

Agnp-Hp Synthesized Using Red Marine Algae *Halymenia Pseudofloresii* and its Pharmacological Activities

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Abstract

The aim of the present investigation is to develop a rapid and ecofriendly method to synthesis AgNPs using aqueous extract of a red marine algae *Halymenia pseudofloresii* (AgNP-Hp) and to evaluated to know the effect of antimicrobial, antioxidant and anticancer activity. The synthesized silver nanoparticles were confirmed the SPR peak at 430nm by UV-Spectrum. The face-centered cubic (FCC) structure of silver was confirmed by using XRD analysis. The FTIR spectrum used to analyzed the active bio compounds from in the AgNP-Hp. The synthesized nanoparticles were rectangular and cubic in structure and the size range in 20-35nm and size was confirmed by SEM images. The AgNP-Hp have showed antimicrobial activity against wound pathogens. The nanoparticles more susceptible to *P. aeruginosa* (30 mm) followed by *E. coli* (23 mm). The AgNP-Hp showed potential antioxidant activities against DPPH and H₂O₂ radical scavenging inhibition were 79.37% and 74.50% found at 100 µg/mL. The synthesized AgNP-Hp was showed excellent anticancer activity against human colon cancer cells (HT-29). The Half inhibitory (IC₅₀) concentration of AgNP-Hp was found at 18.12 µg/mL. This study revealed that the *Halymenia pseudofloresii* synthesized silver nanoparticles effectively inhibited the cancer cells growth.

Keywords: Red algae, AgNP-Hv, Colon cancer, microbial activity, antioxidant activity.

Introduction

In recent years, nanoparticles synthesis has increased focus across the entire field, including physical, chemical, biological, medicine and environmental studies (Batool et al., 2019). Nanotechnology has opened a huge window in material science of engineering. It focused on the synthesis of nanoparticles, with a dimensional range less than 100nm. It has various chemical

and physical components and most of them were undefined substances. (Ramesh et al., 2018). Nanomaterials provide greater benefits to the environment and more compatibility with excellent applications such as anticancer, antimicrobial, immunosuppressive, larvicidal, bio-catalysis, drug delivery and biosensor (Vijaya anand et al., 2019). The smaller sized particles can synthesis in ordinary laboratory condition and it has various synthesis methods, they are biological, physical and biological. Among those, biological route of synthesis is considered greater efficient than other routes because of low cost and low toxic chemicals (Brahim & Mohammed, 2020). Specifically, silver nanoparticles were gained splendid interest globally in the entire studies, and it is attributed to its useful role of multiplied potentials which include anti-oxidant, anti-bacterial, anti-viral, anti-cancer, anti-malarial and wound recovery properties. It is naturally renewable cost effective and it has enormous activities (Zhang et al., 2020). Currently, enormous research is carrying on AgNPs synthesis through biological method using microbes, plant extract, sponge related extract and some marine and terrestrial microorganisms. Due to the increased biological properties plant mediated silver nanoparticles most effective compared to others. It has the excellent absorption potential and utilized the essential chemical compounds from the plant (Shah et al., 2020).

Globally cancer is a leading cause of death accounting for 10 million death in 2020. Colon cancer is the third most common cancer (1.93 million cases) and second most common cause of death (935000 deaths) in 2020 (WHO-2021). Chemotherapy, radiation therapy, hormone therapy and surgery are the common therapeutic approaches to treat and control most cancers. Nowadays nanotechnology-based treatment and diagnostic methods have demonstrated the potential to improve cancer treatment (Ratan et al., 2020). Cancer nanotechnology has unveiled a modern horizon of interdisciplinary research in medicine, engineering, chemistry and biology focusing on key advances in cancer analysis, detection and treatment (Wang et al., 2010). The Increased occurrence of diseases in each human and animal is due to a number of factors including malnutrition, diet, life style changes, stress, changes in environmental condition and microbial pathogens. These factors directly affect the equilibrium between endogenous antioxidants and reactive oxygen species (ROS) in residing cells and tissues. Generally, cells carry many endogenous antioxidants which prevent normal physiological functions and antioxidant damages. But, the imbalance between free radical production and antioxidant defenses can cause cell death (Kurutas, 2015; Nita & Grzbowski, 2016).

Marine algae or seaweeds are plant like organisms in coastal regions which is usually attached with rocks, sand under sea and dead vegetations. The scale of algae ranges from tiny to large size. However, they are classified beneath the group of multicellular algae. The phytochemical elements of seaweeds have been found in and high antioxidant properties and anticancer properties. They are cost effective, do not require toxic agents and environmentally friendly (Bhuyar et al., 2020). The seaweeds integrated AgNPs has a significant attention on the biological pathway worldwide due to its rich source of carbohydrate and various environmental condition (Hashemi et al., 2020). The literature survey reported that the marine red algae are rich source of phenolic compounds particularly bromophenol. Phenolic compounds identified to possess a wide range of biological potential including antimicrobial, antioxidants, vasodilator activities and anti-inflammatory. Moreover, flavonoids and tannins are the naturally occurring phenolic compounds found in seaweeds (Ibraheem et al., 2016). The red marine algae genus *Halymenia* contains different variety of species which is isolated from Indo-Pacific region.

Halymenia pseudofloresii is one of the red algae species belongs to *Halymeniaceae* family (Hernandez-Kantun et al., 2012). In the present work, we investigated first time silver nanoparticles synthesized using *H. pseudofloresii* was characterized and evaluated for antimicrobial, antioxidant and anticancer activity against colon cancer.

Materials and method:

Chemicals used

Silver nitrate (AgNO_3), 2,2- Diphenyl-1-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2), Dimethyl sulfoxide (DMSO), Luria Bertani broth, Muller Hinton Agar and Rose Bengal agar - HiMedia, India. Dulbecco's modified eagle medium (DMEM), Fetal Bovine Serum (FBS), Trypsin-EDTA, Antimycotic and antibiotic solution were purchased from Gibco, Thermo fisherscientific, India. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) - Sigma Aldrich.

