



# Green chemistry approach for silver nanoparticles synthesis from *Halimeda macroloba* and their potential medical and environmental applications

G. Lavanya<sup>1</sup> · K. Anandaraj<sup>1</sup> · M. Gopu<sup>2</sup> · K. Selvam<sup>2</sup> · T. Selvankumar<sup>2</sup> · M. Govarthanan<sup>3,4</sup> · P. Kumar<sup>5</sup>

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## Abstract

This study aims to synthesize silver nanoparticles (AgNPs) using *Halimeda macroloba* extract and assess their anticancer and photocatalytic properties. Ultraviolet–visible spectrophotometry (UV–visible), Fourier transform infrared (FT-IR) spectroscopy, Transmission electron microscope (TEM), scanning electron microscope-energy-dispersive spectroscopy (SEM–EDX), X-ray photoelectron spectroscopy (XPS), and particle-size distribution (PSD) were used to characterize the synthesized AgNPs. The green-synthesized AgNPs to be spherical in shape with a size of about 50–100 nm. The half-maximal inhibitory concentration (IC<sub>50</sub>) for AgNPs was determined to be 89.5 g/mL against the human hepatoma cell line (Huh-7). Fluorescent microscopy was used to evaluate the morphological alterations of Huh-7 cells stained with acridine orange/ethidium bromide (AO/EtBr), 2'-7'-dichlorofluorescein diacetate (DCFH-DA), and rhodamine 123. Meanwhile, AgNPs exhibited 91.35% photocatalytic activity against methylene blue (MB) after 100 min of exposure to sunlight. Therefore, AgNPs have a strong potential for usage in anticancer activities and photocatalytic organic pollutant destruction.

**Keywords** Green seaweeds · *Halimeda macroloba* · Silver nanoparticles · Anticancer activity · Photocatalytic activity

## Introduction

In decades past, nanotechnology was indeed a notable branch of contemporary research dealing with the manipulation, synthesis, and design of particles with 1–100 nm dimensions. Nanoparticles (NPs) are characterized by their small size and increased surface area, and they are utilized in numerous industries, including cosmetics, food industries, environmental bioremediation, optics, electrical, textile, catalysts, light emitters, photocatalytic, biosensors, energy research, drug delivery, and biological sciences (Ashraf et al. 2019; Behzad et al. 2021; Paiva-Santos et al. 2021; Sana et al. 2021; Wang et al. 2021; de Jesus et al. 2021; Seitkaliyeva et al. 2021; Dawood et al. 2022; Hojjati-Najafabadi et al. 2022). The various types of noble metal nanoparticles, including copper, zinc, titanium, magnesium, nickel, iron, silver, and gold. Among them, silver NPs are widely used in the sectors of cosmetics, nanomedicine, photocatalytic, food processing, biomedical imaging, antibacterial activities, and more due to their unusual stability, conductivity, and targeted medication delivery (Aravinthan et al. 2015; Chinnappan et al. 2018; Balakrishnan et al. 2020; Gopu et al.

✉ K. Anandaraj  
kanandaraj2010@gmail.com

✉ P. Kumar  
kumarp@alagappauniversity.ac.in

<sup>1</sup> PG & Research Department of Microbiology, Shanmuga Industries Arts & Science College, Tiruvannamalai, Tamil Nadu 606 603, India

<sup>2</sup> PG & Research Department of Biotechnology, Mahendra Arts & Science College (Autonomous), Kalippatti, Namakkal, Tamil Nadu 637 501, India

<sup>3</sup> Department of Environmental Engineering, Kyungpook National University, 41566 Daegu, Republic of Korea

<sup>4</sup> Department of Biomaterials, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai 600 077, India

<sup>5</sup> Food Chemistry and Molecular Cancer Biology Laboratory, Department of Animal Health and Management, Alagappa University, Karaikudi 630003, Tamil Nadu, India

2021; Suriyakala et al. 2021; Sampath et al. 2021; Heine-mann et al. 2021; Swathilakshmi et al. 2022).

Numerous techniques, including hydrothermal, micro-wave, electrochemical, laser ablation, chemical radiation, ball milling, and green chemistry, have been utilized to create silver nanoparticles (AgNPs) (Aksomaityte et al. 2013; Rabinal et al. 2013; Ellouzi et al. 2021; Ozlem Saygi and Usta 2021). Recently, efforts have been made to design non-toxic and eco-friendly green method for the synthesis of AgNPs. Green chemistry procedures, which are an alternative to chemical and physical processes, have a number of benefits for the synthesis of AgNPs, including reduced time consumption, a slow kinetics ratio, cheap cost, and environmental friendliness. Biological synthesis of AgNPs can be accomplished from plants (Hojjati-Najafabadi et al. 2021) Sengottaiyan et al. 2016; Ameen et al. 2019), bacteria (Ameen et al. 2020), fungi (Popli et al. 2018), actinomycetes (Sowani et al. 2016), cow milk (Lee et al. 2013), panchakavya (Govarthan et al. 2014), oil cake (Govarthan et al. 2016a, b), yeast (Korbekandi et al. 2016), and algae (Muthusamy et al. 2017; Gopu et al. 2021). Among these, AgNPs synthesis by marine green seaweeds is more appropriate, since they develop quickly and produce more biomass at a cheaper cost than other organisms. A number of marine seaweeds have been used such as *Gelidium corneum* (Yılmaz Öztürk et al. 2020), *Spyridia filamentosa* (Valarmathi et al. 2020), *Sargassum wightii* (Selvaraj et al. 2020), *Lobophora variegata* (Kitharian et al. 2021), *Ulva armoricana* (Massironi et al. 2019), *Sargassum muticum* (Trivedi et al. 2021), and *Porphyra yezoensis* (Xu et al. 2019) for the reduction of AgNO<sub>3</sub> to the synthesis of AgNPs. Seaweeds contain bioactive substances, such as fatty acids, carotenoids, polysaccharides, sterols, and oligopeptides, which have a wide range of biological effects and they perform AgNPs synthesis (Massironi et al. 2019).

