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Inhibitory effect of *Azadirachta indica* phytochemicals against acetylcholinesterase (AChE) of *Aedes aegypti* (Diptera: Culicidae): A computational approach

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Abstract
The detrimental environmental impact and collateral harm associated with synthetic insecticides used in mosquito control programs underscore the urgent need for a paradigm shift. Plant-based natural compounds emerge as a compelling alternative demonstrating efficacy in mosquito control while mitigating ecological damage. This transition to nature-derived solutions addresses immediate challenges and signifies a crucial step towards sustainable and environmentally conscious pest management practices. In this study, we employed computational tools to meticulously evaluate the potential of phytochemicals derived from *Azadirachta indica* as potent inhibitors of acetylcholinesterase, coupled with their intrinsic insecticidal properties specifically against *Aedes aegypti* mosquitoes. The analysis revealed a subset of compounds, particularly azulene, squalene, mevalonic acid, hexadecanedioic acid, and neophytadiene, demonstrating remarkable biological activity. These compounds exhibited dual functionality as acetylcholinesterase inhibitors and effective agents with insecticidal properties, as predicted by the PASS webserver. Further substantiating their efficacy, the docking scores for these compounds were notably favorable, ranging from -7.1 to -6.2 kcal/mol. These compelling findings underscore the promising applicability of these phytochemicals in the targeted control and management of *Aedes* mosquito populations, thereby addressing the associated health risks and diseases.

Keywords: Aedes, acetylcholinesterase, neem, bioinsecticide, phytochemicals, insilico

Introduction

Vector borne diseases persist as a significant public health challenge worldwide in tropical and subtropical regions. As arthropod vectors mosquitoes play a critical role in transmitting numerous formidable pathogens. Their bites induce allergies and disrupt sleep, underscoring the profound impact of these vectors on human health [1]. These tiny vectors spread deadly diseases including malaria, dengue fever, Zika virus, and chikungunya. The impact of mosquito-borne diseases on global health cannot be overstated, with millions of people affected yearly. In the ongoing battle against these diseases, one of the primary strategies involves using pesticides to control mosquito populations [2]. Pesticides serve as crucial tools in the fight against mosquitoes, aiming to disrupt their life cycle and reduce their population. The molecular targets of these pesticides are carefully selected to interfere with essential biological processes in mosquitoes while minimizing harm to non-target organisms. Understanding molecular mechanisms involved in mosquito physiology has led to the developing of pesticides that specifically target these disease-carrying vectors [3]. Among the diverse mosquito species, *Aedes aegypti* is a particularly notorious vector. This species is infamous for transmitting diseases such as dengue fever, Zika virus, and chikungunya. *Aedes aegypti* mosquitoes are well-adapted to urban environments, thriving close to humans. This proximity increases the risk of disease transmission, making effective control measures imperative [4]. Acetylcholinesterase (AChE) emerges as a critical molecular target for managing the mosquito population. AChE is an enzyme critical for mosquitoes' proper functioning of the nervous system [5]. Pesticides that act on AChE disrupt the standard transmission of nerve signals, leading to paralysis and, ultimately, the death of the mosquito. The development of pesticides targeting AChE reflects a nuanced understanding of mosquito biology. By specifically disrupting the nervous system of mosquitoes, these pesticides provide a targeted and efficient means of controlling disease transmission [6]. However, as with any pest control strategy, the use of synthetic pesticides demands careful consideration as they might disrupt ecological balance and share a friend-foe relationship. *Azadirachta indica*, commonly known as neem, is revered for its versatile applications in traditional medicine and agriculture, particularly its potent insecticidal properties. The richness of neem lies in its diverse phytochemical composition, with azadirachtin being a standout compound [7]. Azadirachtin serves as a formidable insect growth regulator, as a deterrent to feeding and disrupting the moulting process in insects. This disrupts their life cycle, inhibiting maturation and reproduction [8][9]. Additionally, neem contains other vital phytochemicals like nimbin, nimbidin, salannin, meliantriol, and vepinin, each contributing to the overall insecticidal efficacy [10]. Remarkably, neem-based insecticides exhibit low environmental impact, degrading rapidly and minimizing harm to non-target organisms. Neem's role in pest management, particularly against disease vectors like mosquitoes, underscores its significance as a natural, eco-friendly alternative to synthetic pesticides. The ongoing exploration of neem's bioactive compounds holds promise for sustainable and effective pest control strategies. In this study, we employed various computational approaches to decipher the AChE inhibitor activity of various phytocompounds present in neem.