Collection of Seaweeds

The red seaweed, *Halymenia pseudofloresii* were collected from Mandabam coast (Lat. $09^\circ 17'$ N; Long. $79^\circ 08'$ E), Ramanathapuram, Tamil Nadu, India. Seaweed was thoroughly washed using tap water to remove other sticky and dust particles later rinsed with distilled water, and then they were dried in the shade for a week. The dried seaweed was finely ground using a mixer grinder and stored in sterile container for further uses (Fig. 1).

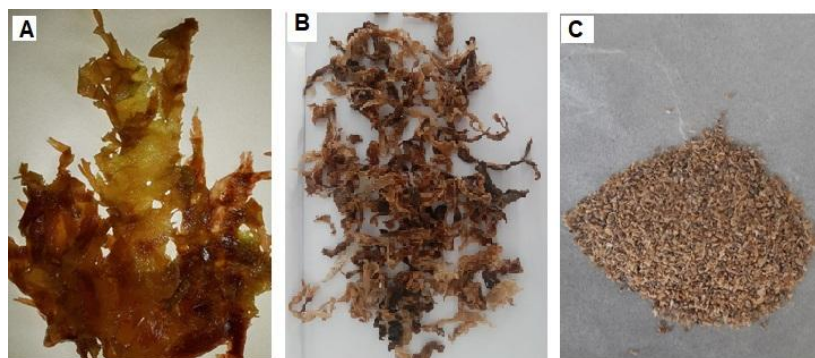


Fig 1- Collection of *Halymenia pseudofloresii* A. Fresh seaweed, B. Dried seaweed and C. Seaweed powder

Preparation of seaweed extract and synthesis of AgNPs

1g of Algae powder was weighed accurately and boiled with 100 mL distilled water for 15 minutes. After that the extract was filtered through Whatmann No. 1 filter paper. Then 10 mL of filtrate was added to 90 mL of 1mM Silver nitrate solution. Then the reaction mixture kept on magnetic stirrer at 30°C for 4h.

Characterization of synthesized AgNPs

The Surface plasmon resonance (SRP) absorption bands had been measured using UV-visible Spectroscopy (Perkin-Elmer MA, USA), range between 350-750 nm. The possible functional groups in the synthesized silver nanoparticles were analyzed using Fourier Transform

Infrared (FTIR Perkin-Elmer, MA, USA) spectra in the range of 4000-400 cm^{-1} . The crystal structure of biosynthesized silver nanoparticles was confirmed by X-Ray Diffraction pattern (XRD-Bruker AXS, Inc., Madison, USA). The size and shape of the nanoparticles were identified by using Scanning Electron Microscope (SEM).

Antioxidant activity

DPPH radical scavenging activity

In the DPPH radical scavenging activity, 0.1 mM DPPH solution was prepared with in ethanol and the biosynthesized AgNPs were added at concentrations (10, 20, 30, 40 and 50 $\mu\text{g/ml}$) individually. After 30 minutes of incubation the purple color solution was changed in to color less solution. Then the absorbance was measured at 517 nm using UV- spectroscopy. The percentage of inhibition was calculated by using the following equation.

Percentage of Inhibition = $[(A_b - A_s) / A_b] \times 100$
 A_b - absorbance of the blank and A_s - absorbance of the AgNPs.

H₂O₂ Scavenging assay

The H₂O₂ scavenging power of the biosynthesized AgNPs was assessed by the following method. 40mM H₂O₂ solution was prepared using pH 7.4 phosphate buffer. Synthesized AgNPs was taken at different concentrations (10, 20, 30, 40 and 50 mg/mL) were added individually to 0.6 mL of 40mM H₂O₂ solution. Then the mixture was incubated for 10 minutes and the absorbance of the reaction mixture was recorded using UV- Spectroscopy at 230 nm.

Percentage of Inhibition = $[(A_b - A_s) / A_b] \times 100$
 A_b - Absorbance of the blank and A_s - Absorbance of AgNPs.

Antimicrobial activity

The microbial activity of synthesized AgNPs nanoparticles were determined by agar well diffusion method against wound pathogens. The test culture *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were maintained in Luria Bertani broth. Well growth 24 hrs cultured species were swabbed on the freshly prepared Muller Hinton Agar for bacteria and Rose Bengal agar for fungus. The 4 wells with a diameter of 6 mm was made using gel puncture. Using a micropipette, 25 μL , 50 μL and 100 μL of Biosynthesized silver nanoparticles and 30 μL standard antibiotic were loaded into each respective well and the plates were incubated at 37°C for 24 h. The diameter of zone of inhibition was measured in millimeter, which was recorded as an inhibitory effect against pathogens.

In vitro-cytotoxic activity by MTT assay

HT-29 cell line was purchased from National Centre for Cell Science (NCCS), Pune, India and the cells were grown on DMEM (Dulbecco's Modified Eagle's Medium) medium supplemented with 10% FBS, 1% antibiotic and antimetabolic solution in to CO₂ incubator at 37°C. These cell lines were maintained in T-25 tissue culture flasks and 3 days once cells were sub cultured.

The cytotoxicity effect of synthesized nanoparticle was evaluated through MTT assay using different concentrations of AgNPs (3.125 to 50 $\mu\text{g/mL}$) on the cell culture media. Each well added with a desired concentration of nanoparticles and incubated for 24 h at 37 °C. A control group was also run along with it. After incubation, the wells were washed with phosphate buffer

saline (PBS) followed by added MTT solution into each well and incubated for 4 h in CO₂ incubator. Later, the crystals formed were solubilized by adding 50 µl DMSO to each of 96 well and kept in dark for 10–15 min, so that the crystals would completely solubilize. The optical density of the solutions was measured at 450 nm, and the viability of cells was calculated by using the formula as OD of nanoparticles/OD of Control × 100. The experiment was conducted in triplicate to interpret the result.

Results

Synthesis of silver nanoparticles using *Halymenia pseudofloresii*

The synthesis of silver nanoparticles performed by the reduction of 1mM silver nitrate (AgNO₃) with *H. pseudofloresii* extract. Respectively, at the initial stage of reaction the solution remains colorless after 30 minutes of heating condition the solution started to change its color rapidly. The reduction of silver ions into silver nanoparticles were visually confirmed by the color change of colorless solution to deep brown color after 4h of reaction (shown in Fig. 2.) The synthesis of AgNPs using *H. pseudofloresii* was abbreviated as AgNP-Hp.



