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Antimicrobial and biochemical characterization of a C-type lectin isolated from pearl spot (*Etroplus suratensis*)

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ABSTRACT

The present study reveals purification and characterization of a C-type lectin from the serum of pearl spot, *Etroplus suratensis* (*Es-Lec*). The *Es-Lec* was purified by affinity chromatography with mannose coupled sepharose CL-4B column and it exhibits single band with a molecular weight of 75 kDa in SDS-PAGE. The surface morphology of purified *Es-Lec* displays the homogeneous nature of protein. A distinct peak with a retention time of 2.958 min was appeared in high performance liquid chromatography (HPLC), X-ray diffraction (XRD) analysis expresses a single peak at 31.837Å and MALDI-TOF peaks which shows the purity and crystalline nature of the protein respectively. Functional analysis of purified *Es-Lec* exhibits yeast agglutination activity against *Saccharomyces cerevisiae* and has the ability to agglutinate the human erythrocytes, which was observed by light microscopy and haemagglutination inhibition was also done. In addition, purified *Es-Lec* showed the broad spectrum of antibacterial activity against Gram negative *Vibrio parahaemolyticus* and *Aeromonas hydrophila*. Antibiofilm potential of purified *Es-Lec* against selected Gram-negative bacteria exhibited the disruption of biofilm architecture at the concentration of 50 μg ml^{-1} and also it exhibited antiviral and anticancer activity.

1. Introduction

Lectins are glycoproteins characterized by their capability to attach carbohydrates like mannose, galactose, lactose, *N*-acetyl glucosamine, *N*-acetyl galactosamie, fucose, and rhamnose with significant specificity [1]. They possess at least one carbohydrate recognition domain (CRD) that specifically and reversibly binds to different sugar moieties present on the surfaces of pathogens, and lectins are present in almost all organisms including plants, animals, viruses, bacteria, cyanobacteria and yeasts [2–5].

Lectins are involved in many biological functions including cell adhesion, phagocytosis, complement activation and innate immunity [6]. In fish, lectins are reported in serum, gills, surface mucus, egg surfaces and other organs [7–10,19]. Based on their structure, binding specificities and calcium dependency, lectins are classified into different families like C-type lectins, F-type lectins, galectins, intelectins, rhamnose binding lectins, I-type lectins, Lily-type lectins etc. [11]. C-type lectin (CTL), one of major lectins in fish, is characterized by Ca^{2+}-dependent binding to mono and oligosaccharides, and they are classified in several groups; collectins, proteoglycan core proteins, selectins directly or indirectly involved in immune function [12]. Various types of CTLs of diverse carbohydrate specificities have been identified in various fish species including; rainbow trout (*Oncorhynchus mykiss*), catfish (*Silurus asutus*), common carp (*Cyprinus carpio*), Japanese eel (*Anguilla japonica*), fugu (*Takifugu rubripes*) and zebrafish (*Danio rerio*) [13–18]. Different types of lectins, their roles and the tissue distribution of lectins in the fishes is comprehensively reviewed by Preetham et al. [19].

In fish, mannose-binding proteins (MBP) or mannose-binding lectins (MBL), a CTL play an important role in innate immunity and disease resistance [20]. Although mannose binding lectin (MBL) have been reported in rainbow trout, channel catfish (*Ictalurus punctatus*), common carp, Nile tilapia (*Oreochromis niloticus*), rohu (*Labeo rohita*) etc. [13,17,21–23] their exact role in agglutination and antimicrobial

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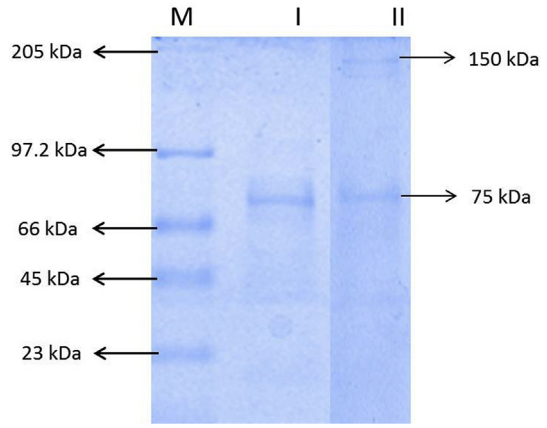

Fig. 1. Polyacrylamide gel electrophoresis (10%) in the presence of sodium dodecyl sulfate (SDS) of *Es-Lec* purified by Mannose-Sepharose CL 4B affinity chromatography. Lane M contains Medium molecular weight marker. Lane I contains reducing pattern and Lane II contains non-reducing pattern of purified *ES-Lec* from *Etroplus suratensis* serum. The protein bands were stained with coomassie brilliant blue – R 250. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

activity is not well established.

Pearl spot (*Etroplus suratensis*) is the state fish of Kerala with high economic value and high flesh quality. They are endemic to peninsular India and Sri Lanka, but the landings of pearl spot have declined drastically during the last years due to disease [24]. Currently no information is available about the immune system and immune response in pearl spot. Therefore, the present study addressed to characterize the immune response in pearl spot, with focus on isolation and characterization of C-type lectin from serum, and substantiate its agglutination and antimicrobial properties.

2. Materials and methods

2.1. Sample collection and preparation

Thirty live and healthy pearl spot (*Etroplus suratensis*) fish ranging from 100 to 150 g in weight were obtained from a hatchery at Vaikom, Kerala, India. Prior to sampling were the fish anesthetized by using MS-222, and blood was collected and extracted in aseptic conditions by cardiac puncture from each fish using gauge 23 hypodermic needles and diluted with 1:1 anticoagulant solution (0.45 M NaCl, 0.1 M glucose, 30 mM sodium citrate, 26 mM citric acid, 10 mM EDTA, pH 7.5). A total of 30 ml blood was pooled for purification of lectin. The blood sample was incubated at 37°C for 4 h and then kept at 4°C overnight. After coagulation, the blood was centrifuged at 1000 rpm for 10 min at 4°C and the supernatant (serum) was collected into fresh tubes and stored at −20°C until further use.

