RESEARCH PAPER

In Silico Identification of Putative Fruit Crop-Derived Antibacterial Peptides Against *Aeromonas hydrophila*

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ABSTRACT

Aquaculture is a fish-producing subsector of agriculture that has a significant role in India's economy and livelihood. The major threat to fish production is the outbreak of bacterial infections that creates severe losses every year. *Aeromonas hydrophila* is an opportunistic invader of human and aquatic animals, ubiquitously present in the aquatic environment. Antibiotics are the common control measure, but their indiscriminate use has resulted in the development of resistance by the pathogen. As an alternative to antibiotics, Antimicrobial Peptides (AMPs) are used nowadays to tackle antimicrobial resistance. This study aims to identify the putative plant-derived antibacterial peptides as a valid drug candidate against multidrug-resistant bacteria, *A. hydrophila*, using bioinformatic approaches. Out of 10953 AMP sequences retrieved from Mendeley covering different fruit crops, 764 and 326 sequences were detected with antimicrobial and antibacterial activity, respectively. They were shortlisted to 11 antibacterial peptides (ABPs) based on non-toxic, non-allergenic, non-hemolytic and bioactive stable proteins. The protein-peptide interaction study revealed that all ABPs have positive binding affinity towards the virulent factors of *Aeromonas*. Hence, the predicted ABPs derived from fruit crops have the bactericidal effect and further wet-lab experiments are required to verify the findings.

Keywords: Antibacterial peptide, fruit-derived peptides, in-silico analysis, *Aeromonas*

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INTRODUCTION

Aquaculture is a fish-producing subsector of agriculture that has a significant role in India's economy and livelihood. India ranks second in global fish production, next to China, producing 9.4 million tonnes of food fish in 2021[1]. The bulk of fish production is inland aquaculture, carps and other freshwater fishes, which holds around 87% share of aquaculture production. The high stocking density of fish culture environments forces aquatic organisms to be vulnerable to pathogens[2], particularly bacteria, which leads to severe productive losses[3]. Vulnerability to pathogens and the development of resistance or multi-resistance by the pathogen against drugs are one, among the factors that influence the production. In addition, high-density intensive aquaculture suppresses the immune system of fish, thereby increasing the chance of disease incidence and transmission. *Aeromoniasis* is a highly infectious bacterial disease caused by the rod-shaped gram-negative bacterium *Aeromonas hydrophila*, which commonly infects carps[4]. Infection is primarily found in tropical and semitropical countries and, to some extent, in temperate countries[5]. It is a facultative anaerobic bacterium in freshwater habitats that infects and causes a high mortality rate of aquatic animals[6]. It creates abscesses and ulcers in the liver, kidney and spleen and hemorrhages in the gills and anus[7]. Furthermore, they are an important threat to humans, causing diarrhea[8], respiratory tract infection, gastroenteritis[9], cellulitis[10], sepsis[11], food poisoning, necrotizing fasciitis[12], various diseases and secondary infections[13], as they were able to transmit from infected fish and contaminated water[14].

A. hydrophila possesses multifactorial pathogenicity, viz., secretion of adhesion molecules, cytotoxin, lipases, proteases, hemolysin, biofilm formation[15], slime formation, production of aerolysin, enterotoxins, cytosine and gelatinase[16]. The hemolytic toxins play a significant role in its pathogenicity establishment[17]. Particularly, aerolysin and hemolysin produced from *A. hydrophila* cause hemolysis, gastroenteritis and diarrhea[18], which

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were used in this study. Aerolysin, a hemolytic pore-forming enterotoxin, possess cytotoxic activity[17,19]. It is formed once the C-terminal of inactive proaerolysin is activated by the protease enzyme. Aerolysin is a highly conserved gene in the *Aeromonas* family expressed in different strains of *A. hydrophila*[20]. It binds to the host cell membrane, forms a homo-heptameric pore, creates an osmotic imbalance, activates G-protein and finally causes cell death[19]. After reaching the cell membrane of the host, it produces a transmembrane pore with oligomeric subunits to break the barrier of cell membrane permeability, leading to cell damage and lysis[21]. Hemolysin, a multifunctional enzyme and enteric toxin encoded by gene *ahh1*, possess hemolytic activity and is responsible for the virulence of *A. hydrophila* by binding to host cells and causing cell damage[22]. The elimination of the two-component hemolytic system, which includes aerolysin and hemolysin, reduces the pathogenicity of *A. hydrophila*. Currently, antibiotics are the widely used drugs to cure *A. hydrophila* infections. However, overuse and injudicious application lead to various complications such as drug residual effect, drug resistance development[23], environmental pollution and human health issues. Recent reports on antibiotic resistance development on *A. hydrophila* were noted against antimicrobials such as amoxicillin, cephalexin, doxycycline, penicillin, piperacillin, and teicoplanin[24]. Ultimately, this results in restricted therapeutic effect of antibiotics against *Aeromonasis*. Though several drugs were developed to treat *A. hydrophila* hemolytic toxin, such as aerolysin[25] and hemolysin[26], the effect of AMPs has been less studied.

The antimicrobial peptide-based drug discovery is an emerging area for researchers as an efficient and feasible alternative to antibiotics due to their characteristics such as minimal toxicity and high target specificity[27], high solubility, bioactivity and metabolic stability[28]. Antimicrobial peptides (AMPs) play a major role in the immune system of multicellular organisms. They act against various microorganisms such as bacteria, fungi, and some viruses[29]. And also, it enhances the host's innate immunity, indirectly reduce the pathogenicity[30]. Natural AMPs kill bacteria through pore formation, cell membrane disruption, and stimulate cellular content efflux[31-32]. However, utilizing plant-derived antimicrobial peptides as antibacterial peptides (ABPs) is still scarce. The traditional method of drug discovery was time-consuming and expensive, and hence, computational method is used to identify effective drugs in a short time[33]. This paper focused on screening and identification of putative plant-derived antibacterial peptides as a valid drug candidate against multidrug-resistant bacteria *A. hydrophila* using bioinformatic approaches.

