RESEARCH PAPER

Discovery of Novel Peptides Targeting Anopheles Gambiae Using In Silico Approaches

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ABSTRACT
Pharmaceuticals that employ peptides, or short chains of amino acids, as therapeutic agents are known as peptide-based drugs. These drugs offer a fresh method of treating a range of illnesses by mimicking or blocking the actions of natural proteins, hormones, or enzymes. Human, animal, and bird infections are spread by Anopheles gambiae. Humans suffer from several severe health problems as a result. The effectiveness of the peptide derived from Boerhavia diffusa against Anopheles gambiae was determined in this investigation. We use in silico techniques to analyse the new peptide's insecticidal characteristics. Protein-peptide docking investigations using automated in silico methods were part of the study's methodology. Using sophisticated 3D macromolecular visualization tools, all the data were explained. The effectiveness of the new peptide against Anopheles gambiae (African malaria mosquito) was amply demonstrated by the full docking data. The new peptide (SFQALLERIYFHVKIEYLVKVLTKNCRIILWLFKDPFTHYIRYQGKSILS) (https://www.modelarchive.org/doi/10.5452/ma-4whza) was ultimately found to have superior inhibitory activity against Anopheles gambiae. As a result, the de novo peptide functions as a possible treatment for Anopheles gambiae.
Keywords: *Peptide, Anopheles gambiae, drug docking, computational protocols*
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INTRODUCTION

Due mostly to their great efficiency in transmitting *Plasmodium falciparum*, the most deadly type of malaria, members of the *Anopheles gambiae* mosquito complex have likely caused more human deaths than any other animal [1-2]. Four key factors make this mosquito complex an extremely effective disease vector: (1) they are frequently quite numerous, (2) they bite humans a lot, (3) they are very prone to infection, and (4) they have a long lifespan (for a mosquito) [3]. At least eight morphologically similar species, the majority of which are largely zoophilic, make up the *Anopheles gambiae* species complex. *Anopheles gambiae* and *Anopheles coluzzii*, two of the most closely related members of the complex, are the main vectors of malaria because of their propensity to feed on humans, their longevity, the fact that they sleep within traditional thatch-roofed homes, and their relative abundance[4-6]. While *Anopheles coluzzii* is restricted to West Africa, *Anopheles gambiae* is found across much of sub-Saharan Africa[7]. They share a lot of behavioral characteristics and are the complex's most recently divergent members. Members of the complex may be expanding their range, as evidenced by the recent report of *Anopheles coluzzii* from East Africa[8].

Soon after dusk, adult anophelines emerge from the pupae and wait for a few minutes before drying themselves and

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inflating their wings before taking off[9]. The direction and strength of the wind affect the dispersal flight. Mosquitoes may be carried by the wind if there is a strong dominating breeze, but dispersal may be random if there is little or no wind[10]. With a maximum flight speed of 1.4–1.8 ms−[13], 80% of *Anopheles gambiae* on open savanna fly less than one meter above the ground[11-12]. Circadian rhythms regulate flight, which happens at night[13-14].

Small-molecule drug design and discovery have found success with computational docking techniques. In the area of peptide therapies, comparable initiatives are underway [15-16]. However, the far more flexible and bigger peptide molecules are typically not effectively modeled by the docking techniques created for small-molecule interactions[17]. New methods for protein–peptide docking have been developed quickly because of the interest in peptide therapies[18-19] and are increasingly being used in the drug discovery and design process[20-27]. *Plasmodium falciparum* parasites are spread via the main and most effective mosquito vector of malaria in Sub-Saharan Africa, *Anopheles gambiae*. These anthropophilic (human-biting) mosquitoes are a primary target for control techniques like ITNs because they thrive in human surroundings and are therefore very successful at spreading the disease, mostly through nocturnal, indoor feeding. In our current research work, we have developed a peptide-based medicine which was analyzed using molecular docking studies to control mosquitoes.