Material and methods

1. Prediction and validation of target protein

The AChE sequence from *Aedes aegypti*, obtained from the Uniprot database [11] (ID: Q8MYC0), served as the protein query for homology modelling using SWISS-MODEL [12] (http://swissmodel.expasy.org). The 3D structure of AChE was evaluated using the crystal structure of *Anopheles gambiae* AChE in complex with PMSF (ID: 5ydj.1.A) as a template. Various methods were employed to analyze the internal consistency and stability of the AChE-modeled structure. PROCHECK [13] and MolProbity [14] programs were utilized to assess the stereochemical quality, examining the residues' placement in the favoured zone of the Ramachandran plot. The GMQE (Global Model Quality Estimate), Ramachandran plot, and MolProbity score were employed to re-evaluate the protein assembly for its quality and reliability.

2. Ligand retrieval and preparation

The molecular structures of phytochemicals found in neem were acquired in sdf file format from the PubChem database, which offers comprehensive details about organic compounds, including their molecular structure, formula, and molecular weight (MW) (https://pubchem.ncbi.nlm.nih.gov/source/15751).
Compound information is available in Table 2. Ligand preparation was conducted using the OpenBabel [15] tool within PyRx 0.8 [16] and ligand energy minimization was performed utilizing the mmff94 force field. The sdf file format of the ligands was converted to pdbqt format to render it executable.

3. Molecular Docking

A computational study involving molecular docking was conducted, employing neem extract compounds as the ligand group and Acetylcholinesterase (AChE) as the macromolecule of interest. The investigation utilized the AutoDock Vina [17] tool within PyRx 0.8 to perform molecular docking. This computational approach allows for analyzing the interaction between the neem's phytochemicals and the AChE macromolecule, providing insights into potential binding affinities and structural arrangements. AutoDock Vina in PyRx 0.8 ensures a systematic and efficient exploration of ligand-macromolecule interactions in the context of the specified research objectives.

4. Visualization of Docking Result and interaction

After the molecular docking process, the ligand orientation with the most favourable negative score (docking score) was selected as the hit ligand for the target molecule. Subsequently, Discovery Studio 4.5 [18] was employed to visualize and display the best-docked position, facilitating an in-depth exploration of the interactions between the ligand and the target macromolecule. This step aid comprehension of specific binding modes, spatial orientations, and potential molecular interactions that contribute to the observed docking score, aiding in interpreting the ligand's affinity and suitability for the target.

5. Prediction of biological activity of the compound

The PASS web server [19] (http://www.pharmaexpert.ru/passonline) was utilized to predict the biological activities of the chosen compounds. The PASS analysis utilizes multilayer atom neighbour descriptors and aids in assessing the chemical's effects solely based on its molecular formula. This implies that the biological behaviour of the pharmaceutical is entirely determined by its chemical structure. Utilizing multilayer atom neighbour descriptors enhances the analysis, allowing for a more comprehensive evaluation of potential biological activities associated with the molecular structure of the compounds under investigation.

