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Research Article

BIOINFORMATICS EXPLORATION OF ROYAL JELLY-DERIVED PEPTIDES OF HONEYBEE AS POTENTIAL INHIBITORS OF SELECTED MAJOR VIRULENCE FACTORS IN *NEISSERIA GONORRHOEAE*

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ABSTRACT

Objectives: *Neisseria gonorrhoeae* has become a substantial worldwide health threat as antibiotic resistance increases. The urgent need for alternative treatments is underscored by the lack of a vaccine and the increasing prevalence of drug-resistant strains. Royal jelly (RJ), a beehive product rich in proteins and bioactive compounds, has garnered attention for its latent therapeutic applications. RJ antimicrobial properties have driven the exploration of new approaches to combat drug-resistant pathogens.

Methods: Bioinformatics approaches were employed for modeling RJ derived-peptide and to analyze the structure, physicochemical properties, antimicrobial potential, and docking interactions of the three peptides (Apisimin, royalisin (defensin-1), and Major RJ protein 1 (MRJP-1) with the major outer membrane protein (PorB) and Type IV major pilin protein PilE from *N. gonorrhoeae* membrane protein.

Results: Distinct motifs were identified for every peptide modeled from RJ by structural analysis. Peptides showed diverse physicochemical characteristics and could impact their biological functions. Machine learning predicted antimicrobial activity for defensin-1 and MRJP-1. Docking simulations suggested stronger interactions between PorB and the peptides compared to PilE. Defensin-1 could be an important peptide in developing novel antimicrobial agents for targeting *N. gonorrhoeae*. Nonetheless, *in vitro* investigations are necessary.

Conclusion: This study underscores the imminent potential of immunoinformatics tools in discovering innovative antimicrobial solutions, including membrane disruption, and emphasizes their role and possible routes of action in developing alternative treatments to overcome this persistent pathogen.

Keywords: *Neisseria gonorrhoeae*, Antibiotic resistance, Royal jelly, Antimicrobial peptides, Immunoinformatics, Drug discovery.

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INTRODUCTION

Gonorrhea is a serious global health concern caused by *Neisseria gonorrhoeae*, a Gram-negative bacterium. This sexually transmitted infection (STI) is quickly transmitted due to poor treatments and the increase in antibiotic resistance [1]. Untreated gonorrhea can cause serious problems, such as infertility, inflammation, ectopic pregnancy, and death [2,3]. Increasing the number of highly drug-resistant bacteria highlights the urgent need for novel treatment techniques.

The absence of a vaccine exacerbates the problem [4,5]. Urgent development of new treatment options is crucial to prevent untreatable gonorrhea in the future [6]. *N. gonorrhoeae* is a highly adaptable bacterium that can easily cause infection by attaching to cells lining the body and avoiding the immune system [7]. The bacteria have special features, including hair-like structures (pili) for sticking to cells and a protective coating lipooligosaccharide (LOS) to hide from the immune system [8]. It also releases proteins that can control how cells behave [9].

The outer membrane of *N. gonorrhoeae* is one of the most important factors affecting how well it can cause disease [10]. Many important proteins that increase the pathogenicity of the bacteria are found in this membrane, including Rmp, PorB [3,11], and Opa proteins [12], allow the pathogen to penetrate host tissues, avoid detection by the immune system, and trigger a persistent infection. Adding to the above, the outer membrane contains LOS and type IV pili, both functionally as potent pathogen-associated molecular patterns [13], interact with the immune system of the host, and causing responses of inflammation that may contribute to tissue damage and disease development [14]. Targeting outer membrane components is essential to developing novel treatments that address the rising problem of antibiotic-resistant gonorrhea. PorB and PilE were specifically selected for further investigation because they represent two of the most functionally distinct and structurally accessible virulence factors of *N. gonorrhoeae*.

Honey bees (*Apis mellifera* L.), like all living species, rely on proteins to form the basic building blocks of their bodies and perform vital functions. Honey bees get protein from pollen, royal jelly (RJ) (queen development), and bee bread (pollen storage) [15], which is needed for growth, repair, and other body activities. Nurse bees feed Honey bee larvae a particular food product called RJ, produced by the head glands. In addition to RJ, propolis derived from honey or stingless bees has been reported to contain diverse bioactive compounds-particularly phenolics and flavonoids-with strong antioxidant, antimicrobial activities, and therapeutic potential [16,17].

RJ is a nutrient-rich substance, a superfood. RJ primary components are proteins, peptides, lipids, and phenolics. Proteins make up over half of RJ's dry weight, with major RJ proteins (MRJPs) being the most abundant component, comprising 80–90% of the total protein content [18]. Other beneficial compounds, including peptides, contribute to RJ's activities for medicinal properties [19]. RJ has demonstrated antimicrobial properties attributed primarily to its protein and peptide constituents. These bioactive compounds have shown inhibitory effects against a range of microorganisms [20-24]. Due to the presence of peptides including apidaecin, defensin, hymenoptaecin, and jelleine, RJ has shown antibacterial effects.

The crux of the problem is the tedious and expensive method for identifying protein structures experimentally [25]. Techniques such as X-ray crystallography and NMR spectroscopy are very specialized and resource-intensive. The delay in obtaining structural information severely restricts drug research efforts. Protein structure model development was improved by metagenomics, homology modeling, and AlphaFold [26-28]. It is important to understand that there are some limitations on these computational predictions. However, experimental validation is still necessary for drug discovery, where accuracy is important.

The lack of a vaccine and the increasing prevalence of antibiotic resistance necessitate novel therapeutic approaches. This study aims to examine the possibility of using RJ honey bee peptides to prevent the negative effects of *N. gonorrhoeae*, mainly targeting the cell membrane proteins. Therefore, we hypothesize that specific RJ-derived peptides, namely, Apisimin, Defensin-1, and MRJP-1, can bind to and inhibit key virulence factors of *N. gonorrhoeae*, PorB and PilE, and thus represent promising candidates for novel antimicrobial development.

