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Research Article

Computational identification of microbial metabolites as potential inhibitors of mosquito juvenile hormone binding protein for vector control

Jincy A. George
Department of Zoology, St. Joseph's University, Bengaluru, India
Febin Antony
Department of Computer Science, Westford University College, Sharjah
Upasana Sridharan
Department of Chemistry, University of California, Davis California, United States
Kuppusamy Alagesan Paari*
Department of Life Science, CHRIST (Deemed to be University), Bangalore, India

*Corresponding author. E-mail: paari.ka@christuniversity.in

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Abstract
A group of acyclic sesquiterpenoids, that form the Juvenile hormone is crucial in the developmental physiology of insects. *Aedes aegypti* is crucial in spreading fatal diseases such as dengue, and dengue hemorrhagic fever. The mosquito undergoes several stages of development, from the egg to the adult stage, utilizing its innate immunity system and juvenile hormone proteins. Thus, targeting the juvenile hormone-binding proteins can potentially inhibit the developmental stages of the mosquito. The mosquito juvenile hormone binding protein (mJHBP) of *Aedes aegypti* was obtained from the RCSB (PDB). The study identified that *Talaromyces islandicus* and *Bacillus velezensis* produced secondary metabolites that act as efficient ligand complexes. The secondary metabolites were procured from PubChem and docked to the binding sites of mJHBP. Among the 26 listed ligand compounds, oxalic acid, decyl 3,5-difluorophenyl ester, oxalic acid 3,5-difluorophenyl undecyl ester, and p-octylacetophenone were found to have higher binding affinity, marking their efficiency in inhibiting the protein. Normal mode analysis studies were performed using iMODs to analyze the B-factor, variance, covariance, and Eigenvalues of the docked protein-ligand complexes. The Absorption, Distribution, Metabolism and Excretion (ADME) properties of the efficient ligand molecules were analyzed using the Swiss ADME tool to segregate potential drug candidates. Targeting the mJHBP complex using the microbial metabolite ligand molecules can inhibit the development of the mosquitoes. The work enlightens the futuristic development of potential candidates in the production of insecticides. The literature confirms it is the first of its type to utilize microbial bio compounds as ligands targeting the mJHB protein complex.

Keywords: ADME analysis, Bacillus velezensis, Juvenile hormone binding protein, microbial metabolites, molecular docking, Talaromyces islandicus.

INTRODUCTION

Vector-borne diseases play a pivotal role in decreasing the human population. Mosquitoes are crucial vectors in carrying viruses that lead to several diseases. Several species of mosquitoes are present that host different viruses. Multiple strategies are employed to control these vectors, including insecticides and other sanitization methods. Most of the strategies are not proven to be successful upon implementation. Some of the strategies, such as the use of pyrethroids and other classes of insecticides, have resulted in adverse side effects. Insecticides are found to act as endocrine-disrupting chemicals (EDC), and the chemicals are detected in human test samples of breast cancer in women. The accumulation of chemicals in the human and animal systems has been reported. The increased concentration of these chemicals in the environment, resulting from exposure, has led to harmful effects (Mnif *et al.,* 2011). Some of the classes of insecticides include pyrethroids and neonicotinoids. The pyrethroids class of compounds is effective in mosquito population control, but the seepage of these compounds into water bodies has resulted in harmful effects on the aquatic environment, such as loss of schooling, neurotoxicological effects, immunotoxicity, and genotoxicity (Farag *et al.,*

2021). Similarly, neonicotinoid-based pesticides are also prevalent in mosquito control. There has been 100% mortality reported with exposure to neonicotinoids. The chemical is found to alter mammalian brain functioning. Brain developmental issues, damage in cognition etc., are reported. The candidates with potential exposure to these pesticides have also shown reproductive defects such as less pregnancy rate, reduction in sperm count, etc. (Farag *et al.,* 2021; Omkar and Bhupendra, 2016). Therefore, the use of certain classes of compounds that do not exhibit adverse side effects must be incorporated into mosquito control strategies. Studies have shown that the usage of microbial metabolites can help defect the insect development stages. The study has been designed to analyze the bioactive compounds produced by microorganisms and their *in vitro* analysis of inhibiting the mJHBP in *Aedes aegypti*.

The corpora allata of the insect brain produce and releases the Juvenile hormones (JH) into the hemolymph of the insects. Juvenile hormones are molecules with high lipophilicity. It is reported to be a protein with a terpenoid structure, featuring the methyl ester of the epoxy farnesoic acid group (Omkar and Bhupendra, 2016). Essential molecules that are required for the undisturbed changes during the development of the insect stages from egg to adult stage. These essential molecules are triggered and maintained by the juvenile hormones. The juvenile hormones are produced through metabolic pathways, specifically the mevalonate pathway (Lombard and Moreira, 2010). The pathway of juvenile hormone synthesis in the insect system involves thirteen major reactions, each involving different enzymes. The pathway is studied in two different sections, including the primitive path involving mevalonate. This pathway begins by using acetyl-CoA to produce farnesyl, a process facilitated by eight different enzymes. The mevalonate pathway is followed by the juvenile hormone pathway, in which the farnesyl utilizes five enzymes to form juvenile hormones (Noriega, 2014). The JH is studied to be exhibited in 6 different homologs in insects, with the presence of epoxide and alpha, beta-unsaturated methyl ester. In *A. aegypti*, the juvenile hormone plays a crucial role in the developmental stages. The hormone binds to the protein that regulates metamorphosis (Kamita and Hammock, 2010).

The juvenile hormone plays a major role in regulating behavioral traits, stress-induced responses, diapause, and reproductive traits (Ramos *et al.,* 2020). The reduction or absence of these hormones results in zero hatchability of the mosquito eggs, and furthermore, hemocyte production is inhibited, preventing the larvae from transitioning to the pupal stage (Kim *et al.,* 2020). The mJHBP in *Aedes aegypti* belongs to the family of odorant binding proteins (OBPs) that are found to bind to two classes of juvenile hormones, such as JH II and JH III. Its abundance was studied in the adult mosquito and is important in maintaining reproductive efficiency in adults (Kim *et al.,* 2017). The juvenile hormone thus marks its importance in the development and propagation of the mosquito.