Fig 2-*Halymenia pseudofloresii* synthesis silver nanoparticles. A. Initial color B. final color change.

Characterization of Silver nanoparticles

UV visible Spectroscopy analysis of AgNP-Hv

UV spectroscopy is an excellent analytical tool used to confirm the silver nanoparticles synthesis. The silver nanoparticles were synthesized with in 4h of warm condition. The synthesized AgNP-Hp was analyzed periodically at 350-550nm wave length. The maximum surface resonance peak (SPR) was found at 430nm (Fig. 3). This analysis confirmed the formation of silver nanoparticles from aqueous extract of *H. pseudofloresii*.

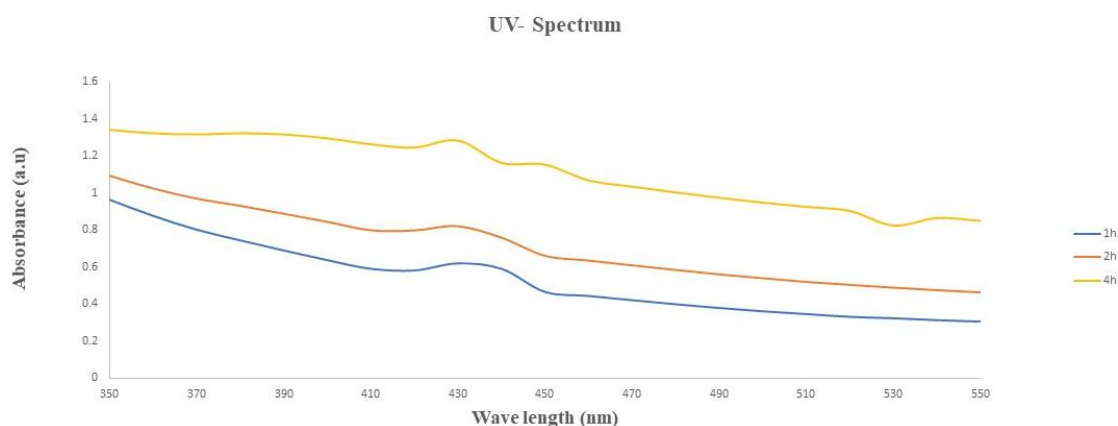


Fig 3- UV analysis of AgNP-Hp

XRD analysis

The crystalline nature of AgNPs derived from *H. pseudofloresii* was proved by powder X-ray diffraction analysis. The XRD results of biosynthesized AgNP-Hp was shown in Fig. 4. Based on the finding, AgNP-Hp clearly showed face-centered cubic (FCC) structure and diffraction patterns was exhibited at 2θ value of 27.98, 32.37, 38.32, 46.41 and 57.61 were well matched with their corresponding reflection patterns (110), (123), (111), (231) and (311) respectively. The measurement of AgNP-Hp was corresponded with the JCPDS, File No. 04-0783. The mentioned results of algae synthesized AgNP-Hp was crystalline condition.

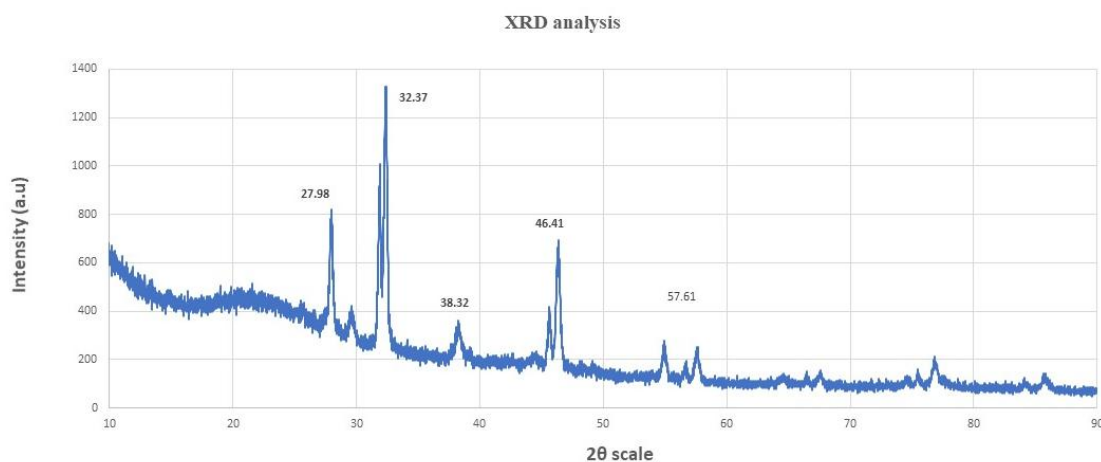


Fig. 4- XRD analysis of AgNP-Hp

FTIR analysis

The FTIR analysis was used to identify the biomolecules present in the aqueous algae extract synthesized AgNP-Hp. Fig. 5 represented the spectra of the synthesized AgNP-Hp resulting in peaks at 673, 1642, 2039, 2167, 2367, 3303 and 3744 cm^{-1} . The strong peak at 673 cm^{-1} denotes the vibrations of the halo compounds (C-Cl or C-Br stretching). The presence of medium peak 1642 cm^{-1} represented the C-C=C symmetric stretching of conjugated alkene groups (1-Propane). The vibration due to the N=C=S stretching of isothiocyanate group has been observed in the 2039 cm^{-1} region. The strong peak at 2167 cm^{-1} is found to be due to the alkynes group of propyne ($\text{HC} \equiv \text{C}-\text{CH}_3$). The absorbance peak at 2367 cm^{-1} represented the O=C=O stretching of carbonic dioxide. The presence of primary and secondary amines groups on N-H stretching was observed at 3303 cm^{-1} . The peak corresponding to the O-H vibration of the alcohol group was observed at 3744 cm^{-1} .