METHODS

Structural modeling, characterization, and validation of *N. gonorrhoeae* proteins and Honey bee-derived peptides

The major outer membrane protein P.IB (PorB) (UniProt ID: P18195) and Type IV major pilin protein PilE (UniProt ID: P11764) sequences from *N. gonorrhoeae* were retrieved from UniProt Data Bank (https://uniprot.org/). Three Honey bee-derived peptides, an apisimin, royalisin (defensin-1), and MRJP-1, respectively, were retrieved from UniProt Data Bank (Table S1) for further bioinformatics analysis. To convert the peptide to FASTA format, a web tool (http://avermitilis.ls.kitasato-u.ac.jp/readseq.cgi) was used. The Honey bee-derived peptide structures were refined using ModRefiner (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) [29]. The automated protein structure homology modeling server (https://swissmodel.expassy.com) was performed based on sequence similarity with peptide-shared identities of 0.58, 0.77, and 0.95% GMQE, respectively (Table S2). All modeled structures were further refined with PEP-FOLD3 (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOL), following the best model selected for further analysis. Refined structures were validated used structural analysis and verification server v6.0. Visualization and analysis of the final models were conducted using Discovery Studio Visualizer version 21.1.0.20298 and UCSF Chimera X, following the generated model structure.

In silico Assessment of physicochemical properties in Honey bee-derived peptides

The physicochemical characteristics of peptides are considered to be important to understanding how they behave and their biological functions. ProtParam tool on the ExPASy server (https://web.expasy.org/protparam/) has been implemented to assess the physicochemical features of the three peptides [30]. The tool employs the N-end rule to predict protein stability based on the N-terminal amino acid sequence. The peptides' half-lives have been estimated.

Prediction of antimicrobial activity in Honey bee-derived peptides

The web tool, an Anti-microbial peptides (CAMPR3) (http://www.camp3.bicnirrh.res.in/predict/) was used to predict the antibacterial properties of apisimin, defensing-1, and MRJP-1 selected peptides. This online website uses three machine learning models – support vector machine (SVM), Random Forest, and artificial neural network (ANN) – to identify peptides as antimicrobial peptides (AMPs) or non-AMPs.

Predicting the bioactivity of honey bee-derived peptides

We used the ProSA-web server (http://distilldeep.ucd.ie/PeptideRanker) [31] to evaluate the quality of the modeled peptide structures. This web-based application evaluates protein and peptide structures and assigns a z-score indicating the structure's overall quality. Negative z-scores suggest a greater resemblance to native protein structures. A lower z-score indicates the modeled peptide structure is more similar to an actual protein structure, suggesting a more desirable and useful conformation.

Predict toxicity differences among Honey bee-derived peptides

Toxicity predictions were analyzed using the CSM-Toxin web tool (http://biosig.lab.uq.edu.au/csm_toxin/) [32]. The amino acid sequences of Honey bee-derived peptides were uploaded in FASTA format, and the toxicity results in predictions and associated probability scores were evaluated.

Protein-ligand docking

To evaluate the quality of anticipated interactions for protein-protein and protein-DNA/RNA complexes, HDOCK uses in-house assessment methods. Protein-peptide docking was performed using the HDOCK server (http://hdock.phys.hust.edu.cn/). The prepared protein and ligand structures were uploaded to the server for docking calculations. We used the default parameters of the HDOCK server for all docking simulations.

Binding pocket and interaction site characterization

The structural and physicochemical features of the predicted binding pocket in the protein-peptide complex were analyzed using the Proteins.plus web server (https://proteins.plus/) under the DoGSiteScorer module. The docked complex obtained from the HDOCK server was uploaded in PDB format for pocket detection and residue-level characterization. DoGSiteScorer automatically identifies potential binding sites based on a difference-of-Gaussian filter and computes a comprehensive set of geometric and physicochemical descriptors, including pocket volume ($Å^3$), surface area ($Å^2$), depth (Å), and enclosure. The pocket with the highest enclosure and volume values was selected as the most probable active site.

RESULTS

Structural diversity in Honey bee-derived AMPs

For further knowledge of the structural foundation of antimicrobial activity, the target peptides' 3D structures were generated by using homology modeling (Fig. 1) and (Table S2 and S3). Subsequent investigation indicated that the peptides have distinct structure-related characteristics. According to a structural study, apisimin conforms to α-helical and coil structures. Another AMPs, royalisin, showed a structure built up of linear regions with α-helices. On the other hand, MRJP-1 demonstrated a more intricate structure with β-sheet and α-helical parts, as well as arrows pointing in the direction of extensions. The three peptides had different structural quality levels, as seen by their Z-scores in the ProSA-web study (Fig. 1b, d, and f). The three peptides have different structural characteristics, as shown by MolProbity analysis (Fig. 2 and Table S4), with apisimin showing the best overall geometry. While acceptable, this value is lower than expected for a high-quality structure in Defensin-1. MRJP-1 showed a moderate number of clashes and Ramachandran outliers.

Structural diversity in *N. gonorrhoeae* proteins

The distribution of amino acid residues within the allowed and core areas of the Ramachandran plot demonstrated that the vast majority of the residues in the targeted proteins displayed a favorable backbone conformation (Fig. 3). ProB 3D structure, through Ramachandran plot analysis, showed 95.3% of residues are in the most favored regions, and 4.7% are in allowed regions. The Ramachandran plot analysis scores became 0% outlier regions for PilE protein.

