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Cymodocea serrulata-capped silver nanoparticles for battling human lung cancer, breast cancer, hepatic cancer: Optimization by full factorial design and *in vitro* cytotoxicity evaluation

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ABSTRACT

In recent times, there has been growing interest in nanoparticles (NPs) synthesized through biological means due to their ease of production and their potential applications in the field of biology. This study presents an environmentally friendly method for the biogenic synthesis of silver nanoparticles (AgNPs) using the leaf extract of *Cymodocea serrulata* as both a reducing agent and a capping agent. Various physico-chemical and microscopic techniques were employed to comprehensively characterize the biogenically produced AgNPs. The results of these characterization studies confirmed the formation of spherical, stable, and crystalline AgNPs with an average size of 30.5 ± 2.5 nm. Furthermore, the antibacterial assessment revealed the remarkable antibacterial properties of these biogenically synthesized Ag NPs, even at exceedingly low concentrations ranging from 50 to 100 μg/mL. The IC_{50} values for the biogenically synthesized AgNPs against different human cancer cell lines, such as A549, MDA-MB-231, HepG2, and MCF-7, were determined to be 93.4 ± 4.5, 82.5 ± 3.7, 87.6 ± 4.1, and 57.3 ± 2.5 μg/mL, respectively. Most notably, the biogenically synthesized Ag NPs exhibited significant anti-inflammatory activity, as evidenced by their IC_{50} value of 30.08 ± 1.4 μg/mL, as assessed through the HRBC membrane stabilization method. These *in vitro* findings strongly suggest that AgNPs fabricated through biogenic processes using *Cymodocea serrulata* leaf extract hold promise as potential therapeutic candidates for combating bacterial infections, cancer, and inflammatory conditions.

1. Introduction

In recent years, nanotechnology has garnered vast attention as a new research area, offering distinctive features and wide-ranging applications in various fields such as biomedicine, sensors, cosmetics, and food technology [1]. Nanoparticles (NPs) are nanometer-sized particles ranging from 1 to 100 nm and possess unique physical, chemical, and magnetic properties, making them suitable agents for novel biomedical applications [2]. Among the different types of NPs, noble metallic NPs, including gold, silver, zinc,

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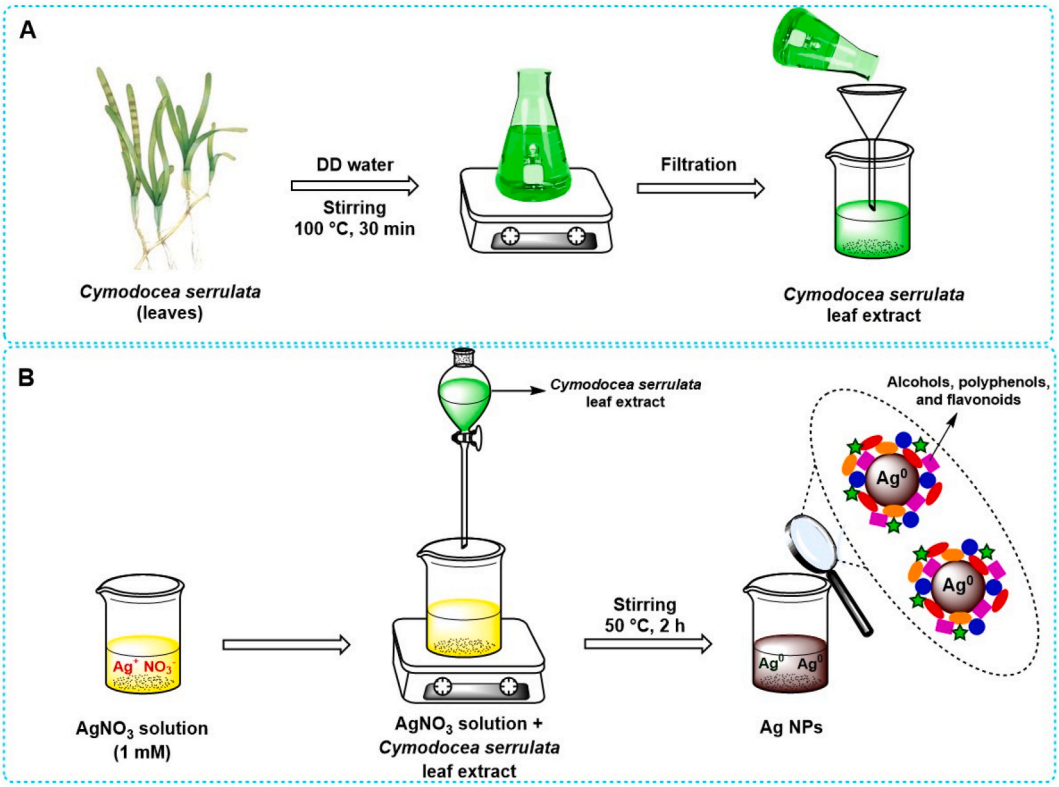

