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Research Article

IDENTIFICATION AND PHARMACOLOGICAL PROFILING OF PLANT PEPTIDES AS INHIBITORS OF *CLOSTRIDIUM BOTULINUM* NEUROTOXINS A AND B

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ABSTRACT

Objective: *Clostridium botulinum* causes botulism which is a neuroparalytic disease caused by neurotoxins that are caused all over the world. This disease is perceived as a food born disease caused by a potent neurotoxin. Plant peptides are those referred to as proteins at a length of small than 100 amino acids. Plants have antimicrobial properties present in almost all plant species. These plant peptides are classified based on their primary structures.

Methods: Molecular docking was implemented to evaluate the efficacy of nine phytocompounds from *C. botulinum* against the target proteins (3nf3, 2npo). PyRx, a Virtual Screening software was utilized for molecular docking which allowed the inspection of three-dimensional protein structures and the identification of potential binding sites.

Result: On analyzing the molecular docking result of puroindoline, impatiens exhibited the best binding affinity toward the two target proteins.

Conclusion: The safe and efficient treatment for some facial pleats and lines is botulinum toxin. High levels of patient and physician satisfaction are linked to its use. This paper has extensively revised three aspects the binding activity, models, and their phytochemical properties. The most significant results involve solvent interactions, van der Waals interactions, electrostatic interactions, and hydrogen bonding were analyzed and concluded in this research paper.

Keywords: Botulism, Neurotoxins A, Neurotoxins B, Peptides, Molecular docking.

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INTRODUCTION

Botulism [1] is an uncommon but deadly condition caused by toxins that target the body's nerves, causing difficulty breathing, muscle paralysis, and even death. The bacteria *Clostridium botulinum*, as well as *Clostridium butyricum* [2] and *Clostridium baratii* [3], can produce this toxin. It is an obligate anaerobic Gram-positive bacillus that produces spores [4]. The ubiquitous clostridial spores in soil are extremely heat, light, dryness, and radiation resistant. Three different types of human botulism diseases can occur foodborne illness, newborn botulism, and wound contamination. Depending on how the toxin was produced, these types change. Usually, food contamination is generally from improperly home-canned vegetables [5] or fermented fish, which is the most prevalent source of the preformed toxin. Type A [6] toxins are to blame for 50% of foodborne outbreaks in the US. Type A for canned foods and type E for poorly fermented fish items are the most often isolated neurotoxins. Infants are the most prevalent victims of human botulism. Transmission of botulism normally happens when the bacterium, neurotoxin, or spores are ingested. If the organism is consumed, it then replicates in the stomach which germinates and releases a neurotoxin [7].

A neurotransmitter called acetylcholine [8], which is necessary for communication between motor neurons and muscle cells, is inhibited by botulinum toxin in the neurological system. All forms of botulism result in paralysis, which normally begins in the facial muscles and progresses to the limbs. In its most severe forms, botulism causes respiratory failure and the paralysis of the breathing muscles. Due to this potentially fatal consequence, all suspected cases of botulism are handled as medical emergencies, and public health officials are typically involved in the investigation to determine the cause and take action to stop more cases from developing.

Peptides are crucial for numerous metabolic activities and play a crucial role in basic physiological functions. Peptide chains are created by combining amino acids with similar fundamental structures; they can grow to become oligopeptides when they have 10–20 amino acid chains. Peptides are important for intercellular communication in eukaryotes, acting as neuropeptides, hormones, and growth factors. They are also found in the body's defensive mechanism. Moreover, some peptides function as "hormones" [9] for bacteria, enabling bacterial communities to orchestrate multicellular activity such as biofilm formation. Certain peptides, such as defensins, are recognized for their significance in medicine. Plant peptides can serve a variety of functions and have been divided into two main categories. Proteins are broken down into two types of peptides: (1) non-bioactive peptides [10], which are predominantly produced by proteolytic enzymes [11] to recycle the proteins, and (2) bioactive peptides which are primarily released by enzymatic activities and are encrypted in the parent proteins structures. Peptides play a variety of roles in plant development, growth, and stress responses. They perform important roles in cell-to-cell communication, disrupt signaling and response systems, or act as antimicrobial agents. According to conventional knowledge, most antimicrobial peptides (AMPs) naturally attack bacteria, fungi, and enveloped viruses; nevertheless, some of these peptides also bind to poisons.