In particular, the calcified green seaweed *Halimeda macroloba* genus *Halimeda* with harder thallus texture due to its high concentration of minerals. Calcium carbonate and bioavailable micronutrients are abundant in *Halimeda* (Chiarathanakrit et al. 2019). To the best of our knowledge, this is the first report describing the biosynthesis method of AgNPs using green algae, *H. macroloba* extract. The following are the goals of our research: (i) biosynthesis of AgNPs using green seaweed *H. macroloba* extract, (ii) characterization of biosynthesized AgNPs with spectroscopic and electron microscopic methods, (iii) evaluation of in vitro anticancer activity of biosynthesized AgNPs against liver cancer (Huh-7) cells, and (iv) photocatalytic behavior for the degradation of methylene blue (MB) dye was investigated.

## Experimental methods and materials

### Materials

Silver nitrate (AgNO<sub>3</sub>) (99.9%), methylene blue (MB), dimethyl thiazolyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) (99.5%) were analytical grades supplied by Himedia Laboratories Pvt. Ltd., (Mumbai, India). All chemicals were used without purification, and deionized water was employed to make solutions. Before being used in research, the glassware was cleaned with deionized water and dried in the hot air oven.

### Collection and preparation green seaweed extract

The marine green algae *Halimeda macroloba* was collected from the coastal areas of Tuticorin (Lat. 8.7874°N; Long. 78.1983°E), Tamil Nadu, India. The collected green marine alga was washed with sterile water for four-to-five times to remove epiphytic organisms, necrotic fragments, and other debris. The washed marine alga was shade dried and ground into a fine powder using a domestic grinder. The alga powder (10.0 g) was combined with 100 mL distilled water and heated at 100 °C for 20 min. The algae extract was filtered through Whatman No. 1 filter paper after cooling and stored at 4 °C used for phytochemical analysis and AgNPs' synthesis.

### Phytochemical analysis of *H. macroloba*

Phytochemical screening was carried out according to standard procedure described elsewhere (Harborne 1998).

### Bioinspired synthesis of AgNPs by *H. macroloba* extract

AgNPs' synthesis was carried out according to Ameen et al. (2019) with slight changes. Briefly, 4 mL of the green algae extract was mixed with 96 mL of 1 mM AgNO<sub>3</sub> solution and the resulting greenish mixture was incubated for 24 h at room temperature. The reduction of Ag<sup>+</sup> was seen as a change in color from light green to dark brownish. The pellet was dispersed in double-distilled water and dried in a lyophilizer after centrifugation (Centrifuge, Eppendorf 5810R) at 15,000 rpm for 15 min.

### Characterization of AgNPs

The reduction of AgNO<sub>3</sub> into AgNPs was investigated using UV–visible spectroscopy (Evolution-201, Thermo, USA). The probable interaction of functional groups

**Table 1** Phytochemical screening of *H. macroloba* extract

S. no.	Phytochemicals	Aqueous extract
1.	Alkaloids	–
2.	Phenolic	+++
3.	Flavonoids	–
4.	Carbohydrates	++
5.	Tannins	+
6.	Saponins	+

+++ High; ++ Medium; + Low; – Absent

involved in the reduction and stability of AgNPs was investigated using Fourier transform infrared (FT-IR; Nicolet iS5, Thermo, USA) spectroscopy. The morphology and size of green-synthesized AgNPs were determined through Transmission electron microscopy (TEM, Tecnai 10, USA) and scanning electron microscope (Jeol JSM 6390 model). In addition, energy-dispersive X-ray (EDX) connected to SEM was used to determine the elemental composition of AgNPs. The size distribution was measured by a laser particle-size analyzer (Nanotrak Wave II, Microtrac Inc, USA). X-ray diffraction (XRD) analysis was performed using a Philips X-pert Pro diffractometer, UK, which was used to study the crystalline state of AgNPs. X-ray photoelectron spectroscopy (XPS, Specs Spectrometer) was used to determine the chemical information on the surface of synthesized AgNPs (Aravinthan et al. 2015).

