Indian Journal of Geo Marine Sciences
Vol. 52 (08), August 2023, pp. 371-380
DOI: 10.56042/ijms.v52i08.11020

Antimicrobial activity of the crude peptide extracts from Blackfin sea catfish *Arius jella* Day, 1877

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Received 04 January 2022; revised 22 August 2023

Most Antimicrobial Peptides (AMPs) reported from fish possess antimicrobial activity. The study reports the antibacterial potential of the crude peptide extract from *Arius jella*, Blackfin sea catfish. The crude peptide extract was purified by a three-step process, namely acetic acid-acetone precipitation, Sep-pak®C-18 solid-phase purification, and cation exchange chromatography. The disc diffusion assay revealed that the crude extract obtained through the modified acetic acid-acetone precipitation method exhibited antimicrobial activity against both Gram-negative and Gram-positive bacteria in all tested cases. The Sep-Pak purification yielded 5, 40, and 80 % fractions of the peptide, and these fractions underwent cation exchange chromatography. The peptide fractions (purified) were further tested for antimicrobial activity by broth microdilution assay. The fractions Aj5-5, Aj40-4, and Aj80-2 showed the highest activity against *Bacillus cereus*; Aj5-5, Aj40-5, and Aj80-2 against *Vibrio alginolyticus*; and Aj5-1, Aj40-1, and Aj80-4 showed highest activity against *Staphylococcus aureus*. All the fractions tested were found to be potent against at least one of the pathogens tested. This is the first report of the isolation of AMPs from *Arius jella*. Further purification and characterization of the peptides could potentially unveil novel therapeutic agents to combat pathogens and would be a substantial contribution to the field of aquaculture and human health management.

[**Keywords**: Antimicrobial peptides, *Arius jella*, Crude peptide, Host defence peptides, Innate immunity]

Introduction

Antimicrobial peptides (AMPs) are integral components of the innate immune system and play a pivotal role in protecting the fishes from microbial intrusion. AMPs possess low molecular weight with both hydrophobic and hydrophilic side chains[1]. These side chains make AMPs soluble in the aquatic environment[2]. The most promising aspect of AMPs is their limited susceptibility to the development of resistance among microorganisms[3]. This feature is may be due to their distinguished mode of action. The primary mode of action for AMPs involve the plasma membrane, where they adopt their native three-dimensional amphiphilic structure, leading to the disruption of bacterial cells[3,4].

Antimicrobial peptides are broadly distributed in animals, encompassing both vertebrates and invertebrates[5-18]. Pleurocidin from winter flounder possesses activity against an extensive spectrum of both Gram-positive and Gram-negative bacteria[19]. Palteobagrin and grammistins are antimicrobial peptides derived from the secretions of the skin of *Pelteobagrus fulvidraco* and *Pogonoperca punctata*, respectively[20-21]. Paradaxin, is a toxic secretion from glands located at the base of the anal and dorsal fins of *Pardachirus marmoratus*[22]. They form pores on the plasma membrane, thereby disrupting the ionic transport of the epithelial osmoregulatory system[22-23]. In HeLa cells, cell death was induced by paradaxin, which explained the antitumor activity of the peptide[24]. Hepcidin from fishes and amphibians performs dual functions of an antimicrobial peptide and iron regulator[25]. Defensin from *Epinephelus coioides* possesses dual antiviral activity against Singapore Grouper iridovirus (SGIV) and Viral Nervous Necrosis Virus (VNNV)[26]. Cathelicidins were isolated from the rainbow trout and Atlantic salmon[27-28]. Two HLP, histone-like proteins from channel catfish, HLP-1 and 2 exhibited activity against *Saprolegnia parasitica*, a fish fungal pathogen.

AMPs from fishes are not confined to skin or gills. They were extracted from different body parts of the

fish. They were isolated from the mucus layer (secretion) of intestine[19,30], head, kidney[31], skin, gills[32-33], and epithelial mucosal layer[34].

