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Application Of Wasps' Defensins As Anti-Fungal Therapeutic Molecules: An In Silico Comparative Study

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Abstract

Animal peptide toxins as part of chemical arsenal for predation and/or protection that can safeguard host from pathogenic infections. Hymenopterans generate toxic bactericidal or bacteriostatic molecules, called defensins are small multifunctional, linear, polycationic peptides causing pain, have antimicrobial effects. Defensins are active against gram-positive, gram-negative bacteria and fungi. The present *in silico* study aims to predict the physicochemical attributes like molecular weight, theoretical pI, amino acid composition, extinction co-efficient, estimated half-life, instability index, aliphatic index and grand average of hydropathy (gravy) of 09 different wasps' defensins using Expasy Protparam tool. The secondary structures of the toxins were predicted using psi-Blast-based PSIPRED, SOPMA tools revealing % α helix, extended β strand, random coil and ambiguous state reflecting a comparative physico-chemical parameters of these defensins. 3D Homology modelling of these toxins was accomplished through Swiss-model webserver and validated through ProSA-web, QMean4 determining Z score, PROCHEK establishing the 3D models of these defensins. Use of InterPro, CDD, ToxDL, PrDOS software predicted protein family, conserved domain, protein toxicity, protein disorder respectively. CYSCON and CYSPRED tools predicted cysteine-cysteine bonds. Docking of the nine (09) wasps' defensins individually with fungal cellwall component 1,4 Beta-D-Glucan was done by DockThor webserver resulting negative affinity scores reflecting strong binding between the defensins and 1,4 beta-D-Glucan indicating that the mentioned wasps' defensin molecules might be used as potential antifungal therapeutic molecules binding to 1, 4 Beta-D-Glucan indicating an avenue to antifungal drug discovery.

KEYWORDS: Wasp, Defensin, in silico, Docking, Anti-fungal

1. INTRODUCTION:

Arthropods are one of the biggest phyla with some orders bearing various toxins and venoms within class Insecta, Order Hymenoptera includes some social insects like ants, bees and wasps among which the wasps bear a certain toxin termed as Defensins [1]. These are cationic antimicrobial molecules and carry-on leucocyte chemotaxis, cytolysis and inflammatory reactions as well [2, 3, 4]. As social wasp bite injects venom causing hypersensitivity reaction i.e., anaphylaxis in human, thus bears a great medical and clinical implication. Defensins being antimicrobial in nature may have wide range of applications as the anti - microbial agents [5]. Defensins have been shown to have cytotoxic effect against gram-positive bacteria [6, 7] as well as some gram-negative bacteria species [8]. Furthermore, CvDef1 defensin from a parasitoid wasp Cotesia *vestalis* showed antimicrobial activity against gram-positive and gram-negative bacteria [9]. The present study is aimed at the *in silico* prediction and analysis of physicochemical attributes, i.e., molecular weight, theoretical PI, instability index, aliphatic index and Grand Average of Hydropathy (GRAVY) etc. of nine different defensins of two wasps species ; *Nasonia vitripennis and Trichomalopsis sarcophagae*.

Further, secondary structure prediction of those defensins were carried out to understand the % α-helix, extended β-strand, random coil and ambiguous state. 3D Homology modelling of those Defensin proteins, and subsequent validation and confirmation of predicted 3D models were done [10]. Prediction of Cysteine Bonding state, protein disorder, toxicity, protein family, conserved domain was also made. Multiple Sequence Alignment and phylogenetic tree analysis of these Defensins were carried on simultaneously**.** Many of the insect defensins have anti-fungal properties [11]**.** Glucan is the most important structural polysaccharide of the fungal cell wall and represents 50–60% of the dry weight of this structure [12]

These defensins may be employed as antifungal agents that would bind with fungal cell wall component such as 1, 4 Beta-D-Glucan [13,14]. Docking of the nine (09) wasp defensins individually with fungal cell wall component 1, 4 Beta-D-Glucan was done through DockThor docking webservers. Various *in silico* docking parameters would reveal quality of docking [15, 16]. The *in silico* docking results would indicate that the mentioned wasps' defensins might be used as potential Antifungal therapeutic molecules against 1, 4 Beta-D-Glucan leading to an avenue to the probable antifungal drug discovery [17].

2. MATERIALS AND METHODS:

Various bioinformatic software tools that were employed for the *in silico* work are as follows:

a. Sequences of the wasps' nine defensins were retrieved from Uniport database (www.expasy.org/sprot) [18].
b. Expasy ProtParam tool to compute the physicochemical properties of Defensins [19].
c. PSI-blast-based secondary structure prediction PSIPRED, SOPMA adopted to characterize and predict secondary structure of defensins [20, 21].
d. 3D Homology modelling of these Defensins were computed through SWISS-MODEL tool [22].
e. 3D Model Validation using ProSA , QMEAN4 programs determining Z score and PROCHECK software tools [23-25].
f. Cysteine Bonding state prediction through CYSPRED and CYSCON software tools [26,27]
g. InterPro, CDD webserver, ToxDL, and PrDOS software were used for protein family prediction, conserved domain prediction, protein toxicity prediction, protein disorder prediction respectively [28-31].
h. The molecular structure of 1, 4 Beta-D-Glucan is being mined from the compound repository Pubchem [32].
i. Docking of the said nine (09) wasp defensins individually with fungal cell wall component 1, 4 Beta-D-Glucan was done through DockThor docking webservers [33].
j. Toxin Codes were given for each Uniprot ID for each defensin molecules (Table 1).

