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ENRICHED SPIRULINA: A POTENT ANTIBIOTIC AGAINST MARINE AND HUMAN PATHOGEN *VIBRIO PARAHAEMOLYTICUS* OPTIMIZED BY REDUCING SILVER NANOCRYSTALS FROM FUNCTIONAL MOLECULES

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ABSTRACT: Organic reduction of nanomaterials is an inexpensive and ecologically feasible method;The major challenge of this method is the optimization. The reduction of metal ions with the conjugation involves either by the extraction from natural materials like plants, algae, fungus, and bacteria or the micro-organisms,which includes the intracellular synthesis of these particles. The present investigation deals with the green synthesis of silver nanoparticles (SNPs) reduced by *Spirulina platensis.* The reduced nanoparticle by the microorganism was materialized to inhibit the pathogenic *Vibrio parahaemolyticus* isolated fromthe aquaculture system of Tamil Nadu. The highest distribution percentage of *V. parahaemolyticus* was recorded in the farm water collected from shrimp farms ofNagapattinam than in the Cuddalore district. The presence of secondary metabolites in qualitative estimation and in GCMS revealed the presence of 32 compounds in both acetone and methanol extract. The essential fattyacidseicosane was found to have antibiotic potency against shrimp infected with pathogenic bacteria. The synthesized *Spirulina*SNPs were

characterized by a biophysical technique like UV-Vis spectrophotometer DAX, XRD, transmission electron microscopy and scanning electron microscopy. The analysis revealed a spherical structure without any agglomerates whose reduction absorption was at 00-4nm. The well diffusion method, it showed inhibition of human and marine pathogen *V. parahaemolyticus,* whose inhibitory zone was at 15.30± 2.21 and 15.19±2.48 mm respectively.Thus, the *Spirulina* mediated silver nanoparticles are found to be a simple, economical, and eco-friendly biomaterial to be a potent antibiotic against the shrimp bacteria.

Keywords: Aquaculture,*Spirulina,*silvernanoparticles,*Vibrio parahaemolyticus,*antibiotics

INTRODUCTION

Aquaculture is the main food-producing sector worldwide to makes an enormous contribution to the economic and social well-being of the dominant fisheries nations (Flegel 2012; Raja *et al.* 2015;Naylor *et al.* 2021). Among that, shrimp aquaculture is one of the major industries which have rapidly grown during the past three decades in subtropical and tropical lowlands of America and Asia (Briggs *et al.*2005). A shrimp species hasan enriched quantity of proteins, essential amino acids, essential fatty acids, and micronutrients and possesses medicinal values (Gunalan *et al.* 2013). Large-scale marine aquaculture has been associated with environmental issues worldwide because ofthe accelerated development and high stocking density. During, the mid-1990s the corporate sector has taken the initiative to run the shrimp farming.Unfortunately, the high-risk factor of viral and bacterial diseases and less production of shrimps due to a decrease in the area used for farming were the major challenges. It was reported that, corporate sector diminished shrimp farming activity from 11, 80,900 ha to 1, 75, 680 ha, which is hardly 15 percentage of the total prospect (Rico *et al.* 2012; Singh *et al.* 2017).

In 1993, there was an outbreak of disease that reduced the Chinese shrimp production, The diseases created several problems for the development of Thai shrimp between 1996 and 1997. The severity of shrimp diseases was acknowledged in Ecuador in 1999 and in Brazil in the year 2006. Moreover, bacterial diseases are the most serious threat and often caused mass mortality in shrimp larvae (Ganesh *et al.* 2010), and *Penaeus vannamei*(Biju and Gunalan 2016). In marine shrimp farming, this is particularly evident in the disquieting increase in vibriosis bacterial disease incidence as shrimp farmers around the world are coping with extreme damage (Chiu *et al.* 2007 and Flegel *et al.* 2008).Vibriospecies, including *V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum*and *V. splendidus*, are known to induce severe infections in aquaculture livestock, especially shrimp (Jayasree *et al.* 2006). They are not only a major economic threat to marine shrimp /farming but represent a public health concern as they are easily transmitted to humans through the consumption of seafood (Fuenzalida *et al.* 2007; Lee *et al.* 2008;).

Conventional approaches, such as the use of disinfectants and antimicrobial drugs to control diseases, have had limited success in the prevention or cure of aquatic diseases. The massive usage of commercially available antibiotics results in the natural emergence of antibiotic-resistant bacteria, which can transfer their resistance genes to other bacteria that have never been exposed to the antibiotics (Fair and Tor 2014). The widespread emergence of antimicrobial resistance acquired by Vibrio speciesagainst various antibiotics worldwide has become a major challenge(Giamarellou 2010). Thishas paved the way in developing a novel antibiotic approach to fight against bacterial infections on shrimp (Rice 2008; Freire-Moran *et al.* 2011).

Many of the experimental studies have emphasized the shrimp physiological properties of important therapeutic compounds in *Spirulina*(Ozdemir *et al.* 2004; Khan *et al.* 2011). The use of plants and algae for the synthesis of nanoparticles could be advantageous when combined with nanotechnology over other environmentally benign biological processes, the occurrence of several compounds in higher plants is well recorded as having an antioxidant function in biological systems, while in microalgae it is recordedless (Colla *et al.* 2007). Exploring the components in microalgae in the utilization of the nanoparticles synthesis to combat against various pathogenic

microbial species is yet to be explored. Hence based on these facts, the present investigation was aimed to synthesis the silver nanoparticles on micro algae (*Spirulina platensis*) to explore its effect on bacteria *Vibrio parahaemolyticus*affecting Shrimps with the following issues (a) the exploration of essential components in *Spirulina platensis* using GCMS. (b) Synthesis of *Spirulina* mediated silver nanoparticles. (c) Characterization of the silvernanoparticles using various biophysical techniques like UV, FTIR, XRD, FESEM, and TEM. (d) Assessing the antimicrobial potency against *Vibrio parahaemolyticus* has been done.