Physicochemical characterization of Honey bee-derived peptides

The physicochemical characteristics of peptides are considered to be important to understanding how they behave and their biological functions. The web-based ProtParm tool has been implemented to assess the physicochemical features of the three peptides (Table 1). The three peptides were predicted to be stable. MRJP-1 differed from the other two peptides in terms of its hydrophobicity, charge, and light absorption properties. Apisimin was shown a smaller size and lower molecular weight and could have moderate solubility, and a slightly

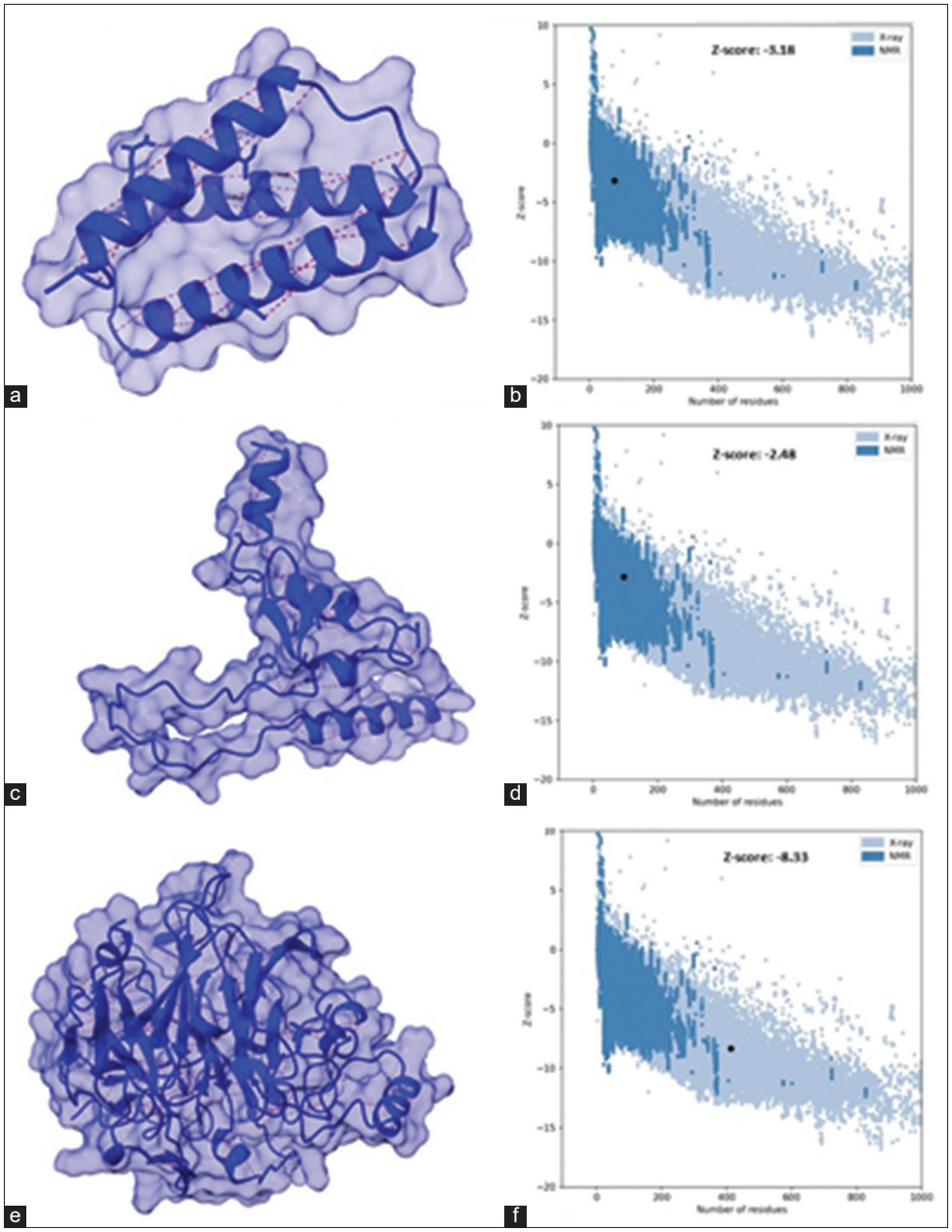

Fig. 1: Predicted 3D structures of honey bee-derived peptides with relaxed constraints (a, c, e): Peptide backbones are depicted in ribbon format for apisimin, defensin-1, and major royal jelly protein 1, respectively. Total hydrogen bonds (a: 61, c: 51, e: 335) are shown as dashed red lines. Constraints were relaxed by 0.4 Å and 20°. (b, d, f): Peptides Z-scores

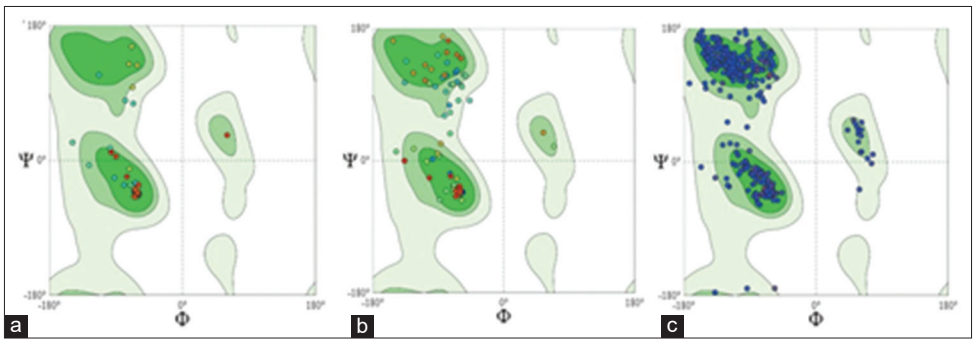

Fig. 2: Ramachandran plot analysis of honey bee-derived peptide conformations. (a): Apisimin, (b) defensin-1, and (c) major royal jelly protein 1

acidic isoelectric point. Defensin-1 is larger than apisimin and showed similar solubility and net charge properties. MRJP-1, a stimulant with a much higher molecular weight and larger size, had adequate solubility at pH 7 despite having a much lower net charge and a more