3. RESULTS AND DISCUSSION:

3.1. RESULTS:

Table 1a. Sequences of nine Defensins from wasps were retrieved from Uniport database (www.expasy.org/sprot).

Sl. No.	Name of venom	Source	Uniprot ID	Toxin Code	Peptide Sequence	Sequence length (No. of amino acid)
1	Antimicrobial peptide Def1-1	*Nasonia vitripennis (wasp)*	D0EZK4_NASVI	1_ NASVI	MKLLLVVAFIAVAVTAGLSIPLNEFEDLVDFQDWDEAAVDEDAGVRQRRVTCDLLSFGGVVGDSACAANCLSMGKAGGSCNGGICECRKTTFKELWDQRFG	101
2	Defensin 2-2a		I1ZEL0_NASVI	2_ NASVI	MKVLVVLAACAVFAGAFGATRIRDGYEDPVFEILGDDIKRDGDNAETVDATDDLSPIKESSDDPTELVQPSYRDRRFSCDVLSFQSKWVSPNHSACAVRCQAQRRKGGKCKNGDCVCR	118
3	INVERT_ DEFENSINS domain-containing protein		K7J0P7_NASVI	3_ NASVI	MKVLVVLAACAVFAGAFGATRIRDGYEDPVFEILGDDIKQDGDNAETVDATDDLSPIKESSDDPTELVQLSYRVRRFSCDVLSFQSKWVSPNHSACAVRCLAQRRKGGKCKNGDCVCR	118
4	INVERT_ DEFENSINS domain-containing protein		K7J0P5_NASVI	4_ NASVI	MKFLTVFAVSALVASAYGASLDVYDGPVNFDGESRLGQDVRELSYDGNLDLEQPSTRARRFTCDVLSFKSAWISPNDSASAVRCLAQNRKGGTCKNGNCECHD	103
5	INVERT_ DEFENSINS domain-containing protein	*Trichomalopsis sarcophagae (wasp)*	A0A232EKP9_9HYME	1_HYME	MKLLLVVAFIAVAVTAGLSIPLNEFEDVVDFQDWDEAAVDEDAGVRQRRVTCDLLSFGGVVGDSACAANCLSMGKAGGRCNGGICECRKTTFKDLWDQRFG	101
6	INVERT_ DEFENSINS domain-containing protein		A0A232F8C1_9HYME	2_ HYME	MKVLVVLAGCAVFVGAFGATTIHDGYEDPVFEIQGDDIKEDGDNAETVDATDDLSPIKESSDDPTDVSPSYRARRFSCDVLSFQSKWVSPNHSACAVRCLAQRRKGGKCKNGVCVCR	117
7	INVERT_ DEFENSINS domain-containing protein		A0A232F7T5_9HYME	3_ HYME	MKVLVVLAVCSLVASAYGASLGVFDGPVYFDDETLASLEARFQLDHRDLSGKLAERKNLRVSLQKNSTQKTNLSLDLSLVEQPSFRARRFTCDVLSFKSMWVSPNHSACAVRCLAQRRKGGKCKNGVCVCR	131
8	INVERT_		A0A232EKR8_9HYME	4_ HYME	MKFLIIAVFSAMVVSAALSLPLDELEDLVDVQDWDEAAVDDNAGIRQRRVTCDLLSFGGKVGDSACA	101

Sl. No.	Name of venom	Source	Uniprot ID	Toxin Code	Peptide Sequence	Sequence length (No. of amino acid)
	DEFENSINS domain-containing protein				ANCLSMGKAGGSCNRGVCQCR KTTFADLWNKRFG	
9	INVERT_ DEFENSINS domain-containing protein		A0A232F8G5_ 9HYME	5_ HYME	MKFLTVFAVCALVASAYGASLDV YDGPVNFDGETRLGQDVLELSY EGKLDLEQPSIRARRFTCDVLSF KSAWISPNDSACAVRCLAQNRK GGTCKNGNCECHD	103

Table 1b. Structure of 1,4 beta-D-Glucan (https://pubchem.ncbi.nlm.nih.gov).

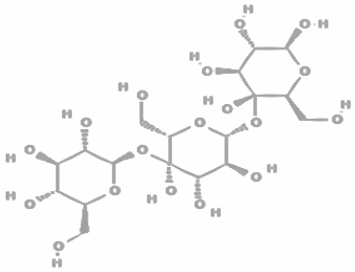
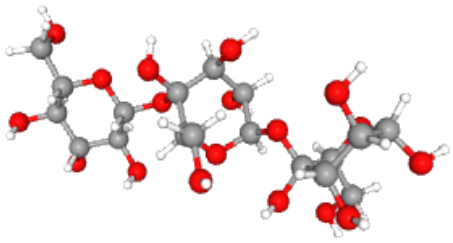
PubChem CID	Molecular Formula (Molecular weight)	2D Structure	3D Structure
53477911	$C_{18}H_{32}O_{18}$ **(536.4)**		

Table 2. Physicochemical properties of nine Defensins from wasps.

