Journal of King Saud University – Science 34 (2022) 101771

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Seaweed polysaccharide mediated synthesis of silver nanoparticles and its enhanced disease resistance in *Oreochromis mossambicus*

Sundaram Thanigaivel [a], Sundaram Vickram [a], Vinayagam Saranya [b], Huma Ali [c], Saud Alarifi [d], Jeevan Kumar Reddy Modigunta [e], Krishnan Anbarasu [f], Rajasekhar Lakshmipathy [g], Karunakaran Rohini [h,i,*]

[a] Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai Tamil Nadu, India
[b] BCX Bioorganics, Krishnasagara Village, Attibele, Bengaluru, Karnataka, India
[c] Department of Chemistry, Maulana Azad National Institute of Technology, Bhopal, India
[d] Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia
[e] Department of Polymer Science and Engineering, Department of IT-Energy Convergence (BK21 FOUR), Chemical Industry Institute, Korea National University of Transportation, Chungju 23769, Republic of Korea
[f] Department of Bioinformatics, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai, Tamil Nadu, India
[g] Department of Chemistry, KCG College of Technology, Chennai, Tamil Nadu, India
[h] Unit of Biochemistry, Faculty of Medicine, AIMST University, Semeling, Bedong, Kedah, Malaysia
[i] Centre of Excellence for Biomaterials Engineering, AIMST University, Semeling, Bedong 08100, Malaysia

ARTICLE INFO

Article history:
Received 6 October 2021
Revised 20 November 2021
Accepted 13 December 2021
Available online 17 December 2021

Keywords:
Seaweed polysaccharides
Nanoparticles
Disease resistance
Fish pathogen
Tilapia fish

ABSTRACT

Objective: Preliminary aim of this research work focuses on testing the efficacy of silver nanoparticles synthesised using water soluble polysaccharides extracted from seaweeds against pathogenic fish bacteria. The emergence of various virulence pathogens during the culturing period leads to mass mortality and influence the loss of aquaculture production.
Methods: The intervention of nanotechnology in the field of aquaculture improves the growth and immunity of aquatic animals when the part of nanomaterials mixed with fish feed. Sometimes the green synthesis of silver nanoparticles prepared from the seaweed polysaccharide play a vital role in the disease controlling strategies in aquaculture farming. The nanoparticle formation and confirmation was charecterized by Ultra Visible Spectroscopy (UV–Vis), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), Dynamic Light Scattering (DLS).
Results and Conclusion: Biologically synthesized silver nanoparticles have been used as an alternative method for commercial antibiotics to control such pathogenic infection with 70–80% of survival rate. Polysaccharides of Caulerpa racemosa was used as a reducing agent in this present study. Particularly the phytochemicals are involved in the silver salt reduction process. Total polyphenol, total flavonoids, total antioxidant, hydroxyl radical scavenging assay and DPPH was found to be 12.32 mg/g, 18.44 mg/g, 38.12 mg/g equivalence, 82.2% and 49.05% respectively. Biologically synthesised AgNps capable of preventing the *Pseudomonas aeruginosa* infection in tilapia, it was proved by *in vitro* and *in vivo* antibacterial activities. Present study provided the ample achievement and these findings found to be effective in controlling the *Pseudomonas* infection in tilapia fishes.

1. Introduction

Algae are considered to be the potential source for various medicinal properties and had been used for different biomedical applications. The types of algae contain numerous polysaccharides compounds and each have different reason to explore for specific biological application (Wijesekara et al., 2011). The sulfated polysaccharides especially are the most important compounds isolated from brown, red and green seaweeds. Carrageenan from red algae, fucoidon form brown algae, and ulvanz from green algae

* Corresponding author.
E-mail address: rohini@aimst.edu.my (K. Rohini).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101771
1018-3647/

are the commonly existing groups which may potentially contain bioactive metabolites exhibiting antimicrobial, antitumor, antimutagenic, anticoagulant, anti-inflammatory, immunomodulatory, antiviral and antithrombotic activities (Raposo et al., 2015). Polysaccharides are essential macromolecules widely distributed in the nature and most abundantly present in the marine environment (Wijesekara et al., 2011). Polysaccharides have been well known by their incomparable use in food industries, biomedical fields and also in biopharmaceutical applications. Polysaccharides are reported to have excellent properties which include non-toxic, hydrophilicity, bio compatibility, biodegradability and other biological properties such as anticancer and antimicrobial activities were also reported against various pathogenic microorganism (Raposo et al., 2015). Aquaculture is the fast-growing sector. The production system has enlarged enormously because of the worldwide importance of the protein resources from the aquaculture and fisheries producing sectors (Zhang et al., 2021). Due to the over exploitation of production and requirement this system has attained greater importance (Austin and Austin, 2016). The requirement of animal proteins, especially derived from fish receive global interest and the need of protein requirement for the human consumption depends on fisheries sector (Alghabshi et al., 2018). Excessive requirement of fish proteins makes the aquaculture industry is one of the fastest growing sector in the global concern, over use and production of aquaculture negatively influence in the physiological, biological factors in fish community (Austin and Austin, 2016; Thanigaivel et al., 2019). The *in vivo* model used in the study is *Oreochromis mossambicus* which commercially known as Tilapia that is cultured worldwide throughout the year for the proteinaceous flesh content and also for their improved nutritional availability. Environmental factors such as poor hygiene nutrient depletion and overcrowding which hamper the fish production (Zhang and Zhu, 2017). The emergence of opportunistic pathogens during the climatic changes affects the fish production adversely. Pathogen *Pseudomonas aeruginosa* is one the opportunistic bacteria which cause huge hindrance in the commercial cultivation of fish (Thanigaivel et al., 2021).