Fig. 1. Schematic illustration of (A) preparation of *Cymodocea serrulata* leaf extract, (B) biogenic synthesis of AgNPs using *Cymodocea serrulata* leaf extract.

and others, have attracted pharmaceutical attention due to their multifunctional therapeutic potential [3]. Moreover, conventional NP synthesis methods involve the use of hazardous chemicals, resulting in toxicity issues. Therefore, alternative methods for synthesizing metal NPs using plant secondary metabolites have gained noteworthy attention. These methods are cost-effective, eco-friendly, highly efficient, have short reaction times, and produce metal NPs with low side effects, clinical adaptability, and biocompatible properties [4–7]. Additionally, plants contain biologically active secondary metabolites such as polyphenols, proteins, alkaloids, flavonoids, and terpenoids. These secondary metabolites possess effective reducing capabilities, stabilizing and capping potentials, and therefore play an imperative role in the biosynthesis of metal NPs with predefined properties.

Among the various types of biogenically synthesized metal NPs, silver nanoparticles (AgNPs) have emerged as superior metal NPs in recent decades due to their distinctive physicochemical and biological properties [8,9]. In fact, Ag exhibits excellent disinfectant as well as bacteriostatic effects. The bacteriostatic effect of Ag particles is enhanced when reduced to the nanometer level, making AgNPs highly suitable for antibacterial applications [10]. Although Ag has some toxic effects at higher concentrations, lower concentrations of Ag exhibit good stability, better catalytic potential, considerable biocompatibility, and remarkable therapeutic activities [11]. Compared to bulk Ag, AgNPs offer the advantage of controlled release [12]. Notably, AgNPs exhibit potent anticancer and antibacterial activities [13]. The anticancer activity of AgNPs is mainly derived from their ability to disrupt telomerase stability mechanisms, interfere with the mitochondrial respiratory chain, induce the formation of reactive oxygen species (ROS) above a threshold level, inhibit ATP synthesis, cause DNA damage, and ultimately inhibit the growth of cancer cells [14–17]. Furthermore, AgNPs possess several advantages over other forms of Ag, such as negligible toxicity, highly effective biological activities at very low concentrations, and no known side effects [18–20]. While many investigations have been conducted on biogenic AgNPs synthesis, the potential anti-inflammatory and anticancer activities of AgNPs are not widely reported. Therefore, in the present study, we have selected *Cymodocea serrulata*, commonly known as sea grass, to biogenically synthesize AgNPs. *Cymodocea serrulata* possesses potent medicinal properties, including antimicrobial, antioxidant, cytotoxicity, and antifouling activities [21–23].

In this article, we report the biogenic synthesis of AgNPs using leaf extract of *Cymodocea serrulata* as a reducing and capping agent. Furthermore, we explore the anti-inflammatory, anticancer, and antibacterial activities associated with these biogenically synthesized AgNPs. This study highlights the environmentally friendly development of a new nano formulation with effective chemotherapeutic applications.

2. Experimental section

2.1. Materials

The Leaves of *Cymodocea serrulata*were collected from local seashore of Rameshwaram, Tamil Nadu, India. Silver nitrate ($AgNO_3$) was procured from Merck in analytical grade. Double distilled (DD) water was used in throughout the experimental section. All other solvents were obtained in the analytical grade and did not require any additional purification process.

2.2. Preparation of Cymodocea serrulata leaf extract

The Fresh leaves of *Cymodocea serrulata*(15 g) were collected as well as washed with DD water for several times. These clean leaves were crushed with grinder [24]. Then 250 ml of DD water was added into the ground mass of leaves then heated at 100 °C for 30 min under the condition of constant magnetic stirring (Fig. 1A). Lastly, the leaf extract was filtered through the WhatmanNo.1 filter paper and then stored at 4 °C [25].

2.3. Biogenic synthesis of silver nanoparticles (AgNPs)

The prepared *Cymodocea serrulata* leaf extract(10 mL) was added drop wise into the $AgNO_3$ solution (1 mM, 100 mL) (Fig. 1B) [26–28]. This mixture was then heated at 50 °C and continuously stirred for above 2 h. The colour of the mixture changed from the light green to the dark brown indicated the successful AgNPs formation [29–32]. This mixture was transferred into centrifuge tube and then centrifuged for 15 min at 13000 rpm. The separated AgNPs washed with DD water and followed by acetone to remove the residual parts of leaf extractand then dried at a room temperature for a day [33–35].