Name of Venom	Source – Wasp	Toxin Code	MW(Da)	Thr. pI	Asp + Glu) (-) R*	(Arg + Lys) (+) R*	II	EC	AI	GRAVY
Antimicrobial peptide Def1-1	*Nasonia vitripennis*	1_ NASVI	10794.31	4.41	15	9	35.77	11375	89.80	0.225
Defensin 2-2a		2_ NASVI	12961.56	5.36	19	17	48.12	8855	69.41	-0.432
INVERT_ DEFENSINS domain-containing protein		3_ NASVI	12918.62	5.34	18	16	48.65	8855	78.47	-0.251

Name of Venom	Source – Wasp	Toxin Code	MW(Da)	Thr. pI	Asp + Glu) (-) R*	(Arg + Lys) (+) R♣	II	EC	AI	GRAVY
INVERT_ DEFENSINS domain-containing protein		4_ NASVI	11186.44	5.22	13	11	41.79	10220	70.10	-0.320
INVERT_ DEFENSINS domain-containing protein	*Trichomalopsis sarcophagae*	1_HYME	10835.37	4.51	15	10	32.54	11375	88.81	0.192
INVERT_ DEFENSINS domain-containing protein		2_ HYME	12646.18	5.05	18	14	45.64	8855	70.77	-0.280
INVERT_ DEFENSINS domain-containing protein		3_ HYME	14484.82	9.50	11	20	30.63	8855	90.00	-0.063
INVERT_ DEFENSINS domain-containing protein		4_ HYME	10835.43	4.91	13	11	37.82	11375	87.92	0.143
INVERT_ DEFENSINS domain-containing protein		5_ HYME	11229.71	5.25	13	11	38.46	10345	77.67	-0.128
Mean			11988.05	5.51	15	13.22	39.94	10012.22	80.33	0.10156
Min			10794.31	4.41	11	9	30.6	8855	69.4	-0.432
Max			14484.82	9.5	19	20	48.7	11375	90	0.225

MW-Molecular weight; **Thr. pI**- Theoritical pI; **R*** -Negatively charged amino acids; R♣ - Positively charged amino acids; **II** – Instability index; **EC** – Extinction coefficient; **AI** – Aliphatic index

Table 3. Prediction of Secondary structures of nine Defensins from wasps.

Sl. No.	Name of venom	Toxin Code	Source - Wasp	A - Helix (%)	Extended β-strand (%)	Random coil (%)	Ambiguous state (%)
1.	Antimicrobial peptide Def1-1	1_ NASVI	*Nasonia vitripennis*	44.55	11.88	32.67	0.00
2.	Defensin 2-2a	2_ NASVI		33.05	15.25	43.22	0.00
3.	INVERT_ DEFENSINS domain-containing protein	3_ NASVI		37.29	16.10	36.44	0.00
4.	INVERT_ DEFENSINS domain-containing protein	4_ NASVI		32.04	9.71	53.40	0.00
5.	INVERT_ DEFENSINS domain-containing protein	1_HYME	*Trichomalopsis sarcophagae*	48.51	11.88	33.66	0.00
6.	INVERT_ DEFENSINS domain-containing protein	2_ HYME		28.21	23.93	39.32	0.00
7.	INVERT_ DEFENSINS domain-containing protein	3_ HYME		39.69	19.08	34.35	0.00
8.	INVERT_ DEFENSINS domain-containing protein	4_ HYME		56.44	6.93	29.70	0.00
9.	INVERT_ DEFENSINS domain-containing protein	5_ HYME		40.78	14.56	35.92	0.00
Mean				39.50125	14.36889	37.63111	0.00
Range		**Min**		56.44	23.93	53.4	0.00
		Max		28.21	6.93	29.7	0.00

Fig. 1. Secondary Structure (cartoon diagram) of nine Defensins from wasps.

Table 4. Cysteine bonding state prediction of nine Defensins from wasps.

Sl. No.	Toxin Code	Protein type	No. of Cysteine bonding state (their position) *	No. of Cysteine non-bonding state and their position*	Paired Cysteine position**
1	1_ NASVI	Antimicrobial peptide Def1-1	5 (CYS: 52,66,80,85,87)	------	SSBOND#1: 52-80 SSBOND#2: 66-85 SSBOND#3: 70-87
2	2_ NASVI	Defensin 2-2a	5 (CYS:96,110,115,117)	2 (CYS: 10,79)	SSBOND#1: 79-110 SSBOND#2: 96-115 SSBOND#3: 100-117
3	3_ NASVI	INVERT_DEFENSINS domain-containing protein	5 (CYS:96,110,115,117)	2 (CYS: 10,79)	SSBOND#1: 79-110 SSBOND#2: 96=115 SSBOND#3: 100-117
4	4_ NASVI		5 (CYS: 52,66,80,85,87)	------	SSBOND#1: 52-80 SSBOND#2: 66-85 SSBOND#3: 70-87
5	1_HYME		5 (CYS:78,95,109,114,116)	1 (CYS: 10)	SSBOND#1: 78-109 SSBOND#2: 95-114 SSBOND#3: 99-116

Sl. No.	Toxin Code	Protein type	No. of Cysteine bonding state (their position) *	No. of Cysteine non-bonding state and their position*	Paired Cysteine position**
6	2_ HYME		4 (CYS: 63,94,99,101)	------	SSBOND#1: 63-94 SSBOND#2: 84-101
7	3_ HYME		5 (CYS:92,109,123,128,130)	1 (CYS: 10)	SSBOND#1: 92-123 SSBOND#2: 109-128 SSBOND#3: 113-130
8	4_ HYME		5 (CYS: 52,66,80,85,87)	------	SSBOND#1: 52-80 SSBOND#2: 66-85 SSBOND#3: 70-87
9	5_ HYME		5 (CYS: 63,80,94,99,101)	1 (CYS: 10)	SSBOND#1: 63-94 SSBOND#2: 80-99 SSBOND#3: 84-101

***Using CYSPRED Software tool; **Using CYSCON Software tool**

Table 5. Protein Family Prediction of nine Defensins from wasps.