In order to ensure the health conditions of fishes during all seasons, commercial formulations of natural products have been to stimulate the immune responses against the predatory pathogens (Wang et al., 2016). To combat such pathogenic infections various other commercial drugs were used such as veterinary medicines and antibiotics formulations which in turn cause resistance to the specific pathogen. In addition the use of such chemicals and its residuals cause side effects to the fish consumers (Reverter et al., 2014). Regular use of antimicrobial drugs led to a serious problem in the aquatic environment because of the rapid spread of antibiotics and drug resistant in aquatic environment. Therefore, the present study investigates the use of biologically synthesized silver nanoparticles from *C. racemosa* would act as an effective alternative to the commercial antimicrobial drugs for controlling and preventing the bacterial infection. There are plenty of studies which focus on the use and mediation of natural plants and plant derived compounds for the immunostimulation and disease resistance properties in aquaculture operations. The count and number of literatures in the field aquaculture and greenery approaches with herbal, plant leaves and seaweeds have been increased in last few decades (El-Boshy et al., 2014).

The silver nanoparticles synthesized using polysaccharides obtained from marine algae are capable of exhibiting various functions and biological activities due to the presence of components such as alginate, fucoidan, laminarin etc. and these were demonstrated in various marine mammalians study to control the bacterial infection though the biological assessments (EBSCOhost, 2021). Similarly the use of metallic silver as an antibacterial agent against various pathogenic organisms have also been discussed in various studies (Yudiati et al., 2016). This type of treatment in this study using sliver nanoparticles mediated approach to aquatic animals will also improve the immunity and enhance the growth, health of aquaculture animals (Sundaram et al., 2020).

This present research delineating the disease resistance efficacy of silver nanoparticles synthesized from seaweed polysaccharides fractions of Caulerpa racemosa against opportunistic *Pseudomonas* infection in tilapia fish.

This AgNPs will enhance the immunostimulatory behavior of fishes against the bacterial pathogen upon the immersion method of bacterial infections and nanoparticle challenge study to determine the percentage of survival rate of fish for the commercial and large scale cultivation of *Oreochromis mossambicus*.

2. Materials and methods

2.1. Collection and maintenance of fish

Healthy fingerlings of tilapia (*Oreochromis mossambicus*) were procured from a local fish farm in Walajapet, Tamil Nadu the local fish farm. The average weight of the fishes were about 8–10 g, they were maintained under lab condition with proper aeration and maintained with necessarily feeding conditions, transported fishes initially acclimatized with treated water lab water. Fishes were starved before starting the experiment.

2.2. Seaweed collection and processing

The seaweed *C. racemosa* was collected from Gulf of Mannar, Coastal region Mandapam (Latitude 9°17′ N; Longitude 79°08′ E), Tamil Nadu, India. Impurities and debris present in the sea weeds were removed by washing in water. Then the seaweed was powdered and processed by maceration method (Thanigaivel et al., 2015).

2.3. Antioxidant and radical scavenging activity

Determination of antioxidant and radical scavenging activities of polysaccharides extracted from seaweeds is presented. It was performed according to the protocol described by (Thanigaivel et al., 2015) have been used for this study in different types of macro algae.

2.4. Extraction of polysaccharide from seaweeds

The detailed method of polysaccharide extraction is followed by the modified protocol of (Phillipson, 2001). Collected macro algae was processed based on the prescribed protocol to remove the unwanted debris attached to plant and then dried. Initially dried leaves were powdered for 100 g then suspended in 1000 ml of ethanol and distilled water in the proportion of 8:2. Sample mixture was then mixed vigorously and filtered using nylon cloth. These filtrates were extracted with 1000 ml of ethyl acetate and then filtered. Further collected residues from ethyl acetate soaked in 500 ml of hot ethanol which is heated up to 60 °C till the extract becomes transparent. Colorless extract was centrifuged and supernatant was discarded, residue was dissolved with boiling sodium chloride (1% w/v). Crude polysaccharides were obtained by precipitating with ethanol solvent, the sediments were washed with acetone and ether by sequential process at 60 °C. Finally, water soluble polysaccharides were obtained through rotary vacuum evaporator, collected dried polysaccharide were further stored in −20 °C.

2.5. Biosynthesis of silver nanoparticles

Polysaccharides of *C. racemosa* was used for the biosynthesis of AgNps. The samples mixture of 10 mg/ml of polysaccharide fraction was added in 100 ml of double distiled water and kept under stirring condition for 30 mins at 50 °C. 1 mM of silver nitrate solution was added to the reaction mixture. After the period of interaction and 15 min of incuation the change in the dark brown colour of sample was observed and visually confirmed the presence of silver nanoparticles, further the yield was centirfuged to remove the aggregates and silver ions were purified and utilized for the study (Thanigaivel et al., 2021).

2.6. Characterization of AgNPs

The reductions of Ag + ions were observed biometrically through UV visible spectroscopy. The optical density of the reaction mixture containing silver nanoparticles was determined by the absorption peak. The absorption peak was recorded at the wavelength between 300 and 700 nm (UV-1800, Shimadzu, Singapore).

Dynamic light scattering technique was used for the particle size anlaysis using the colloidal suspension. The density or size of the nanoparticles was measured on Brookhaven Instrument (model 90 Plus) particle size analyzer. This was used to determine the particle size distribution.

Scanning Electron Microscope (SEM) analysis was employed to determine the external morphology and approximate size of the synthesized nanoparticles using (Hitachi S-4500) instrument (Sathiyanarayanan et al., 2013).