To target these mosquitoes' *in vitro* analysis is considered using bioinformatics and molecular docking. The mJHBP is a major protein in the mosquito's developmental stages and is highly targeted using multiple ligand molecules. Using bioactive compounds, such as the secretory compounds of microbial sources, as a candidate to target the juvenile hormone binding protein is a major area of this study. The involvement of bioinformatics tools such as PubChem (https://pubchem.ncbi.nlm.nih.gov/), RCSB (https://www.rcsb.org/structure/5V13), Open Babel, Universal Force Field, PyRx, PyMol, Discovery Studio Lab and iMODS (https://imods.iqfr.csic.es/) has served in retrieving, processing, analyzing and molecular docking of the ligands and protein. The manuscript emphasizes the potential compounds in inhibiting the mosquito juvenile hormone binding protein. The manuscript outlines the preliminary parameters, including the inhibition potential of microbial bio-compounds. The study has also highlighted the drug-forming capabilities of potential bio compounds, such as adsorption and toxicity distribution. The manuscript thus serves as the basis for further development of products that can be applied to their futuristic purposes.

MATERIALS AND METHODS

Sample extraction and GC-MS

Talaromyces islandicus was inoculated into potato dextrose broth, and *Bacillus velezensis* was inoculated into nutrient broth; both were then incubated. Both microorganisms were incubated and processed using chloroform and then subjected to gas Chromatography-Mass spectrometry using a Shimadzu GCMS-QP2010SE with helium gas as the carrier. The compounds obtained were analyzed and identified using the National Institute of Standards and Technology (NIST) library (Mehta *et al.,* 2021).

Protein preparation

The protein data bank (https://www.rcsb.org/structure/5V13), was accessed to obtain the 3D structures of the Juvenile hormone binding protein (PDB DOI: 10.2210/pdb5V13/pdb). PyMol software (http://www.pymol.org) was used to visualize the 3D structure of the target proteins, along with the water molecules and the associated native ligand complex, which were removed (Kirar *et al.,* 2022; Seeliger and de Groot, 2010). Furthermore, the targeted protein energy was altered by adding charges to it using Autodock vina tools using PyRx software. The protein structure was

converted to the 'pdbqt' format to facilitate ligand-protein docking.

Physiochemical properties of the protein

A Swiss Bioinformatic Portal known as the Expasy tool was utilized to analyze and interpret the physicochemical properties of proteins, such as the number of amino acids, theoretical pI, extinction coefficient, grand average of hydropathy, estimated half-life, instability index, and aliphatic index (https://www.expasy.org/).

Ligands selection

All microbial secondary metabolite compounds were investigated to analyze their potential as drug candidates by examining their physical properties using Lipinski's Web server. Lipinski's rule of five encompasses five principal characteristics of a ligand, including molar refractivity, molecular mass, number of hydrogen bond donors, log P, and number of hydrogen bond acceptors. The ligand compounds that justified Lipinski's rule of five were further moved into ligand preparation.

Preparation of ligands

The microbial secondary metabolite compounds were procured from the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/) based on their properties that satisfied the Lipinski rule of five. The SDF format files of the ligand compounds were converted to PDB format by using the Open Babel software. Torsion root, correcting angles, assigning charges and optimization using UFF (Universal force field) are some of the additions made to the ligand compound to prepare its binding possibilities. The PDBQT-formatted files of the prepared ligand compounds were obtained to analyze the 3D atomic coordinates before the docking procedure.

Active site prediction

The active site of the targeted protein is a crucial criterion before docking protein-ligand interaction. The potential sites at which the inserted ligand can bind were predicted using an active site prediction server maintained by the Supercomputing Facility for Bioinformatics and Computational Biology at the Indian Institute of Technology, Delhi (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp).

Molecular docking

Autodock Vina tools, available in the PyRx virtual screening open-source software (National Biomedical Computation Resources), were utilized for protein–ligand docking studies. The MJHBP and the microbial secondary metabolite compounds were traced for molecular docking using Vina wizard control. The active site of the selected protein is identified and drawn into the grid area to enhance the binding efficiency of the ligand compounds. Autodock vina performed molecular docking to obtain the binding energy (Seeliger and de Groot, 2010). Based on the binding energy and interaction between the protein complex and the ligand compounds, they are identified.

Normal mode analysis

The docked complex with the best binding affinity was selected for analysis of its stability, flexibility, and other protein-ligand interactions using iMODs software (https://imods.iqfr.csic.es/). Using the normal mode analysis of the docked complex can predict the deformability, B-factor residues, eigenvalues, amino acid motion directions, variance percentages, residue correlation indices, and atomic indices that depict elasticity (Kirar *et al.,* 2022).

ADME/T analysis

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/T) properties analyze the capability of the ligand compound to act as a potential drug candidate. The ligands compounds were also selected based on their pharmacodynamic and pharmacokinetic effects on the human system. SwissADME (http://www.swissadme.ch/) was used for conduct this study.

RESULTS

Gas chromatography-mass spectrometric analysis revealed the presence of several compounds in the *Talaromyces islandicus* and *Bacillus velezensis* extracts. The chromatogram recorded by Gas chromatography-mass spectrometry indicated several peaks. *Talaromyces islandicus* (Fig. 1) and *B. velezensis* (Fig. 2) yielded a total of 95 peaks and multiple different bio compounds.

These bio compounds were screened for bioactivity and efficiency as ligand molecules to target the protein. The selection from the 40 bioactive compounds with varying percentage peak areas was based on Lipinski's rule of five, which shows the efficacy of the bio compound in targeting the active site of the Juvenile hormone protein.

Ligand selection

The bioactive compound that acts as a ligand must possess properties to be developed into a drug. These properties are analyzed using the Lipinski rules. The molecular mass, lipophilicity, molar refractivity, hydrogen bond donors and acceptors. Twenty-eight bioactive compounds followed Lipinski's rule of five, with a molecular mass of less than 500 Dalton, a LogP value of less than 5, and a molar refractivity between 40 and 130. Hydrogen bond donors with fewer than 5 and hydrogen bond acceptors with fewer than 10 were screened, as depicted in Table 1.