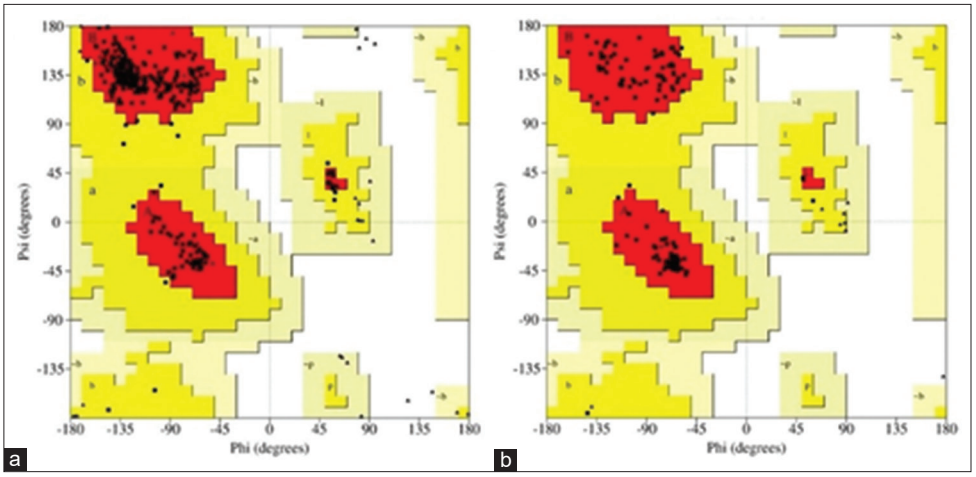

Fig. 3: Ramachandran plot analysis of *Neisseria gonorrhoeae* protein structures. (a): Major outer membrane protein (PorB); (b): Type IV major pilin protein PilE

Table 1: Physicochemical analysis of the three selected honeybee-derived peptides

Peptide	No. AA	MW/g/mol	Theoretical pI	Negative charged*	Positive charged**	Extinction coefficients $M^{-1}cm^{-1}$***	Estimated half-life			Instability index	Aliphatic index	GRAVY ****
							In vitro/h[a]	*In vivo*/h[b]	*In vitro*/h[c]			
Apisimin	78	7946.39	6.27	14	13	7365	30	>20	>10	27.99	151.03	1.253
Defensin-1	95	10717.38	6.27	14	13	7365	30	>20	>10	35.04	77.05	−0.081
MRJP-1[d]	348	48885.59	8.90	32	36	48820	30	>20	>10	22.79	72.61	−0.373

*Total number of negatively charged residues (Asp+Glu); **Total number of positively charged residues (Arg+Lys); ***assuming all Cys residues are reduced, at 280 nm measured in water; [a]mammalian reticulocytes; [b]yeast; [c]*Escherichia coli*; **** Grand average of hydropathicity; Major royal jelly protein 1

acidic isoelectric point. In mammalian reticulocytes, all three peptides were predicted *in vitro* half-life of 30 h. Their estimated *in vivo* half-lives in yeast and *Escherichia coli* were also more than 20 h and 10 h, respectively. These results point to the three peptides' comparatively stable nature.

Machine learning prediction of AMPs

To predict the selected AMPs potential, three machine learning classifiers were employed: SVM, Random Forest, and ANN. Defensin-1 and MRJ-1 1 were consistently predicted as AMPs by SVM and Random Forest classifiers (Table 2).

Bioactive properties of honey bee-derived peptides

In addition, an *in silico* analysis using PeptideRanker was performed to predict antibacterial activities (Table 2). Defensin-1 was the most promising candidate, with the highest predicted bioactivity, followed by apisimin. In contrast, MRJP-1 shows a low value of antibacterial prediction according to the PeptideRanker score.

Differential toxicity profiles for Honey bee-derived peptides

CSM-Toxin predicts defensin-1 as highly toxic, while apisimin and major royal jelly protein 1 could predict it as non-toxic (Table 3). Apisimin remains the most hydrophobic protein (Fig. 4).

Docking score and confidence score analysis

The top-ranked docking pose for each protein-peptide pair was selected (Table 4). The HDOCK score, a complex score reflecting the scoring function between the protein and peptide, the confidence score provided by the HDOCK server were used to assess the reliability of the docking results. Defensin-1 has the highest predicted affinity with ProB, followed by major RJ protein 1, and apisimin (Fig. 5), meanwhile, defensin-1 and apisimin presented closer interactions core with Type IV major pilin protein PilE (Fig. 6).

Table 2: Bioactivity and AMPs prediction for honeybee-derived peptides

Peptide name	Bioactivity	SVM*	Random forest
Apisimin	0.631479	0.516	0.321
Defensin-1	0.999701	0.997	0.979
MRJP-1**	0.224597	1.00	0.959

*Support vector machine; (A value between 0 and 1 indicating the confidence of the prediction). **MRJP-1: Major royal jelly protein 1, AMPs: Antimicrobial peptides

Binding pocket characterization of PorB and PilE with Apisimin

Analysis of the predicted Apisimin-binding sites revealed two distinct pocket architectures (Tables S5 and S6). The site on PorB is a compact, highly apolar pocket (volume: 835.01 $Å^3$; apolar ratio: 0.71), characterized by a flat geometry (ellipsoid c/a: 0.09) and a strong hydrophobic character (hydrophobicity ratio: 0.51), largely composed of valine, isoleucine, and leucine residues. In stark contrast, the binding site on PilE is substantially larger and more accessible (volume: 1838.37 $Å^3$; enclosure: 0.08), with a balanced chemical composition featuring a near-equal mix of apolar (0.47) and polar/charged residues. This equips the PilE pocket with a much greater capacity for polar interactions, as evidenced by its high count of hydrogen bond acceptors (106) and donors [32], suggesting a different and potentially more versatile binding mode for Apisimin on PilE compared to the predominantly hydrophobic interface on PorB.

