution was added to form the TEM grid, subsequently desiccated under a lamp[31].

Antimicrobial and MIC Assay: The antimicrobial activity and MIC of the green synthesized AgNPs were conducted against 11 microbial strains; 4 Gram-positive bacterial strains, including *S. aureus* (MTCC 96), *S. mutans* (MTCC 497), *M. luteus* (MTCC 106) and *S. epidermidis* (MTCC 435); 5 Gram-negative bacteria strains, including *E. coli* (MTCC 443), *V. cholerae* (MTCC 3906), *P. aeruginosa* (MTCC 424), *E. aerogenes* (MTCC 111) and *K. pneumoniae* (MTCC 109) and 2 fungal strains including *A. niger* (MTCC 404) and *C. albicans* (MTCC 227) using the well-diffusion method. The microbial strains were collected from the Microbial Type Culture Collection (IMTECH, Chandigarh, India).

Sterile Petri plates (Himedia, India) were utilized to pour the 25 mL of Mueller Hinton (Himedia, India) agar as well as potato dextrose agar (Himedia, India) and were left to solidify. 100 µl of the aforementioned bacteria were cultured for 18±2 h (with the optical density (OD) set to 0.6). 100 µl fungal spore suspension (105 cfu/mL) of mycelial fungal strain and 18±2 h-old yeast strain (105 cfu/mL) were transferred on agar surface of media and then sterile cotton swab spreader was used to create a culture lawn. The 6 mm wells were prepared on the agar surface using a sterile borer.

Different concentrations 0 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL and 0 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL of AgNPs were prepared in sterile water to check the antimicrobial sensitivity testing and MIC of biosynthesized AgNPs and introduced into 6 mm wells. The positive control for bacterial and fungal strains was azithromycin 30 µg/ml (Hi media, India) and clotrimazole 30 µg/mL (Hi media, India) and sterile water loaded well served as a negative control. The bacterial plates were incubated at 37±2 °C for 24±2 h and fungal plates at 30±2 °C for 48–72 h in different incubators, The diameter of the inhibition zone around the well was measured in millimeters (mm) using the antibiotic zone scale (Himedia, India) to calculate the antimicrobial efficiency and MIC of AgNPs against microbial strains. The experiment was carried out three times[3].

***In vitro* Cytotoxicity Assay**

Preparation of HCT116 and HeLa Cell Suspension: The HCT116 and HeLa cancer cell lines were obtained from the National Center for Cell Science (NCCS), India. Following the discard of the culture medium, a subculture of HCT116 and HeLa cells in Dulbecco's Modified Eagle's medium (DMEM) (Himedia, India) was trypsinized separately. The disaggregated cells in the flask were treated with 25 mL of DMEM containing 10% FCS (Himedia, India). The cells were gently floated in the liquid using a pipette before being homogenized.

Seeding of Cells: AgNPs of *Valonia utricularis* at varying concentrations including 0 µg/mL (Control), 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL, were added to each well of a 24-well culture plate along with 1 mL of

the homogenized cell suspension. The plates were then incubated at 37°C in a humidified CO_2 incubator with 5% CO_2 concentrations. The cells were examined under an inverted tissue culture microscope following a 48-hour incubation period. Cell confluence of 80% was used for the cytotoxic assay.

MTT Assay: The cytotoxicity assay was carried out with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) procured from Central Drug House in India. In viable cells, mitochondrial enzymes convert MTT to purple formazan, which correlates with cell viability but declines with cytotoxicity. After 48 hours of incubation, MTT (5 mg/mL) was applied and incubated for 2 hours at room temperature. The solution was removed, formazan crystals were dissolved in 100 µL of DMSO and absorbance was measured at 570 nm using a Readwell Touch microplate reader[26].

Statistical Analysis: All data were analyzed using the MS Excel, with data (n=3) expressed as mean ± standard deviation. Statistical analysis was conducted using Student's t-test and one-way ANOVA. Statistical significance was set at $p < 0.05$.

Results and Discussion

Biosynthesis of Silver Nanoparticles: Employing an eco-friendly approach, AgNPs were biosynthesized using an aqueous extract obtained from *Valonia utricularis* seaweed and 0.01 M silver nitrate solution. As seen in fig. 1, there was a discernible shift in the solution color from light green to dark brown which was the result of reduction of silver ions by green marine macroalgae (*Valonia utricularis*), providing the visual confirmation of green synthesis of AgNPs.

UV-Visible Spectroscopy Analysis: The extraction of *Valonia utricularis* marine green algae was used to synthesize AgNPs, as demonstrated by the UV-visible absorption maxima seen in the regions of 428 nm (0.679 OD) (Figure 2). The band indicated that the solution of AgNPs absorbed in the range of 400–450 nm[3,17].

