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Plant-derived Antimicrobial Peptides from *Ocimum tenuiflorum* against *Streptococcus pneumoniae* and its role in treating infections: *In Silico* Approach

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Abstract. Antimicrobial resistance is one of the growing global concerns that has resulted in the need for new and effective antimicrobials. A medicinal plant, *Ocimum tenuiflorum* is used to predict the potential antimicrobial peptides (AMPs) via computational (*in silico*) approach. The protein sequences of *Ocimum tenuiflorum* were searched using various bioinformatics tools to identify the peptide regions that are likely to have potential to become an antimicrobial peptide. The predicted peptide was also evaluated for physicochemical properties, structure and molecular docking to test the interaction of the peptide with the targeted bacteria i.e *Streptococcus pneumonia*. This study demonstrates that *in silico* approach is feasible for the low cost, high throughput screening of peptides before *in vitro* experimentation. The results indicate that the peptide from *Ocimum tenuiflorum* can be considered as a source of natural AMPs. It is better to check the antimicrobial activity of the peptides experimentally to check its effectiveness and application against multi-drug resistant pathogen.

Keywords: Antimicrobial resistance, antimicrobial peptides, computational tools, *Ocimum tenuiflorum*

1. Introduction

Antimicrobial resistance (AMR) is a global health challenge and severely reducing the efficacy of conventional antibiotics. Bacterial pathogens have evolved a variety of resistance mechanisms, making many treatment strategies ineffective. This issue has been accelerated by the inappropriate use, over-prescription and misuse of antibiotics in both human and veterinary medicine. According to the World Health Organization (WHO), there has been a rise in resistance to antibiotics, with many infections now resistant to first-line drugs As a result, multidrug-resistant (MDR) and extensively drug-resistant

(XDR) bacterial strains are on the rise, representing a significant public health challenge (Sinha & Upadhyay, 2025). To address this global challenge, there is a pressing need for alternative and renewable sources of antimicrobial agents. Natural products have historically been an important source of bioactive compounds with a wide range of pharmacological activities. These compounds are generally less toxic, more biocompatible and less prone to resistance than synthetic antibiotics. In particular, plant-derived antimicrobials have attracted considerable interest because of their structural diversity and potency against resistant pathogens (Angelini, 2024; Shin et al., 2018). Secondary plant metabolites such as flavonoids, alkaloids and terpenoids, have been widely used for centuries to treat infections. These molecules are naturally produced by plants as a part of their defence mechanism and have a role in fighting microbial pathogens, making them potential drug leads (Helmy et al., 2023; Yang et al., 2018). Apart from these small chemicals, plant-derived antimicrobial peptides (AMPs) have also been identified as promising antibiotic substitutes. AMPs usually kill microbes by disrupting their membrane or interfering with their internal processes, resulting in cell death. Critically, their mode of action lessens the risk of developing resistance and shows low toxicity to the host (Mousa et al., 2025; Talapko et al., 2022).

The plant family Lamiaceae is one of the most significant groups of medicinal plants, comprising over 7,000 species across 236 genera. Members of this family are widely recognized for their extensive therapeutic applications and rich phytochemical profiles. The genus within the family Lamiaceae e.g. *Mentha*, *Ocimum*, *Origanum*, *Rosmarinus* and *Thymus* yield a range of secondary compounds such as alkaloids (including flavonoids, tannins, and quinones) and terpenes that impart their therapeutic properties. Recent studies have highlighted their antimicrobial potential, making them a promising source for novel drug candidates (Singh et al., 2025; Uikey, 2024). Among these, *Ocimum tenuiflorum* (holy basil or Tulsi) has been extensively used in traditional medicine due to its antimicrobial, anti-inflammatory, and immunomodulatory properties. Despite its well-documented phytochemical profile, the peptide-based antimicrobial potential of this plant remains relatively unexplored, particularly through computational approaches. Therefore, the present study focuses on the *in silico* screening and design of a protein-derived peptide from *Ocimum tenuiflorum*, followed by its structural and functional characterization using advanced bioinformatics tools. Furthermore, molecular docking analysis was performed to evaluate the interaction between the designed peptide and a selected bacterial target protein. This integrated computational approach provides a cost-effective strategy for identifying novel peptide candidates with potential therapeutic applications against resistant pathogens.

2. Materials and Methods

2.1 Sequence Retrieval and Antimicrobial Activity Prediction Tools

The amino acid sequence of the chloroplast ribosomal protein L23 (Accession: YP_009673976) was retrieved from NCBI (https://www.ncbi.nlm.nih.gov). The sequence was downloaded in FASTA format and used as the source for designing antimicrobial peptide candidates. Overlapping truncated fragments (10–30 amino acids) were generated using a sliding-window approach.

2.2 Antimicrobial Activity Prediction

Antimicrobial Activity was predicted and evaluated using *in-silico* prediction tools, including APD3 (https://aps.unmc.edu/), CAMPR4 (https://camp.bicnirrh.res.in/) and DBAASP (https://dbaasp.org/).

2.3 Tools used for Characterization

Physicochemical properties were characterized using the PepCalc online tool to assess its net charge, molecular mass, isoelectric point, stability, solubility, and overall suitability as an antimicrobial candidate. The cytotoxicity of the designed peptide was evaluated using the ToxinPred server (reference). The hemolytic potential of **t**he peptide was analyzed using DBAASP (Al-Khdhairawi et al., 2023) and HemoPI 2.0 (Rathore et al., 2025). HLP server (https://webs.iiitd.edu.in/raghava/hlp/) was used to assess the half-life stability of the peptide. Structure prediction tools such as PEP-FOLD3 were used.

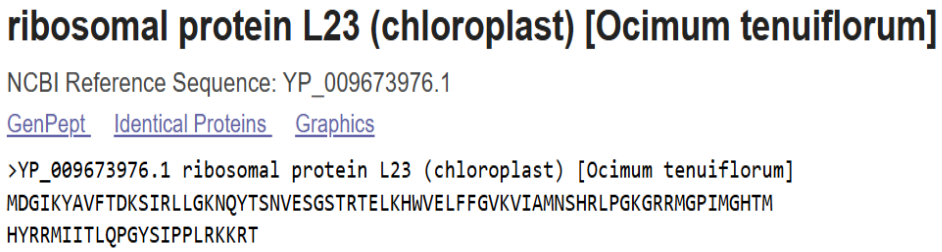
2.4 Molecular docking

Molecular docking was performed using HADDOCK 2.4 (High Ambiguity Driven Protein–Protein Docking) to evaluate the binding affinity, interaction profile of the designed novel peptide against the target protein of *Streptococcus pneumoniae* and compare its efficiency with LL37 (positive control) and scramble sequence (negative control). penicillin-binding protein 2x (PDB ID: 1QMF) of *Streptococcus pneumoniae* was selected for the study.