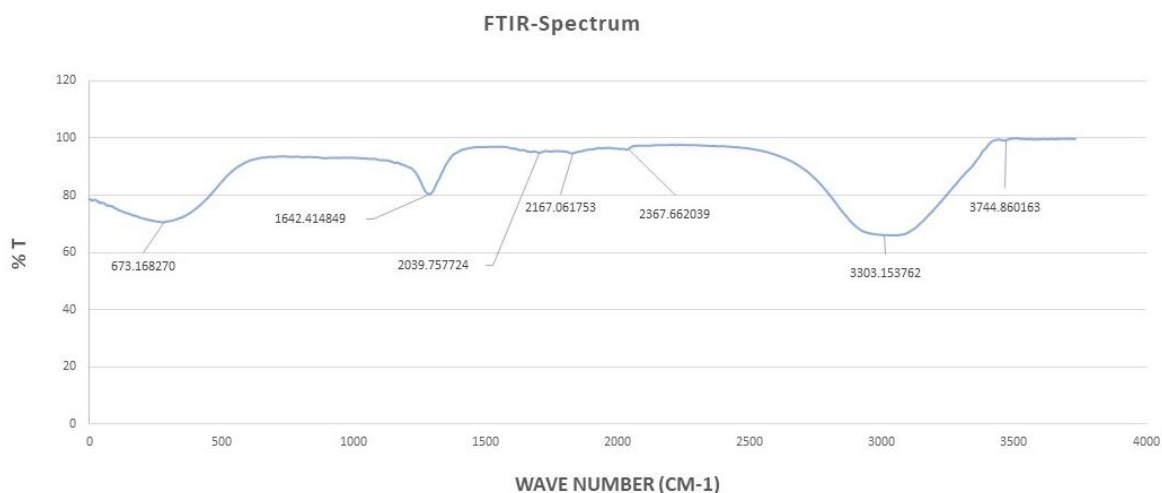


Fig. 5- FTIR analysis of AgNP-Hp

SEM analysis

The synthesized AgNP-Hp particles were observed to be well dispersed as seen in the scanning electron microscope. The size range of the particles was observed in the range of 20-35 nm. Fig 6 showed the biosynthesized AgNP-Hp particles were mostly cubic and rectangular in shape and some particles are round shaped.

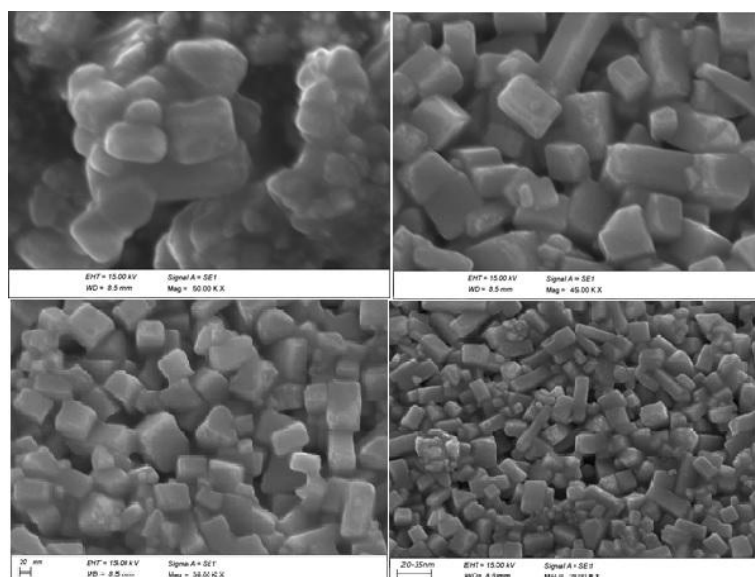


Fig6- SEM images of AgNP-Hp

Antimicrobial activity of synthesized AgNP-Hp

The antimicrobial efficiency of *H. pseudofloresii* synthesized AgNPs was evaluated based on the zone of inhibition observed in the agar plates with the respective pathogen by agar well diffusion method (Table 1) (Fig. 7). The AgNP-Hp had inhibited the growth of *Pseudomonas aeruginosa* (Gram positive bacteria), *Staphylococcus aureus* *Escherichia coli* (Gram negative bacteria) and *Candida albicans* (Fugus) at different concentrations (Fig. 8). Among the 4 pathogens AgNP-Hp more susceptible against *P. aeruginosa* (30 mm) and *E. coli* (23 mm), less susceptible to *S. aureus* (21 mm) and *C. albicans* (20 mm). The maximum concentration of AgNP-Hs showed maximum zone of inhibition. This study revealed that the synthesized AgNP-Hs has excellent antimicrobial activity against wound pathogens.

Table 1: Antimicrobial activity of AgNP-Hp

Pathogens	Control (Ab)	AgNP-Hs (25 μg)	AgNP-Hs (50 μg mL)	AgNP-Hs (100 μg mL)
<i>P. aeruginosa</i>	40mm	21mm	26mm	30mm
<i>S. aureus</i>	25mm	14mm	18mm	21mm
<i>E. coli</i>	35mm	18mm	20mm	23mm
<i>C. albicans</i>	33mm	10mm	13mm	20mm

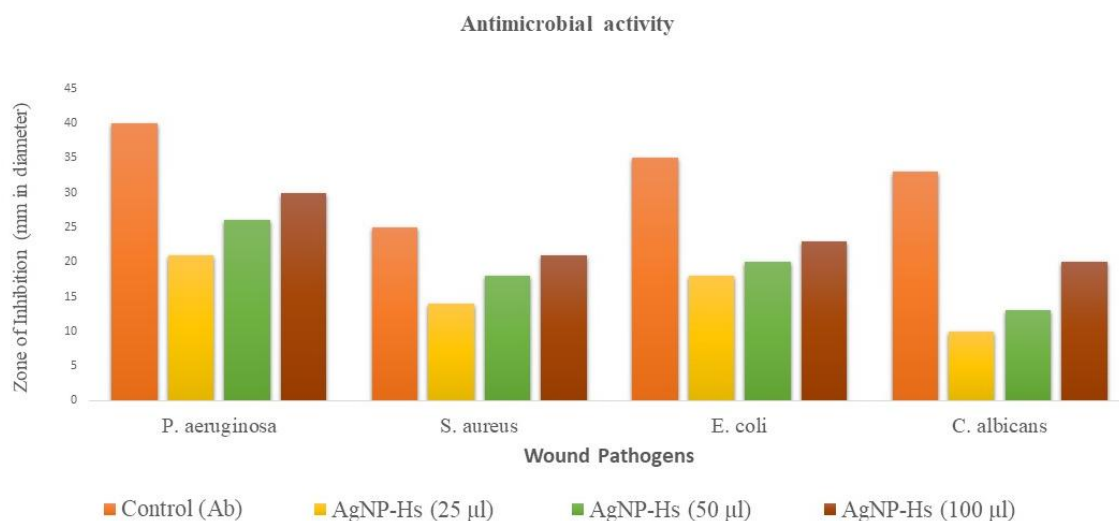


Fig. 7- Antimicrobial activity of AgNP-Hp against wound pathogen.

