International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Novel Antimicrobial Peptides Derived from Urease-2 of Jack Beans for Treatment of Indian Priority Pathogens

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Abstract:
Antimicrobial peptides (AMPs) are short-chain amino acid sequences that play crucial roles in the host immune response. *Canavalia ensiformis*, commonly known as the jack bean, has long been recognized as a valuable protein source for biochemical investigations. Jaburetox, a jack bean protein, is a potent antifungal agent and biopesticide. However, it also exhibits neurotoxic effects. Therefore, it cannot be used as a potential drug for the treatment of bacterial diseases. This study focused on identifying small and nontoxic antibacterial regions within Jack Bean Urease 2, a specific isoform of Jack Bean Urease. The antimicrobial peptides derived from this enzyme may show inhibitory effects against pathogens such as *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* ATCC27853, *Salmonella typhimurium* ATCC14028, *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 25923. Comprehensive assessments of physicochemical, medicinal chemistry, and ADMET properties were conducted to identify the most suitable candidates for in vivo testing against the Indian Priority Pathogen List of Pathogens.

Keywords: Antimicrobial Resistance, Antimicrobial Peptides, *In silico* approach, Pharmacokinetic

Introduction

Antimicrobial Resistance (AMR) has become a global burden and major public health threat [1]. According to the Centers for Disease Control and Prevention (CDC), Antimicrobial Resistance alone claimed at least 1.27 million human deaths worldwide and overall, 5 million deaths in 2019[2]. To identify the organisms of top priority, WHO joined hands with Department of Biotechnology (India) and outlined antibiotic-resistant “priority pathogens” called Indian Priority Pathogens List (IPPL) that consists of *Enterococcus sp*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species* [3]. In line with this, the recent Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2022 emphasizes alarming resistance rates among prevalent bacterial pathogens, reporting median rates of 42% for third-generation cephalosporin-resistant *Escherichia coli* and 35% for methicillin-resistant *Staphylococcus aureus* in 76 countries as major threats[4]. Furthermore, a predicted two-fold increase in resistance is expected by 2035 [5]. These patterns of increased antibiotic resistance necessitate the exploration of alternative antimicrobial agents.

Antimicrobial peptides (AMPs) are short-chain amino acid sequences that play key roles in host immune defense [6]. AMPs are low-molecular-weight proteins that exhibit broad-spectrum activity against microorganisms, such as bacteria, fungi, viruses, and parasites [7]. They have rapid effects on many resistant bacteria, and the chances of bacteria developing resistance against AMPs is low [8]. Antimicrobial peptides are ubiquitous. They are found in all living organisms including bacteria, fungi, plants, animals, and mammals [9]. Plants harbor antimicrobial peptides for defensive purposes such as antifungal, antiviral, and antibacterial agents [10]. This study explored the AMPs produced from the phytotoxic protein Urease II in jack beans.

Jack beans (*Canavalia ensiformis*) have long been a promising source of proteins for biochemical research [11]. *C. ensiformis* has three isoforms, Jack Bean Urease (JBU), Jack Bean Urease II (JBURE-IIb), and canatoxin. Urease enzymes are metalloenzymes that catalyze the hydrolysis of urea to release ammonia and carbon dioxide [12]. The ureases of these enzymes exhibit several biological properties independent of their ureolytic activity, such as blood platelet activation, interaction with glycoconjugates, and insecticidal activity [13], [14]. The extensive presence of ureases and CNTX-like proteins in leguminous plants may indicate their significant physiological role, which is probably related to plant defense [15], [16], [17].

The cDNA sequence of JBURE-II was used as a template to produce the recombinant enterotoxin peptide, jaburetox-2Ec [18]. Although the purified recombinant protein JBURE-IIb did not exhibit any ureolytic activity, it inhibited the growth of *Penicillium herguei*, a phytopathogenic fungus that is susceptible to urease. Additionally, it showed enterotoxin properties and inhibited diuresis in the kissing bug *Rhodnius prolixus* [19]. It has antifungal and anti-insecticidal properties[20], [21], [22]. Jaburetox has been suggested as an alternative to agricultural pest control using genetically modified crops.

However, it must be noted that the antibacterial properties of none of the three isoforms of the jack bean urease have been explored. Additionally, when these proteins were injected intraperitoneally or intracutaneously (LD_{50} = 2 mg/kg) into mice, dyspnea, ataxia, hypothermia, coma, and death within 0.5-24 hr of injection [13], [23]. They also exhibit neurotoxicity, exocytosis, and pro-inflammatory effects [24]. Thus, the entire protein sequence cannot be used as an antimicrobial agent for human administration. Therefore, in this study, we screened for antibacterial regions from one of the isoforms of jack bean urease: jack bean urease 2.

