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Efficacy of compounds used in mosquito repellents (DEET, picaridin, prallethrin and IR3535) against odorant binding protein (OBP20) of *Anopheles gambiae*: A molecular docking study

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ABSTRACT: The study is to use AutoDock software to determine the binding affinity or binding energy of DEET, picaridin, prallethrin, and IR3535 components with the odorant receptor of the *Anopheles gambiae* say (Diptera, Culicidae) mosquito species. The binding energy (ÄG) of prallethrin was determined to be highest at -10.55 kcal/mol followed by picaridin at -7.1 kcal/mol, DEET at -6.57 kcal/mol and IR3535 at -5.6 kcal/mol being the lowest among all. By comparing their binding energy levels after AutoDocking, it is to decide which mosquito repellent is the most effective. © 2023 Association for Advancement of Entomology

KEY WORDS: AutoDocking, olfactory receptor, binding energy, efficacy

INTRODUCTION

The most significant carrier of *Plasmodium falciparum* malaria in the world, female *Anopheles* mosquitoes, largely uses olfactory cues to locate their human hosts. A component of human sweat triggers a response in the female-specific protein AgOr1 of the *Anopheles gambiae,*which belongs to a family of putative odorant receptors. Odorant-binding proteins (OBPs) serve as a bridge between odorant receptors, which are found in olfactory structures of the mosquito's peripheral sensory system (primarily the antennae and maxillary palps), and the air medium that broadcasts chemical signals, serving as the first relay in semiochemicals reception in mosquitoes. OBPs are hypothesised to be involved in the transfer of odorants to odorant receptors (ORs) for the particular signal transduction of behaviorally active odorants (Venthur and Zhou, 2018). These proteins might be used as molecular targets for the creation of mosquito attractants. Dipteran OBPs lack the extended C-terminus needed to occupy the binding pocket at low pH because they are shorter (125 amino acid residues). *An. gambiae* is the only mosquito whose OBP structure has been documented (Leite *et al*., 2009). AgamOBP1 is a member of the medium subclass and is 125 residues long with six cysteines and three disulfide linkages. It also features an extended C-terminal section that is buried inside the protein core and forms a wall with the internal cavity (Cali and Persaud, 2020).The odors emanating from human skin and sweat serve as the *An. gambiae's* primary means of locating its hosts. These odours cause the insect to react in a certain way to OBP. *Anopheles gambiae* OBP

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20 or AgamOBP20, a particular form of OBP, has recently been defined. During the height of its host-seeking behaviour, the female mosquito's antennae exhibit high levels of this OBP, which suggests that it may be involved in olfactory sensing. Hydrophobic residues make up the majority of the AgamOBP20's binding site.

It is believed that the amino acids Leu106, Leu107, and Met53, which have been identified as possible critical residues, are crucial for the interaction between the protein and the ligand. Important considerations for the way the ligand interacts to AgamOBP20 are the steric restriction and hydrophobic interaction. The molecular characteristics and parameters discovered may be used to create novel insecticides and repellents that can interfere with AgamOBP20's function and cause *An. gambiae* to behave differently when looking for a host (Janeiro *et al*., 2016).The major goal of this study is to use AutoDock software to determine the binding affinity or binding energy of DEET, picaridin, prallethrin, and IR3535 components with the ORs of the *An. gambiae*.

MATERIALS AND METHODS

Softwares required: The following softwares were downloaded and installed from online sources which were used to carry out our molecular docking procedure:

- Open Babel GUI (http://openbabel.org)
- UCSF Chimera (http://www.cgl.ucsf.edu/chimera/)
- BIOVIA Discovery Studio
- MGL Tools
- AutoDock 4.2.6

Ligand Retrieval: Ligands were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The three-dimensional (3D) structures of the chemical compounds were obtained from PubChem database in the form of SDF files (structure-data files). For molecular docking, the following five ligands were retrieved:

- DEET- PubChem CID 4284); IUPAC name: N,N-diethyl-3-methylbenzamide
- Picaridin (PubChem ID 125098); IUPAC name: butan-2-yl 2-(2-hydroxyethyl) piperidine-1-carboxylate
- Prallethrin (PubChem ID 9839306); IUPAC name: (2-methyl-4-oxo-3-prop-2-ynylcyclopent-2-en-1-yl)2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate
- IR3535 (PubChem ID 104150); IUPAC name: ethyl 3-[acetyl (butyl) amino] propanoate
- PG4 (Substance SID 7889818; Compound CID 8200); IUPAC name: Tetraethylene glycol

DEET, Picaridin, Prallethrin, and IR3535 were the test ligands among the aforementioned ligands, whilst PG4 was the co-crystal ligand of the template protein OBP20.