MATERIALS AND METHODS

Purchase and maintenance of Spirulina

*Spirulina*stock culture was procured from the commercial farm atPalladam, Coimbatore, Tamil Nadu (**Fig. 1**) and the cultures were maintained at a temperature of 25±1°C in the light intensity of 40 µE / m²/s, photoperiod of 16:8 (Light and Dark) for 20 days.The cells were harvested and centrifuged at 10000 rpm for 5 to 10 minutes and the algal cells were dried at 100°C for 30 min for further use.

Screening of shrimp pathogenic bacteria from shrimp farm water samples

The water from the shrimp farm in Nagappattinam and Cuddalore districtswas collected in sterile polypropylene containers for microbiological analysis in the laboratory.The samples were serially diluted and were plated on to Thiosulfate-citrate-bile salts-sucrose (TCBS) agar medium at 28±2°C for 24 to 48 h.The spread plate technique was employed in the enumeration of total presumptive *Vibrio spp*. In respective samples as described by Beneduce*et al*.(2010). Hundred microliters of each pre-enriched homogenates with appropriate sample dilution (1:10, 1:100, and 1:1000) were spread in duplicates onto the tryptone soy agar (HiMedia, Mumbai) and incubated at 37°C for 18–24 h. After incubation, the total colony count is determined and their concentrations in the original shrimp in cfu/mL were calculated.

To isolate*V. parahaemolyticus,* thecolonies were further purified by repeated streaking of the isolates on Tryptone Soy Agar (TSA) and added with 1.5% NaCl and incubated at 37°C for 18 h. The pure *V. parahaemolyticus*bacterial isolates were maintained (in TSA+NaCl) at 4°C with 15% glycerol at -70°C (Thorstenson and Ullrich, 2021).

Qualitative analysis of *S. platensis*

The aqueous extract of *S. platensis* was subjected to various qualitative analysis for ensuring the presence of active compounds of *S. platensis,*whose protocol is as follows: (i) Alkaloids (Santhi *et al.* 2011): Aqueous extract of *S. platensis* was added with 1% HCl along with 6 drops of Mayer's reagent followed by Drangendroff's mixture for the presence of precipitation to indicatethe presence of alkaloids. (ii) Flavonoids (Beknal*et al.* 2010): The aqueous extract was added with a few drops of 10% lead acetate to obtain yellow precipitation.(iii) Cardiac glycosides (Santhi *et al.* 2011): Aqueous extract was added to 2mL of glacial acetic acid (GAA) containing one drop of ferric chloride solution and 1mL of concentrated sulphuric acid. This gives abrown ring to indicate the presence of a deoxy sugar characteristic. The colour changes toa brown ring from a violet ring. (iv) Steroids (Khan *et al.* 2011):Extract (1mL) + 1mL chloroform + 2-3 drops of conc. H_2SO_4gives pink colouration. (v) Tannins (Thenmozhi *et al.* 2010): Theextract was added on to 1% lead acetate, it leads to yellow colorationindicating the presence of tannins in the sample. (vi) Terpenoids (Chakraborty 2019) *S. platensis*extract was dissolved in 10mL of chloroform and filtered through Whatman filter paper No.1.Additionally, 2mL of acetic anhydride and 3 drops of concentrated H_2SO_4 were added.Finally, the presence of steroids was confirmed with the appearance of the blue/green ring.

Gas Chromatography Mass Spectrometry (GC-MS) analysis

The methanolic extracts of *S. platensis* samples were analyzed inGas chromatography mass spectrometry (GC-MS), Shimadzu Mass Spectrometer-2010 series system (SMS Lab, Chennai) with an AB inno-wax column (60 m × 0.25 mm id and film thickness 0.25 μm). For GC-MS detection, ionization system with ionization energy of 70 eV was used. The Helium gas was used as a carrier gas at a flow rate of 1.2 ml per minute. Injector with mass transfer line of temperature was set at 270 and 280°C thephyto compounds were identified and the mass spectra obtained were matched with the inbuilt library (NIST/Wiley).

Synthesis and Characterization of *S. platensis* reduced AgNPs

Aqueous silver nitrate (Sigma-Aldrich, Mumbai)of concentration 1 mM, is added with 150 ml of *S. platensis* extract. Thiswas kept in dark to avoid photo activation of $AgNO_3$ at room temperature (Muthusamy *et al.* 2017). Then obtained dark brown color solution during the heating process was characterized with the following methods (see **Fig. 2**).