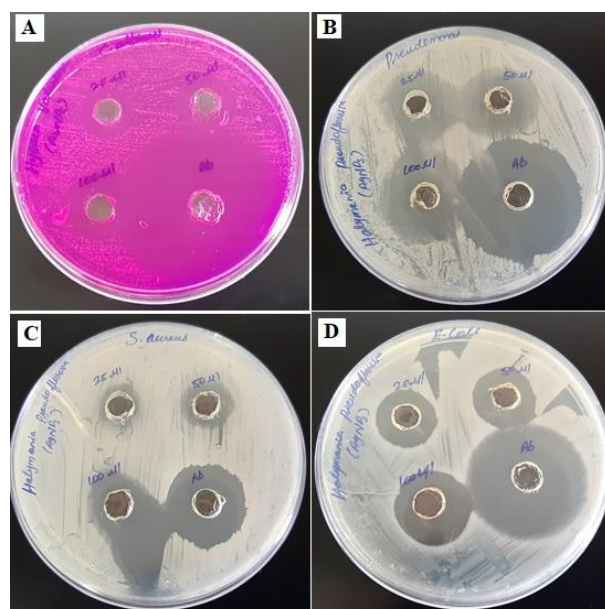


Fig 8 - Antimicrobial activity of AgNP-Hp against A. *Candida albicans*, B. *Pseudomonas aeruginosa*, C. *Staphylococcus aureus* and D. *Escherichia coli*

Antioxidant activity of synthesized AgNP-Hp

The antioxidant potential of *H. pseudofloresii* derived AgNP-Hp was analyzed by DPPH and H_2O_2 radical scavenging assay. The DPPH reducing activity of AgNP-Hs was assessed based on colour change. The DPPH scavenging activity of synthesized nanoparticles exhibited potent inhibition when compared with standard ascorbic acid. Increasing concentration of AgNP-Hp and ascorbic acid (10 -50 µg/mL) showed higher inhibition of DPPH radicals (Fig. 9). Maximum inhibition was (79.37%) found at 100 µg/mL of AgNP-Hp.

The H_2O_2 radical scavenging activity of AgNP-Hp was quantified by spectrophotometrically. The concentrations of test samples at 50 $\mu\text{g/mL}$ inhibition were found to be 74.50% and 69.66% for the AgNP-Hp and ascorbic acid, respectively. Fig. 10 showed the percentage inhibition was gradually increased and it is dose dependent manner. In the hydrogen peroxide scavenging assay proved the biosynthesized silver nanoparticles has more scavenging activity than standard vitamin C.