File Conversion from SDF to PDB: Using the software Open Babel (http://openbabel.org), the ligands that were retrieved from the PubChem database in the form of SDF files were then translated to PDB (Protein data bank) file format. SDF was chosen as the output format, and PDB was chosen as the input format. The input name of the file contained the ligands in their SDF form. The file was named ligand.pdb in the output file and saved to the desktop. The "Convert" button was then clicked. The ligands were eventually prepared for docking after being converted to PDB format.

Retrieval and Preparation of Protein: In the RCSB database (http://www.rcsb.org/pdb/), the 3D structure of the odorant binding protein was looked up. The search results included odorant binding proteins from a number of different organisms, including *Drosophila melanogaster, Aedes aegypti, Anopheles gambiae* and *Bombyx mori*. The odorant binding protein from *An. gambiae* was chosen to perform the molecular docking process. The protein macromolecule known as AGAP005208-PA and an already bound specific

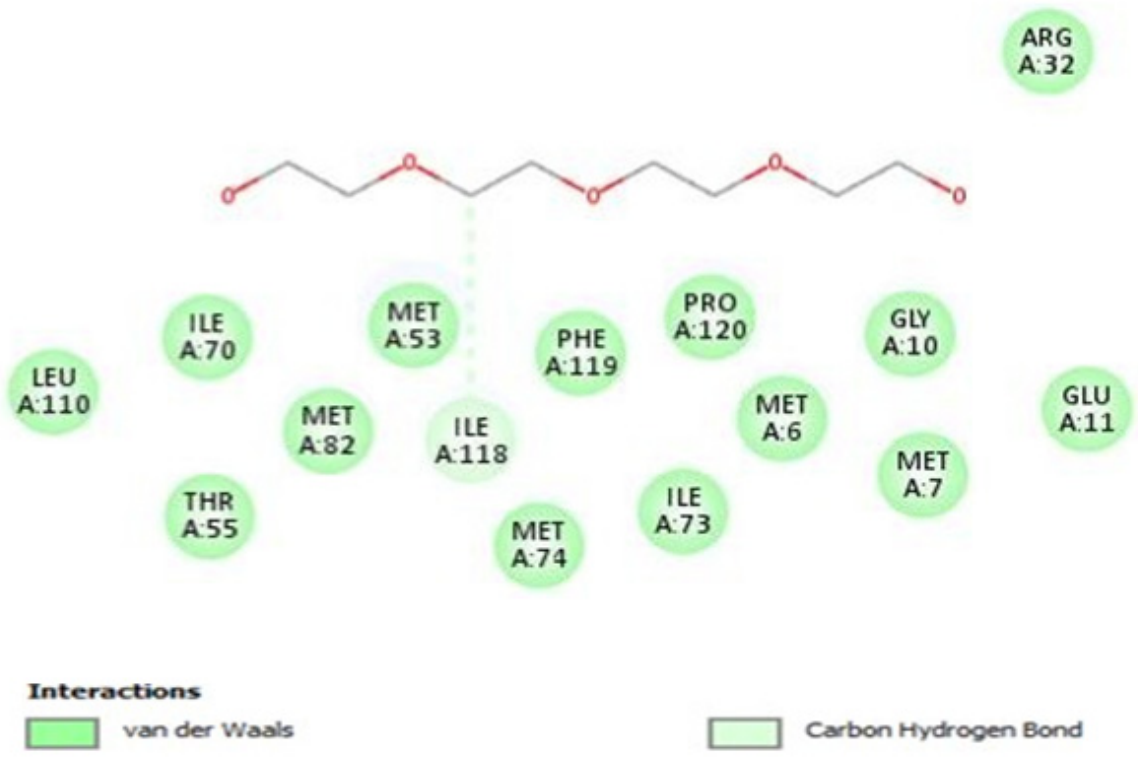

Fig.1 BIOVIA Discovery Studio image showing 2D interactions of co-crystal ligand (PG4) with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20

ligand PG4 or polyethylene glycol were both present in the chosen 3D structure of *Anopheles gambiae's* odorant binding protein (PDB ID 3V2L).