MATERIALS AND METHODS

We followed two main steps in computational identification of potential ABPs against *A. hydrophila*, viz., virtual screening of AMPs with antibacterial property and molecular docking studies of identified ABPs. A detailed workflow of the study was illustrated in Fig.1. The antimicrobial peptide (AMP) sequences are retrieved from our previous work, which were submitted in the Mendeley[34-36] (https://data.mendeley.com) and duplicate sequences were removed. From the 10953 AMPs derived from 15 different fruit crops (such as apple, almond, ber, blueberry, carambola, chestnut, date palm, jack fruit, mango, peach, pecan nut, pistachio nut, raspberry, strawberry, and walnut), only the peptides with 1-20 amino acid sequences were taken for the study. Further, the antimicrobial and antibacterial property of these sequences were analyzed using CAMP R3 (Collection of Anti-Microbial Peptides)[37] (http://www.camp.bicnirrh.res.in/predict/) and AntiBP 3.0 (https://webs.iiitd.edu.in/raghava/antibp3/predict.php)[38] based machine learning model, respectively. The physicochemical properties were computed using ProtParam in ExPASy[39] (https://web.expasy.org/protparam/) and the APD tool[40] (https://aps.unmc.edu/prediction). Further, the toxicity, allergenicity, hemolytic and bioactive potential of the predicted antibacterial peptide were analysed by ToxinPred[41] (https://webs.iiitd.edu.in/raghava/toxinpred/multi_submit.php) with a threshold value of 0.5, AlgPred 2.0[42] (https://webs.iiitd.edu.in/raghava/algpred2/batch.html) with a threshold value of 0.3, HemoPI2.0[43] (https://webs.iiitd.edu.in/raghava/hemopi2/prediction.html), and PeptideRanker[44] respectively. The peptides predicted with toxic, hemolytic, allergenic and non-bioactive were removed before 3D structure determination using PEPFOLD3.0[45] (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3) for molecular interaction with predicted ABPs (Fig.2). The experimentally validated protein structure of proaerolysin (PDB ID: 1PRE), a precursor of aerolysin[18] derived from the XRD method with 2.8Å resolution, was downloaded from the RCSB portal (https://www.rcsb.org/structure/1PRE). However, there was no experimentally validated 3D structure for hemolysin from *Aeromonas* species, and hence, the model was developed from the sequence (NCBI id: APJ14743.1) from the NCBI database (https://www.ncbi.nlm.nih.gov/protein/APJ14743.1/) and used in SWISS-MODEL[46] (https://swissmodel.expasy.org/interactive) to generate template-based homology modelling. The per cent identity, maximum coverage (52.77%), and similarity of 30-50 % between the target and template sequences, as well as a Global Mean Quality Estimation (GMQE score

0.75) near 1, were chosen as parameters to ensure the quality of the modelled structure. The Ramachandran plot was created using PROCHECK in the PDBsum[47] platform for validation of the model (Fig.2). A molecular docking study was carried out using CLUSPRO 2.0[48] (http://cluspro.bu.edu/login.php) with default docking parameters. The interaction of docked complexes was visualized through pyMOL[49].

RESULTS AND DISCUSSION

Antimicrobial and antibacterial activity of peptides: A total of 10953 AMP sequences were retrieved from Mendeley, covering different fruit crop categories, namely tropical, subtropical, temperate, and nut crops. Out of 10953 AMPs, 6797 AMP sequences containing an amino acid range of 1 to 20 residues were utilized in the study. The retrieved sequences were analyzed for various activities such as antimicrobial, antibacterial, bioactivity, toxicity, hemolytic and allergenicity in different computational models. Around 764 sequences were detected with antimicrobial activity in all three statistical models, namely Random Forest (RF), Support Vector Machine (SVM) and Artificial Neural Network (ANN), without duplication of sequences. A total of 326 AMPs were found to have antibacterial activity, which were predicted in AntiBP 3.0. Then, 316 ABPs, 258 ABPs and 118 ABPs were predicted to be non-toxic, non-hemolytic and non-allergenic ABPs, respectively. Only 93 ABPs were found to be non-toxic, non-hemolytic, and non-allergenic ABPs and were shortlisted for physicochemical property analyses. The peptide with an instability index greater than 40 was considered an unstable protein, and those with lesser instability indices were considered stable, indicating that they may have low bioavailability and a short half-life[50]. Since stability is an important factor for AMPs, predicted ABPs were further shortlisted based on stability. Based on physicochemical properties, only 31 ABPs were found to be stable and subsequently tested for their bioactivity. A total of 11 ABPs were shortlisted to have all the characters making them promising candidates for therapeutic investigation (Table1).

