Biocompatibility assessment of silver chloride nanoparticles derived from Padina gymnospora and its therapeutic potential

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Abstract

The objective of the present work was to improve the biological activity of Padina gymnospora. In the current study, silver chloride nanoparticles have been synthesized using the aqueous extract of Padina gymnospora and further characterized by ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, x-ray powder diffraction, scanning electron microscopy, energy dispersive spectroscopy, transmission electron microscopy and atomic force microscope. Further, the hemolytic activity and eco-toxicity of silver chloride nanoparticles analyzed. The synthesized silver chloride nanoparticles were found to be mono-dispersed and spherical with an average size of 11.5–32.86 nm. The particles showed an anticancer effect in a dose-dependent manner against breast cancer cell line (MCF-7 cell lines) (IC50 = 31.37 μg ml−1). In addition, it showed the larvicidal activity against Aedes aegypti at a lower dose (3.92 μg ml−1) than that of the aqueous extract (13.01 μg ml−1). Nanoparticles also exhibited greater antimicrobial activity for both bacterial and fungal pathogens. The synthesized silver chloride nanoparticles showed a maximum zone of inhibition, i.e., 31 mm for Candida albicans followed and 27 mm for vancomycin resistance Enterococcus faecalis. The results suggest the possible use of synthesized silver nanoparticles with P. gymnospora as therapeutic agent for breast cancer, dengue vector control and as antimicrobial agent.

1. Introduction

Evolving nano-based therapeutics has received substantial attention over the last two decades [1]. The fusion of silver nanoparticles with natural resources is looked at as an eco-friendly and cost-effective approach that bounces progression over the other Physico-chemical methods [2–5]. Nanotechnology, a rapidly growing field which involves the production and use of nanomaterials in various research areas [6–8]. At the present time, researchers are aimed to design the synthesis of nanoparticles for various applications which includes therapeutics and diagnostics purposes, based on their cellular mechanisms and the advanced technology in nanomaterials [1]. Silver ions have the capability to inhibit the bacterial multiplication, by binding and denaturing bacterial DNA, thus affecting the ribosomal subunit protein and some enzymes important for bacterial cell growth by penetrating the cells [6, 9, 10]. Silver nanoparticles, the most striking metal nanoparticles which have anti-tumour efficacy [11–13], antimicrobial [14–16], and adjuvanticity [17]. In biomedical and clinical research studies, the preparation of nanoparticles with the desired properties has become a topic of considerable importance for researchers in order to achieve biocompatibility, biosafety and substantial drug localization to the cells [17–19].
Besides, silver-based nanoparticles have much more attention due to their enhanced property of physico-chemical and biological characteristics based upon the nanoparticles preparation by using additional elements [16]. In the middle of associated silver materials, silver chloride is essential in the application of wound healing, antimicrobial, and acts as a preservative of drinking water for long-term storage in the tank [8, 20]. An earlier report suggested that the green synthesized nanoparticle showed activity against the Ae. aegypti [21]. Silver chloride nanoparticles (AgClNPs) also have various photochemical and biomedical applications such as fabricating antiseptic catheters, bone cement, and fabrics owed to the antibacterial activity. While silver chloride (AgCl) a bearable source of silver ions which has a potent agent for treating infection. Owing to their small size, AgCl based nanoparticle can easily penetrate into the cell membrane and cause cell malfunction by accumulation in intracellular. Thus, they possess the toxic to bacteria compared to the bulk materials [22]. In spite of these advantages, the silver chloride nanoparticles (AgClNPs) preparation is limited to such methods as micro-emulsion technique, ultrasound irradiation, matrix-based technique or mixing silver nitrate controlled sized [23–25]. Several protocols have been reported for the AgClNPs synthesis using microorganisms such as bacteria, fungi and algae [15, 26].

Recent research focused on marine algae for the nanoparticle synthesis, because of their bioactive metabolites [27]. Extract of seaweed consists of bioactive compounds includes phenols, ascorbic acid, flavonoids, polyphenolic, alkaloids and terpenes are they also possess reductase which act as a reducing agent [28]. Previous reports also revealed that the marine algae might be a good biological agent for nanoparticle synthesis [29, 30]. Also, few researches shown the synthesis and characteristics features of AgNPs from the marine algae of Padina pavonia [30, 31]. Eventhough, several protocols have been reported for the AgClNPs synthesis. Since the utilization of marine resources for AgClNPs synthesis is yet too pursued. To this extent, very few reports were available under the criteria of the synthesis of AgClNPs from marine resources and their application in medicinal field [32].

