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Insilico STUDIES ON ANTIMICROBIAL PEPTIDES (AMPs) FROM *Calliphora vicina,* THE BLOW FLY OF FORENSIC IMPORTANCE

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ABSTRACT

The forensic flies colonize and feed on carcasses and cadavers and draw their nourishment from the dead and decomposed matter. They find significance in forensic studies as nutrient recyclers, disease vector and reveal association with pathogenic microorganisms. Despite their importance in forensic studies, their innate immune defense molecules like antimicrobial peptides (AMPs), have not been well characterized. Therefore, we characterized the AMPs from forensic fly, *Calliphora vicina*. using MSA, NCBI Tree Viewer, I-TASSER, Peptide cutter, NetPhos 3.1, Prot param Expasy *insilico* tools, for prediction of homology and evolutionary relationships, structure, function, potential cleavage, phosphorylation sites and physicochemical properties. We report for the first time the differences in the structures of *C. vicina* AMPs and the presence of nucleic acid-binding domain in Cecropin, at aspartic acid (D19), glutamine (Q15), histidine (H16) probably contributing to the antiviral immune responses. This *in-silico* study highlights the diversity of AMPs in forensic flies and reveals their indepth structural information. Further, these AMPs partially explain their association with pathogenic microorganisms.

Keywords: Antimicrobial peptide, *Calliphora vicina,* cecropin

INTRODUCTION

Insects are known to possess a strong innate immune system with a very premature adaptive immune system (AIS) that protects them against infections. The insect's innate immune system largely comprises of physical barriers like integuments and peritrophic membranes, cellular and humoral responses mediated by immune cells, receptors and molecules like antimicrobial peptides (AMPs) that are secreted into the insect hemolymph (Ruppert *et al.,* 2003; Muller, 2008; Rosales, 2017). Infection by foreign pathogens is sensed by specific receptors, including peptidoglycan recognition proteins (PGRPs) and β-glucan recognition proteins (βGRPs). Parasite ligand (L)-host receptor (R) interaction leads to the activation of signaling pathways, activating the genes encoding molecules including AMPs, thereby initiate the downstream signaling reaction.

Forensic entomology involves the study of a special group of insects called ‘forensic flies’ that invade decomposed cadavers and reveal succession on carcasses. The forensic flies are helpful

in legal investigations related to the understanding of post-mortem interval, so are important in forensic-and medical-sciences. The forensic flies are exposed to pathogens and harsh niche conditions, thrive on dead decayed habitat and environment. *Calliphora vicina* (*C. vicina*) belongs to the family Calliphoridae. The members of Calliphoridae feed on corpses and are commonly called 'blow and bottle flies'. These flies were first by identified in 1830 by a French entomologist Jean Baptiste Robineau Desvoidy. Ranging from 10-11 mm in length, they reveal the metallic blue-grey colour of the thorax and abdomen. Calliphoridae files are constantly exposed to different pathogens due to their survival and succession in cadavers and survival on nutrients derived from post-degradation of organic matter. Therefore, it is imperative to understand the immune molecules in them that protect them against infectious agents. The perusal of literature reveals that no study has been conducted to understand the immune molecules in forensic flies despite their large role in a vast domain of agriculture, forensic, medical, and veterinary sciences (Hore *et al.*, 2017; Ghosh *et al.*, 2018). The present study was aimed to elucidate the structure-function of AMPs that are known to play a role in combating the infection process.

MATERIALS AND METHODS

Retrieval of peptide sequences of AMPs

The sequences of antimicrobial peptides (AMPs) of *C. vicina* were obtained from Genbank NCBI (*https://www.ncbi.nlm.nih.gov/protein/?term=antimicrobial+peptide+Calliphora+vicina*). The methods used in *insilico* analysis of retrieved AMP's is summarized in Fig. 1.

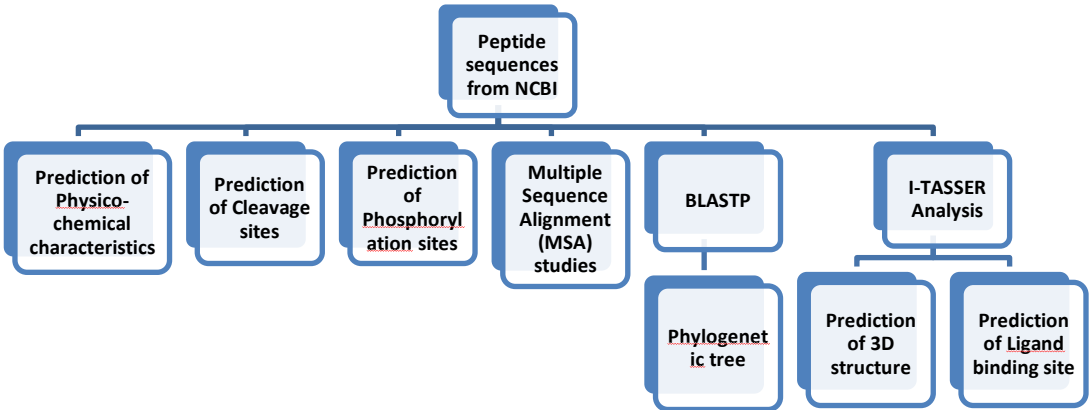

Fig. 1: Methodology used in *insilico* analysis of AMPs from *C. vicina*. *Insilico* tools of MSA, NCBI Tree Viewer, I-TASSER, Peptide cutter, NetPhos 3.1, Prot param Expasy *insilico* tools, were used to study *C. vicina* AMP predicting homology and evolutionary relationships, structure, function, potential cleavage, phosphorylation sites and physicochemical properties, respectively.