Physicochemical properties of predicted ABPs: The physicochemical properties, such as molecular mass, net charge, isoelectric point, instability index, aliphatic index, grand average of hydropathicity (GRAVY) index, amphipathicity, hydrophobic ratio, and Boman index, were calculated for the shortlisted 11 ABPs (Table 2). The molecular weight of ABPs ranged from 1193.42 Da to 2395.92 Da. The net charge ranged from +3 to +6.25. All predicted ABPs were cationic in nature. The cationic nature would favor a positive interaction with negatively charged microbes and also lead to greater binding affinity towards microbial membranes. A report states that more than 2 net charges are needed for the amphipathic nature[51]. The amphipathicity of ABPs ranges from 0.86 to 1.42. The amphipathic nature was essential for antibacterial property as it favors non-covalent membrane interaction and bacterial cell death[52]. The aliphatic index of ABPs ranged from 0 to 121.88. Isoelectric point is an important factor as it affects the solubility of AMPs. The isoelectric point ranges from +9.9 to +12.03, indicating that ABPs have a wide range of pH from neutral to alkaline. Almost half of the AMPs under study have an aliphatic index more than 40; the highest aliphatic index, 121.88, was exhibited by ABP5. Almost all ABPs have an aliphatic index of more than 40. A greater aliphatic index indicates higher thermal stability. The hydrophobic ratio of ABPs ranges from 25 to 71%. The hydrophobic nature facilitates the electrostatic interaction of the peptide with the negatively charged cell membrane of bacteria[53], forms a pore and alters mitochondrial flexibility and depolymerize, finally causing the death of bacteria[54]. GRAVY value ranges from -1.636 to +0.181 and is formulated by the sum of hydropathy values of all amino acids divided by the number of amino acid residues in the query sequence. Positive and negative GRAVY is an indications of hydrophobicity and hydrophilicity, respectively. Almost all ABPs have negative GRAVY, showing a more hydrophilic nature. The Boman index of ABPs varies from 0.73 to +3.37 Kcal/mol. The protein binding potential was measured in terms of the Boman index. A greater Boman index indicates greater binding affinity. Based on amino acid composition, AMPs can be divided into Cystine-rich peptides, Proline-rich, glycine-rich and tryptophan-rich peptides. In this study, two ABP sequences were found to be rich in lysine, namely ABP9 and ABP11. Lysine contains an amine group at its side chain, which improves interaction with negatively charged bacterial cell membranes, particularly the phosphatidylglycerol, lipopolysaccharides and lipoteichoic acid and moves peptides in the plasma membrane lipid bilayer[55]. Apart from lysin, two tryptophan-rich ABPs (ABP6 and ABP11) were identified. ABP11 is rich in lysine and tryptophan. The peptide rich in tryptophan tends to be a strong antibacterial peptide[56]. The ABPs with amino acids such as cysteine, lysine and tryptophan are scavengers of reactive oxygen species[57].

Protein-peptide interaction: Molecular docking of protein-peptide interactions was carried out to predict the conformations and binding affinity of identified ABPs with the virulence factor of the pathogen. The protein-peptide interaction was studied using CLUSPRO (Table 2; Fig.3). The more negative the binding score, the stronger binding affinity. All the ABPs showed a good range of cluster scores for all the targets. ABP6 has a higher cluster score (-1062.1) towards the proaerolysin target, producing 11 h-bonds, followed by ABP3 (- 942.3) with 7 h-bonds, and the least was ABP4 (-762.5). For the target hemolysin, ABP4 showed the highest score (-856)

with 7 h-bonds, and ABP2 showed the lowest score (-680.3) with 10 h-bonds. The docking score of protein-peptide complexes varies depending on the biological systems, but most peptide-bacterial protein interaction studies highlighted a binding score less than -700 as a strong binding affinity[58-59]. ABP11 (WWWGFRKK), derived from the peach, has a high antibacterial score (0.74), bioactive score (0.98) and good cluster scores of -933.4 and -822.3 for proaerolysin and hemolysin targets, respectively. Among the shortlisted ABPs, ABP11 is considered the superior peptide across almost all antibacterial properties.

CONCLUSION

Aeromonas hydrophila is an opportunistic pathogen that causes productive losses each year in aquaculture. AMPs are well-known alternatives to antibiotics, as they are highly target-specific and have minimal toxicity. The plant-derived AMPs against A. *hydrophila* were not explored much, and hence, this study aimed to identify antibacterial peptides from plants and test them against the virulence factor of *A. hydrophila*. As a result, 11 nontoxic, non-allergenic, non-hemolytic, bioactive and stable ABPs were identified from 10953 sequences retrieved from our previous work. The molecular docking studies revealed that all the identified ABPs have strong binding affinity for the hemolytic toxins responsible for *A. hydrophila* 's pathogenicity. The study also hinted that, among the identified ABPs, ABP11 have the highest potential to inhibit *A. hydrophila*, and further wet-lab experiments, viz., antimicrobial susceptibility test, time-kill assay, biofilm inhibition, cytotoxicity, hemolysis and stability assay, are required to verify the findings.

REFERENCES

1. FAO. Fishery and Aquaculture Country Profiles. India, 2023: Country Profile Fact Sheets [Internet]. Rome: Food and Agriculture Organization of the United Nations; updated 2023 Oct 19 [cited 2026 Feb 17]. Available from: https://www.fao.org/fishery/en/facp/ind?lang=es

2. Bao L, Chen Y, Li H, Zhang J, Wu P, Ye K, Ai H, Chu W. Dietary *Ginkgo biloba* leaf extract alters immune-related gene expression and disease resistance to *Aeromonas hydrophila* in common carp *Cyprinus carpio*. *Fish Shellfish Immunol*. 2019;94:810–818.

3. Abdel-Latif HM, Dawood MA, Menanteau-Ledouble S, El-Matbouli M. The nature and consequences of co-infections in tilapia: A review. *J Fish Dis*. 2020;43(6):651–664.

