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MOSQUITO LARVICIDAL ACTIVITY OF COUMARIN ($C_{29}H_{42}O_8$) ISOLATED FROM *Eucalyptus deglupta* LEAVES AND ITS MOLECULAR DOCKING STUDIES AGAINST *Aedesaegypti* L. AND *Culex quinquefasciatus* SAY. (DIPTERA: CULICIDAE)

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AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

Several species of mosquitoes serve as the crucial vector of transmitting disease causing microbes to humans. In particular, *Aedesaegypti* transmits dengue fever, chikungunya fever, zika fever viruses and *Culex quinquefasciatus* transmits filariasisnematode. Mosquito control with plant isolated compounds would be more suitable to avoid the ill effects of chemical insecticides. In the present study, ethanol extract of *Eucalyptus deglupta* leaves weresubjected to column chromatography and isolated a compound and tested on *Cx. quinquefasciatus* and *Ae. aegypti* mosquito larvae and molecular docking analysis were performed to find the site of action.Based on the ^{1}H NMR and ^{13}C NMR spectrum, the isolated compound containing coumarin unit connected with tetrahydrofuran and fatty acid attached through ester linkages was confirmed. The presence of ester carbonyl, aromatic, polyol and aliphatic protons and carbons were also identified from ^{1}H and ^{13}C NMR spectrum. The active compound was identified as (3,4-dihydroxy-5-{[(3,6,8-trimethyl-2-oxo-2H-chromen-7-yl) oxy]methyl}oxolan-2-yl)methyl 3,7-dimethylnonanoate (Mol.weight: 518.6 g/mol; Mol. Form.: $C_{29}H_{42}O_8$). The isolated compound was found to be the most effective, which showed LC_{50} and LC_{90} values of 3.99ppm, 7.76ppm against *Cx. quinquefasciatus* and 4.99 ppm, 10.78 ppm against *Ae. aegypti* larvae, respectively. The docking studies recorded significant binding to the two-mosquito docking model. The results of the present study show that the compound can be used for controlling the larvae of *Cx. quinquefasciatus* and *Ae. Aegypti* mosquitoes.

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Keywords: *Culex quinquefasciatus; Aedesagypti; Eucalyptus deglupta*.

1. INTRODUCTION

Harmful insects are causing several diseases to humankind. Mainly, mosquitoes are medically important insects responsible for transmitting a wide range of pathogens to humans [1]. Various methods control these vector mosquitoes. Many insecticides are been used in mosquito control programmes and these insecticides belong to the class of organochlorines, organophosphates, synthetic pyrethroids, and carbamates [2]. However, these insecticides are effective against vector mosquitoes, they pose many health issues [3]. Also, these insecticides affect other organisms in the environment and disturb the ecosystem [4]. Further, mosquito vectors started developing resistance to these chemical insecticides [5]. Due to these, the mosquito population is increasing and transmitting the pathogens to a large number of human being [6].

Keeping these disadvantages of chemical insecticides, researchers search for a potent and safe larvicidal agent [7,8]. Many researchers, started screening plant extracts to identify useful compounds for the control of vector mosquitoes [9] and plant isolated compounds will not have any side effects on human health and safe to the environment [10,11]. In the present study, a coumarin compound from the ethanol extract of *Eucalyptus deglupta* leaves was isolated and the structure of the compound was identified using spectroscopic analysis. Further, the mosquito larvicidal efficacy was assessed against the fourth stage larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

2. MATERIALS AND METHODS

2.1 Isolation of Compound

The compound obtained from the active ethanol extract of *Eucalyptus deglupta* leaves by elution with ethanol: methanol (90:10) solvent mixtures using column chromatography. The compound was subjected to ^{13}CNMR, ^{1}HNMR, FT-IR, and UV spectroscopic technique. The obtained spectral data were analyzed and the compound structure was elucidated.

2.2 ^{1}HNMR and ^{13}C NMR Spectra

^{1}H NMR and ^{13}C NMR of the isolated compound is recorded using DMSO-d6 as a solvent and TMS as an internal standard. ^{1}H NMR spectrum was recorded at 500 MHz, and ^{13}C NMR spectrum was recorded at 100 MHz using the Bruker Advance III spectrometer at room temperature. The chemical structure of the compound is deduced from respective proton and carbon chemical shifts correlated with the expected group of a chemical compound.

2.3 Test Mosquitoes

The third in star stage larvae of *Ae. aegypti* and *Cx. quinquefasciatus* mosquito subjected in the present study were collected from Entomology Research Institute laboratory (ERI); mosquitoes did not expose to any pathogens or microorganisms, any insecticides, or repellent chemicals. The mosquito colony rearing conditions at the laboratory were: 28 ± 1^{o}C; 70 - 75% Relative Humidity and 11 ± 0.5 hours photoperiod.

2.4 Larvicidal Activity

Larvicidal activity of the isolated compound was studied following the procedure described by the World Health Organization [12] with small changes. The larvicidal activity was carried out with the compound by four different concentrations i.e. 0.5 cppm, 1.0 ppm, 1.5 ppm and 2.0 ppm. These concentrations were prepared with the DMSO and each concentration was maintained with five replications for treatment and control. In each replication, 99 mL of water with twenty larvae of *Ae. aegypti* and 1 mL of DMSO in which extract was dissolved (Total 100 mLl). Five control with DMSO without compoundwas maintained along with the experiment. Similar experiments were carried out with *Cx. Quinquefasciatus* mosquito larvae. Azadirachtin and temephos (positive control) were also tested at the sameconcentration. Five replications of control (without any extract) were also maintained. The total dead larvae were documented after 24 hours of experimental time. The percentage of mortality was determined for each concentration of the compoundusing the following formula.