The 2D interactions of bounded co-crystal ligand (PG4) with OBP20 of *Anopheles gambiae* were visualized in BIOVIA Discovery Studio (Fig.1). The amino acid residues which showed Vander Waals interaction were Met6A, Met7A, Gly10A, Glu11A, Arg32A, Met53A, Thr55A, Ile70A, Ile73A, Met74A, Met82A, Leu110A, Phe119A and Pro120A. On the other hand, the amino acid residue which showed Carbon Hydrogen Bonds was Ile118A.

Following retrieval, the protein structure was opened in the UCSF Chimera programme (http://www.cgl.ucsf.edu/chimera/) to get rid of its co-crystal ligand (PG4) before docking. It was discovered that Chain A was the binding chain for the co-crystal ligand, hence Chain A was chosen. Once the co-crystal ligand had been chosen from the Residue dropdown list, it was deleted from the protein structure. The newly modelled protein structure without the co-crystal ligand was saved which was then ready for docking.

Docking: The ligands and the protein were prepared for docking by using Auto dock Tools (ADT). Using the Lamarckian Genetic Algorithm, the docking software Auto Dock 4.2.6 was utilised for the investigation of protein-ligand complexes (LGA). The template protein (*An. gambiae* OBP20) and its co-crystal ligand PG4 were initially docked. Then DEET, Picaridin, Prallethrin and IR3535 were molecularly docked with the *A. gambiae* OBP20 odorant binding protein receptor. The software Auto Dock 4.2.6 makes advantage of free binding energy to score the ligand-protein complexes (10 numbers). The 2D interactions between the ligand and the amino acids in the protein's LBD (Ligand Binding Domain) were visualised using BIOVIA Discovery Studio (Fig.1). Docking was carried out individually, and

Table 1. Grid box measurements of the LBD of receptor protein used for docking

Protein	Ligand	Gridboxdetails				
		Dimensions	Spacing	Coordinates		
				X	Y	Z
Anopheles gambiae OBP20	DEET	60×60×72	0.375Å	2.083	1.056	-7.222
	Picaridin	56×50×68	0.375Å	3.222	2.083	-4.861
	Prallethrin	58×64×70	0.375Å	1.806	0.167	-5.667
	IR3535	50×52×72	0.375Å	3.139	2.833	-5.306

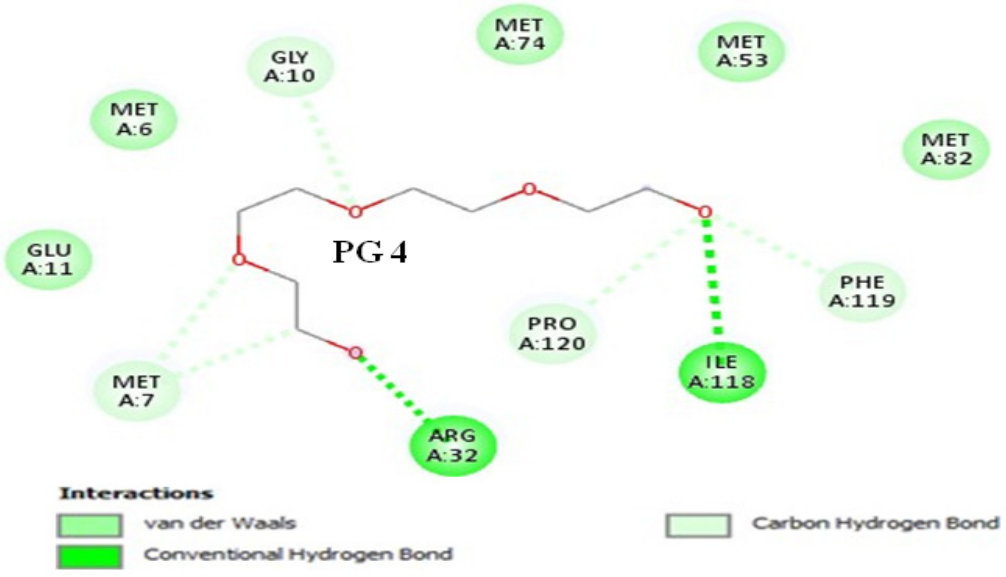

Fig. 2 BIOVIA Discovery Studio image showing 2D interactions of co-crystal ligand (PG4) with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

measurements of every grid box used for each protein-ligand docking (Table 1).