FTIR Spectra was obtained for the polysaccharide compounds which was used as a reducing agent to synthesis AgNPs. This techniques was operated from the range of 400 to 4000 cm^{-1}. Thereafter freeze dried purified suspension was used in the form of powder and resuslts were obtained using Schimadzu FT-IR using standard KBr pellet method (El-Rafie et al., 2013).

2.7. Bacterial culture

Bacterial pathogen of *Pseudomonas aeruginosa* purchased from Microbial type culture collection (MTCC-424) was used in the present study, strain was procured from IMTECH, Chandigarh, India. The culture was tested microbiologically to re confirm the characteristics through biochemical analysis as described in Bergy's manual of systemic bacteriology to avoid the cross contaminations during handling or culturing processes (Nariya et al., 2011).

2.7.1. In vitro antibacterial activity

Antibacterial activity of AgNPs was determined by antimicrobial assays. Well diffusion and disc diffusion were performed according to the modified method of (Thanigaivel et al., 2014). 25 micro liter of the AgNPs was loaded onto the agar well. Miliqwater was kept as a control. In disc diffusion, ciprofloxacin was used disc which found to be effective against the study pathogen, and it is kept as a standard. Nanoparticles loaded disc was used as test to determine the zone of inhibition (Baueraw, 1966).

2.7.2. Pathogenicity experiment

Pathogenicity of *P. aeruginosa in* tilapia was carried out by bath exposure for the experimental pathogenicity by dispersing different dilution of bacterial culture in the glass tank containing 50 L of treated water, after post incubation period from 0th the 15 days the mortality rates were calculated to determine the percentage of mortality of the healthy fishes exposed for bacterial pathogenicity. The detailed methodology was already reported in our earlier study (Thanigaivel et al., 2019).

2.7.3. Confirmation of pathogenicity

Confirmation of the pathogenicity was carried out by re-isolating the specific bacterial disease-causing pathogen from the moribund fish to satisfy Koch's postulates. The infected parts of fish were cut and homogenized. The samples were inoculated on to nutrient agar culture medium by spread plate technique for the confirmation of the bacterial pathogen.

2.7.4. In vivo treatment of bacterial disease using AgNPs

In vivo treatment study using the synthesized silver nanoparticles was carried out against the pathogenic bacteria *P. aeruginosa* to study its potential antibacterial activity against the disease causing pathogen, immersion route was followed to treat the bacterial infection. The method of (Thomas et al., 2014) was followed. Fishes in the size range of 12 ± 0.5 g measured per tank/ bacterial dosage were arranged in the glass tanks. Fresh water was used throughout the experiment with the proper biochemical and physiochemical parameters. The bacterial culture was diluted serially with different dilutions ranging from 10^{-1} to 10^{-7} μl and the bacterial culture was added to the tank containing 1L of water. 30 μl of the synthesised AgNps solution with 12 nM concentration was added to the experimental tanks to check the efficacy of the nanoparticles against the pathogenic microorganism.

2.8. Statistical analysis

Data obtained for the study is made with mean of ±SE of 10 fishes. Percentage mortality value is the mean ± SE of 10 fishes, in triplicates (30 fishes in total). All pairwise comparisons of means at particular day post treatment were done by one-way analysis of variance (one-way ANOVA) with Tukey's aposteriori test.

2.9. Results and discussion

The seaweeds collected from the coastal region were processed and polysaccharides were extracted and they have been tested for the antioxidant capacity to fight against the free radicals. Free radical formations are initiated in the cells of human and animal systems during extensive stress or cell damages caused by internal or external factors. The radical scavenging properties of *C. recemosa* were tested. The assays such as total polyphenol, total flavonoids, Total antioxidant assay with the Folin–Ciocalteu, quercitin, ascorbic acid were used respectively as a standard for the determination of the activity, free radical scavenging assays such as hydroxyl radical scavenging assay and DPPH assays were also performed. Total polyphenol, total flavonoids, total antioxidant, hydroxyl radical scavenging assay and DPPH was found to be 12.32 mg/g,

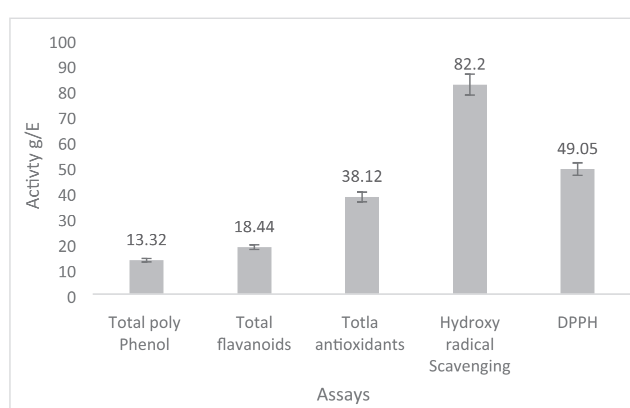

Fig. 1. Antioxidant and free radical scavenging activities of seaweed polysaccharides.

18.44 mg/g, 38.12 mg/g equivalence, 82.2% and 49.05% respectively. The results are shown in the (Fig. 1).