2.2. Purification of lectin from pearl spot serum

Lectin was purified according to the method described by Coelho et al. [22] with slight modifications. Briefly, mannose coupled sepharose CL-4B column was used for the purification of *Es-Lec*. The column was extensively washed with TBS/$CaCl_2$ buffer (10 mM Tris–HCl, 150 mM NaCl, 10 mM $CaCl_2$, pH 7.4). The pearl spot serum

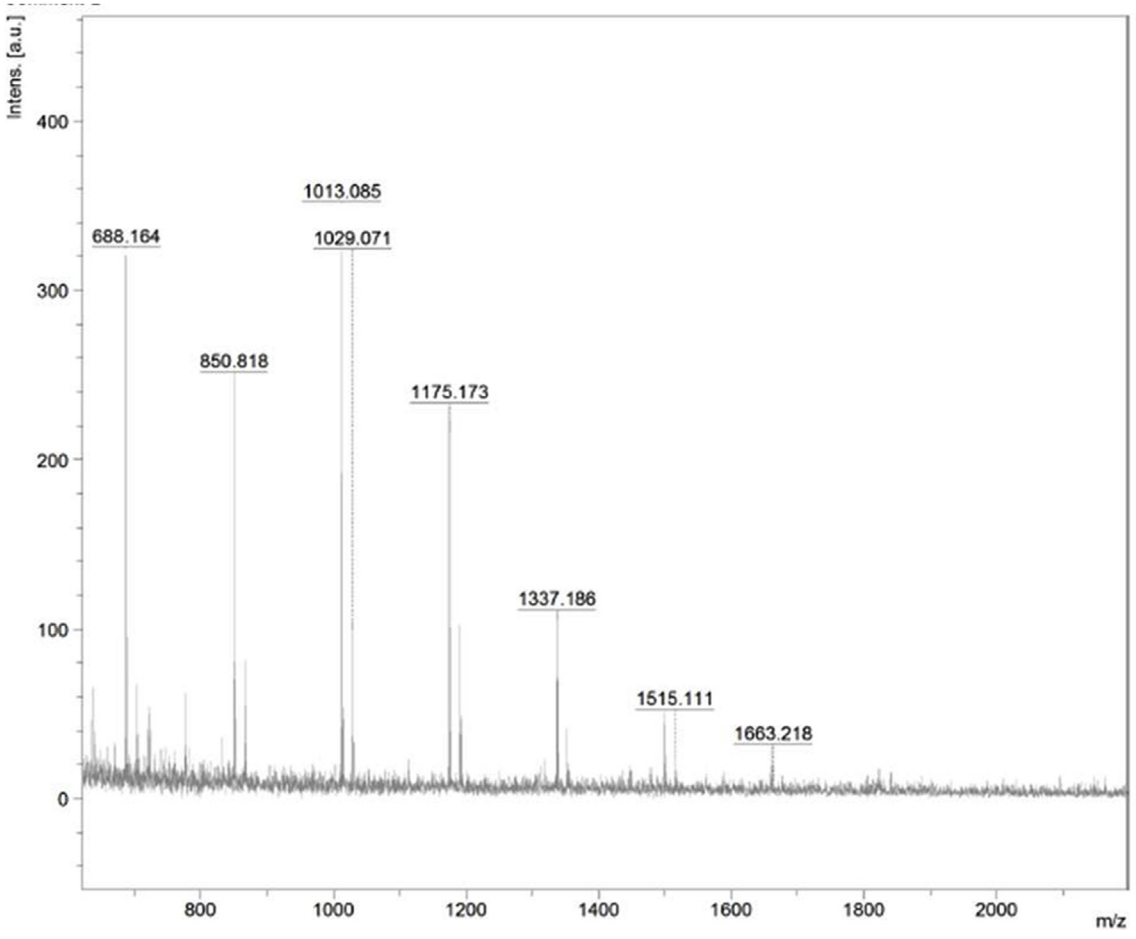

Fig. 2. MALDI–TOF/TOF MS peptide analysis of 75 kDa Es-*Lec* from *Etroplus suratensis*.