Plant peptides represent a promising area of research for the development of novel antimicrobial agents with potentially lower toxicity and greater effectiveness compared to traditional antibiotics. In addition, these are less likely to induce antimicrobial resistance, a growing concern with the overuse of traditional antibiotics. These peptides often have a broad spectrum of activity against a range of pathogenic microorganisms and *in silico* techniques [12], such as molecular docking and virtual screening, can be used to predict the potential efficacy of plant peptides as antimicrobial agents. These

methods can help identify peptides with the greatest potential for activity against specific microbial pathogens, allowing for more targeted experimental testing in the future.

METHODS

Retrieval of peptide sequence

Nine different AMPs are used to identify pharmacological plant peptides to inhibit botulism neurotoxins A and B. The FASTA sequence of all nine AMPs was gathered from the Uniprot database [13]. Uniprot Ids for the following AMPs are as follows: (1) Puroindoline (P33432), (2) Thaumatin-like Protein (G5DC91), (3) Thionin (C0HJH9), (4) Defensin (Q01524), (5) Non-specific ligand transfer protein (P82900), (6) Hevein (P02877), (7) Impatiens (O24006), (8) Snakin (B2ZAW4), and (9) Cyclotide (B3EWH5).

Retrieval of target proteins from protein data bank (PDB) and purification

The target proteins for neurotoxins A and B were taken from the PDB [14]. The target used for neurotoxin A is 3NF3 (PDB DOI-10.2210/pdb3NF3/pdb) which had a good resolution of 2.40A using the X-ray diffraction method [14] which was published on 2010-06-09. Whereas the target used for neurotoxin B is 2NP0 (PDB DOI-10.2210/pdb2NP0/pdb) which had a resolution of 2.64A using the same method used for neurotoxin A which was published on 2006-10-26. The target proteins were extorted from the same organism *C. botulinum*. Then, the neurotoxin that is the protein was purified in the BIOVIA Discovery studio [15].

Homology modeling of peptide

The Fasta sequence of all 9 AMPs was individually loaded to the Swiss model [16]. Each peptide had different templates, so, based on the high QMEAN value, one of the templates was selected. The QMEAN, GMQE, and Ramachandran statics for each AMPs are tabulated. Then for each AMP Swiss models were downloaded which are required for the protein-ligand interactions [17].

Physiological properties of plant peptide

The physiological properties of each plant peptide were analyzed using the Protparam [18] tool. The Protparam tool is a tool that allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY).

Molecular docking of plant peptides with local protein

In this research, molecular docking is the main significant approach for determining the active interactions between the purified protein and the ligands. The molecular docking of the nine ligands was performed using the H DOCK software's molecular docking engine. H DOCK [19] is a template-based modeling and a free docking for protein-protein, protein-DNA/RNA complexes.

Purified proteins were uploaded into H DOCK as a macromolecule, and plant phytocompounds [20] were added as ligands. Then, the protein-ligand interacted molecules were downloaded from the web server.

Visualization

BIOVIA Discovery Studio Software is a structure visualization tool used to visualize two-dimensional and three-dimensional structures. The docked structures were downloaded from PyRx and visualized using DS BIOVIA Discovery Studio software. The docking complexes were analyzed for the interactions of the ligands with amino acids in the protein's binding pocket [21]. The 2D and 3D structures of the interactions were generated.

RESULTS

Retrieval of peptide sequence

The antimicrobial plant peptides [22] for botulism were researched and identified each AMP Uniprot ID, number of amino acids, plant source, and Fasta sequence of all nine peptides were gathered from the Uniprot database and tabulated (Table 1).