Protein analysis, preparation and active site prediction

The targeted protein selected was the Mosquito juvenile hormone-binding protein. The protein belongs to the class of odorant-binding proteins (OBP). The physicochemical parameters indicated that the protein was composed of 288 amino acids, with a molecular weight of 32,871.69 Daltons and a theoretical pI of 5.22. the percentage composition of the protein is listed in Table 2. The atomic composition was analyzed, and it was found that there were 1451 atoms of carbon, 2193 atoms of Hydrogen, 401 atoms of nitrogen, 446 atoms of oxygen, and 15 atoms of Sulfur. It depicted a molecular formula as follows: $C_{1451}H_{2193}$ $N_{401}O_{446}S_{15}$. Extinction coefficient of 52995 with the absorbance percentage of 0.1% with the assumption of all Cysteine residues are reduced. The instability index was computed to be 45.32, which classifies the protein as unstable under test tube conditions. The Aliphatic index was estimated to be 61.39, which categorizes the protein as hydrophobic. The grand average hydropathicity was estimated to be -0.613, which confirms that the protein is non-polar and hydrophobic in nature.

The increased titre of 20-hydroxyecdysone triggers a decrease in the Juvenile hormone (JH) titre, which results in the progression through developmental stages. The active sites identified using the SCFBio active prediction server were found to be similar to those predicted in the literature (Kim *et al.*, 2017). Our results show that the amino acids Ile140, Trp 50, Trp 53, Val 51, Leu 37, Tyr 33, Phe 144, Val 34, Leu 30, Ala 281, Trp 278,

Table 1. List of ligands selected based on Lipinski's rule of five

PubChem ID	Molecular Mass (Daltons)	Hydrogen bond donors	Hydrogen bond acceptor	Log P	Molar refractivity
14260	196.00	0	0	4.430879	74.91695
25913	210.00	0	0	4.73717	80.38397
8209	214.00	0	0	4.234259	79.53075
17095	182.00	0	0	4.124589	69.44999
95039	232.00	0	1	3.982849	78.27149
95908	181.00	0	2	1.883	53.41399
6420363	328.00	0	4	3.935659	82.397
6420726	342.00	0	4	4.24195	87.864
6420727	356.00	0	4	4.54824	93.33099
6420812	278.00	0	3	4.175259	88.07649
6422271	243.00	0	4	2.392679	67.093
6422273	285.00	0	4	3.311549	83.49399
6422275	257.00	0	4	2.698969	72.55999
66344	232.00	0	1	3.944839	78.93249
676803	225.00	1	3	0.9659	55.43859
6782	278.00	0	4	3.460459	77.33599
567684	255.00	3	4	-0.16329	59.32939
567711	225.00	2	2	0.02603	52.63619
575005	227.00	1	4	0.17072	52.47709
545303	276.00	0	3	3.767529	83.65849
17161	220.00	0	2	3.192459	68.79499
3026	278.00	0	4	3.439459	77.44599
346148	284.00	0	3	4.37224	92.11948
155294296	183.00	0	1	2.667789	61.79049
1263351	268.00	0	1	1.18188	77.74349
139874	264.00	0	3	3.745249	79.64449
11006	226.00	0	0	5.146641	89.58598

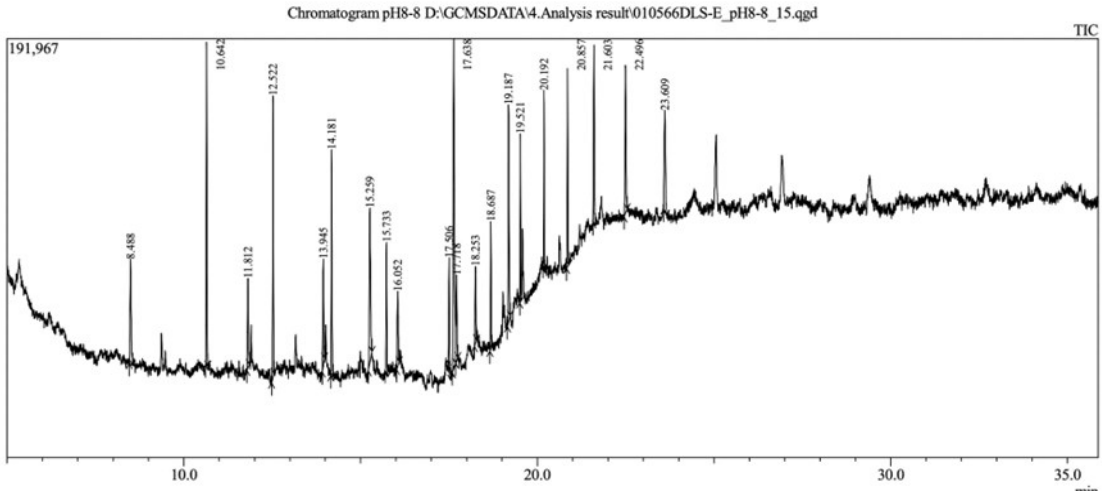

Fig. 1. *Chromatogram indicating the fungal secondary metabolites with the retention time measured in minutes at the X-axis and signal intensity measured in arbitrary units (AU) at the Y-axis*

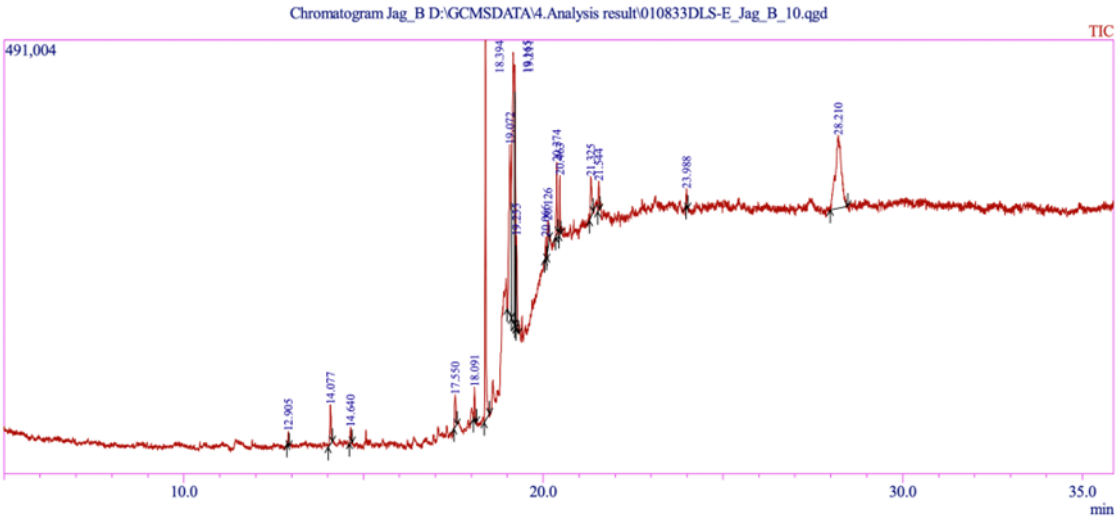

Fig. 2. *Chromatogram indicating the bacterial secondary metabolites with the retention time measured in minutes at the X-axis and signal intensity measured in arbitrary units (AU) at the Y-axis*