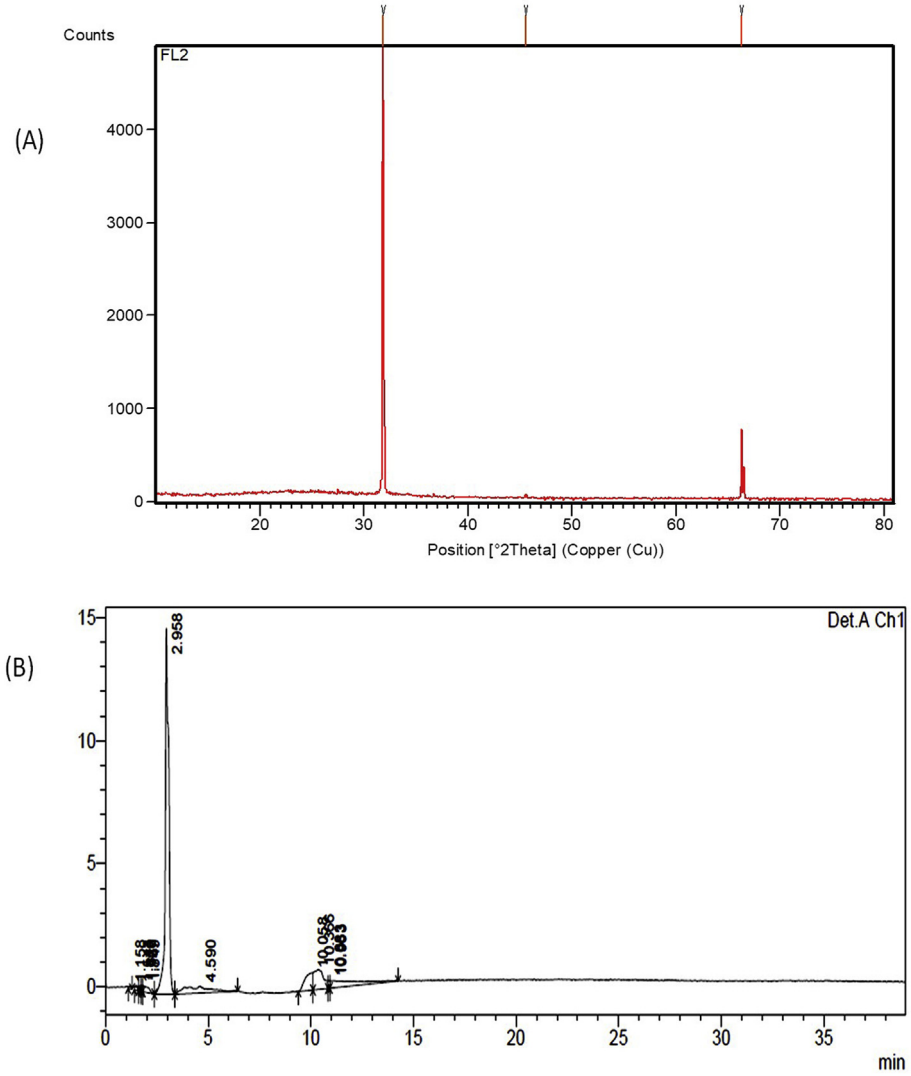

Fig. 3. Biochemical characterization of *Es-Lec*. **(A)** XRD analysis of purified *Es-Lec* showed one diffraction peak at 31.837$\mathring{2}$ which shows the purity and crystalline nature of protein. The lattice constant calculated from this pattern is 2.81085 Å which explains the crystalline nature of the purified lectin. **(B)** The HPLC analysis of purified *Es-Lec* showing a single peak with a retention time at 2.958 min which indicates the purity of the sample.

was equilibrated with an equal volume of TBS/$CaCl_2$ buffer. Followed by this equilibration, approximately 20 ml of the equilibrated sample was gently applied into a mannose coupled sepharose CL-4B column. Purified fractions were eluted with elution buffer (10 mM Tris HCl, 140 mM NaCl, 3 mM EDTA, pH 8.0) containing EDTA.

2.3. Characterization of purified Es-Lec

2.3.1. SDS-PAGE analysis

Polyacrylamide gel electrophoresis (10%) was performed on eluted fractions under reduced conditions as described by Laemmli [26]. After electrophoresis, the gel was stained with coomassie brilliant blue (GE Healthcare Bio-Sciences, India). The molecular mass of the purified *Es-Lec* was determined by comparison of its electrophoretic mobility with those of molecular mass marker proteins (Takara BIO INC, Japan). Total protein concentration was determined by Lowry's method [27] using bovine serum albumin (BSA) as standard.

2.3.2. Mass spectrum analysis, high performance liquid chromatography and X-ray diffraction analysis

The purified 75 kDa protein was excised from SDS-PAGE gel and subjected to trypsin digestion described by Sivakamavalli and Vaseeharan [44] with required modifications. In brief, the gel bands corresponding to *Es-Lec* were finely cut and added to 1.5 ml eppendorf tubes containing stain removal solution (100 mM ammoniumbicarbonate: 50% acetonitrile (1:1)) vortexed for 30 min. After complete removal of stain, the gel pieces were dehydrated with 100% acetonitrile (ACN). Then, the gel pieces were subjected to reduction and alkylation. Trypsin solution was formulated in 40 mM NH_4HCO_3 and added to each eppendorf containing gel pieces and stored at ice cold condition for absorption of trypsin. After absorption, NH_4HCO_3 was added and incubated at 37 °C for 12–16 h. The digestion was stopped by adding 4% formic acid. The peptides were extracted by washing gel pieces 3 times with 4% FA in 50% ACN. The resultant peptides were analyzed by MALDI-TOF/TOF MS (Shimadzu, Biotek Axima Performance).

HPLC separation was carried out using a reversed phase C18 column (7.8 mm × 30 cm) previously equilibrated with TBS-I at a flow rate of 0.8 ml min^{-1}. HPLC system (Zorbax Bio-series GF-250, Du Pont, Willington, DE, USA), was used for the homogeneity analysis. In order to determine the spatial distribution of atomic coordinates and the arrangement of atoms, an XRD analysis (XRD, Scintag-SDS 2000) was performed on purified *Es-Lec* at 40 kV/20 mA, using continuous scanning 2θ mode. The average grain size and shape of the purified *Es-Lec* was determined using Scherrer's formula [$d = (0.9\lambda/\beta \cos\theta)$, where d