Retrieval of target proteins from PDB and purification

The interactions of protein and ligand proteins played an important role which was gathered from PDB. The proteins 3nf3, 2npo had a great catalytic activity which is present in the *C. botulinum* species. The 3nf3 proteins have 1296 amino acids and 2npo has 1291 amino acid residues and share a common cofactor [23] that's is Zn2+. The structure of these proteins was taken from PBD by pasting the Fasta sequence and purified using the BIOVIA Discover Studio by adding the polar and neutralizing the protein which is depicted in Fig. 1.

Homology modelling of peptide

In the case of homology modeling of peptides, each peptide sequence was pasted and certain templates were chosen based on the Q MEAN Disco value [24], coverage, and Ramachandran statistics. The Ramachandran plot was analyzed for each peptide by checking the Molprobity, Bad Bonds, and Bad Angles. The Swiss model info of each peptide is tabulated (Table 2) and depicted (Fig. 2).

Physiological properties of plant peptide

To understand the physical and chemical properties of each plant peptide, the sequence of all the peptides was added to the Protparam database and the results are shown in Table 3.

Molecular docking of plant peptides with local protein

In this docking study, nine ligands were docked in H DOCK software against two protein targets: 3nf3 and 2npo. After docking, based on the docking score, confidence score Ligand root mean square deviation [25], as seen in Table 4, and the best docking peptides were chosen for the visualization process (Table 4).

After docking our ligands with targeted proteins, the binding affinity, center energy, and lowest energy were recorded. Among nine ligands screened, the top 4 ligands were selected with the lowest energy binding for each protein, namely, 3nf3 with Puroindoline and Impatiens and 2npo with Puroindoline and Impatiens.

Visualization

The selected ligands were subjected to visualization using BIOVIA Discovery Studio Visualizer using certain tools present in it. All the proteins and ligands are displayed in certain colors and H-bond interactions, non-bond interactions, and salt bridges are tabulated (Tables 5 and 6), the structures of all the protein-ligand interactions are shown in (Figs. 3 and 4).

Ligand shows major interactions with HIS and ARG amino acids which have seven hydrogen bonds, nine salt bridges, and 159 non-bonded contacts.