RESULTS AND DISCUSSION

Docking of co-crystal ligand (PG4) with *Anopheles gambiae* OBP20: PG4 exhibited ligand binding interactions with OBP20 of *An.gambiae*. After docking, the most favourable protein-ligand complex which was obtained had a binding energy (ÄG) of -3.89 kcal/mol. Two amino acid residues demonstrated conventional hydrogen bonding with LBD of OBP20 of *An. gambiae*, four amino acid residues demonstrated carbon-hydrogen bonding, and five amino acid residues shown Van der Waals interaction (Fig. 2, Table 2).

Table 2. 2D interactions between the co-crystal ligand (PG4) and the ligand binding domain (LBD) of *Anopheles gambiae* OBP20

VanderWaalsInteraction	CarbonHydrogenBonds	Conventional HydrogenBonds
Met6A	Met7A	Arg32A
Glu11A	Gly10A	Ile118A
Met53A	Phe119A	
Met74A	Pro120A	
Met82A		

Docking of DEET with *Anopheles gambiae* OBP20: DEET demonstrated ligand binding interactions with OBP20 of *An. gambiae* (Fig. 3).

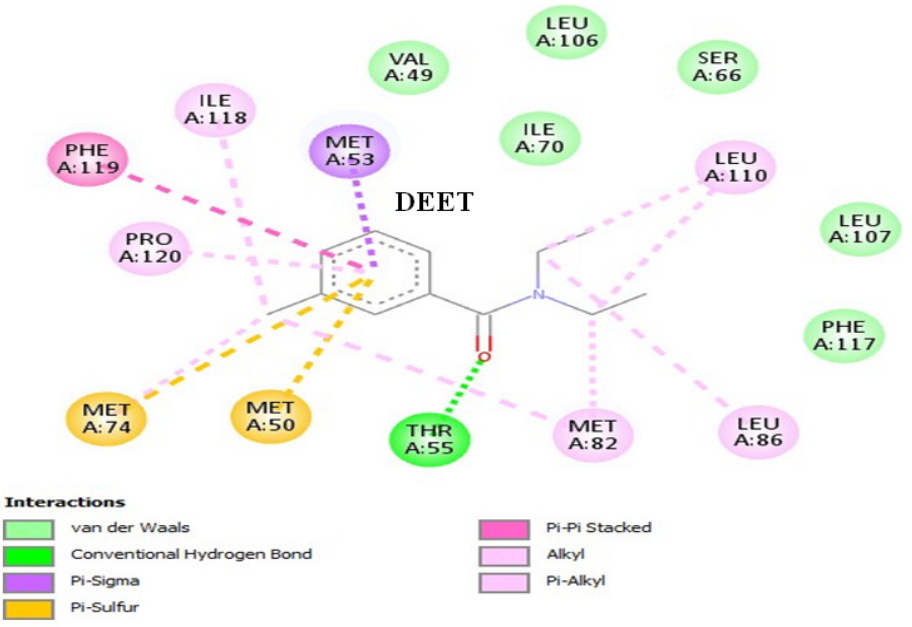

Fig. 3 BIOVIA Discovery Studio image showing 2D interactions of DEET with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

Table 3. 2D interactions between DEET and *Anopheles gambiae* OBP20

Vander Waals Interaction	Conventional Hydrogen Bonds	Pi-Sulfur Interaction	Pi-Sigma Interaction	Pi-PiStacked Interaction	Alkyl Interaction	Pi-Alkyl Interaction
Ile70A	Thr55A	Met74A	Met53A	Phe119A	Leu110A	Met82A
					Ile118A	
					Pro120A	

After docking, the most favourable protein-ligand complex which was obtained had a binding energy (ÄG) of -6.57 kcal/mol. As listed in Table 4, one amino acid residue demonstrated Van der Waals interaction, one demonstrated conventional hydrogen bonding, one demonstrated Pi-Sulfur interaction, one demonstrated Pi-Sigma interaction, one demonstrated Pi-Pi stacked interaction, three demonstrated alkyl interaction, and one demonstrated Pi-Alkyl interaction with LBD of OBP20 of *An. gambiae* (Table 3).

Docking of Picaridin with *Anopheles gambiae* OBP20: Picaridin demonstrated ligand binding interactions with OBP20 of *An. gambiae* (Fig.4). The protein-ligand combination with the best docking results has a binding energy (ÄG) of -7.1 kcal/mol. One amino acid residue exhibited Van der Waals interaction, two exhibited conventional hydrogen bonding, three exhibited alkyl interaction, and three exhibited Pi-Alkyl interaction with LBD of OBP20 of *An. gambiae* (Table 4).

