Journal of Asia-Pacific Entomology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Full length article

Molecular docking studies of natural alkaloids as acetylcholinesterase (AChE1) inhibitors in *Aedes aegypti*

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ARTICLE INFO

Keywords:
Vector Mosquito
Natural polymers
Aedes aegypti
Acetylcholinesterase

ABSTRACT

Mosquitoes are medically important insects worldwide. They serve as a primary vector for transmitting several major diseases like dengue and chikungunya, chiefly spread through *Aedes aegypti,* a prominent mosquito vector. The present study focused on evaluating the inhibitory effect of natural alkaloids on the acetylcholinesterase present in *Ae. aegypti* using molecular docking studies. A total of 25 different alkaloids were selected as ligands and their docking ability with an Acetylcholinesterase 1 (AChE1) recept or found in *Ae. aegypti* was performed by AutoDock. Results indicated that alpha-solanine had the best fit into the AChE1 binding pocket with a minimum binding energy of −8.13 kJ/mol. Among the different alkaloids tested, it is suggested that alpha-solanine would serve as the best inhibitor of AChE1 in *Ae. aegypti*.

Introduction

Global warming will increase the abundance of vector mosquitoes, which will increase the transmission and spread of infectious organisms intropical and subtropical countries, as well as high-altitude regions, thus affecting increasing populations each year (Sutherst, 2004; Ramasamy and Surendran, 2012). Vector-borne diseases will be a severe public health issue worldwide (CIESIN, 2007; Bosseche and Coetzer, 2008; WHO, 2012; Cecilia et al., 2014). The diseases arboviral dengue and chikungunya are primarily transmitted by *Aedes aegypti* mosquitoes (Cauchemez et al., 2004; Roth et al., 2014). Vector species resistance to insecticides is generally due to a detoxification and decreased target protein (Liu, 2015). Furthermore, few studies stated that the mutationsin the voltage sodium channel gene produce a phenotype known as knockdown resistance. These mutations give rise to pyrethroids (PY) resistance, which has been related to fitness costs in many insects including *Ae. aegypti* (David et al., 2018; Leong et al., 2019). Hence, finding new mosquitocides and understanding their mode of action would contribute to our ability to control mosquito species and develop resistance.

Natural phytochemical compounds, such as alkaloids, pregnane glycosides (cynanchoides), stilbenes, triterpenes, ursane, and xanthones, have served as useful botanical sources for identifying new insecticides (Baskar and Ignacimuthu, 2012; Duraipandiyan et al., 2015; Baskar et al., 2018; Pavunraj et al., 2021). Many studies have focused on identifying new compounds that can control insect populations, including alkaloids that show promise for pest control. In general, alkaloids impede all biological processes and interfere with cellular and

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https://doi.org/10.1016/j.aspen.2021.05.011
Received 31 July 2020; Received in revised form 5 May 2021; Accepted 17 May 2021
Available online 25 May 2021

physiological functionsby modifying redox reactions, regulating hormone activity, affecting neural signals, or negatively impacting reproduction (Chowański et al., 2016). Acetylcholinesterase is an essential enzyme in insects (Colovic et al., 2013) that hydrolyses the cholinergic synaptic neurotransmitter acetylcholine (ACh) in the central nervous system [CNS].

Many alkaloids found in plant extractsare toxic to the larvae of insects (Maheswaran et al., 2019),while several other metabolites inhibit AChE activity (Kong et al., 2019).Several researchers have been used the Quantitative structure–activity relationship (QSAR) model to study the inhibitory activities of AChE inhibitors (Araújo et al., 2011; Chaudhaery et al., 2009). They employed efficient feature-selecting tools and corrected feature-selection strategies to determine the structural properties of inhibitors that determined their inhibition potency against AChE. A review of bioactive compounds in the *Solanaceae* family with larvicidal activity, including those that have been subjected to molecular docking analysis to determine the inhibitory ability of the bioactive compounds against AChE at the molecular level, has been reported (Chowański et al., 2016). Sustainable effective integrated management of mosquito vectors requires innovative techniques and strategies. The present study was designed to identify the natural alkaloid that potentially inhibit AChE activity in *Ae. aegypti* using a molecular docking approach. The overall goal is to develop a new tool to minimize the spread of mosquito vector species and/or contain their populations when the risk of transmitting an infectious disease is high.

Materials and method

Selection of ligands

Twenty-five different kinds of alkaloids reported from plant species in the *Solanaceae* such as 2-dodecanone, 2-pentadecanone, 2-tridecanone, 2-undecanone, 4-aminobutanoic acid, alpha-solanine, anabasine, atropine, calystegine B4, capsaicin, chaconine, chlorogenic acid, foliumin, hyoscyamine, leptine I, nicotine, rutin, salpichrolide A, salpichrolide C, salphichrolide G, scopolamine, solamargine, solasonine, tomatidenol, and tomatine were selected for this study. The structural information of these selected compounds was retrieved through PubChem (Chowański et al., 2016). The obtained files format in *.*sdf*wereloaded into ChemDraw 12.0 and converted into *.*mol* file for further evaluation of their chemical structures.