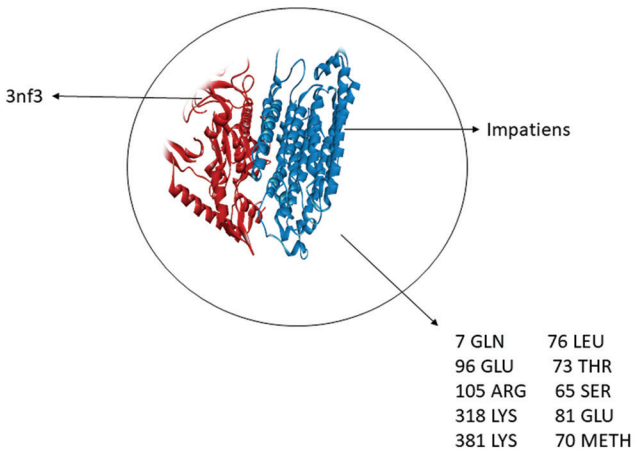


Table 1: Retrieval of plant peptides

Antimicrobial peptide	Uniport ID	Amino acids	Plant source	Fasta sequence
Puroindoline	P33432	148	Triticum aestivum (Wheat)	>sp|P33432|PUIA_WHEAT Puroindoline-A OS=Triticum aestivum OX=4565 GN=PINA PE=1 SV=2 MKALFLIGLLALVASTAFAQYSEVVGSYDVAGGGGAQQCPVETKLNSCRNYLLDRCSTMK DFPVTWRWWKWWKGGCQELLGECCSRLGQMPPQCRCNIIQGSIQGDLGGIFGFQRDRASK VIQEAKNLPPRCNQGPPCNIPGTIGYYW
Thaumatin-like Protein	G5DC91	207	Manilkara zapota (Sapodilla plum) (Achras zapota)	>sp|G5DC91|TLP1_MANZA Thaumatin-like protein 1 OS=Manilkara zapota OX=3741 GN=TLP1 PE=1 SV=2 ATFDVVNQCTFTVWAGASPGGGKQLDQGQTWTITVAPGSTKARIWGRTGCNFDANGQGKC QTGDCNGLLQCQGYGSPPNTLAEFSLNQPNNLDYVDISLVDGFNIPMDFSPAAAGVCKDI RCATDITAQCPAELQAPGGCNNPCTVYKTNEYCCTNGQGTCGPTALSKFFKDRCPDAYSY PQDDPTSLFTCPAGTNYKVVFCPNLDA
Thionin	C0HJH9	35	Nigella sativa (Black cumin)	>sp|C0HJH9|THNW1_NIGSA Thionin NsW1 (Fragment) OS=Nigella sativa OX=555479 PE=1 SV=1 KSCCKNTLGRNCYNTCRFMKKPRKTCSGLCGCKIS
Defensin	Q01524	100	spruce plants	>sp|Q01524|DEF6_HUMAN Defensin-6 OS=Homo sapiens OX=9606 GN=DEFA6 PE=1 SV=1 MRTLTILTAVLLVALQAKAEPLQAEDDPLQAKAYEADAQEQRGANDQDFAVSFAEDASSS LRALGSTRAFTCHCRRSCYSTEYSYGTCTVMGINHRFCCL
Non-specific ligand transfer protein	P82900	96	Triticum aestivum (Wheat)	>sp|P82900|NLT2G_WHEAT Non-specific lipid-transfer protein 2G OS=Triticum aestivum OX=4565 PE=1 SV=2 MAGMMKKQVVTALMLALVVLAAAPGGARAACQASQLAVCASAILSGAKPSGECCGNLRAQ QGCFCQYAKDPTYGQYIRSPHARDTLTSCGLAVPHC
Hevein	P02877	204	Hevea brasiliensis (Para rubber tree) (Siphonia brasiliensis)	>sp|P02877|HEVE_HEVBR Pro-hevein OS=Hevea brasiliensis OX=3981 GN=HEV1 PE=1 SV=2 MNIFIVVLLCLTGVAIAEQCGRQAGGKLCPNNLCCSQWGWCGSTDEYCSPDHNCQSNCKD SGEGVGGGSASNVLATYHLYNSQDHGWDLNAASAYCSTWDANKPYSWRSKYGWTAFCGPV GAHGQSSCGKCLSVTNTGTGAKTTVRIVDQCSNGGLDLDVNVFRQLDTDGKGYERGHITV NYQFVDCGDSFNPLFSVMKSSVIN
Impatiens	O24006	333	Impatiens balsamina (Balsam)	>sp|O24006|AMP_IMPBA Antimicrobial peptides OS=Impatiens balsamina OX=63779 GN=AMP PE=1 SV=1 MVQKGVVFGVLLILFICSTLTSADSKPNPTKEEEPAKKPDEVSVKSGGPEVSEDQYRHRC CAWGPGRKYCKRWCANAEEAAAAIPEASEELAQEEAPVYSEDQWGRRCCGWGPGRRYCVR WCQNAEEAAAAIPEATEKAQEAPVYSEDQWGRRCCGWGPGRRYCVRWCQNAEEAAAAVAI PEASEKAQEGPVYSEDQWGRRCCGWGPGRRYCVRWCSNAADEVATPEDVEPGQYGRRCCN WGPGRRYCKRWCHNAAEEATLKAFEEEAAREQPVYSEDQWGRRCCGWGPGRRYCRRWCQS AEEAAAFQAGEVTASLMLIMFKACPCMGPVPSV
Snakin	B2ZAW4	104	Capsicum annuum (Capsicum pepper)	>tr|B2ZAW4|B2ZAW4_CAPAN Antimicrobial peptide snakin OS=Capsicum annuum OX=4072 GN=Sn PE=2 SV=1 MAISKALLASLFLSLLLLEQVQSIQTDHVASNAISEAAYSYKKIDCGGKCSARCRLSSRP RLCKRACGTCCARCNCVPPGTSGNTQTCPCYANMTTHGNRRKCP
Cyclotide	B3EWH5	79	Petunia hybrida (Petunia)	>sp|B3EWH5|CYCA_PETHY Cyclotide phyb-A OS=Petunia hybrida OX=4102 PE=1 SV=1 MVGVNSLRSALYLIVLILFVQLTYFSDARVMDVDLSRAFLPLTGIGCGESCVWIPCVSAA IGCSCSNKICYRNGIIPKK