Following Anitha *et al.*(2016), the synthesis of *S. platensis* mediated silvernanoparticles wasconfirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV–vis, at the wavelength of 300–800 nm in a UV-3600 Shimadzu spectrophotometer at 1-nm resolution. Furthermore, the reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min, and the resulting pellet was dissolved in deionized water and filtered through a Millipore filter (0.45 μm). Surface groups of the nanoparticles were qualitatively confirmed by using FTIR spectroscopy (Bruker model, TENSOR 37), with spectra recorded by a Perkin Elmer Spectrum 2000 FTIR spectrophotometer. After freeze-driedthe nanoparticles, thestructure and composition were analyzed by XRD analysis (Rigaku RINT 2100 series). The sample (25μl)was sputter coated on a copper stub, and the morphology of *S. platensis* –AgNP was investigated using a Hitachi s-3500N SEM-EDX. Finally, the Transmission Electron Microscopic analysis was performed by a TEM, JEM-1200EX, 3μL by coating the nanoparticle sample on the carbon coated copper grid for making a thin film of sample on the grid. The extra sampleswere removed using a cone of blotting paper and sequentially kept in the grid box.

Antibacterial assay

The Minimum inhibitory concentration (MIC) of green synthesized silver nanoparticle of *S. platensis* against *V. Parahaemolyticus* was studied by the method ofwell diffusion given by Subramanian *et al.* (2017)The test *Vibrio* strain was grown overnight at 37°C in a medium containing alkaline peptone water prepared in 10^{-1} (0·2) dilution. The sample was seeded in three concentrations (10, 50 and 100μmm) filled with 10ml of Mueller Hinton agar (MHA) medium in the Petri plates. In this medium, wells were cut and filled with 10μl of live suspension of *vibrio*culture with a cell density of 10^5ml CFU^{-1} h and observed for the zone of clearance.The Petri plates were incubated at room temperature (28 ±1°C) for 24h and observed for the zone of inhibition which was measured in millimeters(mm).

RESULTS

Vibrio parahaemolyticus is a pathogen with a universal allocation but its densities in the environment and seafood vary depending on the season, location, sample type, and analytical methodology employed (Martinez-Urtaza*et al.* 2008;Zarei *et al.* 2012). Hence,two shrimp farm water samples collected from at Cuddalore and Nagapattinam districts were used forenumerating the presence of *Vibrio* colonies. From the total colonies, the pure culture of *V. Parahaemolyticus*were isolated and cultured for further use(Fig. 3).From these results, it could be concluded that, more risk factors are associated in samples collected from Nagapattinum and Cuddalore districts. The colony count of *V. Parahaemolyticus*was were found to be high in Nagapattinam(58 %) shrimp farms than in Cuddalore (56%) shrimp farms as given in Table 1.

The various primary and secondary metabolites in *S. Platensis*extract was analyzed using qualitative methods and confirmed the presence of Alkaloids, Terpenoids, Tannins, Saponins, Flavonoids, and Steroids (Table 2) respectively. The GC-MS (Fig.4 A&B) analysis was subjected to for the quantification of active components of *S. platensis* in methanolic extract of *S. platensis,* showed around 16 bioactive components (1-Tridecene, Sulfurous acid, 2-ethylhexyl isohexyl ester, Heptadecene, 1-Pentadecene, Octane, 9-Octadecenoic acid (Z), Methyl Ester, Hexadecanoic acid, Methyl Ester, 3-Eicosene, (E)-,6,9,12-Octadecatrienoic acid, 11,14-Eicosadienoioc acid, Methylester, Oxirane, 1,2,3,4-Tetrakis 0 (Trimethylsilyl), 2-Undecene, 9-methyl, Tetracosanoic acid, methyl ester,1,2-benzene dicarboxylic acid) respectively, which is showed in Table 3. Among these compounds, the major compound identified as fatty acid(Eicosane) 42.11% which would be responsible for antibacterial potency.

S. platensis mediated silver nanoparticles have been synthesized using reduction method and the particles were characterized using various biophysical techniques.The reaction mixture changes color from light green to dark reddish-brown, which indicates the formation of AgNPs whose UV-Visible absorption spectrum, recorded a strong, broad peak located at 450 nm (Fig.5A). Further, the synthesized AgNPs acquired high precipitation of silver particles on the cell surface when analyzed inFTIR (Fig 5B) (Perkin Elmer Spectrum 2000) which reveals the presence of the functional groups in *S. platensis* and the nanoparticles conjugated with *S. platensis.* The broad, sharp, and narrow stretching frequency was identified as 3788, 3425, 2927, 2343, 1721, 1657, 1641, 1631, 1547, 1535, 1501, 1461, 1408, 1067, 668, 566 cm^{-1}respectively. These bands may correspond to the various active functional groups like alcohol (O-H), Alkanes (C-H), Carboxylic acids and Alpha, Beta-unsaturated aldehydes (C=O), N-O asymmetric stretch alkyl halides (C-Cl stretch), aromatics (C-C stretch), aliphatic amines (C-N stretch) which are comprehensively postulated in Table 4.

The morphology of element of nanoparticles was determined by SEM and is shownin the corresponding micrographs in Fig.6A. With the critical analysis, it has been observed that silver nanoparticles of *S. platensis* were slightly agglomerated, mostly spherical in shape, and found throughout the region. The TEM (Fig. 7) confirmed the spherical nature of the particles and was observed not forming big agglomerates. This indicates the monodispersed nature of NPs reduced by *S. platensis*. The EDX spectrum indicated that, the reaction product was composed with the presence of the pure silver metal (55.08% of purity)with strong signals given in fig 6B.