Multiple sequence alignment (MSA) and clustal Omega

MSA enables alignment of *Calliphora vicina* AMP protein sequences, and homology and evolutionary relationships were inferred using clustal Omega tool. Clustal Omega tool was used to seed guide trees and Hidden Markov Model (HMM) profile techniques was applied to generate multiple sequence alignments (Larkin *et al.*, 2007).

Phylogenetic tree

In this study, peptide sequences of *C. vicina* AMP were subjected to the protein-protein BLAST tool (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*) that identifies the similarities between biological sequences. A phylogenetic tree was constructed by Neighbour joining method using NCBI tree viewer with a graphical display for phylogenetic trees (*https://www.ncbi.nlm.nih.gov/tools/treeviewer/*).

Prediction of protein structure and function

Iterative Threading ASSEmbly Refinement (I-TASSER) is an online tool (*https://zhanggroup.org/I-TASSER/*) using a hierarchical approach for the prediction of the structure and function of AMPs. It uses the identification of structural templates from the protein databank (PDB) by using multiple threading approach LOMETS, with atomic models generated by using iterative template-based fragment assembly simulations. Function insights of target are then derived by protein function database BioLiP (Roy *et al.,* 2010). C-score is the confidence score of the prediction ranging from (0-1) wherein a higher score is indicative of a more reliable cluster size indicates templates in a cluster. Lig Name is the name of possible binding ligand from BioLiP database (*https:// zhanggroup.org/BioLiP/*) Rep is indicative of a single complex structure with the most representative ligand in the cluster, listed in the Lig Name column. Mult is the complex structures with all potential binding ligands in the cluster.

Prediction of cleavage sites

Peptide cutter was used to predict the potential cleavage sites cleaved by proteases or chemicals in AMP peptide sequence, revealing the cleavage site positions (*https://web.expasy.org/peptide_cutter/*).

Prediction of phosphorylation sites

The NetPhos 3.1 server uses algorithms that enable the prediction of serine, threonine or tyrosine phosphorylation sites in these AMPs (Blom *et al.,* 1999).

Prediction of physicochemical properties

C. vicina AMPs were studied using the Prot param Expasy tool (*https://web.expasy.org/protparam/*) and physicochemical properties of AMPs were detected which included amino acid composition, atomic composition, aliphatic index, extinction coefficient, estimated half-life, instability index, molecular weight, theoretical isoelectric point (pI), and grand average of hydropathicity (GRAVY) [Gasteiger *et al.,* 2005]. The extinction coefficients were expressed in M^{-1} cm^{-1} at 280 nm measured in water and indicated the concentration of Cys, Trp and Tyr amino acids. The instability index (II) indicated the stability of protein. A protein with II score of < 40 was predicted as stable. But a protein with II score of > 40 indicated that the protein may be unstable. The aliphatic index (AI) indicated the stability of a peptide/protein for a wide temperature range. The grand average hydropathy (GRAVY) value was calculated as the sum of hydropathy values of all the amino acids in a given peptide or protein sequence, divided by the number of residues in the sequence. The low range of GRAVY value indicated the possibility of better interaction with water.

RESULTS AND DISCUSSION

The peptide sequences, together with their accession number of NCBI, are listed in Table 1. The MSA study revealed the diversity in the sequences of AMPs, although they were obtained from the same *C. vicina* (Fig. 2). This indicated the robust nature of immunity conferred by diverse AMPs, enabling them to combat infection although they survive on dead/decayed organisms. The phylo-genetic tree revealed the similarity of AMPs with those of other arthropod flies of forensic significance like flesh fly (*Sarcophaga bullata* and *Sarcophaga peregrina*) [family Sarcophagidae], stable fly (*Musca domestica* and *Stomoxys calcitrans*) [family Muscidae], common green bottle fly (*Lucilia sericata*) and blowfly (*Protophormia terraenovae*) causing myasis in livestock [belonging to blowfly family Calliphoridae], fruit fly (*Rhagoletis zephyria*) and apple maggot (*Rhagoletis pomonella*) [the member of genus *Rhagoletis* under family Tephritidae], melon fly, an agricultural pest (*Zeugodacus cucurbitae*), oriental fruit fly (*Bactrocera dorsalis* and *Bactrocera latifrons*), [members of family Tephritidae], mosquitoes (like *Culex pipiens, C. quinquefasciatus, Scaptodrosophila lebanonensis* under family Drosophilidae) and ants (Fig. 3).