Sl. No.	Toxin Code	Source - Wasp	InterPro		CDD	Remarks
			IPR Domain-name; IPR Entry no.; position	Pfam Domain - name; Pfam Entry no.; position	CD Domain-name; CD Entry no.; position	
1	1_ NASVI	*Nasonia vitripennis*	Knottin (IPR036574) 51 – 92 AA	Defensin_2 (PF01097) 58 – 88 AA	DEFL_Defensin_ like (cd21806) 51-88AA	Scorpion toxin-like, invertebrate/fungal
2	2_ NASVI		Defensin (IPR001542) 75 – 118AA (IPR001542)	Defensin_2 (PF01097) 85 – 118AA	DEFL_Defensin_ like (cd21806) 78-118AA	Invertebrate/ Fungal Arthropod defensin
3	3_ NASVI		Defensin (IPR001542) 75 – 118 AA	Defensin_2 (PF01097) 84 - 118	DEFL_Defensin_ like (cd21806) 78-118AA Defensin_2 86-118AA	Invertebrate/fungal Arthropod defensin
4	4_ NASVI		Defensin (IPR001542) 70 -101AA	Defensin_2 (PF01097) 70 –101 AA	DEFL_Defensin_ like (cd21806) 62-102AA	Arthropod defensin Invertebrate/fungal
5	1_HYME	*Trichomalops is sarcophagae*	Defensin (IPR0015420) 49 – 90 AA	Defensin_2 (PF01097) 58 - 89 AA	DEFL_Defensin_ like (cd21806) 51-88AA Defensin_2 56-88AA	Invertebrate/ fungal Arthropod defensin

Sl. No.	Toxin Code	Source - Wasp	InterPro		CDD	Remarks
			IPR Domain-name; IPR Entry no.; position	Pfam Domain - name; Pfam Entry no.; position	CD Domain-name; CD Entry no.; position	
6	2_ HYME		Defensin (IPR001542) 74 – 117 AA	Defensin_2 (PF01097) 84 – 117AA	DEFL_ Defensin_ like (cd21806) 77-117AA Defensin_2 85-117AA	Arthropod defensin Invertebrate/ fungal
7	3_ HYME		Defensin (IPR001542) 88 – 131AA	Defensin_2 (PF01097) 100 -131AA	DEFL_Defensin_ like (cd21806) 91-131AA	Arthropod defensin Invertebrate/fungal
8	4_ HYME		Defensin IPR001542 48-89AA	Defensin_2 (PF01097) 57-88AA	DEFL_Defensin_ like (cd21806) 51-88AA	Arthropod defensin Invertebrate/fungal
9	5_ HYME		Defensin IPR001542 71-101AA	Defensin_2 (PF01097) 71-101AA	DEFL_ Defensin_ like (cd21806) 62-102AA	Arthropod defensin Invertebrate/fungal

Table 6. Protein Disorder Prediction nine Defensins from wasps.

Sl. No.	Toxin Code	Predicted Residues	Numbered Disordered Regions	Number Residues Disordered	Longest Disordered Region	Overall Percent Disordered	Average Prediction Score	Prediction Disordered Segment	Average Strength
1	1_ NASVI	101	3	10	7	9.90	0.2030	[30]-[36] [98]-[99] [101]-[101]	0.5833 0.7420 0.7054
2	2_ NASVI	118	2	46	31	38.98	0.4157	[37]-[67] [97]-[110]	0.7944 0.7212
3	3_ NASVI	118	2	45	32	38.14	0.4149	[35]-[66] [98]-[109]	0.8517 0.7022
4	4_ NASVI	101	3	11	8	10.89	0.2062	[29]-[36] [98]-[99] [101]-[101]	0.6129 0.7398 0.7054
5	1_HYME	117	2	50	36	42.74	0.4497	[31]-[66] [97]-[109]	0.8850 0.6874
6	2_ HYME	103	2	4	3	3.88	0.1940	[44]-[46] [101]-[101]	0.5229 0.6302
7	3_ HYME	131	2	33	20	25.19	0.3017	[47]-[66] [112]-[123]	0.8015 0.6683
8	4_ HYME	101	3	21	18	20.79	0.2342	[18]-[35] [98]-[99] [101-[101]	0.6356 0.6729 0.5994
9	5_ HYME	103	2	16	15	15.53	0.2074	[37]-[51] [101]-[10]	0.6130 0.6302

Table 7. Protein Toxicity Prediction of nine Defensins from wasps.