P. gymnospora is marine algae that belongs to the family Dictyotaceae. It has a brown and darker morphology at the base and the fan-shaped lobe has split into wedge-shaped pieces. It is used as a prominent natural wound-care product [33]. In the present study, it was initiated to explore the application of green synthesized AgClNPs by using seaweed P. gymnospora (Pg-AgClNPs) in the medical application as anticancer, antibacterial and larvicidal activity. In addition to that, the toxicity effect and the eco-toxicity of Pg-AgClNPs were assessed in Artemia nauplii.

2. Materials and methods

2.1. Plant materials and chemicals

Seaweeds (Padina gymnospora) were collected from Gulf of Mannar region, Tuticorin located (8.76°42′ N; 78.13°48′ E) at the Southeast coast of India by handpicking and rinsed immediately with water to take away all kinds of epiphytes and other impurities (i.e., sand, mollusks, seagrasses, etc). Then they were packed in sterilized ziploc bags and transferred to the laboratory. Silver nitrate, Cell culture medium, trypsin, and FBS were procured from HiMedia, Mumbai, India. Other chemicals/reagents used in this study were purchased from HiMedia, Mumbai, India.

2.2. Preparation of seaweed extract

The collected seaweeds were cleaned with de-ionized water and allowed to shade dry for seven days. They were made into powder form using a blender. For the seaweed extraction, the ten grams of algal powder was added into every three Erlenmeyer flasks containing 100 ml of double distilled water and mixed well and kept for 5 h without disturbance. Then they were filtered through Whatman No.1 filter paper and were used for the synthesis of silver nanoparticles.

2.3. Synthesis of nanoparticles from seaweed extract

For AgNP synthesis, silver is used as a precursor. The AgNO₃ and extracts of P. gymnospora selected for of green synthesis of nanoparticles. Previously, AgNO₃ (1 mM) dissolved in distilled water (100 ml). The extract of P. gymnospora was added to AgNO₃ solution in 2:10 ratio and incubated in a dark room for a night. After reduction, the incubated solution was centrifuged at 10 000 rpm for 15 min at 4°C. The obtained residues were washed several times to remove un-reacted materials.

2.4. Physico-chemical characterization of synthesized nanoparticles

2.4.1. UV–vis spectra analysis

The colour change was observed in the silver nitrate solution incubated with an aqueous extract of P. gymnospora. Bioreduction of AgNO₃ ions in the solution was monitored by periodic sampling of aliquots...
(0.1 ml) of aqueous component and measuring the Ultraviolet-Visible spectroscopy (UV–vis spectra) of the solution in 10-mm optical path length quartz cuvettes with a UV-1601 (Shimadzu, International, Co. Ltd, Tokyo, Japan) spectrophotometer at a resolve of 1 nm between 400 and 800 nm with a scanning speed of 1856 nm min⁻¹.

2.4.2. XRD analysis
The x-ray powder diffraction (XRD) was done to analyze the crystallite size of synthesized nanoparticles by the biological method. Samples were prepared by making a thin film of powder with ethanol on a glass plate and the measurement was performed using an Ultima IV—Rigaku diffractometer with CuKα radiation (λ = 1.540 Å, 45 kV and 30 mA) Tokyo, Japan.

2.4.3. FT-IR analysis
Fourier-transform infrared spectroscopy (FT-IR) analysis was performed using a FTIR-8400S- spectrometer (Shimadzu, International, Co. Ltd, Tokyo, Japan) to verify possible interaction of chemical bonds between bio-molecules of seaweed and silver nitrate solution. Samples were scanned from 400–4000 cm⁻¹ with potassium bromide pellets. Characteristic peaks of plain synthesized nanoparticles, seaweed extract and were expressed in a reciprocal wavelength (cm⁻¹).

2.4.4. HR-TEM analysis
Conventional High-resolution transmission electron microscope (HR-TEM) analysis was performed in (HR-TEM-JEOL-2100 + JEOL India Pvt. Ltd, New Delhi, India) using a magnification of 46 000×. The samples were prepared by the formvar resin grid method. Briefly, a 0.5% w/v suspension of prepared synthesized nanoparticles were sprayed onto a formvar resin-coated TEM grid and air-dried for 10 min before the observation. The morphology of nanoparticles complexes was photographed.