Minimization of energy of ligands using MM2 and MOPAC

The energy of the retrieved ligands was minimized using MM2 (Molecular Mechanics) and MOPAC (Molecular Orbital PACkage) modules in Chem 3D Pro 12.0. The RMSD reference was minimized up to 0.0001, and the resulting properties were computed for the 25 selected ligand molecules. The ligand structure files were saved after energy minimization.

Sequence Retrieval, physicochemical Characterization, and secondary structure prediction of AChE1

The protein sequence of AChE1 from *Ae. aegypti* was obtained from the sequence ID Q8MYC0 in UniProt (http://www.uniprot.org/uniprot/Q8MYC0) and saved in FASTA format. The physicochemical properties including molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) of *Ae. aegypti*'s AChE1 were computed using Protparam software (https://web.expasy.org/protparam/). The SOPMA tool was utilized to predict the secondary structures of AChE1 (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html).

Homology modelling of AChE1

The three-dimensional structure of AChE1 (*Ae. aegypti*) was modeled by homology modelling in the automated workspace of SWISS-MODEL (https://swissmodel.expasy.org/).6ARY _A (PDB ID) was considered as a template structure for modelling AChE1 protein. After submitting the protein sequence, the server searches for suitable templates in the Protein Data Bank based on the best BLAST and HH blits scores.The quality of the identified templates was assessed based on their properties. Templates were then sorted based on their quality ranking, and the protein structure was modeled using ProMod3 (Waterhouse et al., 2018). The obtained 3D structure was subjected to alignment and super imposed on the template structure. SAVES server-based Ramachandran plot was used for PROCHECK analysis to get the stereochemical qualities of the model (Laskowski et al., 1993).

Prediction of the binding pocket in AChE1

The probable binding sites of preferred target receptors in AChE1 were searched using Q-site Finder to predict the ligand-binding site. CASTp analysis (Dundas et al., 2006) was utilized to analyze cavities within the AChE1 hydrolase. The CASTp program was used to calculate the empty concavities *i.e.* pockets on the protein surface based on the cross-section of the depressions found in the protein structure. The area and volume of the selected pockets were then calculated.

Molecular docking of AChE1 with alkaloid molecules

The docking of different ligands with AChE1 receptors was analyzed using AutoDock v4.1.2 (Sanner, 1999). A searching grid, extended above preferred ligands,was used for analyzing docking potential. The addition of polar hydrogen to ligand molecules, assignment of Kollman charges, the addition of atomic solvation, assignment of Gasteiger-type polar hydrogen charges, merging of nonpolar hydrogen with carbon, the setting of internal degrees of freedom, and torsion were all employed in the docking analysis. The docking of all ligands with the Acetylcholinesterase model was analyzed, where AChE was constant and the ligands were variable. The complete AChE receptor was used for blind docking consideration. All of the atom types and affinity maps were present. A grid spacing of 0.375A° grid was used for the electrostatic map. An analysis utilizing populations consisting of 150 individuals at a 0.02 mutation rate for ten generations was conducted based on the Lamarckian genetic algorithm. Different complexes were ranked based on the predicted binding energy of the ligand. Cluster analysis, based on root mean square deviation values, was performed and the most populated cluster with the lowest energy conformation was considered an authenticated solution. AutoDock software was employed for analyzing hydrogen bonding and hydrophobic interactions between docked ligand molecules and their target. The protein structure was first to fit into the grid box using different X, Y, Z coordinates. The Autogrid software predetermines the grid box of the target protein. AutoDock allows flexible rotatable side chains based on their interactions with the ligand molecule.

Results

Secondary structure prediction of AChE1 protein

SOPMA analysis predicted various secondary structures, including alpha-helix, extended strands, beta-turn, and Random coil. However, other secondary structures were absent in the predicted primary structure of the AChE1 protein. Results of the physicochemical characterization and secondary structure prediction of the AChE1 protein are provided (Table 1). The percentages of the different secondary structures are listed (Table 2).

Table 1
Physico-chemical properties of *Aedes aegypti* acetylcholinesterase (AChE1) revealed using Protparamsoftware.

S.No	Parameters	
1.	Number of amino acids	91
2.	Molecular weight (Mw)	10716.71
3.	Number of negatively charged residues	16
4.	Number of positively charged residues	9
5.	Total number of atoms	1442
6.	Theoretical	4.76
7.	pIEstimated Half	7.2
8.	life Instability	hours47.
9.	Index Aliphatic	88 44.95
10.	Index GRAVY	-1.077

Table 2
Percentage of different secondary structures in *Aedes aegypti* acetylcholinesterase (AChE1) predicted using SOPMA software.