Fig.8 showed the X-ray diffraction spectrum of synthesized silver nanoparticles by using aqueous extract *S. platensis*. The reflections were observed in the Braggs XRD pattern at 2θ = 38.48; 44.95; 65.71 and 77.18. These reflections clearly indicated the presence of observed peaks (at 111, 200, 220, and 311 sets) of lattice planes, and further, it can be indexed as a face-centered-cubic (FCC) structure of Ag. Therefore, the present study clearly showed that the X-ray diffraction pattern of AgNPs formed crystalline structures.

Finally, the antimicrobial potency of *S. platensis* reduced silver nanoparticles was tested against shrimp pathogen *V. parahaemolyticus*. The range was expressed as inhabitation zone of 15.19±2.4 and 16.67±1.3mm, when inoculated with 10, 50, and 100μmmrespectively. This is shown inFig.9. This clearly indicates that, the silver nanoparticles have the potential to for control of the pathogenic *V. parahaemolyticus* of shrimp pathogens.

DISCUSSION

The *Spirulina platensis* mediated silver nanoparticles antibacterial results showed excellent inhibition against the human and marine *Vibrio parahaemolyticus* isolated from the aquaculture system of Tamil Nadu. Shrimp farming is a blooming aquaculture industry which altered every time due to disease causing bacterial pathogens in the soil and water which causes bacterial diseases in the aquatic animals (Chellapandian*et al.* 2021) and their associated people. The few strains of *Vibrio parahaemolyticus* species are human pathogens, causing gastroenteritis (Broberg *et al.* 2012) it can be inhibited by *Spirulina platensis* mediated silver nanoparticles.

Overall, 55.7% and 58.3% of *Vibrio parahaemolyticus*arepresent in our study area Cuddalore and in Nagappattinam district which is higher than 20 percent recorded by Gámez-Bayardo*et al.* 2021. Also, Silvester*et al.*(2015)reported 71.6 % in the Cochin estuary and 53.3 % in the shrimp farm. Biju and Gunalan (2016)reportedthat 29.01% *Vibrio spp.* were isolated from *Penaeus vannamei*from three major shrimp farm villages of Nagappattinam district and the detection rate of *V. parahaemolyticus* reported in shrimp has 6.7% (Gámez-Bayardo*et al.* 2021), and 4.28% from marine shrimp and 2.5% from freshwater shrimp samples (Patel *et al.* 2018). According to Borowsky *et al.* (2007), the quantification of microorganisms present in the samples is important for assessing the risk to consumers.

The gram-negative *Vibrio parahaemolyticus*is usually found in tropical and temperate coastal waters, as well as in shrimp aquaculture (Norma *et al.* 2009). Also, Zulkifli *et al.* (2009) mentioned a high marine temperature between 25 and 35 °C, resulting in the distribution of *V. parahaemolyticus* all year round. The shrimp ponds with lower dissolved oxygen and higher unionized ammonia level provide *Vibrio* spp. opportunity to enter and cause mortality (Elgendy*et al.* 2015). In the present investigation, the colony count of the *Vibrio* spp. was found to be high in the Nagappattinam district than in the Cuddaloredistrict, due to the temperature variation as suggested by Naylor (2021). The difference in temperature in the environment, contributes to the high counts of *V. parahaemolyticus* in the wet markets as seen in our study. Seafood such as fish should be kept in cold conditions during transit and storage to reduce the risk and level of *Vibrios*(Elhadi *et al.* 2004). Sudha *et al.* (2014) have also reported higher contamination of shellfishes with pathogenic *Vibrios* isolated from samples collected from the roadside stalls compared to markets in Cochin, India. Hence the percentage of *V. parahaemolyticus* distribution depends on the temperature and postulates the differential distribution among the districts selected for the present study.Among the marine microorganisms, *S. platensis*, a well-known cyanobacterium is an exceptionally rich source of bioactive compounds (Rastogi *et al.* 2015) in killing cancer cells. It has been reported to have antimicrobial, anti-inflammatory, and antiviral properties (Vijayakumar and Menakha 2015). The major secondary metabolites (Alkaloids, Terpenoids, Tannins, Saponins, Flavonoids, and Steroids) isolated from this *S. platensis* can be utilized for the antibiotic potency. These Gram-negative photosynthetic prokaryotes produce bioactive secondary metabolites as quorum sensing inhibitory compounds and as a chemical defense against invading pathogens (Mayer and Hamann 2000). Agustini*et al.* (2015) indicated the absence of alkaloids in *Spirulina*, while Ali *et al.* (2014) reported qualitative and quantitively presence of phenolic compounds and total alkaloids like the present study for *S. platensis*. And, Kannan *et al.* (2014) has reported the presence of similar phytochemical constitutions in the same species hence there is not many phytochemical compounds difference found.

The GC-MS analysis showed the phytochemicals of secondary metabolites present in the *S. platensis*. The algal species showed 16 bioactive components in methanol extract and in all these compounds eicosanoids (fatty acids) dominated the account. The similar result was also studied by Jubie and Dhanabal(2012) who isolated 15 compounds in *S. Platensis* acetone extract which contains essential fatty acids like gamma linolenic, stearic, myristic, linoleic, heptadecanoic acid, etc. Therefore, the composition of individual fatty acids has been found to influence the retention time and molecular weight that controls the pathogenic microbes. The results of the current study, it is suggested that the silver nanoparticles derived from *S. platensis* help in reducing the effect of shrimp pathogen infection. This may also be a potential alternative to chemicals in the control of pathogenic microbes and ensure sustainable growth of the industry.