Materials and Methods

Screening of Antimicrobial Peptide motifs from Urease-2 of Jack Beans

Canatoxin (Urease-2) is 840 a.a. long protein. (accession id: E6Y5X0) The entire sequence was screened for the presence of potential AMPs using $CAMP_{R4}$ (accessed on 06.02.2024). During screening, AMPs with lengths of 20, 30, 40, and 50 a.a. were generated. A few hundred AMPs generated via this process were subjected to further filtration based on their probability of being AMPs. For lengths ranging from 20 to 40 a.a., AMPs with a probability above 0.9 were selected. AMPs with a probability ≥ 0.85 were selected for 50 a. a. length. Seventy-five AMPs were first checked for similarities with previously reported AMPs using APD3 and DBAASP (accessed on 06.02.2024). Additionally, they were weeded by checking their antimicrobial activity against eight bacteria. The test bacteria used were *Bacillus subtilis* ATCC 6633, *E. coli* ATCC25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC14028, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC700603, and *Pseudomonas aeruginosa* ATCC27853.

Machine learning approaches, data on peptide sequences, and bacterial genomes (DBAASP, accessed on 06.02.2024) were used to predict the antimicrobial activities. Only the peptides that exhibited antibacterial activity against one or more bacteria were further evaluated.

Evaluation of the physicochemical properties of the peptides

The identified potential antibacterial peptides were evaluated for their physicochemical properties that influence their activity. Hemolytic activity was assessed using HemoPI (accessed on 0.7.02.2024). GRAVY, instability, and aliphatic indices are determinants of hydrophobicity, hydrophilicity, stability under standard conditions, and the thermostability of peptides. These properties were verified using Expasy Protparam (accessed on 07.02.2024). The Boman index, a factor predicting the interaction of peptides with other proteins, was assessed using the APD3 tool (accessed on 08.02.2024). The potential trigger for class I immunogenicity was checked using IEDB (accessed on 08.02.2024).

Estimation of medicinal chemistry and ADMET properties

Drug development may fail because of undesirable pharmacokinetics, absorption, distribution, metabolism, excretion, and toxicity of the peptides. The medicinal chemical and ADMET properties of the strains were evaluated using ADMETlab2.0 (accessed on 08.02.2024).

Results and Discussion

Screening of Antimicrobial Peptide motifs from Urease-2 of Jack Beans

The $CAMP_{R4}$ tool generates AMP sequences of varying lengths (20, 30, 40, and 50 amino acids). This tool identifies AMP motifs with probabilities ranging from 1 to 0. This research focused primarily on AMPs with probabilities greater than or equal to 0.9 for all lengths except those with lengths of 50 amino acids, where motifs with probabilities above 0.85 were considered. A detailed list of all generated peptide motifs is provided in the Supplementary Document S1. This study listed the predicted AMPs with potent antibacterial properties **(Table 1)** and confirmed that none of the peptides were reported in the APD3 or DBAASP databases. All AMPs were screened for potential antimicrobial activity (Figure 1). Twenty-eight AMPs displayed potency against one or more bacterial species **(Table 2)**. Many of the 28 AMPs showed a broad spectrum of action **(Table 2)**. Forty-seven AMPs did not exhibit any antibacterial activity. None of the AMP acted against *Klebsiella pneumoniae* ATCC700603 or *Pseudomonas aeruginosa* ATCC27853.

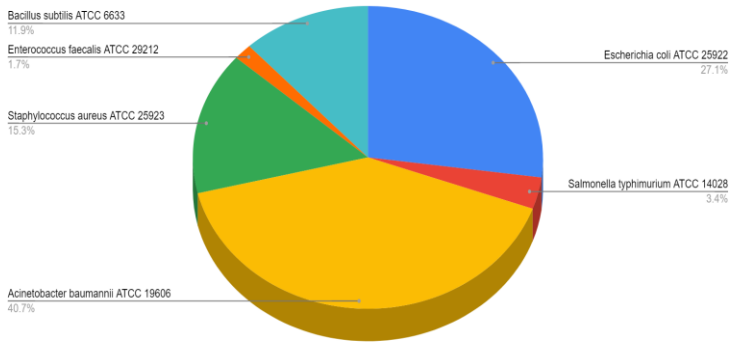


Fig 1 Percentage of Antimicrobial Peptides classified based on their potential antibacterial activities.