4. Sanyal KB, Mukherjee D, Guchhait A, Dash G. Phenotypic and molecular identification of bacterial species in Indian major carps and exotic carps from South 24 Parganas, West Bengal, India. *Int J Curr Microbiol Appl Sci*. 2018;7(1):534–547.

5. Ngugi CC, Oyoo-Okoth E, Muchiri M. Effects of dietary levels of essential oil extract from bitter lemon (*Citrus limon*) fruit peels on growth, biochemical, haemato-immunological parameters and disease resistance in juvenile *Labeo victorianus* fingerlings challenged with *Aeromonas hydrophila*. Aquac Res. 2017;48(5):2253-2265. doi:10.1111/are.13062.

6. Starliper CE, Ketola HG, Noyes AD, Schill WB, Henson FG, Chalupnicki MA, et al. An investigation of the bactericidal activity of selected essential oils to *Aeromonas* spp. J Adv Res. 2015;6:89.

7. Constantino CF, Armijo OA, Osorio SD, Chávez SL. Infección por *Aeromonas hydrophila* e *Ichthyophthirius multifiliis* en trucha (*Oncorhynchus mykiss*, Walbaum) y tilapia (*Oreochromis aureus*, L.) de un centro de acopio de Morelos, México. Estudio patológico. Vet Mex. 1997;28:59-62.

8. Bravo L, Morier L, Castaneda N, Ramirez M, Silva M, Castro-Escarpulli G. Aeromonas: an emerging pathogen associated with extraintestinal infection in Cuba. Rev Cubana Med Trop. 2003;55(3):208-209.

9. Gracey M, Burke V, Robinson J. Aeromonas-associated gastroenteritis. Lancet. 1982;320(8311):1304-1306. doi:10.1016/S0140-6736(82)91510-0.

10. Seshadri R, Joseph SW, Chopra AK, Sha J, Shaw J, Graf J, et al. Genome sequence of *Aeromonas hydrophila* ATCC 7966T: jack of all trades. J Bacteriol. 2006;188(23):8272-8282.

11. Park SY, Nam HM, Park K, Park SD. *Aeromonas hydrophila* sepsis mimicking *Vibrio vulnificus* infection. Ann Dermatol. 2011;23(Suppl):S25-S29.

12. Hong CK, Chen HC, Su WR, Lee PY. Rapid progression and mortality of necrotizing fasciitis caused by *Aeromonas hydrophila* in a patient with type I open tibial fracture. Kaohsiung J Med Sci. 2018;34:307-309.

13. Bargui H, Marzouk M, Ben Hadj A, Ali MH. Aeromonas spp. human infection: retrospective study in the region of Sousse, 2011-2015, Tunis. Med. 2017;95(4):257-261.

14. Devi KR, Srinivasan R, Kannappan A, Santhakumari S, Bhuvaneswari M, Rajasekar P, et al. In vitro and in vivo efficacy of rosmarinic acid on quorum sensing mediated biofilm formation and virulence factor production in *Aeromonas hydrophila*. Biofouling. 2016;32(10):1171-1183.

15. Rasmussen-Ivey CR, Figueras MJ, McGarey D, Liles MR. Virulence factors of *Aeromonas hydrophila*: in the wake of reclassification. Front Microbiol. 2016;7:1337

16. Asmat A, Gires U. The occurrence of aerolysin-positive *Aeromonas hydrophila* strains in sea water and

associated with marine copepods. In: Proceedings of the Regional Symposium on Environment and Natural Resources; Kuala Lumpur, Malaysia; 2002. p. 495-502.

17. Wang G, Clark CG, Liu C, Pucknell C, Munro CK, Kruk TM, et al. Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. J Clin Microbiol. 2003;41:1048-1054. doi:10.1128/JCM.41.3.1048-1054.2003.

18. Fivaz M, Abrami L, Tsitrin Y, Van der Goot FG. Aerolysin from *Aeromonas hydrophila* and related toxins. In: van der Goot FG, editor. Pore-forming toxins. Berlin: Springer; 2001. doi:10.1007/978-3-642-56508-3_3.

19. Zhang L, Ma L, Yang Q, Liu Y, Ai X, Dong J. Sanguinarine protects channel catfish against *Aeromonas hydrophila* infection by inhibiting aerolysin and biofilm formation. Pathogens. 2022;11:323.

20. Osman K, Aly M, Kheader A, Mabrok K. Molecular detection of the *Aeromonas* virulence aerolysin gene in retail meats from different animal sources in Egypt. World J Microbiol Biotechnol. 2012;28:1863-1870. doi:10.1007/s11274-011-0915-z.

21. Dong J, Zhang L, Liu Y, Xu N, Zhou S, Yang Y, et al. Luteolin decreases the pathogenicity of *Aeromonas hydrophila* via inhibiting the activity of aerolysin. Virulence. 2021;12:165-176.

22. Takahashi E, Ozaki H, Fujii Y, Kobayashi H, Yamanaka H, Arimoto S, et al. Properties of hemolysin and protease produced by *Aeromonas trota*. PLoS One. 2014;9(3):e91149.

23. Bulfon C, Volpatti D, Galeotti M. Current research on the use of plant-derived products in farmed fish. Aquac Res. 2015;46:513-551.

24. Zhu W, Zhou S, Chu W. Comparative proteomic analysis of sensitive and multidrug resistant *Aeromonas hydrophila* isolated from diseased fish. Microb Pathog. 2019;139:103930.

25. Vijai S, Pallavi S. Inhibition of oligomerization of aerolysin from *Aeromonas hydrophila*: homology modeling and docking approach for exploration of hemorrhagic septicemia. Lett Drug Des Discov. 2009;6:215-223.