FTIR Analysis: The presence of a certain functional groups interacted and reduced the AgNPs in the *Valonia utricularis* seaweed which was examined using the FTIR spectra (Figure 3). The peak attributed to AgNPs was due to (O-Si-O network and ring opening) vibration, which was analyzed at 528 to 567 cm^{-1}. The observed band at 620 cm^{-1} shifted to the lower frequency at 600 cm^{-1}, suggesting the stretching of the alkyl group's C–Cl bond. The vibration of carboxylic acids shifted the O-H stretching AgNPs from 3292 to 3337 cm^{-1} (Figure 3). In this study, the swift capping and reduction of silver ions into AgNPs may have been facilitated by the presence of proteins and flavonoids in the water based extract of green marine algae *Valonia utricularis*[5].

Figure 1: (A) Aqueous extract of *Valonia utricularis* (B) Silver nitrate solution (C) AgNPs synthesized from seaweed aqueous extract of *Valonia utricularis*

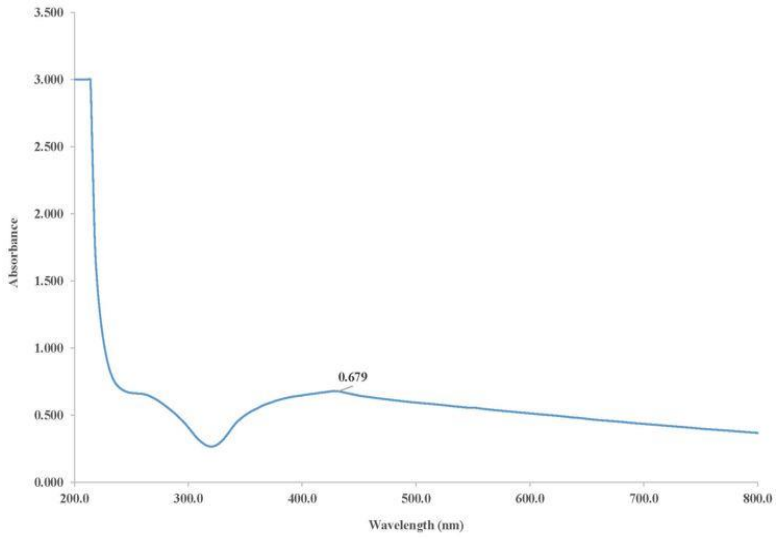

Figure 2: Using the aqueous extract of the seaweed *Valonia utricularis*, AgNPs in the UV-visible spectrum

XRD Analysis: X-ray diffraction (XRD) was used to characterize the manufactured AgNPs utilizing extract from *Valonia utricularis* seaweed in order to verify the particles' silver content and provide structural details. The AgNPs XRD pattern is displayed in figure 3. The primary peaks of the pattern are located at (2 theta) 38.19, 44.37, 64.56 and 77.47 respectively. These correspond to the (111), (200), (220) and (311) planes. It is discovered that the predominant pattern of green-synthesized AgNPs has a face-centered cubic geometry by comparing JCPDS (file no: 89-3722)[5,24].

FESEM Analysis: The AgNPs FESEM pictures are displayed in figure 4. AgNPs appeared to be spherical in shape and even surface morphology. The current study's particle size histogram falls between 50 and 60 nm in size.

TEM Analysis: TEM was used to examine the AgNPs that were biosynthesized in terms of size and shape. The TEM micrograph (Figure 4) indicates that the created AgNPs were found spherical in shape with average size of about 60 nm.

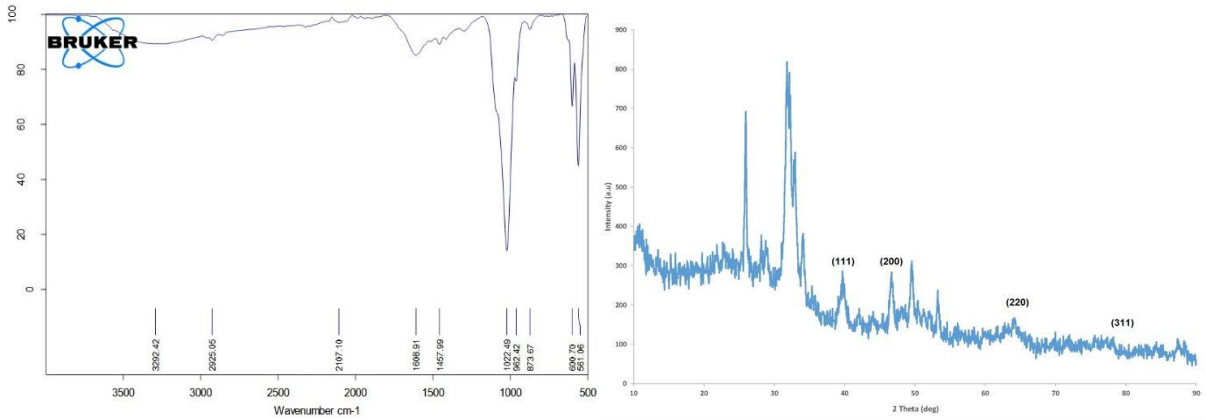

Figure 3: The FTIR and XRD spectra revealed the biosynthesized AgNPs using the aqueous extract obtained from *Valonia utricularis.*