Result and discussion

1. Structure prediction and validation

The homology modelling of AChE was conducted using the SWISS-MODEL simulated protein modelling server powered by ProMod3, an open structure comparative modelling tool. The crystal structure of *Anopheles gambiae* AChE in complex with PMSF (ID: 5ydj.1.A) served as the template for the 3D modelling, with a high sequence identity of 96.51% between the query and template sequences (Figure 1). The resulting AChE model is presented in Figure 2 (a). The Z-score is employed to evaluate and confirm the accuracy of the modelled structure of the queried protein. Z-scores close to 0.0 indicate a structure resembling the native state, while a commonly accepted criterion suggests that a "QMEAN" Z-score below -4.0 reflects poor model quality. The graph depicts protein length on the x-axis and the "QMEAN" score on the y-axis, with each point representing an experimental protein structure. Black dots correspond to structures with a "QMEAN" score within 1 standard deviation of the mean (|Z-score| between 0 and 1), grey dots represent structures with a |Z-score| between 1 and 2, and light grey dots represent structures farther from the mean. A red star indicates the model under examination. In the case of the predicted structure of AChE, its Z-score falls within the grey zone, indicating its acceptability (Figure 3). MolProbity is a web-based service crafted explicitly for validating structural models and assessing model quality for proteins and nucleic acids at global and local levels. It plays a crucial role in evaluating the precision of molecular models, offering valuable insights into various structural parameters on both broad and specific scales. This tool proves indispensable for researchers and structural biologists, aiding them in refining their models to ensure accurate representations of proteins and nucleic acids. The predicted AChE structure obtained a MolProbity score of 0.74, and a Global Model Quality Estimate (GMQE) value of 0.83 was reported for the structure. GMQE is a quality measure that considers both the alignment between the objective and the template, as well as the structure of the template. The GMQE value falls within the range of 0 to 1, where a score closer to 1 indicates high accuracy in the modelled structure, and conversely, a lower score implies lower accuracy. The reliability and endorsement of the proposed homology model are substantiated by the GMQE score of 0.83 obtained for AChE, emphasizing its credibility and acceptability. The UCLA SAVES 6.0 server was employed to evaluate the stereochemical quality of a protein structure through Ramachandran analysis, residue-by-residue geometry examination, and an overall structure geometry assessment using PROCHECK. The Ramachandran plot revealed that 95.9% of amino acids resided in the most favoured region (Figure 2b). All these examinations collectively affirm the acceptability of the modelled structure of AChE.

Fig 1: Sequency alignment of query protein sequence (AChE) with template Protein sequence (Crystal structure of Anopheles gambiae AChE in complex with PMSF). Sequence alignment Identity was found to be 96.51%

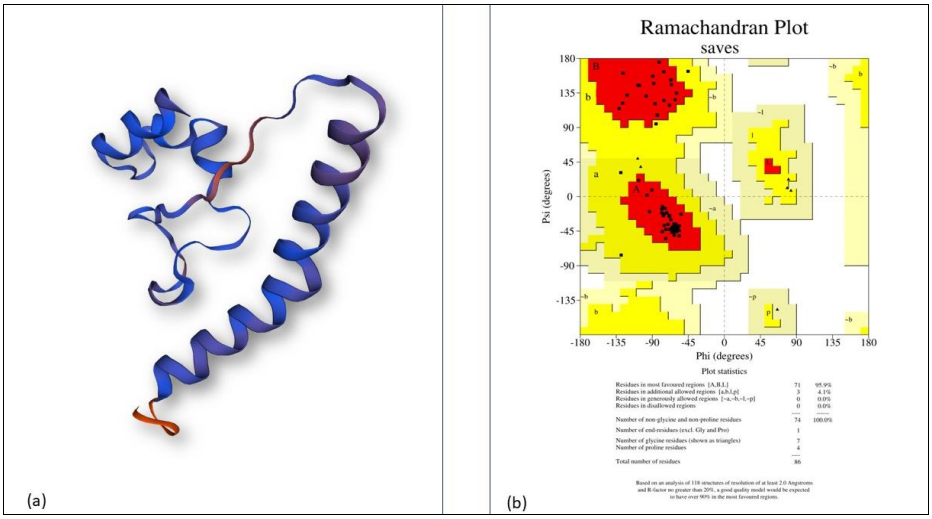

Fig 2: (a) Predicted 3D structure of AChE using SWISS-MODEL webserver (b) Ramachandran plot For modelled AChE structure. It was observed through Ramachandran analysis that 95.9% of the Amino acid residues were in most favoured region thus validating its acceptability for further a