In general, silver has greater chemical stability and unique catalytic activity when combined with the plant species it will have greater potency in their application without any residual effects. The UV-vis spectra can provide valuable information on the shape, size, and distribution of nanoparticles based on Surface Plasmon Resonance (SPR) bands. For instance, the appearance of the Ag peak at a shorter wavelength in the UV-vis spectra reveals the small size of Ag-NPs that

were formed, while a longer wavelength indicates bigger Ag-NPs (Mashwani *et al.* 2016).Hence in the present investigation, the UV spectra of synthesized silver nanoparticles was recorded at 450nm which corroborates with the findings of Anitha *et al.* (2012). It was suggested that the reduction method of silver nanoparticles reduced at 450nm respectively. In general, the peaks (at 450nm) obtained by colloidal silver nanocomposite in the specified region will be due to the excitation of the surface plasmon vibrations between the functional groups of the *S. platensis* which may facilitate the reduction of Ag^{+} to Ag^{0}. Nandhagopal *et al.*(2015) studied the UV-vis spectrophotometry, where the peak was detected at 425 nm.The reduction might be due to the primary and secondary metabolites which play an active role as (Anitha *et al.* 2012) reducing agents in biological agents (Benelli 2016a; Benelli 2016b).

FTIR measurements of greener microalgae mediated AgNPs was carried out to identify the possible biomolecules responsible for the stabilization of silver nanoparticles. The FTIR analysis revealed various functional groups present in the reduction process in which the bands obtained at 3788 and 3425cm^{-1}, assumes the presence of alcohol group (OH stretch) which always have bonded with oxygen and hydrogen for its functionality (Niraimathi *et al.* 2013). The unsaturated hydrocarbons which mean, more bonds will be present between carbon atoms are the C-H stretch (alkene groups)wereat 2927 and 2343 cm^{-1}. The C-N and N-H bonds present in 1631 and 1461cm^{-1} postulates the presence of the glycosides and amines of the proteinsin*S. platensis*was in support withthe findings of Prakash*et al.* (2013).The presence of flavonoids and proteins may facilitate the reduction of silver ions in to silver nanocomposite was suggested by Zuas*et al.*(2014). The durable bands at 1547, 1535, and1501 cm^{-1}, could be due to C=C stretching in the aromatic ring, confirming the presence of the aromatic group which was in accordance with the aromatic rings obtained fromthe silver nanoparticles studied by Al-Otibi*et al.* (2021).Further the presence of C=O stretch, and N-O asymmetric stretch in the FTIR analysis predicted the presence of nitro compounds which may help in the reduction of the nanoparticles (Silverstein *et al.* 1991). From this analysis, it has been studied that the functional groups will help in the poly dispersion of the nanoparticles whose vibration frequency are at 668 and 566 cm^{-1} respectively. Similarly, Xie *et al.*(2007) also reported that carboxyl and hydroxyl groups from the extract of microbial species are involved in the biosynthesis of AgNPs.

The shape of the particlesobtained is in accordance with the spherical to oval AgNPs with average particle size of 17.9 and 26.4 nm for *S. platensis* and *A. variabilis*, respectively (Ismail *et al.* 2020). Thismight be due to the polydispersity index of the nanoparticles through SEM and TEM studies (Ardani*et al.* 2017). These measurements are fundamentally different and are based on the hydrodynamic and dry radius of particles, respectively (Kaasalainen *et al.* 2017). The phase of the silver metal was confirmed in the EDAX pattern of synthesized silver nanoparticles. The intense signal was obtained as a peak for silver and a weak signal was acquired for the copper in the present investigation. From this, it has been confirmed that the reduction of silver ion by the molecules present in *S. platensis* is feasible.Thesilver nanocrystals usually display a characteristic visual absorption peak approximately at 3KeV due to surface plasmon resonance (Kaviya *et al.* 2011)

The presence of pure metal peak for silver suggested the confirmation of the presence of silver. The XRD for the synthesized Ag NPs exhibits the behavior diffraction features with 2θ at 38.48, 44.95, 65.71, and 77.18° which represents the Bragg's reflections (111), (200), (220), (311), and (422) respectively (Baraka *et al.* 2017; Bryaskova*et al.* 2011).Biological effectiveness of NPs enhances due to increase in specific surface area and surface energy andused for various therapeutic purpose (Danaei*et al.* 2018). Silver has long been documented as having an inhibitory effect on many bacterial strains and microorganisms (Mahdieh *et al.* 2012) even at a very low concentration and they inhibit the growth of antibiotic-resistant bacteria. The charge of bacterial cell wall is negative because of dissociation of carboxylic groups on the cell surface (Parveen *et al* 2018). Weak positive charges present on AgNPs are attracted towards negative charges (Raffi *et al.* 2008). In contrast, Sondi and Salopek-Sondi (2004) and Schultz *et al.*(2000) suggested that the antibacterial effects of AgNPs on bacteria depended on the concentration of AgNPs and are closely related to the

development of pits on cell wall of bacteria. AgNPs interact with the thiol groups of bacterial proteins and may retard the replication of DNA (Sondi and Salopek 2004).