3. Results and discussion

3.1 Source Selection and Antimicrobial Activity Prediction

The amino acid sequence of the chloroplast ribosomal protein L23 (Accession: YP_009673976) of *Ocimum tenuiflorum* comprised of 93 amino acid residues (Fig 1). A small ribosomal protein with 93 amino acid residues, suggests that it is involved in crucial structural and functional roles within the ribosome, such as in the assembly and function of the ribosome during protein synthesis. The small size of L23 is in agreement with other known chloroplast ribosomal proteins, which often have conserved structures that interact with the rRNA and nascent proteins (Kramer et al., 2002; Zoschke & Bock, 2018). Functionally, small proteins like L23 are ideal candidates for peptide development, as they often contain short conserved sequences which may be bioactive. These can be readily searched and refined for antimicrobial activity in silico. Besides, the small size of the protein also allows for effective *in silico* analysis, such as sequence prediction, protein structure modelling and peptide design. In addition, antimicrobial peptide like sequences have been identified in ribosomal proteins in the past, suggesting they can be used as starting points for new peptide design (Hurtado-Rios et al., 2022; Pulido et al., 2018).Therefore, the discovery of a 93-amino-acid-long L23 protein offers an ideal and logical template for the development of small bioactive peptides in this study.


ribosomal protein L23 (chloroplast) [Ocimum tenuiflorum]

NCBI Reference Sequence: YP_009673976.1

GenPept Identical Proteins Graphics

>YP_009673976.1 ribosomal protein L23 (chloroplast) [Ocimum tenuiflorum]
MDGIKYAVFTDKSIRLLGKNQYTSNVESGSTRTELKHWVELFFGVKVIAMNSHRLPGKGRRMGPIMGHTM
HYRRMIITLQPGYSIPPLRKKRT

Fig 1: Ribosomal protein L23 sequence from *Ocimum tenuiflorum* (NCBI accession YP_009673976.1)

3.2 Antimicrobial Activity Prediction

Out of all the truncated fragments analysed for antimicrobial activity (data not shown), 16-amino-acid peptide fragment **(**GMPKVSIRLAFNVHGK**)** derived from the chloroplast ribosomal protein L23 of *Ocimum tenuiflorum* exhibited the highest antimicrobial probability. This peptide was selected as a result of the power of computational truncation approaches in

pinpointing bioactive regions within large proteins. These techniques are increasingly being used to discover cryptic antimicrobial peptides hidden within functional proteins, which may not be apparent during traditional experimental screening (Agüero-Chapin et al., 2022; Aronica et al., 2021; Zasloff, 2002). Based on these findings, this peptide was selected for further study. The antimicrobial peptide designer (APD3) database classified the peptide as a cationic antimicrobial peptide, exhibiting a high positive charge (+3) and amphipathic characteristics (Fig 2a). Similarly, collection of Anti-Microbial Peptides (CAMPR4) prediction using the SVM classifier indicated a probability score above the AMP threshold i.e. 0.87, confirming strong antimicrobial potential (Fig 2b). The constructed peptide GMPKVSIRLAFNVHGK is a fundamental point to the computational screening platform where antimicrobial motifs can be predicted resulting in potential new peptide-based interventions against *Streptococcus pneumoniae* related infections. The therapeutic potential of plant-derived molecules is supported by the report that the leaf extracts of *Ocimum tenuiflorum* have the ability to inhibit the growth of *S pneumoniae* (Haidari et al., 2023). The APD3 categorisation of the selected peptide as a cationic antimicrobial peptide also suggests its potential activity. The overall positive charge (+3) is a key characteristic of many antimicrobial peptides, as it allows electrostatic interactions with negatively charged bacterial surfaces. Moreover, the amphipathic structure of the peptide in which the hydrophobic and hydrophilic residues are spatially separated facilitates membrane insertion and disruption, a common mechanism of action of many antimicrobial peptides (Lee et al., 2015; Poojitha et al., 2023). Consistently with this, a high probability (0.87) was found in the prediction from the CAMPR4 using the support vector machine (SVM) classification model, which is above the AMP threshold. This consistency between distinct computational tools increases the trust in the prediction and prevents false positives. It is considered a good practice in peptide screening to use multiple prediction tools, so that the chances of identifying the potential peptides of interest are maximised (Gawde et al., 2023). Importantly, the physicochemical characteristics such as size, charge and amphipathicity - of the new peptide are similar to those of other known broad-spectrum, low resistance-inducing, antimicrobial peptides. This suggests the new peptide may induce bacterial cell death by targeting the membrane, causing rapid death. This is especially important for pathogens like *Streptococcus pneumoniae*, which are known to be difficult to treat due to antibiotic resistance. Moreover, the antibacterial activity of *Ocimum tenuiflorum* is validated by experimental reports showing that the leaves of the plant can suppress *Streptococcus pneumoniae* (Haidari et al., 2023; Srichok et al., 2022). This serves as a biological confirmation of the computational results and the importance of plant-derived molecules in the discovery of new antimicrobials. To summarise, the discovery of the peptide GMPKVSIRLAFNVHGK is an important step towards computational peptide drug discovery. Its potential antimicrobial activity, as predicted by several in silico models

and its favourable physicochemical properties underline its suitability for further molecular structure design and docking, followed by experimentation against *Streptococcus pneumoniae* infections.

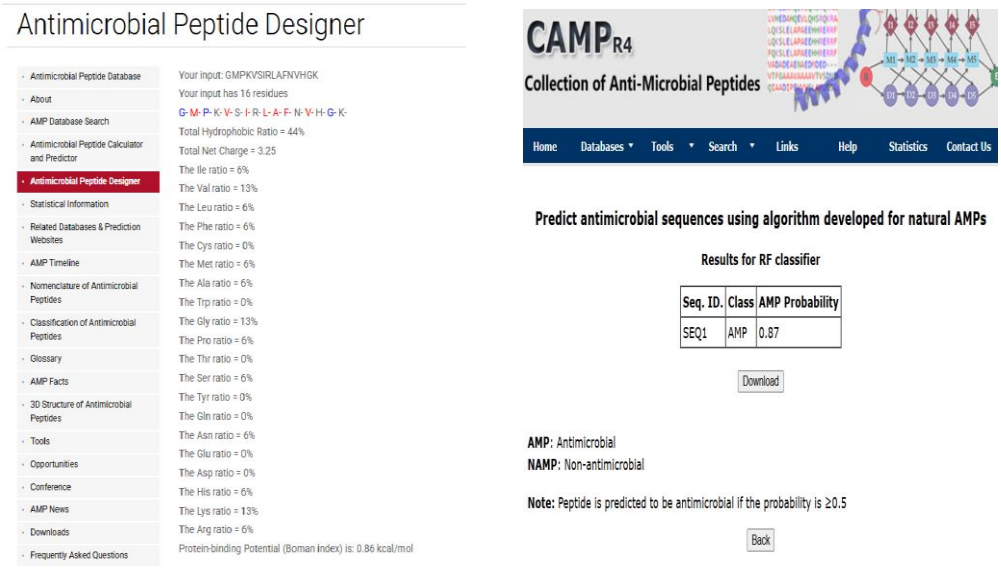


Fig. 2. Analysis of the designed peptide using two AMP prediction tools. (a) APD3 output showing the peptide's physicochemical characteristics. (b) CAMPR4 RF classifier result predicting the peptide as antimicrobial.

3.3 Physicochemical Characterization

The 16 amino acids peptide fragment consisted of approximately 1754.11 g/mol and an isoelectric point (pI) of 11.58, indicating a strongly cationic nature at physiological pH (Fig 3a-b). The peptide displayed a moderate hydrophobicity ratio (44%), suggesting potential amphipathic behaviour, which is essential for membrane insertion and disruption. The selected peptide has good water solubility and cationic charge (+3), making it suitable for biological interaction in aqueous environments along with former mentioned features indicating the strong interaction with negatively charged bacterial cell membrane. The peptide contains a high proportion of positively charged residues (lys), which contribute to electrostatic attraction toward negatively charged bacterial membranes, a key feature of antimicrobial peptides. Pfalzgraff et al. (2018) and Hsu et al. (2024) emphasized that small, amphipathic peptides with high positive charge are promising therapeutic candidates due to their biocompatibility and membrane-targeting selectivity (Hsu et al., 2024; Pfalzgraff et al., 2018).