Phe 269, Pro 55, Leu 72, Val 68, Tyr 64, Ser 69, Leu 74, Tyr 129, Val 65 and Tyr 133 form possible binding sites of Juvenile binding hormone in mosquito development (Fig. 3).

Protein-Ligand docking

The microbial bioactive compounds were docked against the mosquito juvenile hormone in *Aedes aegypti* to identify compounds that can inhibit the protein, thereby disrupting mosquito development. To compare the docked structures, the Root Mean Square Deviation (RMSD) value of the imposed ligand and protein structure was analyzed. The RMSD value less than 1.5 Å was refined from the set of ligands that was docked with the mJHBP. The binding affinity of the ligands to the mJHBP is listed in Table 3.

Protein-ligand docking of the mJHBP with the selected ligands resulted in high binding affinity with compound 14 (-8.8 kcal/mol) and compound 19 (-8.8 kcal/mol), followed by compound 13 (-8.7 kcal/mol). The binding affinity is calculated based on hydrogen bond and hydrophobic bond interactions (Fig. 4). Oxalic acid, decyl 3,5-difluorophenyl ester interacting with the residues Tyr64 (green) forming hydrogen bonds (yellow) of length 3.0 Å, Oxalic acid, 3,5-difluorophenyl undecyl ester interacting with the residues Trp53 and Tyr148 (green) forming hydrogen bonds (yellow) of length 2.9, 3.0 and 3.2 Å. d) p-octyl acetophenone formation of non-polar interaction with residues (orange).

Normal mode analysis (NMA)

The interaction between the protein-ligand complex of p -octylacetophenone (A), Oxalic acid, decyl 3,5-diflurophenyl ester (B) and Oxalic acid,3,5-diflurophenyl undecyl ester (C) was analyzed using iMODS. The interaction resulted in seven parameters of analysis. i) The amino acid motion direction. The interaction revealed that the residues of amino acids exhibit mobility,

as confirmed by normal mode analysis. The binding of the ligand to the complex was demonstrated (Fig. 5).

ii)Eigenvalue represents the stiffness in motion of the protein-ligand complex. It is helpful to analyze the energy required to deform the complex structure completely. The lower the eigenvalue, the higher the deformability of the complex structure. The compound p-octylacetophenone (A) showed an eigenvalue of 3.677195 e-07, Oxalic acid, decyl 3,5-diflurophenyl ester (B) showed an eigenvalue of 2.499837 e-07 and Oxalic acid,3,5-diflurophenyl undecyl ester (C) showed an eigenvalue of 6.935835 e-07 (Fig. 6 A, B and C) iii) The deformability is high at regions with high hinges in the graph. The distortion of the atoms is represented in the graph at the hinge areas (Fig. 6D, E, and F). iv) The variance of the protein-ligand structure is found to be inversely proportional in all three docked molecular complexes of individual normal modes of analysis (Fig. 6 G, H and I). v) the b factor represents the stable mobility and average amplitude of the 3D conformer structure of the docked complex. It also depicts the average root mean square (RMS) of the protein-ligand complex (Fig. 7A, B, and C). vi) The graph of the elastic network is reported concerning the atomic index value. The interactions between atomic residues (amino acids) are represented by a dot, which corresponds to the springs between the interactions. Darker the grey area or the grey dot, the more the stiffness of interaction b between the two atoms in correspondence Fig. 7 D, E and F). (vii) The graph of covariance is represented mainly using three colours such as red, blue and white. The area of white represents the uncorrelated matrix of the complex. The red and blue areas represent the correlated and anti-correlated variance of the docked complex (Fig. 7G, H, and I) (López-Blanco *et al.,* 2017).

ADME properties

ADME/T (absorption, distribution, metabolism, excretion, and toxicity) and physiochemical properties of the selected three bio compounds were analyzed. The data has been provided in the supplementary file (Table S1). The toxicity analyzed is represented in Table 4. The selected bioactive compounds have demonstrated efficient human intestinal absorption (HIA), Caco-2 permeability values, and 20% bioavailability, as well as P-glycoprotein inhibitor and substrate properties. In vitro absorption properties are marked with the standard reference to the absorbance levels in the Caco-2 cell line for the intestinal absorption of drug materials (Natesh *et al.*, 2021). The distribution properties are studied to analyze the passage of drug compounds through the human system via blood to the liver for processing. The three major criteria for analyzing the drug's transverse movement through the bloodstream are volume distribution, Blood-Brain Barrier crossing ability, and plasma Binding Protein values. The metabolism of the drug is analyzed using the Cytochrome P450 family, which contains enzymes that can break down the drug molecules in the liver and remove them through urine (Glue and Clement, 1999). The study indicated that the three compounds selected can metabolize cytochrome P450 family enzymes. Elimination properties are crucial in understanding the rate of removal of the drug residue and the clearance of the drug. The half-life time (T 1/2) and the clearance rate indicate the safety level of drug removal from the human system. The bioactive compounds showed a half-life of less than 2 hours and clearance from the body. Toxicity evaluation of the drug can predict the levels of toxicity it can cause on the liver, skin, etc. The hERG values indicate that the drug poses no threat to cardiac functionality, and the AMES (Ames Mutagenicity) shows that the candidate does not enhance any possible genetic damage. The skin sensitization values indicate the candidates' ability to cause no effects on human skin.