These results were supported by (Ramasubburayan et al., 2015) who reported that the potential antioxidant activity of *Caulerpa scalpelliformi* showed the maximum of 21.34 ± 0.05 mg/ml. Further described that the antioxidant activity of methanolic extract of *C. antennina* was significantly lower when compared to the antioxidant activity of *C. scalpelliformis*

The present study utilized the silver nanoparticles synthesized form C. racemosa was found to be one of the effective in controlling the *P. aeruginosa* bacterial infection in *Oreochromis mossambicus* fish with notable percentage of survival which was obtained through lab scale challenge method it was found to be above 70–80%. This survival data gives the clear idea about the nanoparticles exactly works against the test organism. It also suggested as prophylactic methods for controlling the emergence of microorganisms. Similarly (Cui et al., 2016; Rafi et al., 2020) had conducted silver nanoparticles synthesis using polysaccharides extracted from marine macro algae they proved the different combinations of AgNPs showed good antibacterial activity against gram –ve and gram + ve bacteria such as *E-coli, S. aureus* (Wijesekara et al., 2011, Raposo et al., 2015; Ummat et al., 2020).

The sulfated polysaccharides especially are the most important compounds isolated from brown, red and green seaweeds. Carrageenan from red algae, fucoidon form brown algae, and ulvan from green algae are the commonly groups which may potentially contain bioactive compounds exhibiting antimicrobial, antitumor, antimutagenic, anticoagulant, anti-inflammatory, immunomodulatory, antiviral and antithrombotic activities(Déléris et al., 2016). Instance case the carrageenan extracted red algae are used as effective immunomodulatory compounds and sodium alginated known to be the good proteinaceous compound extracted from brown both were excreted the disease resistance activity against grouper fish *Epinephelus fuscoguttatus.* Similar studies were also conducted by synthesizing AgNPs from the plant extract *Boerhavia diffusa* against fish bacterial pathogen *Flavobacterium branchiophilum* and the study was found to be very effective in controlling infections (Saranya and Sudhakaran, 2020).

Biosynthesis of silver nanoparticles using polysaccharides were prepared and confirmed by the color change from brownish yellow to dark brown. The intensity of this brown color is well developed during incubation period and is responsible for the excitation of the surface plasmon resonance (SPR). The reduction of Ag^{+} ion was characterized by the presence of polysaccharides in the seaweed extract. The solution mixture showed the characteristic absorbance peak of AgNPs at 420 to 430 nm. The absorbance of silver nanoparticles is shown in the (Fig. 2).

This supports the published data of (Kannan et al., 2013; Das et al., 2020) who reported the absorbance of silver nanoparticle at 420 nm. The size characterization of biosynthesized silver nanoparticles was achieved primarily with Dynamic light scattering technique, which exhibited the size distribution of 148 nm. The results are shown in (Fig. 3). This indicates that the incidence of light on the particles initiated in the Brownian motion. This method partially confirms the particle size range at nanoscale. This result supports the report of (Islam, 2019) who determined the structure and size of the nanoparticle synthesized from sophoro lipids by varying the effects of physiological parameters.

Further the SEM analysis of the nanoparticle was studied to determine the structure and accurate size of the nanoparticle, our present study had the average mean size of AgNPs was found to be 88 ± 0.5 nm as shown in the (Fig. 4). This micrograph revealed the particles present in the colloidal suspension of the silver nanoparticle which exhibited spherical shape. The similar seaweed of *Caulerpa racemosa* medicated synthesis have exhibited the 10 nm sized; spherical shape of silver nanoparticles against *Staphylococcus aureus and Pseudomonas mirabilis* as per the conducted by the (Roy et al., 2019). The average mean diameter of the particle size was found to be around 125 ± 0.2 nm. These results support the result of (Islam, 2019; Kasture et al., 2008). The detailed reports of morphology and size of the nanoparticles are essential for various biomedical applications like targeted drug delivery and nano drug formulations. Present study results also agreed with the published report of (Sakhare et al., 2022) who studied the synthesis of silver nanoparticles by *Aeromonas paniculata.* The results revealed an average particle size of 55 nm with a spherical shape.

FTIR spectrum of polysaccharide extracted from *C. racemosa* was shown in the (Fig. 5). Theses measurement was performed to identify the functional groups involved in the reduction of Ag + ions for the yield of interacted AgNPs made out of polysaccharide compounds. It denotes the presence of functional groups in the interacted and un-interacted form of polysaccharides with silver and without silver upon reduction process. The extracted polysaccharides showed nearly 10 peaks which contains 864, 1034, 1049, 1078 1184, 1487, 1683, 2926, 2925, 3334 cm^{-1}. These absorption frequencies are denoted for the representation of OH group of the algal polysaccharides the bands between the ranges of 2926, 2925, can be assigned to the alkane C H stretching and secondary amines. The bands near the ranges of 1487, 1683 cm^{-1} are assigned to the C O O group.

The absorption bands observed at 1654 and 1683 cm^{-1} can be assigned to the amide groups of protein or to the carbonyl stretching groups of the algal polysaccharides. This characterization techniques is one of the ample tool for the better identification of different functional groups present in the sample mixture based

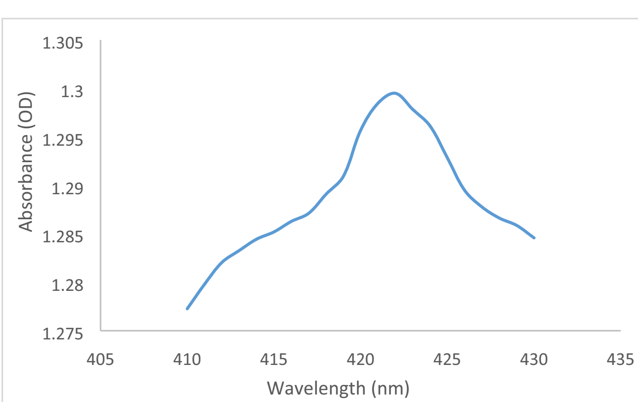

Fig. 2. Peak at 422 nm confirms the presence of AgNPs through ultra-violet visible spectroscopy.