Fish AMPs play an important role in first line of defence against a broad spectrum of pathogens, carrying significant implications. The widespread use of antibiotics in aquaculture has led to bacterial resistance issues. The potential of AMPs as an antibiotic alternative makes them a compelling tool, as they pose a limited risk of resistance development. They also show their activity in combating other pathogens like viruses. In addition to their antimicrobial role, fish AMPs exhibit various noteworthy but less-explored features and capabilities, including serving as antioxidants, active components in immunogenic drugs, and also as adjuvants. *Arius jella* Day, 1877, is primarily located in the coastal marine waters, estuaries, and tidal rivers. Its main diet consists predominantly of invertebrates. This species is also a significant food source, sustaining subsistence fisheries in both lagoons and estuaries.

The current study focuses on the isolation, purification, and detection of antimicrobial potential of the peptides from *Arius jella*, Blackfin sea catfish.

Materials and Methods

Sample collection

The fish sample *Arius jella* (Fig. 1), commonly known as Blackfin sea catfish, was used for this study. The fish was collected from Thevara, Cochin backwaters, Kerala. The collected fish had a length of 20 cm and 150 g of weight. The sample was preserved at -20 °C until the extraction of peptides.

Peptide (Crude) extraction

The acetic acid-acetone precipitation method[35], with minor modification, was employed for the extraction of the crude peptide. The samples were cut into small pieces, weighed and subjected to homogenization with 10 % acetic acid. Acetic acid was used at a ratio of 1:5 (w/v). The homogenized sample was left to incubate overnight at 4 °C. Following this, the homogenate was centrifuged at 13500 rpm for 30 min at 4 °C. Two volumes of ice-cold acetone were then added to the supernatant, and the mixture was stored overnight at 4 °C. Later, the centrifugation was done to collect the crude peptide precipitate at 13500 rpm (4 °C) for 30 min. Post-centrifugation, the supernatant was discarded, and the protein (crude) pellet was collected and stored at -80 °C until needed.

Fig. 1 — Photograph of experimental species *Arius jella*

Disc diffusion analysis

A disc diffusion assay[36] was done to test the antimicrobial activity of the crude peptide. The crude peptide collected was reconstituted in sterile MilliQ water at a concentration of 200 mg/mL. The reconstituted sample was loaded onto discs (Whatman No. 1) with 6 mm diameter. These sample-laden discs were placed on nutrient agar plates containing microbial strains (1×10^8 cells/ml). Two Gram-positive and nine Gram-negative bacterial cultures were selected for the test. They were *Escherichia coli* (MTCC 483), *Edwardsiella tarda* (MTCC 2400), *Pseudomonas aeruginosa* (MCCB 119), *Vibrio alginolyticus* (VKF44), *Vibrio harveyi* (MCCB 284), *Vibrio cholera* (MCCB 129), *Vibrio parahaemolyticus* (MCCB 133), *Vibrio proteolyticus* (M10W1), and *Vibrio vulnificus* (WV13) (Gram-negative); and *Bacillus cereus* (MCCB 101) and *Staphylococcus aureus* (MTCC 3061) (Gram-positive). The plates underwent a 30-min cooling period at 4 °C before being incubated overnight at 37 °C. The plates were examined for the presence of a zone of inhibition.

When the bacterial strain is susceptible to the antimicrobial agent, it results in the development of a zone of inhibition on the agar plate. Conversely, if the bacterial strain is resistant to the antimicrobial agent, no observable zone appears.

Solid phase extraction

The crude peptide underwent solid-phase extraction using Sep-Pak® C-18 cartridges (Waters). The sample were dissolved in trifluoroacetic acid (TFA-0.1 %), and the cartridges were prepped with the same solution for equilibration. The crude protein sample was loaded, and subsequently, a wash with 0.1 % trifluoroacetic acid (TFA) was performed. The analyte trapped in the cartridges was then eluted using 6 ml of 5, 40, and 80 % acetonitrile in 0.1 % TFA,

respectively. The collected fractions were subjected to lyophilisation and were stored at -80 °C.

Cation exchange chromatography

Further purification of the Sep-Pak® (lyophilized) fractions - 5, 40 and 80 % was done by cation exchange chromatography. Cation exchange chromatography was conducted utilizing the Duo-Flow-Fast Protein Liquid Chromatography (FPLC) system from Bio-Rad. The purification column employed for this process was the UNOTM QI (Q1 BioRad). Two solvents were employed as the mobile phase: Solution A, consisting of 25 mM Tris-HCl, and Solution B, composed of 1 M NaCl in 25 mM Tris-HCl. The lyophilized fractions were reconstituted in HPLC-grade water. At first, the equilibration of both the column and baseline was performed using Solution A. Following the equilibration, sample loading was carried out with a set solvent flow rate of 2 mL/min. A linear gradient elution was executed, ranging from 0 to 50 % of Solution B. The chromatography was monitored at four different wavelengths *viz*. 215, 225, 260, and 280 nm using Quad Tec detector (Bio-Rad). Each peak was individually collected as distinct fractions using a fraction collector. Subsequently, the collected fractions underwent lyophilisation and were stored at -80 °C.