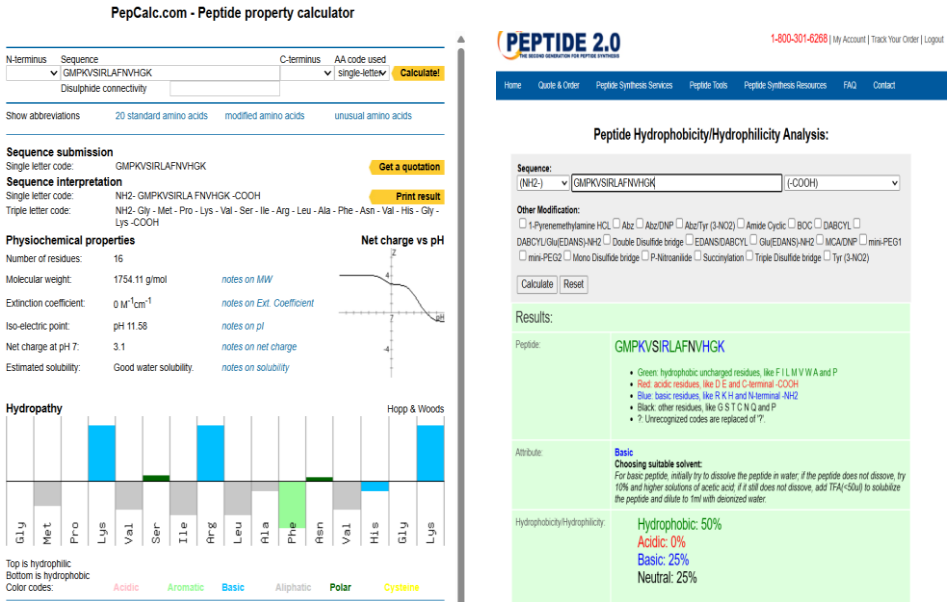


Fig. 3. Pepcalc and peptide 2.0 tool shows a) amphiphilic nature of peptide b) hydrophobic ratio of peptide

3.4 Cytotoxicity Prediction and Haemolytic Prediction

The prediction result by ToxinPred indicated that the peptide is non-toxic toward mammalian cells**,** suggesting its safe biological profile and biocompatible nature for further therapeutic or biomedical applications (Fig 4). The non-toxic prediction supports the potential of peptide. Besides, DBAASP predicts value of 0.83 further suggesting that the peptide is non-hemolytic and is safe for mammalian cells (Fig 5). The greater the size of peptide, higher is its correlation with the hemolysis. Hemolysis assays are thus a fast and accurate initial screening method for toxicity of peptide especially in case of membrane-active antimicrobials (Greco et al., 2020; Robles-Loaiza et al., 2022). Differences in the membrane of the erythrocytes such as difference in lipid/protein ratios, transmembrane proteins, aquaporins and Na^+/K^+/ATPase content can be a cause of difference in osmotic fragility and toxicity (Farag & Alagawany, 2018).

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Therefore, it is expected that peptides with a low hemolytic activity in these assays have good biocompatibility and low potential of disrupting mammalian membranes (Olsen et al., 2010; Wan et al., 2022).Therefore, the peptide can be considered biocompatible**,** an important property for its possible therapeutic and antimicrobial applications**.** This feature is essential for developing peptide-based therapeutics with minimal adverse effects on host cells.

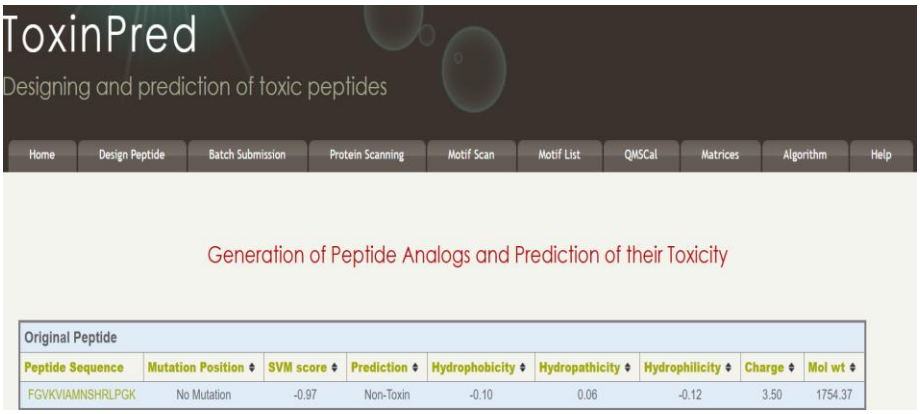

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Fig. 4. Result shows the non-toxic nature of the peptide as predicted by ToxinPred

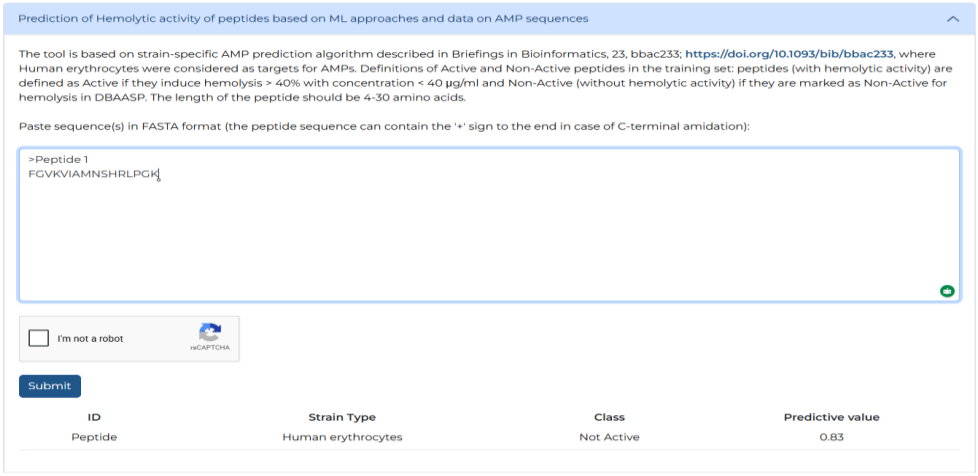
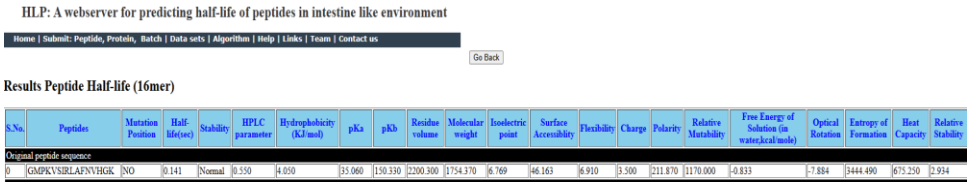


Fig. 5. Selected peptide sequence is not active against human erythrocytes with predictive value 0.85

3.5 Half-Life stability

Half-life stability of peptide sequence GMPKVSIRLAFNVHGK was shown as 0.14 seconds. The hydrophobicity (4.050 Kj/mol) of peptide sequence suggests the strong non-polar interactions with net charge 3, which may affect its solubility in biological environments (Fig 6). The peptide has higher molecular weight (1754.37 g/mol) with lower isolelectric point (6.7) represents it's different behaviour at different pH levels. The peptide is more favorable for dissolution in water due to lower free energy of solution -0.833 Kcal/mole). The short half-life (predicted to be 0.14 seconds) indicate the natural susceptibility of unmodified antimicrobial peptides to fast proteolytic degradation, especially at simulated enzymatic conditions. This could reduce the potential of oral delivery, but this kind of rapid turnover is typical of native AMPs and is not a reason that they cannot be

used in topical or localized treatment where the rapid antimicrobial response is adequate (Shao et al., 2019). Moreover, the rational amino acid substitutions or cyclizations may be done to increase stability, and, therefore, the currently predicted results can be evaluated as a screening phase before optimising the peptide and testing it in the laboratory (Pei et al., 2025).