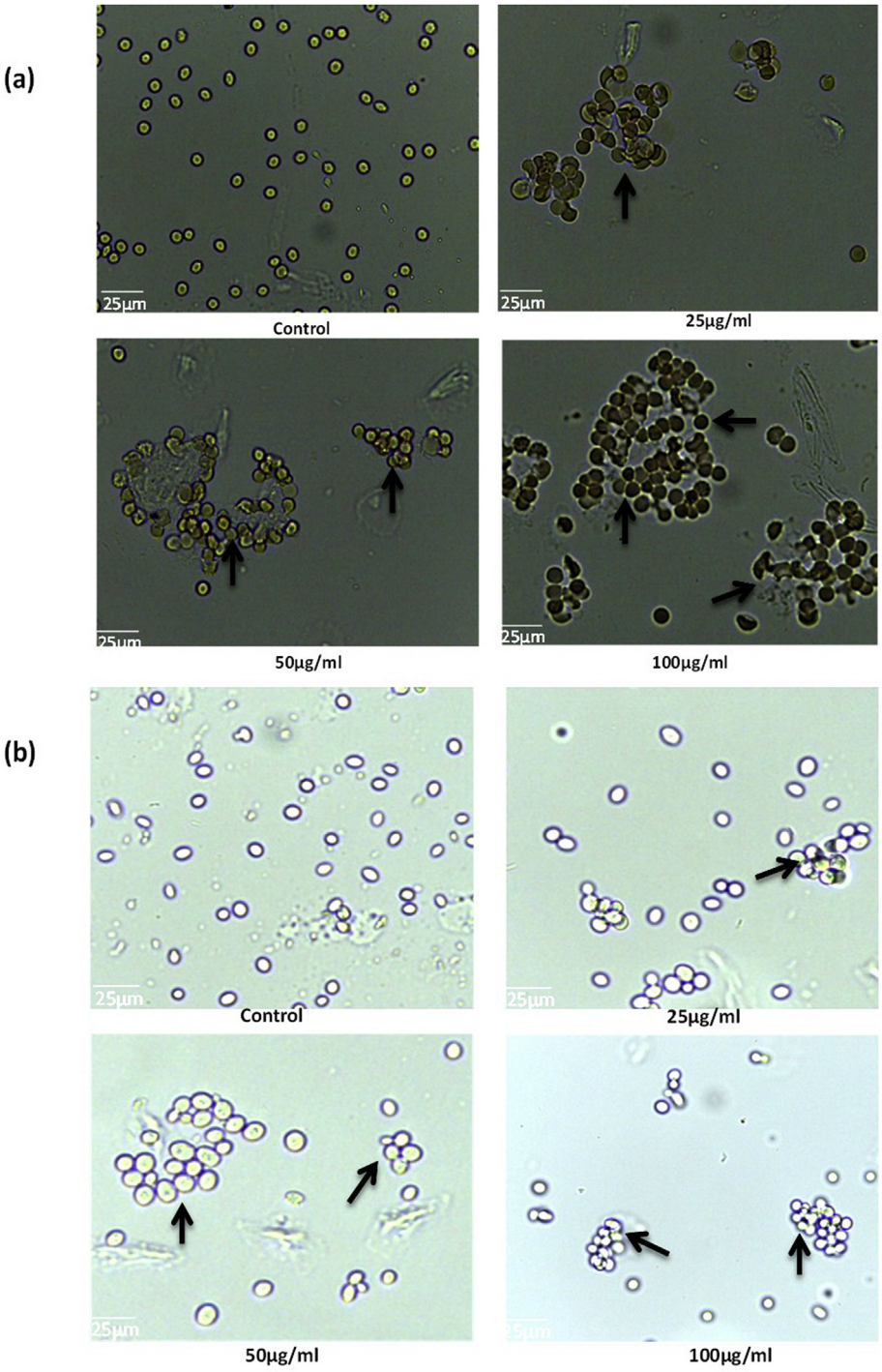

Fig. 4. (a) Haemagglutination assay with against 2% human RBC suspension and **(b)** yeast agglutination assay of *Es-Lec* at different concentrations. Highest concentration (100 µg/ml) showed maximum agglutination. Black arrows indicate the agglutination.

is the mean diameter of purified *Es-Lec*, λ is the wavelength of the X-ray radiation source, and β is the angular FWHM of the XRD peak at the diffraction angle (θ).

2.4. Functional analysis

2.4.1. Haemagglutination assay

The haemagglutination assay (HA) was performed in microtiter plates according to Correia and Coelho [25]. Lectin preparations (50 µl) were serially two fold diluted in PBS before addition of 50 µl 2% (v/v) suspension of human erythrocytes. In control well, lectin was replaced by BSA. The titer was expressed as the highest dilution exhibiting haemagglutination. The HA was defined as the inverse of the last dilution at which the sample still showed haemagglutination. Specific HA (SHA) was defined as the ratio between the titer and protein concentration (milligrams per milliliter). The haemagglutinated titres of *Es-Lec* were visualized by light microscopy at the magnification of 40X (Leica DMIL).

2.4.2. Effect of different temperatures and Ca^{2+} on lectin HA

Thermal stability was evaluated by keeping the inhibitory assay set up (*Es-Lec* and 2% erythrocyte suspension) at various temperatures

Table 1
Minimal inhibitory concentration corresponds to the lowest carbohydrate concentration able to neutralize hemagglutinating activity of *Es-Lec*. The values are expressed in millimolar and the highest carbohydrate concentration used was 250 mM. *N*-acetyl glucosamine, mannose and glucose could inhibit the haemagglutination at a very low concentration.

Sugar	Maximum Concentration of sugar (mM)	Minimum inhibitory Concentration of sugar (mM)
2-Deoxy D-glucose	250	0.24
Maltose	250	0.24
N-Acetyl glucosamine	250	0.06
Mannose	250	0.06
Glucose	250	0.06
Fucose	250	–
Rhamnose	250	–
N-Acetyl galactosamine	250	–
Methyl α D galacto pyranoside	250	–
Sucrose	250	–
Arabinose	250	–
Xylose	250	–
Galactose	250	–
Ribose	250	–
Lactose	250	–
Raffinose	250	–

(4 °C, 25 °C, 37 °C) for 30 min. To determine Ca^{2+} dependence, *Es-Lec* was incubated overnight in TBS (pH 8.0) containing 25 mM ethylene diamine tetraacetic acid (EDTA). Aliquots of the lectin solution were incubated with different concentrations of Ca^{2+} (5, 10, 15, 20 mM) in TBS, placed in 96-well microtiter plates, and incubated overnight at room temperature. HA was measured for all experiments using 50 µl of a 2% (v/v) suspension of gluteraldehyde-treated human erythrocytes.

2.4.3. Carbohydrate-binding specificity

The evaluation of hemagglutinating activity inhibition used *Es-Lec* and carbohydrates (*N*-Acetyl glucosamine, maltose, *N*-Acetyl galactosamine, 2-Deoxy D-glucose, rhamnose, mannose, glucose, methyl α D galacto pyranoside, sucrose, arabinose, xylose, galactose, ribose, lactose, raffinose). The assay was performed according to Correia and Coelho [25] in 96-well microtiter plates. The inhibitory assays were similar to the hemagglutinating assay with the addition of the incubation step (60 min, at 25 °C) to provide a lectin–inhibitor interaction before erythrocyte addition. Minimal inhibitory concentrations were determined and corresponded to the lowest carbohydrate concentration able to neutralize hemagglutinating activity of *Es-Lec*. The highest carbohydrates concentrations used were 250 mM.

2.4.4. Yeast agglutination assay

To determine the ability of agglutination of *Es-Lec* to yeast cells (*Saccharomyces cerevisiae*), 50 µl of different concentrations of the purified *Es-Lec* was added to a U-shaped 96-well microtitre plate containing equal volume of Tris buffer. The same volume of a suspension of yeast (10^6 cells ml^{-1}) was then added to the wells and incubated for 4 h at 25 °C. In control, purified *Es-Lec* was replaced by BSA. The pattern of agglutination was monitored with an inverted light microscope (40X) (Leica DMIL).

2.4.5. Bacterial agglutination assay

The bacterial agglutination by purified *Es-Lec* against *V. parahaemolyticus* and *A. hydrophila* was evaluated. Different concentrations of the purified *Es-Lec* were added to a 96-well microtitre plate and an equal volume of Tris buffer was added. In control, purified *Es-Lec* was replaced by BSA. The same volume of a suspension of bacterial cells (10^6 cells ml^{-1}) was then added to the wells and incubated for 30 min and then the pattern of agglutination was monitored with an inverted light microscope (10X) (Leica DMIL).