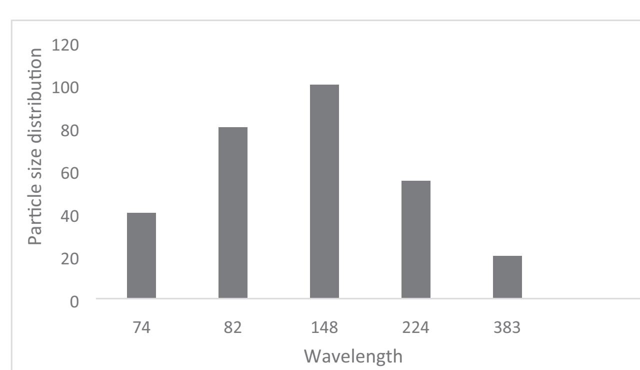

Fig. 3. Dynamic light scattering image for the identification of average particle size distribution of AgNPs synthesized using polysaccharides.

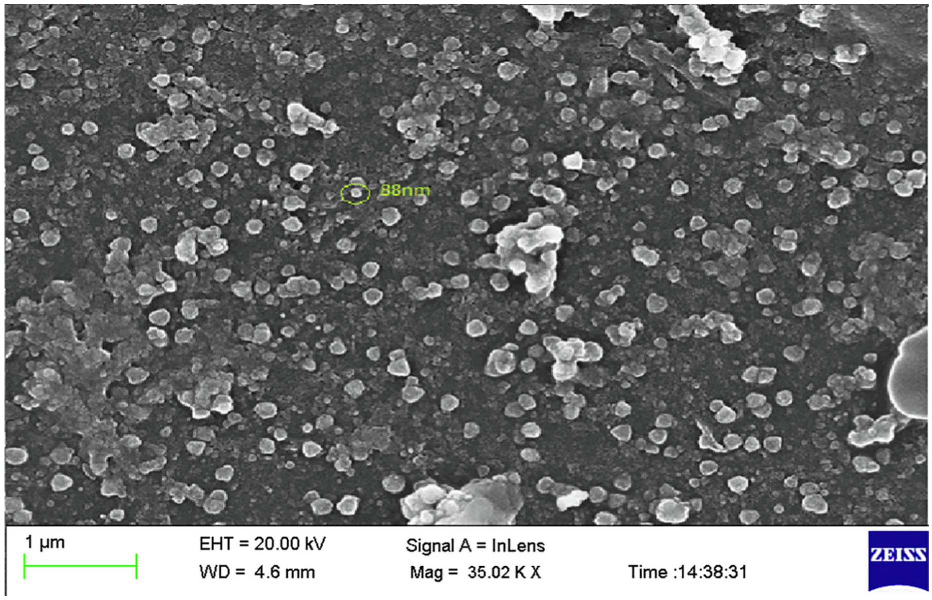

Fig. 4. Scanning electron microscopic image of AgNPs.

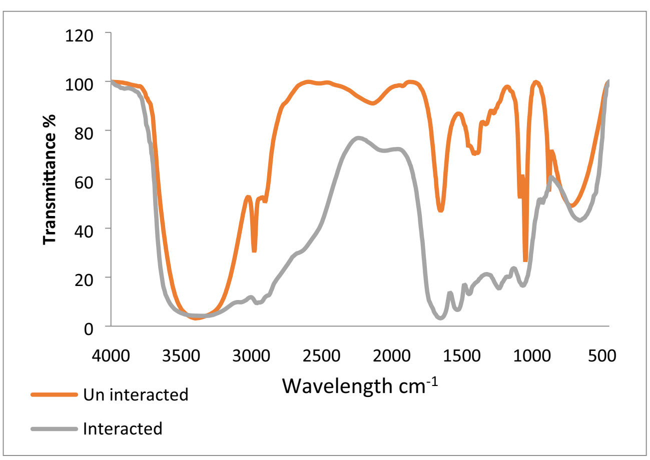

Fig. 5. FTIR Spectroscopy of un interacted -Polysaccharide extract, interacted-AgNPs formation.

on these observations our study Carbonyl, amino acids, alkyl and proteins and vitamins groups containing various phytocompounds such as polyphenol, flavanoids, alkaloids, terpenoids have strong ability to bind with metal ions which is reduced through the biological synthesis could facilitate the bio layer covering metal nanoparticles (AgNPs), found to be responsible for the reduction of Ag+ ions and our FT-IR spectrum resembles the polyols, heterofucans and sulfated polysaccharides extracted from *C. racemosa*. Many of the polysaccharides obtained from our study are matched with (El-Rafie et al., 2013; Gómez-Ordóñez and Rupérez, 2011; Huang et al., 2011).

Antibacterial activity of the nanoparticle was studied against the fish pathogen *P. aeruginosa*. The activity of nanoparticles in both well diffusion and disc diffusion was found to be effective against the tested pathogen. The zone of inhibition for the synthesized silver nanoparticle was found to be 15 mm. The commercial antibiotic ciprofloxacin showed comparatively 18 mm zone of inhibition. Disease controlling efficacy of this nanoparticle was further confirmed through the *in vivo* activity and the obtained percentage of mortality through experimental pathogenicity achieved about 100% at the end of week in the bacterial dilutions whose bacterial concentration is high, and wisely the percentage of mortality was maintained less when compared to higher dilutions shown in the (Fig. 6). The results of the present study showed that there was about 80% of cumulative survival rate was observed in the fish which was treated with synthesized silver nanoparticle as shown in (Fig. 7). The inhibitory effect of the silver nanoparticles against fish pathogen was reported in the studies carried out by (Raju et al., 2021) using *Aeromonas hydrophilia.* This coroborates with the present study. Use of such novel antimicrobial agents promotes the health and disease management in the aquaculture production.