HLP: A webserver for predicting half-life of peptides in intestine like environment

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Results Peptide Half-life (16mer)

S.No.	Peptides	Mutation Position	Half-life(sec)	Stability	HPLC parameter	Hydrophobicity (KJ/mol)	pKa	pKb	Residue volume	Molecular weight	Isoelectric point	Surface Accessibility	Flexibility	Charge	Polarity	Relative Mutability	Free Energy of Solution (in water,kcal/mole)	Optical Rotation	Entropy of Formation	Heat Capacity	Relative Stability
Original peptide sequence																					
0	GMPKVSIRLAFNVHGK	NO	0.141	Normal	0.550	4.050	35.060	150.330	2200.300	1754.370	6.769	46.163	6.910	3.500	211.870	1170.000	-0.833	-7.884	3444.490	675.250	2.934

Fig. 6. Result showing half life of 16 mer peptide

3.6 Structure Prediction and Visualization

The primary structure of selected peptide of 16 amino acids GMPKVSIRLAFNVHGK showed a linear chain of amino acids (Fig 7a). The Secondary structure of peptide showed the selected peptide has extended strands and coiled structure with blue N terimal and red for C terminal (Fig 7b). The tertiary structure of peptide showed coiled structure the compact conformation without any beta sheets and alpha-helices, its structural stability and potential biological activity (Fig 7c). The structural characterisation of the chosen 16-amino acid peptide (GMPKVSIRLAFNVHGK) gives important insights into its conformational behaviour and potential functional implications. The primary structure is the sequence of amino acids related together in a linear chain. This enables the folding and structure. Secondary structure analysis also suggested that the peptide is predominantly present as extended strands with random coil regions in between, suggesting a flexible and less ordered conformation rather than a rigid secondary structure. Such flexibility is often associated with peptides that show dynamic interaction with biological targets. The tertiary structure showed that the peptide adopts a compact conformation with coiled regions dominating; no obvious α-helices or β-sheets were observed. The absence of well-defined secondary structural elements suggests that the peptide may have characteristics of intrinsically disordered peptides (Wang, 2015). Such structural plasticity is of particular importance for membrane-active peptides enabling them to interact with diverse targets and environments. The structural features of the selected peptide, its coil-rich secondary

and tertiary organization with no rigid folding, support its potential functional significance in general. These properties may provide improved adaptation in interaction and biological activity, indicating its potential use as a bioactive peptide.

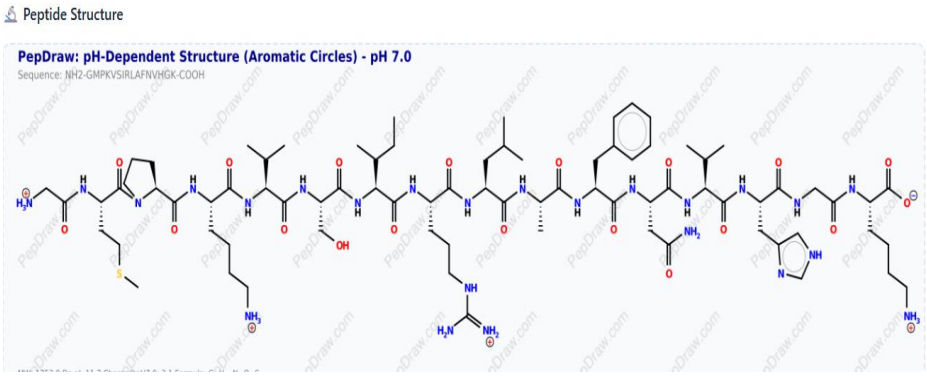
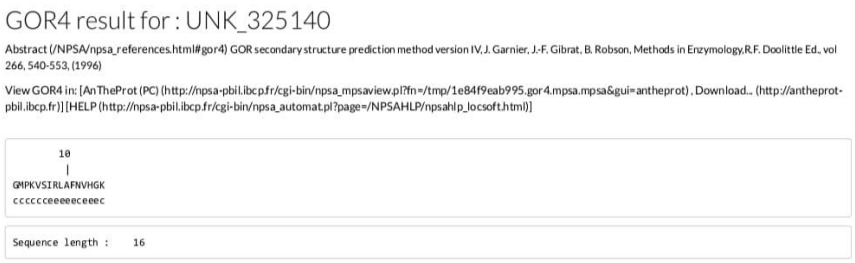


Fig. 7(a). Linear chain of amino acid residues of selected peptide draw by using PepDraw.



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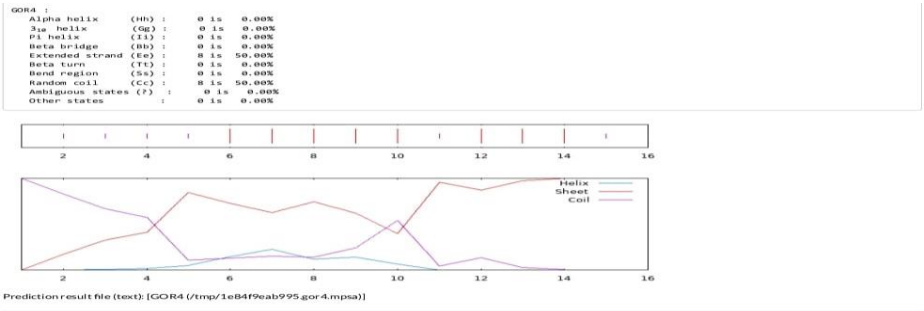

Fig. 7(b). Secondary structure of amino acid residues of selected peptide draw by using PepDraw.

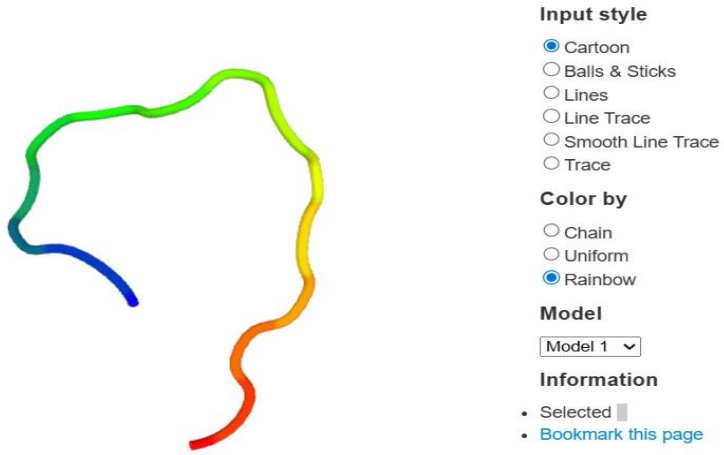

Fig. 7(c). The tertiary structure is represented in a cartoon style with a rainbow gradient, representing simple coiled structure of peptide from red (C-terminal) to blue colour (N-terminal).