Seq ID	Position	Sequence	Class	AMP Probability
CANA_20_1	763-782	AFVSKAALDLGVKVLYGLNK	AMP	0.98
CANA_20_2	751-770	GTLGKAGSALSIAFVSKAAL	AMP	0.98
CANA_20_3	209-228	TVNLVSIGGNKIIRGGNAIA	AMP	0.97
CANA_20_4	764-783	FVSKAALDLGVKVLYGLNKR	AMP	0.97
CANA_20_5	137-156	IICADGRLTLNPGRKAVFLK	AMP	0.97
CANA_20_6	773-792	GVKVLYGLNKRVEAVSNVRK	AMP	0.97
CANA_20_7	760-779	LSIAFVSKAALDLGVKVLYG	AMP	0.97
CANA_20_8	765-784	VSKAALDLGVKVLYGLNKRV	AMP	0.97
CANA_20_9	208-227	KTVNLVSIGGNKIIRGGNAI	AMP	0.97
CANA_20_10	762-781	IAFVSKAALDLGVKVLYGLN	AMP	0.96
CANA_20_11	142-161	GRLTLNPGRKAVFLKVVNHG	AMP	0.96
CANA_20_12	772-791	LGVKVLYGLNKRVEAVSNVR	AMP	0.96
CANA_20_13	138-157	ICADGRLTLNPGRKAVFLKV	AMP	0.96
CANA_20_14	759-778	ALSIAFVSKAALDLGVKVLY	AMP	0.96
CANA_20_15	351-370	GIIKADIGIKDGLIASIGKA	AMP	0.96
CANA_20_16	774-793	VKVLYGLNKRVEAVSNVRKL	AMP	0.95
CANA_20_17	757-776	GSALSIAFVSKAALDLGVKV	AMP	0.94
CANA_20_18	10-29	KISLHNAGFLAQKRLARGVR	AMP	0.94
CANA_20_19	707-726	IWKPSFFGAKPDIVIKGGSI	AMP	0.94
CANA_20_20	670-689	NFRIKRYIAKYTINPAIVNG	AMP	0.93
CANA_20_21	139-158	CADGRLTLNPGRKAVFLKVV	AMP	0.93
CANA_20_22	706-725	VIWKPSFFGAKPDIVIKGGS	AMP	0.91
CANA_20_23	761-780	SIAFVSKAALDLGVKVLYGL	AMP	0.91
CANA_20_24	705-724	LVIWKPSFFGAKPDIVIKGG	AMP	0.91
CANA_20_25	12-31	SLHNAGFLAQKRLARGVRLN	AMP	0.91
CANA_20_26	758-777	SALSIAFVSKAALDLGVKVL	AMP	0.9
CANA_20_27	777-796	LYGLNKRVEAVSNVRKLTKL	AMP	0.9
CANA_20_28	350-369	TGIIKADIGIKDGLIASIGK	AMP	0.9

Table 1 AMPs Predicted by CAMP$_{R4}$ Tool: AMPs of lengths 20, 30, 40, and 50 a.a. with AMP probabilities and class. Cana - *Canavalia ensiformis* (source of Urease 2), length of AMP, Sequence number

Seq ID	Position	Sequence	Class	AMP Probability
CANA_20_29	776-795	VLYGLNKRVEAVSNVRKLTK	AMP	0.9
CANA_30_1	754-783	GKAGSALSIAFVSKAALDLGVKVLYGLNKR	AMP	0.99
CANA_30_2	756-785	AGSALSIAFVSKAALDLGVKVLYGLNKRVE	AMP	0.99
CANA_30_3	757-786	GSALSIAFVSKAALDLGVKVLYGLNKRVEA	AMP	0.98
CANA_30_4	753-782	LGKAGSALSIAFVSKAALDLGVKVLYGLNK	AMP	0.98
CANA_30_5	751-780	GTLGKAGSALSIAFVSKAALDLGVKVLYGL	AMP	0.98
CANA_30_6	759-788	ALSIAFVSKAALDLGVKVLYGLNKRVEAVS	AMP	0.98
CANA_30_7	752-781	TLGKAGSALSIAFVSKAALDLGVKVLYGLN	AMP	0.97
CANA_30_8	763-792	AFVSKAALDLGVKVLYGLNKRVEAVSNVRK	AMP	0.96
CANA_30_9	15-44	NAGFLAQKRLARGVRLNYSESVALIASQIL	AMP	0.95
CANA_30_10	755-784	KAGSALSIAFVSKAALDLGVKVLYGLNKRV	AMP	0.95
CANA_30_11	758-787	SALSIAFVSKAALDLGVKVLYGLNKRVEAV	AMP	0.95
CANA_30_12	10-39	KISLHNAGFLAQKRLARGVRLNYSESVALI	AMP	0.95
CANA_30_13	700-729	GKLADLVIWKPSFFGAKPDIVIKGGSIAWA	AMP	0.95
CANA_30_14	762-791	IAFVSKAALDLGVKVLYGLNKRVEAVSNVR	AMP	0.94
CANA_30_15	210-239	VNLVSIGGNKIIRGGNAIADGPVNEANCKA	AMP	0.94
CANA_30_16	687-716	VNGISQYVGSVEVGKLADLVIWKPSFFGAK	AMP	0.94
CANA_30_17	209-238	TVNLVSIGGNKIIRGGNAIADGPVNEANCK	AMP	0.93