Antimicrobial activity screening

The lyophilized fractions were subjected to testing for antimicrobial activity using broth microdilution assay[37] with slight modifications. The microdilution assay was conducted in 96-well microtiter plates. Bacteria in a mid-logarithmic phase were diluted to a concentration of 10^4 CFU/ml in 50 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) buffer at pH 6.8. Microtiter wells were loaded with 10 µl of FPLC fractions and 10 µl of the diluted bacterial suspension, followed by incubation at room temperature for two hours. HEPES buffer was used as the blank in the experiment. For the assay, a set of negative and positive controls were included. The negative control consisted of 10 µl of bacterial suspension and 10 µl of 50 mM Tris-HCl. The positive control comprised 10 µl of bacterial suspension along with antibiotics (1 mg/mL) such as Ampicillin and Chloramphenicol. Following 2-hour incubation at room temperature, 80 µl of Mueller-Hinton (MH) broth was added to each well. Subsequently, absorbance was measured at a wavelength of 600 nm using a microplate reader (Tecan, USA) after further incubation at 37 °C for an additional 16 h. The percentage of inhibition was calculated as *Inhibition % = 100 - Growth %*; where, *Growth % = (OD of Test / OD of Control) × 100*. The percentage of inhibition was determined by calculating the average from three independent experiments, each conducted in triplicate.

Results

Disc diffusion analysis

Initial antimicrobial potential of the crude peptide from *A. jella* was performed by disc diffusion assay. The activity was tested against 11 microorganisms *i.e.*, two Gram-positive and nine Gram-negative strains. Maximum activity was observed against *B. cereus* (3.1 mm), followed by *S. aureus* (2.9 mm), *V. alginolyticus* (2.3 mm) and *P. aeruginosa* (2.2 mm). Among the tested strains, the peptide exhibited lowest activity against *V. vulnificus* (0.7 mm) (Table 1).

Cation exchange chromatography

Crude peptide fractions (5, 40, 80 %) obtained by Sep-Pak were further purified by cation exchange chromatography using FPLC. The 5 % Sep-Pak fraction produced six fractions designated as Aj5-1, Aj5-2, Aj5-3, Aj5-4, Aj5-5, and Aj5-6 (Fig. 2a). Additionally, FPLC of the 40 % Sep-Pak fraction resulted in five fractions labelled Aj40-1, Aj40-2, Aj40-3, Aj40-4, and Aj40-5 (Fig. 2b). Furthermore, the FPLC of the 80 % Sep-Pak fraction yielded three fractions namely Aj80-1, Aj80-2, and Aj80-3 (Fig. 2c).

Broth microdilution assay

The fractions Aj5-5 (96.5 %), Aj40-4 (95.05 %) and Aj-80-2 (95.24 %) exhibited notable activity

Table 1 — Antimicrobial activity by disc-diffusion assay of crude peptide from *A. jella*

Sl. No.	Bacteria	Zone of inhibition in mm
1.	*Pseudomonas aeruginosa*	2.2
2.	*Escherichia coli*	1
3.	*Edwardsiella tarda*	1.5
4.	*Vibrio harveyi*	1.2
5.	*Vibrio alginolyticus*	2.3
6.	*Vibrio proteolyticus*	1.1
7.	*Vibrio vulnificus*	0.7
8.	*Vibrio cholera*	0.9
9.	*Vibrio parahaemolyticus*	1.1
10.	*Bacillus cereus*	3.1
11.	*Staphylococcus aureus*	2.9