3.7 Structure of Target Protein

The target protein Penicillin binding protein (PBP2x**)** (PDB ID: 1QMF**)** from *Streptococcus pneumoniae* was selected for the study. Penicillin-Binding Protein, PBP2x is the first protein to undergo mutations when the bacteria is exposed to β-lactam antibiotics (like penicillin or cephalosporins)**.** The Ramachandran plot analysis revealed

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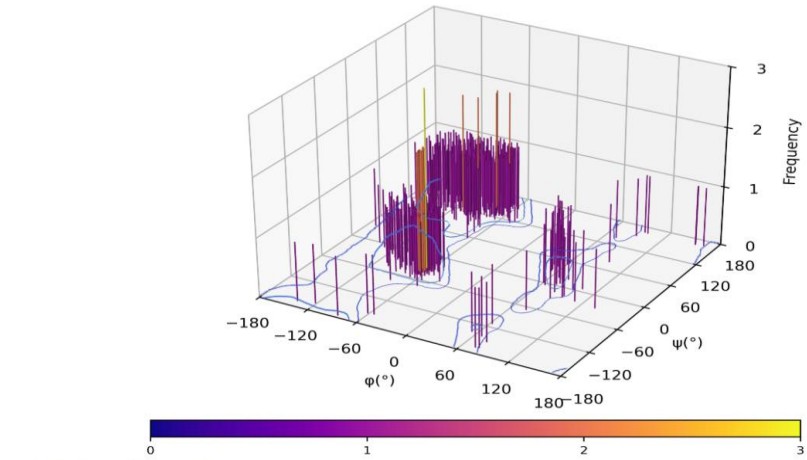
that 95.4% of the residues were located in the most favoured regions, while 4.17 % fell within the additionally allowed regions. This distribution is indicative of good stereochemical quality and structural integrity of the protein model. Only 0.36% of residues occupied generously allowed regions, and importantly, no residues were observed in the disallowed regions, indicating a well-refined and geometrically acceptable model. The dihedral angle parameters (phi–psi, chi1–chi4) and main-chain covalent geometry (bond lengths and bond angles) demonstrated favourable stereochemical characteristics (Fig 8a-c). Favoured regions with high occupancy are indicative of energetically stable backbone conformations and are a hallmark of well resolved protein structures (Ramachandran et al., 1963). Furthermore, the dihedral angles (φ–ψ and χ1–χ4) and main-chain covalent geometry (bond lengths and bond angles) displayed values within the acceptable ranges, confirming the absence of steric hindrances or unusual conformations. Such favourable stereochemical parameters are essential for the validation of protein structures prior to molecular docking or simulation studies, as they ensure realistic molecular interactions and binding predictions (Naqvi et al., 2018).The structural analysis of the target protein Penicillin-Binding Protein 2x (PBP2x) (PDB ID: 1QMF) from *Streptococcus pneumoniae* confirms the reliability and suitability of the model for further downstream studies. PBP2x is a major transpeptidase in bacterial cell wall biosynthesis and is well known to be one of the major targets of mutation under selective pressure of β-lactam antibiotics. These mutations are strongly associated with the development of antibiotic resistance, particularly against penicillins and cephalosporins (Dabhi et al., 2024). Overall, the high-quality structural validation of PBP2x indicates that the model is geometrically sound and appropriate for further computational studies. The validated structure, due to its central role in ß-lactam resistance, provides a reliable framework for studying ligand binding, peptide interaction and potential inhibitor design.



8 a

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Results

Ram Plot Analysis
JobID RamPlot69edccfdb7a6b 2026-04-26 13:59:52.202372
Total Residue 551
Ramachandran plot (Standard) statistics
Favoured: 526 (95.463%)
Allowed: 23 (4.174%)
Disallowed: 2 (0.363%)
Disallowed Residues
SN. PDB ID Chain Residue Residue No PHI PSI
1. RECEPTOR A ALA 554 -62.83 -156.46
2. RECEPTOR A GLU 556 -55.82 -155.42

8 b

Ramachandran plot (six categories) statistics
Favoured: 525 (95.281%)
Allowed: [illegible]%)
Disallowed: 2 (0.363%)
Disallowed Residues
SN. PDB ID Chain Residue Residue No PHI PSI
1. RECEPTOR A ALA 554 -62.83 -156.46
2. RECEPTOR A GLU 556 -55.82 -155.42
Ramachandran plot (Seven categories) statistics
Favoured: 528 (95.281%)
Allowed: 20 (4.356%)
Disallowed: 3 (0.363%)
Disallowed Residues
SN. PDB ID Chain Residue Residue No PHI PSI
1. RECEPTOR A ALA 554 -62.83 -156.46
2. RECEPTOR A ASP 555 73.16 138.58
3. RECEPTOR A GLU 556 -55.82 -155.42

8 c

Fig. 8. (a)–(c). (a) Ramachandran plot of the modeled peptide structure prepared using RAMPLOT, indicating the distribution of residues within favoured, allowed and disallowed regions (b) Statistics summarizing the locations of the residues with 95.28% of the residues in the most favoured regions and none in the disallowed region (c) Statistics analysis of the model representing the stereochemical parameters.

3.8 Molecular Docking of novel peptide against *Staphylococcus aureus*

The docking results indicate that the novel peptide (Cluster 8) demonstrates superior binding energetics compared to both the controls (to what??), achieving the most favorable HADDOCK score of -92.0 +/- 8.1, which is significantly lower than the Positive Control's -68.8 +/- 2.5 and the negative Control's -87.0 +/- 1.3. While the positive control (LL37) exhibits the strongest statistical reliability with a Z-Score of -2.7 and the largest buried surface Area (1945.9 +/- 117.9), the Novel Peptide maintains a highly competitive Z-Score of -2.2 and a substantial interface area of 1677.9 +/- 70.7 (fig 9a-c).

Energetically, the binding of the positive control is most heavily driven by electrostatic energy (-219.0 +/- 50.5), whereas the novel peptide shows a more balanced profile with strong electrostatics (-162.5 +/- 14.9) and comparable Van der Waals energy (-51.0 +/- 5.6). Notably, the novel peptide appears more structurally compatible with the binding site, as evidenced by its significantly lower restraints violation energy (96.0 +/- 79.6) compared to the high violation energy of the positive Control (393.7 +/- 59.4) and the Negative Control (163.6 +/- 24.1) (Table 1). Overall, the Novel Peptide shows high-affinity binding that is more energetically favorable than LL37, while maintaining much better structural integrity within the docking constraints. Overall, The experimental molecular docking analysis revealed that the novel designed peptide is stable in terms of binding and structurally compatible with the PBP2x target protein.