MATERIALS AND METHODS

The plant *Boerhavia diffusa* and its novel antigenic peptide was derived to assess its role against *Anopheles gambiae.* Molecular docking was conducted using PDB-IHM database to assess the peptide-protein docking. In silico drug discovery method was done to determine the interaction of compounds with Cytochrome C Oxidase subunit I in *Anopheles gambiae.* Using computational analysis peptide-receptor interactions and its 3D conformation was visualized using discovery studio software.

RESULTS

1.1. Peptide-Protein Docking

To conduct molecular docking experiments, the structure of the novel peptide (ma-4whza) (https://www.modelarchive.org/doi/10.5452/ma-4whza) was taken from the PDB-IHM database.

The Cytochrome c Oxidase Subunit 1 (COX1) (*Anopheles gambiae*) protein sequence was made public by the NCBI database (https://www.uniprot.org/uniprotkb/P34838/entry#sequences). (Figure 1)

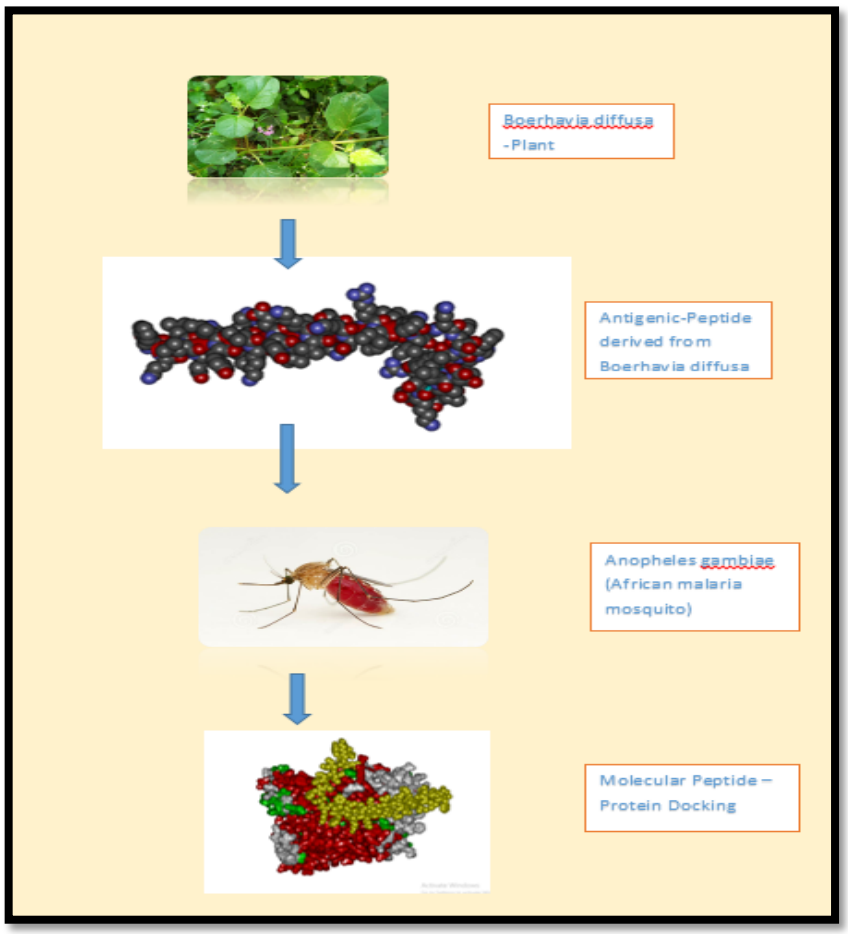

Figure 1: The above pictorial representation depicts the major steps involved in our current research work

Docking of Drugs

One popular in silico drug discovery method, "molecular docking," looks at how tiny compounds interact with Cytochrome c Oxidase subunit 1 in *Anopheles gambiae* to determine their optimal conformations. Docking tests were conducted using peptides and the Cytochrome-C Oxidase subunit 1 (COI). The PatchDock server (https://bio3d.cs.huji.ac.il/webserver/patchdock/) supplied the 3D docked file format of the ligand and receptor sequence (Cytochrome-C Oxidase subunit 1) in order to predict the 3D interaction of the receptor and ligand molecules. The research of the Discovery studio program also shed light on H-bond interactions (Figure 2 and 3).