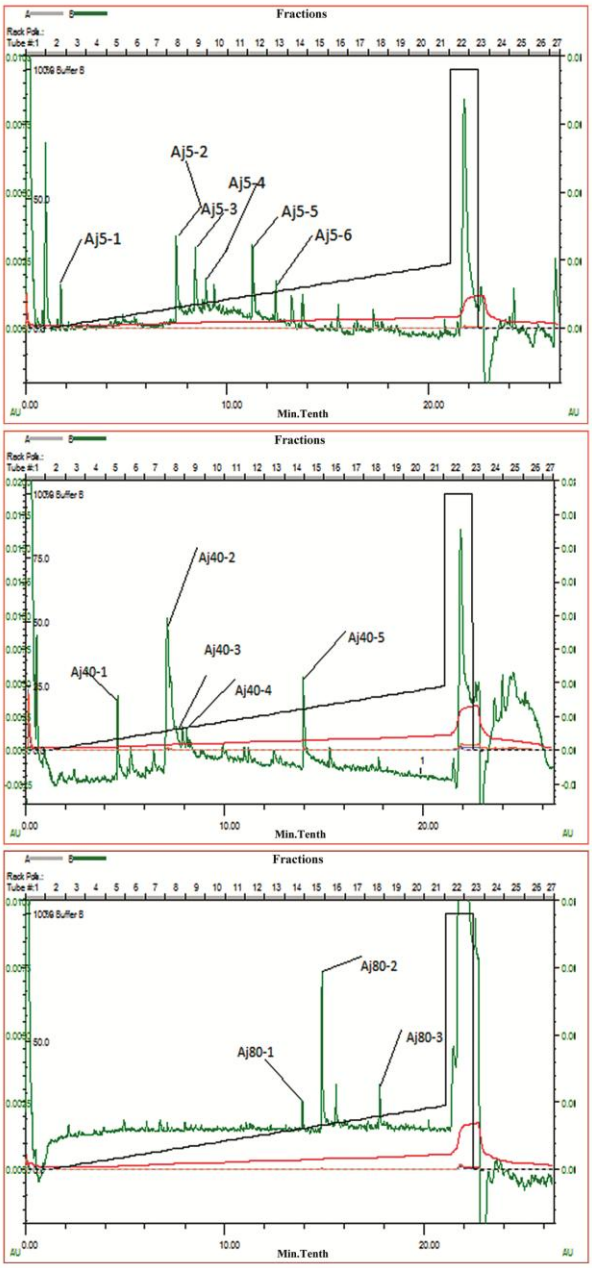

Fig. 2 — Chromatogram for *A. jella* peptide extract using FPLC: a) 5 % Sep-Pak fraction, b) 40 % Sep-Pak fraction, and c) 80 % Sep-Pak fraction

against *B. cereus*. In the case of *V. alginolyticus*, Aj5-5 (97.37 %), Aj40-5 (97.63 %), Aj80-2 (96.61 %) showed inhibition. Aj5-1 (68.25 %), Aj40-1 (66.67 %) and Aj80-1 (68.82 %) showed higher inhibition against *S. aureus* (Tables 2 – 4).

Discussion

Antimicrobial Peptides (AMPs) are anticipated to function as integral components of the initial host defence mechanisms. They are identified from plants to animals and insects to mammals[38-41]. Pathogenic bacteria were developing resistance to conventional antibiotics and this calls a higher demand for identification and purification of antimicrobial peptides with different modes of action[42]. Identification of peptides with antimicrobial potential provides a better understanding of the immune system of organisms. This approach leads to the development of new pharmaceutical drugs and better monitoring of the aquaculture industry[43]. The current study deals with the extraction of peptides with antimicrobial potential from Blackfin sea catfish *Arius jella*.

Bioactive peptides derived from marine fishes showcase diverse biological activities, including antimicrobial, antioxidant, immunostimulation and cancer metastasis inhibition[44-45]. The production of AMPs depends on their sensitivity to pH, temperature, and enzymes and they are easily denatured during the production process[46]. Many studies favour the direct extraction and batch fermentation for the production of AMPs. Different methods have been used for isolation, purification and characterization of AMPs[47]. The methods include precipitation alone[48-50], precipitation and RP-HPLC[51], precipitation, gel permeation and reverse phase HPLC[52], precipitation and HPLC[13], and precipitation and ion exchange chromatography[53]. Many studies on AMPs were carried out in their crude form[49,54]. Misgurin, an AMP purified and characterized from loach, *Misgurnus anguillicaudatus* was purified to homogeneity in three steps, *i.e.* by heparin affinity chromatography, reverse phase (RP)-HPLC and gel permeation HPLC[55]. Misgurin demonstrated activity against a broad spectrum of both Gram-positive and Gram-negative bacteria. Crude peptides from *A. jella* were extracted and characterized by three-step protocol involving acetic acid-acetone precipitation, solid phase extraction by Sep-Pak column and cation exchange chromatography using FPLC system, and the peptide fractions exhibited considerable antimicrobial activity. Park *et al.*[55] found misgurin was approximately six times more efficient than magainin 2 from *Xenopus laevis*. Further, purification and characterization of crude extract by HPLC, MALDI, NMR, X-ray crystallography could reveal more purified and characterized AMPs from *A. jella*.