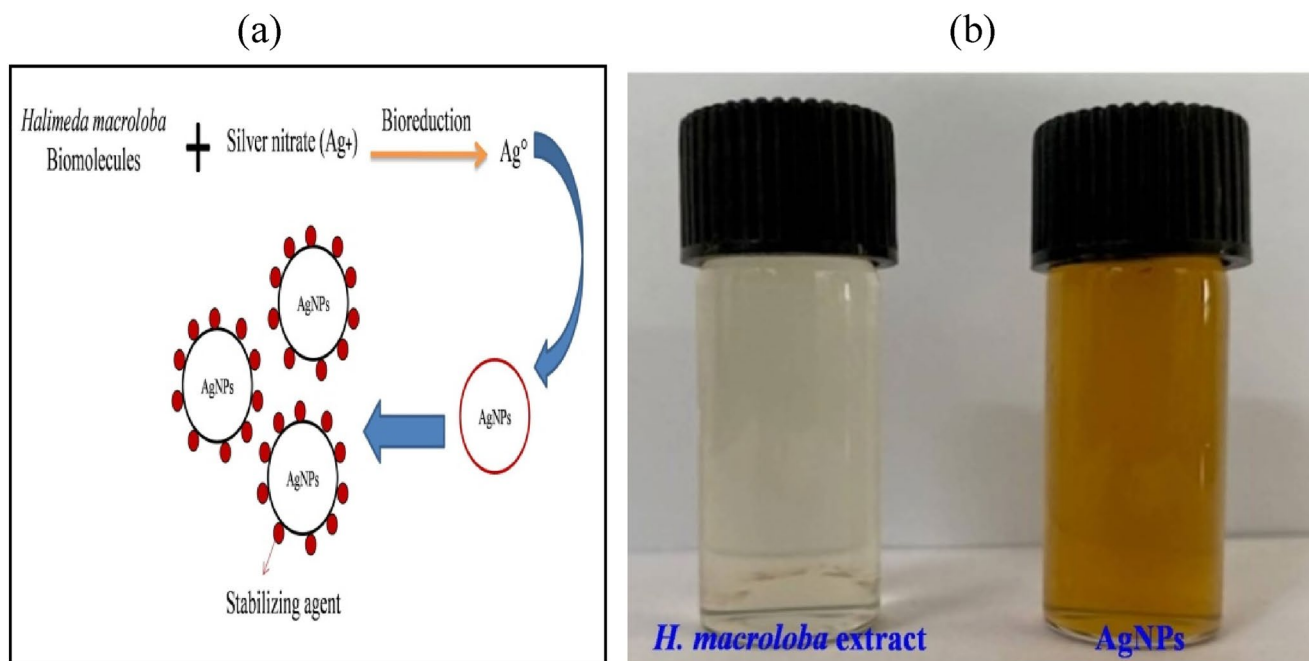
## In vitro cytotoxicity of algae-based AgNPs

MTT (dimethyl thiazolyltetrazolium bromide) assay was used to test the *in-vitro* cytotoxicity of the green algae-based synthesis of AgNPs against breast human hepatoma (Huh-7) cells based on the methodology adopted by Kumar et al. (2014). Briefly, Huh-7 cells ( $1 \times 10^5$  cells/well) were seeded onto the 96-well plates at 37 °C for 24 h. After that, cells were cultured at 37 °C for 24 h with 0–100 g/ml of *H. macroloba* extract and AgNPs. Thereafter, 10  $\mu$ l of MTT solution (0.5 mg/mL) was added to each well and the incubation period was extended for another 4 h. The formation of purple-colored formazan crystals was slowed by the addition of 100  $\mu$ l of dimethyl sulfoxide (DMSO) followed by monitoring their absorbance at 590 nm with a microtiter plate reader (Bio-Rad, USA).

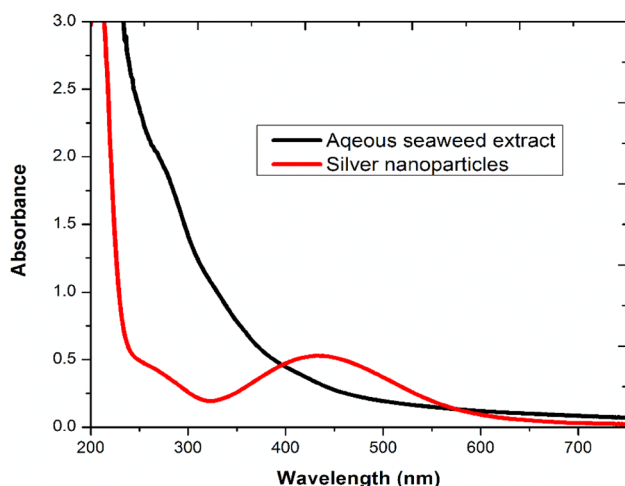
## Morphological staining

### Evaluation of apoptosis by acridine orange and ethidium bromide (AO/EtBr) staining

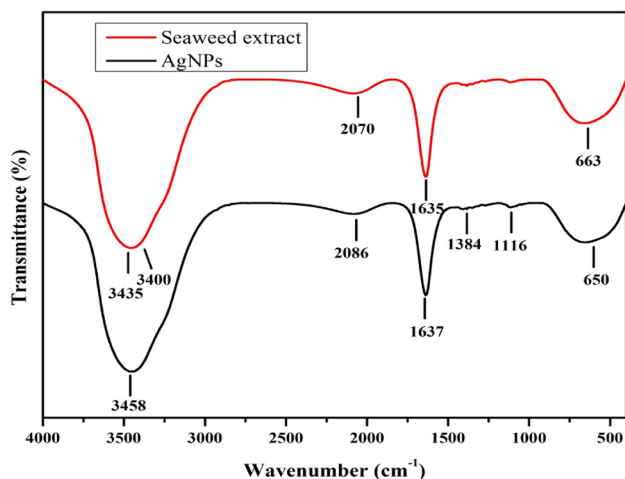
The morphology and membrane permeability of AgNPs-treated Huh-7 cells were investigated using the AO/EtBr dual staining technique (Puja et al. 2020). Briefly, Huh-7 cells ( $1 \times 10^5$  cells/well) were cultured in 6-well plates and treated for 24 h with AgNPs at a concentration of 90.12 g/