CYTOCHROME P450 SEQUENCE

> P34838 · COX1_ANOGA

[*Anopheles gambiae (African malaria mosquito)*]

MSRQWLFSTNHKDIGTLYFIFGAWAGMVGTSLSILIRAELGHPGAFIGDDQIYNVIVTAHAFMIFFMVMPIMI

GGFGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLISSSMVENGAGTGWTVYPPLSSGIAHAGASVDL

AIFSLHLAGISSILGAVNFITTVINMRSPGITLDRMPLFVWSVVITAVLLLLSLPVLAGAITMLLTDRNLNTSFFD

PAGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKETFGNLGMIYAMLAIGLLGFIVWAHHMFTV

GMDVDTRAYFTSATMIIAVPTGIKIFSWLATLHGTQLTYSPAMLWAFGFVFLFTVGGLTGVVLANSSIDIVLHD

TYYVVAHFHYVLSMGAVFAIMAGFVHWYPLLTGLTMNPTWLKIQFSIMFVGVNLTFFPQHFLGLAGMPRRY

SDFPDSYLTWNVVSSLGSTISLFAILYFLFIIWESMITQRTPAFPMQLSSSIEWYHTLPPAEHTYAELPLLTNNF

Figure 2: Cytochrome P450's amino acid sequence obtained from the UniProt database

BOERHAVIA DIFFUSA PEPTIDE

SFQALLERIYFHVKIEYLVKVLTKNCRIILWLFKDPFTH YIRYQGKSILS

Figure 3: Amino acid sequence of the peptide obtained from *Boerhavia diffusa.*

3.3 Peptide-Receptor interactions

Discovery Studio Software, a molecular visualization tool, was used to display the 3D structure along with cytochrome P450 and molecular drug docking studies on peptides. (Figures 4 – 10)

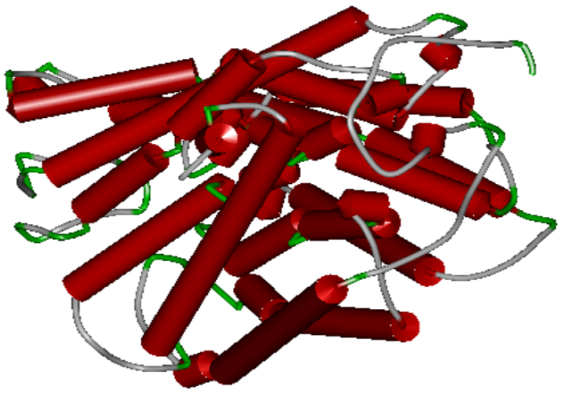

Figure 4: The 3D structure of cytochrome-C oxidase subunit 1 (COX1) in *Anopheles gambiae* as displayed by Discovery Studio program. The different atoms are represented by different colors.

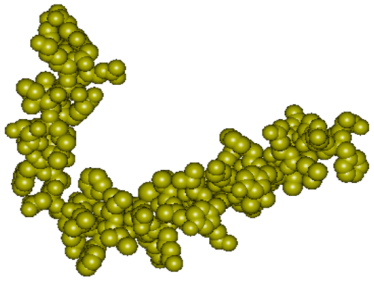

Figure 5: The 3D structure of a novel peptide using the Discovery Studio software's Space fill model view

Solution number	Score	Area	ACE	PDB file	Show/hide
1	13898	1785.6	-209.99	result.1.pdb	☑
2	13344	1729.2	-428.19	result.2.pdb	☐
3	13302	2022.2	-473.31	result.3.pdb	☐
4	13244	1633.0	-493.39	result.4.pdb	☐
5	13160	2101.3	-547.0	result.5.pdb	☐
6	12980	2129.0	-330.52	result.6.pdb	☐
7	12378	1736.5	-411.67	result.7.pdb	☐
8	12298	1782.4	-323.36	result.8.pdb	☐
9	12268	2074.3	-527.99	result.9.pdb	☐

Figure 6: PatchDock server results showing the de novo peptide's complex form with *Anopheles gambiae* Cytochrome c Oxidase Subunit 1 (COX1) and the associated binding scores