CANA_30_18	211-240	NLVSIGGNKIIRGGNAIADGPVNEANCKAA	AMP	0.93
CANA_30_19	761-790	SIAFVSKAALDLGVKVLYGLNKRVEAVSNV	AMP	0.93
CANA_30_20	11-40	ISLHNAGFLAQKRLARGVRLNYSESVALIA	AMP	0.93
CANA_30_21	673-702	IKRYIAKYTINPAIVNGISQYVGSVEVGKL	AMP	0.92
CANA_30_22	199-228	SVRFEPGDHKTVNLVSIGGNKIIRGGNAIA	AMP	0.92
CANA_30_23	760-789	LSIAFVSKAALDLGVKVLYGLNKRVEAVSN	AMP	0.92
CANA_30_24	351-380	GIIKADIGIKDGLIASIGKAGNPDIMNGVF	AMP	0.91
CANA_30_25	767-796	KAALDLGVKVLYGLNKRVEAVSNVRKLTKL	AMP	0.91
CANA_30_26	748-777	PMYGTLGKAGSALSIAFVSKAALDLGVKVL	AMP	0.91
CANA_30_27	750-779	YGTLGKAGSALSIAFVSKAALDLGVKVLYG	AMP	0.91

Table 1 (continued from page 5): AMPs Predicted by CAMP$_{R4}$ Tool: AMPs of lengths 20, 30, 40 and 50 a.a. with AMP probabilities and class. Cana- *Canavalia ensiformis* (source of Urease 2), length of AMP, Sequence number

Seq ID	Position	Sequence	Class	AMP Probability
CANA_30_28	699-728	VGKLADLVIWKPSFFGAKPDIVIKGGSIAW	AMP	0.91
CANA_30_29	766-795	SKAALDLGVKVLYGLNKRVEAVSNVRKLTK	AMP	0.91
CANA_30_30	343-372	SAVIIDYTGIIKADIGIKDGLIASIGKAGN	AMP	0.9
CANA_30_31	344-373	AVIIDYTGIIKADIGIKDGLIASIGKAGNP	AMP	0.9
CANA_30_32	12-41	SLHNAGFLAQKRLARGVRLNYSESVALIAS	AMP	0.9
CANA_30_33	674-703	KRYIAKYTINPAIVNGISQYVGSVEVGKLA	AMP	0.9
CANA_30_34	773-802	GVKVLYGLNKRVEAVSNVRKLTKLDLKLNN	AMP	0.9
CANA_40_1	754-793	GKAGSALSIAFVSKAALDLGVKVLYGLNKRVEAVSNVRKL	AMP	0.98

CANA_40_2	756-795	AGSALSIAFVSKAALDLGVKVLYGLNKR VEAVSNVRKLTK	AMP	0.98
CANA_40_3	751-790	GTLGKAGSALSIAFVSKAALDLGVKVLY GLNKRVEAVSNV	AMP	0.98
CANA_40_4	752-791	TLGKAGSALSIAFVSKAALDLGVKVLYGL NKRVEAVSNVR	AMP	0.97
CANA_40_5	757-796	GSALSIAFVSKAALDLGVKVLYGLNKRV EAVSNVRKLTKL	AMP	0.96
CANA_40_6	753-792	LGKAGSALSIAFVSKAALDLGVKVLYGL NKRVEAVSNVRK	AMP	0.94
CANA_40_7	755-794	KAGSALSIAFVSKAALDLGVKVLYGLNK RVEAVSNVRKLT	AMP	0.93
CANA_50_1	678-727	AKYTINPAIVNGISQYVGSVEVGKLADLV IWKPSFFGAKPDIVIKGGSIA	AMP	0.89
CANA_50_2	754-803	GKAGSALSIAFVSKAALDLGVKVLYGLN KRVEAVSNVRKLTKLDLKLNNS	AMP	0.88
CANA_50_3	751-800	GTLGKAGSALSIAFVSKAALDLGVKVLY GLNKRVEAVSNVRKLTKLDLKL	AMP	0.87
CANA_50_4	750-799	YGTLGKAGSALSIAFVSKAALDLGVKVL YGLNKRVEAVSNVRKLTKLDLK	AMP	0.86
CANA_50_5	677-726	IAKYTINPAIVNGISQYVGSVEVGKLADL VIWKPSFFGAKPDIVIKGGSI	AMP	0.86

Table 1 (continued from page 6): AMPs Predicted by CAMP$_{R4}$ Tool: AMPs of lengths 20, 30, 40 and 50 a.a. with AMP probabilities and class. Cana- *Canavalia ensiformis* (source of Urease 2), length of AMP, Sequence number