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sp|C0HJY0.1|PRP_CALVI      FVDRNRI---PRSNNGPKIPII-SNP------------------------------  22
sp|C0HJX8.1|CEC_CALVI      -------GW-LK-----KIGKKIGRVGQHTRD----ATIQGLAVAQQAANVAATAR-  39
sp|C0HJX7.1|DEFI_CALVI     ----------------ATCDLLSGTGANHSACAAHCLLRGNRGGYCNGKAVCVCRN  40
sp|C0HJX9.1|DIP_CALVI      --DSKPLNLVLPKEEPPNNPQTYGGGGGS-RKDDFDVVLQGAQEV-----------  42
sp|P83412.1|AFN_CALVI      -------------------HGVSGHGQH-GV-------HG---------------  13
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Fig. 2: MSA analysis of AMPs from *C. vicina*

Table 1: Peptide sequences and *Calliphora vicina* AMP obtained from NCBI (*https://www.ncbi.nlm.nih.gov/protein/?term=antimicrobial+peptide+Calliphora+vicina*)

Name	Accession No.	Sequence
Proline-rich peptide, partial [*C vicina*]	C0HJY0.1	FVDRNRIPRSNNGPKIPIISNP
Diptericin, partial [*C. vicina*]	C0HJX9.1	DSKPLNLVLPKEEPPNNPQTYGGGGGSRKDDFDVVLQGAQEV
Defensin [*C. vicina*]	C0HJX7.1	ATCDLLSGTGANHSACAAHCLLRGNRGGYCNGKAVCVCRN
Cecropin [*C. vicina*]	C0HJX8.1	GWLKKIGKKIGRVGQHTRDATIQGLAVAQQAANVAATAR
Alloferon-1 [*C. vicina*]	P83412.1	HGVSGHGQHGVHG