The relative energy analysis of peptide-protein interactions gives important insights into the binding behaviour and stability of the designed peptide against the target Penicillin Binding Protein 2x (PBP2x). The positive control peptide, LL-37, exhibited binding mainly driven by strong electrostatic interactions (−219.0 ± 50.5), indicating that charge–charge interactions are the dominant factor in the stabilisation of its complex with the protein. Although electrostatic dominance may enhance initial binding, it can also indicate decreased flexibility and a reliance on ionic interactions, which may sometimes impede structural adaptability in the binding pocket. In contrast, the novel peptide showed a more balanced energetic profile, dominated by significant electrostatic contributions (−162.5 ± 14.9) with similar Van Der Waals interactions (−51.0 ± 5.6). This balance indicates that,

in addition to long-range electrostatic forces, short-range hydrophobic and dispersion interactions contribute significantly to binding stabilisation. Such an energy distribution is often associated with more stable and biologically relevant complexes, since it reflects optimal complementarity between the ligand and the binding site (Du et al., 2016). One of the main differentiating factors was the restraints violation energy, with the novel peptide showing much lower values (96.0 ± 79.6) than the positive control (393.7 ± 59.4) and the negative control (163.6 ± 24.1). Lower violation energy indicates fewer steric clashes and better conformity to the geometric constraints of the binding pocket, indicating better structural compatibility. On the other hand, the high violation energy of the positive control signifies probable strain in the complex, which can affect the long-term stability and binding efficiency. Overall, the results suggest that the novel peptide can bind with high affinity and maintain its structural integrity in the docking environment. The better balance of the interaction energies and the lower conformational strain indicates a more favourable and realistic binding mode compared to LL-37. This agrees with previous studies showing that effective peptide inhibitors are usually based on a synergistic contribution of electrostatic and van der Waals interactions and minimal structural distortion upon binding (Du et al., 2016). In summary, the molecular docking results are in favour of the potential of the designed peptide as a stable and structurally compatible ligand for PBP2x. The favourable energy binding profile and lesser geometric violations emphasise its potential as a candidate for further validation including molecular dynamics simulations and experimental assays.

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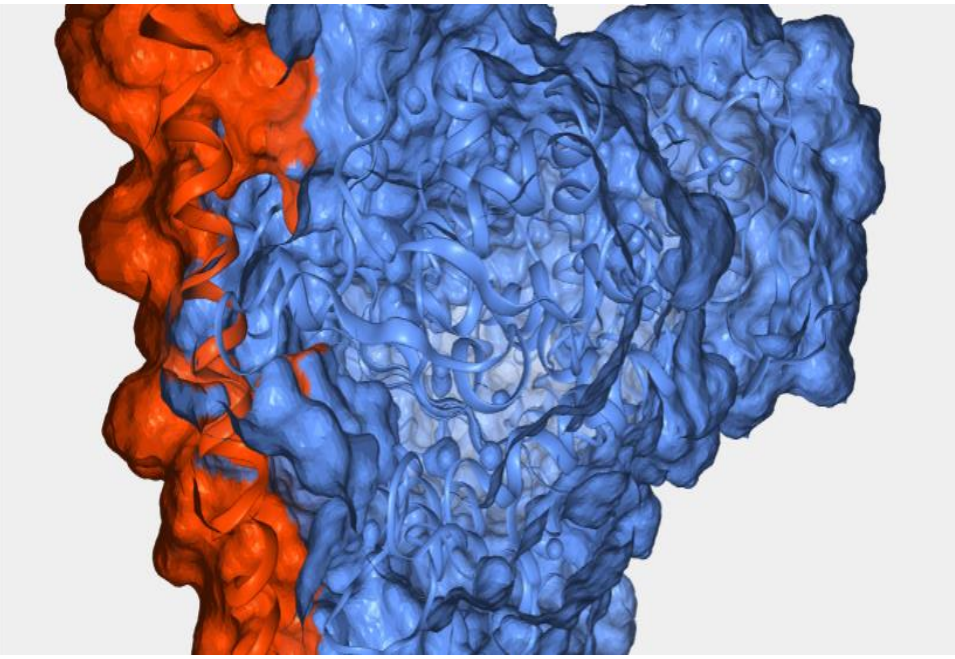

Fig. 9(a). Molecular docking of positive control i.e LL-37 against PBP2x

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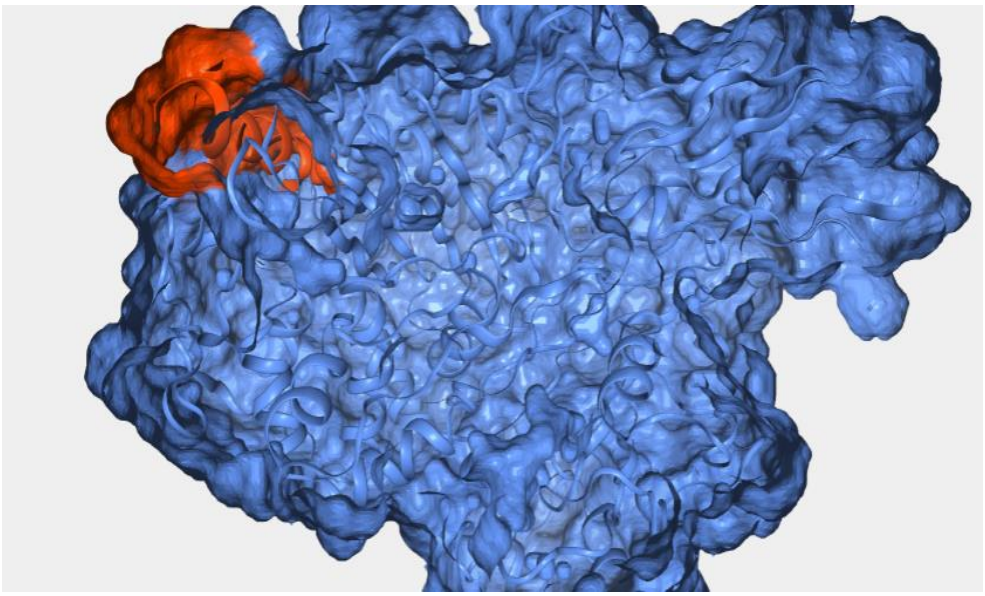

Fig. 9(b). Molecular docking of designed peptide against PBP2x

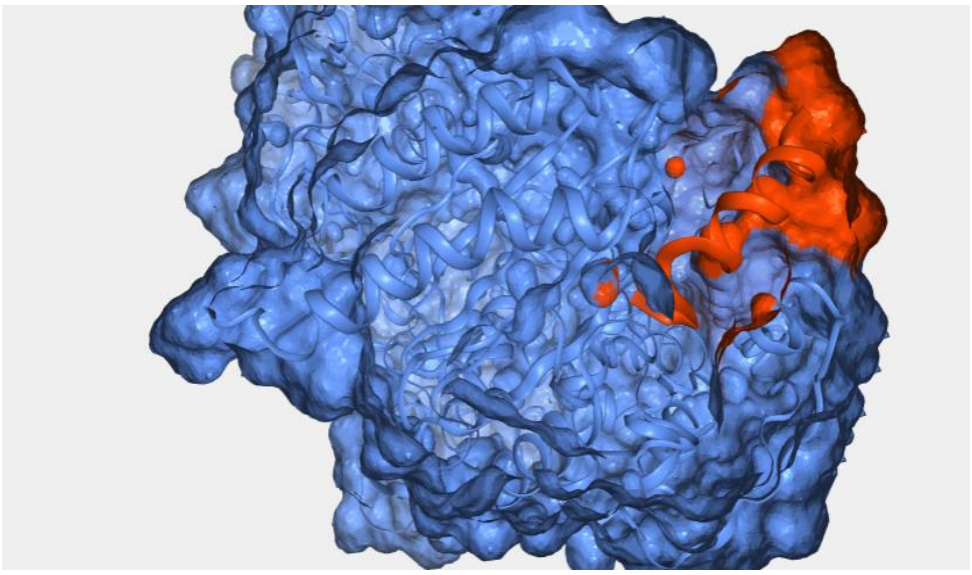

Fig. 9(c). Molecular docking of negative control against PBP2x

Table 1. Docking Parameters of LL-37, Novel Peptide, and Scrambled Control against PBP2a