2.4.5. SEM and EDX analysis
For analysing the morphological characteristics, the synthesized nanoparticles were uniformly spread and sputter coated with platinum using an ion coater for 120 s, and then observed under a scanning electron microscopy (SEM) (EVO18-CARL ZEISS, USA). Energy dispersive spectroscopy (EDX) is a technique used to identify the elemental composition of the specimen or an area of interest thereof. The system works as an integrated feature of the SEM which was done by using Quantax 200 with X Flash® 6130(Bruker India Scientific Pvt. Ltd Bangalore, India).

2.4.6. AFM analysis
A thin film of the sample was prepared on a coverslip by dropping 0.1 ml of the sample on the slide and allowed to dry for 30 min. The slides were then scanned with an atomic force microscope (AFM), (Park systems XE-100 AFM system, Suwon, Korea). The AFM characterization was carried out in ambient temperature in non-contact mode using silicon nitrate tips with varying resonance frequencies.

2.5. Anti-proliferative effects
2.5.1. MTT-based cytotoxicity assay
The cytotoxic effect of synthesized nanoparticles against human tumor cell lines was determined by a rapid colorimetric assay, using 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and compared with untreated controls. MCF-7 cells were seeded in 96-well plates in 100 μl of medium containing 5% FBS, at plating density 10 000 cells/well and incubated at 37 °C, 5% CO₂, 95% air and relative humidity (100%) for 48 h prior to addition of compounds. After 48 h, compounds at various concentrations were added and incubated at 37 °C, 5% CO₂, 95% air and relative humidity (100%) for 48 h. After the treatment, the cells were incubated with MTT solution (100 μl, 0.5 mg ml⁻¹) for 4 h and solubilized with DMSO (150 μl). The absorption was recorded at 545 nm in an ELISA reader. The cell survival data were normalized as a percentage of the untreated control (set as 100% viability). All of the experiments were conducted in triplicate for concordant values [33].

2.5.2. Assessments of cell death through Flow cytometry
Flow cytometry study was carried out using propidium iodide staining. Breast cancer cell line (MCF-7) was seeded in 6 well microtiter plates. After 24 h, the medium was discarded, and the nanoparticle’s calculated concentration was added in each well and incubated for 48 h at 37 °C. Cells were washed with PBS for several times and trypsinized. The cells were then suspended in 1 ml of PBS and centrifuged at 1000 rpm for 3 min. The supernatant was discarded and the pellet obtained was vortexed at the lower speed. Then, 1 ml of PBS was added again and centrifuged at 1000 rpm for 6 min. The supernatant was discarded and the pellet was resuspended in
250 μl of PBS. The obtained cell suspension was stored in 0.8 ml of ethanol and incubated at 4 °C for 30 min. To the sample (volume), 10 μl of propidium iodide was added and incubated at room temperature for 30 min and protected from light. Flow cytometry was determined immediately using a flow cytometer (BD, Sasc, Jazz Trademark, US).

2.6. Mosquito rearing
The egg rafts of Ae. aegypti obtained from the Vector Control Research Centre, Madurai, Tamil Nadu India. Larvae of Ae. aegypti reared in enamel trays containing dechlorinated water. The larvae fed with a finely powdered mixture having a 3:1 ratio of dark biscuits and dry yeast. Then larvae were observed and monitored for III instar stages, and it used for the larvicidal activity.

2.6.1. Larvicidal activity
The toxic activity of different concentrations (2, 4, 6, 8 and 10 μg ml⁻¹) of Pg-AgCNPs was evaluated against the III instar of Ae. aegypti. The aqueous extract of P. gymnospora tested at various concentrations (5, 10, 20, 30, and 60 μg ml⁻¹) against the larvae. Mortality was recorded after 24 and 48 h of post-exposure [34]. The results obtained were subjected to statistical analysis. The dose-dependent response data were concerned to probit analysis for finding the LC₅₀, upper, and lower confidence limit at 95% [35].

2.7. Anti-microbial activity
The antimicrobial activity of synthesized nanoparticles was done by the well diffusion (or) Kirby- Bauer methods in Muller Hinton Agar (MHA) medium. The sterilized MHA medium at 121 °C at 15 lbs for 20 min poured into sterile Petri plates. After solidification, pathogens were swabbed using a sterile cotton swab over the surface of the medium. After 15 min the wells were made using cork-borer on agar medium. 100 μl of three different concentrations (1 mg ml⁻¹, 2 mg ml⁻¹ and 3 mg ml⁻¹; prepared in 50% Dimethyl sulfoxide (DMSO) of nanoparticles were loaded at three different wells. Zone of inhibition were measured after 24 h of incubation at 37 °C.