26. Wang T, Zhang P, Lv H, Deng X, Wang J. A natural dietary flavone myricetin as an α-hemolysin inhibitor for controlling *Staphylococcus aureus* infection. Front Cell Infect Microbiol. 2020;10:330. doi:10.3389/fcimb.2020.00330.

27. Rani N, Saravanan V, Lakshmi PTV, Annamalai A. Inhibition of pore formation by blocking the assembly of *Staphylococcus aureus* α-hemolysin through a novel peptide inhibitor: an in silico approach. Int J Pept Res Ther. 2014;20:575-583.

28. Craik DJ, Fairlie DP, Liras S, Price D. The future of peptide-based drugs. Chem Biol Drug Des. 2013;81:136-147. doi:10.1111/cbdd.12055.

29. Hancock REW, Sahl HG. Antimicrobial and host defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006;24:1551-1557.

30. Yeung ATY, Gellatly SL, Hancock RE. Multifunctional cationic host defence peptides and their clinical applications. Cell Mol Life Sci. 2011;68:2161-2176.

31. Olivieri C, Buonocore F, Picchietti S, Taddei AR, Bernini C, Scapigliati G, et al. Structure and membrane interactions of chionodracine, a piscidin-like antimicrobial peptide from the icefish *Chionodraco hamatus*. Biochim Biophys Acta Biomembr. 2015;1848:1285-1293.

32. Shabir U, Ali S, Magray AR, Ganai BA, Firdous P, Hassan T, et al. Fish antimicrobial peptides (AMPs) as essential and promising molecular therapeutic agents: a review. Microb Pathog. 2018;114:50-56.

33. Umadevi P, Manivannan S, Fayad AM, Shelvy S. In silico analysis of phytochemicals as potential inhibitors of proteases involved in SARS-CoV-2 infection. J Biomol Struct Dyn. 2022;40(11):5053-5059.

34. Vijayalakshmi K, Senthilkumar S, Manivannan S, Shelvy S. Discerning of antimicrobial peptides (AMPs) from whole genome sequenced nut crops. Mendeley Data. 2021;Version 3. doi:10.17632/zhthnd824r.3.

35. Jinto J, Shelvy S, Manivannan S. Discerning of antimicrobial peptides from whole genome sequenced temperate fruit crops. Mendeley Data. 2021;Version 1. doi:10.17632/njzkvs89xm.1.

36. Nivethakumar S. Discerning of antimicrobial peptides (AMPs) from whole genome sequenced tropical and subtropical fruit crops. Mendeley Data. 2021;Version 1. doi:10.17632/p6gdn5bg57.1.

37. Waghu FH, Barai RS, Gurung P, Idicula-Thomas S. CAMPR3: a database on sequences, structures and signatures of antimicrobial peptides. Nucleic Acids Res. 2016;44(D1):D1094-D1097. doi:10.1093/nar/gkv1051.

38. Bajiya N, Choudhury S, Dhall A, Raghava GPS. AntiBP3: a method for predicting antibacterial peptides against Gram-positive/negative/variable bacteria. Antibiotics (Basel). 2024;13:168. doi:10.3390/antibiotics13020168.

39. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, et al. Protein identification and analysis tools on the ExPASy server. In: Walker JM,

editor. The Proteomics Protocols Handbook. Totowa (NJ): Humana Press; 2005. p. 571-607.

40. Wang G, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res. 2016;44(D1):D1087-D1093.

41. Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Raghava GPS. In silico approach for predicting toxicity of peptides and proteins. PLoS One. 2013;8(9):e73957. doi:10.1371/journal.pone.0073957.

42. Sharma N, Patiyal S, Dhall A, Pande A, Pannu R, Raghava GPS. AlgPred 2.0: an improved method for predicting allergenic proteins and mapping of IgE epitopes. Brief Bioinform. 2021;22(4):bbaa294.

43. Rathore AS, Kumar N, Choudhury S, et al. Prediction of hemolytic peptides and their hemolytic concentration. Commun Biol. 2025;8:176. doi:10.1038/s42003-025-07615-w.

44. Mooney C, Haslam NJ, Pollastri G, Shields DC. Towards the improved discovery and design of functional peptides: common features of diverse classes permit generalized prediction of bioactivity. PLoS One. 2012;7:e45012.

45. Lamiable A, Thévenet P, Rey J, Vavrusa M, Derreumaux P, Tufféry P. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. Nucleic Acids Res. 2016;44(W1):W449-W454.

46. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 2018;46(W1):W296-W303. doi:10.1093/nar/gky427.

47. Laskowski RA, Jabłońska J, Pravda L, Vařeková RS, Thornton JM. PDBsum: structural summaries of PDB entries. Protein Sci. 2018;27(1):129-134. doi:10.1002/pro.3289

48. Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, Yueh C, et al. The ClusPro web server for protein-protein docking. Nat Protoc. 2017;12(2):255-278.

49. Schrödinger LLC. The PyMOL Molecular Graphics System, Version 3.0. New York: Schrödinger LLC; 2023.

50. Dziuba B, Dziuba M. Milk proteins-derived bioactive peptides in dairy products: molecular, biological and methodological aspects. Acta Sci Pol Technol Aliment. 2014;13(1):5-26.

51. Hancock RE. Peptide antibiotics. Lancet. 1997;349(9049):418-422.

52. Tossi A, Sandri L, Giangaspero A. Amphipathic, α-helical antimicrobial peptides. Pept Sci. 2000;55:4-30.

53. Huang HW. Action of antimicrobial peptides: two-state model. Biochemistry. 2000;39:8347-8352.

54. Patel S, Akhtar N. Antimicrobial peptides (AMPs): the quintessential "offense and defense" molecules are more than antimicrobials. Biomed Pharmacother. 2017;95:1276-1283.