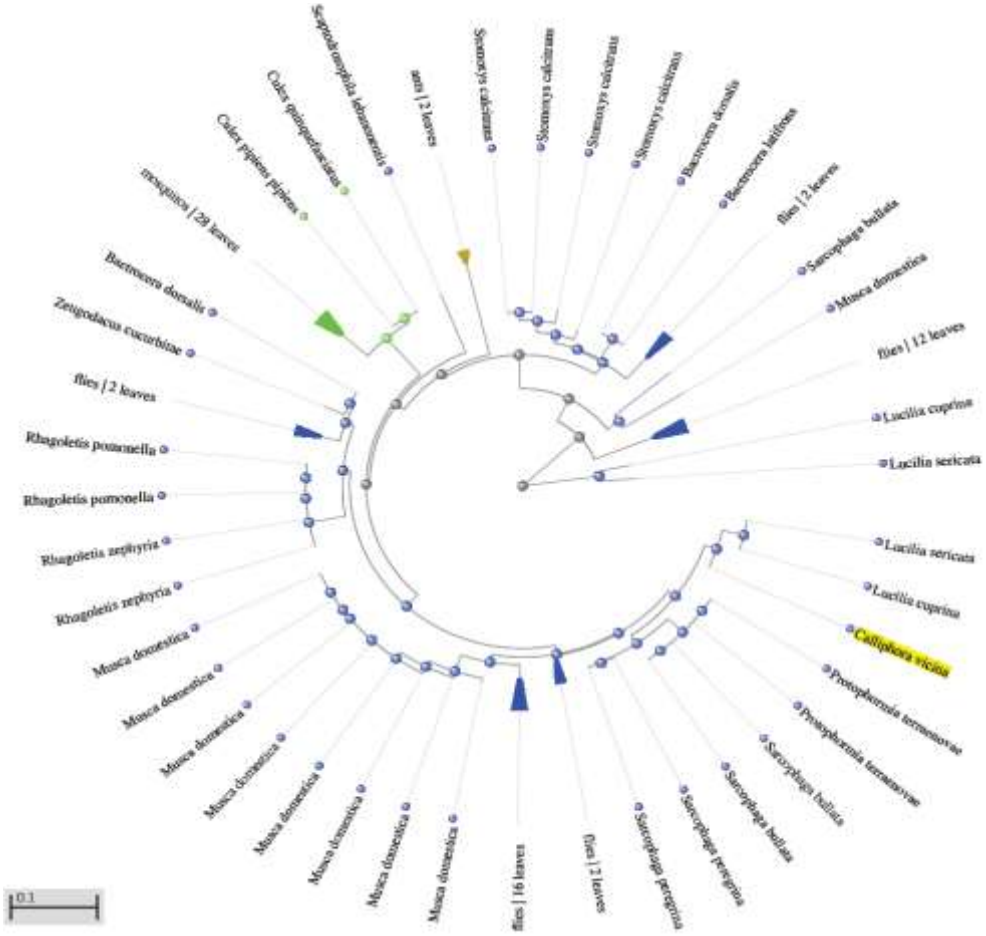


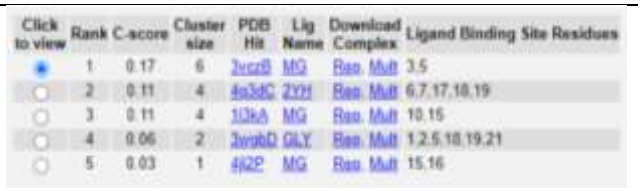
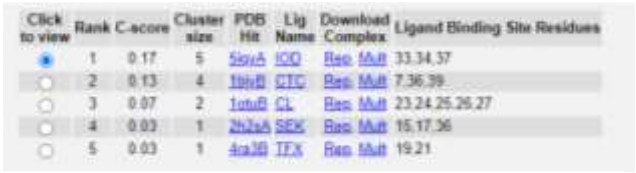
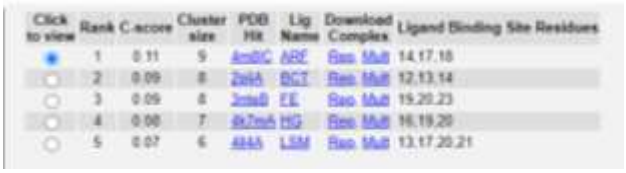
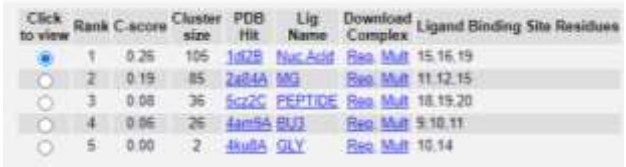
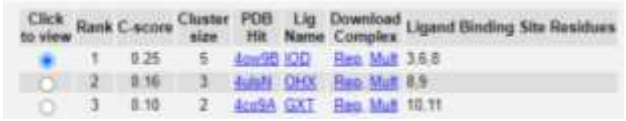
Fig. 3: Phylogenetic tree analysis for AMPs from *C. vicina*

The Fig. 4 revealed differences in the structures of AMPs of *C. vicina*, which were obtained from submitting peptide sequences in ITASSER platform. The predicted difference in structures is

indicative of robustness of innate immune system in *C. vicina* in their capability of targeting a wide range of pathogen, thus reveal their probable diverse roles.

The Fig. 5 revealed the differences in ligand-binding sites of AMPs from *C. vicina*. Proline-rich peptide, partial (*C. vicina)* revealed the ligand-binding sites at amino acid aspartic acid (D3) and asparagine (N5). Diptericin, partial (*C. vicina)* revealed the ligand-binding sites at aspartic acid (D33), valine (V34) and glutamine (Q37). Defensin (*C. vicina)* revealed the ligand-binding sites at serine (S14), alanine (A17) and alanine (A18); while Alloferon-1 (*C. vicina)* revealed the ligand-binding sites at valine (V3), histidine (H6) and glutamine (Q8) (Fig. 5 A-E, Table 2). Cecropin (*C. vicina)* revealed a nucleic acid (NA) as ligand- binding site at aspartic acid (D19), glutamine (Q15), histidine (H16). This is the first report of nucleic acid ligand-binding sites in Cecropin AMP from *C. vicina*.

Table 2: Prediction of structure and function of *Calliphora vicina* AMP

NCBI accession/ reference	I Tasser Id	Estimated C score	Estimated TM-score	Estimated RMSD	Ligand binding sites
Proline-rich peptide, partial *[C. vicina]*	S593853	-1.78	0.50±0.15	4.5±3.0Å	
Diptericin, partial *[C. vicina]*	S593698	-2.55	0.42±0.14	7.5±4.3Å	
Defensin *[C. vicina]*	S593551	-0.02	0.71±0.12	2.3±1.8Å	
Cecropin *[C. vicina]*	S593426	-1.17	0.57±0.15	4.4±2.9Å	
Alloferon-1 *[C. vicina]*	S593213	-1.53	0.53±0.15	3.0±2.1Å	

The predicted peptide cutting sites in AMP from *C. vicina* revealed differences in their susceptibility to different enzymes (Table 3). The Alloferon-1 (*C. vicina*) revealed the susceptibility to peptide cutting only by enzymes such as chymotrypsin-high specificity (C-term to [FYW], not before P) proteinase K and thermolysin remained inert to most other enzymes to which other AMPs were susceptible (Table 3). Most of the AMPs were susceptible to cleavage by enzymes including Arg-C proteinase, Asp-N endopeptidase, Asp-N endopeptidase + N-terminal Glu, chymotrypsin-high specificity (C-term to [FYW], not before P), chymotrypsin-low specificity (C-term to [FYWML], not before P), clostripain, formic acid, hydroxylamine, LysC, LysN, pepsin (pH 1.3), pepsin (pH>2), proteinase K, thermolysin and trypsin. Diptercin in addition revealed cleavage by glutamyl endopeptidase, proline-endopeptidase and staphylococcal peptidase I. Defencin revealed susceptibility to cleavage by NTCB (2-nitro-5-thiocyanobenzoic acid) additionally. Cecropin in