AMPs: Antimicrobial peptides

Table 2: Homology modeling of plant peptides

Peptide name	Uniport id	GMQE	Q MEAN Disco	Template	Ramachandran statistics (%)
Puroindoline	A0A7D5G7M5	0.21	0.61	2ds2.1.B	96.88
Thaumatin like-protein	G5DC91	0.88	0.84	4l2j. 1.A	95.57
Thionin	C0HJH9	0.76	0.70	1wuw. 1.A	93.94
Defensin	C0HJZ1	0.44	0.64	2gl1.1.A	94.2
Non-specific ligand transfer	P82900	0.58	0.73	1tuk. 1.A	100
hevein	P02877	0.15	0.73	1wkx. 1.A	100
impatiens	A0A0K1Z7J5	0.85	0.80	7wff. 1.B	97.8
Snakin	B2ZAW4	0.46	0.77	5e5q. 1.A	98.33
Cyclotide	C0HLN8	0.82	0.78	7rii. 1.A	89.29

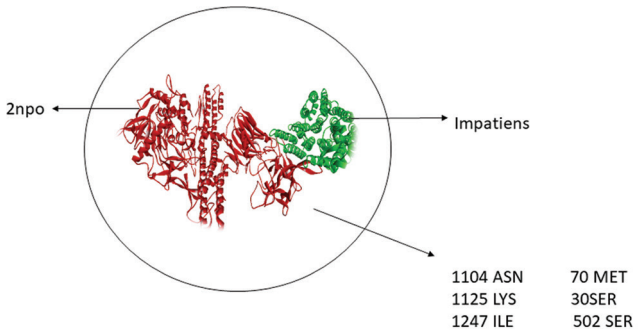
Table 3: Physiological properties of plant peptides

Peptide name	Amino acid	Molecular weight	Theoretical pI	Extinction Co-efficient	Instability index	Aliphatic Index	GRAVY
Puroindoline	164	18196.83	7.44	34950	55.27 (classified protein)	70.24	−0.282
Thaumatin- like protein	207	2122.41	4.44	2693.	24.86 (classified protein)	54.73	−0.312
Thionin	35	3933.75	9.67	1490	10.15 (Classified protein)	33.43	−0.2060
Defensin	72	8307.74	7.53	6990	24.80 (Classified protein)	73.06	−0.078
Non-specific ligand transfer	96	9831.55	8.89	4470	41.36 (classified protein)	81.56	0.308
Hevein	204	21859.28	5.63	44920	26.05 (classified protein)	63.53	−0.256
Impatiens	515	57325.48	5.32	76780	41.17 (classified protein)	117.77	0.675
Snakin	104	11176.03	9.24	4470	37.42 (classified protein)	71.44	−0.138
Cyclotide	30	3179.77	8.32	2980	27.88 (classified protein)	52.00	0.427

The major interactions with 3nf3 protein and impatiens

Ligand containing hydrogen bonds is represented in this figure which shows major interactions with GLN and LYS amino acids which have seven hydrogen bonds, two salt bridges, and 176 non-bonded contacts.

Ligand containing hydrogen bonds is represented in this figure which shows major with ASP and GLN amino acids and has six hydrogen bonds, one salt bridge, and 293 non-bonded contacts.



The major interactions with 2npo protein and Impatiens

Ligand containing hydrogen bonds is represented in this figure which shows major with ASN, LYS, and GLN amino acids and has 3 hydrogen bonds and 187 non-bonded contacts.