Silver nanoparticles exhibit effective antimicrobial properties compared to other nanoparticles because of their extremely large surface area, which offers improved interaction with microorganisms (Wang *et al.* 2017).In the current findings, the silver nanoparticles reduced using *S. platensis* showed that the range of inhabitation zone between 15.19±2.48mm and 16.67±1.37mm, respectively. This clearly indicates the biogenic silver nanoparticles have the potential forcontrol of the pathogenic *V. parahaemolyticus*, a shrimp pathogen. The algal species has contained fatty acids which may be responsible for inhibiting pathogenic bacteria *V. parahaemolyticus* which is supported by the results of Martinez-Castanon *et al.*(2008) who reported that the fatty acids in *S. platensis* have been more effective against bacterial species (see Fig. 6). Similarly, Sudha *et al.* (2011)and Abdo *et al.* (2012) have reported that the *S. platensis* individually and combined with silver nanoparticles exhibited more anti-bacterial activity against human pathogens.

CONCLUSION

The current study demonstrates that the silver nanoparticles derived from *S. platensis* can control the *V. parahaemolyticus bacteria*from causing harm to shrimps. The pathogenic bacteria, *Vibrio parahaemolyticus*, produces a variety of deleterious diseases in shrimp and thus causes heavy economic damage to the shrimp industry which yields a considerable foreign exchange to our country. *Vibrio* spp. was found to be high in the Nagappattinam district than in the Cuddalore district. The produced AgNPs were then confirmed by different techniques: UV–vis, FTIR, and GC-MS spectroscopy, SEM-EDAX, SEM, TEM imaging techniques, and XRD analyses. The synthesized AgNPs showed better antibacterial activity against *Vibrio* spp. with the range was expressed as inhabitation zone of 15.19±2.4 and 16.67±1.3 mm, when inoculated with 10, 50 and 100 μmmrespectively. The EDX spectrum indicated that, the reaction product was composed with the presence of the pure silver metal (55.08% of purity) with strong signals. This spirulina silver nanoparticles may also be a potential alternative to chemicals control of pathogenic microbes and ensure sustainable growth of the aquaculture industry.

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Fig. 1 Farm processing of *Spirulina platensis*: A) Mass culture B) Drying

Fig. 2 Synthesis of *Spirulina platensis* mediated silver nanoparticles: A) Spirulina extract and its nanoparticle solution B) Dried nano powder

Fig. 3 Pure culture of *Vibrio parahaemolyticus* isolated from shrimp farm water

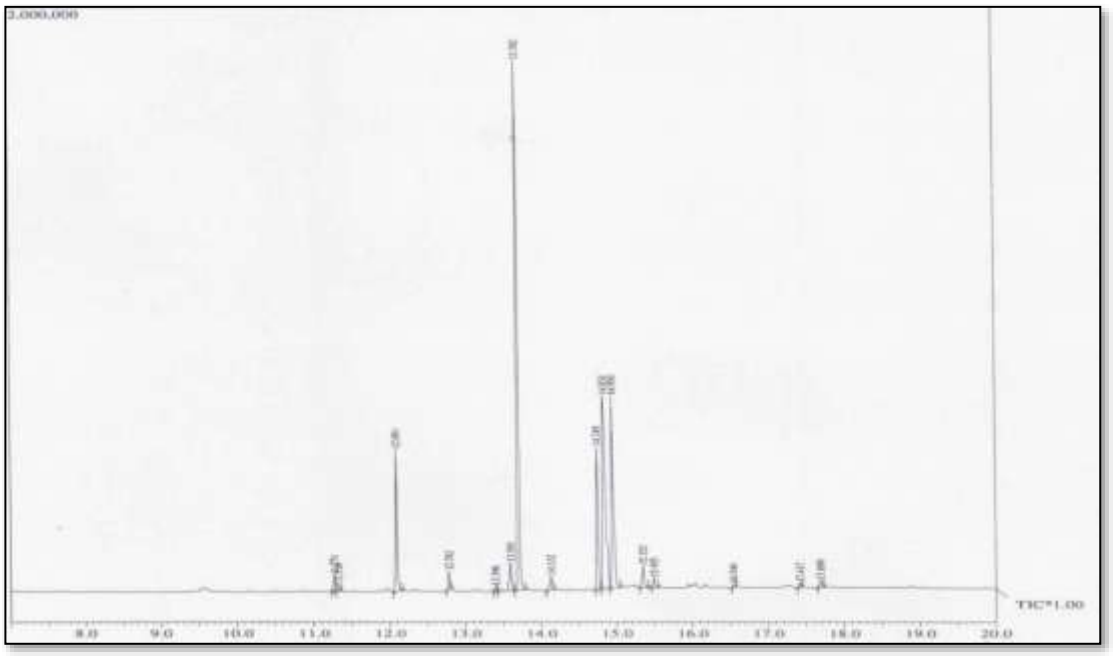

Fig. 4 GCMS peak profiling of the active components of methanolic extract of *Spirulina platensis*