**Fig. 1** a Bioreduction and stability of AgNPs using *H. macroloba* extract and b visual examination of AgNPs formation



**Fig. 2** UV–Vis adsorption spectra of *H. macroloba* extract and AgNPs



**Fig. 3** FT-IR spectrum of *H. macroloba* extract and AgNPs

mL ( $IC_{50}$ ). The cells were rinsed in phosphate-buffered saline (PBS) and stained with a 2  $\mu$ L combination of AO/EtBr (100 g/mL) for 5 min after the medium was removed. A fluorescence microscope (Accu-Scope, EX310, USA) was used to examine the cells at a magnification of 20X.

### Measurement of intracellular ROS

2'-7'-Dichlorofluorescein diacetate (DCFH-DA) staining was used to determine the amount of intracellular ROS activities in Huh-7 cells. Briefly, Huh-7 cells ( $1 \times 10^5$  cells/well) were cultured in 6-well plates and treated for 24 h with AgNPs at a concentration of 90.12 g/mL ( $IC_{50}$ ). The cells were washed in PBS and stained with 100  $\mu$ L of DCFH-DA (50  $\mu$ M) for 15 min under dark. A fluorescence microscope (Accu-Scope,

EX310, USA) was used to examine the cells at a magnification of 20X.

### Assessment of changes in mitochondrial membrane potential ( $\Delta\Psi_m$ ) (MMP)

Huh-7 cells were cultured and treated for 24 h with AgNPs at a concentration of 90.12 g/mL ( $IC_{50}$ ). The cells ( $1 \times 10^5$  cells/well) were washed in PBS and stained for 1 h in the dark at 37 °C with rhodamine 123 dye. The mitochondrial membrane potential was examined under a fluorescence microscope (Accu-Scope, EX310, USA).

### Photocatalytic degradation of methylene blue (MB) dye

The photocatalytic degradation efficiency of the synthesized AgNPs was assessed by MB with sunlight irradiation (Kayalvizhi et al. 2020). The catalyst AgNPs, weighing about 10 mg, was ultrasonically dispersed in 100 mL of MB for 10 min. The pH and temperature of the dye solution has been observed to be 7.0 and  $30 \pm 2$  °C. The mixed solution was stirred evenly for 30 min in the dark to balance the absorption desorption equilibrium. The solution was sunlight irradiated at given time intervals and extracted in a cuvette to measure the absorption spectrum was estimated using a UV–Vis spectrophotometer (Evolution-201, Thermo, USA). The photocatalytic degradation of MB was monitored at regular intervals of 10 min. The photocatalytic degradation potential of MB was calculated using the following equation:

$$\text{Photodegradation of MB (\%)} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100\%, \quad (1)$$

where  $A_0$  is the adsorption equilibrium concentration of the solution, and  $A_t$  is the concentration of solution at time  $t$ .

## Results and discussion

### Phytochemical analysis

A phytochemical screening study was performed to detect the presence of phytochemicals in aqueous leaf extract of *H. macroloba*. Table 1 showed the presence of high phenol, carbohydrates, tannins, and saponins in the aqueous leaf extract of *H. macroloba*. As a result, it is reasonable to assume that phenolic compounds and reducing sugar have a greater capacity to bind  $Ag^+$  ions and may function as a reducing agent, primarily responsible for the bioreduction of  $Ag^+$  to  $Ag^0$  and stability of AgNPs (Fig. 1a) (Ramkumar et al. 2017; Arumai Selvan et al. 2018; Govindappa et al. 2021).