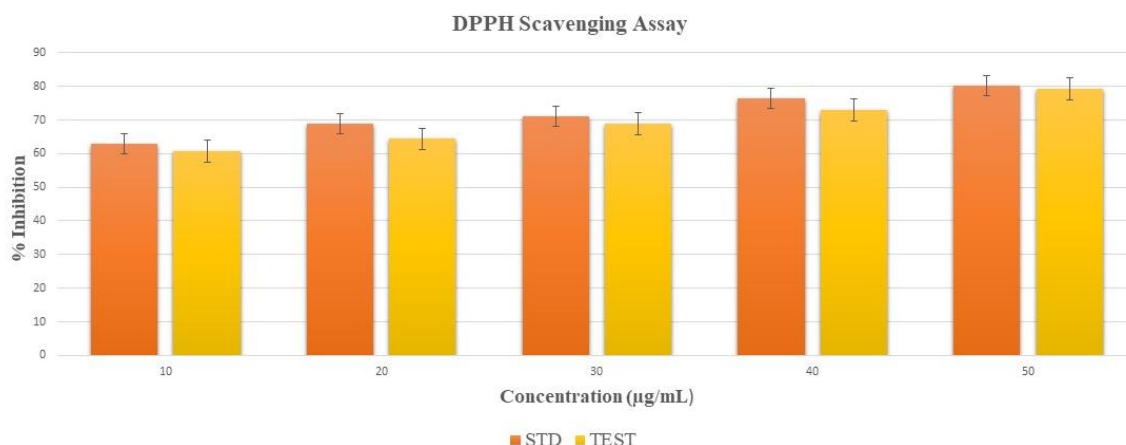


Fig 9- DPPH free radical scavenging assay

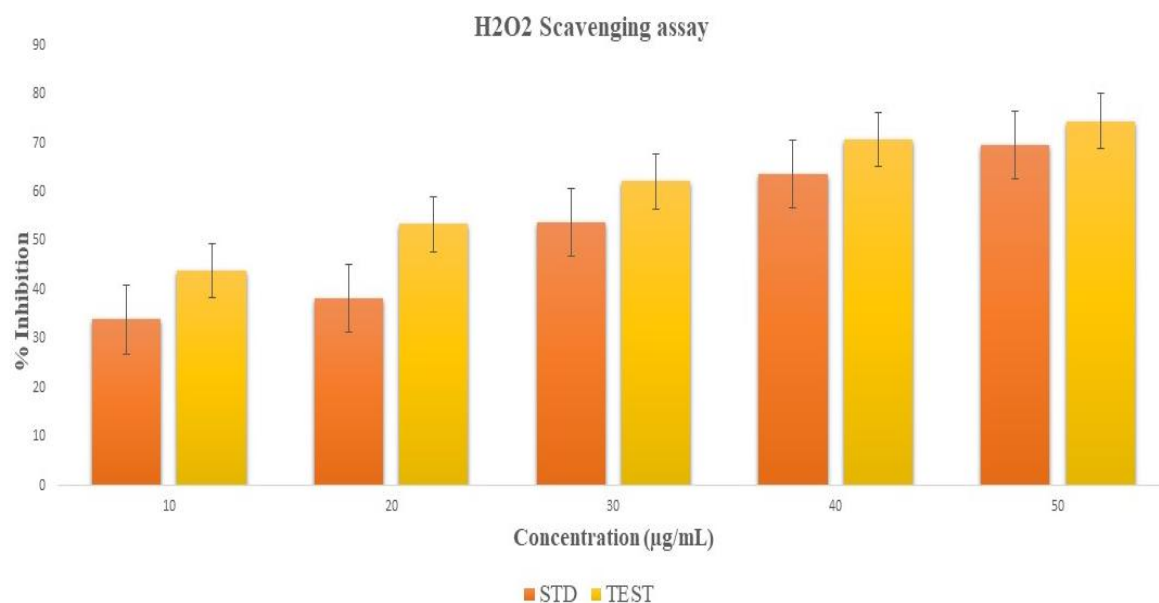


Fig10- Hydrogen Peroxide scavenging activity.

Invitro cytotoxic activity of AgNP-Hp

The cytotoxic activity of synthesized AgNP-Hp was tested in human colon cancer HT-29 cells by MTT assay. The cells were treated with AgNP-Hp at concentration range from 3.125 to 50

$\mu\text{g/mL}$ and the untreated cells considered control well. The obtained results were represented in Table 4. The percentage of inhibition the cells were increased in dose dependent manner (Fig. 12). The IC_{50} value of AgNP-Hp was clearly found around $18.12 \mu\text{g/mL}$. The dosage effect of AgNP-Hp on HT-29 cell lines shown in Fig. 11. This invitro screening of AgNP-Hp showed potential cytotoxicity activity against human colon cancer cells.

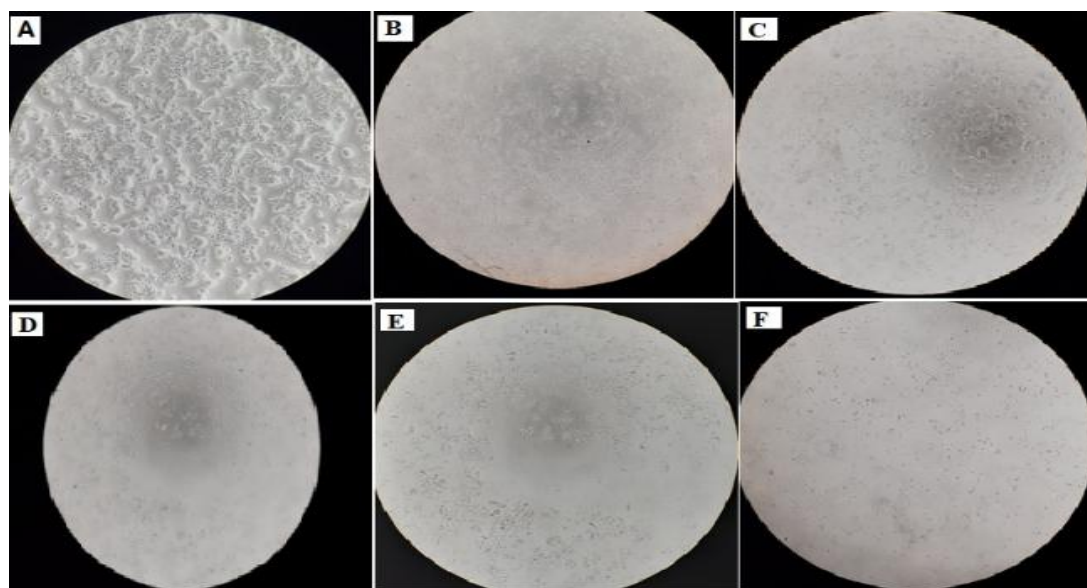


Fig 11- AgNP-Hp treated cells in different concentration. A. Control, B. $3.125 \mu\text{g/mL}$, C. $6.25 \mu\text{g/mL}$, D. $12.5 \mu\text{g/mL}$, E. $25 \mu\text{g/mL}$ and F. $50 \mu\text{g/mL}$

Table 4 Cytotoxic activity of AgNP-Hp.

Concentration ($\mu\text{g/mL}$)	Absorbances (O.D)	Average (O.D)	Viability cells	% Inhibition
Control	0.601, 0.623, 0.656	0.626	100	100%
3.125	0.424, 0.414, 0.403	0.413	65.97	34.03%
6.25	0.333, 0.318, 0.317	0.322	51.43	48.57%
12.5	0.304, 0.298, 0.305	0.302	48.24	51.7%
25	0.288, 0.274, 0.292	0.284	45.36	54.64%
50	0.256, 0.230, 0.194	0.226	36.10	63.9%