AMPs derived from the haemoglobin of *Ictalurus punctatus* were effective against parasitic (*Ichthyophthirius multifiliis*, ich) infection[56]. In this

Table 2 — Inhibition by 5 % FPLC fractions of crude peptide from *A. jella* by broth microdilution assay

Sl. No.	FPLC fractions of 5 % Sep-pak purified peptide	% of inhibition of *V. alginolyticus*	% of inhibition of *B. cereus*	% of inhibition of *S. aureus*
1	Aj5-1	-72.83	49.13	68.25
2	Aj5-2	10.2	29.73	65.03
3	Aj5-3	93.82	86.35	64.05
4	Aj5-4	64.4	-28.24	64.08
5	Aj5-5	97.37	96.5	62.56
6	Aj5-6	93.91	85.08	61.07

Table 3 — Inhibition by 40 % FPLC fractions of crude peptide from *A. jella* by broth microdilution assay

Sl. No.	FPLC fractions of 40 % Sep-pak purified peptide	% of inhibition of *V. alginolyticus*	% of inhibition of *B. cereus*	% of inhibition of *S. aureus*
1	Aj40-1	25.25	-19.62	66.67
2	Aj40-2	90.61	30.87	66.07
3	Aj40-3	84.01	41.34	59.58
4	Aj40-4	96.36	95.05	55.47
5	Aj40-5	97.63	87.53	61.36

Table 4 — Inhibition by 80 % FPLC fractions of crude peptide from *A. jella* by broth microdilution assay

Sl. No	FPLC fractions of 80 % Sep-pak purified peptide	% of inhibition of *V. alginolyticus*	% of inhibition of *B. cereus*	% of inhibition of *S. aureus*
1	Aj80-1	79.1	45	68.82
2	Aj80-2	96.61	95.24	66.77
3	Aj80-3	63.21	-45.3	64.27

experiment, only three peaks from RP-HPLC were purified, and suggested that many peaks might possess strong antibacterial or antiparasitic activity. Kumar *et al*.[57] tested the antimicrobial activity of different tissues such as gills, skin, blood, liver, kidney, intestine, and ovary of *Channa striata*. Totally 48 fractions were collected by solid-phase extraction, and antimicrobial activity was tested against both Gram-positive and negative bacteria like *S. aureus*, *B. cereus*, *E. coli*, *Klebsiella pneumonia*, etc. Extracts from gill and blood exhibited more activity than other tissue fractions. The antimicrobial activity of *C. striata* epidermal mucus was also documented by Wei *et al*.[58] and Ebran *et al*.[38]. The whole body of *A. jella* was used for crude peptide extraction. The above-mentioned reports confirm the distribution of AMPs in different parts of the fish body. Hence, AMPs from gills, blood, skin, intestine, muscle tissues, and kidney of *A. jella* could be extracted through purification procedures. The extracts from herring skin and internal organs reported by Pampanin *et al*.[59] were a potential reservoir of small molecular weight peptides with higher bioactive properties. Different tissues from salmon were found to prevent bacterial growth and possessed radical scavenging activity[60]. Piscidin 4 from hybrid striped bass tissues also exhibited antibacterial activity[61]. Kumar *et al*.[62] reported a small molecular weight peptide with antioxidant properties. The peptide was isolated from horse mackerel viscera protein. Potential protein hydrolysate was obtained through in vitro gastrointestinal digestion using the FPLC method. The antioxidant peptide efficiently worked as a free radical scavenger and inhibited lipid peroxidation. This helps the organisms from oxidative damage. A peptide with antioxidant activity was also reported from the yellow fin sole[63]. However, further research is needed to fully understand the antioxidant properties of *A. jella* AMPs.