Sl. No.	Toxin Code	Score	Toxic domain name	Toxic domain position
1	1_ NASVI	0.7499646	IPR003614	50 – 90 AA
2	2_ NASVI	0.01760792	IPR003614	75 – 120 AA
3	3_ NASVI	0.01729618	IPR003614	75 – 120 AA
4	4_ NASVI	0.7306411	IPR003614	50 – 90 AA
5	1_HYME	0.025115572	IPR003614	75 – 120 AA
6	2_ HYME	0.36280227	IPR003614	61 – 102 AA
7	3_ HYME	0.005755974	IPR003614	90 – 130 AA
8	4_ HYME	0.7825295	IPR003614	49 – 90 AA
9	5_ HYME	0.2856705	IPR003614	61 – 102 AA

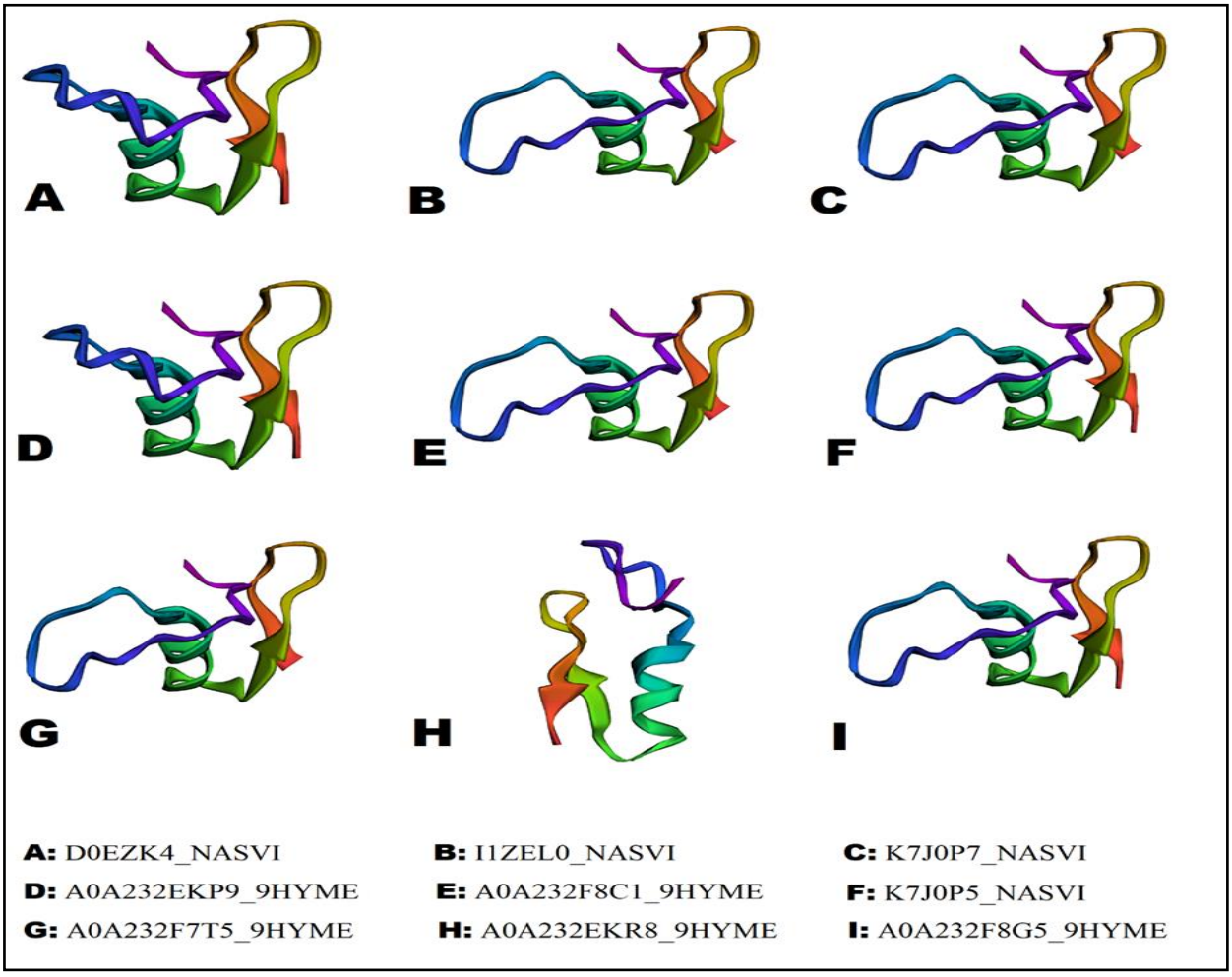


Fig. 2. Homology Models of nine Defensins from wasps.