2.8. Minimum Inhibitory Concentration (MIC) of synthesized nanoparticles
The experiment was carried out in 96 well plate. 100 μl of serially diluted synthesized nanoparticles (512, 256, 128, 64, 32, 16, 8, 4, 2, 1 mg l⁻¹ in Muller- Hinton Broth) were taken in 96 well plate. 100 μl of commercially available antibiotic was taken in a separate well. 5 μl of 12 h grown test pathogens (105 to 10⁶ cfu ml⁻¹) were added separately to all the wells except negative control. Plates were incubated at 37 °C for 16 h. After incubation, 10 μl of freshly prepared MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (5 mg ml⁻¹) was added to all the wells and incubated for 10 min 100 μl of DMSO solution was added as the solubilizing agent and incubated for 15 min. Optical density (OD) was taken at 595 nm and the percentage of cell death was calculated and MIC were noted.

2.9. Hemolytic assay
The hemolytic activity of synthesized nanoparticles was determined in fresh anti-coagulated human blood cells. The blood cells were washed twice with phosphate buffered saline (PBS), and diluted to 10% with PBS. 800 μl of diluted nanoparticles (different concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 μg ml⁻¹ of phosphate buffered saline) were taken in micro-centrifuge tubes. PBS alone serves as a negative control and 1% Triton X-100 serves as a positive control. 200 μl of Red Blood Cell (RBC) suspension was added to all the tubes and incubated for 1 h at 37 °C. All the tubes were centrifuged at 2000 rpm for 5 min and supernatant was read at 540 nm. The percentage of hemolysis was calculated as:

\[
\% \text{ of hemolysis} = \frac{AS - AN}{AP - AN} \times 100
\]

Where, AS = Abs of the sample treated, AP = Abs of positive and AN = Abs of Negative control respectively.

2.10. Toxicity assessment on Artemia nauplii
The acute eco-toxicity assay was performed by following the guidelines from OECD (Organisation for Economic Co-operation and Development) 2004 [36] with slight modifications. Artemia brine shrimp cysts were obtained from the Central Marine Fisheries Research Institute- Tuticorin, Tamil Nadu, India. The 48 hold Artemia nauplii exposed to different concentrations of synthesised nanoparticles (10–150 μg ml⁻¹). Each concentration was tested against A. nauplii ten number in a 24 well plate. The result was observed for 48 h without feed and aeration all over the experiment. The mortality rate of A. nauplii was taken undercount and lethality concentrations (LC₅₀ & LC₉₀) were determined by Probit analysis [35].
2.11. Statistical analysis

One-way analysis of variance (ANOVA) followed by a Tukey post-hoc test was used for MTT analysis. Probit analysis followed by Finney method. These statistical analyses were performed using Statistical Package for the Social Sciences 16.0 package Chicago, USA. Data were presented as mean ± SD and P values <0.05 was considered to be statistically significant.

3. Result and discussion

Nanotechnology plays a diverse role in medical sciences and pharmaceutical industries in recent years. Most silver nanoparticles are used as anticancer, antimicrobial agents and have been reported for many biomedical applications [11–13]. To the extent that, much research was not done under the criteria of the synthesis of nanoparticles from marine resources and about their application in medicinal field. Therefore, the marine algae, *P. gymnospora* were used for synthesising nanoparticles and their applications in the biomedical field have been investigated. The green synthesized nanoparticles were confirmed by the formation of the dark brown colour from the yellow colour in 24 h of incubation (figure 1). Similar colour change has also been observed on the synthesis of AgClNPs using an aqueous extract of *Sargassum plagiophyllum* [32]. The colour change is due to the reduction of Ag⁺ and Cl⁻ ions to form the AgClNPs [37]. Then it was optically confirmed by UV-spectrophotometric analysis. A strong surface plasmon resonance peaks were obtained around 377 nm.
The peaks were obtained at around 400 nm indicates the formation of AgNPs [38, 39]. The maximum absorbance indicates the relative size of the nanoparticle, where the higher number corresponds to the larger particle size [40]. The presence of chloride ions is confirmed by EDAX. EDAX analysis revealed the signal for silver (56.6%) along with signals of other elements B, C, O, Na, Si, and Cl. Thus, it confirms the chloride content be the source for the formation of AgClNPs [32, 41]. The presence of other element indicates the solution P. gymnospora AgClNPs. On the other hand, the presence of other factors stabilizes the AgNPs [42].