S. No	Secondary Structure	Number of Amino Acids	Percentage
1.	Alpha Helix	29	31.87
2.	310helix	0	0
3.	Pi Helix	0	0
4.	Beta Bridge	0	0
5.	Extended strand	18	19.78
6.	Betaturn	7	7.69
7.	Bendregion	0	0
8.	Random Coil	37	40.66
9.	Ambiguousstates	0	0
10.	Otherstates	0	0

Homology modelling of AChE1

Amino acid content occupying the position in *Ae. aegypti* acetylcholinesterase (AChE1) was listed (Table 3). Ninety-one amino acids were submitted in the SWISS-MODEL package. After the modelling process, the results showed the modeled structure of AChE1 (Fig. 1). The modeled residues ranged from 1 to 91. The best template selected by the SWISS-MODEL server was 6ARY_A with a resolution of 2.26 Å and asequence identity of 95.60%. The QMEAN Z-score was −1.02. C-beta interaction energy score was 0.73, and the pair-wise energy of all atoms was 0.67. The solvation energy and torsion angle energy scores were −1.39 and −0.79, respectively. The E-value of the BLAST target-template alignment was 0.0001 using 25 as a minimum template size and 60 as minimum sequence identity. The E-value indicated by the HH Search Target-template alignment was 0.0001. Minimum template size and minimum sequence identity were 50 and 0.3, respectively. No ligands were included in the model.

Table 3
Amino acid content occupying PocketID#1 in *Aedes aegypti*acetyl cholinesterase (AChE1).

S.No.	Amino acids in the binding pocket	Position
1.	Asp	14,19, 64
2.	Lys	15, 50
3.	Val	17, 60
4.	Gly	18, 51, 59, 63, 79
5.	Phe	22, 87
6.	Thr	23, 46, 58
7.	Val	26, 69
8.	Asn	27, 52, 67
9.	Ala	30
10.	Met	42, 61
11.	Tyr	43, 45, 68, 80
12.	Leu	44, 78
13.	His	47, 62
14.	Arg	48
15.	Ser	49
16.	Pro	53
19.	Glu	65, 84
20.	Ile	91

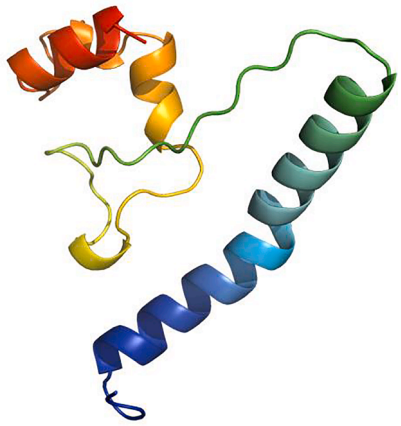

Fig. 1. Predicted three-dimensional structure of *Aedes aegypti* acetylcholinesterase (AChE1).

The modeled AChE1 protein was validated using the SAVES web server. A Ramachandran Plot validated the quality of the modeled protein generated using Procheck software, which revealed that 94.8% of the residues occupy a favored region and 5.2% were in the allowed region (Fig. 2a). No residueswere found to occupy the outlier region. The overall quality of the protein model predicted using the ERRAT (Colovos and Yeates, 1993) software program was 100 (Fig. 2b). Seven binding pockets were identified in the AChE1 model, of which only one pocket, illustrated as greensphere (Fig. 3), was chosen for further study (Table 4). The area and volume of the selected binding pocket were 393.227 and 367.295 respectively.

The docked conformation was opened using the **.dlg* file (docking log file). The derived binding energy (the sum of Intermolecular energy), internal energy, and torsional energy are presented (Table 5). Alpha-solanine, tomatidenol, and salphichrolide A were the alkaloid molecules determined to have the lowest binding energy required for docking with AChE1 (Figs. 4, 5 & 6). Alpha-solanine showed hydrogen bond interaction with the acidic polar amino acid Glu72 and a polar amino acid Asn67 and showed the hydrophobic interaction with the nonpolar amino acids Leu74 and Leu78. Tomatidenol showed hydrogen bond interaction with the acidic polar amino acids Glu65 and Asp14. Additionally, it showed the hydrophobic interaction with the polar amino acids Tyr43, Tyr45, His62, and a nonpolar amino acid Val60. Salphichrolide A showed hydrogen bond interaction with the polar and nonpolar amino acids Tyr45 and Val26. Also, it showed the hydrophobic interaction with nonpolar, polar and acidic polar amino acids Phe29, Ala30, Tyr45, and Glu65, respectively.The docking interaction figures were depicted using Pymol and PLIP server (Salentin et al., 2015)