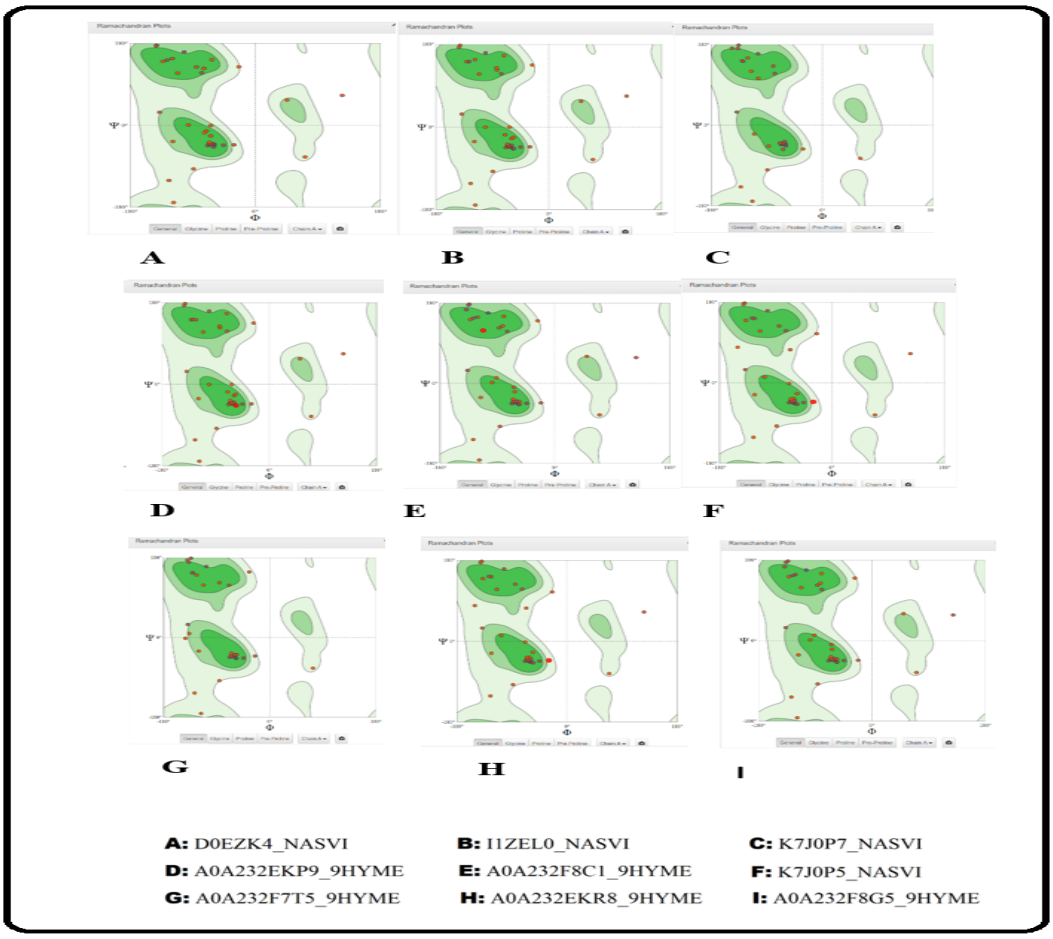


Fig 3. Ramachandran plot of nine Defensins from wasps.

Table 8. Validation of 3D Homology Models of nine Defensins from wasps

Sl. NO.	Toxin Name	Toxin Code	PROSA Z- Score	QMEAN4 value	PROCHECK	
					Core (%)	Allowed region (%)
1	Antimicrobial peptide Def1-1	1_ NASVI	-4.08	-2.69	75.9	20.7
2	Defensin 2-2a	2_ NASVI	-4.78	-2.68	75.0	19.4
3	INVERT_DEFENSINS domain-containing protein	3_ NASVI	-4.54	-2.31	75.0	19.4
4		4_ NASVI	-4.15	-2.57	83.3	10.0
5		1_HYME	-4.34	-2.25	72.2	22.2
6		2_ HYME	-4.75	-2.48	73.0	21.0
7		3_ HYME	-4.67	-2.17	75.0	19.4
8		4_ HYME	-5.12	-3.67	67.7	29.0

Sl. NO.	Toxin Name	Toxin Code	PROSA Z- Score	QMEAN4 value	PROCHECK	
					Core (%)	Allowed region (%)
9		5_ HYME	-4.91	-2.25	73.0	21.6