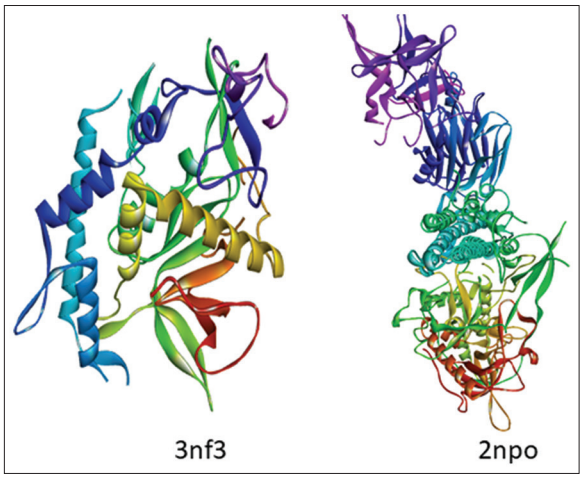


Fig. 1: Botulism neurotoxins A and B protein structures

DISCUSSION

C. botulism is an anaerobic bacteria which are viable and produces toxins in food which may cause foodborne botulism. This disease was not only caused by spoiled food consumption but also by infected wounds which would cause paralytic botulism, which is not distinguishable from normal botulism. Botulism neurotoxins (BoNTS) are produced by a heterogeneous group of clostridia bacteria that differ widely in genetic and metabolic characteristics. BoNTs are the most potent protein toxins and their toxicity depends on the route of entry into the human body. The multiplication of this bacteria and the formation of toxins occur in products with low oxygen content and more frequently in lightly preserved foods and inadequately processed, home-canned food. When these spores enter the gut and pass to the stomach, the spores may mature and cause infant botulism [26]. About 20% of infant botulism can be caused due to the intake of honey or corn syrup. Although mainly a foodborne intoxication [27], human botulism can also be caused by intestinal infection with *C. botulinum* in infants, wound infections, and inhalation. There are certain benefits of taking botulism toxin injection increases the quality of life, increased confidence, and increased the mobility of the limbs. The effectiveness of the botulism is temporary but the muscles get relaxed within 3–4 days and will remain in state for 3–4 months. This disease is very harmful because these toxins are the most lethal substances which can block nerve functions which can lead to blockage of nerve, respiratory functions, and paralysis. Intestinal botulism is the most common form of botulism where children under 12 get this disease in most common, but adults who have gastrointestinal issues also suffer from this disease. The common symptoms of this disease in adults are dry mouth, nausea, vomiting, visual disturbances, muscle weakness, and breathing difficulties as in the case of children suffering from constipation, poor sucking and feeding, inability to control movements, and feeble cry. Precautions are to avoid giving honey to babies under 12 months of age and take care of when preparing, handling, and storing solid food for babies.

Table 4: Docking score, confidence score, and ligand RMSD of each peptide

	Peptide-3 nf3			Peptide-2npo		
Peptide	**Docking score**	**Confidence score**	**Ligand RMSD (Å)**	**Docking score**	**Confidence score**	**Ligand RMSD (Å**
Puroindoline	−213.74	0.7816	23.42	−284.95	0.9370	93.65
Thaumatine like protein	−207.26	0.7586	62.64	−260.32	0.9008	86.07
Thionine	−231.86	0.8372	68.61	−266.32	0.9110	54.58
Defensin	−208.59	0.7635	29.17	−222.75	0.8108	90.67
Hevein	−205.25	0.7512	34.29	−224.37	0.8157	80.00
Non-specific ligand transfer	−203.50	0.7446	23.03	−246.92	0.8742	65.10
Cyclotide	−209.23	0.7658	20.24	−223.27	0.8142	86.58
Snakin	−258.38	0.8973	40.84	−263.33	0.9061	77.44
Impatiens	−3.14.56	0.9641	54.67	−298.00	0.9507	124.74