The FTIR analysis of P. gymnospora showed that the strong band at 1190 cm$^{-1}$ attributed to the vibration of C–O time as the presence of the alcoholic group. The medium to the weak band obtained at 2111.91 cm$^{-1}$ assigned to wave of C≡C as the presence of the alkyne group. The bending and medium band obtained at 2751.27 cm$^{-1}$ and 2861.2 cm$^{-1}$ attributed to C–H as the presence of the alkyne group. The medium and broad

![Figure 3. EDAX analysis P$_2$AgClNPs. EDAX analysis reveals the signal for silver (56.6%) along with signals of other elements B, C, O, Na, Si, and Cl.](image)

![Figure 4. (a): FTIR analysis of the aqueous extract of P.gymnospora. The presence of the amine group and alcohol group in P. gymnospora (b): FTIR analysis of P$_2$AgClNPs. It reveals that the presence of functional groups such as alcohol, amines, amides methyl, alkanes, halides and aliphatic compounds.](image)
band received at the stretch of 3399.3 cm$^{-1}$ associated due to the vibration of N–H as the presence of the amine group (figure 4(a)). On the other hand, $P_g$-AgClNPs showed the peak of C–O bend shifted from 1190 cm$^{-1}$ to 1091.63 cm$^{-1}$ and the peak or N–H bend shifted from 3399.3 cm$^{-1}$ to 3142.79 cm$^{-1}$ (figure 4(b)). This data reveals that the alcoholic and amine groups are involved in the synthesis of $P_g$-AgClNPs. In this sense, it is possible to carry out the reduction of Ag$^+$ ions by $P$. gymnospora extract; it confirms the presence of organic compounds associated with the synthesis of AgClNPs. The presence of functional groups such as alcohol, amines, amides methyl, alkanes, halides, and aliphatic compounds confirmed the synthesis of AgClNPs [38, 43, 44]. These functional groups are acting as biomolecules for stabilizing, capping, and reducing agent of AgNPs [45, 46, 43]. In this sense, it is possible to carry out the reduction of Ag$^+$ ions and Cl$^-$ ions by $P$. gymnospora extract; it confirms the presence of organic compounds associated with the synthesis of $P_g$-AgClNPs.

The crystalline nature of synthesized $P_g$-AgClNPs is obtained by XRD analysis. The XRD analysis exhibits the crystalline structure as obtaining diffraction peaks of (111), (200), (220), (311), (222) and (400) planes which located at 27.87°, 32.35°, 46.20°, 54.45°, 57.45° and 67.49° (figure 5). Moreover, the XRD spectrum is compared with the standard (JCPDS file no. 85-1355) and confirms the identity of the synthesized nanoparticles. There is
no extra peak indicates that the high purity of prepared \textit{Pg}-AgClNPs and the strong peak intensity shows a high crystallinity of the sample. The grain size was calculated as 30.39 nm. These similar reports were obtained in previous studies as 27.68°, 32.13°, 44.34°, 46.02°, 54.58°, 57.23°, 67.15°, 74.11° and 76.38° which corresponds to (111), (200), (111), (220), (311), (222), (400), (331) and (420) respectively [8] confirming the formation of AgClNPs according to diffraction standards (JCPDS file no. 85-1355) [32]. The absence of additional reflection indicates that \textit{Pg}-AgClNPs lattice was not affected by other molecules in seaweed extract. Similar XRD pattern was also reported in synthesised AgClNPs with \textit{Escherichia coli} cell-free supernatant and \textit{Bacillus subtilis} [20, 44]. SEM micrograph shows nanoparticles round like structure (figure 6) and the morphological features were obtained by HR-TEM analysis; it reveals that \textit{Pg}-AgClNPs are mono-dispersed spherical shape with the average size of 32.866 nm (figure 7). AFM micrographs indicate that the formulated \textit{Pg}-AgClNPs possess a spherical shape and the calculated sizes in the range of 15.5 to 21.38 nm (figure 8). It characterizes the interaction between the nanomaterials and the supported lipid bilayers in real-time. The particle size is varied from 10 to 28 nm. Previous study reported that the AgClNPs were spherical in shape and the size of the particles were around 18–42 nm [32].

