Insilico Construction of Plasmid Containing Antimicrobial Peptides for Food Pathogens Along with Anticancer Peptides

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Abstract: The aim of this research is to identify novel antimicrobial peptides (AMPs) in fish species using bioinformatics tools, aiming to discover candidates for anticancer therapeutics and pathogen interaction. AMPs are small, positively charged molecules crucial in innate immunity, capable of killing pathogens and modulating host defenses, with some showing promise as anticancer agents. This study focuses on constructing plasmids containing AMPs with dual functionalities: combating food pathogens and exhibiting anticancer properties. Proteomic data from UniProt was analyzed, focusing on AMPs from snakehead murrel. Prediction tools like AMP Scanner, iAMPpred, AntiBP3, and CAMPR3 identified potential AMP candidates. Antigenicity and allergenicity of selected AMPs were evaluated using tools such as SVMTrip, Vaxijen, and AlgPred. Structural modeling and docking studies with CXCR1 and E.coli O157:H7 highlighted the potential of these AMPs, with promising results suggesting effective binding. These findings indicate that these AMPs could be valuable in food preservation and cancer therapy. Further studies could explore the *in vivo* efficacy of these peptides. This research underscores the promise of AMPs in medicine and food preservation, addressing antibiotic resistance and exploring their potential applications in cancer therapy.

Keywords: Antimicrobial Peptides (AMPs), docking, plasmids, structural modeling

I INTRODUCTION

Antibiotic resistance has become a global health crisis, necessitating the search for alternative therapeutic strategies. Antimicrobial peptides (AMPs) have emerged as promising candidates due to their potent ability to kill pathogens and modulate host immune responses. These small, positively charged molecules are found across various organisms and play a crucial role in innate immunity by combating a wide range of microbes, including bacteria, viruses, parasites, and fungi. In addition to their antimicrobial properties, some AMPs have shown potential as anticancer agents, offering a dual-functional approach to addressing both infectious diseases and cancer[36].

The significance of AMPs in innate immunity is underscored by their efficiency and rapid action. Unlike the acquired immune system, which relies on the time-consuming production of antibodies, AMPs are part of the innate immune system, enabling a quick and energy-efficient response to microbial invasion[25]. This is particularly crucial for organisms that lack a lymphocyte-based immune system, such as insects, which rely on synthesizing antibacterial compounds to combat invading microorganisms. AMPs have been identified in diverse sources, including microorganisms, plants, invertebrates, fish, amphibians, reptiles, birds, and mammals, each with specific roles in their respective host organisms[3].

AMPs can be categorized based on their amino acid sequences, net charge, protein structure, and source. Key subgroups include anionic and cationic alpha helical AMPs, cationic beta-sheet AMPs, and extended cationic AMPs[25]. These peptides exert their antimicrobial effects primarily by disrupting microbial membranes, a strategy that limits the potential for resistance development. Notably, bacteriocins, a type of AMP, exhibit narrow or broad antibacterial activity and can be synthesized by ribosomes or non-ribosomes. Examples like nisin, used as a natural food preservative, and

copsin, which disrupts bacterial cell wall biosynthesis, highlight the diverse applications of AMPs[14],[41]. CXCR1, also known as C-X-C motif chemokine receptor 1, is a crucial protein in cancer biology. It serves as a marker for cancer stem cells (CSCs), which are a subset of cells within tumors that possess the ability to self-renew and drive tumor growth[23]. Additionally, CXCR1 plays a role in the inflammatory tumor microenvironment, promoting tumor progression and metastasis[33]. As a receptor for interleukin-8 (IL-8), CXCR1 is vital for neutrophil chemotaxis, aiding in the creation of a pro-inflammatory environment that supports cancer progression[42]. Inhibiting CXCR1 has shown potential in disrupting cancer cell proliferation and metastasis, making it a promising therapeutic target . Furthermore, high CXCR1 expression is associated with poor prognosis in various cancers, highlighting its clinical relevance[54].

Escherichia coli O157:H7 is a particularly harmful strain of E. coli. It is one of the Shiga toxin producing types of E. coli (STEC), known for producing toxins that can cause severe foodborne illness (CDC, 2020). Infections often lead to severe, acute hemorrhagic diarrhea and abdominal cramps. In some cases, it can also cause hemolytic-uremic syndrome (HUS), a serious condition that can lead to kidney failure, especially in young children, the elderly, and those with compromised immune systems[38]. The primary mode of transmission is through consumption of contaminated food or water, with common sources including undercooked ground beef, raw milk, and raw vegetables[62]. Preventing infection involves proper food handling, thoroughly cooking meat, and practicing good hygiene to reduce the risk of contamination[69].