RMSD: Root mean square deviation

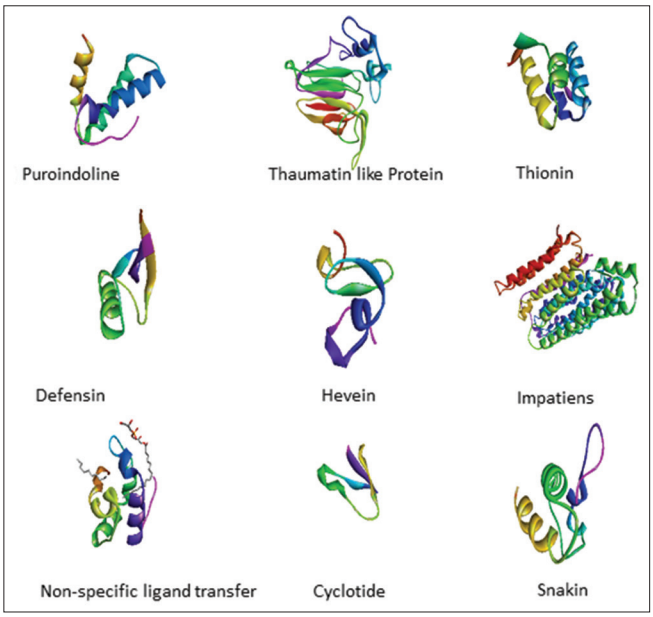


Fig. 2: Botulism neurotoxins A and B plant peptides

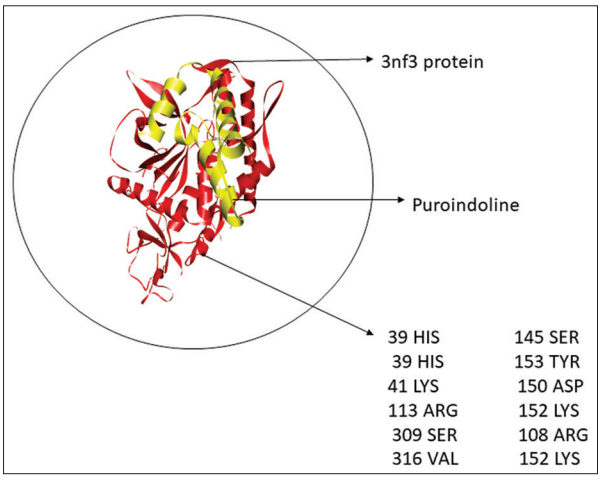


Fig. 3: The major interactions with 3nf3 protein and puroindoline

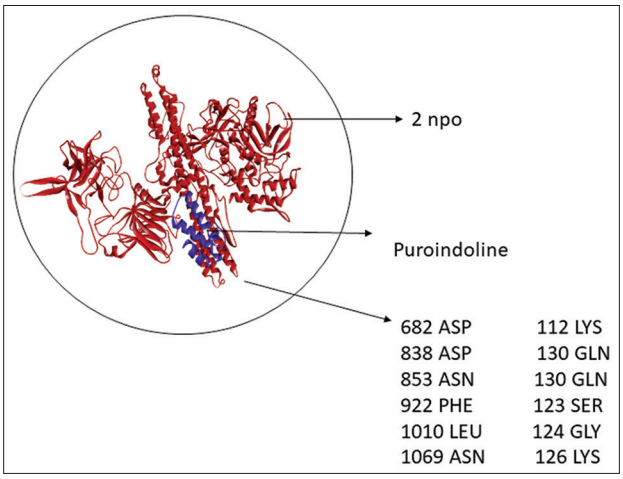


Fig. 4: The major interactions with 2npo protein and puroindoline

AMPs are a major area of research around the world, but there are still a lot of pressing design and application problems that need to be resolved. The use of AMPs is constrained by several variables. Potential AMPs can be developed further by the interaction of interdisciplinary fields such as biology, materials science, chemistry, bioinformatics, molecular informatics, and pharmacy. The mechanism of AMPs and different targets helps experimental designs to produce more robust systemic and scientific demonstrations. These antimicrobial pant peptides act as a defense mechanism to the plants and are isolated from the roots, leaves, stems, flowers, seeds, etc., which contain antibiotic compounds and some medicinal uses. For the creation of unique antimicrobial drugs that may be less harmful and more effective than conventional antibiotics, plant peptides constitute a promising area of research. As a result of the abuse of conventional antibiotics, these are also less likely to lead to the development of antimicrobial resistance. *In silico* methods such as molecular docking and virtual screening can be used to anticipate the potential efficacy of plant peptides as antimicrobial drugs. These peptides frequently have a broad spectrum of activity against a variety of pathogenic microbes. With the aid of these techniques, peptides with the greatest potential for activity against particular microbial infections can be identified, enabling future more focused experimental testing. When compared to other kingdoms, plant AMPs exhibit more diversity and abundance. With their enormous quantity and isoform diversity, plants may contain many AMP classes that have not yet been identified. Even in the omics era, the genomic and peptide structure of AMPs might vary, with only a few important residues being conserved. This makes identification, classification, and comparison difficult. Since amino acids are used to make AMPs, altering their structure (including library creation and screening), and immobilizing them on surfaces is quite simple. Fully synthetic peptides can be created using chemical synthesis or recombinant expression systems. These synthetic AMP sources can be used to modify natural AMPs as well as create brand-new synthetic AMPs. These alterations have the potential to alter the targets for AMPs and increase their protease stability. The main method of death was the permeabilization of the bacterial cell membrane by AMP. To kill microorganisms by disrupting the membrane with enough channels