DISCUSSION

N. gonorrhoeae is a serious problem emerging everywhere because the bacteria are getting potent and no longer fend off antibiotics. We urgently need new novel methods as of right now, there is no approved

Table 3: Predicted toxicity of bee proteins using the CSM-toxin model

Peptide	Toxicity	Probability	AA* liphatic	AA aromatic	AA non-polar	AA polar	AA charged	Net charge	Hydrophobicity
Apisimin	Non	0.19	42	2	49	29	7	−1.06	1.25
Defensin-1	Toxic	0.94	27	12	55	40	30	−1.09	0.08
MRJP-1**	Non	0	112	52	215	217	103	−14.6	35

*Amino acid; **MRJP-1: Major royal jelly protein 1

Table 4: Protein-ligand docking score from HDOCK server

Protein	Peptides	HDOCK score	Confidence score
PorB*	Apisimin	−316.4	0.9654
	Defensin-1	−334.7	0.9757
	MRJP-1**	−333.2	0.9750
PiLE**	Apisimin	−231.7	0.8368
	Defensin-1	−227.6	0.8253
	MRJP-1	−212.4	0.7768

*Major outer membrane protein; **MRJP-1: Major royal jelly protein 1; **Type IV major pilin protein

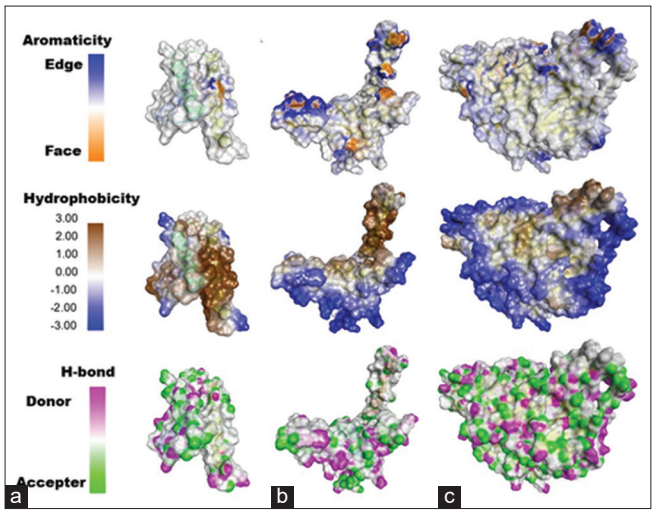

Fig. 4: Surface properties of royal jelly-derived peptides (Aromaticity, Hydrophobicity, and Hydrogen Bonding); (a) Apisimin; (b) Defensin-1; (c) Major royal jelly protein 1

vaccination to prevent gonorrhea; instead, ongoing research is being done to find novel vaccines [4,5]. Besides recent research, RJ has become a valuable ingredient in various industries, including pharmaceuticals, cosmetics, and food [33].

N. gonorrhoeae bacteria have hair-like structures on their surface (PilE), such as Type IV pili (Tfp), which are required for infection. Due to their structure, the bacteria can adhere to human cells and evade the defenses of the body. Early studies found a connection between pili and the disease-causing ability of the bacterium [7]. However, since the bacteria can quickly alter the structure of these pili, studies to create a vaccine targeting pili have failed. The PilE pocket was large and polar in nature (hydrophobicity ratio=0.38; apolar/polar amino acid ratio=0.47/0.36), with increased numbers of hydrogen bond donors [32] and acceptors (106), implying a more flexible and solvent-exposed interface.

PorB is an important key protein in the outer membrane of *N. gonorrhoeae* that regulates the passage of ions and nutrients into the bacteria [34]. PorB, the major porin of the outer membrane, is highly conserved and plays dual roles in nutrient transport and immune evasion, making it a critical determinant of bacterial survival and host interaction. Therefore, focusing on PorB and PilE provides a rational and mechanistically relevant approach to exploring the interaction of AMPs with the most stable and functionally exposed virulence determinants of *N. gonorrhoeae*. Based on a Ramachandran plot study, Type IV major pilin protein and the major outer membrane protein displayed strong backbone conformations with minimal deviations from a predicted dihedral angle. The large number of residues found in the Ramachandran plot's central regions and the absence of Ramachandran outliers provide evidence.

Apisimin has antimicrobial effects [35,36], and could increase monocytes growth. According to Bíliková and colleagues, the presence of cysteine does not always correlate with a peptide's potential to inhibit bacterial growth. Furthermore, apisimin is rich in valine and serine, and the presence of albumin in combination with apisimin might contribute to additional cellular activities.

Among the numerous RJ-derived peptides, Apisimin, Defensin-1, and MRJP-1 were selected for this study because they represent three structurally and functionally distinct classes of antimicrobial and immunomodulatory molecules. Royalisin (Defensin-1) previously showed antimicrobial activities against a limited Gram-positive bacterium and some effect on Gram-negative bacteria [37,38]. Importantly, honey bees contain exosome-like extracellular vehicles enriched with AMPs, MRJP1, defensin-1, and jellein-3, vesicles were demonstrated antibacterial and antibiofilm properties, and therefore, suggesting their potential in the prevention of dental caries [39,40]. Fujiwara *et al*. demonstrated defensin-1 could play a role in protecting honey bee from bacterial infections [38]. The combined selection of these three peptides therefore captures a representative spectrum of RJ's antimicrobial potential – from small, hydrophobic, membrane-active peptides (Apisimin, Defensin-1) to larger multifunctional glycoproteins (MRJP-1) – allowing a comparative analysis of how peptide size, charge, and hydrophobicity influence interactions with *N. gonorrhoeae* virulence proteins. Our bioinformatics research indicates that defensin-1 is primarily hydrophobic. This structure is consistent with the amphipathic structure found in other antibacterial proteins [38], indicating a possible mechanism of action that involves disrupting membranes. Moreover, defensin-1 and apisimin showed a high likelihood of antibacterial action according to PeptideRanker analysis. MRJP-1, on the other hand, showed a low anticipated antibacterial potential.