The majority of the peptide fractions exhibited activity against the pathogens. Growth inhibition of *S. aureus* was observed by all the fractions. Among 5 % Aj fractions, the most potent fractions were Aj5-5, Aj5-6 and Aj5-3. Among all the 40 % fractions, Aj40-5 and Aj40-4 showed maximum activity against all the pathogens. Aj80-2 was the fraction more active against *S. aureus*, *B. cereus*, and *V. alginolyticus* than the other two 80 % Aj fractions. It is hypothesized that certain compounds contributing to the microorganism's growth promotion may be present in the 5 % and 80 % FPLC fractions. The absence of inhibition, which was noted in the initial disc

diffusion assay, may be attributed to the synergistic effect of these compounds when combined with the 40 % FPLC fractions present in the crude peptide sample[53].

The initial crude peptide sample encompassed active compounds from various fractions. In the liquid growth inhibition assay, certain purified fractions (5, 40, and 80 % FPLC fractions) were identified to demonstrate growth-promoting activities against *V. alginolyticus* (Aj5-1) and *B. cereus* (Aj5-4, Aj40-1, Aj80-3). It is hypothesized that the combined synergistic effect of growth-inhibiting and growth-promoting properties within the diverse fractions of the crude peptide sample against these strains contributed to the absence of inhibition observed in the initial disc diffusion assay.

Fish mucus is considered as an important component in the innate immune response. The mucus of fishes was used as a medicine for centuries[47]. Piscidins (pleurocidins, dicentracins, moronecidins, chrysopsins) were reported as the conventional AMPs found in fish mucus[19,64,65]. Piscidins were normally present at sites of microbial invasion such as skin, gills and gastrointestinal tract[66]. Hellio *et al*.[67] studied the antibacterial and antifungal activity of fish epidermal mucus and epidermis. The mucus extract of *Periophthalmodon schlosseri* exhibited antibacterial activity against eight human pathogens (*K. pneumoniae*, *Bacillus anthracis*, *Proteus mirabilis*, *E. coli*, *Salmonella typhi*, *S. aureus*, *P. aeruginosa*, and *V. cholerae*) and four fungal strains (*Candida albicans*, *Aspergillus flavus*, *Trichoderma longibrachiatum* and *Mucor* sp.)[68]. Manikantan *et al*.[69] also studied the activity of the skin mucus of *Epinephelus tauvina.* This was active against human pathogens (*S. typhi*, *E. coli*, *P. mirabilis*, *K. pneumoniae*, and *S. aureus*) and fish pathogens (*V. parahaemolyticus*, *Pseudomonas fluorescens*, *A. hydrophila*, *V. alginolyticus*, and *V. harveyi*). The hagfish is considered one of the most primitive groups of fishes in terms of evolution. Potential components from the epidermal mucus of hagfish were extracted through acid extraction, partially purified using Sep-Pak C18 cartridges, and further fractionated using liquid chromatographic methods. The fractions from Sep-Pak showed activity against *S. enterica* and *E. coli*[51]. The antibacterial activity observed in fish mucus is likely attributed to the presence of antibacterial glycoproteins. These glycoproteins may exert their antibacterial effect by creating large pores in the target membrane of bacteria[38]. The mucus extract from *Puntius sophore* exhibited noteworthy effectiveness in inhibiting bacterial biofilms. Moreover, the extract demonstrated the ability to disrupt preformed biofilms and hinder the adhesion properties of the tested strains, including *E. coli*, *B. subtilis*, *S. aureus*, and *P. aeruginosa*. The results suggest that the mucus from *P. sophore* possesses significant potential as an antibacterial and antibiofilm agent, offering an alternative to conventional antibiotics or other pharmaceutical agents[18].

The disc diffusion assay of *A. jella* crude peptide fraction after acetic acid-acetone precipitation showed potential activity against all the tested pathogens. All the *Vibrio* strains *viz*., *V. cholera*, *V. alginolyticus*, *V. proteolyticus*, *V. harveyi*, *V. vulnificus* and *V. parahaemolyticus* displayed inhibition towards the crude Aj extract. The AMPs found in the epidermal mucus of *A. jella* contribute to its antimicrobial activity. Even though fish mucus has antimicrobial properties, there hasn't been much attention paid in terms of human health-related research[70].