55. Killian JA, Von Heijne G. How proteins adapt to a membrane-water interface. Trends Biochem Sci. 2000;25:429-434.

56. Feng X, Jin S, Wang M, Pang Q, Liu C, Liu R, et al. The critical role of tryptophan in the antimicrobial activity and cell toxicity of the duck antimicrobial peptide DCATH. Front Microbiol. 2020;11:1146.

57. Maestri E, Marmiroli M, Marmiroli N. Bioactive peptides in plant-derived foodstuffs. J Proteomics. 2016;147:140-155. doi:10.1016/j.jprot.2016.03.048.

58. Okella H, George JJ, Ochwo S, Ndekezi C, Koffi KT, Aber J, et al. New putative antimicrobial candidates: in silico design of fish-derived antibacterial peptide-motifs. Front Bioeng Biotechnol. 2020;8:604041.

59. Eshtiaghi S, Nazari R, Fasihi-Ramandi M. Molecular docking, anti-biofilm and antibacterial activities and therapeutic index of mCM11 peptide on *Acinetobacter baumannii* strains. Curr Microbiol. 2023;80(6):191.

Fig.1. Detailed outline of the shortlisting ABPs

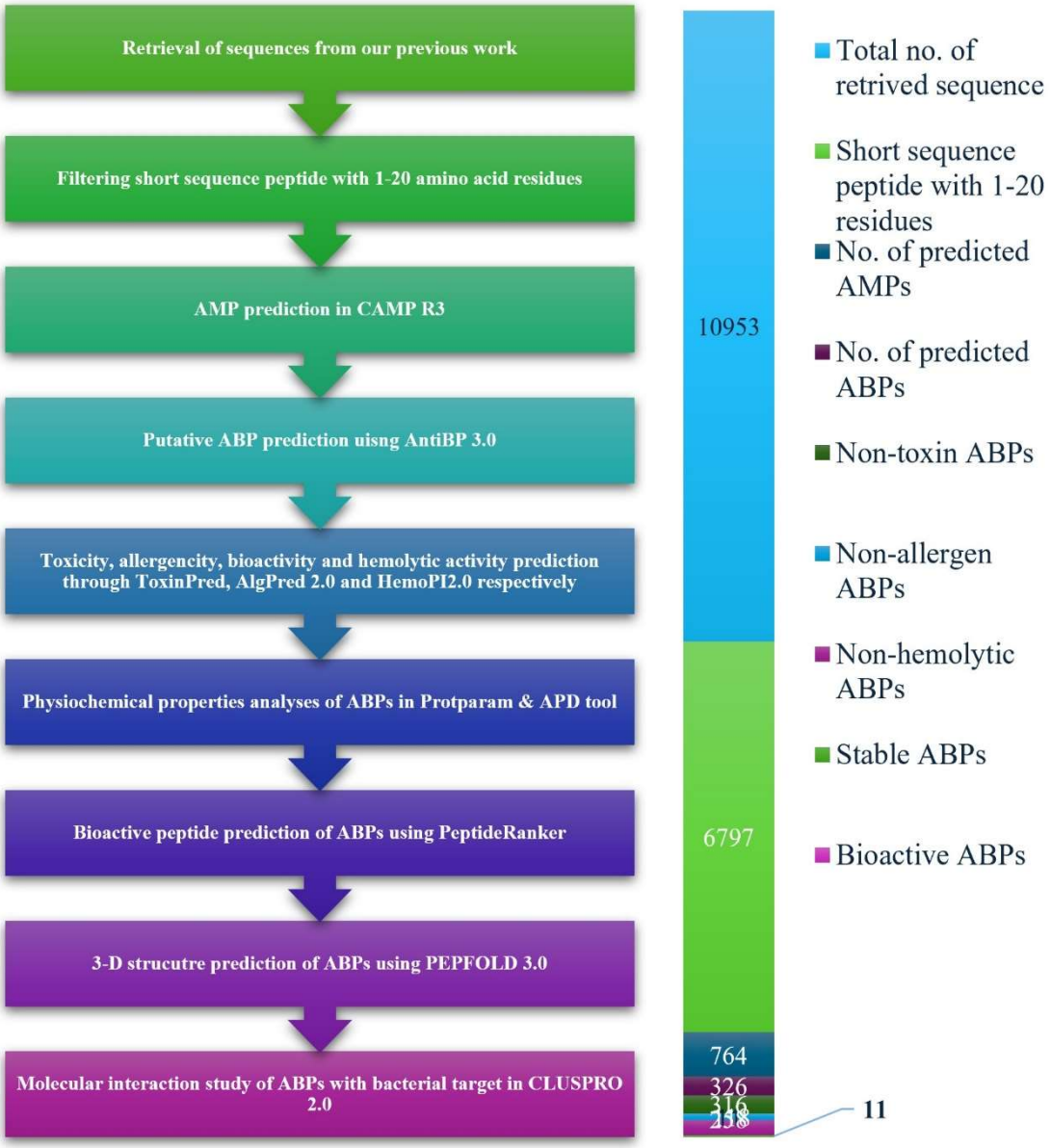

Fig.2. 3D-structure prediction Antibacterial peptide and their protein targets

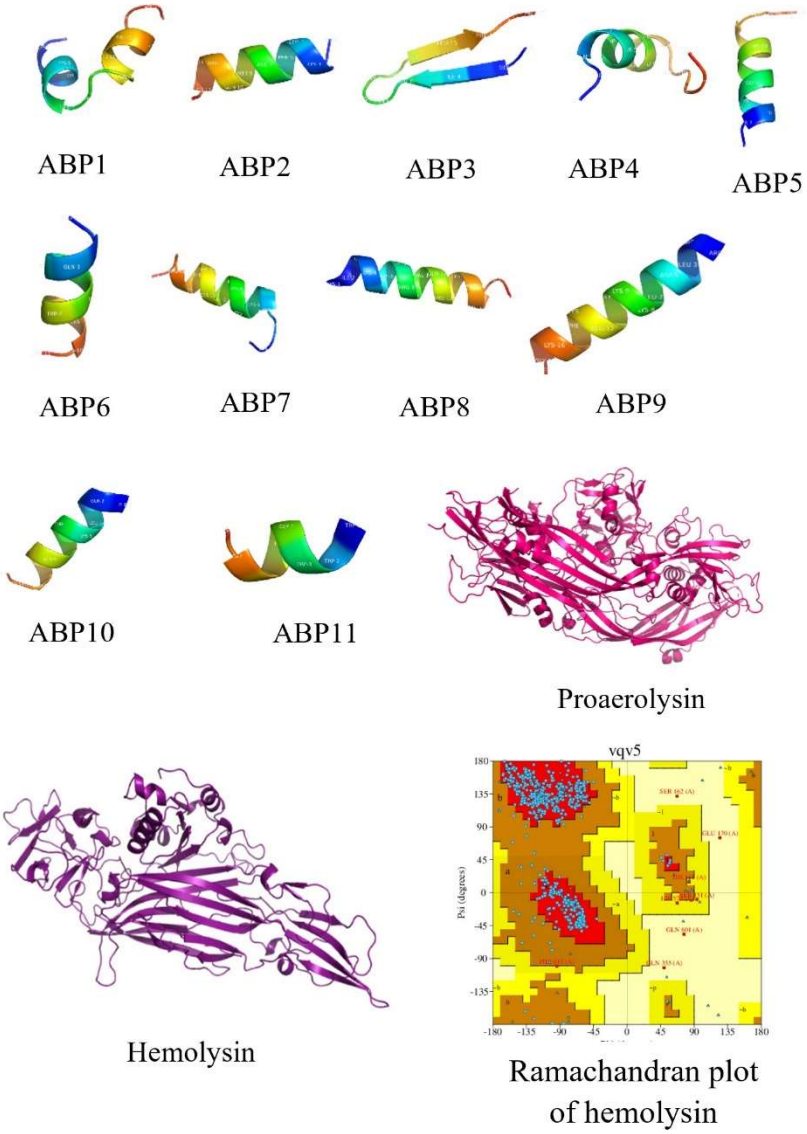

Fig.3 Molecular interactions of protein target of proaerolysin and hemolysin with antibacterial peptide

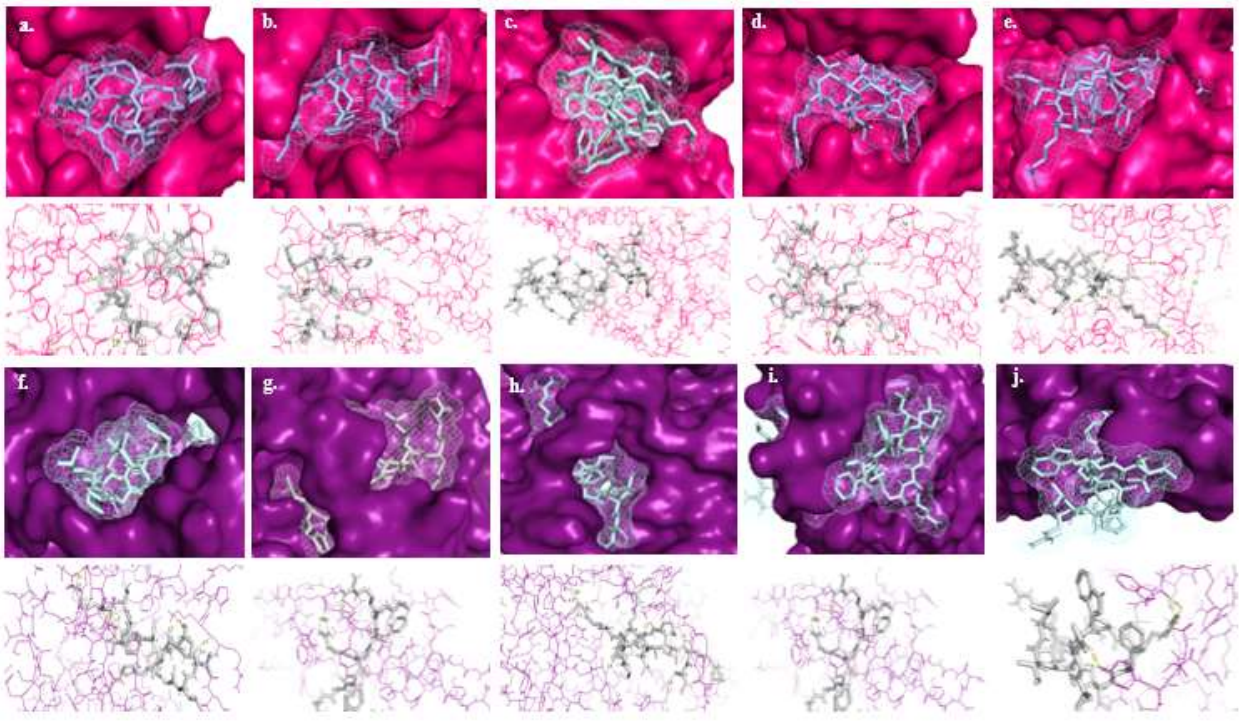

Fig. 3. Docking images of proaerolysin with a. ABP11, b. ABP4, c. ABP10, d. ABP5, e. ABP2; hemolysin with f. ABP11, g. ABP4, h. ABP10; i. ABP5, j. ABP 2