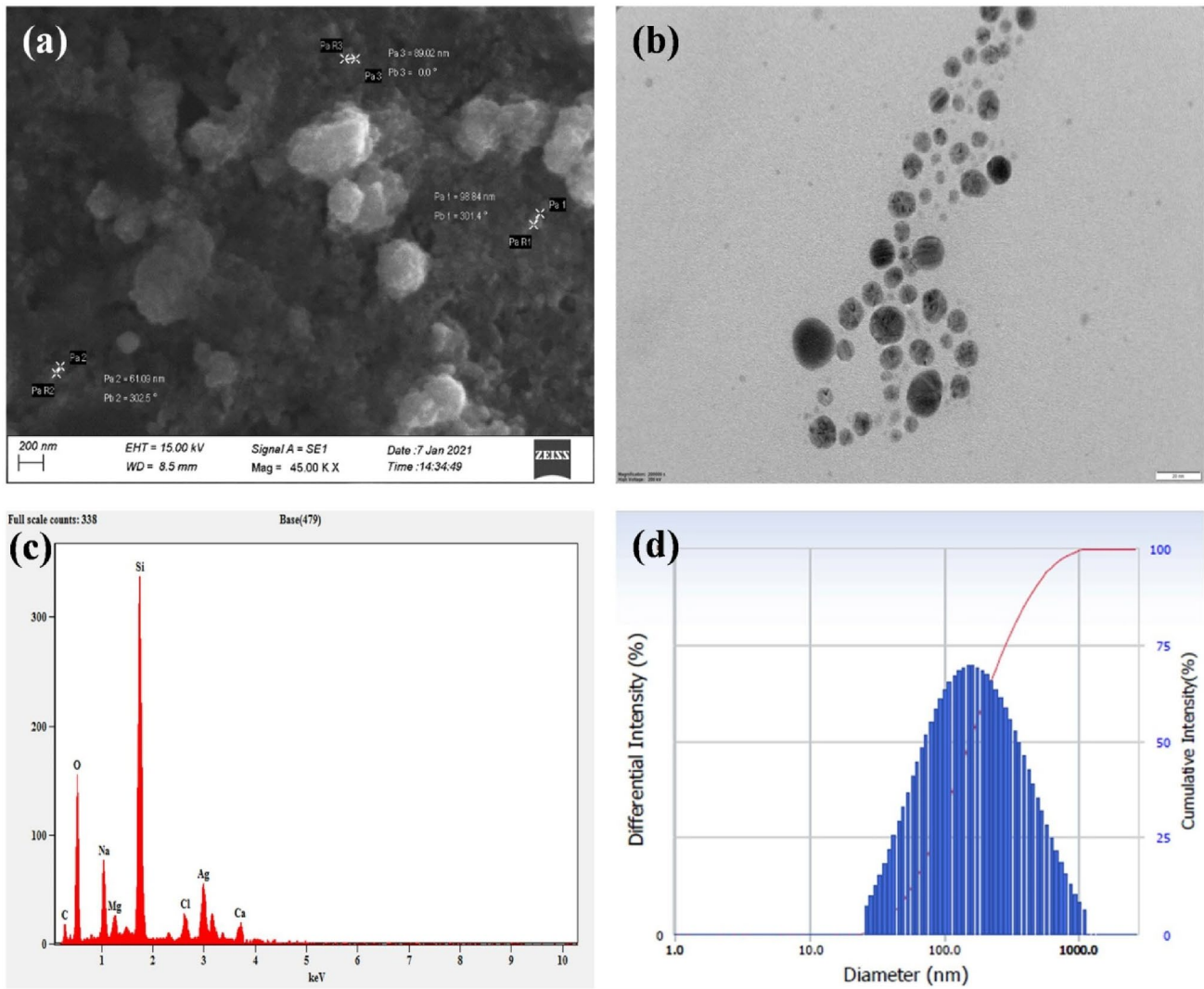


Fig. 4 a SEM micrograph, b TEM micrograph, c EDAX spectrum of Ag, and d particle-size histogram of AgNPs

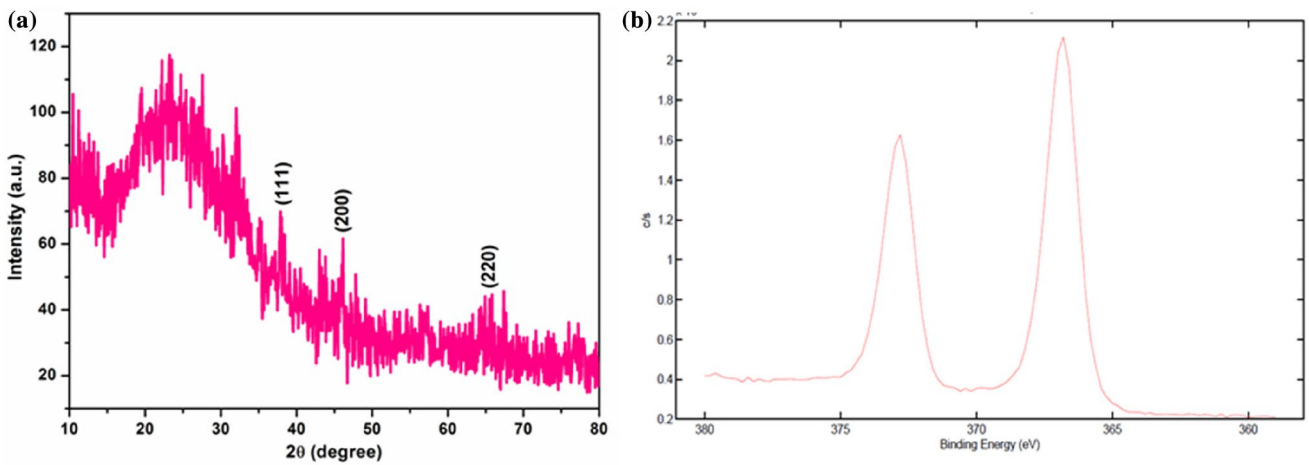
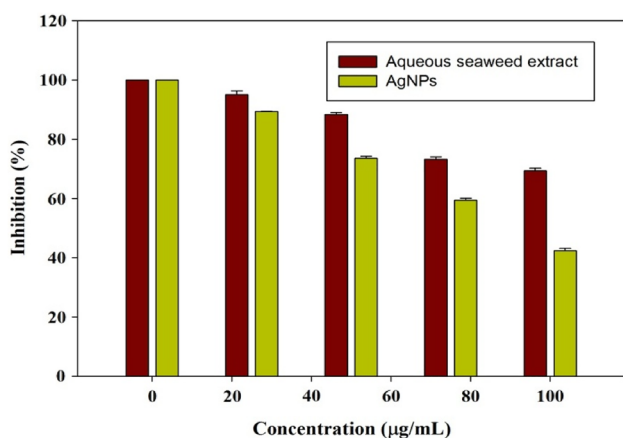