Evaluation of physicochemical properties of peptides

Physicochemical properties are the determining factors for the pharmacokinetic properties of peptides. Potential drug candidates should exhibit minimal or no activity against RBCs. All the AMPs exhibited intermediate hemolysis, with probabilities ranging from 0.4-0.6 (Figure 2a). The peptides were classified as hydrophobic or hydrophilic using the GRAVY scale. This is the ratio of the average hydrophobicity of a peptide to its length. CANA_20_18, CANA_20_25, CANA_30_29, and CANA_30_34 had negative values. These findings suggest that these peptides are hydrophilic, whereas the others are hydrophobic (Figure 2b). A drug molecule should be stable under standard conditions (instability index) and at elevated temperatures (aliphatic indices) (Figure 2c and d, respectively). None of the peptides had an instability index above 40 and could therefore be considered stable at room temperature. Additionally, the aliphatic indices of the peptides were higher, indicating the thermostability of all candidates. Drugs may interact with other proteins and thus can be multifunctional. All peptides 20 a.a. in length, except for CANA_20_18, had a negative Boman index and, therefore, no interaction with other proteins. All other peptides had Boman indices ranging from to 0-2. This range corresponds to moderate interactions with

other proteins (Figure 2e). The threat of triggering a class I immune response was also determined. All peptides, except CANA_30_1 and CANA_30_6, had scores less than 0.3, and were therefore non-immunogenic (Figure 2f). CANA_30_1 and CANA_30_6 had scores of 0.61 and 0.43, respectively. Therefore, these candidates may trigger an immune response.

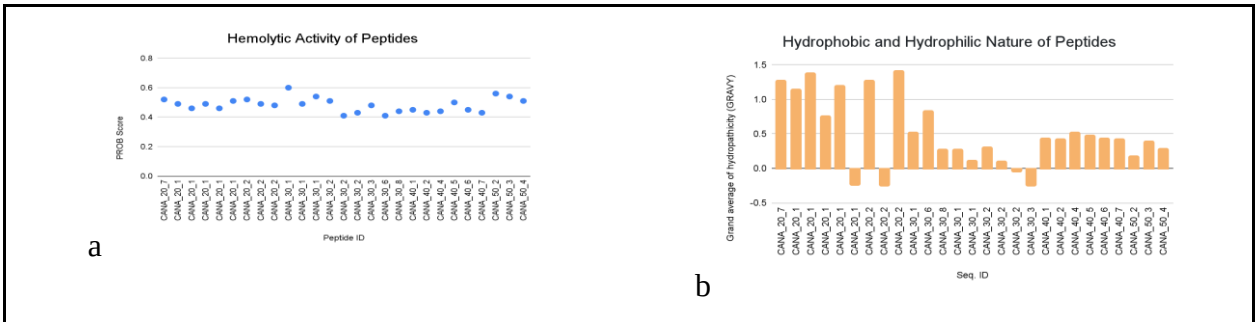
Evaluation of the ADMET properties of AMPs

Understanding the pharmacokinetics and toxicity of proposed drug molecules early in the drug development process is important. This approach saves considerable time and money in in vivo assays. The Absorption, Distribution, Excretion, and Toxicity (ADMET) properties of the peptides were studied using ADMET Lab 2.0 and are listed in Tables 3 and 4. The intestinal absorption of orally administered drugs is very important. The absorption of the peptides was evaluated in two different cell lines: Human Intestinal Cells (HIA) and Human Colon Epithelial Cancer Cells (Cacoa-2). All candidates showed positive HIA values and highly negative Caco-2 values. This finding indicated that the selected candidates could be effectively absorbed by the intestinal lining. In addition, all AMPs showed suboptimal blood-brain barrier penetration. Plasma Protein Binding (PPB) is the ability of a drug to interact with other molecules in blood. A capacity higher than 90% indicates a low therapeutic index, whereas a capacity lower than 90% indicates greater therapeutic potential. All AMPs have a high therapeutic potential. Thirteen AMPs had volume distributions within the desired range, while the others had negative values. Xenobiotic metabolism is based on the capacity of a drug to act as a substrate or inhibitor of the cytochrome P450 genes CYP1A2 and CYP3A4. All the candidates were classified as non-inhibitors. This finding suggests that the metabolism of these drugs was efficient. The peptide clearance rate was very low. The probability of having a half-life above 3 h is also close to 0.9 for all AMPs. This implies that each peptide had a longer half-life. The effects on Human Ether-a-go-go-related genes are a measure of cardiotoxicity. None of the peptides were cardiotoxic. None of the peptides are responsible for Drug Induced Liver Injury (DILI) or serve as potential carcinogens. The peptides used in this study also showed no risk to skin sensitivity.