The \textit{Pg}-AgClNPs were evaluated for antiproliferative activity against breast cancer cell line (MCF-7 cell lines) and the cytotoxicity to the normal (Vero) cell line was also studied. \textit{Pg}-AgClNPs showed a cytotoxic effect on breast cancer cells in a dose-dependent manner. The IC\textsubscript{50} values of \textit{Pg}-AgClNPs were found to be 31.37 \(\mu\text{g} \text{ml}^{-1}\) for MCF-7 cells and 421.46 \(\mu\text{g} \text{ml}^{-1}\) for Vero Cells (non-tumor cell) (figure 9). Interestingly, the synthesized \textit{Pg}-AgClNPs had negligible cytotoxicity effect on Vero non-tumour derived cell line. Growth inhibition related to cell cycle arrest and apoptosis was determined in flow cytometry using propidium iodide. MCF-7 cells treated with IC\textsubscript{50} concentration of AgClNPs increased the percentage of apoptosis in G0–G1 phase (5.16% when compared with control 1.39%) (figure 10). An increase in the percentage of apoptotic cells clearly indicates the induction of programmed cell death by AgClNPs treatment. The \textit{Pg}-AgClNPs induces cell death through the mitochondrial pathway (scheme 1). Earlier studies reported that AgNPs mediated cell death is independent of p53 [15, 47]. Previously, reported that the biosynthesis of AgClNPs induces cytotoxicity and apoptosis on human breast cancer cells through the up-regulation of apoptotic genes [15].

The larval bioassays performed by aqueous extract of \textit{P. gymnospora} and \textit{Pg}-AgClNPs against larvae of \textit{Aedes aegypti}, a dengue vector. The extract of \textit{P. gymnospora} showed LC\textsubscript{50} of 34.92, 21.61 and 11.82 \(\mu\text{g} \text{ml}^{-1}\) in 24, 48 and 72 h, respectively. The synthesized \textit{Pg}-AgClNPs showed LC\textsubscript{50} of 7.15 \(\mu\text{g} \text{ml}^{-1}\) in 24 h, 6.06 \(\mu\text{g} \text{ml}^{-1}\) in 48 h and 3.61 \(\mu\text{g} \text{ml}^{-1}\) in 72 h (table 1). Among these, the \textit{Pg}-AgClNPs showed higher larvicidal activity against \textit{Ac.
aegypti with low concentration compared to the aqueous extract of P. gymnospora. The toxicity mechanisms of mosquito mortality on the nanoparticle treatment were studied recently. It is hypothetically suggested that the mechanism of toxicity against mosquito larvae by the penetration of nanoparticles through the exoskeleton. In intracellular space, nanoparticle degrades the enzymes and organelles, and it leads to the loss of cellular function and cell death [48–50]. By the accumulation of nanoparticles in the midgut region of the mosquito, larva causes damage in the midgut, cortex region, and epithelial cells. Similar to seaweed mediated synthesis of nanoparticles, several plants mediated synthesized nanoparticles were showed mosquito larval control [51, 52].

Figure 8. (a), (b): AFM analysis of Pg-AgClNPs. The obtained micrographs indicate that the formulated Pg-Ag NPs possess a round shape and the calculated sizes in the range of 15.5 to 21.78 nm.
Compared to pathogenic bacteria and fungi, AgClNPs act as the best antifungal activity (figure 11) (table 2). Moreover, the MIC also investigated for human pathogens by using Pg-AgClNPs at the range of 1 mg ml$^{-1}$ by the serial dilution method (table 3). Among all the pathogens, Candida albicans and VREF showed the best minimum inhibition level at the concentrations of 8 mg l$^{-1}$ and 32 mg l$^{-1}$, respectively. Different variation and great impact on antimicrobial properties were predominately used in pharmaceuticals and drug industries [52].

Figure 9. The IC$_{50}$ values Pg-AgClNPs against breast cancer cells were found 31.37 $\mu$g ml$^{-1}$ for MCF-7 and 421.46 $\mu$g ml$^{-1}$ for Vero Cells (non-tumor cell).