Discussion

Several insecticides including organophosphates, carbamates, and also insect repellent, N,N-Diethyl-*meta*-toluamide (DEET) have been known to target AChE, which eventually leads to the accumulation of acetylcholine at the synapses and causes the death of mosquitoes (Singh and Singh, 2000; Aygun et al., 2002). The enzyme AChE has been inhibited using the Limonoids like azadirachtin from neem *in vitro* in *Nilaparvata lugens* (Nathan et al., 2008). Further studies on Pyrimidine Trione Furan (PTF) substituted chemical compounds have been reported to show inhibitory activity on AChE1 in *Culex pipiens* and *Anopheles gambiae* mosquitoes insensitive to organophosphate and carbamates (Alout et al., 2012). The compound 1-nitro-2-phenylethane isolated from *Aniba canelilla* plant oil has been inhibitory action on AChE of electric eel (Silva et al., 2014). In this present study, 25 different

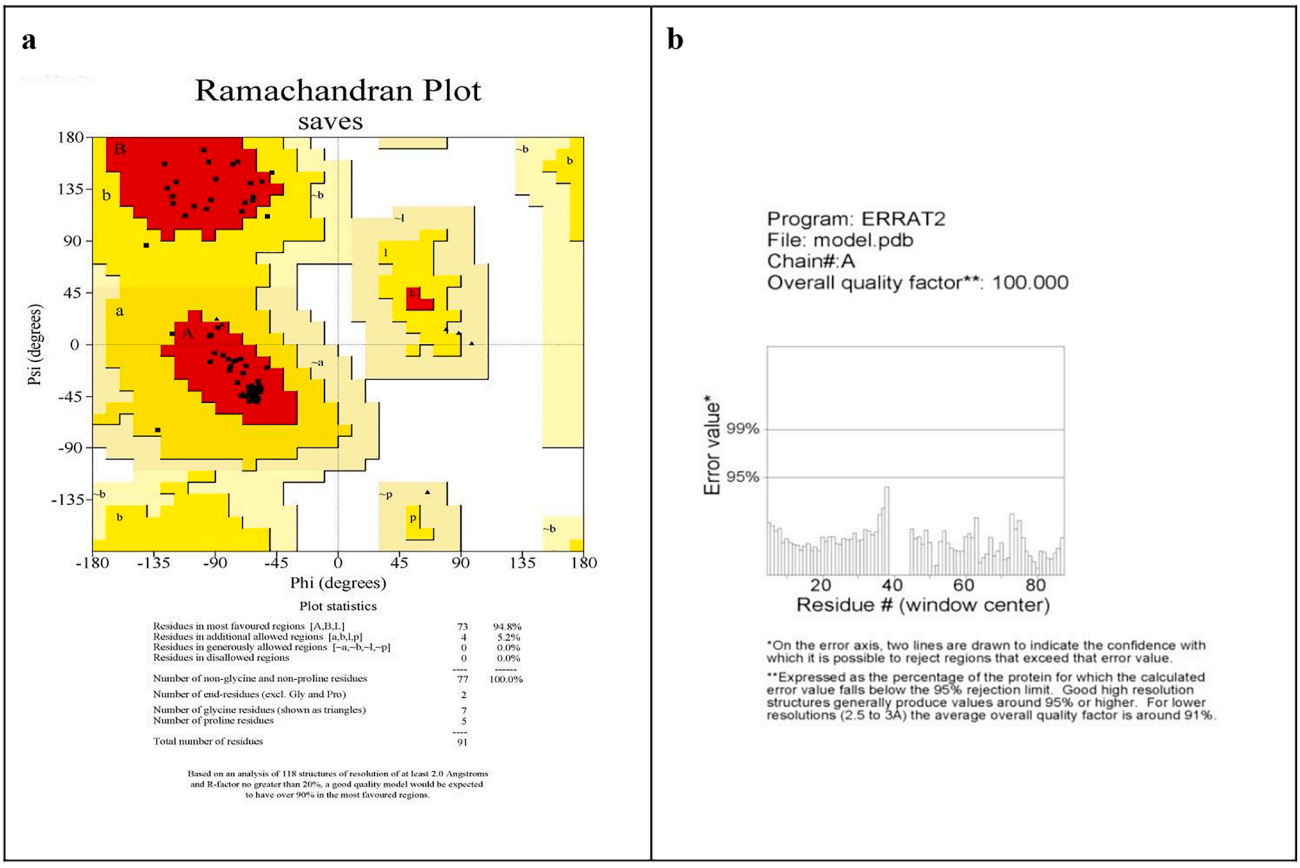

Fig. 2. a) Ramachandran plot, b) ERRAT structural quality of the predicted model of *Aedes aegypti* acetylcholinesterase (AChE1).