Seq. ID	***Escherichia coli* ATCC 25922**	***Salmonella typhimurium* ATCC 14028**	***Acinetobacter baumannii* ATCC 19606**	***Staphylococcus aureus* ATCC 25923**	***Enterococcus faecalis* ATCC 29212**	***Bacillus subtilis* ATCC 6633**
CANA_20_18	✓		✓		✓	✓
CANA_30_1	✓					
CANA_30_8	✓		✓	✓		
CANA_30_10	✓		✓			
CANA_30_25	✓		✓			✓
CANA_30_29	✓		✓			✓
CANA_30_34	✓		✓			✓

CANA_40_1	✓		✓	✓		
CANA_40_2	✓		✓	✓		
CANA_40_4	✓					
CANA_40_5	✓		✓	✓		
CANA_40_6	✓		✓	✓		
CANA_40_7	✓		✓	✓		
CANA_50_2	✓	✓	✓	✓		✓
CANA_50_3	✓	✓	✓	✓		
CANA_50_4	✓		✓	✓		
CANA_20_7			✓			
CANA_20_10			✓			
CANA_20_14			✓			
CANA_20_15			✓			
CANA_20_17			✓			
CANA_20_23			✓			
CANA_20_26			✓			
CANA_30_6			✓			
CANA_30_12			✓			
CANA_30_20			✓			
CANA_20_25						✓
CANA_30_12						✓