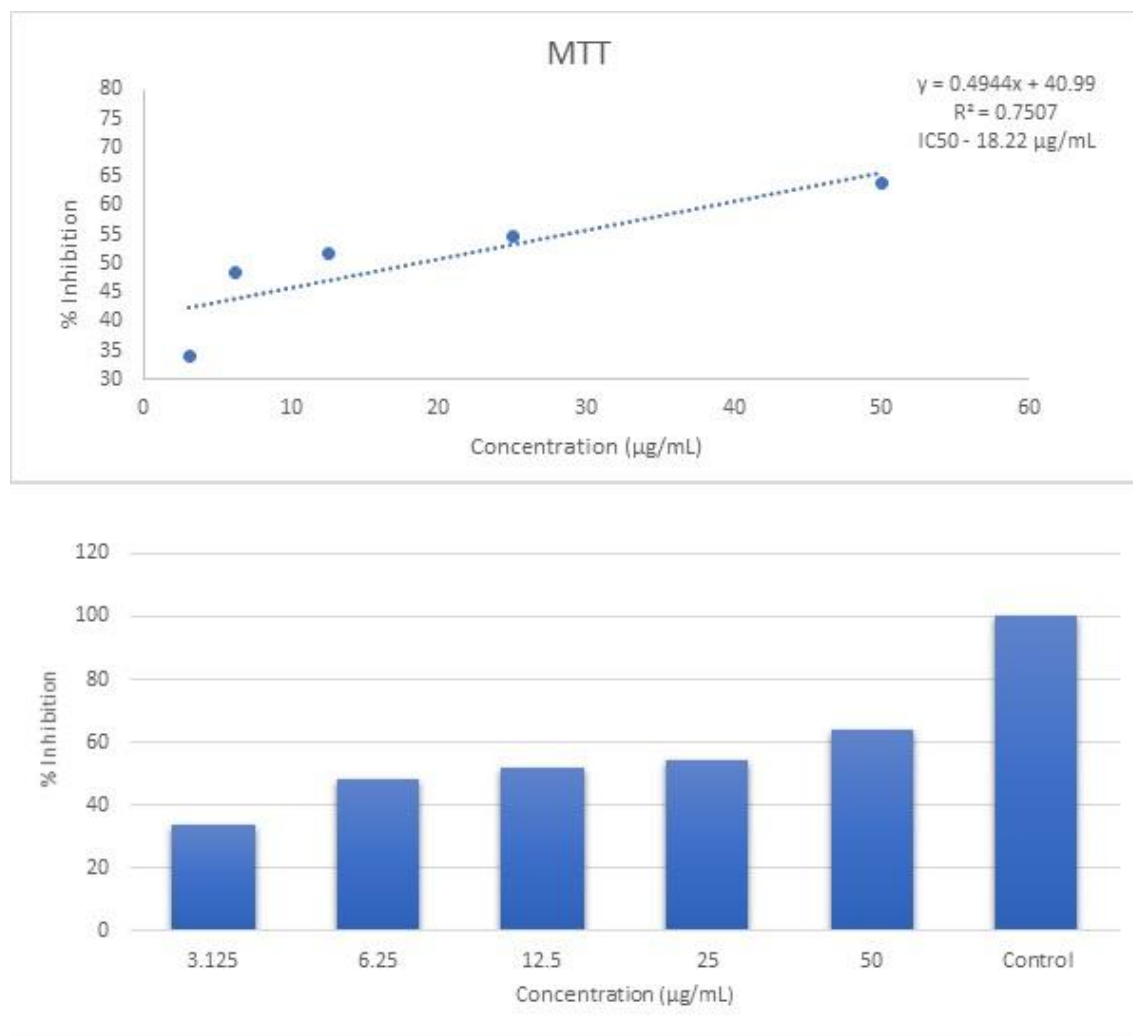


Fig 12- Cytotoxic activity of AgNP-Hp against colon cancer.

Discussion

The nanoparticles were successfully prepared from the aqueous extract of marine red algae *Halymenia pseudofloresii* as a reducing and stabilizing agent. The dark brown color indicated the formation of silver nanoparticles. After 4 h of incubation signature peak of formed AgNP-Hp was observed at 430nm using UV spectroscopy. The color changes of reaction mixture due to the excitation of SPR vibration surrounding the metal nanoparticles. The changes in the SPR peaks because of the dimensions, shapes and composition of the bioactive molecules in the aqueous extract used in synthesis process. (Abdelhafez et al., 2020). Valarmathi et al., 2020 have reported that the silver nanoparticles synthesized from marine red algae *Apyridia filamentosa* found SPR peak at 420nm. *Gracilaria corticate* synthesized AgNPs showed peak at 424nm (Rajivganthi et al., 2020). Dexit et al., 2018 observed the peak at 480nm in marine green algae *Ulva flexuosa* derived silver nanoparticles. Compare to previous reports our AgNP-Hp was formed rapidly and the SPR peaks effectively confirmed the formation of silver nanoparticles in reaction solution.

The XRD pattern of AgNP-Hp exhibited diffraction peaks corresponding to (110), (123), (111), (231) and (311) respectively. These peaks indicating the crystalline nature of silver nanoparticles. The additional intense peaks at 2θ angle of AgNP-Hp, the involvement of biomolecules present in the algae extract react with AgNO₃ solution which involved in the AgNPs synthesis (Govindappa et al., 2018.) The crystallization of bioorganic phase obtained on the surface of AgNPs. Our results were moderately similar with the finding of silver nanoparticles synthesized using marine algae *Chaetomorpha linum* (Acharya et al., 2021; Bhayur et al., 2020) and *Ecklonia cava* (Venkatesan et al., 2016).

The FTIR analysis was used to identified the biomolecules present in the aqueous algae extract synthesized AgNP-Hp. The spectra of the synthesized AgNP-Hp resulted in peaks at 673, 1642, 2039, 2167, 2367, 3303 and 3744 cm⁻¹. The absorbance peak at 673, 1642 and 2367 cm⁻¹ were identified the halo compounds, alkene groups and carbonic dioxide. The marine cyanobacterium *Phorminium formosum* mediated silver nanoparticles found the similar molecules (Elkomy et al., 2020). The N-H stretching was observed at the peak 3303 cm⁻¹ Similar result reported by Sinha et al., 2015. The Small bands found at 2300–2336 cm⁻¹ may indicate the presence of C≡C stretching (Aboelfetoh et al., 2017). The peak 3744 cm⁻¹ corresponding to O-H group of alcohol. Similarly result found in marine red algae *Acanthophora specifera* synthesized silver nanoparticles. The group of secondary metabolites and some protein in the marine algae has possibility to cover or reduce in metal nanoparticles (Ibraheem et al., 2016).

According to SEM images, the morphology of synthesized AgNP-Hp was mostly in cubic, rectangular and some particles are round shaped. The size of nanoparticles range between 20-35nm. Previously similar result was found in *Achillea millefolim* L. extract synthesized silver nanoparticles (Yousaf et al., 2020). As the increased temperature results in the formation of irregular shaped nanoparticles in size 5-200 nm. Likewise, the shape-controlled silver nanoparticles can develop in biological method by different temperature (Bhuyar et al., 2020).