DISCUSSION

The study highlights the significance of exposing potential biological compounds that act as ligands or drug molecules that can inhibit the mosquito juvenile hormone binding protein The mosquito juvenile hormone binding protein plays a highly significant role in the growth and development of mosquitoes. It plays a crucial role in the development of the mosquito. Therefore, the study focuses on bioactive molecules that can target mJHBP and act as an effective method for controlling mosquitoes that cause the spread of uncontrolled, fatal diseases.

The presence of the juvenile hormone in the haemolymph maintains the instar stages of the mosquito (Devillers *et al.,* 2014). The mJHBP is known to bind to the juvenile hormone that is crucial in regulating the metamorphosis between the stages of a mosquito. It is highly exhibited at the egg hatchability and pupal hatchability stages. A previous study by Araujo *et al.* (2020) reported the isolation of fungal compounds, such as those from *Aspergillus* sp., that can inhibit the activity of Juvenile hormone-binding protein in mosquitoes and possess good larvicidal activity. The study has reported the efficiency of replacing the components in insecticides using microbial secondary metabolites (Araujo *et al.,* 2020).

The active site predicted by the present study includes twenty-one amino acid entities that form the active site grid. This region was further confirmed by a similar study conducted by Kim et al. (2017). The area of interaction exhibits hydrophobic interactions involving amino acids as predicted by the study (Kim *et al.,* 2017). Similar studies conducted by Da Costa *et al*. (2022) revealed the interaction between several compounds (M01, M02, M03, M04, and M05) and the mosquito

Table 2. Amino acid percentage composition

Sl no	Amino acid	Number	% composition
1	Alanine (A)	29	10.1
2	Arginine (R)	17	5.9
3	Asparagine (N)	17	5.9
4	Aspartic acid (D)	19	6.6
5	Cysteine (C)	10	3.5
6	Glutamine (Q)	12	4.2
7	Glutamic acid (E)	24	8.3
8	Glycine (G)	15	5.2
9	Histidine (H)	5	1.7
10	Isoleucine (I)	9	3.1
11	Leucine (L)	17	5.9
12	Lyssine (K)	17	5.9
13	Methionine (M)	5	1.7
14	Phenylalanine (F)	16	5.6
15	Proline (P)	12	4.2
16	Serine (S)	18	6.2
17	Threonine (T)	11	3.8
18	Trptophan (W)	6	2.1
19	Tyrosine (Y)	13	4.5
20	Valine (V)	16	5.6

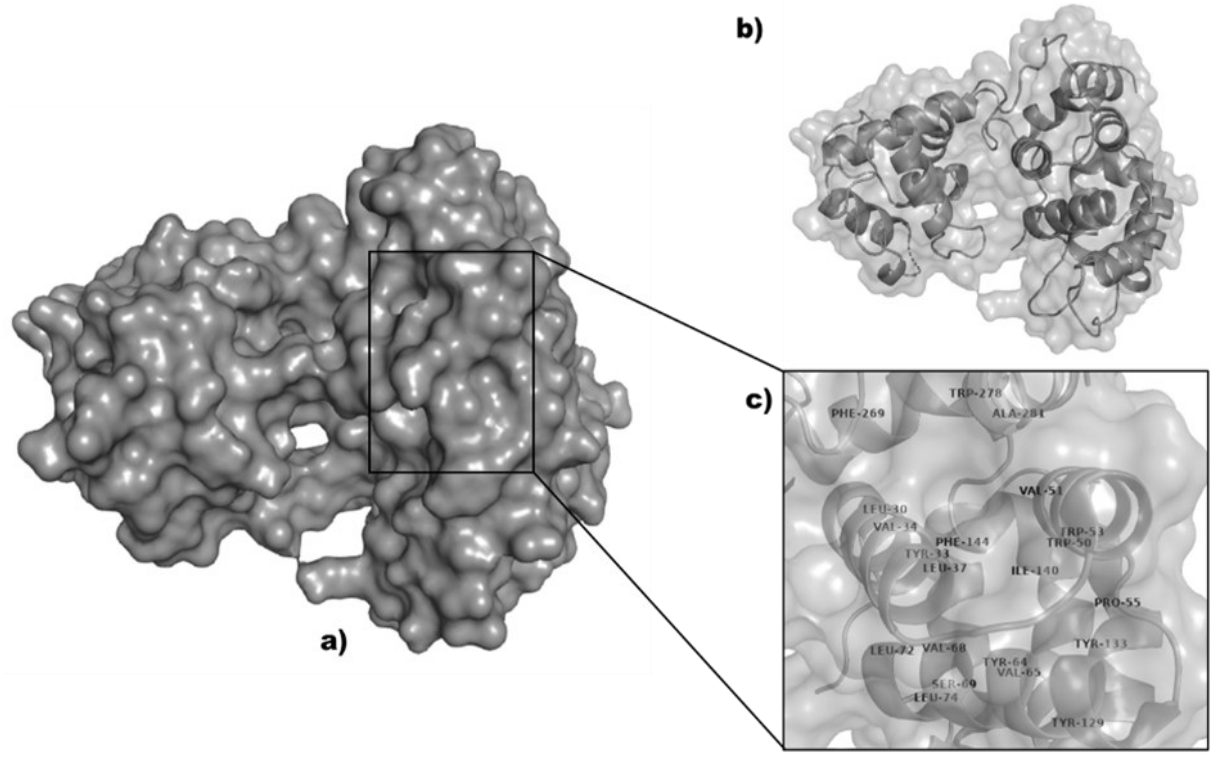

Fig. 3. *Active site for ligands in 5v13 protein. The binding site representing the (a) surface, (b) chains and (c) grid area focused to unveil the amino acid residues interacting in <5.0 Å vicinity*

Table 3. List of ligand's binding affinity to the targeted mJHBP and its RMSD values