Table 2 Antimicrobial activities of AMPs generated from Urease 2



a b

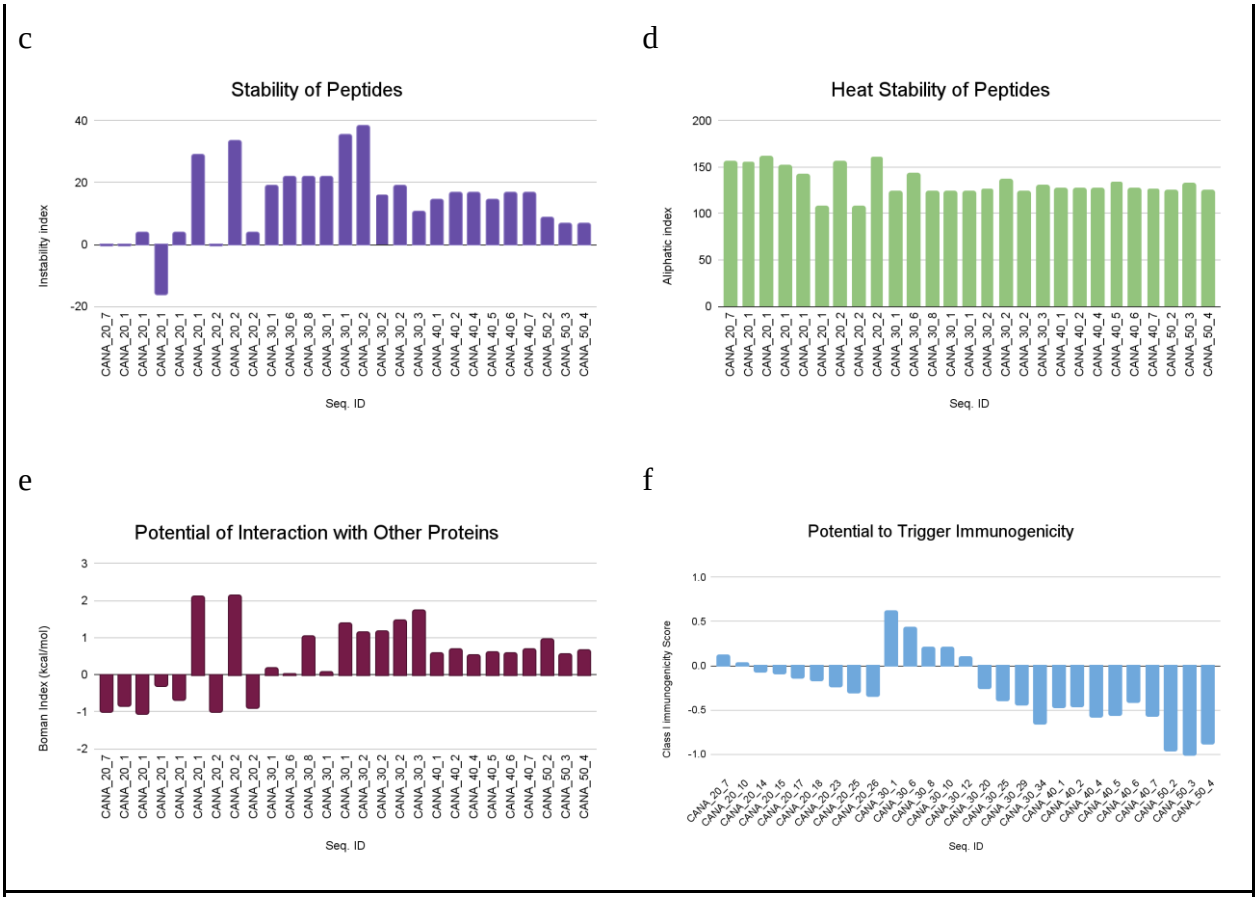


Fig 2 Physicochemical properties of AMPs a. Hemolytic Activities of Peptides. The PROB score indicates a peptide's probability (0-1) to be hemolytic. '0' indicates the probability of being the least hemolytic and '1' hemolytic. b. Potential to Trigger Immunogenicity: Values above 0.3 suggest Class I immunogenicity c. The stability of Peptides in the test tube is essential. Candidates with an instability index >40 are unstable. d. The heat stability of Peptides is calculated using an aliphatic index. Heat stability is directly proportional to index values. The higher the index value, more the stability of the peptide. e. Hydrophobicity and Hydrophilic Nature of AMPs. Negative and positive values indicate globular (hydrophilic) and membranous proteins (hydrophobic) peptides, respectively.

	Absorption		**Distribution**			**Metabolism**			
Seq ID	HIA	Caco-2	BBB	PPB%	VDss	CYP1A2-inh	CYP1A2-sub	CYP3A4-inh	CYP3A4-sub
CANA_20_7	1	-8.201	0.008	44.42	0.468	0	0	0.017	0
CANA_20_10	0.999	-8.279	0.009	38.73	0.522	0	0	0.011	0
CANA_20_14	1	-8.271	0.008	47.71	0.478	0	0	0.011	0
CANA_20_15	1	-8.717	0.014	32.78	0.335	0	0	0	0
CANA_20_17	1	-8.852	0.01	22.84	0.322	0	0	0.001	0
CANA_20_18	0.973	-6.326	0.016	31.03	0.207	0	0	0.001	0

CANA_20_23	1	-8.356	0.008	45.15	0.484	0	0	0.012	0
CANA_20_25	0.865	-6.365	0.02	26.65	0.224	0	0	0	0
CANA_20_26	1	-8.445	0.007	34.73	0.332	0	0	0.003	0
CANA_30_1	1	-8.933	0.003	31.98	0.047	0	0	0	0
CANA_30_6	1	-9.086	0.002	41.64	0.116	0	0	0	0
CANA_30_8	1	-8.794	0.002	29.03	-0.004	0	0	0	0
CANA_30_10	1	-8.542	0.002	38.01	0.044	0	0	0	0
CANA_30_12	0.999	-7	0.003	47.46	-0.062	0	0	0	0
CANA_30_20	0.999	-7.518	0.003	49.75	0.021	0	0	0	0
CANA_30_25	1	-8.608	0.003	26.62	-0.123	0	0	0	0
CANA_30_29	1	-8.895	0.003	23.42	-0.181	0	0	0	0
CANA_30_34	1	-8.463	0.003	21.23	-0.206	0	0	0	0
CANA_40_1	1	-9.702	0.001	29.36	-0.382	0	0	0	0
CANA_40_2	1	-9.566	0.001	30.79	-0.401	0	0	0	0
CANA_40_4	1	-9.923	0	33.94	-0.319	0	0	0	0
CANA_40_5	1	-9.598	0.001	31.03	-0.424	0	0	0	0
CANA_40_6	1	-9.512	0.001	31.67	-0.363	0	0	0	0
	HIA > 0.3: HIA positive	Optimal: higher than −5.15	Optimal: 0.04–20 L/kg	≥0.1: BBB positive and	PPB < 90%: optimal PPB >	>0.5: inhibitor <0.5: non	>0.5: substrate <0.5: non	>0.5: inhibitor <0.5: noninhibit	>0.5: substrate <0.5: nonsubstrat

	HIA < 0.3: HIA negative			<0.1: BBB negative	90%: low therapeutic index	inhibitor	substrate	or	e

Table 3 Absorption, Distribution, and Metabolism properties of AMPs

Sequence ID	Absorption		Distribution			Metabolism			
	HIA	Caco-2	BBB	PPB%	VDss	CYP1A2-inh	CYP1A2-sub	CYP3A4-inh	CYP3A4-sub
CANA_40_7	1	-9.466	0.001	31.31	-0.427	0	0	0	0
CANA_50_2	1	-10.52	0	21.13	-0.877	0	0	0	0
CANA_50_3	1	-10.705	0	27.32	-0.77	0	0	0	0
CANA_50_4	1	-10.525	0	33.51	-0.661	0	0	0	0
	HIA > 0.3: HIA positive HIA < 0.3: HIA negative	Optimal: higher than −5.15	Optimal: 0.04–20 L/kg	≥0.1: BBB positive and <0.1: BBB negative	PPB < 90%: optimal PPB > 90%: low therapeutic index	>0.5: inhibitor <0.5: non inhibitor	>0.5: substrate <0.5: non substrate	>0.5: inhibitor <0.5: noninhibitor	>0.5: substrate <0.5: nonsubstrate

Table 3 Absorption, Distribution, and Metabolism properties of AMPs

Discussion

Urease 2, a protein derived from the jack bean, is a source of novel AMP stretches. AMPs derived from this toxin showed potential effects against *Bacillus subtilis* ATCC 6633, *E. coli* ATCC25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC14028, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC700603, and *Pseudomonas aeruginosa* ATCC27853. All molecules fulfill most of the requirements of the ADMET properties. Further molecular engineering may help identify more stable peptides derived from native peptides. CANA_20_18 is the most suitable AMP candidate for in vivo studies.