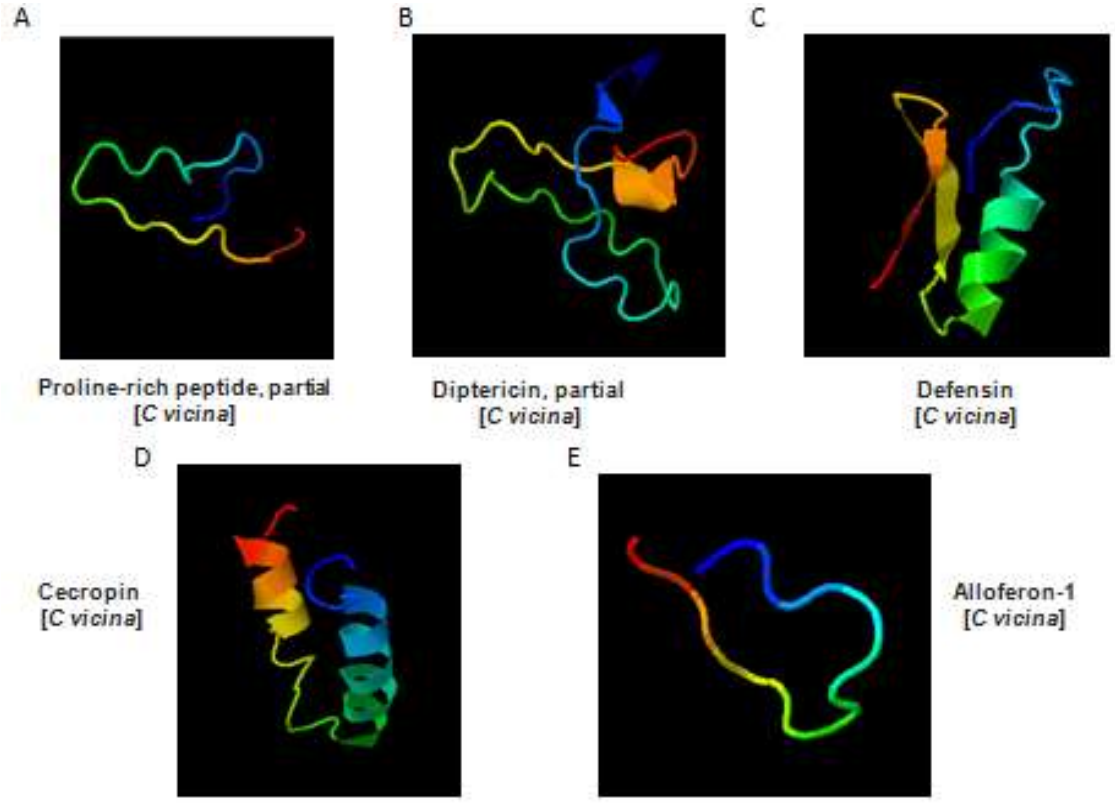


Fig. 4: Predicted structure derived from the sequence of AMPs from *Calliphora vicina*