2.4.6. Biofilm inhibition assay

The effect of purified *Es-Lec* on biofilm-forming Gram-negative bacteria (*Aeromonas hydrophila* and *Vibrio parahaemolyticus*) was tested using 24-well polystyrene plates containing glass pieces immersed in Luria Bertani broth inoculated with the bacterial suspension of 1% inoculum from overnight cultures (10^7 CFU ml^{-1}). Different concentrations of *Es-Lec* were introduced into the wells and incubated at 28° C for 48 h. In control, purified *Es-Lec* was replaced by BSA. For examining the biofilm, the media were discarded and weakly adherent cells were removed by thorough washing with deionized water and allowed to air dry before staining. The biofilm were stained with 400 ml of 0.4% crystal violet (w/v) for 10 min. Stained glass pieces were inspected under light microscopy at the magnification of 40X. Similar experiment was executed to analyze the biofilm inhibition in 3D view by confocal laser scanning microscopy (Carl Zeiss LSM 710 Germany) with 0.4% acridine orange (0.4 g in 100 ml).

2.4.7. Antiviral assay

The antiviral activity of *Es-Lec* against CyHV-2 was also assessed. In short, confluent monolayers in 96-well plates were pre-incubated with 20 µl of 100 times diluted virus suspension (100 PFU/100 µl) for 1 h at 25 °C. Then 0.2 ml of maintenance medium containing appropriate serially diluted concentrations of the test sample was added. Cell control and virus control were run simultaneously. After 72 h of incubation, the cells were observed for cytopathic effects. MTT assay was also performed with serially diluted Es-Lec. Previously, the possibility of cytotoxicity of purified *Es-Lec* against uninfected koicarp gill cell lines was ruled out by cytotoxicity assay.

2.4.8. Anticancer activity

To evaluate the anticancer activity of the *Es-Lec*, an MTT assay was performed against the MDA-MB-231 breast cancer cell lines. Cells were seeded onto 96-well plates at a density of 1×10^5 cells per well. In control, purified *Es-Lec* was replaced by BSA. The MTT assay was performed in triplicate for *Es-Lec* at different concentrations.

2.5. Statistical analysis

All experiments were performed in triplicates (n = 3). Data are presented as mean ± standard error mean (S.E.M.) of control and treated samples. The data were subjected to one way analysis of variance (ANOVA) and the significance of differences between means were calculated by Tukey's HSD test and the significance accepted at $P^{<} 0.05$.

3. Results

3.1. Purification of lectin from pearl spot

Lectin from serum of pearl spot revealed a single band of approximately 75 kDa in 10% SDS-PAGE under reducing and non-reducing conditions (Fig. 1).

3.2. Mass spectrum analysis, HPLC and X-ray diffraction analysis

MALDI-TOF/TOF peaks of peptides after trypsin digestion corresponding to the *Es-Lec* was obtained (Fig. 2). The XRD analysis of purified *Es-Lec* showed one diffraction peak at 31.8372̊ (Fig. 3a) which shows the purity and crystalline nature of protein. The lattice constant calculated from this pattern was 2.81085 Å which explains the crystalline nature of the purified lectin. A single distinct peak with a retention time of 2.958 min was detected in high performance liquid chromatography (HPLC) (Fig. 3b).