	Excretion		Toxicity				
Seq ID	CL (mL/min/kg)	T12	hERG	DILI	Ames	Carcinogenicity	SkinSen
CANA_20_7	0.466	0.914	0.001	0.004	0.002	0.021	0.039
CANA_20_10	0.268	0.852	0	0.003	0.002	0.043	0.059
CANA_20_14	0.405	0.903	0	0.008	0.001	0.03	0.03
CANA_20_15	0.566	0.933	0	0	0.001	0.29	0.056
CANA_20_17	0.15	0.919	0	0.006	0.002	0.017	0.067
CANA_20_18	-0.08	0.886	0.001	0	0.011	0.021	0.078
CANA_20_23	0.459	0.903	0	0.005	0.001	0.037	0.044
CANA_20_25	-0.17	0.823	0.001	0	0.013	0.027	0.077
CANA_20_26	0.505	0.898	0	0.006	0.002	0.026	0.047
CANA_30_1	-1.426	0.946	0	0	0.002	0.009	0.054
CANA_30_6	-1.239	0.935	0	0.001	0.001	0.014	0.024
CANA_30_8	-1.654	0.933	0	0	0.004	0.015	0.03
CANA_30_10	-1.269	0.93	0	0	0.002	0.009	0.042
CANA_30_12	-1.342	0.929	0	0	0.003	0.015	0.035
CANA_30_20	-1.229	0.916	0	0	0.002	0.013	0.033
CANA_30_25	-1.363	0.938	0	0	0.003	0.012	0.033
CANA_30_29	-1.608	0.952	0	0	0.004	0.015	0.034
CANA_30_34	-1.863	0.909	0	0	0.008	0.025	0.031
CANA_40_1	-3.211	0.963	0	0	0.001	0.002	0.019

CANA_40_2	-3.18	0.956	0	0	0.001	0.003	0.011
CANA_40_4	-3.004	0.947	0	0	0.001	0.002	0.013
CANA_40_5	-3.097	0.957	0	0	0.001	0.002	0.012
CANA_40_6	-3.099	0.956	0	0	0.001	0.003	0.019
CANA_40_7	-3.097	0.956	0	0	0.001	0.002	0.012
CANA_50_2	-5.203	0.971	0	0	0.001	0.003	0.014
CANA_50_3	-4.702	0.979	0	0	0	0.001	0.01
CANA_50_4	-4.783	0.975	0	0	0	0.001	0.009
	High: >15 Moderate: 5–15 Low: <5	Long half-life: >3 h Short half-life: <3 h	>0.5: blocker <0.5: nonblocker	>0.5: hepatotoxic <0.5: nonhepatotoxic	>0.5: positive <0.5: negative	>0.5: carcinogen <0.5: noncarcinogen	>0.5: sensitizer <0.5: nonsensitizer

Table 4 Excretion and Toxicity of the peptides studies.

Conflict of Interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interests in the subject matter or materials discussed in this manuscript.

Ethics Approval: This study did not involve the use of animals. Therefore, ethical approval was not required for this study.

Authors Contribution

Mugdha Belwalkar-Conceptualization, Data Analysis & Investigation, Original Draft Preparation
Aditi Thakkar - Screened for unique protein to be analyzed and Original Draft Preparation
Varsha Shukla - Scientific advice
Anushree Lokur - Scientific advice and manuscript review

Acknowledgement: The authors acknowledge Dr. Mayuri Rege for the Scientific advice.

Funding:n No funding was received for this study.

Data Avaialability:

All the data supporting the findings of the above research are available in the following links:

https://docs.google.com/spreadsheets/d/1ks3SgII9i4oXfwoFj1vXs66xWYAM5j0ntXj5VsGwgVY/edit?usp=sharing
https://drive.google.com/file/d/1j1Q_ykQ5FXLHO80HVfMfBraVcInQTqVA/view?usp=sharing
https://drive.google.com/file/d/1rGMrbcXPcg2NE2GlZBbNkVkdzKV0GPdT/view?usp=sharing
https://drive.google.com/file/d/14pdY9nr-DRJPABbxpMn7Ktr9o_TOod6q/view?usp=sharing
https://drive.google.com/file/d/1O3EuK0ezlADsAwolQMpZo-yapCvoJyqz/view?usp=sharing

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