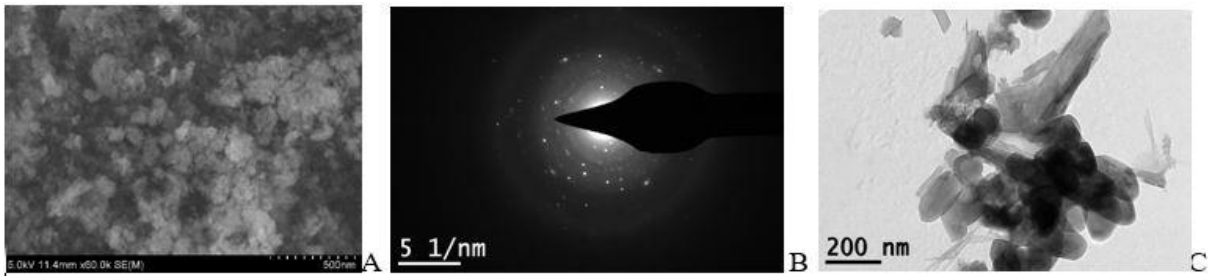

Figure 4: Using an aqueous extract of *Valonia utricularis*, the (A) SEM (B, C) TEM pictures of green generated AgNPs

Table 1
Antimicrobial efficacy of biosynthesized AgNPs using aqueous extract of *Valonia utricularis* against selected human pathogenic strains of bacteria and fungi. ZOI: Zone of inhibition
***Azithromycin as a positive control for bacterial strains *Clotrimazole as a positive control for fungal strains**

Bacterial Strain	**ZOI (mm)**					**ZOI (mm) Standard***
	0 µg/mL	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	30 µg/mL
Escherichia coli	-	23 ± 0.6	24 ± 0.7	25 ± 0.6	26 ± 0.8	14 ± 0.5
Enterobacter aerogenes	-	26 ± 0.7	27 ± 0.8	27 ± 0.6	29 ± 0.9	32 ± 0.8
Klebsiella pneumoniae	-	30 ± 0.6	31 ± 0.7	31 ± 0.8	32 ± 0.7	14 ± 0.5
Micrococcus luteus	-	14 ± 0.5	14 ± 0.6	15 ± 0.7	16 ± 0.6	16 ± 0.5
Pseudomonas aeruginosa	-	25 ± 0.7	26 ± 0.6	27 ± 0.8	29 ± 0.9	14 ± 0.4
Staphylococcus aureus	-	22 ± 0.6	24 ± 0.7	24 ± 0.8	25 ± 0.7	19 ± 0.6
Staphylococcus epidermidis	-	10 ± 0.4	11 ± 0.5	11 ± 0.6	11 ± 0.5	24 ± 0.7
Streptococcus mutans	-	25 ± 0.6	25 ± 0.7	26 ± 0.8	27 ± 0.9	8 ± 0.3
Vibrio cholerae	-	12 ± 0.5	13 ± 0.6	14 ± 0.7	15 ± 0.8	29 ± 0.8
Aspergillus niger	-	10 ± 0.4	11 ± 0.5	12 ± 0.6	14 ± 0.7	20 ± 0.6
Candida albicans	-	7 ± 0.3	8 ± 0.4	9 ± 0.5	11 ± 0.6	15 ± 0.5

Antimicrobial and MIC Assay: The AgNPs synthesized via green methods, employing extracts from *Valonia utricularis* exhibited strong antimicrobial potential against human pathogens at various concentrations (Figure 5). The *Streptococcus mutans and Staphylococcus aureus* were found more susceptible Gram-positive bacterial strains with maximum zone of inhibition 25mm and 22mm for 25 µg/mL concentration of AgNPs solution. The created AgNPs using green chemistry were shown strong antibacterial potential against Gram-negative pathogens, *Klebsiella pneumoniae* (zone of inhibition 30mm for 25 µg/mL) followed by *Enterobacter aerogenes* (zone of inhibition 26mm for 25 µg/mL) than Gram-positive human pathogenic bacteria as shown in table 1. *Candida albicans* was shown slightly more resistance against AgNPs with zone of inhibition (7mm for 25 µg/mL) than *Aspergillus niger* with inhibition zone (10 mm for 25 µg/mL) and other strains of pathogenic bacteria as mentioned in table 1.

The assessment of the minimal inhibitory concentration (MIC) for AgNPs derived from *Valonia utricularis* was conducted against multiple test pathogens, as depicted in (Figure 6). Table 2 demonstrates that the diameter of the inhibition zone enlarged proportionally with the rise in AgNPs concentrations. In this investigation, MIC of green synthesized AgNPs was found to be 12.5 µg/mL for the following microorganisms: *M. luteus, S. epidermidis* (16 mm), *P. aeruginosa* (14 mm), *A. niger, C. albicans* (13 mm), *S. aureus* (12 mm), *E. coli, K. pneumoniae, V. cholerae* (10 mm), *E. aerogenes* (9 mm) and *S. mutans* (8 mm).