Given the rising threat of antibiotic resistance, AMPs offer a promising alternative for developing new antimicrobial therapies. This study focuses on identifying and characterizing AMPs in snakehead murrel (Channa striata), leveraging bioinformatics tools to discover peptides with both antimicrobial and anticancer properties[48]. Proteomic data from UniProt was meticulously analyzed, and prediction tools such as AMP Scanner, iAMPpred, AntiBP3, and CAMPR3 were employed to identify potential AMP candidates[67]. To ensure their safety and therapeutic potential, antigenicity and allergenicity were evaluated using SVMTrip, Vaxijen, and AlgPred[6],[20]. Structural modeling and docking studies were conducted to investigate the interaction of these AMPs with CXCR1 and E. coli O157:H7, revealing promising binding capabilities[27].

The findings of this study suggest that these AMPs could play a significant role in food preservation and cancer therapy, addressing the dual challenges of antibiotic resistance and cancer treatment. Future research should focus on in vivo studies to further validate the efficacy and safety of these peptides, paving the way for their potential application in medicine and food technology[24].

II. LITERATURE REVIEW

Antimicrobial peptides (AMPs) have garnered significant attention as potential therapeutic agents due to their dual functionalities in combating infectious diseases and cancer. These small, positively charged molecules are integral to the innate immune system across various species, including humans and fish, where they serve as frontline defenders against pathogens. AMPs are known for their broad-spectrum antimicrobial activity, targeting bacteria, fungi, viruses, and even cancer cells[66]. Their mechanism of action typically involves disrupting microbial membranes, leading to cell death[7]. Beyond their antimicrobial properties, several AMPs have exhibited anticancer activities by inducing apoptosis, inhibiting angiogenesis, and modulating the immune response.

Advances in bioinformatics have revolutionized the discovery and characterization of AMPs. Tools like AMP Scanner, iAMPpred, AntiBP3, and CAMPR3 have been instrumental in predicting and identifying novel AMPs with high accuracy[21]. These computational methods allow for the efficient screening of large proteomic datasets, facilitating the discovery of peptides with potential therapeutic applications.

Fish species are a rich source of AMPs due to their diverse immune systems and constant exposure to aquatic pathogens. Snakehead murrel (*Channa striata*), in particular, has been studied for its potent AMPs, which exhibit strong antimicrobial and anticancer activities. The unique peptides derived from fish not only enhance our understanding of innate immunity but also provide valuable leads for developing new therapeutics.

The application of structural modeling and docking studies has provided deeper insights into the interaction dynamics of AMPs with their targets. These studies have shown that AMPs can effectively bind to receptors such as CXCR1 and pathogens like E.coli O157:H7, highlighting their potential as therapeutic agents[15]. The use of tools like SVMTrip (SVMTriP: a tool to predict linear antigenic epitopes), Vaxijen (VaxiJen), and predicted antigenic peptide (Immunomedicine Group: Tools >> PREDICTED ANTIGENIC PEPTIDES) further ensures the antigenicity and allergenicity of these peptides, crucial for their safe application in medicine.

While the potential of AMPs in medicine and food preservation is promising, several challenges remain. The stability, bioavailability, and potential resistance mechanisms of AMPs need to be thoroughly investigated. Additionally, in vivo studies are essential to validate the efficacy and safety of these peptides, paving the way for their potential application in clinical and food technology settings.

This literature review highlights the significant progress made in the discovery and application of AMPs, particularly from fish species, using bioinformatics tools. The dual functionality of these peptides in antimicrobial and anticancer activities positions them as valuable candidates in addressing the global challenges of antibiotic resistance and cancer therapy. Future research should continue to focus on overcoming current challenges and exploring the full therapeutic potential of AMPs.

III. RESEARCH METHODOLOGY

This study outlines a comprehensive approach for predicting, analyzing, and validating antimicrobial peptides (AMPs) from the fish specimen *Channa striata*. The methodology consists of several meticulously planned steps to ensure thorough checks for allergenicity, antigenicity, and functionality, followed by structural modeling and refinement. Each phase of the methodology was designed to maximize the reliability and relevance of the findings.