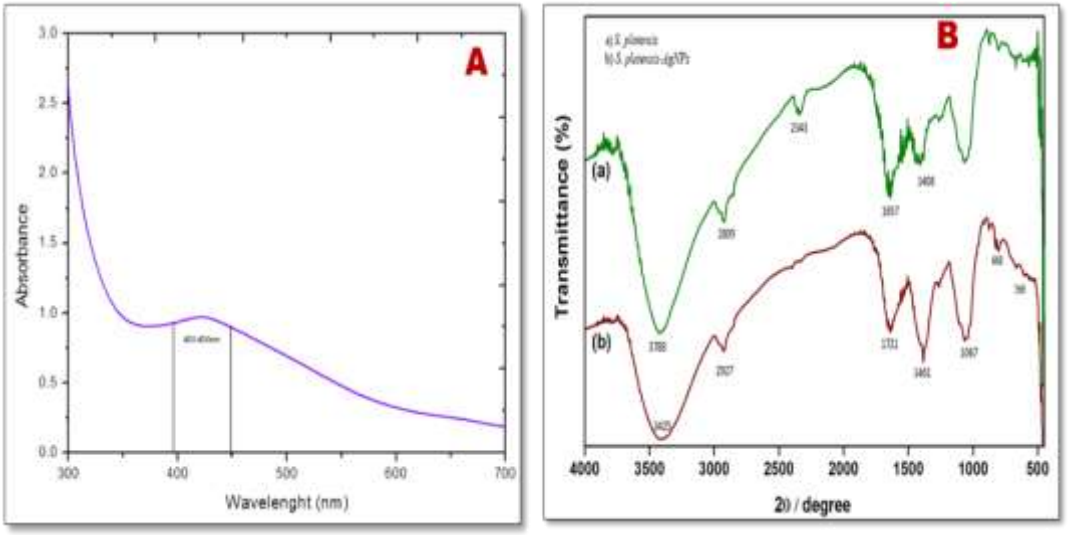

Fig. 5 Absorbance characterization of *Spirulina platensis* synthesized silver nanoparticles: a) UV – Visible spectrum b) FTIR spectrum

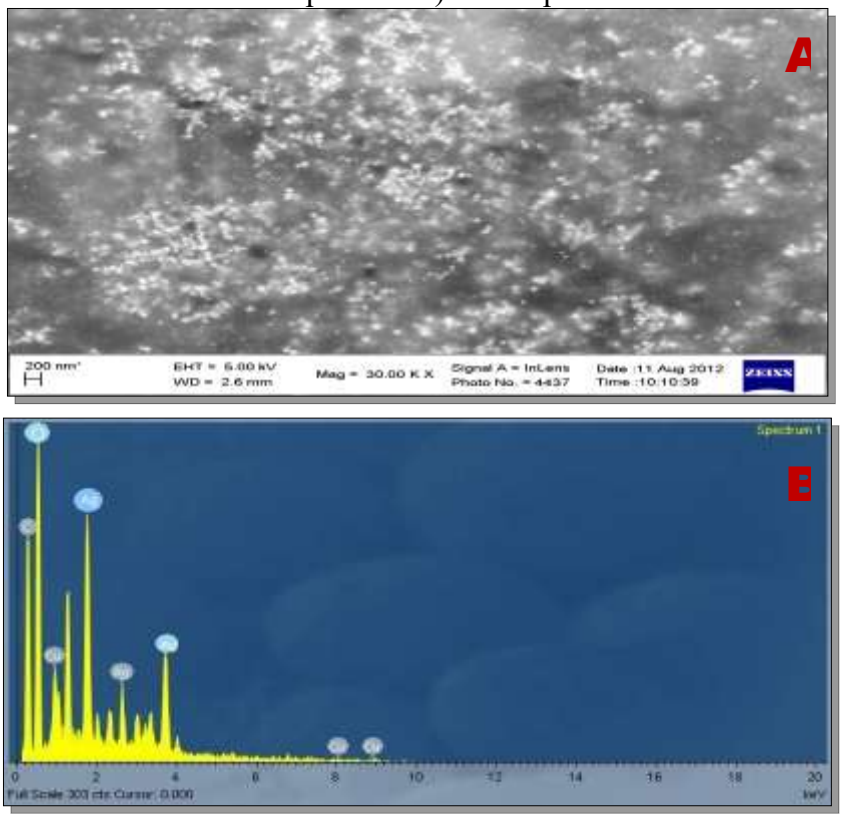

Fig. 6 Morphology on SEM (A) and pure metal distribution in EDAX (B) of silver nanoparticles synthesised using *Spirulina platensis*

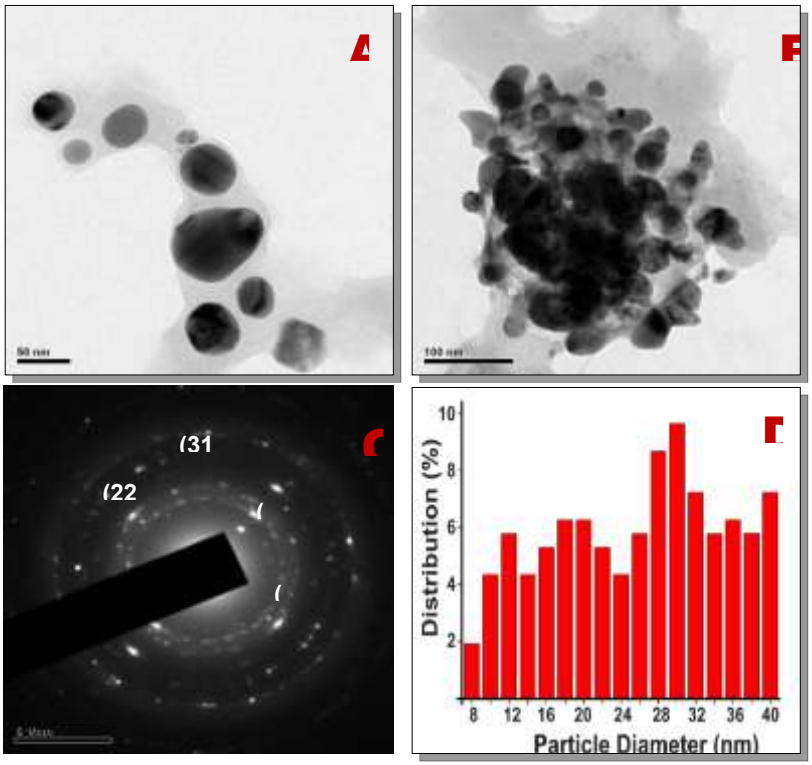

Fig. 7 TEM characterization of silver nanoparticles mediated by *Spirulina platensis* for its Particle distribution with various diameter (A, B, C, D)