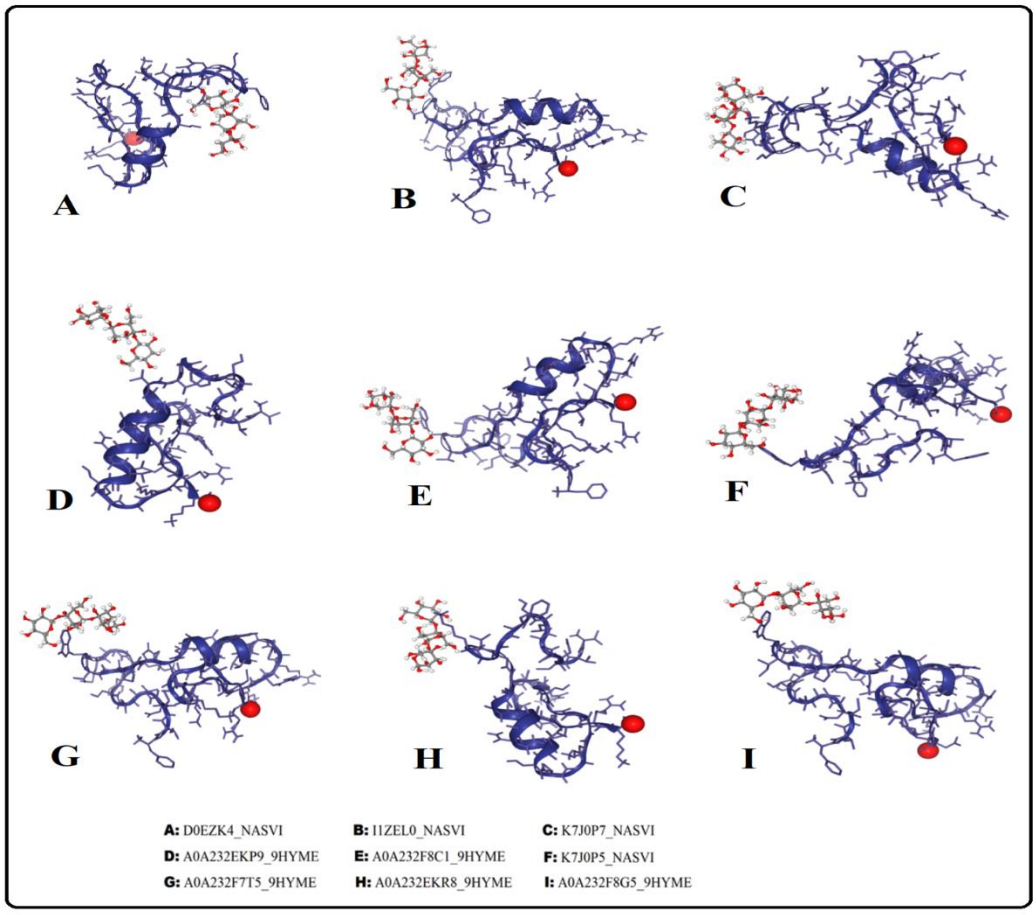


Fig 4. Docking Mode between nine defensins and 1, 4 beta-D-Glucan

Table 9. Results of docking of nine Defensins from wasps with fungal cell wall component 1, 4 Beta-D-Glucan using DockThor webserver.

Sl. No.	Uniprot ID.	Toxin Code	Ligand	Affinity	Total Energy (Kcal)	Interaction Energy (Kcal)	vdW Energy (Kcal)	Elec. Energy (Kcal)	Remarks
1	D0EZK4_NASVI	1_ NASVI	1,4 Beta D- Glucan	-6.648	91.011	-38.155	-7.366	-15.908	Docking Valid
2	I1ZEL0_NASVI	2_ NASVI		-5.856	108.943	-34.826	-2.590	-0.169	Docking Valid
3	K7J0P7_NASVI	3_ NASVI		-6.923	84.783	-36.667	-9.692	-15.746	Docking Valid
4	A0A232EKP9_ 9HYME	4_ NASVI		-5.874	103.157	-45.550	-2.116	-0.315	Docking Valid
5	A0A232F8C1_ 9HYME	1_ HYME		-5.837	108.727	-35.546	-2.815	-0.033	Docking Valid
6	K7J0P5_NASVI	2_ HYME		-6.004	107.223	-42.246	-3.756	-0.615	Docking Valid

Sl. No.	Uniprot ID.	Toxin Code	Ligand	Affinity	Total Energy (Kcal)	Interaction Energy (Kcal)	vdW Energy (Kcal)	Elec. Energy (Kcal)	Remarks
7	A0A232F7T5_ 9HYME	3_ HYME		-6.013	101.046	-29.679	-3.835	-0.709	Docking Valid
8	A0A232EKR8_ 9HYME	4_ HYME		-5.770	109.164	-40.393	-1.598	-0.804	Docking Valid
9	A0A232F8G5_ 9HYME	5_ HYME		-5.995	101.283	-42.286	-3.721	-0.606	Docking Valid

3.2. DISCUSSION:

3.2.1. Basic description of wasps' nine Defensins and fungal 1,4 beta-D-Glucan:

Table 1a, provides the details of different wasps' defensin proteins, their source from two wasps species *Nasonia vitripennis and Trichomalopsis sarcophagae,* primary sequences, Uniprot ID (www.expasy.org/sprot), a code provided for each defensin and peptide length. It *is* observed that, minimum peptide sequence length was observed in 1_ NASVI, 1_HYME, 4_HYME (101) while maximum peptide sequence length was observed for 3_HYME.**Table 1b** shows the 2D and 3D structures of 1,4 beta-D-Glucan (https://pubchem.ncbi.nlm.nih.gov).

3.2.2. Physico-chemical parameters of wasps' nine defensins:

Table 2, shows various physico-chemical data of these defensin molecules that are predicted through ProtParam tool. These properties includes peptide sequence length, molecular weight, isoelectric point (pI), total number of negatively (-R) and positively charged residues (+R), Extinction coefficient, Instability Index (II), Aliphatic Index (AI) and Grand Average of Hydropathicity (GRAVY) for two wasps species viz., *Nasonia vitripennis* and *Trichomalopsis sarcophagae* Theoretical pI values range from 4.41 to 9.50 indicating that tall the defensin molecules are on the acidic side of the scale except 3_HYME which is in the basic side. Local subcellular formation, interaction and melting depends on the isoelectric zone and the number of well-charged and negatively charged residues [34]. PI is the pH value at which the protein is free or the amount of negative and positive costs are equal. Highly charged negative residues are found in 2_NASVI while high positive residues are found in 3_HYME. Proteins with an index of instability (II) <40 are considered stable and those with a value of II > 40 are called unstable [35]. Here the Instability Index values range from 30.60 to 48.70 which means that the protein may be stable or unstable in the test tube as a value of less than 40 is stable in the test tube. Here the high levels of coagulation in the extinction are indicators of the high absorption capacity of the protein under study. Almost all of the global proteins contain large numbers of α-helices and β-sheets / fibres folded into a composite structure stabilized by both polar and lightweight interactions [36]. Aliphatic index plays a role in the thermal stability of proteins. Proteins having high Aliphatic index are stable in temperature. Aliphatic amino acids are also naturally hydrophobic. The Aliphatic index of cytotoxins in the range 66.5 to 84.33 showed that these proteins are stable in temperature and contain high levels of hydrophobic amino acids. The presence of hydrophobic and polar (charged) residues within cytotoxins creates an amphipathic environment for cytotoxins. With the disruption of the biological membrane this is an important indicator of the molecule. The short neurotoxin separates the Aliphatic index of 30.33 to 54.26 [37]. High values of Aliphatic index indicate that the protein is thermo-stable over a wide range of temperatures. Here it ranges from 79.40 to 90 indicating a wide range of temperature range. Good GRAVY values refer to hydrophobicity; negative values refer to hydrophilicity. Here it ranges from -0.432 to 0.225, which means that increasing the positive points indicates the range of hydrophobicity to the hydrophilicity of the proteins being studied.