Parameter	LL-37 (Positive Control)	Novel Peptide	Scrambled (Negative Control)
HADDOCK Score	-68.8 +/- 2.5	-92.0 +/- 8.1	-87.0 +/- 1.3
Z-Score	-2.7	-2.2	-2.0
Electrostatic Energy	-219.0 +/- 50.5	-162.5 +/- 14.9	-143.4 +/- 14.8
Van der Waals Energy	-47.0 +/- 6.0	-51.0 +/- 5.6	-58.2 +/- 3.2
Buried Surface Area	1945.9 +/- 117.9	1677.9 +/- 70.7	1641.7 +/- 42.1
Restraints Violation	393.7 +/- 59.4	96.0 +/- 79.6	163.6+/- 24.1

4. Conclusion and Future Directions

In the present research, a peptide fragment of 16 amino acids (GMPKVSIRLAFNVHGK) was designed based on the chloroplast ribosomal protein L23 of *Ocimum tenuiflorum* and thoroughly analyzed through combined bioinformatics techniques. Extensive physicochemical and structural examination revealed that the peptide has antimicrobial activity compatibility. Molecular docking of Penicillin-Binding protein 2a (PBP2x) of *Streptococcus pneumoniae* showed high binding affinity and fixed patterns of interaction confirming that the modeled peptide possesses reliable geometric integrity and is structurally suitable for downstream molecular docking and interaction studies. PBP2x is the first protein to undergo mutations when the bacteria is exposed to β-lactam antibiotics (like penicillin or cephalosporins)**.** Together, the *insilico* results are positive indicators that the designed peptide may be used as a second-generation antimicrobial agent against β-lactam resistance pathways. Future research must find the minimum inhibitory concentration (MIC) of methicillin-susceptible *Streptococcus pneumoniae in vitro*, followed by in vivo research. Experiments must be conducted to identify its antibacterial activity, mechanism of action, and safety profile. In general, this research can

offer a solid computerized basis for plant-derived antimicrobial peptides establishment as a novel treatment alternative to antibiotic resistance.

References

Agüero-Chapin, G., Galpert-Cañizares, D., Domínguez-Pérez, D., Marrero-Ponce, Y., Pérez-Machado, G., Teijeira, M., & Antunes, A. (2022). Emerging Computational Approaches for Antimicrobial Peptide Discovery. *Antibiotics*, *11*(7), 936. https://doi.org/10.3390/antibiotics11070936

Al-Khdhairawi, A., Sanuri, D., Akbar, R., Lam, S. D., Sugumar, S., Ibrahim, N., Chieng, S., & Sairi, F. (2023). Machine learning and molecular simulation ascertain antimicrobial peptide against Klebsiella pneumoniae from public database. *Computational Biology and Chemistry*, *102*, 107800. https://doi.org/10.1016/j.compbiolchem.2022.107800

Angelini, P. (2024). Plant-Derived Antimicrobials and Their Crucial Role in Combating Antimicrobial Resistance. *Antibiotics*, *13*(8), 746. https://doi.org/10.3390/antibiotics13080746

Aronica, P. G. A., Reid, L. M., Desai, N., Li, J., Fox, S. J., Yadahalli, S., Essex, J. W., & Verma, C. S. (2021). Computational Methods and Tools in Antimicrobial Peptide Research. *Journal of Chemical Information and Modeling*, *61*(7), 3172–3196. https://doi.org/10.1021/acs.jcim.1c00175

Dabhi, M., Patel, R., Shah, V., Soni, R., Saraf, M., Rawal, R., & Goswami, D. (2024). Penicillin-binding proteins: the master builders and breakers of bacterial cell walls and its interaction with β-lactam antibiotics. *Journal of Proteins and Proteomics*, *15*(2), 215–232. https://doi.org/10.1007/s42485-024-00135-x

Du, X., Li, Y., Xia, Y.-L., Ai, S.-M., Liang, J., Sang, P., Ji, X.-L., & Liu, S.-Q. (2016). Insights into Protein–Ligand Interactions: Mechanisms, Models, and Methods. *International Journal of Molecular Sciences*, *17*(2), 144. https://doi.org/10.3390/ijms17020144

Farag, M. R., & Alagawany, M. (2018). Erythrocytes as a biological model for screening of xenobiotics toxicity. *Chemico-Biological Interactions*, *279*, 73–83. https://doi.org/10.1016/j.cbi.2017.11.007

Gawde, U., Chakraborty, S., Waghu, F. H., Barai, R. S., Khanderkar, A., Indraguru, R., Shirsat, T., & Idicula-Thomas, S. (2023). CAMPR4: a database of natural and synthetic antimicrobial peptides. *Nucleic Acids Research*, *51*(D1), D377–D383. https://doi.org/10.1093/nar/gkac933

Greco, I., Molchanova, N., Holmedal, E., Jenssen, H., Hummel, B. D., Watts, J. L., Håkansson, J., Hansen, P. R., & Svenson, J. (2020). Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides. *Scientific Reports*, *10*(1), 13206. https://doi.org/10.1038/s41598-020-69995-9

Haidari, H., Melguizo-Rodríguez, L., Cowin, A. J., & Kopecki, Z. (2023). Therapeutic potential of

antimicrobial peptides for treatment of wound infection. *American Journal of Physiology-Cell Physiology*, *324*(1), C29–C38. https://doi.org/10.1152/ajpcell.00080.2022

Helmy, Y. A., Taha-Abdelaziz, K., Hawwas, H. A. E. H., Ghosh, S., AlKafaas, S. S., Moawad, M. M. M., Saied, E. M., Kassem, I. I., & Mawad, A. M. M. (2023). Antimicrobial Resistance and Recent Alternatives to Antibiotics for the Control of Bacterial Pathogens with an Emphasis on Foodborne Pathogens. In *Antibiotics* (Vol. 12, Issue 2). https://doi.org/10.3390/antibiotics12020274

Hsu, C.-Y., Yousif, A. M., Abullah, K. A., Abbas, H. H., Ahmad, H., Eldesoky, G. E., Adil, M., & Hussein, Z. (2024). Antimicrobial Peptides (AMPs): New Perspectives on Their Function in Dermatological Diseases. *International Journal of Peptide Research and Therapeutics*, *30*(3), 33. https://doi.org/10.1007/s10989-024-10609-7

Hurtado-Rios, J. J., Carrasco-Navarro, U., Almanza-Pérez, J. C., & Ponce-Alquicira, E. (2022). Ribosomes: The New Role of Ribosomal Proteins as Natural Antimicrobials. *International Journal of Molecular Sciences*, *23*(16), 9123. https://doi.org/10.3390/ijms23169123

Kramer, G., Rauch, T., Rist, W., Vorderwülbecke, S., Patzelt, H., Schulze-Specking, A., Ban, N., Deuerling, E., & Bukau, B. (2002). L23 protein functions as a chaperone docking site on the ribosome. *Nature*, *419*(6903), 171–174. https://doi.org/10.1038/nature01047