2.4. Characterization of biogenically synthesized AgNPs

Crystallographic studies of the biogenically synthesized AgNPs were done by X-ray diffractometer, D/Max-IIIC, Japan. The FTIR was performed on Avator 360, America, spectrophotometer. The formation of AgNPs (reduction of Ag ions into AgNPs) was monitored and confirmed by UV–Vis spectrophotometer, Perkin Elmer at different wavelength from 200 to 700 nm. The morphology of biogenically synthesized AgNPs was investigated by Scanning electron microscopy, SEM, FEI QuanTa200, Holland. Transmission electron microscopy (TEM) analysis was performed on Philips/CM12 TEM (120 kV) to measure the size of AgNPs. The hydrodynamic particle size of Ag NPs was found by dynamic light scattering (DLS) method using Zeta sizer instrument, Malvern Instruments, UK.

2.5. Antibacterial assay

The antibacterial activity of the biogenically synthesized AgNPs was assessed using the disc diffusion method.Two gram positive bacteria *Bacillus subtilis (B.Subtilis)* and *Pseudomonas aeruginosa* (*P. aeruginosa*); and 2 g negative bacteria *Staphylococcus aureus* (*S. aureus*) and Escherichia coli (*E. coli*) were used in this assay. First, a bacterial suspension was uniformly spread over on Muller-Hinton Agar. Then, 25 μL of different concentrations of the *Cymodocea serrulata* leaf extract and biogenically synthesized AgNPs (50 and 100 μg/mL) were added in different discs and incubated at 37 °C for 24 h [36]. In this study gentamycin and DMSO were used as a positive and negative control, respectively.The inhibition zone was measured in terms of millimetres (mm) [37].

2.6. Cytotoxicity assay

Anticancer activity of AgNPs against the different types of cancer cell lines such as human lung cancer (A549) cell line, human breast cancer (MDA-MB-231 and MCF-7)cell lines, and a hepatic cancer (HepG2) cell line were assessed by using cytotoxic assay. In this method, the cancer cells were seeded (1×10^4) in a 96-well plate and treated with Dulbecco's modified Eagle's medium, which was supplemented with 10% FBS. Then the cancer cell lines were incubated under 5% CO_2. Cultured A549, MDA-MB-231, HepG2 and MCF-7 cancer cell lines were treated with different concentrations (20, 40, 60, 80 and 100 μg/mL) of biogenically synthesized AgNPs in DMSO. After the treatment of AgNPs, the cells were incubated in the CO_2 incubator for 24 h. After that, the morphology of the cells was observed under a microscope, and then, the cancer cells viability was found by hemocytometer using trypan blue [38].

2.7. In vitro anti-inflammatory assay

The anti-inflammatory activity of biogenically synthesized AgNPs was confirmed by HRBC membrane stabilization method. In this method, the human blood samples were obtained from healthy human volunteers (they did not taken the non-steroidal anti-inflammatory drugs for 2 weeks prior to the assay performed). First, the blood sample was shaken with Alsever's solution and centrifuged at 5000 rpm for 10 min. The separated red blood cells were washed with isosaline. The different concentrations (20, 40, 60, 80 and 100 μg/mL) of *Cymodocea serrulata* leaf (CS) extract and AgNPs were added into red blood cells. Moreover, 2 mL of hyposaline, 1 mL of phosphate buffer solution, and 0.5 mL of HRBC suspension were added [39]. Then, this mixture was incubated and centrifuged at 3000 rpm for 5 min. Finally, the haemoglobin content was measured by spectrophotometrically at 560 nm. In this assay, diclofenac and *Cymodocea serrulata* leaf extract were used as a standard and control, correspondingly [40].

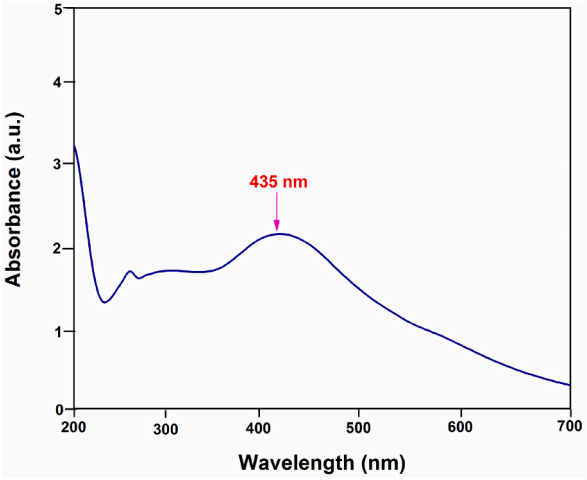

Fig. 2. UV–Vis spectrum of the biogenically synthesized AgNPs.