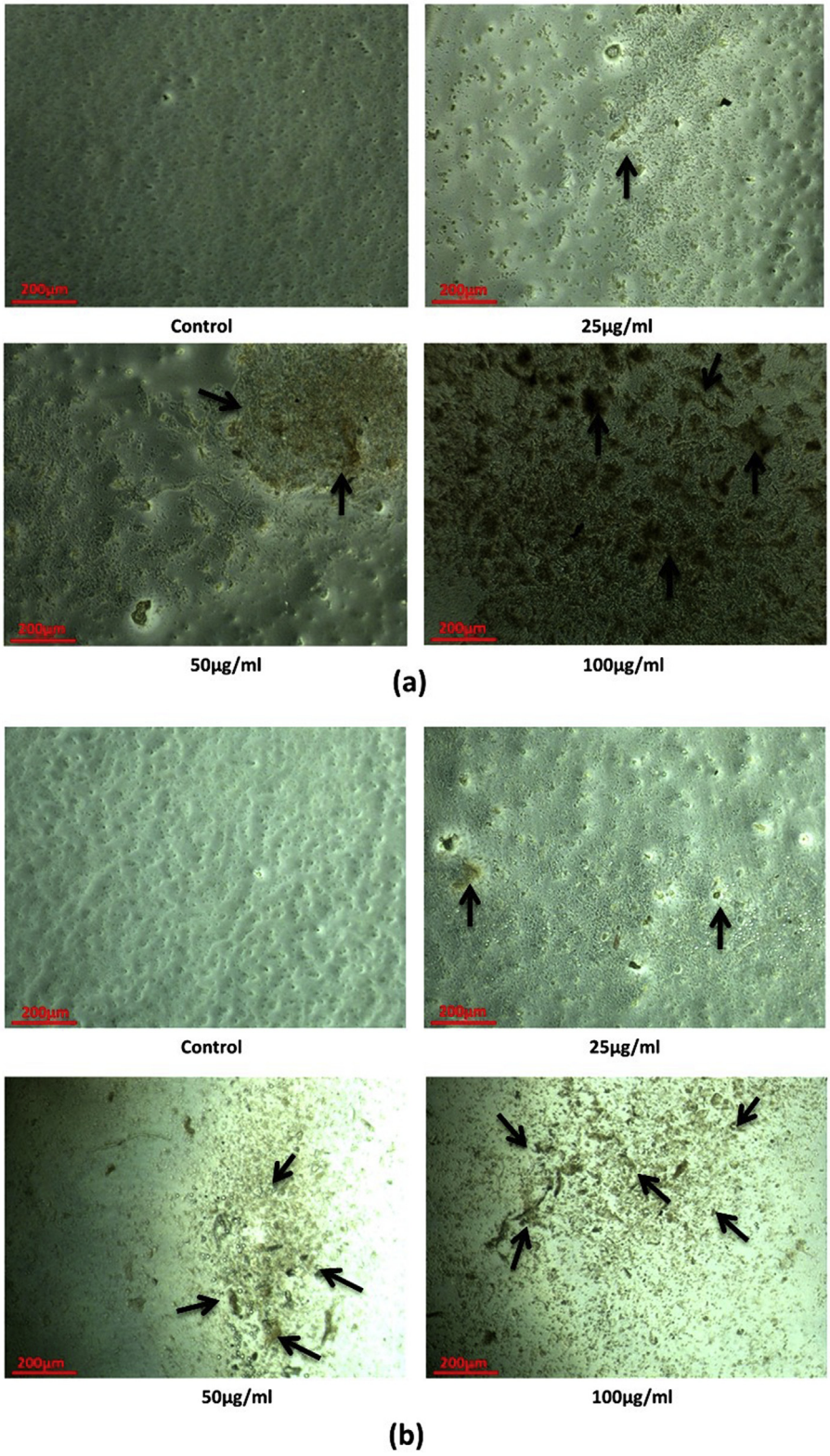

Fig. 5. Bacterial agglutination effect of *Es-Lec* against clinically important **(a)** Gram negative *A. hydrophila* and **(b)** Gram negative *V. parahaemolyticus* demonstrated by light microscopy. Higher concentration of lectin showed maximum agglutination activity. Black arrows indicate the agglutination.

3.3. Haemagglutination assay

Purified *Es-Lec* had the ability to agglutinate human erythrocytes, and agglutination was highest in 100 µg/ml of purified *Es-Lec vs.* the lower concentration (25 µg/ml). No agglutination occurred when purified *Es-Lec* was replaced by BSA (Fig. 4a). The maximum haemagglutination activity was observed at temperatures of 4°C and 25°C and 10 mM calcium ion concentration.

3.4. Carbohydrate-binding specificity

Out of the 15 carbohydrates tested, *N*-acetyl glucosamine, maltose,

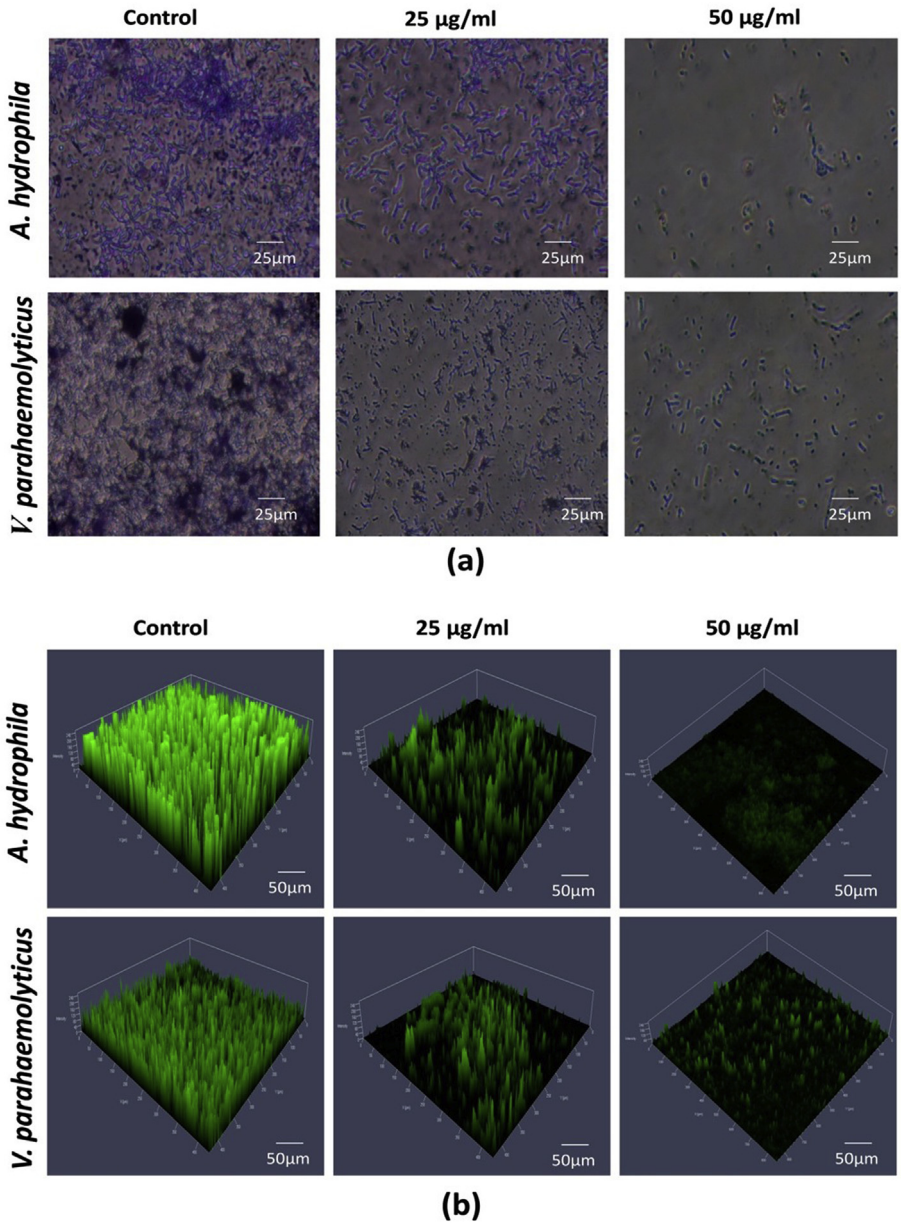

Fig. 6. Antibiofilm effect of *Es-Lec* on biofilm inhibition against clinically important Gram negative *A. hydrophila* and Gram negative *V. parahaemolyticus* with the stain of crystal violet demonstrated by light microscopy **(a)** and with the stain of acridine orange demonstrated by confocal microscopy **(b)**. Higher concentration of lectin showed maximum activity against biofilm formation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2-deoxy D-glucose, mannose and glucose showed inhibitory activity and among these glucose, *N*-acetyl glucosamine and mannose exhibited inhibition at a minimum concentration of 0.06 mM (Table 1).

3.5. Yeast agglutination assay

The purified *Es-Lec* showed ability to agglutinate *S. cerevisiae* and highest agglutination was revealed in 100 µg/ml of purified *Es-Lec* compared to the lowest concentration (25 µg/ml). No agglutination occurred when purified *Es-Lec* was replaced by BSA (Fig. 4b).