Controlling bacterial infection in fishes using the silver nanoparticles with different concentrations of formulated nanoparticles was reported for optimum exposure in *in vivo* treatment of bacterial infection of fish. Prepared nanoparticles were dispersed through immersion of nanoparticles in water containing disease-causing pathogen and experimental fishes which were challenged with these nanoparticles. Disease controlling efficacy of the silver nanoparticles synthesized from the seaweed was optimized for the effective challenge against the fish pathogen. The use of antimicrobial drugs and chemical in aquaculture is banned due to the development of resistance by pathogens. In order to overcome such a situation this kind of biologically synthesized antimicrobial formulation has been preferred. Our study reported that the

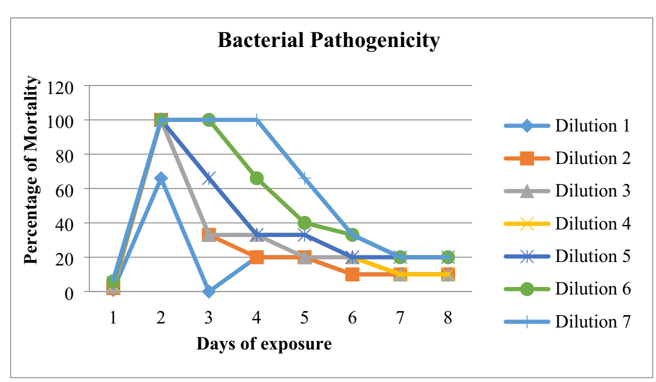

Fig. 6. Bacterial challenge study against healthy fishes for mortality percentage.

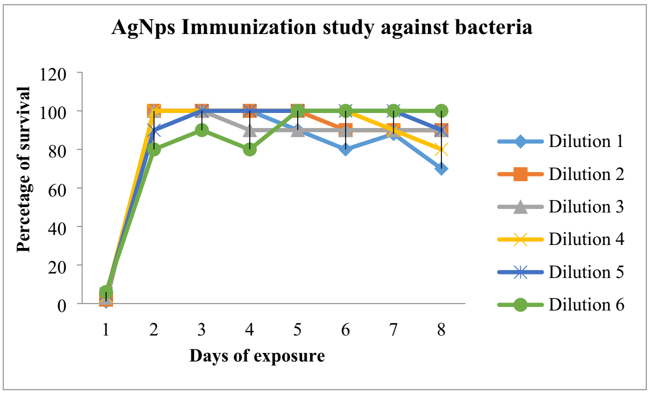

Fig. 7. Challenging of 1 mM concentration of AgNPs 20 μl against experimental fishes exposed to bacterial load of 20 μl from the serial dilutions of bacterial culture 10^{-1} to 10^{-7} μl.

percentage of survival during the successive treatment was found to be nearly 80%. These results corroborated with the report of (Vaseeharan et al., 2010). Similarly such nanoparticles based treatment studies against fish pathogens have been reported by (Vaseeharan et al., 2010) who studied the disease controlling efficacy of biologically synthesized nanoparticles to control the pathogenicity of *Vibio harveyii* in *Feneropenaeus indicus.* The protective efficacy of AgNps was found to be effective in controlling bacterial infection during the *in vivo* treatment with the survival rate of 71% and mortality of 29%.

2.10. Conclusion

In conclusion the use of such biologically synthesized nanoparticle delivery systems needs to be optimized for use in aquaculture disease management system in various commercial forms. The results of the present study will suggest many alternative methods for treating the bacterial infection in fishes by incorporating the bioavailable and bioactive ingredients against various pathogenic organisms. Further studies need to be carried out on how to deliver this biosynthesized nanoparticle in the aquatic environment with suitable carrier. The use of such novel nano antimicrobial drugs with natural bioprotectant methods and their disease targetting efficacy approaches are found to be one of the effective alternative methods of treatment to commercial chemical drugs used in aquaculture and fisheries industries.

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.

Acknowledgment

This research work was funded by Researchers Supporting Project number (RSP-2021/27), King Saud University, Riyadh, Saudi Arabia.

References

Alghabshi, A., Austin, B., Crumlish, M., 2018. Aeromonas salmonicida isolated from wild and farmed fish and invertebrates in Oman. Int. Aquat. Res. 102 (10), 145–152. https://doi.org/10.1007/S40071-018-0195-4.

Austin, B., Austin, D.A., 2016. Bacterial fish pathogens: Disease of farmed and wild fish, sixth edition. Bact. Fish Pathog. Dis. Farmed Wild Fish, Sixth Ed., 1–732 https://doi.org/10.1007/978-3-319-32674-0.

Bauer, A.W., 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45, 149–158.

Cui, C., Lu, J., Sun-Waterhouse, D., Mu, L., Sun, W., Zhao, M., Zhao, H., 2016. Polysaccharides from Laminaria japonica: Structural characteristics and antioxidant activity. LWT 73, 602–608. https://doi.org/10.1016/J.LWT.2016.07.005.

Das, C.G.A., Kumar, V.G., Dhas, T.S., Karthick, V., Govindaraju, K., Joselin, J.M., Baalamurugan, J., 2020. Antibacterial activity of silver nanoparticles (biosynthesis): A short review on recent advances. Biocatal. Agric. Biotechnol. 27,. https://doi.org/10.1016/J.BCAB.2020.101593 101593.