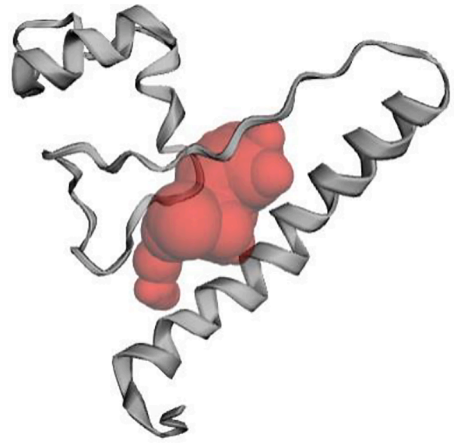

Fig. 3. The binding pocket present in the predicted model of *Aedes* aegypti acetylcholinesterase (AChE1).

Table4
Binding Pocket Information for *Aedes aegypti* acetylcholinesterase (AChE1) from CASTp software.

ID	AREA	VOLUME
1	393.227	367.295
2	72.533	101.920
3	58.450	27.479
4	4.004	0.276
5	0.449	0.049
6	0.985	0.034
7	0.156	0.004

alkaloids were evaluated *in silico* using molecular docking for their ability to interact with AChE1 protein from *Ae. aegypti*. This mosquito carries yellow fever and dengue. Alkaloids are plant-derived pharmacologically and insecticidal active heterocyclic nitrogen compounds. Over 1200 alkaloid compounds have been described, among which strychnine, cocaine, caffeine, nicotine, α-solanine, and α-tomatineare well known (Gupta et al., 2015). Alkaloids derived from closely related plant species possess similar chemical structures.The Solanaceae family includes plant species rich in tropane, pyrrolizidine glycoalkaloids, and indole alkaloids (Chowański et al., 2016).

Secondary structure of AChE1

The physico-chemical properties of the protein model were predicted using the ExPASyProtParam software program. The AChE1 protein from *Ae. aegypti* comprises 91 amino acids, and the predicted molecular weight is 10716.71 Da. The protein was predicted to have a negative charge based on a the oretical pI value of 4.76.

The aliphatic index of 44.95 indicated by the AChE1 protein model describes the relative volume occupied by aliphatic amino acids such as lysine, valine, isoleucine, and leucine.The low aliphatic index of the protein model indicates that the protein does not have a wide range of temperature stability (Gupta et al. 2015). The secondary structural information derived from the AChE1 protein model improves the target-template alignment and modelling of its 3D structure. AChE1 is mainly composed of random coils (40.66%) and alpha helices (31.87%) with a higher score than extended strands. The protein model did not reveal any beta-sheets or turns in the secondary structure of AChE1.

The QMEAN4 score of the AChE1 protein model was 0.45. Values between 0 and 1 indicate a good model and so the obtained AChE1 protein model was used for the docking analyses. The QMEAN Z-score is a measure of the absolute quality of a protein model. The Z-score was

Table 5
Binding energy and inhibition constant obtained for 25 Alkaloids docked with the predicted AChE1 protein model.

S.No.	Molecule	BindingEnergy	kI	Inter molecular Energy	Internal Energy	Torsional Energy	Ref RMS
1.	2-dodecanone	−4.96	229.72 μM	−6.92	−0.68	2.47	78.15
2.	2-pentadecanone	0.08	–	−5.15	1.71	3.29	75.71
3.	2-tridecanone	−3.63	2.18 mM	−5.91	−0.65	2.74	72.32
4.	2-undecanone	−3.23	4.28 mM	−4.77	−0.8	2.2	75.3
5.	4-aminobutanoicacid	−3.71	1.9 mM	−3.43	−1.94	1.37	82.24
6.	Alpha-Solanine	**−8.13**	1.11 μM	−7.1	−6.83	4.66	85.35
7.	Anabasine	−5.32	126.93 μM	−5.73	−0.01	0.27	75.88
8.	Atropine	−6.0	40.26 μM	−6.95	−1.06	1.65	73.41
9.	CalystegineB4	−4.54	471.44 μM	−5.59	−0.91	1.1	75.83
10.	Capsaicin	−5.29	133.28 μM	−7.03	−1.39	2.74	73.27
11.	Chaconine	−5.95	43.37 μM	−5.98	−5.27	4.12	78.2
12.	Chlorogenicacid	−4.18	856.07 μM	−5.31	−2.54	3.02	87.31
13.	Foliumin	−6.2	28.73 μM	−6.69	−3.99	3.57	73.23
14.	Hyoscyamine	−5.38	113.03 μM	−6.65	−0.68	1.65	71.82
15.	LeptineI	−5.87	50.02 μM	−6.65	−5.32	4.66	67.34
16.	Nicotine	−4.9	257.31 μM	−5.18	0.06	0.27	74.97
17.	Rutin	−4.4	593.71 μM	−5.44	−4.82	4.39	80.91
18.	SalphichrolideA	−7.4	3.78 μM	−7.96	−0.64	0.82	76.54
19.	SalphichrolideC	−5.99	41.0 μM	−7.58	−0.95	2.2	73.36
20.	SalphichrolideG	−6.64	13.52 μM	−7.27	−0.82	1.1	75.29
21.	Scopolamine	−5.72	64.6 μM	−7.22	−0.41	1.65	74.87
22.	Solamargine	−6.73	11.66 μM	−6.79	−5.26	4.12	68.46
23.	Solasonine	−5.59	79.97 μM	−5.22	−6.49	4.66	87.74
24.	Tomatidenol	**−8.13**	1.1 μM	−8.22	−0.16	0.27	73.75
25.	Tomatine	−4.72	343.98 μM	−6.08	−7.19	6.31	87.21