Fig. 5 a XRD pattern and b XPS spectrum of AgNPs



**Fig. 6** MTT assay of HUH-7 cells treated with different concentration of synthesized AgNPs

### Visual examination of AgNPs

Visual examination of the reaction media revealed the first sign of AgNPs' synthesis. The pale yellow reaction mixture gradually turned dark brown after adding *H. macroloba* extract to AgNO<sub>3</sub> solution, indicating the green synthesis of AgNPs (Fig. 1b). The development of a unique dark brown color was caused by the activation of surface plasmon resonance (SPR) in AgNPs (Govarathan et al. 2014; Mythili et al. 2018; Narayanan et al. 2021). This suggests a high concentration of phenols, carbohydrates, and other phytochemicals that may be involved in the bioreduction of Ag to AgNPs. Several additional reports have verified the same (Gopu et al. 2021; Sampath et al. 2021).

### UV–Vis and FT-IR spectral analysis

The green-synthesized AgNPs was confirmed using UV–visible spectroscopy from 200–750 nm. The UV–Vis spectrum of synthesized AgNPs revealed a strong absorbance peak at 420 nm due to the stimulation of SPR (Fig. 2). Several studies have shown that the SPR of AgNPs at 410–440 nm corresponds to spherical shape of AgNPs (Govarathan et al. 2014; Chinnappan et al. 2018). FT-IR analysis was used to analyze the functional groups contained in *H. macroloba* extract that are responsible for the synthesis of AgNPs. Figure 3 shows that spectrum of *H. macroloba* aqueous extract showed absorbance peaks at 3435 cm<sup>-1</sup>, 3400 cm<sup>-1</sup>, 2070 cm<sup>-1</sup>, 1635 cm<sup>-1</sup>, and 663 cm<sup>-1</sup>. The major peaks at 3435 cm<sup>-1</sup>

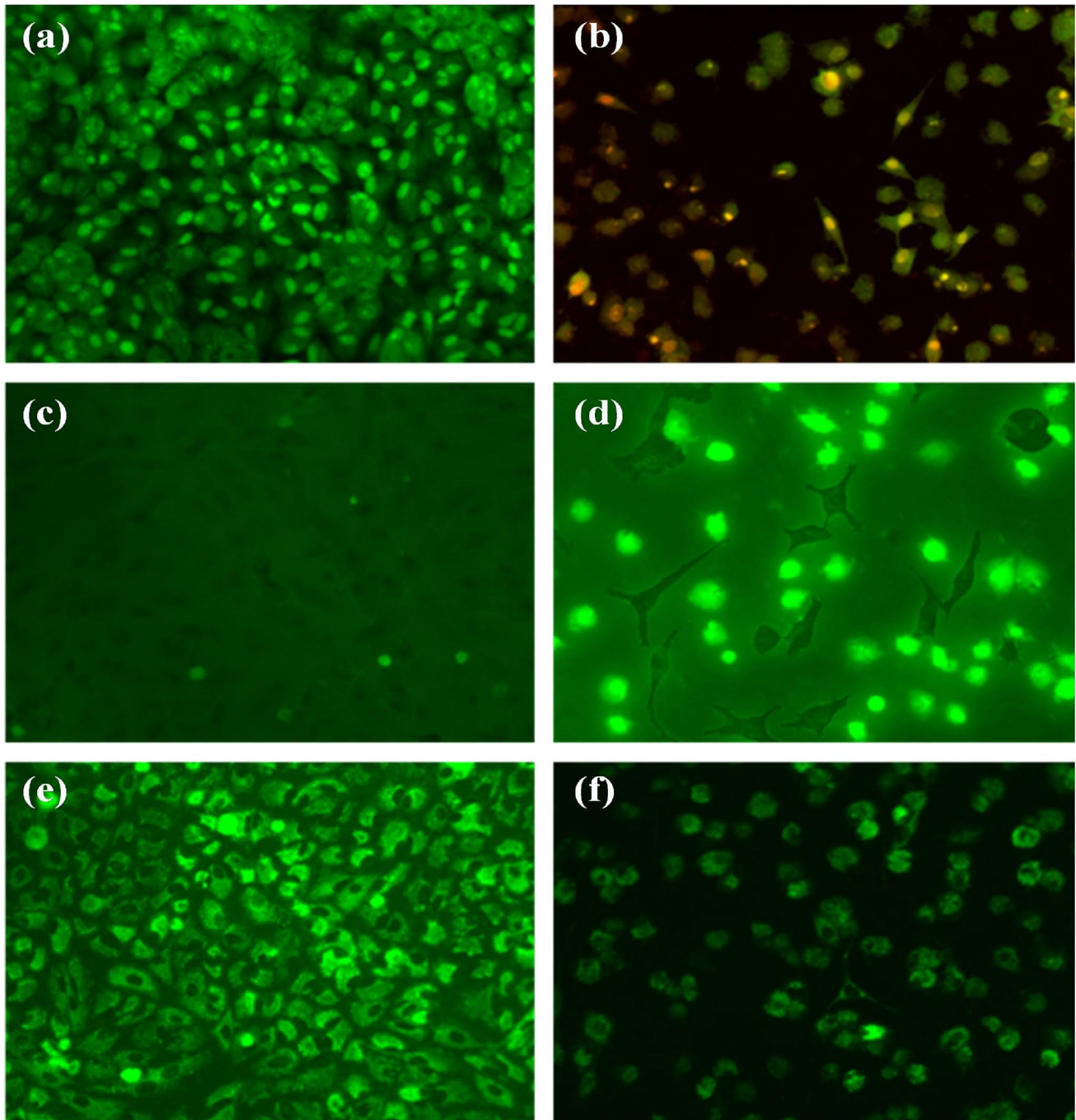
and 3400 cm<sup>-1</sup> corresponds to the presence of hydrogen-bonded N–H-stretching vibrations, polyphenolic O–H stretching, respectively (Lakhan et al. 2020; Babu et al. 2020). The strong peak appeared at 1635 cm<sup>-1</sup> corresponds to the C=C-stretching vibrations of the phenolic group in the extract (Hamedi and Shojaosadati 2019). The spectrum of synthesized AgNPs showed absorbance peaks at 3458 cm<sup>-1</sup>, 1637 cm<sup>-1</sup>, 1384 cm<sup>-1</sup>, 1116 cm<sup>-1</sup>, and 650 cm<sup>-1</sup>. The peak appearing at 1637 cm<sup>-1</sup> corresponds to C=O stretching of carbonyl group that becomes manifest in this range. The shifting and loss of peaks from AgNPs relative to those from *H. macroloba* extract prove the presence of a reaction between the *H. macroloba* and AgNPs (Rajkuberan et al. 2015; Sherin et al. 2020).