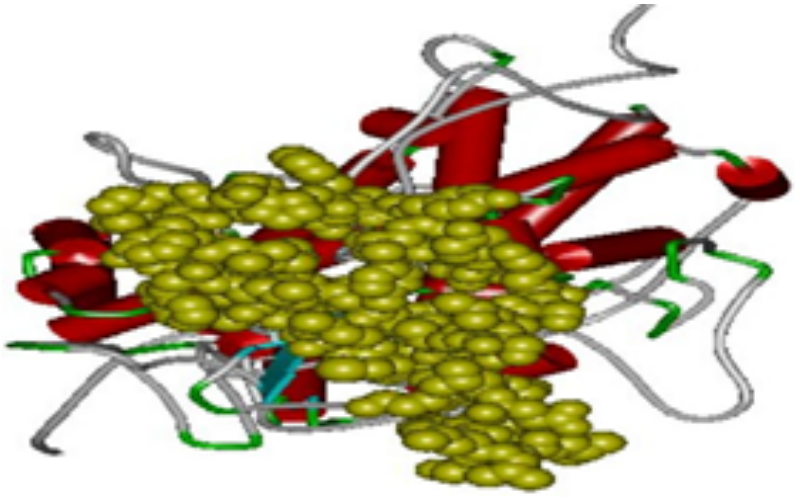

Figure 7: Using the de novo peptide, a surface model produced using Discovery Studio software displays the 3D complex structure of Cytochrome-C Oxidase (COX1) subunit 1

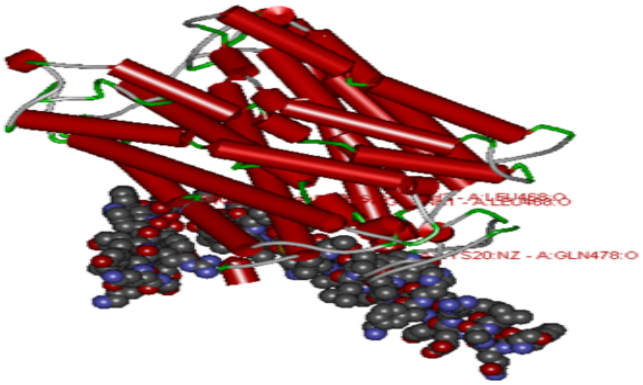

Figure 8: The 3D H-bond interaction view of cytochrome-C oxidase subunit 1 with the de novo peptide generated by Discovery Studio program, marked with the red colour amino acids at the binding site.

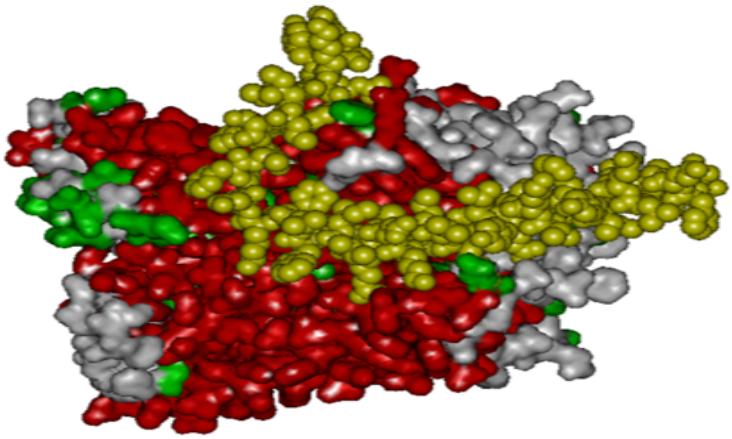

Figure 9: Three-dimensional representation of the Vander Waals interaction between the de novo peptide generated by Discovery Studio software and Cytochrome-C oxidase subunit 1.

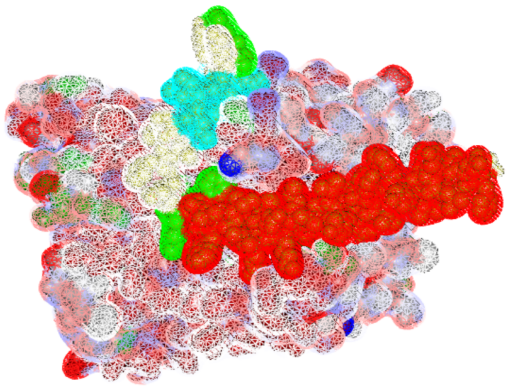

Figure 10: Cytochrome c oxidase subunit 1 and the de novo peptide's electrostatic contact as generated by Discovery Studio software.