Channa striata was chosen for this study due to its well-documented bioactive properties. The selection was based on the hypothesis that this fish species may possess unique antimicrobial peptides that could contribute significantly to medical and environmental applications. Protein sequence was retrieved from UniProt.

To identify potential antimicrobial peptides, several advanced bioinformatics tools were employed. These tools included AMP Scanner[64], iAMPpred, AntiBP3[32], and CAMPR3[65]. Each tool utilizes different algorithms to analyze the sequence data, predicting peptides with antimicrobial properties. The combined use of multiple prediction tools enhances the accuracy and confidence in the identified AMPs.

Safety is a critical factor when developing bioactive compounds. Therefore, the allergenicity of the predicted AMPs was assessed using multiple online tools: AlgPred[52], AllergenFP[18], AllergenTOOL and AllerTOP[17]. These tools evaluate the sequences against known allergens to predict potential allergenic properties. Only peptides predicted to be non-allergenic were selected for further analysis, ensuring the safety of these compounds for potential therapeutic use.

Following allergenicity assessment, the antigenicity of the peptides was evaluated using VaxiJen[20], Predicted Antigenic Peptide[9], and SVMTrip[72]. These tools predict the immune response potential of the peptides, which is crucial for determining their suitability as vaccine candidates or therapeutic agents. Peptides that showed high antigenicity scores were prioritized for further functional analysis.

To explore additional therapeutic potentials, the anti-cancer properties of the peptides were predicted using tools such as AntiCP2 and ACPred[11]. These tools assess the sequences for characteristics common in anti-cancer peptides, broadening the potential applications of the identified AMPs. Peptides showing strong anti-cancer properties were noted for further experimental validation.

Based on the results from the AMP prediction and safety assessments, the most promising sequences were selected for vector construction. These vectors are essential for the genetic and functional analysis of the peptides. The sequences were cloned into appropriate vectors using standard molecular biology techniques, ensuring they are ready for expression and further analysis[53].

A sequence homology search using BLAST[2] ensured the uniqueness and identified similar sequences of AMPs. InterProScan[28] was used to predict the biological roles of the peptides, providing a comprehensive view of their potential activities. The secondary structures of the peptides were predicted using PSIPRED[71], aiding in stability and interaction analyses.

The constructed vectors containing the selected peptide sequences were modeled using I-TASSER (Iterative Threading ASSEmbly Refinement)[71]. I TASSER predicts the 3D structure of proteins based on the sequence data and threading alignments. The predicted models were analyzed to understand the structural conformations and potential functional sites of the peptides.

To ensure the accuracy of the predicted protein structures, verification was carried out using SAVES (Structure Analysis and Verification Server)[35] and ProSA (Protein Structure Analysis)[68]. SAVES includes multiple verification tools like VERIFY3D and ERRAT, which assess the quality of the protein models. ProSA evaluates the overall model quality by comparing it with a database of known structures. Only models that passed these verification checks were selected for further refinement.

The selected protein models were refined using ModRefiner[70] and Galaxy Web[29]. ModRefiner improves the structure by optimizing the atomic interactions, while Galaxy Web provides a suite of tools for further refinement and validation. This step should only be carried out if the protein quality doesn't meet the standards.The refined models were assessed for stability and functional relevance, ensuring that they are suitable for subsequent docking studies.

The final step involved protein docking studies using ClusPro[13], a widely used tool for predicting protein-protein interactions. ClusPro performs rigid body docking and ranks the resulting complexes based on their interaction energy. The docking studies provided insights into the binding interactions and potential efficacy of the peptides, aiding in the identification of promising candidates for further experimental validation.

This comprehensive methodology integrates multiple bioinformatics tools and techniques to predict, analyze, and validate antimicrobial peptides from *Channa striata*. Each step is designed to ensure the reliability and applicability of the findings, contributing to the development of safe and effective antimicrobial agents. The approach not only emphasizes the discovery of novel AMPs but also ensures their safety, functionality, and therapeutic potential through rigorous validation and analysis.

IV. RESULTS

Fig 1. Vector construct includes genes encoding antimicrobial peptides and anticancer peptides, along with regulatory elements.

Fig 2. Vector Map of plasmid in vector builder displays the plasmid VB240620-1431hyv, 6275 base pairs in length. The plasmid is designed for cloning, gene expression and vaccine development and the vaccine construct 1 encodes the target antigen for vaccine development.