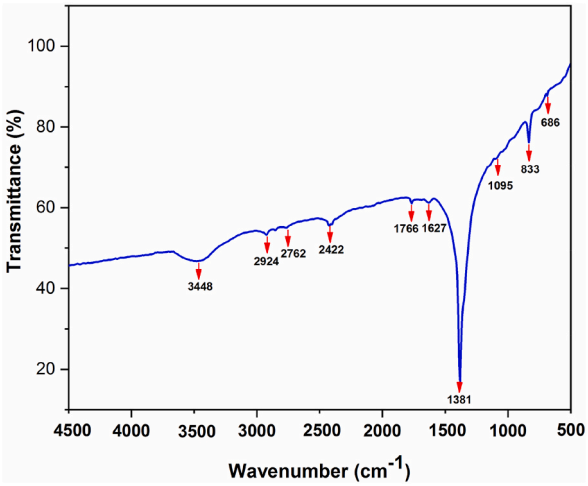

Fig. 3. FTIR spectrum of the biogenically synthesized AgNPs.

2.8. Statistical analysis

The study was carried out in triplicate to guarantee the dependability and reproducibility of the results. Statistical analysis of the data was executed using Origin Pro 8.1 software developed by Origin Lab. A one-way analysis of variance (ANOVA) was applied, followed by a Tukey test to ascertain statistical significance. The results were expressed as the mean ± standard error of the mean (SEM) for each investigation, with a consistent sample size (n) of 3. Statistical significance was determined by a p-value below 0.05, indicating notable distinctions among the compared groups.

3. Results and discussion

3.1. UV–Vis spectral analysis

Using UV–Vis spectroscopy, it was demonstrated that the biogenically synthesized AgNPs exhibit an absorption peak that corresponds to their absorption characteristics.The UV–visible spectrum of the AgNPs produced from the leaf extract of *Cymodoceaserrulata* can be seen in Fig. 2. AgNPs are characterized by a characteristic absorption peak at 435 nm, which is confirmed by the UV–Vis spectrum spectra of AgNPs, which illustrates that AgNPs are formed by reducing Ag salts with *Cymodocea serrulata* leaf extract (a reducing agent) [41].

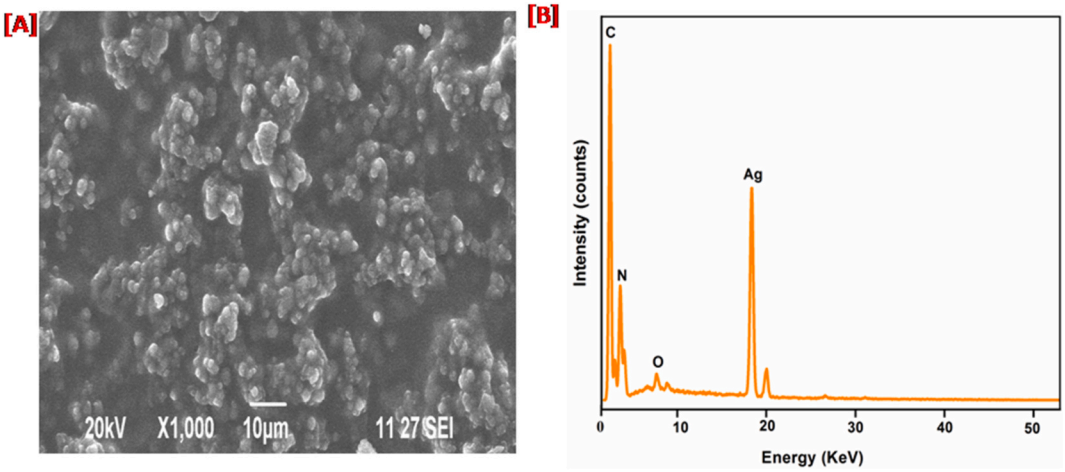

Fig. 4. (A)SEM image and (B) EDX spectrum of biogenically synthesized AgNPs.