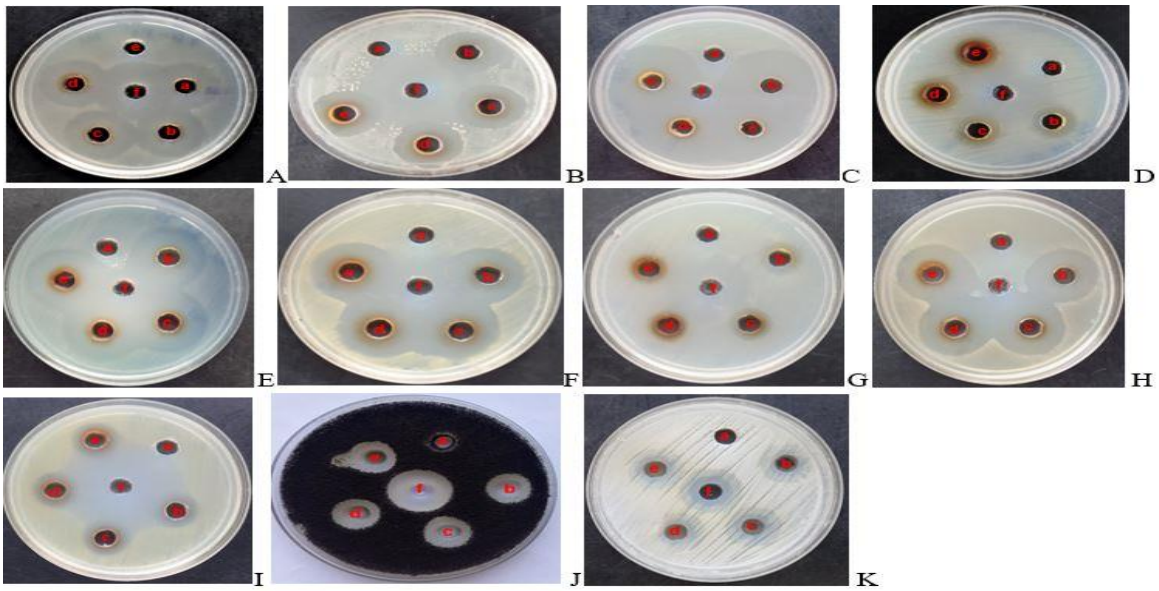

Figure 5: Antimicrobial potential of biosynthesized AgNPs against (A) *E. aerogenes* (B) *E coli* (C) *K. pneumoniae* (D) *M. luteus* (E) *P. aeruginosa* (F) *S. aureus* (G) *S. epidermidis* (H) *S. mutans* (I) *V. cholerae* (J) *A. niger* (K) *C. albicans* at various concentration such as a: 0 µg/mL; b: 25 µg/mL; c: 50 µg/mL; d: 75 µg/mL; e: 100 µg/mL; f: Azithromycin (30 µg/mL)

Table 2
Minimum inhibitory concentration of synthesized AgNPs using aqueous extract of *Valonia utricularis* against selected strains of human pathogenic bacteria and fungi. ZOI: Zone of inhibition
***Azithromycin as a positive control for bacterial strains. *Clotrimazole as a positive control for fungal strains**

Bacterial Strain	**ZOI (mm)**					**ZOI (mm) Standard***
	0 µg/mL	12.5 µg/mL	25µg/mL	50 µg/mL	100µg/mL	200µg/mL
Escherichia coli	-	10 ± 0.5	12 ± 0.6	13 ± 0.6	21 ± 0.8	24 ± 0.9
Enterobacter aerogenes	-	9 ± 0.4	10 ± 0.5	13 ± 0.6	17 ± 0.7	22 ± 0.8
Klebsiella pneumoniae	-	10 ± 0.5	13 ± 0.6	16 ± 0.7	18 ± 0.8	25 ± 0.9
Micrococcus luteus	-	16 ± 0.6	18 ± 0.7	20 ± 0.8	25 ± 0.9	29 ± 1.0
Pseudomonas aeruginosa	-	14 ± 0.5	17 ± 0.7	22 ± 0.8	25 ± 0.9	28 ± 1.0
Staphylococcus aureus	-	12 ± 0.5	14 ± 0.6	16 ± 0.7	19 ± 0.8	24 ± 0.9
Staphylococcus epidermidis	-	16 ± 0.6	19 ± 0.7	22 ± 0.8	24 ± 0.9	28 ± 1.0
Streptococcus mutans	-	8 ± 0.4	9 ± 0.5	10 ± 0.6	14 ± 0.7	18 ± 0.8
Vibrio cholerae	-	10 ± 0.5	12 ± 0.6	14 ± 0.7	19 ± 0.8	23 ± 0.9
Aspergillus niger	-	13 ± 0.5	15 ± 0.6	18 ± 0.7	20 ± 0.8	25 ± 0.9
Candida albicans	-	13 ± 0.5	15 ± 0.6	17 ± 0.7	18 ± 0.8	25 ± 0.9