Table 5: List of peptide interactions with protein 3nf3

Peptide	Hydrogen bond		Bond Distance	Non-bonded		Bond distance	Salt bridges	
	Chain A	Chain B		Chain A	Chain B		Chain A	Chain B
Impatiens	7 GLN	76 LEU	2.57	1 METH	109 THR	3.60	325 GLU	476 ARG
	96 GLU	73 THR	3.03	2 PRO	72 ILE	3.53	381 LYS	81 GLU
	105 ARG	65 SER	2.43	4 VAL	76 LEU	3.29		
	318 LYS	482 ASN	3.24	6 LYS	505 PRO	3.83		
	381 LYS	81 GLU	1.69	7 GLN	509 ILE	3.82		
				13 PRO	504 ASN	3.68		
Puroindoline	39 HIS	145 SER	2.78	1 MET	146 LYS	3.42	41 LYS	150 ASP
	39 HIS	153 TYR	2.92	5 ASN	148 ASN	3.85		
	41 LYS	150 ASP	2.69	37 LYS	148ASM	3.67		
	113ARG	152 LYS	2.72	39 HIS	145 SER	3.43		
	309 SER	108 ARG	2.26	40 ASN	148 SER	3.71		
	316 VAL	152 LYS	2.92	109 THR	153 TYR	3.79		

Table 6: List of major interactions with 2npo protein

Peptide	Hydrogen bond		Bond Distance	Non-bonded		Bond distance	Salt bridges		Bond distance
	Chain A	Chain B		Chain A	Chain B		Chain A	Chain B	
Puroindoline	682 ASP	112 LYS	2.42	480 TYR	157 PRO	3.84	682 ASP	112 LYS	2.42
	838 ASP	130 GLN	1.78	675 ASN	108 ARG	3.77			
	853 ASN	130 GLN	2.49	678 ILE	108 ARG	3.37			
	922 PHE	123 SER	3.10	679 LYS	108 ARG	3.80			
	1010 LEU	124 GLY	3.24	682 ILE	112 LYS	3.21			
	1069 ASN	126 LYS	2.60		112 LYS	3.44			
Impatiens	1104 ASN	70 MET	2.42	991 ARG	27 PHE	3.66			
	1125 LYS	30 SER	3.19	1104 ASN	70 MET	3.52			
	1247 ILE	502 SER	2.50	1107 SER	70 MET	3.60			
				1122 THR	77 PHE	3.46			
				1125 LYS	28 ASP	3.43			
				1240 HIS	62 PHE	3.80			

and holes, it was suggested that AMPs be utilized at concentrations that are high enough. Although it was shown that some AMPs could begin membrane permeabilization at concentrations lower than their MICs, other AMPs could only do so at concentrations higher than their MICs. The discovery that some AMPs can kill their target cells without leading to membrane permeabilization raises the possibility that there are additional killing mechanisms. Recently, it was discovered that intracellularly active AMPs interacted with cell targets. Even a simple change in primary sequence can affect many other physicochemical parameters which are often vital for the activity of an AMP and the range of target cells.

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