Table 1: In silico prediction of antimicrobial, antibacterial, toxicity, allergenicity, hemolytic, and bioactive properties of shortlisted ABPs

ABP No.	ABP sequence	Source of peptide	AMP prediction			Anti BP score	TOXICITY		ALLERGENCITY		Hemolytic prediction		Bio-active score
			SVM score	RF score	ANN classifier		SVM Score	Prediction	ML Score	Prediction	ML Score	Prediction	
ABP1	HCIKKRLQWNTCFARKVC	Carambola	0.75	0.93	AMP	0.58	-0.54	Non-Toxin	0.25	Non-Allergen	0.29	Non-Hemolytic	0.69
ABP2	VSKLFKRTMQRLLK	Carambola	0.82	0.64	AMP	0.63	-1.11	Non-Toxin	0.19	Non-Allergen	0.18	Non-Hemolytic	0.56
ABP3	KGYINLGKGIHGLVFKR	Jack fruit	0.88	0.97	AMP	0.60	-0.62	Non-Toxin	0.27	Non-Allergen	0.18	Non-Hemolytic	0.79
ABP4	KWQRTSWRLKKLGHPKI	Blueberry	0.94	0.91	AMP	0.68	-0.94	Non-Toxin	0.27	Non-Allergen	0.18	Non-Hemolytic	0.59
ABP5	GYLRVGKAIHGLIFKR	Peach	0.96	0.98	AMP	0.63	-1.46	Non-Toxin	0.26	Non-Allergen	0.18	Non-Hemolytic	0.62
ABP6	RTQWWMWRKKL	Chestnut	0.58	0.57	AMP	0.61	-1.31	Non-Toxin	0.24	Non-Allergen	0.18	Non-Hemolytic	0.87
ABP7	IFSSRKCKTVSKTFRGIC	Apple	0.56	0.83	AMP	0.51	-1.58	Non-Toxin	0.29	Non-Allergen	0.18	Non-Hemolytic	0.74
ABP8	KIGKEFKRIVQRIKDFLRN	Apple	0.84	0.80	AMP	0.56	-1.19	Non-Toxin	0.25	Non-Allergen	0.19	Non-Hemolytic	0.52
ABP9	RRLRDFLKKIGEKFKKIGY	Apple	0.60	0.72	AMP	0.60	-1.31	Non-Toxin	0.27	Non-Allergen	0.19	Non-Hemolytic	0.52
ABP10	FQRLKTRMQKAWKW	Chestnut	1.00	0.68	AMP	0.63	-0.96	Non-Toxin	0.28	Non-Allergen	0.18	Non-Hemolytic	0.58
ABP11	WWWGFRKK	Peach	1.00	0.56	AMP	0.74	-1.20	Non-Toxin	0.30	Non-Allergen	0.18	Non-Hemolytic	0.98

Table 2: Physicochemical properties of the shortlisted ABPs and their Molecular interactions with the virulence proteins of Aeromonas hydrophila

ABP No.	ABP sequence	Mol. mass (Da)	pI	Net charge	Hydrophobic ratio (%)	Boman index (kcal/mol)	Instability index	Aliphatic index	GRAVY	Amphipathicity	Pro-aerolysin		Hemolysin	
											Cluster score	No. of H-bond	Cluster score	No. of H-bond
ABP1	HCIKKRLQWNTCFARKVC	2234.73	9.9	5.25	50	2.28	38.37	65	-0.439	1.03	-904	21	-696.5	10
ABP2	VSKLFKRTMQRLLK	1748.21	12.02	5	43	2.41	4.32	104.29	-0.386	1.23	-892.3	13	-680.3	10
ABP3	KGYINLGKGIHGLVFKR	1900.3	10.46	4.25	35	0.73	-4.06	108.82	-0.129	0.88	-942.3	7	-736.7	6
ABP4	KWQRTSWRLKKLGHPKI	2162.61	12.03	6.25	29	2.81	-7.26	68.82	-1.441	1.31	-762.5	13	-856	7
ABP5	GYLRVGKAIHGLIFKR	1828.24	11.1	4.25	44	0.9	17.71	121.88	0.181	0.86	-910.1	9	-790.3	11
ABP6	RTQWWMWRKKL	1618.97	12.02	4	45	3.16	16.05	35.45	-1.636	1.23	-1062.1	11	-825.8	5
ABP7	IFSSRKCKTVSKTFRGIC	2061.49	10.33	5	39	2.13	28.27	59.44	-0.061	0.88	-919.7	5	-815.1	4
ABP8	KIGKEFKRIVQRIKDFLRN	2388.89	11.07	5	37	3.37	13.51	97.37	-0.863	1.29	-887.1	13	-748.5	6
ABP9	RRLRDFLKKIGEKFKKIGY	2395.92	10.64	6	32	3.19	16.04	82.11	-1.047	1.42	-804.3	13	-839.9	6
ABP10	FQRLKTRMQKAWKW	1907.31	12.02	5	43	3.1	-2.16	35	-1.421	1.31	-857.5	6	-733	8
ABP11	WWWGFRKK	1193.42	11.17	3	50	1.88	-4.21	0	-1.575	1.22	-933.4	2	-822.3	3