Fig. 5

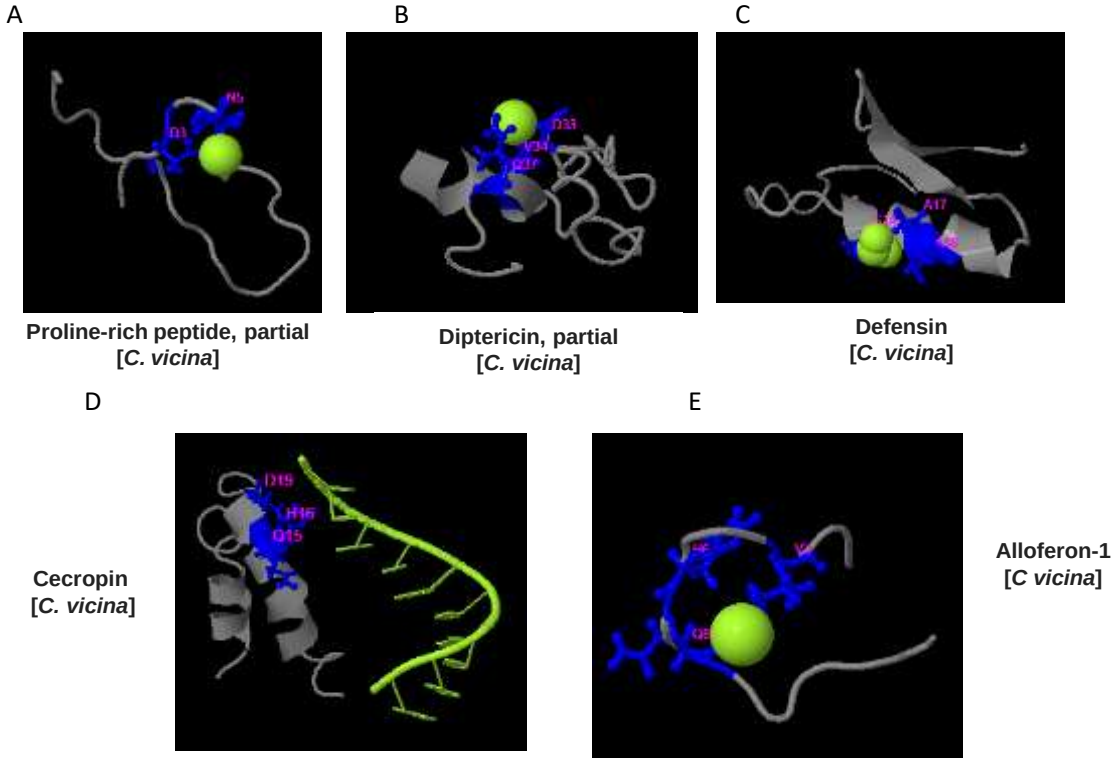


Fig. 5: Ligand-binding sites of AMPs from *C. vicina*

Table 3: Prediction of cleavage sites of *C. vicina* AMPs

Name	Predicted cleavage sites			
	Enzymes	No. of cleav-ages	Position of cleavage sites	Enzymes/ chemicals that do not cleave
Proline-rich peptide, partial *[C vicina]*	Arg-C proteinase	3	4 6 9	BNPS-Skatole, CNBr, Caspase1, Caspase10, Caspase2, Caspase3, Caspase4, Caspase5, Caspase6, Caspase7 Caspase8, Caspase9 Enterokinase, Factor Xa, Glutamyl endo-peptidase, Granzyme B, Iodosobenzoic acid, NTCB (2-nitro-5-thiocyanobenzoic acid), Proline-endopeptidase, Staphylococcal peptidase I, Thrombin, Tobacco etch virus protease
	Asp-N endopeptidase	1	2	
	Asp-N endopeptidase + N-terminal Glu	1	2	
	Chymotrypsin-high specificity (C-term to [FYW], not before P)	1	1	
	Chymotrypsin-low specificity (C-term to [FYWML], not before P)	1	1	
	Clostripain	3	4, 6 and 9	
	Formic acid	1	3	
	Hydroxylamine	1	12	
	LysC	1	15	
	LysN	1	14	
	Pepsin (pH1.3)	1	1	
	Pepsin (pH>2)	1	1	
	Proteinase K	6	1, 2, 7, 16, 18 and 19	
	Thermolysin	3	1, 17 and 18	
	Trypsin	4	4, 6, 9 and 15	
Diptericin, partial *[C vicina]*	Arg-C proteinase	1	28	BNPS-Skatole, CNBr, Caspase1, Caspase10, Caspase2, Caspase3, Caspase4, Caspase5, Caspase6, Caspase7, Caspase8, Caspase9, Enterokinase, Factor Xa, Granzyme B, Hydroxylamine, Iodosobenzoic acid, NTCB (2-nitro-5-thiocyanobenzoic acid), Thrombin, Tobacco etch virus protease
	Asp-N endopeptidase	3	29, 30, 32	
	Asp-N endopeptidase + N-terminal Glu	6	11, 12, 29, 30, 32 & 40	
	Chymotrypsin-high specificity (C-term to [FYW], not before P)	2	21 and 32	
	Chymotrypsin-low specificity (C-term to [FYWML], not before P)	5	5, 7, 21, 32 and 36	
	Clostripain	1	28	
	Formic acid	4	1, 30, 31, 33	
	Glutamyl endopeptidase	3	12, 13, 41	
	LysC	3	3, 11, 29	
	LysN	3	2, 10, 28	
	Pepsin (pH1.3)	7	4, 6, 7, 9, 32, 35, 36	
	Pepsin (pH>2)	9	4, 6, 7, 9, 20, 21, 32, 35, 36	
	Proline-endopeptidase [*]	1	4	
	Proteinase K	15	5, 7, 8, 9, 12, 13, 20, 21, 32, 34, 35, 36, 39, 41, 42	
	Staphylococcal peptidase I	2	12, 41	
	Thermolysin	6	4, 6, 7, 34, 35, 38	
	Trypsin	3	11, 28, 29	
Defensin *[C. vicina]*	Arg-C proteinase	3	23, 26, 39	BNPS-Skatole, CNBr, Caspase1, Caspase2, Caspase3, Caspase4, Caspase5, Caspase6, Caspase7, Caspase8, Caspase9, Caspase10, Enterokinase, Factor Xa, Granzyme B, Hydroxylamine, Iodosobenzoic acid, NTCB (2-nitro-5-thiocyanobenzoic acid), Thrombin, Tobacco etch virus protease
	Asp-N endopeptidase	1	3	
	Asp-N endopeptidase + N-terminal Glu	1	3	
	Chymotrypsin-high specificity (C-term to [FYW], not before P)	1	29	
	Chymotrypsin-low specificity (C-term to [FYWML], not before P)	7	5, 6, 13, 19, 21, 22, 29	
	Clostripain	3	23, 26, 39	
	Formic acid	1	4	
	Hydroxylamine	1	31	
	LysC	1	33	
	LysN	1	32	
	NTCB (2-nitro-5-thiocyanobenzoic acid)	6	2, 15, 19, 29, 35, 37	
	Pepsin (pH1.3)	5	4, 5, 6, 20, 22	