There are nine members of the wider family that the MRJPs are a part of, the most abundant protein components of RJ. MRJP-1, an RJ component, has a variety of biological functions and has been shown to increase cell proliferation in some circumstances [41]. In addition, MRJP-1 affects cytokine production and, therefore, modulates immunological responses [42]. MRJP-1 recently evaluated its antibacterial activity against several bacterial strains, including *Enterococcus faecalis*, *Bacillus pumilus*, *E. coli*, and *Pseudomonas fluorescens* [43,44]. Glycosylated MRJP1 had antibacterial efficacy against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococci [45]. Crucially, jelleins, which are generated from MRJP1, have antibacterial action against both Gram-positive and Gram-negative bacteria as well as yeast [22]. It is thought that these peptides work by rupturing the cell membranes of bacteria [46]. A relative evaluation of the protein-peptide binding strength was calculated by HDOCK scores. The PorB pocket showed a higher hydrophobicity ratio (0.51) and was predominantly composed of apolar residues (71%), particularly valine (n=11), isoleucine (n=5), and leucine (n=4), indicating that hydrophobic interactions likely play a major role in peptide binding. PorB-honey bee-derived peptides docked results demonstrated a higher HDOCK

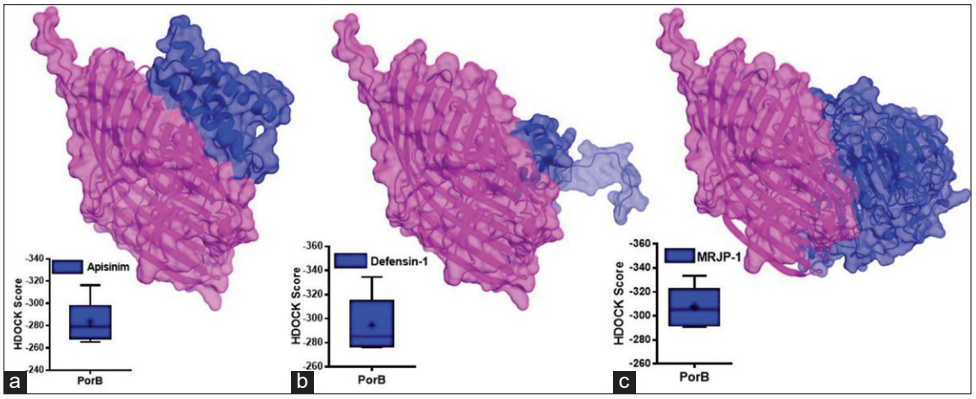

Fig. 5: Major outer membrane protein (PorB) and peptide complex. (a) 3D visualization of the predicted interaction between the apisimin and PorB protein; (b) 3D visualization of the predicted interaction between the defensing-1 and PorB protein; (c) 3D visualization of the predicted interaction between the major royal jelly protein 1 (MRJP-1) and PorB protein. The bottom panel displays a box plot illustrating the distribution of the top ten HDOCK scores obtained for the predicted complexes of PorB with apisimin, defensin-1, and MRJP-1 respectively. Peptides are represented in blue, and protein is displayed in magenta

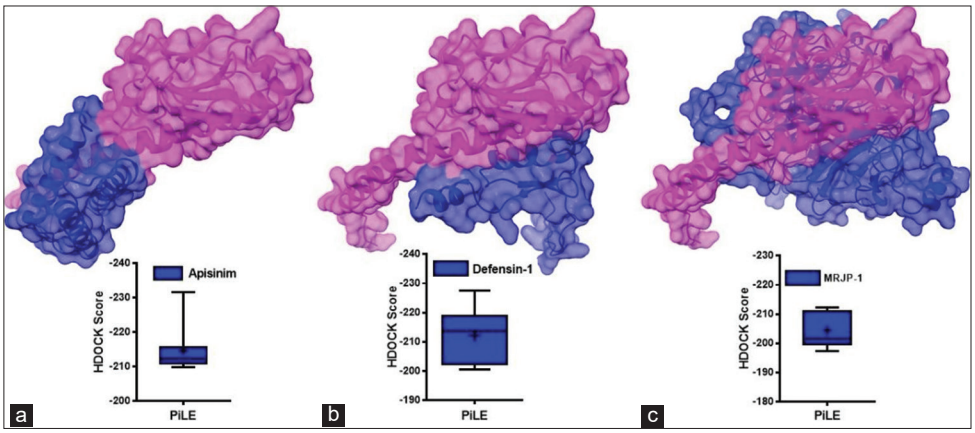

Fig. 6: Type IV major pilin protein (PilE) and peptide complex. (a) 3D visualization of the predicted interaction between the apisimin and PilE protein; (b) 3D visualization of the predicted interaction between the defensing-1 and PilE protein; (c) 3D visualization of the predicted interaction between the major royal jelly protein 1 (MRJP-1) and PilE protein. The bottom panel displays a box plot illustrating the distribution of the top ten HDOCK scores obtained for the predicted complexes of PilE with apisimin, defensin-1, and MRJP-1 respectively. Peptides are represented in blue, and protein is displayed in magenta

score than PiLE, suggesting a strong predicted interaction. Surprisingly, PorB-MRJP-1 has closed values, reflecting a similar binding ability. The presence of both positively (10%) and negatively (8%) charged residues in PilE, absent in PorB, further indicates a higher electrostatic contribution to Apisimin binding.

Crucially, the binding modes we observed are directly correlated with the physicochemical properties of the peptides. The highly hydrophobic nature of Defensin-1 (GRAVY=−0.081, indicating a near-neutral but slightly hydrophilic character that can mask large hydrophobic patches common in amphipathic peptides) provides a mechanistic explanation for its strong predicted binding to the predominantly apolar PorB pocket. This suggests an interaction driven by hydrophobic complementarity, where non-polar residues on Defensin-1 engage with the valine-, isoleucine-, and leucine-rich surface of PorB, potentially disrupting the membrane protein's function. In contrast, the binding of Apisimin to the larger, more polar PilE pocket can be explained by its smaller size and more balanced charge distribution. The polar and electrostatically diverse PilE interface, rich in hydrogen bond donors and acceptors, is well-suited to form specific polar contacts with Apisimin, while its substantial hydrophobic surface area (86 hydrophobic interactions) can still accommodate Apisimin's moderately hydrophobic character (GRAVY=1.253). This dual-character binding-polar specificity, combined with hydrophobic stabilization, may allow Apisimin to interfere with the adhesive function of PilE. The large, acidic MRJP-1, with its substantial negative charge (32 acidic residues) and low overall hydrophobicity (GRAVY=−0.373), would be less compatible with the non-polar PorB pocket, which may explain its comparatively lower predicted binding affinity despite its size, highlighting that affinity is not solely a function of molecular weight but of chemical compatibility. Collectively, these findings suggest that Apisimin binds to a hydrophobic and compact site on PorB, while its interaction with PilE involves a larger, more polar, and electrostatically diverse binding pocket.