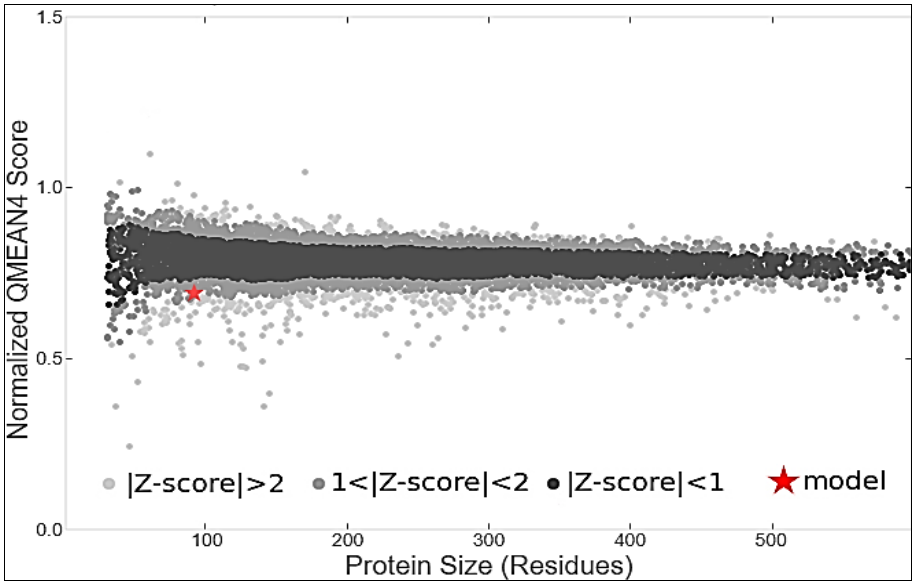

Fig 3: The Z-score for the predicted structure is represented, with a red star indicating that the Model structure falls within the gray area, signifying its acceptability.

2. Docking score of the compounds

The docking study utilized the 3D crystal structure of the predicted Acetylcholinesterase (AChE), employing Autodock Vina from PyRx 0.8 for the analysis. The protein was transformed into a macromolecule, and the selected compounds underwent initial minimization using the mmff94 force field. Subsequently, they were converted to pdbqt format using OpenBabel within PyRx. Blind docking Was executed with a grid box dimension of (39.13 Å × 41.84 Å × 42.36), centred at (-1.31, 76.38, -2.00), and an exhaustiveness setting of 8 by default. Table 1 summarises ligands alongside their respective pubchem ID, molecular weight and their docking scores. Remarkably, all the selected compounds from Azadirachta indica demonstrated favourable docking scores in the study.

Table 1: Phytochemicals with their molecular weight (MW), PUBCHEM ID, and their respective docking score

Phytochemicals	MW (g/mol)	PUBCHEM ID	Docking score (kcal/mol)
Azadirachtin	720.7	5281303	-7.0
Azulene	128.17	9231	-7.1
Epoxyazadiradione	466.6	49863985	-8.4
Gedunin	482.6	12004512	-8.5
Hexadecanedioic acid	286.41	10459	-6.4
Neophytadiene	278.5	10446	-6.2
Squalene	410.7	638072	-6.8
Mevalonic acid	148.16	439230	-5.4
Salannin	596.7	6437066	-6.3
Salannol	556.7	157144	-6.4
Meliantriol	490.7	101650343	-8.9