Déléris, P., Nazih, H., Bard, J.M., 2016. Seaweeds in Human Health. Seaweed Heal Dis. Prev. 319–367. https://doi.org/10.1016/B978-0-12-802772-1.00010-5.

EBSCOhost | 102903107 | Effect of Silver Nanoparticles on Haematological and Protein Metabolic Indices of Carp Fish, Catla catla, treated with Lihocin and A. veronii. Available online: https://web.b.ebscohost.com/abstract?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09767126&AN=102903107&h=uii8XPyBL5ZZ3IlQYgqPpX9IMgR1PZeeSGtmNTRjsvqKQLRMmqNP7oOj7WVD6yu8FOqYi2uNKmelDv01FE7uXg%3D%3D&crl=c&resultNs=AdminWebAuth&resultLocal=ErrCrlNotAuth&crlhashurl=login.aspx%3Fdirect%3Dtrue%26profile%3Dehost%26scope%3Dsite%26authtype%3Dcrawler%26jrnl%3D09767126%26AN%3D102903107 (accessed on Sep 16, 2021).

El-Boshy, M., El-Ashram, A., Risha, E., Abdelhamid, F., Zahran, E., Gab-Alla, A., 2014. Dietary fucoidan enhance the non-specific immune response and disease resistance in African catfish, Clarias gariepinus, immunosuppressed by cadmium chloride. Vet. Immunol. Immunopathol. 162, 168–173. https://doi.org/10.1016/J.VETIMM.2014.10.001.

El-Rafie, H.M., El-Rafie, M.H., Zahran, M.K., 2013. Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. Carbohydr. Polym. 96, 403–410. https://doi.org/10.1016/J.CARBPOL.2013.03.071.

Gómez-Ordóñez, E., Rupérez, P., 2011. FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. Food Hydrocoll. 25, 1514–1520. https://doi.org/10.1016/J.FOODHYD.2011.02.009.

Huang, Y.R., Shiau, C.Y., Chen, H.H., Huang, B.C., 2011. Isolation and characterization of acid and pepsin-solubilized collagens from the skin of balloon fish (Diodon holocanthus). Food Hydrocoll. 25, 1507–1513.

Islam, S., 2019. Integrating green chemistry and sustainable engineering. Integr. Green Chem. Sustain. Eng. 1–692. https://doi.org/10.1002/9781119509868.

Kannan, R.R.R., Stirk, W.A., Van Staden, J., 2013. Synthesis of silver nanoparticles using the seaweed Codium capitatum P.C. Silva (Chlorophyceae). South African J. Bot. 86, 1–4. https://doi.org/10.1016/J.SAJB.2013.01.003.

Kasture, M.B., Patel, P., Prabhune, A.A., Ramana, C.V., Kulkarni, A.A., Prasad, B.L.V., 2008. Synthesis of silver nanoparticles by sophorolipids: Effect of temperature and sophorolipid structure on the size of particles. J. Chem. Sci. 1206, 515–520. https://doi.org/10.1007/S12039-008-0080-6. 2009.

Nariya, P.B., Bhalodia, N.R., Shukla, V.J., Acharya, R.N., 2011. Antimicrobial and antifungal activities of Cordia dichotoma (Forster F.) bark extracts. Ayu 32 (4), 585. https://doi.org/10.4103/0974-8520.96138.

Phillipson, J.D., 2001. Phytochemistry and medicinal plants. Phytochemistry 56 (3), 237–243. https://doi.org/10.1016/S0031-9422(00)00456-8.

Rafi, M.; Syafitri, U.; Wahyuni, W.T. Development botanical reference material View project TiO2 nanosheet View project. **2020**, 10.22146/ijp.755.

Raju, S.V., Sarkar, P., Kumar, P., Arockiaraj, J., 2021. Piscidin, fish antimicrobial peptide: structure, classification, properties, mechanism, gene regulation and therapeutical importance. Int. J. Pept. Res. Ther. 27 (1), 91–107.

Ramasubburayan, R., Sumathi, S., Magi Bercy, D., Immanuel, G., Palavesam, A., 2015. Antimicrobial, antioxidant and anticancer activities of mangrove associated bacterium Bacillus subtilis subsp. subtilis RG. Biocatal. Agric. Biotechnol. 4, 158–165. https://doi.org/10.1016/J.BCAB.2015.01.004.

Raposo, M.F.D.J., De Morais, A.M.B., De Morais, R.M.S.C., 2015. Marine polysaccharides from algae with potential biomedical applications. Mar. Drugs 13, 2967–3028. https://doi.org/10.3390/MD13052967.

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433, 50–61. https://doi.org/10.1016/J.AQUACULTURE.2014.05.048.

Roy, A., Bulut, O., Some, S., Mandal, A.K., Yilmaz, M.D., 2019. Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. RSC Adv. 9 (5), 2673–2702. https://doi.org/10.1039/C8RA08982E.

Sakhare, K., Prasasvi, K.R., Palani, S.G., 2022. Plant and bacteria mediated green synthesis of silver nanoparticles. Green Funct. Nanomater. Environ. Appl. 155–178. https://doi.org/10.1016/B978-0-12-823137-1.00006-3.

Saranya, S.R., Sudhakaran, R., 2020. Report on prevalence of tilapia lake virus infection in tilapia fishes (Oreochromis niloticus). Biocatal. Agric. Biotechnol. 27, 101665. https://doi.org/10.1016/j.bcab.2020.101665.

Sathiyanarayanan, G., Seghal Kiran, G., Selvin, J., 2013. Synthesis of silver nanoparticles by polysaccharide bioflocculant produced from marine Bacillus subtilis MSBN17. Colloids Surfaces B Biointerfaces 102, 13–20.