Scheme 1. Pg-AgClNPs action illustrating the possible mechanism of the delivery system of non combinations induced anti-cancer activity.
Table 1. The larval bioassays performed by aqueous extract of *P. gymnospora* and Green synthesized silver chloride nanoparticle.

| Sample                  | Conc (μg ml$^{-1}$) | Exposure Time(hr) | LC$_{50}$ 95%LCL-UCL | LC$_{90}$ 95%LCL-UCL | Intercept  | $X^2$ Value | pValue |
|-------------------------|---------------------|-------------------|-----------------------|----------------------|------------|-------------|--------|
| Crude Extract           | 5                   | 24                | 34.92 (29.69 ± 43.95) | 61.73 (30.46 ± 86.43) | −1.67      | 3.75        | 0.99   |
|                         | 10                  | 48                | 21.61 (17.48 ± 25.91) | 44.87 (37.98 ± 57.59) | −1.19      | 6.90        | 0.90   |
|                         | 20                  | 72                | 11.82 (7.19 ± 15.35)  | 31.02 (26.18 ± 39.58) | −0.79      | 4.25        | 0.98   |
|                         | 30                  |                   |                       |                      |            |             |        |
|                         | 40                  |                   |                       |                      |            |             |        |
| *Pg*-AgClNPs            | 2                   | 24                | 7.15 (6.30 ± 8.16)    | 11.99 (10.44 ± 14.86) | −1.89      | 2.84        | 0.99   |
|                         | 4                   | 48                | 6.06 (5.19 ± 6.95)    | 10.88 (9.49 ± 13.36)  | −1.61      | 6.19        | 0.93   |
|                         | 6                   | 72                | 3.61 (2.49 ± 4.42)    | 7.82 (6.81 ± 9.56)    | −1.10      | 5.79        | 0.95   |
|                         | 8                   |                   |                       |                      |            |             |        |
|                         | 10                  |                   |                       |                      |            |             |        |

LC—Lethal Concentration, $X^2$—Chi square value; p—significant (0.5).
Compared to pathogenic bacteria and fungi, AgClNPs showed better antimicrobial activity against fungi. Observed microbial growth inhibition by AgNPs is possibly due to the surface and particle size variance [52]. Release of Ag$^{+}$ ions into the microbial cells leads to free radical generation and cells leakage caused by protein denaturation [53, 54]. The small size of AgNPs has a larger surface area, facilitates interaction with the bacterial cell membrane, and altered the cell membrane’s primary function, including permeability and cell respiration, which causes cell apoptosis [22]. Previous study reported that the bacterial cells treated with AgClNPs cause morphological changes and the AgClNPs were distributed on the surface of the bacterial cells [55, 56].

Hemolytic activity of synthesized silver nanoparticles were screened at different concentrations, among them, 50 $\mu$g ml$^{-1}$ concentration showed low hemolysis (table 4, figure 12). At the nanotoxicity level, the investigation of blood compatibility is essential, because the blood cells are affected by nanoparticles directly or indirectly. The erythrocytes circulated to various organs through the cardiovascular system lead to cause DNA damage, cell membrane injury, and congenital malformation [6, 9]. In this condition, the biocompatibility of AgNPs rupturing and releasing of erythrocytes have more attention to analyzing the toxicity level of nanoproducts [52]. This result suggested that the P3-AgClNPs can be safe and also used for biomedical applications.

### Table 2. Antimicrobial activity of green synthesized silver chloride nanoparticle compound against human pathogens.

| Concentration (mg ml$^{-1}$) | Zone of Inhibition (mm) |
|-----------------------------|-------------------------|
| Gram-positive bacteria       | Gram-negative bacteria   | Fungal Pathogen         |
| MRSV VREF                   | E. coli P. aeruginosa   | C. albicans             |
| 1                           | 22 26                    | 14 19 29                |
| 2                           | 22 26                    | 14 21 31                |
| 3                           | 23 27                    | 15 22 31                |

Figure 10. MCF7 cell lines were treated with IC$_{50}$ concentration of Compound. (a) Control treated with IC$_{50}$ concentration of compound increased the percentage of apoptosis in G0–G1 phase (1.39%), (b) MCF7 cells treated with IC$_{50}$ concentration of compound increased the percentage of apoptosis in G0–G1 phase (5.16%). The result indicates that apoptosis was induced in MCF7 cells by P3-AgClNPs in a dose-dependent manner.
The eco-toxicity assessments of $Pg$-AgClNPs were determined on the marine crustacean $Artemia nauplii$. The mortality rate was observed at 48 h, at different concentrations of $Pg$-AgClNPs ($10$, $20$, $50$, $100$, $150 \mu g \, ml^{-1}$). At 48 h, it showed LC$_{50}$ and LC$_{90}$ values of $47.92 \mu g \, ml^{-1}$ and $81.60 \mu g \, ml^{-1}$, respectively.

Figure 11. Zone of inhibition of $Pg$-AgClNPs against human bacterial and fungal pathogens of clinical isolates.