DISCUSSION

The cytochrome c oxidase subunit 1 of *Anopheles gambiae* has an amino acid length of 514 aa (AB332027.2) (Fig 1). We decided to investigate COX1 since it is essential to the study of dengue. Using in silico techniques, the peptide was isolated from *Boerhavia diffusa* and added to PDB-IHM (https://www.modelarchive.org/doi/10.5452/ma-4whza)[28]. The total length of the peptide is 50 aa (Fig.2). Using in silico methods, the structure of our unique peptide was transformed from 2D to 3D. Automated drug docking techniques were used to introduce the chosen target protein from *Anopheles gambiae*. The outcomes unequivocally showed that the proteins are inhibited by the newly discovered peptide. Fig. 4 shows the 3D structure of the new peptide with colored atom identifiers.

The three-dimensional structure of the peptide as observed in the space fill model is depicted in Fig. 3 using colored atoms. The three-dimensional structure was visualized using Discovery Studio, a state-of-the-art molecular visualization tool. The internal Vander Waals force determines how the *Anopheles gambiae* target protein for cytochrome c oxidase subunit 1 binds to the chemical structure. PatchDock is a molecular docking technique that relies on geometry[29]. Its goal is to identify docking changes that produce good complementarity between molecular shapes. Applying such modifications results in minor levels of steric conflicts as well as broad interface areas. Numerous matched local features of the docked molecules with complementary properties are guaranteed to be included in the broad interface. The Connolly dot surface representations of the molecules is separated into concave, convex, and flat patches using the PatchDock algorithm[30].

According to our drug evaluations, the novel peptide against Cytochrome c oxidase subunit 1 (*Anopheles gambiae*) has a higher negative value of -262.21 *kcal/mol* than other medications (Figure 5). According to theoretical Cheminformatics principles, a bigger negative value indicates a good drug-receptor binding relationship. The thorough molecular dynamic studies of Cytochrome c oxidase subunit 1(COX1) against the distinct peptide (Figure 6-9) show that the peptide directly connects with the matching surface binding cavities of the *Anopheles gambiae* protein[31]. Interestingly, we discovered that the (COX1) protein from *Anopheles gambiae* had an overall length of 514 aa. The functional part of the (COX1) sequence is the Cytochrome c oxidase subunit I signature and profile, which is located between amino acids 1-511 [PS50855]. In biological molecules, hydrogen bonds (H-bonds) are among the most important and consequential non-covalent interatomic interactions. They oversee the complementarity of the two strands in DNA and RNA as well as the stabilization of the primary secondary structural components in proteins. The way that enzymes connect with their substrates or products, how antibodies recognize antigens, and numerous other biological processes all depend on H-bonds. The following H-bond interaction amino acid involvement within these borders was clearly shown by the post-docking studies: ARG: 109, PRO: 36, LYS: 520, GLN: 478, ARG: 27, LEU: 468, PHE: 37, THR: 109. All the previously mentioned findings were investigated[32, 33].

CONCLUSION

In this study, the insecticidal capabilities of a peptide produced from the plant species *Boerhavia diffusa* were examined. The de novo peptide was docked with the protein target, which in this case was *Anopheles gambiae* Cytochrome c oxidase subunit 1 (COX1). The collective findings unequivocally demonstrated that the peptide's molecular interactions with *Anopheles gambiae* Cytochrome c oxidase subunit 1 (COX1) occur within the domain ranges. According to our investigation, our modeled peptide with the following sequence (SFQALLERIYFHVKIEYLVKVLTKNCRIILWLFKDPF THYIRYQGKSILS) possesses insecticidal capabilities. It can therefore be used as a peptide-based pesticide. This drug can be used in spays to control mosquitoes because the peptide's source is a natural substance.

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