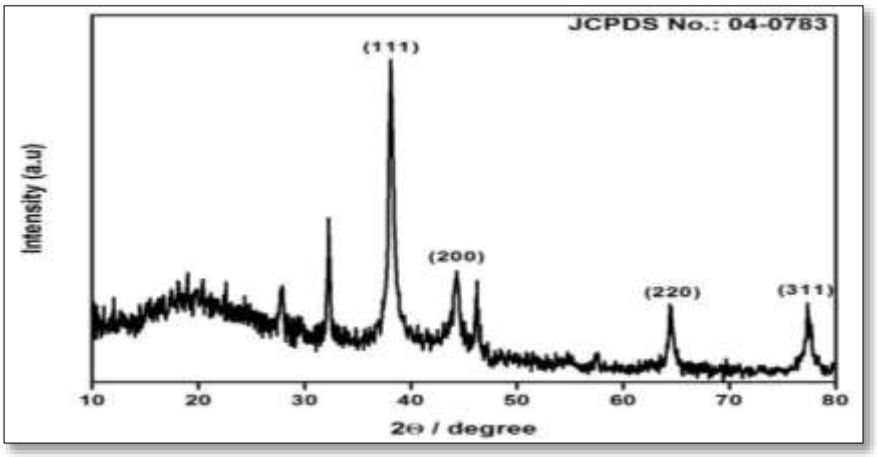

Fig. 8 XRD pattern of silver nanoparticles mediated by *Spirulina platensis*

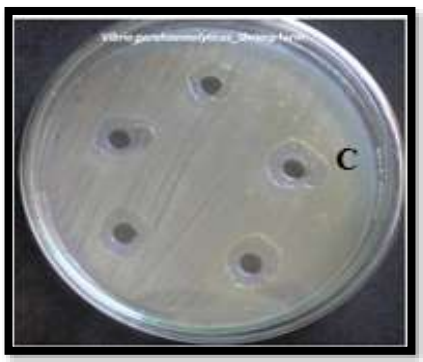

Fig. 9 Zone of inhibition against *vibrio parahaemolyticus* exhibited by silver nanoparticles mediated by *Spirulina platensis*

Table 1 Frequency distribution of *V. Parahaemolyticus* strain in selected districts of Tamil Nadu

Sampling District	**Total Number of other unknown colonies**	**Total Number of *V. Parahaemolyticus***	**Percentage (%) of appearance**
Cuddalore	140	78	55.7
Nagapattinum	173	101	58.3

Table 2 Qualitative assessment of secondary metabolites of *S. platensis*

S.No.	**Phytochemicals**	**Present/Absent**
1.	Alkaloids	+
2.	Flavonoids	+
3.	Glycosides	+
4.	Steroids	+
5.	Tannins	+
6.	Terpenoids	+

Table 3. GCMS quantification of active components of *S. platensis*

Peak	**Retention time (RT)**	**Area**	**Area (%)**	**Active compounds**
1	11.271	106819	1.31	1-Tridecene
2	11.318	41868	0.51	Sulfurous acid, 2-Ethylhexyl isohexyl ester
3	12.091	762878	9.37	Heptadecene
4	12.782	91362	1.12	1-Pentadecene
5	13.396	27954	0.34	Octane
6	13.595	196469	2.41	9-Octadecenoic acid (Z), Methylester
7	13.702	2688125	33.01	Hexadecanic acid, Methyl ester
8	14.132	95738	1.18	3-Eicosene (E)
9	14.749	721661	8.86	6,9,12-Octadecatrienoic acid
10	14.831	1875273	23.03	11,14-Eicosadienoioc acid, Methyl ester
11	14.950	1249522	15.35	Phytol

Table 4 FTIR stretching frequency corresponding bonds with its functional groups

Wavenumbers (cm^{-1})	***Bond***	***Functional groups***
3788.91	O-H stretch, free hydroxyl	Alcohols
3425.49	O-H stretch (alcohols) H-bonded	Alcohols
2927.29	C-H stretch	Alkanes
2343.65	C-H stretch	Alkanes
1721.88	C=O stretch	Carboxylic acids
1657.96	C=O stretch	Alpha, Beta-unsaturated aldehydes
1641.08	-C=C-stretch	Alkenes
1631.02	N-H bend	Primary amines
1547.57	N-O asymmetric stretch	Nitro compounds
1535.68	C-C stretch (in-ring)	Aromatics
1501.66	C-C stretch	Aromatics
1461.31	C-H bend	Alkanes
1408.95	C-C stretch	Aromatics
1067.51	C-N stretch	Aliphatic amines
668.87	C-Cl stretch	Alkyl halides
566	C-Br stretch	Alkyl halides