Docking of Prallethrin with *Anopheles gambiae* OBP20: Prallethrin demonstrated ligand binding interactions with OBP20 of *An. gambiae* (Fig. 5). The protein-ligand complex with the best docking results had a binding energy (ÄG) of -10.55 kcal/

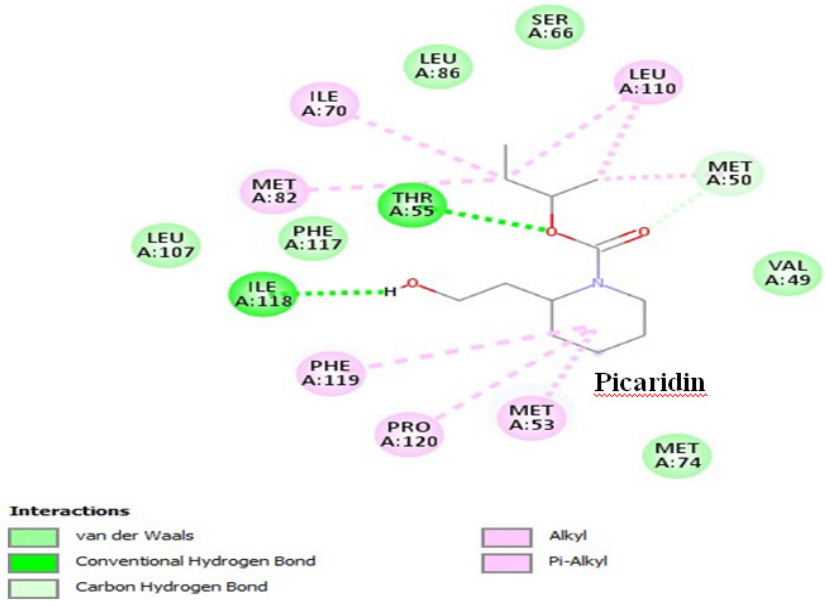

Fig. 4 BIOVIA Discovery Studio image showing 2D interactions of Picaridin with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking.

Table 4. 2D interactions between Picaridin and *Anopheles gambiae* OBP20

Vander Waals Interaction	Conventional Hydrogen Bonds	Alkyl Interaction	Pi-Alkyl Interaction
Met74A	Thr55A	Ile70A	Met53A
	Ile118A	Met82A	Phe119A
		Leu110A	Pro120A

mol. Seven amino acid residues showed alkyl interaction with LBD of OBP20 of *An. gambiae*, six amino acid residues demonstrated Van der Waals interaction, and one residue demonstrated carbon hydrogen bonding (Table 5).

Docking of IR3535 with *Anopheles gambiae* OBP20: OBP20 from *An. gambiae* had ligand binding interactions with IR3535 (Fig. 6). The most favourable protein-ligand combination that could be formed after docking had a binding energy (ÄG) of -5.8 kcal/mol. Five amino acid residues showed alkyl contact with LBD of OBP20 of *An. gambiae,* three amino acid residues demonstrated Van der Waals interaction, one amino acid residue demonstrated typical hydrogen bonding (Table 6).

The obtained results have been interpreted statistically (Fig. 7).

Utilizing Auto Dock 4.2.6, molecular docking was performed for the current study. Finding the strongest component (among those chosen for the study) used in the manufacturing of insect repellents was the main goal of this investigation. This study's findings will help determine which compound is most effective at keeping mosquitoes away, one of the most dangerous insect vectors on the planet. In this study, *An. gambiae*, a mosquito which plays a major role in the transmission of malaria, is the organism against which the efficacies of the chemicals were evaluated.

Following molecular docking, it was discovered that the binding energy (ÄG) of DEET bound to the odorant binding protein of *An. gambiae* was -6.57 kcal/mol. This demonstrates that the DEET chemical is a key component of insect repellents. The efficiency of several commercial mosquito repellent sprays and items containing DEET was investigated in a study conducted by Rodriguez and

Table 5. 2D interactions between Prallethrin and *Anopheles gambiae* OBP20

Vander WaalsInteraction	Alkyl Interactions	Carbon Hydrogen Bonds
Gly10A	Met6A	Pro120A
Arg32A	Met7A	
Met53A	Ile70A	
Thr55A	Ile73A	
Ile118A	Met74A	
Phe119A	Met82A	
	Leu110A	