	Pepsin (pH>2)	6	4, 5, 6, 20, 29	
	Proteinase K	15	1, 2, 5, 6, 9, 11, 15, 17, 18, 21, 22, 29, 34, 35, 37	
	Thermolysin	10	5, 10, 14, 16, 17, 20, 21, 33, 34, 36	
	Trypsin	4	23, 26, 33, 39	
Cecropin *[C vicina]*	Arg-C proteinase	3	12, 18, 39	CNBr, Caspase1, Caspase2, Caspase3, Caspase4, Caspase5, Caspase6, Caspase7, Caspase8, Caspase9, Caspase10, Enterokinase, Factor Xa, Glutamyl endopeptidase, Granzyme B, Hydroxylamine, NTCB (2-nitro-5-thiocyanobenzoic acid), Proline-endopeptidase, Staphylococcal peptidase I, Thrombin, Tobacco etch virus protease
	Asp-N endopeptidase	1	18	
	Asp-N endopeptidase + N-terminal Glu	1	18	
	BNPS-Skatole	1	2	
	Chymotrypsin-high specificity (C-term to [FYW], not before P)	1	2	
	Chymotrypsin-low specificity (C-term to [FYWML], not before P)	4	2, 3, 16, 25	
	Clostripain	3	12, 18, 39	
	Formic acid	1	19	
	Iodosonenzoic acid	1	2	
	LysC	4	4, 5, 8, 9	
	LysN	4	3, 4, 7, 8	
	Pepsin (pH1.3)	4	2, 3, 24, 25	
	Pepsin (pH>2)	5	1, 2, 3, 24, 25	
	Proteinase K	20	2, 3, 6, 10, 13, 17, 20, 21, 22, 25, 26, 27, 28, 31, 32, 34, 35, 36, 37, 38	
	Thermolysin	15	2, 5, 9, 12, 21, 24, 25, 26, 27, 30, 31, 33, 34, 35, 37	
	Trypsin	7	4, 5, 8, 9, 12, 18, 39	
Alloferon-1 *[C vicina]*	Chymotrypsin-high specificity (C-term to [FYW], not before P)	4	1, 6, 9, 12	Arg-C proteinase, Asp-N endopeptidase, Asp-N endopeptidase + N-terminal Glu, BNPS-Skatole, CNBr, Caspase1, Caspase2, Caspase3, Caspase4, Caspase5, Caspase6, Caspase7, Caspase8, Caspase9, Caspase10, Chymotrypsin-high specificity (C-term to [FYW], not before P), Clostripain, Entero-kinase, Factor Xa, Formic acid, Glutamyl endopeptidase, Granzyme B, Hydro-xylamine, Iodoso-benzoic acid, LysC, LysN, NTCB (2-nitro-5-thiocyano-benzoic acid), Pepsin (pH1.3), Pepsin (pH>2), Proline-endo-peptidase, Staphylo-coccal peptidase I, Thrombin, Tobacco etch virus protease, Trypsin
	Proteinase K	2	3, 11	
	Thermolysin	2	2, 10	

addition revealed the susceptibility to cleavage by BNPS-Skatole and iodosobenzoic acid but no cleavage with hydroxylamine (Table 3). This is further indicative of the diversity amongst the AMPs.

Table 4: Predicted phosphorylation sites[@@] of *C. vicina* AMPs

AMP's	Predicted phosphorylation sites
Proline-rich peptide, partial [*C. vicina*]	FVDRNRIPRSNNGPKIPIISNP
Diptericin, partial [*C. vicina*]	DSKPLNLVLPKEEPPNNPQTYGGGGGSRKDDFDVVLQGAQEV
Defensin [*C. vicina*]	ATCDLLSGTGANHSACAAHCLLRGNRGGYCNGKAVCVCRN
Cecropin [*C. vicina*]	GWLKKIGKKIGRVGQHTRDATIQGLAVAQQAANVAATAR
Alloferon-1 [*C. vicina*]	Limitation of tool for protein of less than 15 amino acid

[@@]Peptides < 15 amino acids are excluded from the study since it is the limitation of NetPhos 3.1 Server.

The differences in the predicted phosphorylation sites within AMPs were revealed in Table 4. The S10 of proline-rich peptide, S27 of Diptericin, S7, S14, Y29 of Defensin and T37 of Cecropin revealed potential sites of phosphorylation. Phosphorylation sites of Alloferon-1 could not be detected by the tool due to its smaller size (<15 amino acid).

Of the AMPs studied, diptericin revealed the predicted instability (Table 5) while all other AMPs revealed stability. Proline-rich peptide, Diptericin, Defensin, Cecropin and Alloferon-1 revealed 22, 42, 40, 39 and 13 amino acids, respectively, with molecular weights of 2504.88, 4466.89, 4038.60, 4084.74 and 1265.31 kDa and isoelectric points (pI) of 11.71, 4.39, 8.69, 11.74 and 7.1, aliphatic index of 84.09, 67.14, 68.5, 92.82 and 44.62 and GRAVY scores of -0.832, -0.931, -0.035, -0.262 and -0.823, respectively. Diptericin did not contain any tryptophan (Trp) residue. There was no tryptophan (Trp), tyrosine (Tyr) or cysteine (Cys) in Proline-rich peptide protein and Alloferon-1 as they were not visible by UV spectrophotometry and therefore their extinction coefficient (EC) could not be predicted which remains a limitation of the study.