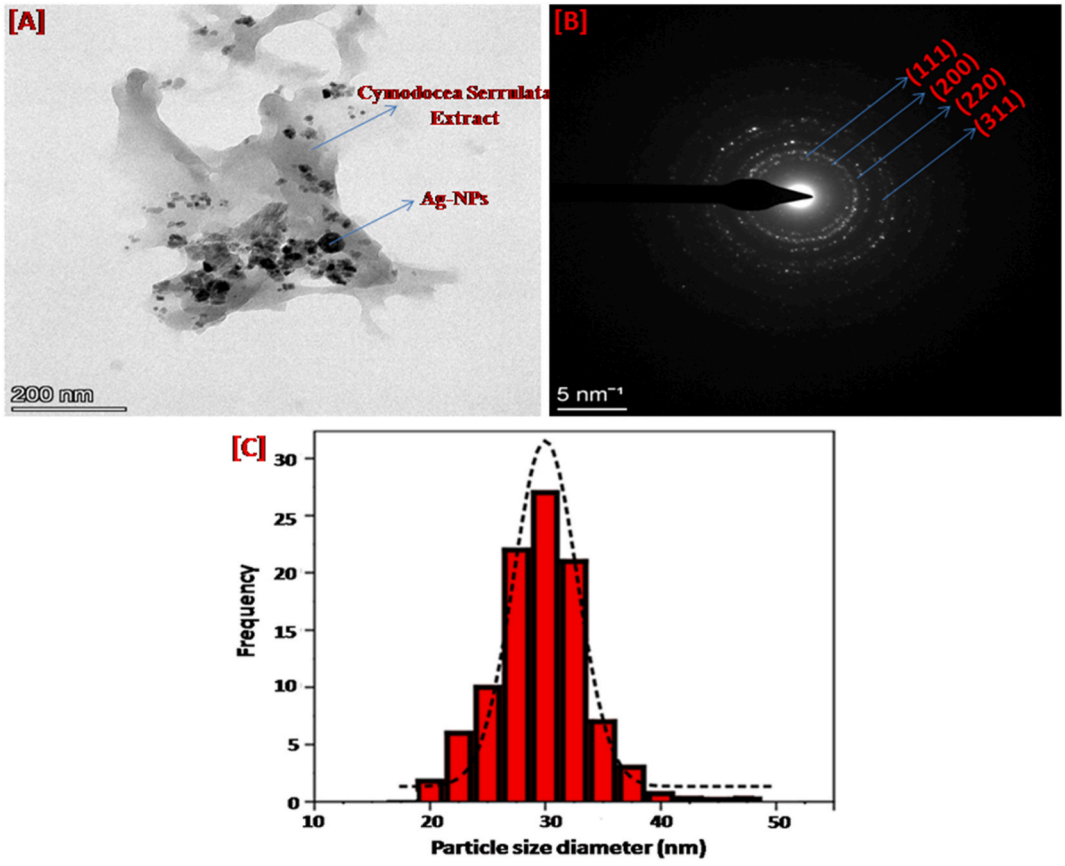

Fig. 5. (A)TEM image (B) SAED pattern and (C) DLS spectrum of biogenically synthesized AgNPs.

3.2. FTIR analysis

A FTIR analysis was conducted within *Cymodocea serrulata* leaf extract with the objective of identifying the biomolecules responsible for the reduction of Ag^{+} ions into AgNPs under the action of its biomolecules. The FTIR spectra of AgNPs comprise the absorption bands at the different wavelengths of 3448, 2924, 2762, 2422, 1766, 1627, 1381, and 1095 cm^{-1}(Fig. 3). A high absorption peak at 3448 cm^{-1} has been associated with the O–H stretching vibrations in alcohols, polyphenols, and flavonoids which should lead

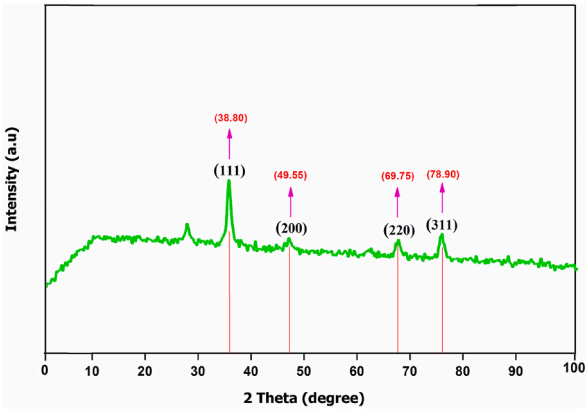

Fig. 6. XRD pattern of the biogenically synthesized AgNPs.

to the absorption peak [42,43]. This leaf extract of *Cymodocea serrulata* represents the presence of aromatic compounds whose stretching vibrations are responsible for the absorption peaks of 2924, 2762, and 2422 cm^{-1}. The C=O, N–H stretching vibrations at 1766 cm^{-1} and 1627 $cm^{-1}$for carbonyl compounds, and amide (I) in proteins, respectively. The C–H stretching vibrations (1381 cm^{-1}) of methylene moieties, the C–N stretching vibrations (1254 cm^{-1}) of aliphatic amines [44] and the C–O stretching vibrations (1095 cm^{-1}) of alcohols and phenols were also observed [45]. These findings unequivocally demonstrate that the presence of alcohols, polyphenols, and flavonoid compounds in the *Cymodocea serrulata* leaf extract plays a pivotal role in both the AgNPs' formation and stability.