3.6. Bacterial agglutination assay

The purified *Es-Lec* could bind and agglutinate *A. hydrophila,* and *V. parahaemolyticus* and agglutination was evident as clumps of bacteria as revealed in 100 µg/ml of purified *Es-Lec vs*. the lowest concentration (25 µg/ml). No agglutination occurred when purified *Es-Lec* was replaced by BSA (Fig. 5a and b).

3.7. Biofilm inhibition assay

The effect of purified *Es-Lec* on biofilm-forming Gram-negative bacteria (*A. hydrophila,* and *V. parahaemolyticus*) was tested and it was evident that the antibiofilm activity of lectin increases by a dose-dependent manner. The *Es-Lec* is more effective against *A. hydrophila* compared to *V. parahaemolyticus* (Fig. 6a and b).

3.8. Antiviral assay

The activity of *Es-Lec* against the fish pathogen CyHV-2 virus was evaluated using koicarp gill cell lines. It was revealed that the lectin could bind to the virus so that the virus was unable to produce any

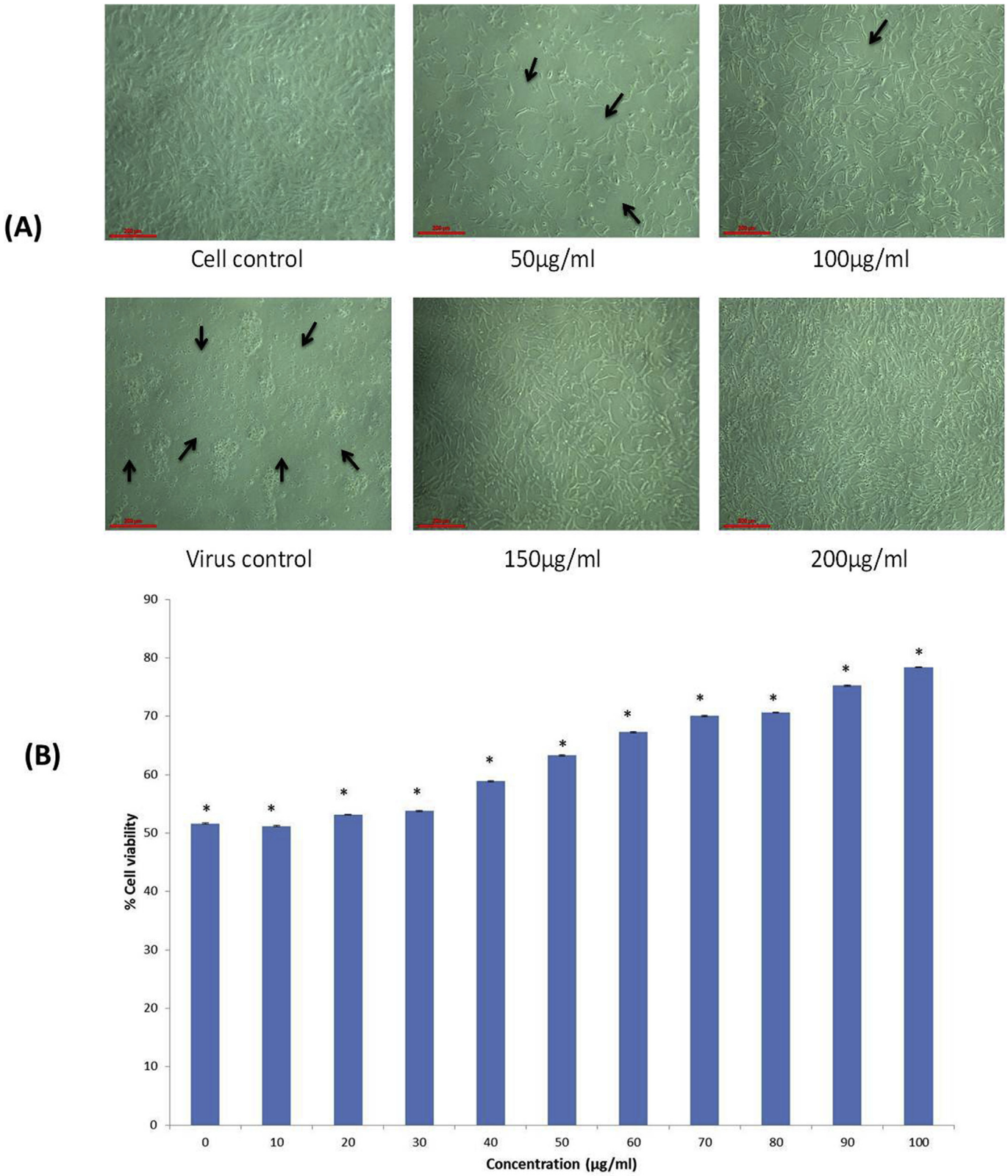

Fig. 7. The antiviral activity of *Es-Lec* on koicarp gill cell line against CyHV-2 virus. *Es-Lec* shows minimal cytopathic effects at a high concentration of 200 µg/ml of lectin when compared to CyHV-2 virus infected control. Black arrows indicate cytopathic effects (vacoulation) **(A).** MTT assay of serially diluted *Es-Lec* **(B).**

cytopathic effects in wells with highest concentration (100 µg/ml) even though they could exhibit some cytopathic effects in wells with lower concentrations (Fig. 7). The purified *Es-Lec* did not have any cytopathic effect on the koicarp (coloured varieties of common carp) gill cell lines studied.

3.9. Cytotoxicity

On increased concentrations, the purified *Es-Lec* exhibited an anti-cancer potential against the tested breast cancer cell line MDA-MB-231 (Fig. 8).