Sl. No.	Ligand	Binding affinity
1	Oxalic acid, 3,5-difluorophenyl undecyl ester	-8.8
2	p-octylacetophenone	-8.8
3	Oxalic acid,decyl 3,5-difluorophenyl ester	-8.7
4	Oxalic acid, 3,5-difluorophenyl nonyl ester	-8.5
5	1.2-benzenedicarboxylic acid, bis(2 methylpropyl) ester	-8.4
6	2,5 cyclohexadien-1-one, 2,6 bis (1,1 dimethylethyl)-4 ethylidene-	-8.2
7	5-(3-ethoxy-4,5-dihydro-isoxazol-5-yl)-5-methyl imidazolidine-2,4-dione	-8.1
8	Oxalic acid monomorpholide, nonyl ester	-8.1
9	4-amino-furazan-3-carboxylic acid(3-morpholin-4-yl-propyl)-amide	-8
10	Methyl 3,5-di-t-butyl salicylate	-7.7
11	Acetamide,N-(5-methylisoxazol-3-yl)-2-morpholin-4-yl-	-7.7
12	N-(Cyclohexyl)succinimide	-7.7
13	Myristic acid glycidyl ester	-7.6
14	Oxalic acid monomorpholide, heptyl ester	-7.6
15	Hexadecane	-7.5
16	2,5-di-tert-butyl -1,4-benzoquinone	-7.3
17	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene -2,8-dione	-7.3
18	1H-(1,2,4)triazole-3-carboxylic acid(2-morpholin-4-yl-ethyl)-amide	-7.3
19	Oxalic acid monomorpholide, hexyl ester	-7.3
20	pentadecene	-7.2
21	Sulfurous acid, hexyl octyl ester	-7.1
21	Tetradecene	-7.1
22	tetradecanol	-7.1
23	(1,2,4)Triazolo(4,3-a)azepine-3-thione,2-morpholin-4-ylmethyl-2,5,6,7,8,9 -hexahydro-	-6.9
24	Tridecene	-6.6
25	Dibutyl phthalate	-6.5
26	4-Oxa-1-azaspiro(5.6)dodecane,2-methyl-	-6.2

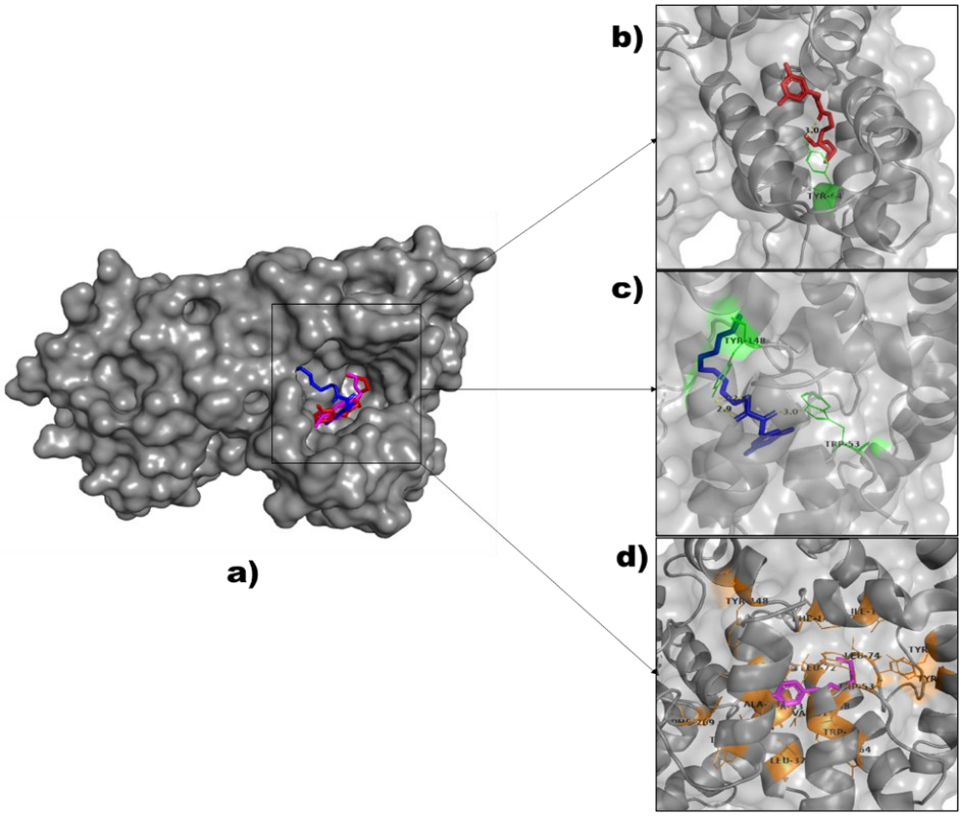

Fig. 4. a) *Binding poses of Oxalic acid, decyl 3,5-difluorophenyl ester (red), Oxalic acid, 3,5-difluorophenyl undecyl ester (blue) and p-octylacetophenone (magenta) to the mJHBP protein (b) Oxalic acid, decyl 3,5-difluorophenyl ester, (c) Oxalic acid, 3,5-difluorophenyl undecyl ester d) p-octylacetophenone*.

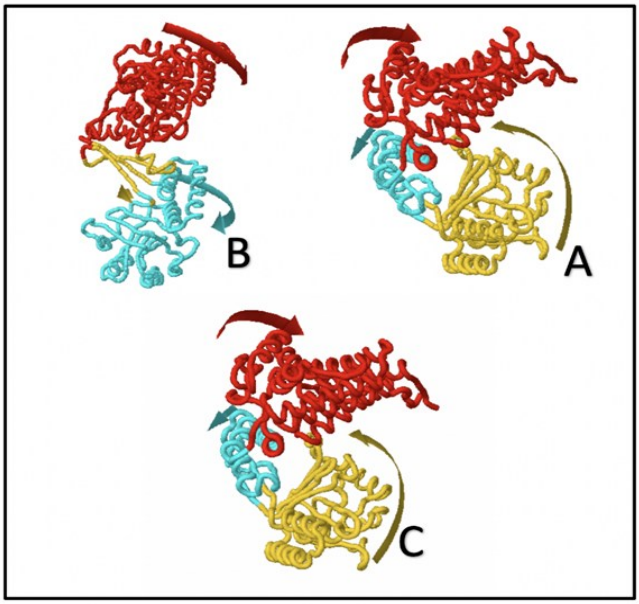

Fig. 5. *Structure of protein-ligand complex interaction and mobility define by arrows of motion direction*

juvenile hormone, MJHBP. The study depicted the binding of compounds at active-site amino acids, such as Tyr 64, Trp 53, Val 65, and Val 68. These compounds are concluded as potential insecticidal agents similar to present study (Da Costa *et al.,* 2022). The active site predictions have helped the study optimise accurate interactions to avoid any false positives and thus targeted the right active site to achieve the protein's functionality. The selected protein must be screened for its efficiency in binding to the ligand molecules. The atomic composition, Extinction coefficient, instability index, Aliphatic index and grand average hydropathicity was predicted using ExPASy tool. The protein indicated its efficiency in down streaming applications, thermal stability and structural reliability in it futuristic applications Studies by Gasteiger *et al.,* 2005 confirms that these parameters are crucial in the selection of the protein for docking studies (Gasteiger *et al.*, 2005).