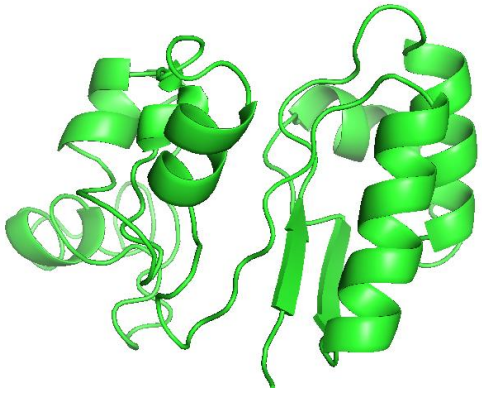

Fig 3. 3D structure of a vector construct modelled using I-TASSER, providing insights into the structural conformation and stability of the vector

construct

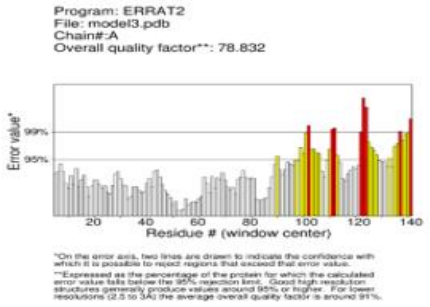

Fig 4. Validation of the predicted protein structure modelled using I-TASSER, assessed by ERRAT2. An overall quality factor above 95% is considered indicative of a good quality model, while values below this threshold suggest that the model may require further refinement.

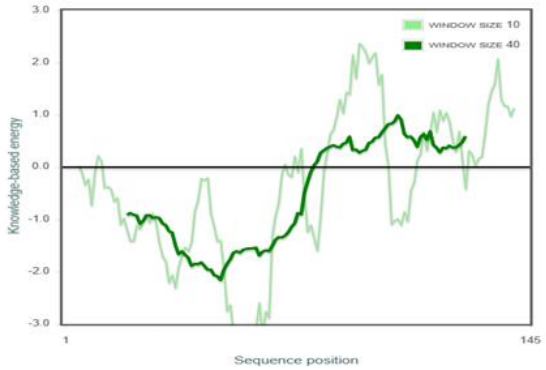

Fig 5. Local model quality assessment of the protein structure modeled using I-TASSER, validated by ProSA-web. The graph shows knowledge-based energy plotted against sequence position for window sizes 10 (light green) and 40 (dark green). Ideally, lower energy values indicate better model quality. The fluctuations in the graph highlight areas where the model may require further refinement to achieve optimal structural stability.

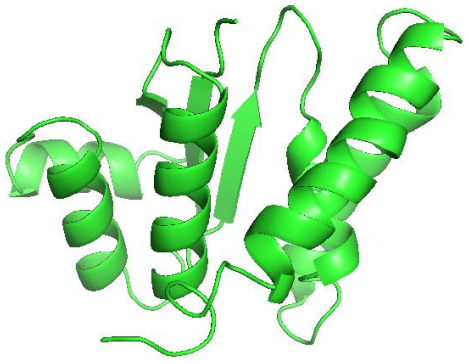

Fig 6. Refined 3D structure of the protein model generated using GalaxyWeb. The refinement process has optimized the folding and spatial arrangement of the protein, enhancing the accuracy of the structural representation.

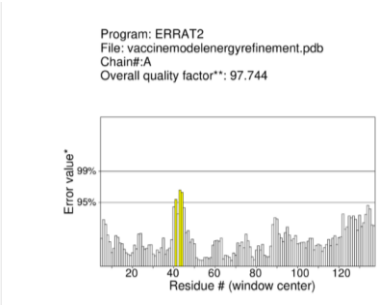

Fig 7. Validation of the refined protein structure using GalaxyWEB, assessed by SAVEServer. The overall quality factor is 97.74%, which indicates good quality.