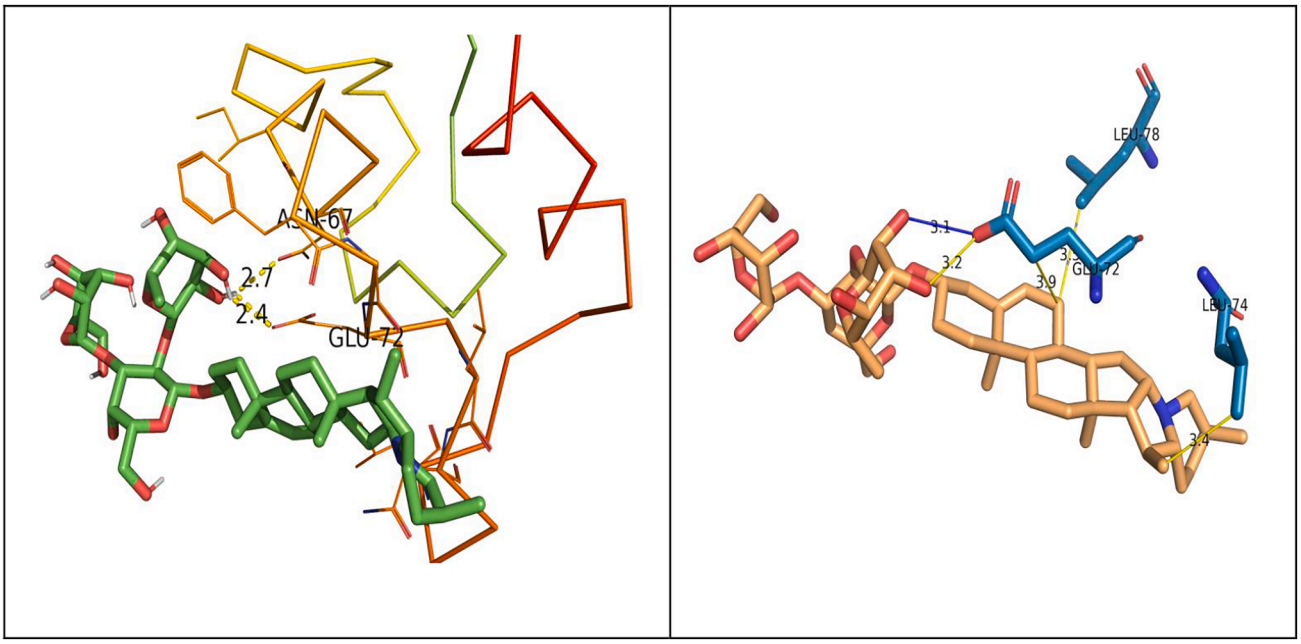

Fig. 4. Docked conformation and possible interactive forces (Hydrogen bond (yellow line) and hydrophobic interaction (blue line)) between the predicted model of *Aedes aegypti* acetylcholinesterase (AChE1) and the ligand, alpha-solanine.. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

calculated by comparing the 'degree of nativeness' observed in the predicted AChE model with template structures. A more negative QMEAN4 Z-score indicates the construction of a low-quality model relative to the template structure (Benkert et al., 2011). In the present study, the QMEAN4 Z-score calculated for the AChE1 protein model was −1.02, indicating that the quality of the predicted model is significantly good compared to the template structure 6ARY_A. The relative mean score deviation (RMSD) value of 0.08 Å was calculated using TM-align software (Zhang and Skolnick, 2005) after super imposition of both template (6ARY_A) and the predicted structure of the test protein (AChE1).