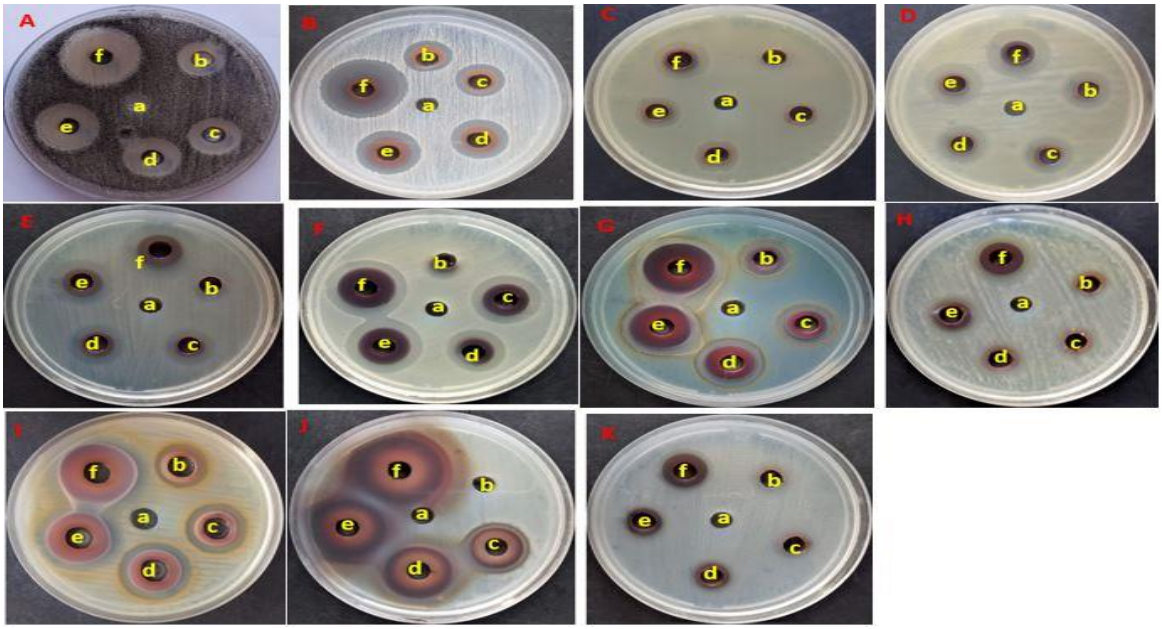

Figure 6: MIC of biosynthesized AgNPs against (A) *Aspergillus niger* (B) *Candida albicans* (C) *Escherichia coli* (D) *Enterococcus aerogenes* (E) *Klebsiella pneumoniae* (F) *Micrococcus luteus* (G) *Pseudomonas aeruginosa* (H) *Staphylococcus aureus* (I) *Staphylococcus epidermidis* (J) *Streptococcus mutans* (K) *Vibrio cholerae* at various concentration such as a: Sterile water; b: 12.5 µg; c: 25 µg; d: 50 µg; e: 100 µg; f: 200 µg