Molecular Docking:The three compounds showed high binding affinity at the binding site of the protein, the binding can inhibit the structural stability of the protein that can in turn alter the functionality of the protein. it was found that the protein and its active site is crucial in the functionality. Similar studies by Singh *et al*., (2025) showed similar binding site in 5V13 protein (Singh *et al.,* 2025). NMA IMODS: The normal mode analysis helps in validating the stability of the protein-ligand complex. It also increases the reliability of the virtual screening technique and reduces false negatives. Studies on SARS-CoV-2 envelope protein were targeted using plant protease ligand compounds. The simulations study using iMODS resulted in predicting the efficiency of the protein-ligand complex by analyzing its stability using the deformability graph. The eigenvalues range concluded the stiffness of interaction, along with the elastic values graph (Kirar *et al.,* 2022).

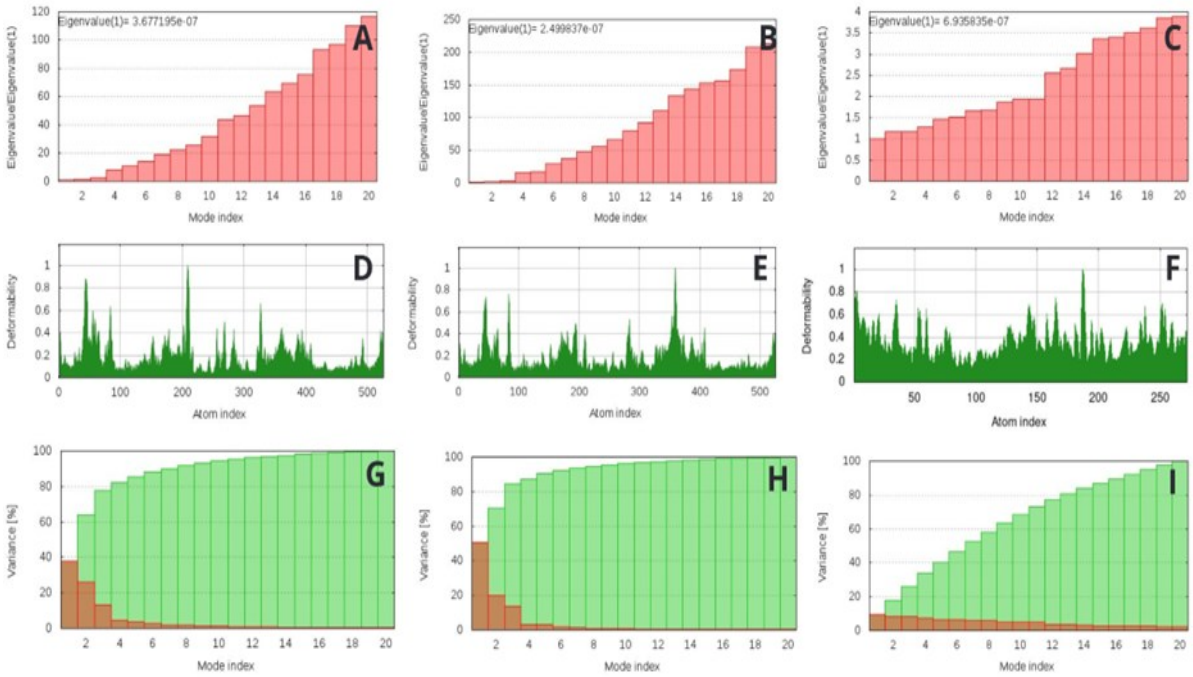

Fig. 6. *Eigen values of the protein-ligand complex of p-octylacetophenone (A), Oxalic acid, decyl 3,5-diflurophenyl ester (B) and Oxalic acid,3,5-diflurophenyl undecyl ester (C). The deformability of the protein-ligand complex of p-octylacetophenone (D), Oxalic acid, decyl 3,5-diflurophenyl ester (E) and Oxalic acid,3,5-diflurophenyl undecyl ester (F). the variance analysis graph protein-ligand complex of p-octylacetophenone (G), Oxalic acid, decyl 3,5-diflurophenyl ester (H) and Oxalic acid,3,5-diflurophenyl undecyl ester (I)*