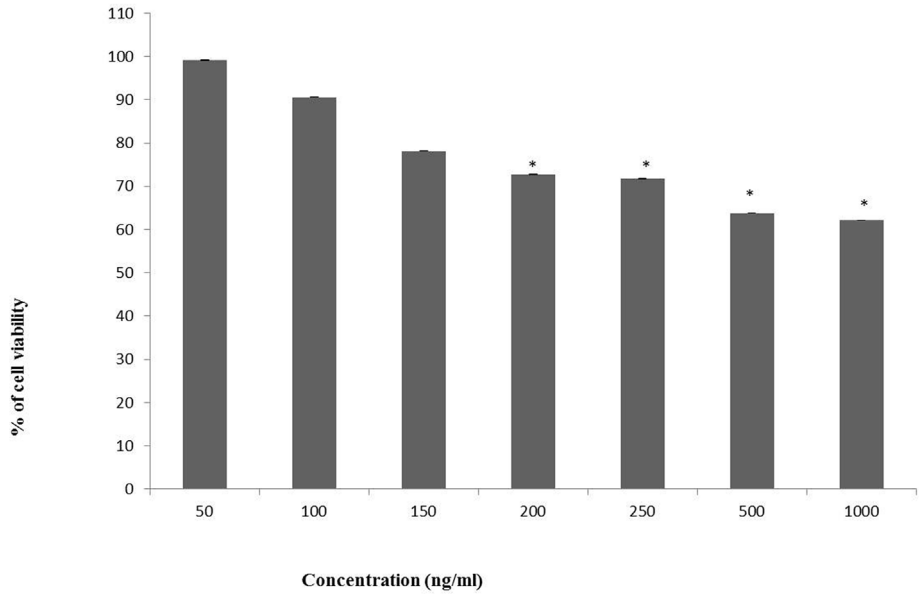

Fig. 8. The cytotoxic effect of *Es-Lec* at different concentrations against breast cancer cell lines MDA-MB-231. Data are presented as mean ± SEM.

4. Discussion

A lectin was purified to homogeneity from the serum of brackish water fish *E. suratensis* by mannose-sepharose affinity chromatography. The enhanced activity of lectin in presence of calcium ions indicates that it is a C-type lectin. Many types of lectins such as mannose binding lectin [25], galactose binding lectin [29], fucose binding lectin [30] pentraxins [31], serum amyloid protein [32], C reactive protein [33] are previously reported in fish sera. The TBS-Ca^{2+} buffer used to purify *Es-Lec* contained calcium which is necessary to bind the lectin to chromatography column and the elution buffer used to elute the lectin from the column contained EDTA which chelates calcium ions thus destabilizing the binding of lectin to the column. Thus, the present study is the first report of isolation and characterization of C-type lectin (*Es-Lec*) from the serum of pearl spot with an estimated molecular mass of 75 kDa. Furthermore, this *Es-Lec* exhibits remarkable antimicrobial activity with antiviral and antibiofilm potential. Previously, different types of fish lectins with a wide range of molecular mass from 17 kDa to 240 kDa with respect to subunit and multimeric molecular masses are already reported [28,30,34–36]. The MALDI-TOF, HPLC, and XRD analyses of *Es-Lec* revealed the purity and crystalline nature, as previously reported by Sivakamavalli and Vaseeharan [44], Jayanthi et al. [37] and Ishwarya et al. [38].

Increased agglutination against 2% erythrocyte suspension and yeast cells were observed with increased concentration of purified *Es-Lec* which suggests that the agglutination has occurred in a dose-dependent manner and lectin could bind to the exposed glycan on the cell surfaces. The result of carbohydrate binding assay reveals that the purified lectin had strong affinity towards glucose, *N*-acetyl glucosamine and mannose as they inhibited haemagglutination at 0.06 mM concentration, while maltose and 2-deoxy D-glucose inhibited haemagglutination at a concentration of 0.24 mM. Thus it can be inferred that *Es-Lec* can recognise primarily the glucose and mannose moieties present on pathogen surface. Similar carbohydrate binding and haemagglutination activity was reported by Silva et al. [39]; Mitra and Das [40].

Most pathogens have the ability to adhere and colonize themselves and to produce antibiofilm before they start infection. The antibiofilm assay results suggests that purified *Es-Lec* from pearl spot could inhibit the colonization and proliferation by the Gram-negative bacteria tested, by disturbing the biofilm architecture which is evident by light microscopy and confocal laser scanning microscopy. This explains the role of lectin in antimicrobial resistance as reported in lectin, *Pp-Lec* from blue swimmer crab (*Portunus pelagicus*) [37]. The *Es-Lec* also exhibited antiviral activity against CyHV-2 virus, a fish pathogenic virus by binding to them and thus inhibiting the virus from attacking the fish cell lines. Recently, Zhang et al. [41] reported the antiviral activity of chicken mannose binding lectin against infectious bronchitis virus and also the *Es-Lec* exhibited an anti-cancer potential against the MDA-MB-231 breast cancer cell lines. The anti-tumor activity of lactose binding molecules in eel skin mucus was previously reported by Kwak et al. [42] and the cytotoxicity of biopolymer on MDA-MB-231 breast cancer cell lines was published by Kumar et al. [43].

Through this study we report the purification of the immune related, C-type lectin from serum of pearl spot through mannose coupled sepharose CL-4B column and characterized by SDS-PAGE, MALDI-TOF, HPLC and XRD analyses. Functional analysis of the purified *Es-Lec* is carried out by different immune assays such as haemagglutination, yeast agglutination, antibacterial and antiviral assays. Even though lectins were reported to possess antibacterial activity, antibiofilm property is not well established in fish lectins. Thus, this is the first report of *Es-Lec* from pearl spot, we establish its strong antibiofilm potential, anti-viral and cytotoxic activity and the further future studies will provide a better understanding on the significance of lectins in innate immune system of pearl spot, and would be helpful to prevent and control of bacterial and viral diseases in fish.

In conclusion, purified *Es-Lec* showed molecular weight of 75 kDa, which was confirmed by SDS-PAGE under reduced conditions. Purity and crystalline nature of *Es-Lec* was further confirmed by HPLC and X-ray diffraction analysis, respectively. Immunological role of purified *Es-Lec* was confirmed by haemagglutination and yeast agglutination. A broad spectrum of antibacterial activity and anti-biofilm activity of purified *Es-Lec* was revealed against the Gram-negative bacteria's *V. parahaemolyticus* and *A. hydrophila*. *Es-Lec* was also active against CyHV-2 virus. Based on these findings, it is concluded that *Es-Lec* is involved in immune response of pearl spot, and gives an insight into pathogen associated pattern recognition mechanism that triggers the lectin pathway of complement activation.

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Appendix A. Supplementary data

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