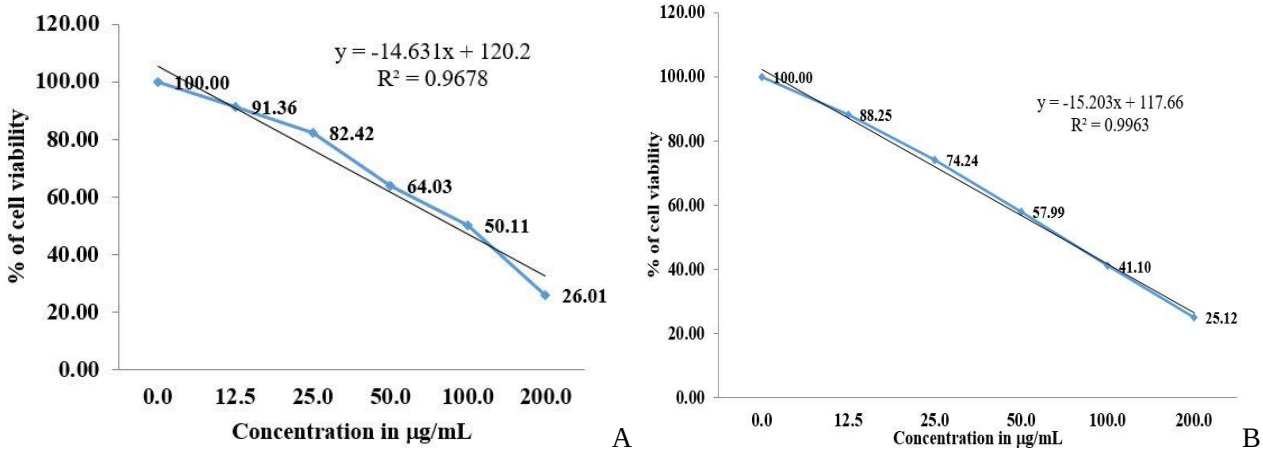

Figure 7: IC50 value of AgNPs against (A) HTC116 (B) HeLa cell lines

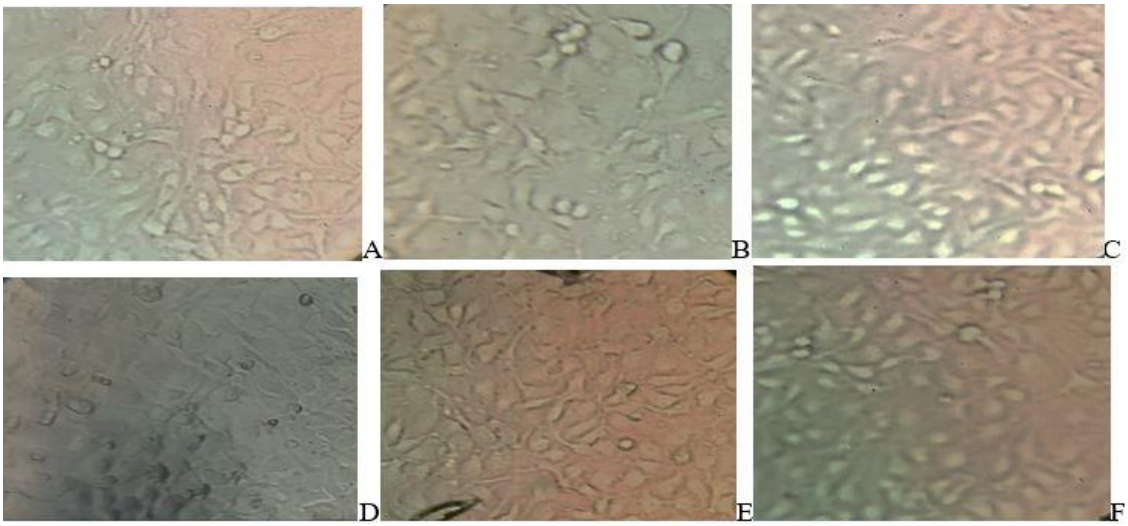

Figure 8: Microscopic images of morphological changes of AgNPs against HCT116 cancer cells at (A) 0 µg/mL (B) 12.5 µg/mL (C) 25 µg/mL (D) 50 µg/mL (E) 100 µg/mL (F) 200 µg/Ml

Table 3
The percent of cell inhibition of AgNPs synthesized using *Valonia utricularis* against HCT116 and HeLa cell line after 48 h. Data are expressed as mean ± SD (n = 3).

Concentration (µg/mL)	0.0	12.5	25.0	50.0	100.0	200.0
Cell Viability (%) of HCT116 Cell lines	100.00 ± 3.00	91.36 ± 2.74	82.42 ± 2.47	64.03 ± 1.92	50.11 ± 1.50	26.01 ± 0.78
Cell Viability (%) of HeLa Cell Lines	100.00 ± 3.00	88.25 ± 2.65	74.24 ± 2.23	57.99 ± 1.74	41.10 ± 1.23	25.12 ± 0.75

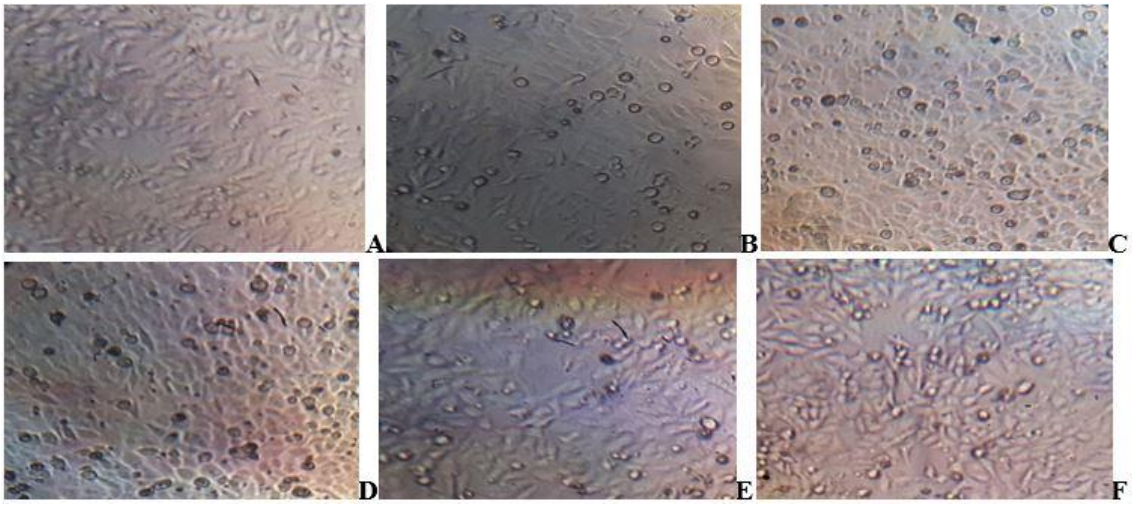

Figure 9: Microscopic images of morphological changes of AgNPs against HeLa cancer cells (A) 0 µg/mL (B) 12.5 µg/mL (C) 25 µg/mL (D) 50 µg/mL (E) 100 µg/mL (F) 200 µg/mL

***In vitro* Cytotoxicity assay:** The AgNPs had a notable dose-dependent cytotoxic effect on HCT116 and HeLa cells, as shown in table 3. At lower concentrations (12.5-25 µg/mL), there was a small reduction in cell viability. However, at a high concentration of 200 µg/mL after 48h treatment, cell survival decreased by 26.01% and 25.12% against HCT116 and HeLa cell lines respectively (Figure 7). The dose-response curves revealed IC_{50} values of 117.62 µg/mL for HCT116 and 105.49 µg/mL for HeLa cells, demonstrating slightly higher sensitivity to the therapy[19].