Sundaram, T., Indu, B., Reddy, C.S., Raj, V.S.S., Priya, S.H., Poojitha, P., Renusree, K., Govindarajan, G., Rajendiran, N., Sundaram, V., 2020. Bio inspired silver nanoparticle synthesis from fish liver oil and its antibacterial activity against shrimp pathogen. IOP Conf. Ser Mater. Sci. Eng. 993,. https://doi.org/10.1088/1757-899X/993/1/012166 012166.

Thanigaivel, S.; Thomas, J.; Vickram, A.S.; Gulothungan, G.; Nanmaran, R.; Rani, D.J. Antioxidant and antibacterial efficacy of Chaetomorpha linum and its

toxicological evaluation for the prophylactic treatment against Pseudomonas aeruginosa infection in Labeo rohita. **2021**, 10.1080/10454438.2021.1961966.
Thanigaivel, S., Vijayakumar, S., Mukherjee, A., Chandrasekaran, N., Thomas, J., 2014. Antioxidant and antibacterial activity of Chaetomorpha antennina against shrimp pathogen Vibrio parahaemolyticus. Aquaculture 433, 467–475. https://doi.org/10.1016/J.AQUACULTURE.2014.07.003.
Thanigaivel, S., Vijayakumar, S., Gopinath, S., Mukherjee, A., Chandrasekaran, N., Thomas, J., 2015. In vivo and in vitro antimicrobial activity of Azadirachta indica (Lin) against Citrobacter freundii isolated from naturally infected Tilapia (Oreochromis mossambicus). Aquaculture 437, 252–255. https://doi.org/10.1016/J.AQUACULTURE.2014.12.008.
Thanigaivel, S., Vidhya Hindu, S., Vijayakumar, S., Mukherjee, A., Chandrasekaran, N., Thomas, J., 2015. Differential solvent extraction of two seaweeds and their efficacy in controlling Aeromonas salmonicida infection in Oreochromis mossambicus: A novel therapeutic approach. Aquaculture 443, 56–64. https://doi.org/10.1016/J.AQUACULTURE.2015.03.010.
Thanigaivel, S., Chandrasekaran, N., Mukherjee, A., Thomas, J., 2019. Protective efficacy of microencapsulated seaweed extracts for preventing Aeromonas infections in Oreochromis mossambicus. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 218, 36–45. https://doi.org/10.1016/J.CBPC.2018.12.011.
Thanigaivel, S., Thomas, J., Vickram, A.S., Anbarasu, K., Karunakaran, R., Palanivelu, J., Srikumar, P.S., 2021. Efficacy of encapsulated biogenic silver nanoparticles and its disease resistance against Vibrio harveyi through oral administration in Macrobrachium rosenbergii. Saudi J. Biol. Sci. 28 (12), 7281–7289.
Thomas, J., Thanigaivel, S., Vijayakumar, S., Acharya, K., Shinge, D., Seelan, T.S.J., Mukherjee, A., Chandrasekaran, N., 2014. Pathogenecity of Pseudomonas aeruginosa in Oreochromis mossambicus and treatment using lime oil nanoemulsion. Colloids Surfaces B Biointerfaces 116, 372–377. https://doi.org/10.1016/J.COLSURFB.2014.01.019.
Ummat, V., Tiwari, B.K., Jaiswal, A.K., Condon, K., Garcia-Vaquero, M., O'Doherty, J., O'Donnell, C., Rajauria, G., 2020. Optimisation of ultrasound frequency, extraction time and solvent for the recovery of polyphenols, phlorotannins and associated antioxidant activity from brown seaweeds. Mar. Drugs 18, 250. https://doi.org/10.3390/MD18050250.
Vaseeharan, B., Ramasamy, P., Chen, J.C., 2010. Antibacterial activity of silver nanoparticles (AgNps) synthesized by tea leaf extracts against pathogenic Vibrio harveyi and its protective efficacy on juvenile Feneropenaeus indicus. Lett. Appl. Microbiol. 50, 352–356. https://doi.org/10.1111/J.1472-765X.2010.02799.X.
Wang, B., Wang, P., Wu, Z.-H., Lu, Y.-S., Wang, Z.-L., Jian, J.-C., 2016. Molecular cloning and expression analysis of IgD in Nile Tilapia (Oreochromis niloticus) in response to Streptococcus agalactiae stimulus. Int. J. Mol. Sci. 17, 348. https://doi.org/10.3390/IJMS17030348.
Wijesekara, I., Pangestuti, R., Kim, S.K., 2011. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carbohydr. Polym. 84, 14–21. https://doi.org/10.1016/J.CARBPOL.2010.10.062.
Yudiati, E., Isnansetyo, A., Murwantoko, Ayuningtyas, Triyanto, Handayani, C.R., 2016. Innate immune-stimulating and immune genes up-regulating activities of three types of alginate from Sargassum siliquosum in Pacific white shrimp, Litopenaeus vannamei. Fish Shellfish Immunol. 54, 46–53.
Zhang, W., Li, C., Guo, M., 2021. Use of ecofriendly alternatives for the control of bacterial infection in aquaculture of sea cucumber Apostichopus japonicus. Aquaculture 545, 737185. https://doi.org/10.1016/j.aquaculture.2021.737185.
Zhang, T., Zhu, M.J., 2017. Enhanced bioethanol production by fed-batch simultaneous saccharification and co-fermentation at high solid loading of Fenton reaction and sodium hydroxide sequentially pretreated sugarcane bagasse. Bioresour. Technol. 229, 204–210.