Antimicrobial activity of the synthesized AgNP-Hp was evaluated against gram-positive bacteria *Staphylococcus aureus*, two gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* and Fungus *C. albicans* by agar well diffusion method. Among the above pathogens Gram negative bacteria showed higher zone of inhibition compared to Gram positive bacteria and fungus. Bhayur et al., 2020 reported the similar results in *Padina sp.* synthesized silver nanoparticles. Silver nanoparticles has a tendency to bind to cell membrane of bacteria coated with Sulphur containing proteins. This mechanism of action might vary because of the cell wall composition of Gram positive and Gram-negative bacteria. The Gram-negative bacterial cell wall contains lipopolysaccharide membrane allows to enter molecules into this layer and hence the nanoparticles easily penetrate into the cell wall and causing damage to the bacterial cells (Yin et al., 2020).The green seaweed *Enteromorpha flexuosa* (wulfen) synthesized AgNPs have comparatively rapid and wide applications to antimicrobial therapy in modern medicine (Yousefzadi et al., 2014). The synthesis of AgNPs by *Gracilaria corticata* effectively inhibited the growth of fungus species *Candida albicans* and *C. glabrata* (Kumar et al., 2013).

Green algae, *Enteromorpha compressa* (Linnaeus) AgNPs showed good antimicrobial activity against human bacterial pathogens like *E. coli*, *K. pneumoniae*, *Pseudomonas sp.*, *S. aureus* and some fungus (Ramkumar et al., 2017). Compare to previous reports our investigation proved the *Halymenia pseudofloresii* synthesized AgNP-Hp effectively control disease causing microbes in lower concentration.

This oxidative stress leads to the cellular damages. Antioxidant rich substances could reduce the free radical production (Fouda et al., 2020). The antioxidant activity of synthesized AgNP-Hp was assayed by DPPH and H₂O₂ free radical scavenging activity. The synthesized AgNP-Hp showed the increasing radical scavenging in dose dependent manner. Maximum inhibition was (79.37%) found at 50 µg/mL of AgNP-Hp which is very closer to standard ascorbic acid inhibition. The antioxidant activity of biological synthesized silver nanoparticles attributed proton donating capability to free radicals due to the presence of bioactive compounds in green source which is acted as capping agents of AgNPs surface (Kumar et al., 2019). *Gracilaria corticate* synthesized silver nanoparticles showed 83% inhibition against 250 µg/mL. Hence the synthesized AgNP-Hv showed greater radical scavenging activity at very low concentration. Mass of uninhibited H₂O₂ results in formation of oxygen free radicals that causes serious damage to cell membrane. The concentration at 50 µg/mL of inhibition were found to be 74.50 and 69.66% for the AgNP-Hp and ascorbic acid respectively. S

In the presence of hydrogen peroxide, the distributed AgNPs will induce hydroxyl radicals. Hydrogen peroxide in the living cells at low volume can dissolve AgNPs and form an oxidative stress (He et al., 2012). Silver nanoparticles can form a high accounts of hydrogen peroxide and they trigger the development of inflammation as a result of strong leakage of cathepsins from unpaired lysosomes and the excretion of K⁺ ions may can contribute to the super oxide and hydrogen peroxide production in the mitochondrial membrane (Govindappa et al., 2018). Our results are in good accordance with a previous report on the hydrogen peroxide scavenging effect of spherical silver nanoparticles synthesized from *Endophytic Streptomyces spp.* (Fouda et al., 2019).

Silver nanoparticles acted as therapeutic agents in cellular sensitizers with pro-apoptotic and pro-antioxidant potential, conjointly functioned as photodynamic therapy against cancer cells. When AgNPs penetrate into cancer cells, a cascade process begins with loss of inner homeostasis and redox instability. A serial free radical wave destructs the mitochondrial and nuclear membrane and generate antioxidant stress. Additionally, silver ions can block the DNA repair enzymes hence the replication suppressed in the S-phase of the cell cycle (Biresaw et al., 2020). *In vitro* cytotoxic activity of *H. pseudofloresii* synthesized AgNPs tested against human colon cancer HT-29 cells. The *in vitro* screening of the AgNP-Hp showed potential cytotoxicity in a dose dependent manner. Half inhibitory concentration of AgNP-Hp was found at 18.12 µg/mL. Recent data reports suggested that the generation of ROS level play an important role in AgNPs inducing cytotoxicity in cancer cells (Du et al., 2016). Venkatadri et al., 2020 studied the synthesized silver nanoparticles from *Zingiber officinale* found IC₅₀ 150.8 µg/mL against HT-29 cells. Marine algae synthesized silver nanoparticles using *Sargassum polycystum* showed potent anticancer activity against colon cancer cells (IC₅₀ 20 µg/mL) (Palanisamy et al., 2017). Devi & Bhimba, 2012 reported the AgNPs synthesized using *Ulva lactuca* has great anticancer activity. Fucoidans, terpenoids and laminarians are the cytotoxic compounds in the seaweeds reported to possess antiproliferative properties.

Conclusion

To the best of our knowledge, we are the first one to developed the silver nanoparticles from red marine algae *Halymenia pseudofloresii*. The synthesized AgNP-Hp was confirmed by characterization technique such as UV-visible spectroscopy, XRD, FTIR and SEM. The SPR

peak was found at 430nm using UV-Spectroscopy. The crystalline nature of synthesized AgNP-Hp was confirmed by XRD. The active compounds in the algae synthesized AgNP-Hp was identified by FTIR spectrum. The morphology and average size of the AgNP-Hp was 20-35 nm, confirmed by SEM images. The AgNP-Hp exhibited good antimicrobial activity against wound pathogens such as *P. aeruginosa*, *S. aureus*, *E. coli* and *C. albicans*. AgNP-Hp showed significant antioxidant activity in DPPH as Hydrogen peroxide scavenging. The nanoparticles effectively inhibited the growth of human colon cancer cells in half inhibition concentration 18.12 µg/mL. The outcome of the research confirmed that the marine algae *Halymenia pseudofloresii* extract responsible for development of silver nanoparticles and exhibited potent pharmacological activities.

Acknowledgments

We gratefully acknowledge Er. A.C.S. Arun Kumar, President, Dr. M.G.R Educational and Research Institute University for providing the necessary facilities and we would like to thank Department of Pharmacology, Saveetha Dental College and Hospital, SIMATS, for providing microbial stains and we like to thank Department of Human genetics, Sri Ramachandra university for providing cell culture laboratory facility. We acknowledge the Nanotechnology Research Centre (NRC), SRMIST for providing the research facilities.

Conflict of interest:

The authors declare that they have no known conflict of interests.

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