3. Protein-ligand interaction

Different types of interaction, such as Van der Waals, pi-alkyl, pi-sigma and hydrogen bonding, emphasize protein-ligand interactions. Azadirachtin, a plant-derived compound in neem, demonstrates a diverse range of molecular interactions with amino acids, underscoring its binding characteristics. Notably, Van der Waals interactions play a pivotal role, involving amino acids like His21, Thr23, Asn25, Phe24, and His62, contributing significantly to the overall stability of the ligand-receptor complex. A hydrogen bond is also established with Tyr43, featuring a specific recognition and binding pattern with a bond length of 2.29 Å. Azadirachtin also participates in Pi-sigma interactions with Tyr43, characterized by an attractive force and a bond length of 2.51 Å. Alkyl interactions with Val26, Ile66, and Val69, with bond lengths of 4.88 Å, 4.62 Å, and 4.28 Å, respectively, contribute to hydrophobic interactions in the ligand-receptor interface. Azulene forms Van der Waals interactions with Tyr45 and Glu65, along with Pi-stacked interactions with Tyr43 (3.67 Å) and Alkyl interactions with Val69 (5.48 Å) and Ile91 (4.65 Å), collectively enhancing the stability and specificity of its binding to target molecules. Epoxyazadiradione engages in Van der Waals interactions with various amino acids, including Asp14, Lys15, Phe22, Thr23, Tyr43, Leu44, Tyr45, Thr46, Gly59, Val60, and Gly63. Carbon-hydrogen bonding occurs with Met61 and Glu65, featuring bond lengths of 3.45 Å and 3.35 Å, respectively. Furthermore, hydrogen bonds form with His62 at a bond length of 1.96 Å. Gedunin's Van der Waals interactions encompass Asp14, Lys15, Gly18, Phe22, Thr23, Val26, Tyr43, Tyr45, Thr46, Val60, Met61, Gly63, and Glu65, while Pi-sigma interactions involve Leu44 (3.59 Å). A hydrogen bond forms with His62 at a bond length of 1.99 Å. Hexadecanedioic Acid demonstrates Van der Waals interaction with Phe22, Thr23, Tyr45, Val60, and Met61. Alkyl interactions occur with Val26 (4.22 Å), Tyr43 (4.21 Å), and Leu44 (5.38 Å). Hydrogen bonds form with His62 (2.73 Å), Gly63 (1.96 Å), and Glu65 (1.90 Å). Neophytadiene engages in Van der Waals interaction with Gly18, Thr23, Tyr45, Val60, Met61, and Glu65. Alkyl interactions involve Phe22 (4.42 Å), Val26 (3.68 Å), Tyr43 (4.77 Å), Leu44 (5.39 Å), and His62 (4.75 Å). Squalene's Van der Waals interaction includes Asp14, Gly18, Phe22, Thr23, Met61, Gly63, and Glu65. Alkyl interactions engage Lys15 (5.28 Å), Val26 (3.70 Å), Tyr43 (4.79 Å), Leu44 (4.92 Å), Tyr45 (5.32 Å), Val60 (4.78 Å), and His62 (4.72 Å). Mevalonic Acid's Van der Waals interactions involve Arg48, Asp64, Asn67, and Tyr68. Alkyl interactions occur with Pro53 (4.38 Å) and Trp54 (4.67 Å). Hydrogen bonds form with Ser49 (2.62 Å), Asn52 (2.68 Å), and Tyr80 (2.10 Å). Salannin's Van der Waals interactions are found with Tyr45, His62, Gly63, and Val69. Alkyl interactions involve Phe22 (5.42 Å), Val26 (4.01 Å), and Ile66 (4.21 Å). Carbon-hydrogen bonding occurs with Glu65 (3.58 Å), and a hydrogen bond forms with Tyr43 (2.17 Å). Salannol exhibits Van der Waals interactions with Tyr45, Glu63, Ile66, and Phe70. Alkyl interactions engage Phe22 (5.20 Å), Val26 (4.06 Å), His62 (4.25 Å), and Val69 (4.04 Å). Pi-anion interactions with Glu65 (4.77 Å) and a hydrogen bond with Tyr45 (2.09 Å) contribute to the ligand's binding. Lastly, meliantriol exhibits Van der Waals interactions with Gly18, Phe22, Thr23, Leu24, Tyr45, Val60, Met61, and His62. Alkyl interactions engage Lys15 (5.11 Å), Val26 (4.12 Å), Ala30 (4.35 Å), and Met42 (4.42 Å). Hydrogen bonds form with Asp14 (2.79 Å), and Pi-sigma interactions occur with Tyr43 (3.97 Å).

4. Predictions of Biological Activity of Compounds

The PASS webserver validated the predicted biological activity of phytochemicals found in neem extract. Among all the docked compounds, azulene, squalene, mevalonic acid, hexadecanedioic acid, and neophytadiene exhibited both AChE inhibitor activity and insecticidal properties. The Pa values for AChE inhibitor activity ranged from 0.653 to 0.866, while for insecticidal properties, the Pa values varied between 0.152 to 516. When Pa exceeds Pi, the reported Pa values suggest a likelihood of potent biological activity.

Conclusion

In this study, we employed a computational approach to assess the potential of phytochemicals present in neem extract as inhibitors of Acetylcholinesterase (AChE) and to explore their insecticidal properties. Strikingly, all 11 selected phytochemicals demonstrated favorable docking scores, suggesting a promising interaction with the AChE enzyme. Furthermore, the interactions between these phytochemicals and the active site of AChE were thoroughly examined and discussed. Further investigation revealed that among the 11 compounds, namely azulene, squalene, mevalonic acid, hexadecanedioic acid, and neophytadiene, exhibited significant biological activity both as AChE inhibitors and possessing insecticidal properties. The docking scores for these five compounds were notably satisfactory, measuring at -7.1, -6.8, -5.4, -6.4, and -6.2 kcal/mol, respectively. Based on our computational findings, it is imperative to conduct additional experimentation involving *in vitro* and *in vivo* assessments to comprehensively investigate the efficacy of these identified phytochemicals.

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