Order Siluriformes to which catfishes belong is the most extensively studied order for antimicrobial activity in its epidermal mucus[71]. Two freshwater catfishes *viz*., *Clarias batrachus* and *Clarias gariepinus* showed wide spectrum of antibacterial activity against both fish and human pathogens[48,49,72]. Bragadeeswaran *et al*.[73] noted that the aqueous mucus extract of *Arius caelatus* inhibited *V. cholerae*, *S. typhi*, *V. parahaemolyticus* and *S. aureus.* Also, the aqueous mucus extract of *Arius maculatus* exhibited activity against a broad range of Gram-positive as well as Gram-negative pathogens[74,75]. Anbuchezhian *et al*.[75] compared the activity of *Mystus gulio* and *Arius maculatus.* Antimicrobial peptides from epidermal mucus of *M. gulio* expressed antibacterial activity than antimicrobial peptides from *A. maculatus*, where peptides from *A. maculatus* showed higher antifungal activity than *M. gulio* peptides. Palteobagrin isolated from yellow catfish exhibited a broad range of antibacterial activity without haemolytic activity[20]. *Clarias gariepinus*, a catfish, possess wound healing capacity in slime coat[76]. The mucus from *Clarias* sp. possess anti-tumour agent[77] and also aids in healing burns[78]. Apart from the above functions, they were reported as antibacterial and antifungal agents[48,72,76,79].

The combination of drugs always has advantages over single agents. The combination of mucus extract

of *P. sophore* and gentamicin (an antibiotic) showed synergistic activity against various microorganisms[18]. To find the effect of drug amalgamation with AMPs, further investigation is required. Apart from the above-mentioned functions of fish-derived peptides, they are used for the development of cosmeceutical products. Chemical or enzymatic hydrolysis of peptides from marine fishes was termed as a safer option for such products[80]. Stabilium Protizen/ Procalm from protein hydrolysate of fish serve as anxiolytic and seacure, a compound from fish hydrolysate protein good for intestinal health has the potential as nutraceuticals. These are some of the examples of commercially used bioactive components from fishes[81].

Antimicrobial peptides typically focus on and attach to bacterial membranes through interactions between peptides and lipids. Numerous AMPs eliminate bacteria by inducing membrane disruption and causing leakage of bacterial contents. Leakage assays involving model membranes support this mechanism. Additionally, certain AMPs exert their antibacterial effects by interacting with DNA or RNA, disrupting processes such as synthesis, replication, and translation[82,83]. The utilization of membrane dyes and fluorescence spectrophotometer plate readers allows for the investigation of how AMPs affect the integrity of specific layers within bacterial membranes when applied to bacterial cells. Viable bacteria exhibit polarized cell membranes characterized by a negative transmembrane potential. Certain cationic AMPs induce bacterial death by depolarizing their membranes[84].

The current study did not address the mode of action, and also could not isolate the specific antimicrobial peptide from the crude extract of *Arius jella*. Therefore, future research involving the identification of the exact antimicrobial peptide and investigating its mode of action, including whether it is membrane-based or involves nucleic acid binding is invited.

Conclusion

Fishes have been confirmed as an abundant source of potent antimicrobial peptides. This research was focused on the antimicrobial potential of crude peptide fractions of Blackfin sea catfish *Arius jella*. The results revealed that the *A. jella* can potentially serve as a source of bioactive peptides effective against specific human and fish pathogens. The further purification and characterization of antimicrobial peptides from *A. jella* may lead to the exploration of new molecules with specific functions. Future investigations on specific AMPs from *A. jella* would be promising in aquaculture and human health-related fields.

Acknowledgements

The authors are grateful to the Director, Centre for Marine Living Resources and Ecology (CMLRE), and Ministry of Earth Sciences (MoES), Govt. of India for the research grant (MoES/10-MLR/01/2012) and scientific support. Authors also thank Director, National Centre for Aquatic and Animal Health (NCAAH) for scientific/ technical support. The first author gratefully acknowledges DST (Department of Science and Technology), Govt. of India, for the award of a fellowship.

Conflict of Interest

Authors of the manuscript declare that they have no conflict of interest.

Ethical Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Author Contributions

MVA carried out the experiment with the support from CRR, KA, PPA & AA. MVA wrote the manuscript. MVA & KA corrected the manuscript.

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