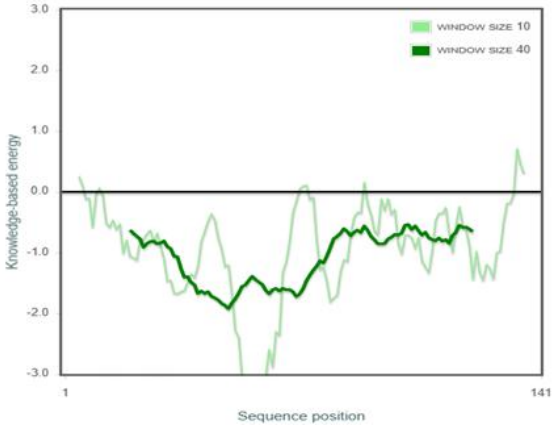

Fig 8. Local model assessment of the refined protein structure using GalaxyWEB, assessed by ProSA web.The dark green line demonstrates a smoother trend compared to the light green line, indicating more stable energy values across the sequence. This analysis is crucial for understanding the stability and reliability of the model across different sequence positions

Cluster Scores

We strongly encourage you to read the FAQ related to these scores before using them.

Cluster	Members	Representative	Weighted Score
0	81	Center	-1275.8
		Lowest Energy	-1531.6
1	54	Center	-1177.2
		Lowest Energy	-1546.1
2	46	Center	-1301.7
		Lowest Energy	-1301.7
3	45	Center	-1168.7
		Lowest Energy	-1339.3
4	43	Center	-1131.3
		Lowest Energy	-1263.9

Fig 9. Cluster scores for the predicted protein structures against CXCR1 using ClusPro. The scores were generated through docking between the refined protein (predicted protein) and CXCR1. Lower scores indicate more stable protein conformations, with Cluster 1's Lowest Energy representative showing the lowest weighted score of -15461.1kcal/mol, suggesting it as the most stable predicted structure.

Cluster Scores

We strongly encourage you to read the FAQ related to these scores before using

Cluster	Members	Representative	Weighted Score
0	73	Center	-659.5
		Lowest Energy	-821.3
1	64	Center	-760.2
		Lowest Energy	-785.3
2	51	Center	-779.3
		Lowest Energy	-818.1
3	45	Center	-715.9
		Lowest Energy	-889.5
4	39	Center	-733.5
		Lowest Energy	-750.1
5	34	Center	-704.6
		Lowest Energy	-716.2

Fig 10. Cluster scores were generated through docking between the refined protein (predicted protein) and EcoliO157:H7 by using ClusPro. Lower scores indicate more stable protein conformations, with Cluster 1's Lowest Energy Representative showing the lowest weighted score of -889.5 suggesting it as the most stable predicted structure.

Figure 1 depicts the vector construct, where the black-colored sequence represents the adjuvant, specifically a 50S ribosomal L7/L12 adjuvant. Sequences screened through various tools for antimicrobial peptides are indicated. The allergenic and antigenic properties were evaluated, and anticancer properties were assessed. Sequences that passed all these evaluations are shown in green, while linkers (EAAAK and GPGPG) are depicted in red. Figure 2 illustrates the vector map of the plasmid, which incorporates the vector construct into the plasmid of a bacterium. After constructing the vector, the 3D structural modeling was performed using I-TASSER, as seen in Figure 3. The initial model's validity was assessed using I TASSER and SAVEServer, with the results presented in Figures 4 and 5. Due to the initial model not meeting the desired quality standards, the protein model underwent refinement using the Galaxy web tool. The refined model is shown in Figure 6. The refined model's validity was further confirmed using SAVEserver and Prosa web, as presented in Figures 7 and 8. Docking studies were conducted to assess the interaction between the modeled protein and target proteins using the ClusPro tool. The docking results for the interaction between the antimicrobial peptide (AMP) and CXCR1 are shown in Figure 9, while those for the interaction between the AMP and E. coli O157:H7 are presented in Figure 10.

V. CONCLUSION

In this study, an *insilico* approach was utilized to design a plasmid containing antimicrobial peptides (AMPs) with dual functionality combating food pathogens and exhibiting anticancer properties. We collected relevant proteomic data from UniProt, focusing on AMPs from snakehead murrel. AMP prediction was performed using multiple tools, including AMP Scanner, iAMPpred, AntiBP3, and CAMPR3. These tools helped identify potential AMP candidates with antimicrobial activity. As AMPs play a crucial role in innate immunity across various organisms, they exhibit inhibitory effects against bacteria, fungi, parasites, and viruses. As antibiotic-resistant microorganisms become more prevalent, AMPs offer promise in medicine, food, animal husbandry, agriculture, and aquaculture. They are also found in dairy products, generated through milk enzymatic hydrolysis. We further evaluated the antigenicity and allergenicity of the selected AMP candidates using various tools: SVMTrip, Vaxijen, AlgPred, AllergenFP, Allercatop, and AllerTop. These evaluations were critical to balance antimicrobial efficacy with safety considerations, ensuring practical applications. We also investigated the presence of anticancer peptides (ACPs) using AntiCp2.0 and ACPred tools, which is essential for assessing immunogenicity, safety, and designing effective vaccines and therapeutic applications.