### SEM, TEM, and PSD characterization

The morphological depiction of synthesized AgNPs was confirmed by SEM micrographs. The SEM micrographs of the AgNPs revealed that they were spherical in form and well dispersed throughout the solution, with aggregation (Fig. 4a). Furthermore, TEM analysis was used to study the surface shape, size, and distribution of the synthesized AgNPs. Figure 4b shows that the green-synthesized AgNPs to be spherical in shape with a size of about 50–100 nm. The biological activity of AgNPs is affected by intrinsic properties, including shape and size. Figure 4c depicts the EDX spectrum at 3 keV, which verified the existence of silver as a significant component element (Lee et al. 2013; Aravinthan et al. 2015; Sengottaiyan et al. 2016; Selvam et al. 2017). Figure 4d shows the particle-size histogram; the average particle size of Ag NPs was determined to be 125.1 nm.

### XRD and XPS analysis

X-ray diffraction was used to examine the crystalline structure of the green-synthesized AgNPs (Fig. 5a). The rather wide XRD pattern indicates the synthesized of AgNPs. The XRD patterns of green-synthesized AgNPs show a significant 2 theta peak at 38.53, 44.26, and 67.23 indexed to the plane (111), (200), and (220), respectively. The plane values are consistent with the findings of face-centered cubic structure from JCPDS card number 01–1164. Figure 5b depicts XPS patterns of synthesized AgNPs. The strong binding energy peak of silver was observed at 367.5 eV and 373.8 eV, corresponding to orbital of 3d<sub>5/2</sub> and 3d<sub>3/2</sub>, respectively. These peaks shows that Ag (0) is

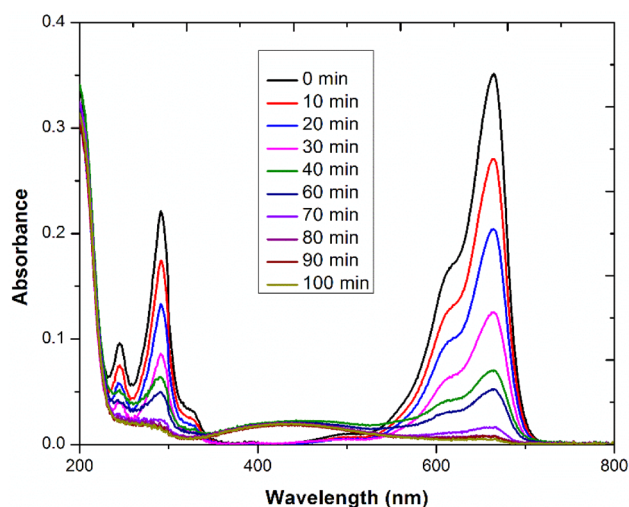


**Fig. 7** AO/EtBr stained morphology (a) before, (b) after AgNPs treated; ROS (c) before (d) after AgNPs treated; MMP (e) before (f) after AgNPs treated

the dominant and Ag (I) is completely reduced. The intensity detected in the energy values 367.5 eV and 373.8 eV shows the existence of Ag (0) and Ag (I) (Kumar et al. 2016; Aygün et al. 2020).