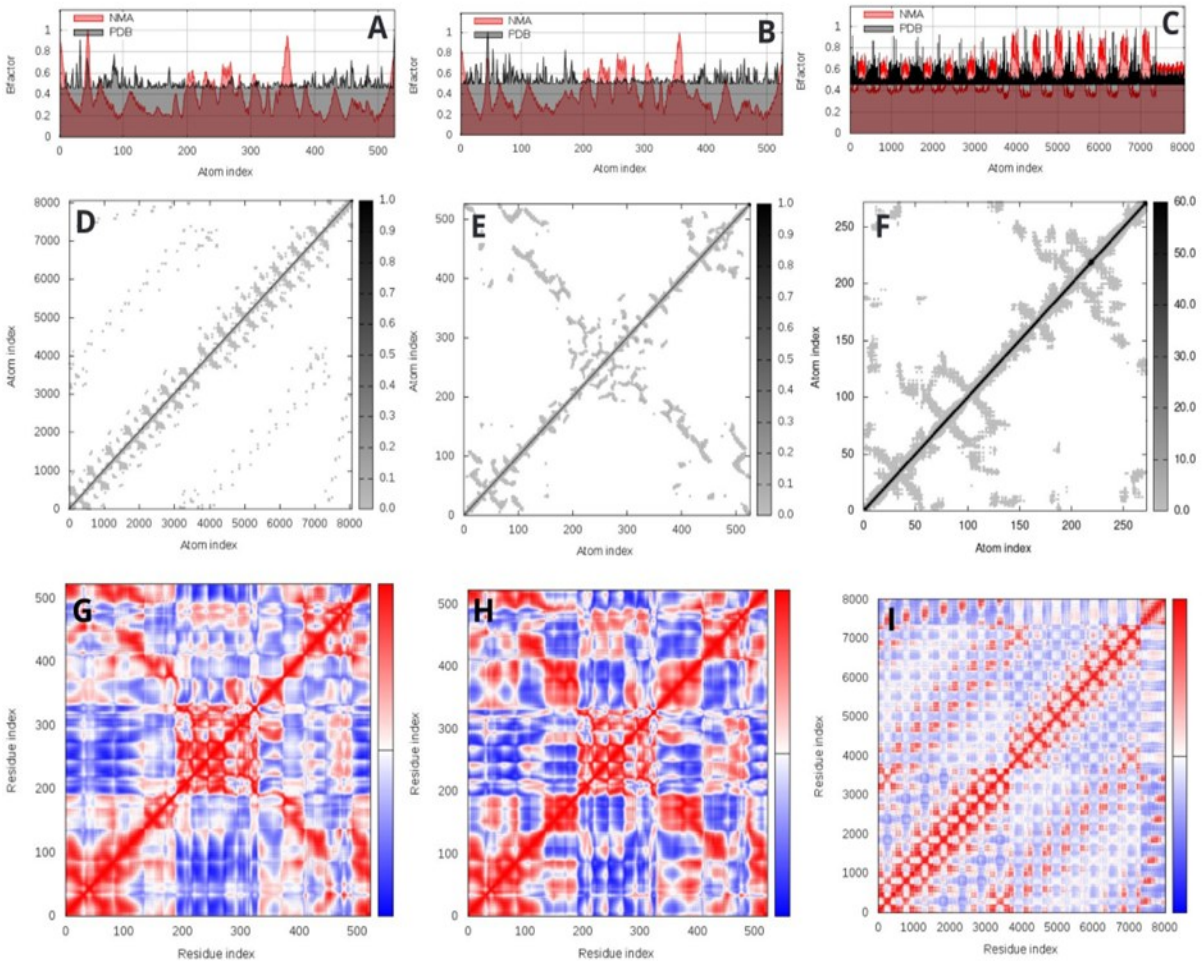

Fig. 7. *B factor graph of the protein-ligand complex: p-octylacetophenone (A), Oxalic acid, decyl 3,5-diflurophenyl ester (B) and Oxalic acid,3,5-diflurophenyl undecyl ester (C). The elastic values of the protein-ligand complex: p-octylacetophenone (D), Oxalic acid, decyl 3,5-diflurophenyl ester (E) and Oxalic acid,3,5-diflurophenyl undecyl ester (F). The covariance analysis graph protein-ligand complex: p-octylacetophenone (G), Oxalic acid, decyl 3,5-diflurophenyl ester (H) and Oxalic acid,3,5-diflurophenyl undecyl ester (I)*

ADME/T analysis helps in analyzing the potential drug like candidates from the bio compounds that were screened. While the docking studies revealed the binding molecule, the ADME analysis helped to identify the compounds ability in a biological system. Studies have also analyzed the toxicity of commercially available insecticidal compounds, such as diflubenzuron, FCX, TEF, DFB, and buprofezin, using the properties of ADME/T analysis methods (Da Costa *et al.,* 2022). The commercially available compounds exceed the toxicity levels, highlighting the relevance of our study in utilizing microbial secondary metabolites for inhibiting mosquito development (Ramos *et al.,* 2020).

Conclusion

Aedes aegypti is the primary vector for carrying and spreading fatal diseases, such as dengue, which must be controlled for the betterment of humanity. Given the drawbacks of using major sanitization and vector management strategies, it becomes crucial to develop an alternative approach to eradicate vector growth completely. Targeting the vector at its molecular level, such as using the vector's important proteins, can help alter its developmental system. The study utilized the mJHBP, a major protein in the juvenile hormone binding receptors, which plays a crucial role in egg hatchability and other developmental aspects of the mosquito. Using bioactive compounds, such as microbial metabolites, can have a potential impact in developing candidates against the vector without any adverse effects on humans. Three compounds analyzed in this study include P-Octylacetophenone, Oxalic acid, decyl 3,5-difluorophenyl ester, and Oxalic acid 3,5-difluorophenyl undecyl ester. These compounds have shown binding affinities of -8.8, -8.7, and -8.8, respectively, marking their ability to bind at the active site of the juvenile hormone-binding protein.

Table 4. Physicochemical properties and toxicity of the selected ligand compounds

	Toxicity							Physicochemical properties		
	hERG	H-HT (Human Hepatotoxicity)	AMES (Ames mutagenicity)	Skin Sen (Skin sensitization)	LD 50 (LD 50 of acute toxicity High-toxicity: 1~50 mg/kg; Toxicity: 51~500 mg/kg low-toxicity: 501~5000 mg/kg	FDAMDD (Maximum Recommended Daily Dose)	Log S (Solubility higher than -4 log mol/L	Log D7.4 (Distribution coefficient D) 1 to 3: Solubility moderate; Permeability moderate; Metabolism low	LogP (Distribution Coefficient P) LogP >3: poor aqueous solubility	
P-octylacetophenone	0.527	-0.436	-0.058	0.789	3229.791 mg/kg	-0.27	0.72	-4.779 Log mol/L	2.966	4.792
Oxalic acid, decyl 3,5-difluorophenyl ester	0.725	0.516	-0.044	-0.337	1638.743 mg/kg	-0.38	0.602	-4.594 Log mol/L	3.14	4.554
Oxalic acid, 3,5-difluorophenyl undecyl ester	0.724	-0.488	-0.044	-0.337	1798. 688 mg/kg	-0.38	0.602	-4.98 Log mol/L	3.169	4.944

Supplementary Information

The author(s) are responsible for the content or functionality of any supplementary information. Any queries regarding the same should be directed to the corresponding author. The supplementary information is available for download from the article's webpage and will not be included in the print copy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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