Morphological Damage Analysis: The viability statistics was further validated by microscopic analysis. At higher doses, AgNPs-treated cells clearly showed evidence of cytotoxic stress, while untreated cells showed normal morphology with intact membranes and adherence. These findings confirm that green-synthesized AgNPs have strong cytotoxic effects on both cancer cell lines (HCT116 and HeLa) in a concentration-dependent manner, as depicted in figures 8 and 9.

Conclusion

The present study successfully synthesized AgNPs utilizing an extract of green seaweed *Valonia utricularis*, paving the way for novel ways for an inexpensive, ecological and less harmful synthesis without the need of hazardous chemical reducers. The biosynthesized AgNPs demonstrated exceptional antimicrobial efficacy and cytotoxicity against multidrug-resistant microbial infections and HCT116 and HeLa cancer cell lines. The development of multi-drug resistance in microorganisms and the scarcity of novel anticancer agents are the global challenges which present a major risk to public health and motivate researchers to look into green marine algae for the production of AgNPs, an efficient treatment substitute for antibiotics and anticancer drugs in the pharmaceutical industry.

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References

1. Ahmad S., Munir S. and Zeb N., Green nanotechnology: A review on green synthesis of silver nanoparticles—An ecofriendly approach, *International Journal of Nanomedicine*, **14**, 5087-5107 **(2019)**

2. Anggraini W., Nisa S.C., Da R.R. and Ma B., Antibacterial activity of 96% ethanol extract of *cantaloupe* (Cucumis melo L. Var. cantalupensis) against the growth of *Escherichia coli* bacteria, *Pharmaceutical Journal of Indonesia*, **5(1)**, 61-66 **(2019)**

3. Anjali R., Ramya S., Kavitha K. and Suresh P., Fabrication of silver nanoparticles from marine macroalgae: characterization and biomedical appraisal, *Colloids and Surfaces B: Biointerfaces*, **213**, 112412 **(2022)**

4. Balaraman P., Balasubramanian B. and Kaliannan D., Phyco-synthesis of silver nanoparticles mediated from marine algae *Sargassum myriocystum* and its potential biological and environmental applications, *Waste and Biomass Valorization*, **11**, 5255-5271 **(2020)**

5. Bibi S., Biosynthesis of silver nanoparticles from macroalgae *Hormophysa triquetra* and investigation of antibacterial activity and mechanism against pathogenic bacteria, *Scientific Reports*, **15**, 24-76 **(2025)**

6. Cañadas A., Martín P., López M. and Serrano M., Green synthesis of antibacterial silver nanocolloids from agro-industrial residues and activity profiling, *Processes*, **6**, 74 **(2022)**

7. Chellapandian C., Ramkumar B. and Puja P., Gold nanoparticles using red seaweed *Gracilaria verrucosa*: Green synthesis, characterization and biocompatibility studies, *Process Biochemistry*, **80**, 58-63 **(2019)**

8. Chaudhary R., Nawaz K., Khan A.K., Hano C., Anjum S. and Abbasi B.H., An overview of the algae-mediated biosynthesis of nanoparticles and their biomedical applications, *Biomolecules*, **10**, 1498 **(2020)**

9. Choudhary S., Kumawat G., Khandelwal M. and Khangarot R.K., Phyco-synthesis of silver nanoparticles by environmentally safe approach and their applications, *Scientific Reports*, **14**, 60195 **(2024)**

10. Chugh D., Viswamalya V.S., Das B. and Rao P., Green synthesis of silver nanoparticles with algae and the importance of capping agents in the process, *Journal of Genetic Engineering and Biotechnology*, **19**, 126 **(2021)**

11. Dubey S.P., Lahtinen M. and Sillanpää M., Tansy fruit mediated greener synthesis of silver and gold nanoparticles, *Process Biochemistry*, **45(7)**, 1065-1071 **(2010)**

12. Dhir R., Verma A., Singh P. and Kaur S., Plant-mediated synthesis of silver nanoparticles: unlocking medicinal and environmental applications, *Journal of Cleaner Production*, **402**, 138549 **(2024)**

13. Fahim M., Shahzaib A., Nishat N. and Jahan A., Green synthesis of silver nanoparticles: a comprehensive review of methods, influencing factors and applications, *Journal of Colloid and Interface Science Open*, **16**, 100125 **(2024)**