### In vitro biocompatibility and anticancer activity against Huh-7 cells

The in vitro biocompatibility of green-synthesized AgNPs was checked. The biocompatibility is



**Fig. 8** Photocatalytic degradation of MB

concentration-dependent, with an increase in AgNPs' concentration resulting in a decrease in biocompatibility. Figure 6 shows the effect of green-synthesized AgNPs with different concentrations (0–100  $\mu\text{g}/\text{mL}$ ) for 24 h onto Huh-7 cells. Cell viability is concentration-dependent, with an increase in AgNPs' concentration resulting in a decrease in cell viability. Biosynthesized AgNPs with an  $\text{LC}_{50}$  value of 89.5  $\mu\text{g}/\text{mL}$  showed significant cytotoxic action. *H. macroloba* aqueous extract alone showed no significant cytotoxic activity against Huh-7 cells. Hence, the AgNPs-treated Huh-7 cells exhibit significant anticancer potential. In a previous work, biosynthesized AgNPs had a dose-dependent anticancer effect on Huh-7 cells (Fageria et al. 2017; Bin-Jumah et al. 2020).

AO/EtBr dual staining methods are commonly used to assess apoptotic changes in cells. Uniform green stained cells signify healthy cells, whereas yellow green granulated granules and orange red cells reflect early and late apoptotic cells, respectively. Figure 7 depicts apoptotic labeling with AO/EtBr which was used to discriminate between living and dead cells. Figure 7a shows the control, the cell nuclei fluoresced consistently green, suggesting that the cells were healthy and the nuclei were intact. Figure 7b shows

that the cells treated with AgNPs at  $\text{IC}_{50}$ , on the other hand, show significant increases in the number of apoptotic cells. The emission of red fluorescence is highly caused by fragmented and condensed DNA within nuclei, implying apoptosis (Puja et al. 2020; Babu et al. 2020; Patel et al. 2021). Figure 7c, d depicts the results of an analysis of the intracellular ROS level in AgNPs-treated cells using the fluorescent dye DCFH-DA. Fluorescence microscopic images revealed increased green fluorescence in cells after treatment with green-synthesized AgNPs. Active mitochondria within cells can be identified with the cationic dye rhodamine 123, which can be easily sequestered by active mitochondria without causing cytotoxicity (Cottet-Rousselle et al. 2011). Figure 7c, d depicts the mitochondrial membrane potential of before and after treated cells with synthesized AgNPs. Treatment with the  $\text{IC}_{50}$  concentration of synthesized AgNPs resulted in a reduction in ATP generation, indicating the presence of deformed mitochondria (Puja et al. 2020).

### Photocatalytic degradation of MB

Figure 8 depicts that the photocatalytic decolorization of MB dye was performed utilizing synthesized AgNPs under direct sunlight irradiation with various incubation times. Under sunlight irradiation, the strength of the absorption peaks progressively declines with increasing duration, with no change in peak location. The highest absorption band of MB in the synthesized AgNPs is around 664 nm (Hamedi and Shojaosadati 2019). This shift in the absorption spectra demonstrates AgNPs' catalytic capability in the degradation of MB to a colorless solution. In less than 100 min, AgNPs degraded MB by about 91.32%. Furthermore, the catalytic capability of produced AgNPs is highly dependent on their size and shape (Ebrahimzadeh et al. 2020; Li et al. 2020; Rajkumar et al. 2021). Comparative data of MB dye degradation by the photocatalyst, AgNPs synthesized using different biological entity including the present work are shown in Table 2. The green-synthesized AgNP is found to be highly efficient photocatalyst in terms of amount of catalyst needed and time taken for degradation of MB dye (Table 2).

**Table 2** Comparison of some parameters for degradation of MB using biogenic AgNPs

S. no.	Biological entity	Reaction time (mins)	Degradation efficiency (%)	References
1.	<i>Chlorella vulgaris</i>	180	96.51	(Rajkumar et al. 2021)
2.	<i>Coleus Vettiveroids</i>	180	70.00	(Ajay et al. 2022)
3.	<i>Cinnamon bark</i>	60	97.00	(Aravind et al. 2022)
4.	<i>Sargassum ilicifolium</i>	180	100	(Devi et al. 2022)
5.	<i>Nostoc carneum</i>	20	92.50	(Borah et al. 2023)
6.	<i>Halimeda macroloba</i>	100	91.35	This work



## Conclusions

The current work focused on the biogenic, simple, eco-friendly, and cost-effective synthesis of AgNPs utilizing *Halimeda macroloba* aqueous leaf extract. FT-IR spectroscopy indicated the participation of functional groups in the bioreduction of  $\text{Ag}^+$  to  $\text{Ag}^0$ , and UV–Vis spectra show the peak at 430 nm typical of AgNPs. TEM and SEM micrographs confirmed the spherical shape and crystalline nature of the green-synthesized AgNPs. XRD reveals crystalline nature, while the EDX spectrum reveals silver. The green-synthesized AgNPs exhibits higher cytotoxicity activity against Huh-7 cells in a dose-dependent manner. In addition, AgNPs showed apoptosis-associated changes, intracellular ROS changes, and effective mitochondrial membrane potential by AO/EtBr staining, DCFH-DA, rhodamine 123 dye. Furthermore, green AgNPs showed significant catalytic activity in the reduction of MB dye. Overall, green-synthesized AgNPs exhibit a wide spectrum of medicinal and environmental remediation characteristics.

## Declarations

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

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