Table 3, portrays *in silico* predictions of the secondary structures of 09 Defensins from 02 wasps species: *Trichomalopsis sarcophagae* and *Nasonia vitripennis* with the help of PSI-PRED, SOPMA tools depicting the highest α-helix, and random coils followed by β-strand displays limited amount of dynamic stability.The secondary structure predicts that approximately or more than 30% of the second structure is made up of alpha helix. This points to the fact that protein toxins are naturally occurring globular [38].

Fig. 1, reveals a cartoon diagram of the comparative secondary structure of 09 defensin molecules from wasp. α helix extensions were found at the beginning of the defensin protein sequences. Homology modelling is useful when the model protein (in the known sequence and unknown structure) is related to at least one single protein with both known sequence and known structure [39].

Fig. 2, shows homology models predicted using SWISS-MODEL software tool. Z-score of all modelled proteins obtained within acceptable scores that point towards a native protein structure pointing towards a good model quality. In the QMEAN Z-score represents a measure of how the model can be compared to test-based structures of the same size (https://tshi.page/ox/notes/techniques.html). Ramachandran plots generated for all defensin molecules through PROCHECK tool also pointed towards a validated and acceptable Homology model produced through Swiss-Model tool.

3.2.3. Cysteine di-sulphide bond prediction of wasps' nine Defensins:
Table 4, shows the cysteine-cysteine binding prediction using CYSPRED and CYSCON webservers. This demonstrates the stability of the binding site with any ligand or other type of molecule, structural relationships - protein activity. Defensins under study, most of them carry 03 pairs of Cysteine in bonded cases, which is a signature of invertebrate Defensins.

3.2.4. Protein Family/Domain Prediction of wasps' nine Defensins:
Table 5, showing defensin proteins family/domain prediction using InterPro and CDD software tools. Results reveals that all 09 defensins have 'Defensin' domains that are part of the invertebrate or specific Arthropod Defensin family. Protein intrinsic disorder is increasingly being identified in proteomics studies. While lack of structure, many disturbance regions have been associated with biological activity.

3.2.5. Protein Disorder Prediction:
Table 6, reveals the disordered regions predicted by the PrDos software tool for the 09 defensin molecules. Of the nine (09) defensin proteins the highest disorderedness is found in 1_HYME with 50 numbers of residues in the disordered state[40].

3.2.7. Predicted Protein Toxicity of wasps' nine Defensins:
Table 7, represents Protein Toxicity of 09 Defensin molecules Prediction by ToxDL Scores and Toxic domain positions obtained are as follows: Highest toxicity score is 0.7499646 in 1_ NASVI for a peptide stretch of 40 amino acids and lowest toxicity score is 0.005755974 for 3_HYME for a peptide stretch of 40 amino acids. All defensin molecules exhibit a predicted Toxicity score less than 01. This is indicative of low toxicity level. Highest toxicity marked 4_HYME with 0.7825295 which is less than 01.

3.2.8. Homology models of wasps' nine Defensins:
Fig. 2, shows Homology Models of wasps' nine defensins by SWISS-MODEL webserver where the 3D protein model is automatically generated by first transferring conserved atom coordinates as defined by the target-template alignment [41].
Table 8, shows Homology model validation scores predicted through ProSA, as well as QMEAN 4 tools with Z-score analysis showing that 3D models are viable and acceptable.
Fig. 3 (G-I), exhibits the Ramachandran plots of nine Defensin molecules generated through PROCHECK software. In Ramachandran plot core or allowed regions are the areas that show preferred regions for Ramachandran plot displays the ψ and φ angle pairs for residues in the protein. PROCHECK results clearly showed that about 93 to 96 % of the amino acid residues for all defensin molecules are in the core and allowed regions. This confirmed the reliability of the model [42].

3.2.9. Docking results of wasps' nine Defensins with fungal 1,4 beta-D-Glucan:
Fig. 4 as well as **Table 9**, portrays Docking results reflected Affinity, Total Energy, Interactional Energy, vdW Energy, Electrostatic Energy generated through the DockThor webserver showing the docked models and results (between defensing molecules and, high negative affinity values obtained from docking of nine (09) wasp defensins with 1,4 beta-D-Glucan indicate strong binding interaction as well as stable target-defensin binding interaction. Highest negative affinity score of -6.923 and with lowest Total Energy of 84.783 Kcal was observed with the defensin with Toxin ID, 3_ NASVI.

4. CONCLUSION:

In silico analysis of 09 defensin proteins from two wasp species were carried out in details. The homology modelled defensin molecules docked with fungal cell wall component 1,4 Beta D Glucan strongly. These defensins thus can have a potential use as an anti-fungal agent.

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CONFLICT OF INTEREST STATEMENT:
The Authors declare that there is no conflict of interest of any short in relation to the paper.

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