Homology modeling has been used to determine the structural and functional properties of the peptides from honey bee. The results showed that the peptides varied in their structural motifs, indicating different modes of action. ProSA-web research revealed that the three peptides have differing levels of structural quality, which could impact the peptides' biological activity [47].

Different properties were identified by physicochemical analysis for each of the three peptides. Variations in the properties could influence different biological roles of honey bee-derived peptides. The predicted stability of the peptides, given by their half-life estimations, suggests the likelihood of relentless antibacterial activity against *N. gonorrhoeae*. A longer half-life could be important for sustained antimicrobial activity [48]. However, this needed to be evaluated against the risk of increased toxicity or bacterial resistance.

AMP prediction was evaluated for the targeted peptides. Using machine learning-based prediction, the results suggested that they could be medicinal. Nevertheless, stress how important it is for having empirical data to back up such assertions. Interestingly, *in silico* toxicity prediction classified Defensin-1 as highly toxic, a result that carries important implications for its therapeutic potential. This prediction is consistent with the peptide's amphipathic and strongly hydrophobic character, which enables efficient disruption of bacterial membranes but may also compromise mammalian cell membranes at higher concentrations. Therefore, Defensin-1 may require optimization to improve its therapeutic index. A notable inconsistency was observed between the peptide prediction tools, where MRJP-1 was classified as an antimicrobial candidate by both SVM and Random Forest algorithms, but received a low bioactivity probability in PeptideRanker. This discrepancy likely reflects methodological differences in the predictive frameworks. PeptideRanker primarily evaluates short, linear peptides based on amino acid composition and motif patterns derived from experimentally validated antimicrobial sequences. In contrast, the SVM and Random Forest models integrate additional physicochemical and structural descriptors that may better accommodate large, multidomain proteins such as MRJP-1. Moreover, MRJP-1 is a glycosylated and multifunctional protein that serves as a precursor for shorter antimicrobial fragments like jelleins, which could contribute to the positive AMP classification in SVM- and RF-based models. Therefore, the lower PeptideRanker score may not necessarily negate MRJP-1's potential antimicrobial relevance but rather highlights the limitations of applying short-peptide prediction models to large, complex protein structures. This emphasizes the importance of experimental validation to confirm computational predictions.

CONCLUSION

Gonorrhea continues to pose an important global health problem as drug resistance grows. This study investigated the potentiality of honey bee-derived peptides as a novel therapeutic approach against *N. gonorrhoeae*, a STI with increasing antibiotic resistance. This study applied a comprehensive computational framework integrating structural modeling, physicochemical characterization, AMPs prediction, and molecular docking to evaluate honey bee–derived peptides as potential inhibitors of *N. gonorrhoeae* virulence proteins PorB and PilE. Among the peptides examined, Defensin-1 exhibited the strongest predicted antimicrobial activity and binding affinity, particularly toward the PorB protein, consistent with its amphipathic and membrane-disruptive nature. However, its high predicted toxicity underscores a critical limitation that may restrict its direct application as a therapeutic without structural modification or formulation strategies to mitigate cytotoxicity. Apisimin, by contrast, demonstrated a favorable balance between binding strength, predicted bioactivity, and low toxicity, suggesting it as the most promising lead candidate for further experimental validation and rational optimization. MRJP-1, while multifunctional and biologically relevant, displayed comparatively weaker predicted activity and higher molecular complexity. Collectively, these findings highlight the potential of honey bee–derived peptides, particularly Apisimin – as a novel scaffold for developing safe and effective anti-gonococcal agents. While this study provides a starting point for future research, *in vitro* and *in vivo*, experiments are required to validate these findings. By understanding the structure-function relationships of these peptides and their interactions with bacterial targets, we can develop novel strategies to combat this persistent pathogen.

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AUTHORSHIP CONTRIBUTION STATEMENT

Maryam Alhumaidi: Conceptualization, data curation, Formal analysis, methodology, writing - original draft, writing - review and editing, Investigation, Project administration, resources, supervision, validation, visualization, funding acquisition.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that have been used are confidential.

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SUPPLEMENTARY MATERIAL

Table S1: Honeybee-derived peptides sequence

Peptide name	UniProt ID	Peptides Sequence
Apisimin	Q8ISL8	MSKIVAVVVLAAFCVAMLVSDVSAKTSISVKGESNVDVVSQINSLVSSIVSGANVSAVLLAQTLVNILQILIDANVFA
Defensin-1	P17722	MKIYFIVGLLFMAMVAIMAAPVEDEFEPLEHFENEERADRHRRVTCDLLSFKGQVNDSACAANCLSLGKAGGHC EKGVCICRKTSFKDLWDKRFG
MRJP-1*	O18330	MTRLFMLVCLGIVCQGTTGNILRGESLNKSLPILHEWKFFDYDFGSDERRQDAILSGEYDYKNNYPSDIDQWHDKIF VTMLRYNGVPSSLNVISKKVGDGGPLLQPYPDWSFAKYDDCSGIVSASKLAIDKCDRLWVLDSGLVNNTQPMCSPK LLTFDLTTSQLLKQVEIPHDVAVNATTGKGRLSSLAVQSLDCNTNSDTMVYIADEKGEGLIVYHNSDDSFHRLTSNTFDYDPKFTKM TIDGESYTAQDGISGMALSPMTNNLYYSPVASTSLYYVNTEQFRTSDYQQNDIHYEGVQNILDTQSSAKVVSKSGVLFFG LVGDSALGCWNEHRTLERHNIRTVA QSDETLQMIASMKIKEALPHVPIFDRYINREYILVLSNKMQKMVNNDFNFDDVNFRIMNANVNELILNTRCENPDNDRTPFKISIHL