Table 6. 2D interactions between LBD of IR3535 and *Anopheles gambiae* OBP20

Vander Waals Interaction	Alkyl Interaction	Conventional Hydrogen Bond
Met74A	Met53A	Thr55A
Ile118A	Ile70A	
	Met82A	
	Leu110A	
	Pro120A	

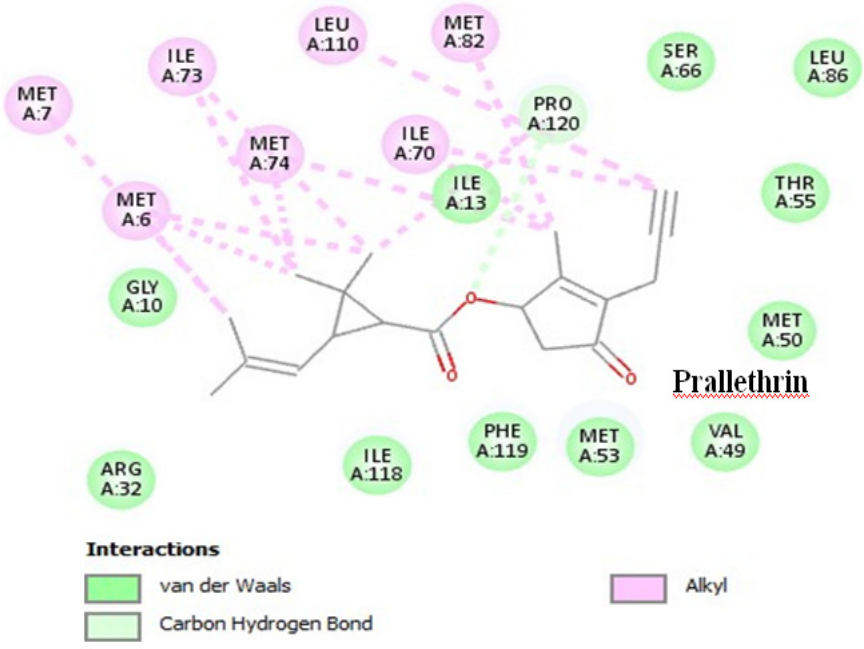

Fig. 5 BIOVIA Discovery Studio image showing 2D interactions of Prallethrin with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

Hansen (2015) which showed that they were effective and lasted for a fair amount of time. DEET-containing products have been proven to be both safe and efficient. The chemical N,N-diethyl-meta-toluamide is known by the acronym DEET.

However, after conducting more docking experiments with several other compounds that are also included in repellents, it could be understood that Prallethrin is the most efficient substance out of the four that were chosen in this study. The binding energy (ÄG) of Prallethrin was found to be -10.55 kcal/mol. It is a synthetic pyrethroid with quick knock-down action against domestic insect pests and vectors. It is utilised in household insecticides to combat cockroaches, houseflies, and mosquitoes (Matsunga *et al.,* 1987). It is most frequently utilised in liquid insect repellents and it is thick yellow to amber liquid. These chemicals, in the form of vapor obstruct mosquitoes' respiratory tracts and chemo receptor. The second most effective compound is Picaridin, which has a binding energy (ÄG) of -7.1 kcal/mol. With a binding energy (ÄG) of -5.8 kcal/mol, IR3535 exhibits the lowest effectiveness.