3.3. Morphological analysis

Using SEM analysis, we were able to determine the morphology of the biogenically synthesized AgNPs, which were made using a synthesis method. As can be seen in Fig. 4A, the SEM image shows the spherical shape of AgNPs with porous surface morphology which makes them appear to be nanoparticles [46]. The AgNPs sample is seen to be an agglomerated form due to the drying effect. It has been confirmed through energy dispersive x-ray (EDX) analysis that *Cymodocea serrulata* leaf extract protects the surface of AgNPs on the cellular level (Fig. 4B). As the EDX spectrum shows, the characteristics of carbon, nitrogen, oxygen, and silver peaks are all present in the spectrum of Ag NPs.

To quantify the size, morphology, and distribution of AgNPs synthesized by biogenically synthesizing them, TEM methods were applied to analyze their size, morphology, and distribution of sizes (Fig. 5A) [47]. As can be seen in the TEM image of the Ag nanoparticles, the biogenically synthesized Ag nanoparticles are evenly dispersed in the matrix of *Cymodocea serrulata*, thereby indicating that the biomolecules of *Cymodocea serrulata* were instrumental in stabilizing the AgNPs. Moreover, the AgNPs produced by the *Cymodocea serrulata* leaf extract are spherical in shape and the size distribution of the particles ranges between 20 and 40 nm. The AgNPs produced by the extract gave a spherical shape to the final product. The average size of AgNPs was 30.5 $\pm$ 2.5 nm, which is further confirmed by DLS analysis (Fig. 5C). This average size of AgNPs is corresponded to the average size assessed based on the XRD analysis.

Fig. 5B shows the SAED pattern of biogenically synthesized AgNPs. The dotted ring patterns are well aligned to the d-spacing values of (111), (200), (220), and (311) diffraction crystal planes of Ag [48]. A crystalline composition, a definite size distribution, and a low degree of agglomeration are evident in our results and indicate that the AgNPs biogenically synthesized are the type to be crystalline.

3.4. X-ray diffraction analysis

The powder XRD analysis of the biogenically synthesized AgNPs contains four diffraction peaks at the 2θ values of 38.80°, 49.55°, 69.75°, and 78.90° as shown in Fig. 6. These peaks correspond to the (111), (200), (220), and (311) crystal planes of Ag, respectively (JCPDS file number: 04-0783), which reflect the FCC crystal structure of the biogenically synthesized AgNPs [49]. The XRD peaks corresponding to the (111), (200), and (311) planes were well comparable to the XRD pattern observed for the other biogenically synthesized AgNPs using various plant extract [50–52]. Moreover the average crystalline size of AgNPs was found to be 31.6 nm, which is supported by the TEM and DLS analysis results.

3.5. Antibacterial activity

The antibacterial activity of biogenically synthesized AgNPs was assessed by disc diffusion method [53] using 2 g-positive bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa* and 2 g-negative bacteria *Staphylococcus aureus* and *Escherichia coli* (Fig. S1). It can be seen from the analysis that the viability of bacteria decreases rapidly while the concentration of AgNPs increases, and in fact, the bacteria achieve a total death when at 100 g/mL the oxygen levels are reduced dramatically. As compared to the standard drug gentamycin, the

Table 1
Antibacterial activity (in terms of inhibition zone (mm)) of AgNPs, *Cymodocea serrulata* leaf extract.

Sample	Inhibition Zone (mm) at 100 μg/mL			
	B. subtilis	*P. aeruginosa*	*S. aureus*	*E. coli*
AgNPs	12 ± 0.5	18 ± 0.7	14 ± 0.5	17 ± 0.8
Cymodocea serrulata leaf extract	R	R	R	R
Gentamycin	4 ± 0.2	5 ± 0.2	7 ± 0.3	5 ± 0.2

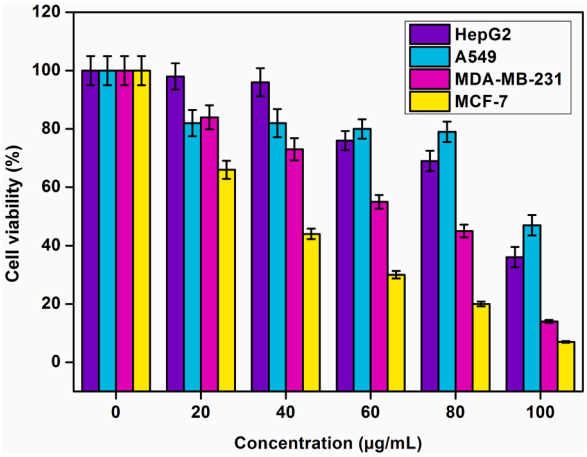

Fig. 7. Cytotoxicity profile of biogenically synthesized AgNPs on the viability of various human cancer cell lines.