The eco-toxicity assessments of $Pg$-AgClNPs were determined on the marine crustacean $Artemia nauplii$. The mortality rate was observed at 48 h, at different concentrations of $Pg$-AgClNPs ($10$, $20$, $50$, $100$, $150 \mu g \, ml^{-1}$). At 48 h, it showed LC$_{50}$ and LC$_{90}$ values of $47.92 \mu g \, ml^{-1}$ and $81.60 \mu g \, ml^{-1}$, respectively.
Figure 12. (a), (b): Hemolytic activities of P. AgClNPs treated against human O(+/−) RBCs.

Figure 13. Toxity determination of A- P. gymnospora extract, B- AgNo3 and C- P. AgClNPs against Artemia nauplii at which arrow marks seeing gut abrasions.

Table 3. Minimum Inhibitory Concentration MIC of Green synthesized silver chloride nanoparticle.

| Nano-Particles | Minimum inhibitory concentration (MIC) (mg l⁻¹) |
|----------------|-----------------------------------------------|
|                | Gram-positive bacteria | Gram-negative bacteria | Fungal Pathogen |
|                | MRSA | VREF | E. coli | P. aeruginosa | C. albicans |
| NP PC          | NP PC NP PC NP PC PC |
| MIC value      | 64 4 | 32 1 | 128 1 | 64 2 | 8 NA |

MRSA: Methicillin resistance Staphylococcus aureus, VREF: Vancomycin resistance Enterococcus faecalis, NP: Nanoparticles, PC: Positive control, NA: Not Appear.

Table 4. Hemolytic activity of synthesized nanoparticles.

| Compound | Concentration of synthesized nanoparticles (μg ml⁻¹) |
|----------|--------------------------------------------------|
| Nanoparticles | 0.78 1.5 3.12 6.25 12.5 25 50 100 |
| 00% 00% 00% 00% 00% 00% 12% 92% |
From our investigation, Pg-AgClNPs found to be eco-toxic, because it gives mortality only at high concentration (figure 13). The increased concentration of AgClNP increases the mortality rate, apoptotic cells, DNA damage, and aggregation in the gut region. On the other hand, the hatching of *Artemia* cysts decreased [57, 58].

**Table 5.** Eco- toxicity assay of *Pg*-AgClNPs on *Artemia* nauplii.

| Sample     | Exposure Time (h) | Conc (μg ml⁻¹) | LC₅₀  | 95% LCL-UCL | LC₉₀  | 95% LCL-UCL | X² (d = 4) |
|------------|------------------|----------------|-------|-------------|-------|-------------|------------|
| *Pg*-AgClNPs | 24               | 10             | 93.06 | (67.36 ± 136.26) | 201.21 | (137.11 ± 626.84) | 0.439     |
|            |                  | 25             |       |             |       |             |            |
|            |                  | 50             |       |             |       |             |            |
|            |                  | 100            |       |             |       |             |            |
|            |                  | 150            |       |             |       |             |            |
|            |                  | 25             |       |             |       |             |            |
|            |                  | 50             |       |             |       |             |            |
|            |                  | 100            |       |             |       |             |            |
|            |                  | 150            |       |             |       |             |            |

LC—Lethal Concentration, X²—Chi square value.

(5). From our investigation, *Pg*-AgClNPs found to be eco-toxic, because it gives mortality only at high concentration (figure 13). The increased concentration of AgClNP increases the mortality rate, apoptotic cells, DNA damage, and aggregation in the gut region. On the other hand, the hatching of *Artemia* cysts decreased [57, 58].

**4. Conclusion**

The active principles of AgClNPs obtained from the seaweed *P. gymnopora* exhibits potent anticancer activity against MCF-7 cells and larvicidal activity against the *Ae. aegypti* without harming the non-target organism. AgClNPs also exhibit antimicrobial activity and biocompatibility to RBC in connection with human welfare. Overall, the synthesis of *Pg*-AgClNPs is considered as an eco-toxic and eco-friendly. It can be effectively used against breast cancer cells. In addition, it controls the *Ae. aegypti* a dengue causing vector, and it may also be used as an antimicrobial agent against the human infectious pathogens.

**Data availability statement**

All data that support the findings of this study are included within the article (and any supplementary files).

**Conflict of interest**

There is no conflict of interest.

**Author contributions**

S I and G R have designed the work. G R executed synthesis and Characterization. G R and N G M executed the biological applications. M S edited the manuscript. S S and A G contributed new reagents and analytical tools. G R drafted the manuscript. S I and N M approved the final version of the manuscript.

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