Homology modeling of AChE1

The predicted structural model of AChE1 was validated using PROCHECK, RAMACHANDRAN PLOT, and ERRAT, all available on the SAVES server. The analysis of the modeled AChE1 protein indicated that 97.7% of the amino acid residues occupy a favoured region while 2.3% are located in the allowed region. No residues were located in the outlier region. The overall quality of the protein model predicted by the ERRAT program was 100. The good quality score of the predicted AChE1 protein model is based on the overall quality score for non-bonded atomic interactions. Binding pockets in the protein structure were predicted by

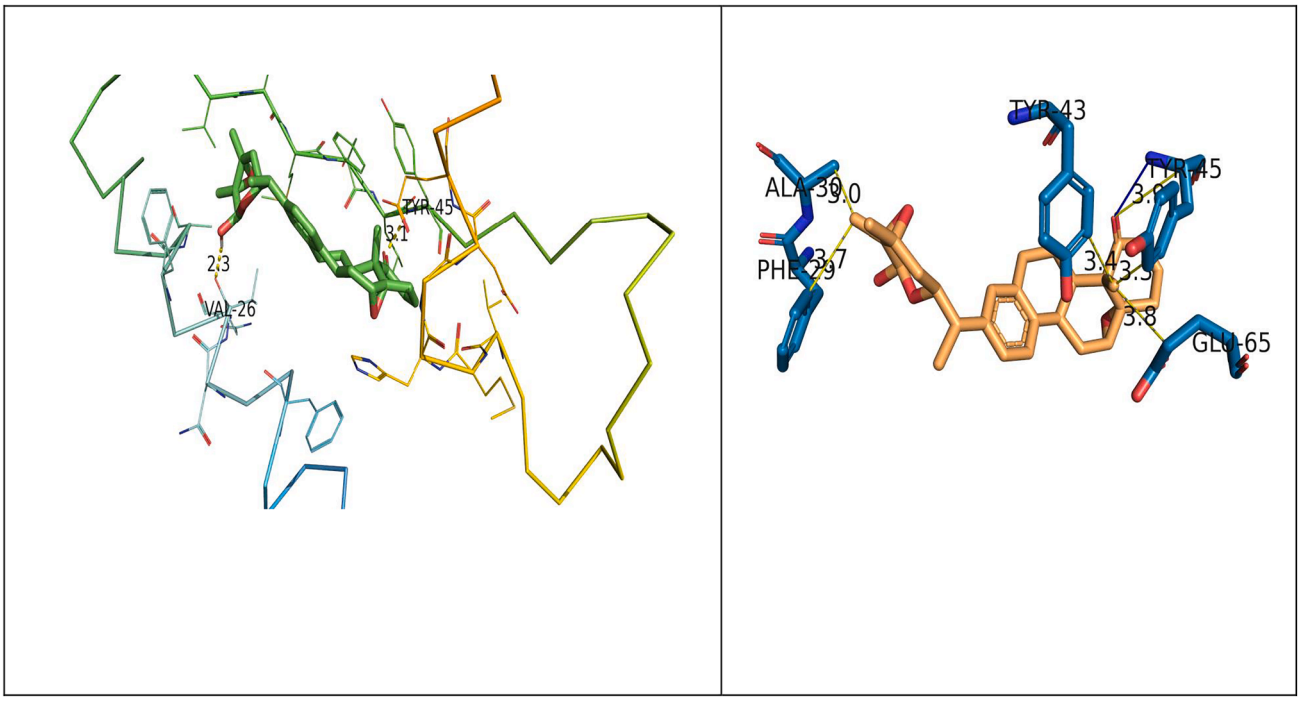

Fig. 5. Docked conformation and possible interactive forces (Hydrogen bond (yellow line) and hydrophobic interaction (blue line)) between the predicted model of *Aedes aegypti* acetylcholinesterase (AChE1) and the ligand, salphichrolide A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