*Major royal jelly protein 1

Table S2: Structural modeling and characterization of honeybee-derived peptides using swissmodel and alphafold

Model	Apisimin	Defensin-1	MRJP-1*
GMQE**	0.58	0.77	0.95
Method	AlphaFold v2-1.00Å	AlphaFold v2	EM 0.00Å
Found By	AFDB search	AFDB search	HHblits
Seq Similarity	0.56	0.63	0.62
Biounit Oligo State	monomer	monomer	homo-dimer
Target Prediction	It is only possible to build a monomer.	It is only possible to build a monomer.	It is only possible to build a monomer.
Seq Identity	96.15	100.00	100.00
Coverage	1.00	1.00	1.00
Range	1-78	1-95	20-432

*:Major royal jelly protein 1, and QSQE is 0.63, **: Global Model quality estimate

Table S3: Structural modeling and characterization of porb* and type iv major pilin protein pile swissmodel and alphafold

Model	PorB	PilE
GMQE**	0.91	0.92
Method	Alphafold v2-1.00Å	Alphafold v2
Found By	AFDB search	AFDB search
Seq Similarity	0.60	0.55
Biounit Oligo State	monomer	monomer
Target Prediction	It is only possible to build a monomer.	It is only possible to build a monomer.
Seq Identity	96.55	84.34
Coverage	1.00	0.99
Range	1-348	1-167

* Major outer membrane protein, ** Global Model Quality Estimate

Table S4: The quality of a predicted protein structure

Protein name	Score*	Clash score	R** favored	R** outliers	Rotamer outliers	C-Beta deviations	Bad bonds	Bad angles
Apisimin	1.00	0.00	94.74%	0.00%	1.52%	0	0/556	1/759[a]
Defensin-1	1.06	0.68	89.25%	2.15%[b]	2.53%	2[c]	0/764	8/1025[d]
MRJP-1***	0.83	0.15	96.35%	0.24%[e]	1.07%[f]	1[g]	0/3370	16/4576[h]

* MolProbity Score; ** Ramachandran; *** Major outer membrane protein; [a]A37 ASP; [b]A38 ALA, A36 GLU; [c]A33 GLU, A85 SER; [d](A27 GLU-A28 PRO), A57 ASP, (A40 ARG-A41 HIS), A41 HIS, A32 PHE, A30 GLU, (A35 GLU-A36 GLU; [e]E196 ASN; [f]E195 CYS, E188 LEU, E348 ASP, E301 ILE; [g]E118 CYS; [h]E399 ASN, E365 HIS, (E72 TRP-E73 HIS), E431 HIS, E63 ASN, (E147 GLN-E148 PRO), E20 ASN, E35 HIS, E217 HIS, E294 HIS, E333 HIS, E117 ASP, E224 HIS, E172 HIS, E40 PHE, E340 ASN

Table S5: Pocket size, composition, and amino acid descriptors of the major outer membrane protein (porb) and apisimin-binding site predicted by the proteins.plus server

Parameter Category	Descriptor	Value
Size and Shape Descriptors	Volume (Å≥)	835.01
	Surface area (Å^2)	1189.13
	Depth (Å)	17.66
	Ellipsoid main axis ratio (c/a)	0.09
	Ellipsoid main axis ratio (b/a)	0.50
	Enclosure	0.12
Element Descriptors	Number of pocket atoms	161
	Number of carbons (C)	117
	Number of nitrogens (N)	21
	Number of oxygens (O)	22
	Number of sulfurs (S)	1
	Number of other elements	0
Functional Group Descriptors	Hydrogen bond donors	11
	Hydrogen bond acceptors	44
	Metals	0
	Hydrophobic interactions	57
	Hydrophobicity ratio	0.51
Amino Acid Composition	Apolar amino acid ratio	0.71
	Polar amino acid ratio	0.29
	Positive amino acid ratio	0.00
	Negative amino acid ratio	0.00
Amino Acid Descriptors	ALA	4
	ARG	0
	ASN	1
	ASP	0
	CYS	1
	GLN	2
	GLU	0
	GLY	0
	HIS	0
	ILE	5
	LEU	4
	LYS	0
	MET	0
	PHE	0
	PRO	0
	SER	4
	THR	1
	TRP	0
	TYR	1
	VAL	11

Table S6: Pocket size, composition, and amino acid descriptors of the type iv major pilin protein (pile) and apisimin-binding site predicted by the proteins.plus server

Parameter Category	Descriptor	Value
Size and Shape Descriptors	Volume (Å≥)	1838.37
	Surface area (Å^2)	2447.10
	Depth (Å)	21.31
	Ellipsoid main axis ratio (c/a)	0.41
	Ellipsoid main axis ratio (b/a)	0.43
	Enclosure	0.08
Element Descriptors	Number of pocket atoms	339
	Number of carbons (C)	228
	Number of nitrogens (N)	51
	Number of oxygens (O)	60
	Number of sulfurs (S)	0
	Number of other elements	0
Functional Group Descriptors	Hydrogen bond donors	32
	Hydrogen bond acceptors	106
	Metals	0
	Hydrophobic interactions	86
	Hydrophobicity ratio	0.38
Amino Acid Composition	Apolar amino acid ratio	0.47
	Polar amino acid ratio	0.36
	Positive amino acid ratio	0.10
	Negative amino acid ratio	0.08
Amino Acid Descriptors	ALA	4
	ARG	2
	ASN	4
	ASP	3
	CYS	1
	GLN	6
	GLU	3
	GLY	3
	HIS	2
	ILE	5
	LEU	9
	LYS	3
	MET	1
	PHE	2
	PRO	0
	SER	7
	THR	3
	TRP	0
	TYR	2
	VAL	13