The AMPs screened through these tools were finalized and used to make a vector construct. The initial part of the vector construct consisted of an adjuvant, followed by linkers that joined the AMPs, and then more AMPs and linkers. Once the construct was prepared, it was incorporated into the plasmid of a bacterium. The vector construct was modeled using I-TASSER and validated using SAVEServer and ProSAweb tools. However, the initial model quality was not satisfactory and was below the threshold value. Therefore, the protein

model was refined using the GalaxyWeb tool. Post-refinement, the model's validity increased from 78.83% to 97.74%, as observed in SAVEServer, where ERRAT score above 95% is considered a good quality model. The Ramachandran plot of the modelled protein showed 6 residues in disallowed regions and post refinement 1 residue in disallowed region(Fig A2 and A3). ProSAweb further confirmed the model quality, although some portions in Figure 5 indicated errors in the later part of the sequence. As seen in Fig A4 and A5 the z score of the protein model was -4.6 and after refinement its -4.92, which indicates that the refinement model is more good, as more less the z score indicates better protein quality.

Once validation was completed, the predicted proteins were docked against CXCR1 and E. coli O157:H7 using the ClusPro tool. The docking studies revealed good binding affinity, with scores less than -800 for both CXCR1 and E. coli O157:H7, indicating stable interactions. However, while the computational models provided significant insights, it is important to acknowledge the limitations inherent in in silico approaches. These models may not fully capture the complexity of in vivo conditions, necessitating further experimental validation. Simulations and wet lab experiments are crucial for substantiating these findings and fully understanding the AMP interactions.

Future research should prioritize in vitro and in vivo studies to confirm the efficacy of these peptides. Additionally, exploring the interaction dynamics over time and under varying conditions could offer deeper insights into the behavior and efficacy of these AMPs. Understanding their in vivo efficacy will be pivotal for their potential application in food preservation and cancer therapy. This study underscores the potential of AMPs designed through an in silico approach, providing valuable insights into their structural and interaction dynamics. The promising results from docking studies highlight their potential for practical applications in combating food pathogens and cancer, paving the way for future experimental validations and therapeutic developments.

VI. FUTURE PROSPECTS

Future research should focus on the in vivo validation of the antimicrobial and anticancer efficacy of the designed plasmid, using relevant animal models to confirm its safety and effectiveness. Ensuring the safety profile and immunogenicity of the AMPs in vivo is crucial to avoid adverse immune responses. Additionally, optimizing the plasmid design to enhance its expression and stability in bacterial hosts, and exploring different promoter and terminator sequences to improve performance, will be essential. Peptide engineering could further enhance the stability, efficacy, and spectrum of activity of the AMPs, improving their pharmacokinetic and pharmacodynamic properties.

Targeting protein-protein interactions (PPIs) through in silico tools offers a promising approach for treating various diseases, including cancer, infectious diseases, and neurodegenerative conditions. High-throughput screening of large peptide libraries, identifying “hotspots” on protein surfaces, and 3D peptide modelling are crucial for designing effective interfering peptides. Hybrid peptides, combining natural and synthetic components, can be created using in silico-based design to combat multidrug-resistant pathogens, leveraging computational approaches to utilize biological resources effectively.

In food preservation, in silico-designed AMPs can be incorporated into plasmids to inhibit spoilage bacteria, molds, and yeasts, thereby extending the shelf life of perishable foods. Evaluating their stability, solubility, and toxicity is essential for designing plasmids to express these AMPs in food matrices. AMPs can also disrupt biofilms formed by foodborne pathogens such as *Salmonella* and *E. coli*, enhancing food safety by preventing bacterial attachment. Prediction of AMPs targeting biofilm components, followed by optimization of peptide length and charge, is crucial for effective biofilm penetration.

For cancer therapy, in silico prediction of AMPs with anticancer properties can help specifically target cancer cells while minimizing damage to healthy tissues. Screening databases for AMPs with tumor-specific receptors and predicting binding affinity using molecular docking simulations are essential steps. AMPs can induce apoptosis in cancer cells by disrupting cell membranes or intracellular processes, inhibiting tumor growth.