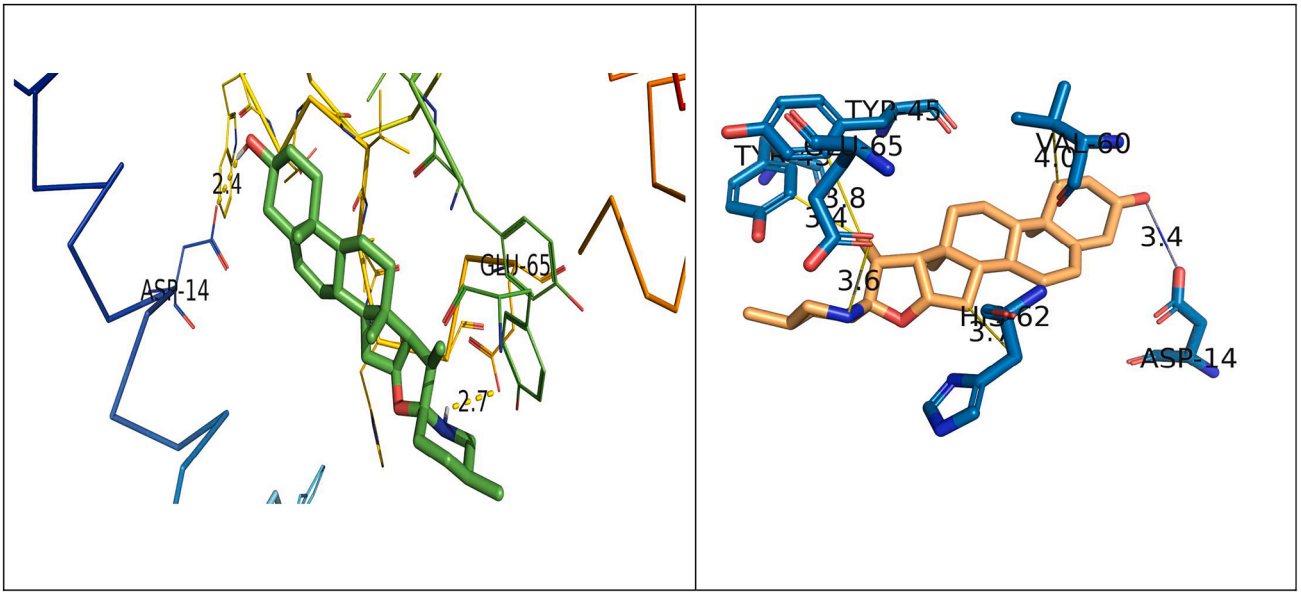

Fig. 6. Docked conformation and possible interactive forces (Hydrogen bond (yellow line) and hydrophobic interaction (blue line)) between the predicted model of *Aedes aegypti* acetylcholinesterase (AChE1) and the ligands, tomatidenol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CASTp.

Molecular docking

Most of the selected alkaloids exhibited anaffinity to the receptor, AChE1, with a minimum energy requirement. Among the 25 different ligand molecules tested for AChE1 inhibition, only three molecules exhibited the potential for a high inhibition rate based on the minimum energy required by the ligand to fit into the pocket in AChE1. The ligand molecules, alpha-solanine, salpichrolide A, and tomatidenol, were found to firmly interact with the binding pocket in AChE1 requiring minimum energy of −8.13 kJ/mol, −8.13 kJ/mol, and −7.4 kJ/mol, respectively. *In vivo* assays have previously demonstrated that administration of the Solanaceous glycoalkaloids, alpha-solanine, and alpha-chaconine, in rabbits inhibited acetylcholinesterase activity (McGehee et al., 2000).

Acetylcholinesterase is an enzyme that hydrolyses the cholinergic synaptic neurotransmitter acetylcholine (ACh). If the drug molecule inhibits the acetylcholinesterase activity, the acetylcholine

concentration will be increased in the synaptic cleft and leads to death (Colović et al., 2013). Three alkaloids Alpha-solanine, salphichrolide A and tomatidenol, showed the interaction with the amino acids Asn (Asparagine) and Tyr (Tyrosine), which are mainly present in the catalytic binding sites of AChE1. In these two amino acids, Asn is essential to the function and development of the brain and Tyr is used to enhance the plasma neurotransmitter levels and helps to initiate the neurotransmitters (Deijen and Orlebeke, 1994; Lieberman et al., 1985). The studied molecules alpha-solanine, salpichrolide A and tomatidenol showed the least binding energy with Asn and Tyr and expressed the strong binding of the drug molecule. Reegan et al. (2016)supported our findings with related AChE1 inhibition on *Ae. aegypti*. Hence, we assume that these molecules might inhibit the acetylcholinesterase enzyme.

Conclusion

In the present investigation, 25 natural alkaloids present in plant species belongs to the Solanaceae were subjected to molecular docking studies with the predicted protein model of AChE, representing the acetylcholinesterase enzyme in *Ae. aegypti* mosquitoes. Among the 25 docked ligand conformations, alpha-solanine was found to fit into the AChE1 binding pocket with a minimum energy of −8.13 kJ/mol. The information gained from this study may assist in the discovery of potential AChE inhibitors against natural alkaloids is alpha-solanine. Therefore, it was considered to be a potential candidate for inhibiting AChE enzyme activity in mosquitoes. Furthermore, the effect of alpha-solanine on AChE1in *in-vitro* conditionsis necessary to understand the physiology of the mosquito population and control measures.

Declaration of competing statement

We declare that we have no conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge MHRD, Government of India, New Delhi for providing financial support for establishing National Centre of Excellence in Statistical and Mathematical Modeling for Bioresources Management in Thiagarajar College, Madurai (F.No. 5-6/2013 – TS-VII). The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research group NO (RGP-271).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aspen.2021.05.011.

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