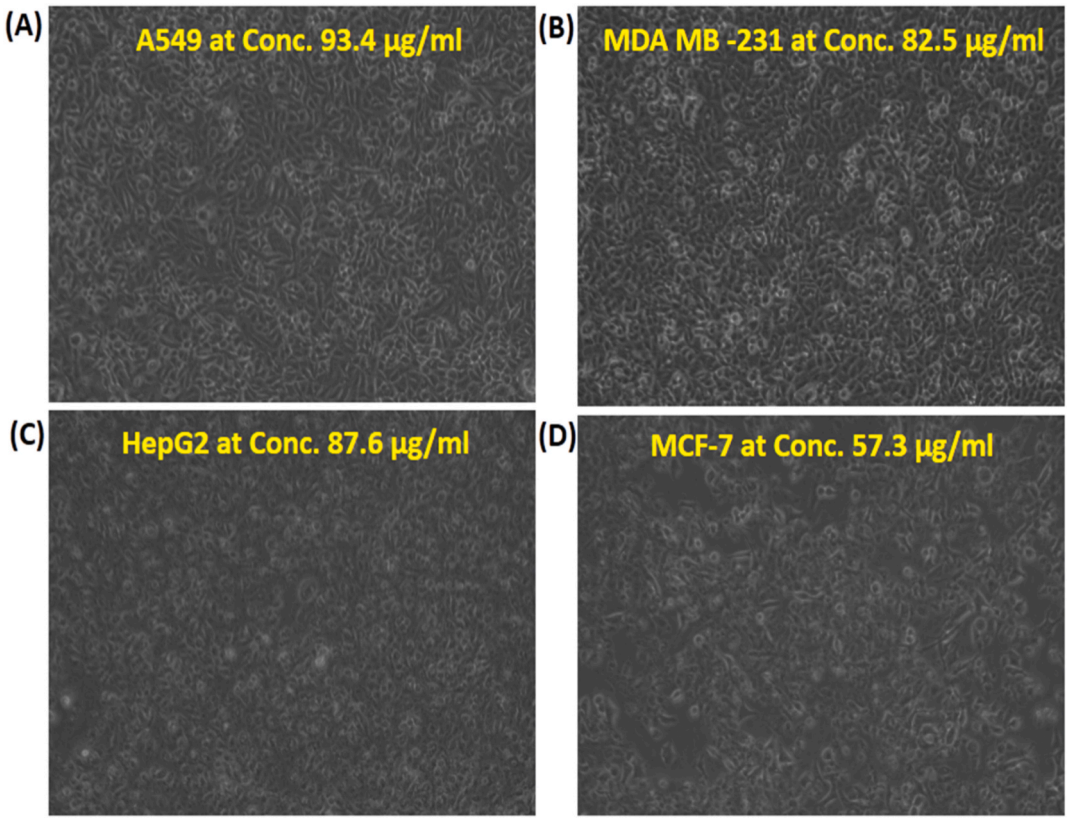

Fig. 8. (A–D)Morphology changes of various cancer cell lines at the IC_{50} concentration of biogenically synthesized AgNPs.

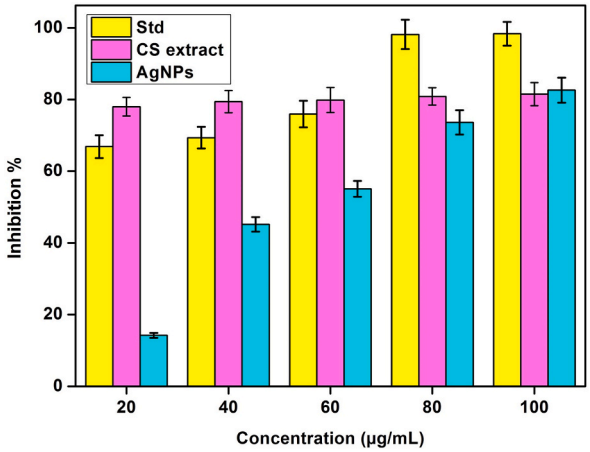

Fig. 9. *In- vitro* anti-inflammatory assay data of biogenically synthesized AgNPs, and *Cymodocea serrulata* leaf extract by HRBC membrane stabilization method.

bacteria are completely killed at 100 g/mL when compared with the concentration of AgNPs. According to our findings, biogenetically synthesized AgNPs have significantly greater antibacterial activity than gentamycin at low concentrations, indicating that they are more effective at low concentrations than the standard drug. It was found that pure *Cymodocea serrulata* leaf extract had low antibacterial activity against all bacteria species in a control experiment (Table 1).