Lee, T.-H., N. Hall, K., & Aguilar, M.-I. (2015). Antimicrobial Peptide Structure and Mechanism of Action: A Focus on the Role of Membrane Structure. *Current Topics in Medicinal Chemistry*, *16*(1), 25–39. https://doi.org/10.2174/1568026615666150703121700

Mousa, W. K., Shaikh, A. Y., Ghemrawi, R., Aldulaimi, M., Al Ali, A., Sammani, N., Khair, M., Helal, M. I., Al-Marzooq, F., & Oueis, E. (2025). Human microbiome derived synthetic antimicrobial peptides with activity against Gram-negative, Gram-positive, and antibiotic resistant bacteria. *RSC Medicinal Chemistry*, *16*(1), 312–323. https://doi.org/10.1039/D4MD00383G

Naqvi, A. A. T., Mohammad, T., Hasan, G. M., & Hassan, M. I. (2018). Advancements in Docking and Molecular Dynamics Simulations Towards Ligand-receptor Interactions and Structure-function Relationships. *Current Topics in Medicinal Chemistry*, *18*(20), 1755–1768. https://doi.org/10.2174/1568026618666181025114157

Olsen, C. A., Ziegler, H. L., Nielsen, H. M., Frimodt-Møller, N., Jaroszewski, J. W., & Franzyk, H. (2010). Antimicrobial, Hemolytic, and Cytotoxic Activities of β-Peptoid–Peptide Hybrid Oligomers: Improved Properties Compared to Natural AMPs. *ChemBioChem*, *11*(10), 1356–1360. https://doi.org/10.1002/cbic.201000232

Pei, Z., Song, Q., Xu, J., Yu, S., & Ma, H. (2025). The Cyclic Antimicrobial Peptide C-LR18 Has Enhanced Antibacterial Activity, Improved Stability, and a Longer Half-Life Compared to the Original Peptide. *Antibiotics*, *14*(3), 312. https://doi.org/10.3390/antibiotics14030312

Pfalzgraff, A., Brandenburg, K., & Weindl, G. (2018). Antimicrobial Peptides and Their Therapeutic Potential for Bacterial Skin Infections and Wounds. *Frontiers in Pharmacology*,

9. https://doi.org/10.3389/fphar.2018.00281

Poojitha, R., Shrivastav, A., Shrivastava, N., Kumar, N., Singh, S. K., & Ranjan, R. (2023). Antimicrobial Peptides and Their Therapeutic Aspect. *International Journal of Livestock Research*, *13*(1).

Pulido, P., Zagari, N., Manavski, N., Gawronski, P., Matthes, A., Scharff, L. B., Meurer, J., & Leister, D. (2018). CHLOROPLAST RIBOSOME ASSOCIATED Supports Translation under Stress and Interacts with the Ribosomal 30S Subunit. *Plant Physiology*, *177*(4), 1539–1554. https://doi.org/10.1104/pp.18.00602

Ramachandran, G. N., Ramakrishnan, C., & Sasisekharan, V. (1963). Stereochemistry of polypeptide chain configurations. *Journal of Molecular Biology*, *7*(1), 95–99. https://doi.org/10.1016/S0022-2836(63)80023-6

Rathore, A. S., Kumar, N., Choudhury, S., Mehta, N. K., & Raghava, G. P. S. (2025). Prediction of hemolytic peptides and their hemolytic concentration. *Communications Biology*, *8*(1), 176. https://doi.org/10.1038/s42003-025-07615-w

Robles-Loaiza, A. A., Pinos-Tamayo, E. A., Mendes, B., Ortega-Pila, J. A., Proaño-Bolaños, C., Plisson, F., Teixeira, C., Gomes, P., & Almeida, J. R. (2022). Traditional and Computational Screening of Non-Toxic Peptides and Approaches to Improving Selectivity. *Pharmaceuticals*, *15*(3), 323. https://doi.org/10.3390/ph15030323

Shao, C., Zhu, Y., Lai, Z., Tan, P., & Shan, A. (2019). Antimicrobial Peptides with Protease Stability: Progress and Perspective. *Future Medicinal Chemistry*, *11*(16), 2047–2050. https://doi.org/10.4155/fmc-2019-0167

Shin, J., Prabhakaran, V.-S., & Kim, K. (2018). The multi-faceted potential of plant-derived metabolites as antimicrobial agents against multidrug-resistant pathogens. *Microbial Pathogenesis*, *116*, 209–214. https://doi.org/10.1016/j.micpath.2018.01.043

Singh, G., Gupta, S., & Gupta, P. (2025). Antibacterials Derived From the Plants of Lamiaceae: Phytochemical Framework and Mechanistic Insights. *Journal of Pure and Applied Microbiology*. https://doi.org/10.22207/JPAM.19.3.42

Sinha, S., & Upadhyay, L. S. B. (2025). Understanding antimicrobial resistance (AMR) mechanisms and advancements in AMR diagnostics. *Diagnostic Microbiology and Infectious Disease*, *113*(2), 116949. https://doi.org/10.1016/j.diagmicrobio.2025.116949

Srichok, J., Yingbun, N., Kowawisetsut, T., Kornmatitsuk, S., Suttisansanee, U., Temviriyanukul, P., & Chantong, B. (2022). Synergistic Antibacterial and Anti-inflammatory Activities of Ocimum tenuiflorum Ethanolic Extract against Major Bacterial Mastitis Pathogens. *Antibiotics*, *11*(4), 510. https://doi.org/10.3390/antibiotics11040510

Talapko, J., Meštrović, T., Juzbašić, M., Tomas, M., Erić, S., Horvat Aleksijević, L., Bekić, S., Schwarz, D., Matić, S., Neuberg, M., & Škrlec, I. (2022). Antimicrobial Peptides—Mechanisms of Action, Antimicrobial Effects and Clinical Applications. *Antibiotics*, *11*(10),

1417. https://doi.org/10.3390/antibiotics11101417

Uikey, J. (2024). REVIEWING OF PLANT BELONGING TO LAMIACEAE FAMILY. *International Journal of Scientific Development and Research*, *9*.

Wan, P., Wang, Y., Guo, W., Song, Z., Zhang, S., Wu, H., Yan, W., Deng, M., & Xiao, C. (2022). Low-Molecular-Weight Polylysines with Excellent Antibacterial Properties and Low Hemolysis. *ACS Biomaterials Science & Engineering*, *8*(2), 903–911. https://doi.org/10.1021/acsbiomaterials.1c01527

Wang, G. (2015). Improved Methods for Classification, Prediction, and Design of Antimicrobial Peptides. In *Methods in Molecular Biology* (Vol. 1268, pp. 43–66). https://doi.org/10.1007/978-1-4939-2285-7_3

Yang, S.-K., Low, L.-Y., Yap, P. S.-X., Yusoff, K., Mai, C.-W., Lai, K.-S., & Lim, S.-H. E. (2018). Plant-Derived Antimicrobials: Insights into Mitigation of Antimicrobial Resistance. *Records of Natural Products*, *12*(4), 295–396. https://doi.org/10.25135/rnp.41.17.09.058

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, *415*(6870), 389–395. https://doi.org/10.1038/415389a

Zoschke, R., & Bock, R. (2018). Chloroplast Translation: Structural and Functional Organization, Operational Control, and Regulation. *The Plant Cell*, *30*(4), 745–770. https://doi.org/10.1105/tpc.18.00016