Identifying AMPs that disrupt mitochondrial function or inhibit anti-apoptotic proteins, followed by optimization of peptide stability and cellular uptake, is vital for effective cancer treatment.

Combination therapy involving plasmids containing AMPs alongside conventional chemotherapy can enhance drug delivery, reduce side effects, and combat drug-resistant cancer cells. Predicting interactions between AMPs and chemotherapeutic drugs and optimizing drug delivery using AMP conjugated nanoparticles are promising strategies. Additionally, AMPs can modulate immune responses, aiding in wound healing and tissue repair, and enhance the host's defense against pathogens in food safety. Predicting AMP interactions with immune receptors and optimizing peptide stability and immunomodulatory effects are essential for harnessing these benefits.

Overall, the integration of computational tools in the design and evaluation of AMPs provides a robust framework for developing novel therapeutic agents with dual functionality. Scaling up production methods for industrial applications and ensuring cost-effectiveness and regulatory compliance will facilitate industrial and clinical translation. Finally, designing and conducting clinical trials to assess the safety and efficacy of AMPs in humans, in collaboration with clinical researchers and healthcare institutions, will be essential to bringing these innovations from the lab to the clinic.

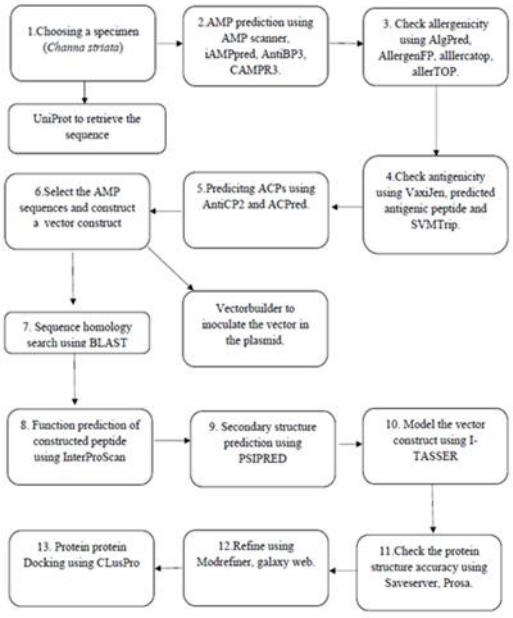

Fig A1. Bioinformatics pipeline. This figure illustrates the comprehensive workflow employed in this study, detailing each step from data collection and preprocessing to analysis and interpretation. The pipeline provides a clear and structured overview of the methodological framework that underpins the research findings presented in this paper

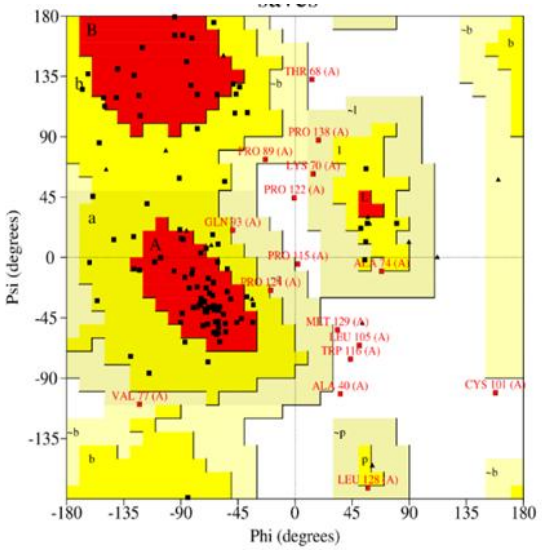

Fig A2. Ramachandran plot which again validates the modelled protein using I-TASSER, this Ramachandran plot is generated using SAVEServer tool which shows the distribution of phi and psi angles for a protein structure, highlighting residues in favoured, allowed and disallowed regions

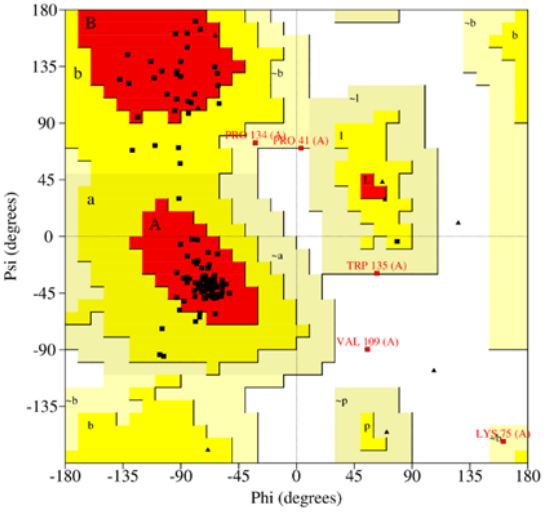

Fig A3. This Ramachandran plot is of refined protein from GalaxyWeb, this plot is generated using the SAVEServer validation tool. It tells a detailed information on the residues of a protein.