3.6. Anticancer activity

To probe the anticancer activity of the biogenically synthesized AgNPs, different concentrations of the AgNPs were treated with various cancer (A549, MDA-MB-231, HepG2, and MCF-7) cell lines. The anticancer assay result shows that there is a steady decrease in cancer cells viability with increasing the concentration of biogenically synthesized AgNPs (Fig. 7). The IC_{50} value of biogenically synthesized AgNPs against the different types of human cancer cell lines was found to be 93.4 ± 4.5 (A549), 82.5 ± 3.7 (MDA-MB-231), 87.6 ± 4.1 (HepG2), and 57.3 ± 2.5 µg/mL (MCF-7). As result, AgNPs effectively kills MCF-7 cancer cells as compared to the rest of cancer cell lines. Moreover the morphology changes of the different cancer cells incubated with AgNPs at IC_{50} concentration was perceived under an inverted optical microscope (Fig. 8(A-D)). The biogenically synthesized AgNPs showed a difference in cytotoxicity towards the different cancer cell lines, this might due to enhanced cellular uptake via endocytosis and retention of AgNPs [54–56]. AgNPs may stimulate reactive oxygen species (ROS) above to the threshold level and perhaps damage cellular component which lead to cell death by apoptosis mechanism [57]. The outcomes of the *in vitro* anticancer investigation provide evidence that AgNPs biogenically produced through the utilization of *Cymodocea serrulata* leaf extract have the potential to emerge as a viable chemotherapeutic option for combating cancer. Moreover, they may offer a solution to the challenges associated with traditional anticancer medications.

3.7. In vitro anti-inflammatory studies

Using biogenically synthesized AgNPs, it was examined whether their anti-inflammatory properties are concentration-dependent. It was found that spectrophotometric measurements at 560 nm were effective in measuring the haemoglobin absorbance both with and without AgNPs, *Cymodocea serrulata* leaf extract, and diclofenac as a standard drug. The biogenically synthesized AgNPs demonstrated more potent anti-inflammatory actions with an IC_{50} value of 30.08 ± 1.4 µg/mL than the *Cymodocea serrulata* leaf extract and standard drug with IC_{50} values of 57.72 ± 2.9 and 197.6 ± 7.4 µg/mL, respectively (Fig. 9). Considering the results presented here, it appears that biogenically synthesized AgNPs need to be considered as a potential anti-inflammatory agent, which is capable of inhibiting inflammation efficiently within the human red blood cells [58]. Our initial *in vitro* investigations into the anti-inflammatory properties of biogenically synthesized AgNPs suggest their potential as a therapeutic option for mitigating inflammatory diseases through the reduction of oxidative stress and inflammation.

4. Conclusion

In conclusion, our research demonstrates the successful and biogenic synthesis of silver nanoparticles (AgNPs) using *Cymodocea serrulata* leaf extract. The biogenically synthesized AgNPs were characterized using various physico-chemical and microscopic techniques. The results of analyses revealed that the presence of alcohols, polyphenols, and flavonoids in the leaf extract of *Cymodocea serrulata* was responsible for the formation and stability of AgNPs. The AgNPs exhibited a spherical shape, with a particle size distribution ranging from 20 to 40 nm. The average crystalline size of the AgNPs, as determined by XRD analysis, was 30.5 ± 2.5 nm. Furthermore, the results of antibacterial study showed that the biogenically synthesized AgNPs displayed potent antibacterial activity,

even at low concentrations. Additionally, AgNPs demonstrated significant cytotoxicity against human breast cancer cells (MCF-7), with an IC_{50} value of 57.3 ± 2.5 μg/mL. Notably, the biogenically synthesized AgNPs exhibited remarkable anti-inflammatory activity, with an IC_{50} value of 30.08 ± 1.4 μg/mL. These findings underscore the potential of biogenically synthesized AgNPs using *Cymodocea serrulata* leaf extract as effective therapeutic agent in the biomedical field, offering superior antibacterial, anticancer, and anti-inflammatory properties.

Author contribution statement

S. Venmani - Conceived and designed the experiments.
Mookkandi Palsamy Kesavan - Analyzed and interpreted the data; Wrote the paper.
Srinivasan Ayyanaar - Performed the experiments; Wrote the paper.
N. Muniyappan - Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20039.

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