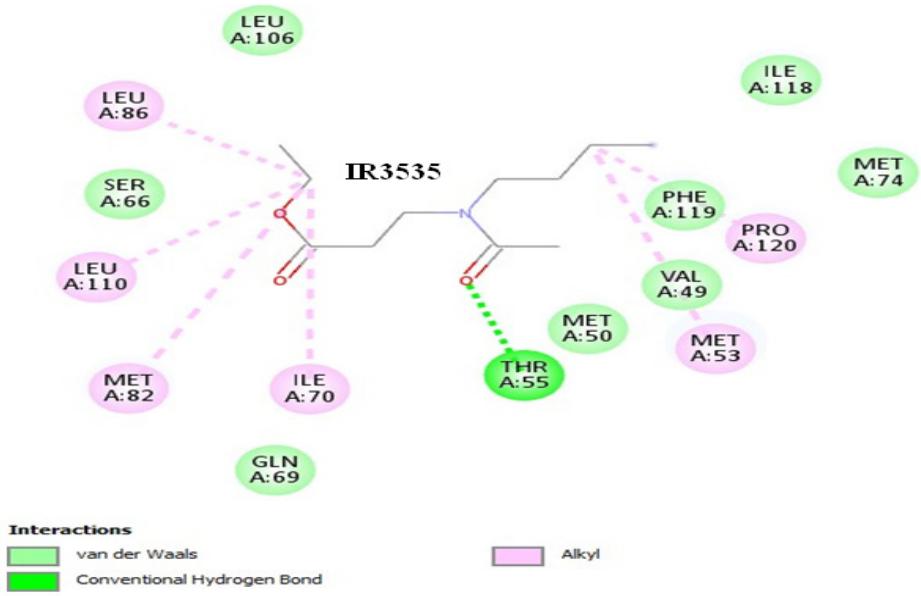

Fig. 6 BIOVIA Discovery Studio image showing 2D interactions of IR3535 with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

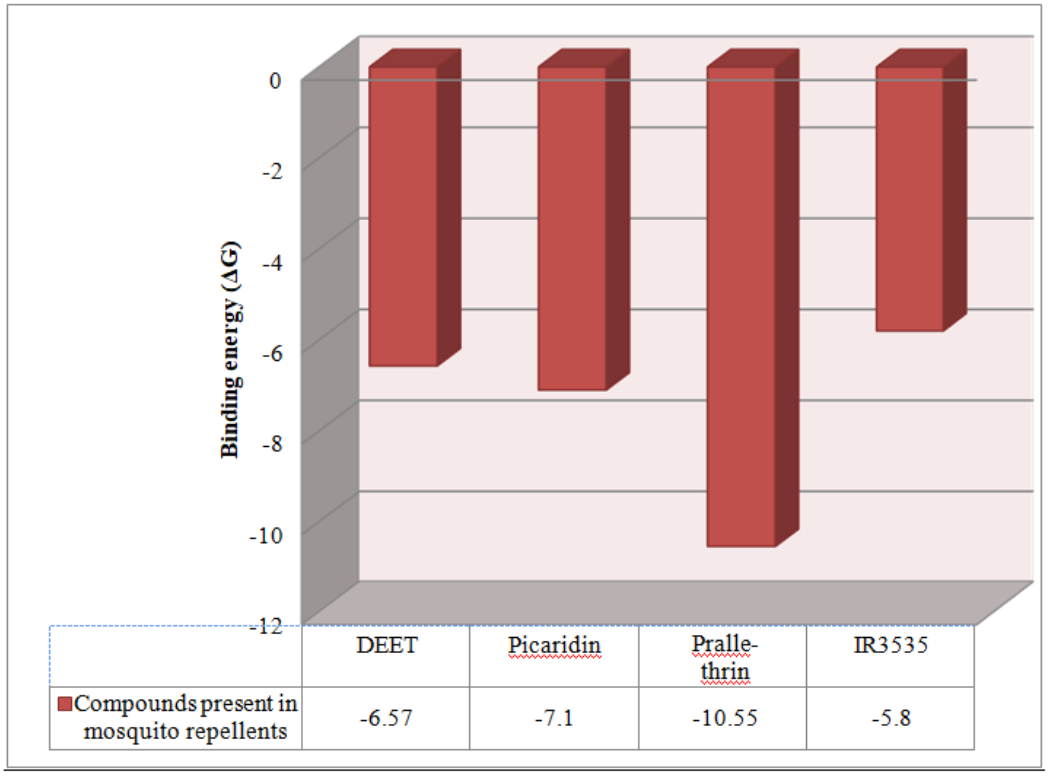

Fig. 7 Statistical data interpretation of binding energy (ÄG) of various chemical compounds present in mosquito repellents. Here, x-axis represents the compounds present in mosquito repellents and y-axis represents of binding energy of each of them

Therefore, DEET is not the only weapon. Dr. Dan Strickman, who oversees the Global Health Program at the Bill and Melinda Gates Foundation and is the author of "Prevention of Bug Bites, Stings, and Disease", claims that products containing the active components Picaridin and IR3535 are equally effective. Picardin has won the advantage, according to Strickman. Mosquitoes may land on individuals using DEET but refrain from biting them. When they use a picaridin-containing product, mosquitoes are less likely to even settle on them. However, Strickman notes that IR3535 repellents don't have the overpowering aroma of other products and are just marginally less effective. The Centre for Disease Control and Prevention recommends repellents containing any of those active components as being secure and reliable. They are easily accessible everywhere in the world.