The knowledge of fly immune system is focused on *Drosophila*, revealing that Toll and IMD (immune deficiency) signaling pathways involve the activation of NFκB-like proteins which are activated in response to the microbes and lead to the transcription of AMP [Lemaitre *et al.*, 2007]. Toll signaling involves the activation of Dorsal and Dif which are NFκB homologs in *Drosophila* and reportedly play a role in combating pathogenic infections of both Gram-positive and Gram-negative bacteria, fungi and viruses. The IMD pathway involves the activation of Relish, an NFκB homolog in *Drosophila*, which leads to the activation of Janus kinase (JNK)-mediated signaling and plays role in combating the infections both by Gram-negative and Gram-positive bacteria (Lemaitre, 2007; Schneider, 2007). However not much is known about the immune system and its function in

Table 5: Physicochemical parameters of *C. vicina* AMPs[@]

Sequences	No. of amino acids	Mole-cular weight	Theoretical pI (iso-electric point)	-R (Asp + Glu)	+R (Arg + Lys)	Extinction coefficient (EC)	Instability index (II)	Aliphatic index (AI)	GRAVY
Proline-rich peptide, partial *[C. vicina]*	22	2504.88	11.71	1	4	--##	6.82 stable	84.09	-0.832
Diptericin, partial *[C.vicina]*	42	4466.89	4.39	7	4	1490; This protein does not contain any Trp residues	63.76 unstable	67.14	-0.931
Defensin *[C.vicina]*	40	4038.60	8.69	1	4	1865	16.61 stable	68.5	0.035
Cecropin *[C.vicina]*	39	4084.74	11.74	1	7	5500	8.19 stable	92.82	-0.262
Alloferon-1 *[C.vicina]*	13	1265.31	7.1	0	0	--##	-22.68 stable	44.62	-0.823

@ using the Prot Param expasy tool (*https://web.expasy.org/protparam/*); [#]This protein is devoid of Trp residues
--##As there are no Trp, Tyr or Cys in the region considered, protein is not visible by UV spectrophotometry.

blow flies. Insects belonging to the family Calliphoridae under order Diptera include blowflies which are of forensic importance as they thrive on dead decayed substances and are always exposed to the pathogens (Tomberlin *et al.,* 2016). They also showed the association with several bacterial pathogens of genus *Aeromonas, Acinetobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Morganella, Proteus, Pseudomonas, Providencia* and *Serratia* (Junqueira *et al.,* 2017). The associated microbiome is recently known to play role in their development and survival regulate physiology and behaviour. However, the current knowledge of bacteria and their interactions with blow fly species is far from complete (Tomberlin *et al.,* 2016) which calls for more research in this domain of biological sciences. Although only a few studies have been conducted on AMPs from *Sarconesiopsis magellanica* and has been exploited for their therapeutic potential (Pérez *et al.,* 2021), the Sarconesin II, Sarconesin from *S. magellanica* (Díaz-Roa *et al.,* 2018, 2019), and AMP complexes from *C. vicina* have been reported to combat antibiotic resistant biofilms (Gordya *et al.,* 2017). Their structure function and characterization are far from being complete to implicate their role in therapeutics use. No study has been conducted so far highlighting the diversity of AMPs in forensic flies.

The immune system of forensic flies (the molecules, interactions and signaling pathways) that enable their survival in a dead/decaying environment is not completely studied. Two observations about these flies were very intriguing. The one being their robust immune system that enables their survival and combating infection by pathogens that they are exposed to in their life cycle in a dead/decayed environment. The other important observation is their association with bacteria and pathogens. From our study the differences in structure are indicative of diversity in the function of AMPs that contribute to the properties of combating different pathogens to which they are exposed during their food and lifecycle in dead/decayed environment. Earlier the diversity in AMPs in leech and earthworm has been reported by Ghosh (2020a,b). *C. vicina* AMPs revealed the similarity in AMPs with other forensic flies under family Calliphoridae, Sarcophagidae, Muscidae, fruit fly, ants and mosquitoes. However, no similarity was observed with the AMPs of Annelids including the leeches and earthworm, indicating that these AMPs bear similarity only to a few arthropod families.

The *insilico* study on *Calliphora* AMP revealed diversity in the structure of AMPs with nucleic acid ligand-binding site in Cecropin AMP which indicate their role in antiviral immune responses. The study revealed the heterogeneity in peptide cutting sites and differences in phosphorylation sites in AMPs while Diptericin revealed predicted instability. The differences in structure and the associated function of AMPs highlight the fact that these flies can combat different pathogens that they are exposed to during their succession and survive on carriers, flesh and decayed matter. This study highlights the diversity in their structure and characterization and there is need to assess their therapeutic value.

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