14. Gahlawat G., Shikha S., Chaddha B.S., Ray Chaudhuri S., Mayilraj S. and Choudhury A.R., Microbial glycolipoprotein-capped silver nanoparticles as emerging antibacterial agents against cholera, *Microbial Cell Factories*, **15**, 25 **(2016)**

15. Ghosh A. et al, Green synthesis of silver nanoparticles and its applications as sensor, catalyst and antibacterial agent, Materials Today: Proceedings, **47**, 1–8 (**2023**)

16. Gidde N.D., Nitalikar M.M. and Raut I.D., Nanocomposites: A review on current status, *Asian Journal of Pharmacy and Technology*, **11(3)**, 231-237 **(2021)**

17. Ganesapandian K. and Kumaraguru A.K., Seaweeds resource in the intertidal and subtidal regions of Pudhumadam Gulf of Mannar, *Seaweed Research and Utilization*, **30**, 97-105 **(2008)**

18. Haiss W., Thanh N.T.K., Aveyard J. and Fernig D.G., Determination of size and concentration of gold nanoparticles from UV-Vis spectra, *Analytical Chemistry*, **79(11)**, 4215-4221 **(2007)**

19. Hamouda R.A., Abdel-Hameed M. and Salem A.M., Assessment of silver nanoparticles derived from brown alga *Sargassum vulgare*: characterization, antimicrobial and anticancer activities, *Marine Drugs*, **22**, 154 **(2024)**

20. Jain D., Daima H.K., Kachhwaha S. and Kothari S.L., Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities, *Digest Journal of Nanomaterials and Biostructures*, **4(3)**, 557-563 **(2009)**

21. Kim S.M., Lee J.H. and Park J., Characterization and antibacterial activity of green-route silver nanoparticles: recent advances and mechanisms, *International Journal of Molecular Sciences*, **25**, 5790 **(2024)**

22. Kuo C.H., Chiang T.F., Chen L.J. and Huang M.H., Synthesis of highly faceted pentagonal- and hexagonal-shaped gold nanoparticles with controlled sizes by sodium dodecyl sulfate, *Langmuir*, **20(18)**, 7820-7824 **(2004)**

23. Kumar V. and Yadav S.K., Plant-mediated synthesis of silver and gold nanoparticles and their applications, *Journal of Chemical Technology & Biotechnology*, **84(2)**, 151-157 **(2009)**

24. Li W.R., Xie X.B. and Shi Q.S., Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*, *Applied Microbiology and Biotechnology*, **85**, 1115-1122 **(2010)**

25. Link S. and El-Sayed M.A., Size and temperature dependence of the plasmon absorption of colloidal gold nanoparticles, *Journal of Physical Chemistry B*, **103(21)**, 4212-4217 **(1999)**

26. Muzakkar Muhammad Zakir, Kuen Novianti Anggraeni Wulandari, Salim La Ode Agus and Nurdin Muhammad, Enhancing the Electrochemical Performance of TiO2/Ti Electrodes through Sulphur Calchogenic Material Doping, *Res. J. Chem. Environ.*, **28(8)**, 51-56 **(2024)**

27. Pirtarighat S., Ghannadnia M. and Baghshahi S., Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment, *Journal of Nanostructure in Chemistry*, **9**, 1-9 **(2019)**

28. Rana A., Yadav K. and Jagadevan S., A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity, *Journal of Cleaner Production*, **272**, 122880 **(2020)**

29. Rodrigues A.S. et al, Advances in silver nanoparticles: a comprehensive review on their potential as antimicrobial agents and mechanisms of action elucidated by proteomics, *Frontiers in Microbiology*, **15**, 1440065 **(2024)**

30. Sharma V.K., Yngard R.A. and Lin Y., Silver nanoparticles: green synthesis and their antimicrobial activities, *Advances in Colloid and Interface Science*, **145(1-2)**, 83-96 **(2009)**

31. Sivalingam A.M., Silva A.R. and Martinez M., Characterization of silver nanoparticles: linking spectral signatures with size and morphology, *Materials Today Advances*, **21**, 100456 **(2024)**

32. Tripathi R.M., Saxena A., Gupta N., Kapoor H. and Singh R.P., High antibacterial activity of silver nanoballs against *E. coli* MTCC 1302, *S. typhimurium* MTCC 1254, *B. subtilis* MTCC 1133 and *P. aeruginosa* MTCC 2295, *Digest Journal of Nanomaterials and Biostructures*, **5(2)**, 323-330 **(2010)**

33. Yildirim A., Mavi A. and Oktay M., Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf ex DC), sage (*Salvia triloba L.*) and Black tea (*Camellia sinensis*) extracts, *Journal of Agricultural and Food Chemistry*, **48(10)**, 5030-5034 **(2000)**

34. Yu C., Tang J. and Liu X., Green biosynthesis of silver nanoparticles using *Eriobotrya japonica* (Thunb.) leaf extract for reductive catalysis, *Materials (Basel)*, **12(1)**, 189 **(2019)**.

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