REFERENCES

Barnard D.R. and Xue R.D. (2004) Laboratory evaluation of mosquito repellents against *Aedes albopictus, Culex nigripalpus* and *Ochierotatus triseriatus* (Diptera: Culicidae). Journal of Medical Entomology 41(4): 726 –730.

Bohbot J.D. and Dickens J.C. (2010) Insect repellents: modulators of mosquito odorant receptor activity. PLoS ONE5(8): e12138.

Cali K. and Persaud K.C. (2020) Modification of an *Anopheles gambiae* odorant binding protein to create an array of chemical sensors for detection of drugs. Science Reports 10: 3890.

Carey A.F., Wang G., Su C.Y., Zwiebel L.J. and Carlson J.R. (2010) Odorant reception in the malaria mosquito *Anopheles gambiae.* Nature 464(7285): 66 –71.

Dickens J.C. and Bohbot J.D. (2013). Mini review: Mode of action of mosquito repellents. Pesticide Biochemistry and Physiology 106(3): 149 –155

Ghavami M.B., Khoeini S., and Djadid N.D. (2020) Molecular characteristics of odorant-binding protein 1 in *Anopheles maculipennis*. Malaria Journal 19**:** 29.

Hallem E.A., Nicole F.A., Zwiebel L.J. and Carlson J.R. (2004) Olfaction: mosquito receptor for human-sweat odorant. Nature 427(6971): 212–213.

Janairo J.I.B., Bravo P.I.K., Morano N.L.G. and Yu D.E.C (2016) In Silico site-directed Mutagenesis of the *Anopheles gambiae*Odorant Binding Protein 20. Nova Biotechnologica et Chimica 15(2): 156 –165.

Konopka J.K., Task D., Afify A., Raji J., Deibel K., Maguire S., Lawrence R. and Potter C.J. (2021) Olfaction in Anopheles mosquitoes. Chemical Senses 46: 21– 23.

LeiteN.R., Krogh R., Xu W., Ishida Y., Iulek J., Leal W.S. and Olivia G. (2009) Structure of an Odorant-Binding Protein from the Mosquito *Aedes aegypti* Suggests a Binding Pocket Covered by a pH-Sensitive "Lid". PLoS ONE 4(11): e8006

Ogoma S.B., Hassan N., Simfukwe E.T., Mseka A., Moore J., Maia M.F., Moore S.J. and Lena L.M. (2014) The Mode of Action of Spatial Repellents and their impact on Vectorial capacity of *Anopheles gambiae sensu stricto.* PLoS ONE 9(12): e110433.

Paluch G., Bartholomay L.and Coats J. (2010) Mosquito repellents: a review of chemical structure diversity and olfaction. Pest Management Science 66(9): 925–935.

Pellegrino M., Steinbach N., Stensmyr M.C., Hansson B.S.and Vosshall L.B. (2011) A natural polymorphism alters odour and DEET sensitivity in an insect odorant receptor. Nature 478: 511–514.

Ray A. (2015) Reception of odors and repellents in mosquitoes.Current Opinion in Neurobiology 34: 158 – 64.

Strickman D., Frances S.P., and Debboun M. (2009) Prevention of Bug Bites, Stings and Disease. Oxford University Press, New York.

Venthur H. and Zhou J.J. (2018) Odorant Receptors and Odorant-Binding Proteins as Insect Pest Control Targets: A Comparative Analysis. Frontiers in Physiology 9: 1163.

Wang G., Carey A.F., Carlson J.R. and Zwiebel L.J. (2010) Molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. Proceedings of the National Academy of Sciences of the United States of America 107(9): 4418 –23.

Wilson D.K., Leal W.S., Ishida Y., Morgan T., and Wogulis M. (2006) The crystal structure of an odorant binding protein from *Anopheles gambiae*: Evidence for a common ligand release mechanism. Biochemical and Biophysical Research Communications 339(1): 157–164

Xia Y., Wang G., Buscariollo D., Pitts J., Wenger R.H. and Zwiebel L. (2008) The molecular basis of

olfactory-based behavior in *Anopheles gambiae* larvae. Proceedings of the National Academy of Sciences of the United States of America105: 6433–6438.

Ziemba B.P., Murphy E.J., Edlin H.T., and Jones D.N.M. (2013) A novel mechanism of ligand binding and release in the OBP 20 from *Anopheles gambiae*. Protein Science 22: 11–21.

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