85.6% of residues are in the most favoured regions, 12.0% of residues are in additional allowed regions and 0.8% of residues are in disallowed regions.

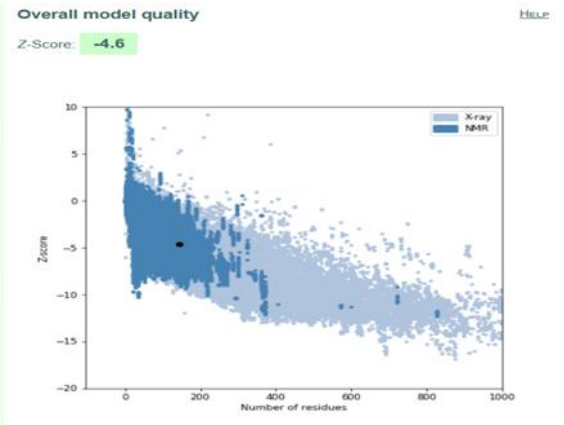

Fig A4. The quality of the protein modelled using I TASSER was validated using ProSAweb so it illustrates the relationship between the number of residues in protein structures and their Z-scores, with lower Z-score indicating better model quality. The models derived from X-ray crystallography has a good quality than NMR.

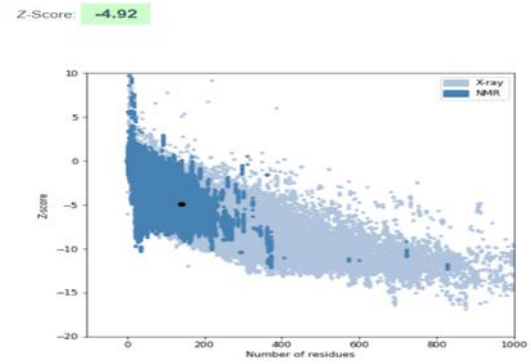

Fig A5. The quality of the refined protein is validated using ProSAweb which reveals a specific high-quality model is highlighted with a z-score, more less the value indicates a high quality model

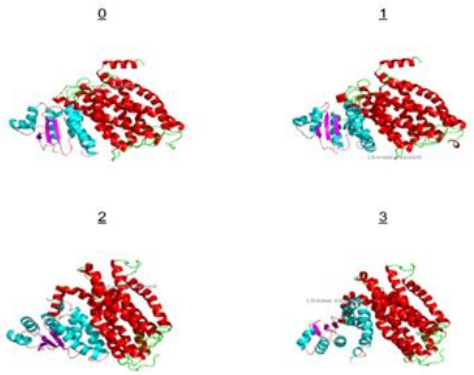

Fig A6. Docking interactions of the predicted antimicrobial peptide (vector construct) protein with CXCR1, showing different binding conformations. Cluster scores indicate binding stability, with lower scores suggesting more stable interactions. Cluster scores for this are represented in Fig 9.

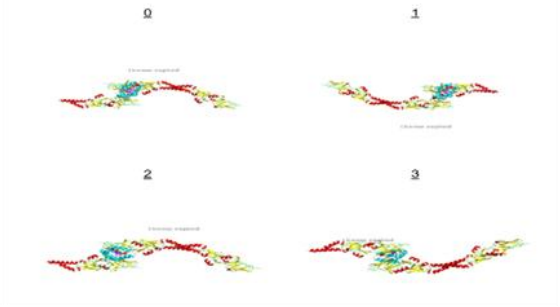

Fig A7. Docking interactions of the predicted antimicrobial peptide (vector construct) protein with EcoliO157:H7, showing different binding conformations. Cluster scores indicate binding stability, with lower scores suggesting more stable interactions. Cluster scores for this are represented in Fig 10.

VIII. ACKNOWLEDGMENT

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