Percentage of Mortality = No. of Dead larvae/ No. of Larvae introduced x 100

Abbott's formula [13] was used to get corrected percentage mortality when control mortality was below 5%:

Corrected percentage of mortality: (1 – *n*) in Treatment/ *n* in Control x 100

2.5 Molecular-Docking Study

2.5.1 Ligand molecules preparation

The ligand molecule structure of the isolated compound and the chemical insecticides, temephos,

were taken from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/) and check their bond orders using Chem Draw Ultra, 12.0. The energy minimization along with the geometrical optimization of the ligand was executed by the server PRO-DRG [14].

2.5.2 Homology modelling and prediction of active sites

Acetyl cholinesterase (AChE1) from *Ae.aegypti*and *Cx. quinquefasciatus* were modelled by homology modelling software Modeller v9.19 [14] and swiss model. The *Ae. aegypti* (Q8MYC0) and *Cx. quinquefasciatus* (Q867X2) protein sequence was retrieved from UniProtKB. The BLASTP was used to analyze the suitable template of AChE1 for both species and homology modeling was done. The SAVES server was used to examine the stereo-chemical assessment of the models by the Ramachandran plot (PROCHECK) analysis [15]. The plausible active binding sites of target proteins were identified by CASTp analysis [16].

2.6 Docking Analysis

The molecular docking simulation was carried out by Auto dock tools [17]. The isolated compound and the standard drug temephos were docked to the modeled target protein AChE1 from *Ae. aegypti* and *Cx. quinquefasciatus* with the molecules treated like a stiff structure and the presence of the flexible ligands. All docking experiments consisted of 10 docking runs with 150 individuals and 500,000 energy evaluations using a Lamarckian Genetic Algorithm. The parameter search was extended in grid points per dimension and a step size of 0.375 Å centred on the binding site of the target protein. The Auto Dock results indicated the mandatory binding spots and docking conformation of the active sites, along with the estimation of its interaction. The docked structure which had the least binding energy, was selected to analyze the mode of binding. The ligand-protein interactions were analyzed by PyMol molecular viewer (The PyMOL Molecular Graphics System, Version 2.1.0 Schrödinger, LLC).

2.7 Statistical Analysis

Dose-response curves were prepared for each derivative with larval and pupal mortality data. Further, larvicidal and pupicidal mortality data were subjected to probit analysis (US EPA probit; version 1.5) to find LC_{50} and LC_{90} values, and the differences were considered significant at $p \leq 0.05$.

3. RESULTS

3.1 Identification of Compound

Fig. 1 is ^{1}H NMR spectrum of the isolated compound. The chemical shift values and their respective results are summarised in Table 1. The ^{1}H NMR spectrum showed peaks in the aromatic region, polyolether and aliphatic regions. The spectrum showed two peaks in the aromatic region at 7.62-7.68 (J=30 Hz) and 7.47-7.50 (J = 15 Hz) revealed two types of aromatic protons. The protons observed in the region from 3.80 ppm – 5.20 ppm indicates the presence of polyol groups. i.e. cyclic ether type of compound. The peaks in the range of 2.00 to 2.30 ppm indicate the presence of acetyl protons connected to the carbonyl group. The protons in the 0.70 to 2.0 region show the compound containing aliphatic side chains. i.e. compound containing the fatty acid group.

Figs. 2 to 4 indicate various regions of the ^{13}C NMR spectrum of the isolated compound to reduce the complexity of the spectrum. As observed in the^{1}H NMR spectrum, the ^{13}C NMR spectrum also showed peaks in carbonyl, aromatic, polyol, and aliphatic region. Fig. 2 showed the carbonyl, aromatic and alkenyl carbons region in the ^{13}C NMR spectrum. The peaks 173.7 and 167.4 ppm indicate that the compound containing two carbonyl groups, and both are ester carbonyl group. The aromatic showed eight aromatic carbons at 145.8, 109.2, 138.5, 117.8, 132.1, 160.5, 126.9 and 119.8 ppm. Fig. 3 shows the expanded polyol region from 40.0 to 85.0 ppm, in which the peaks indicate the presence of hydroxy and ether compounds. In this region, there are six carbons present viz., 61.7, 60.0, 77.0, 70.3, 55.2 and 52.8 ppm. The presence of six carbons indicates that six carbons connected to oxygen. It shows that the compound containing polyol groups. Fig. 4 shows the aliphatic region of the ^{13}C NMR spectrum. The alkyl or aliphatic carbons of the fatty acid chain and other alkyl groups in the compound observed in the 10.0-40.0 ppm region in the ^{13}C NMR spectrum.

3.2 Structure of the Compound

The active compound was identified as (3,4-dihydroxy-5-{[(3,6,8-trimethyl-2-oxo-2H-chromen-7-yl) oxy]methyl}oxolan-2-yl)methyl 3,7-dimethylnonanoate (Mol.weight: 518.6 g/mol; Mol. Form.: $C_{29}H_{42}O_8$) (Fig. 5).

3.3 Larvicidal Activity of the Isolated Coumarin ($C_{29}H_{42}O_8$)

The LC_{50} and LC_{90} values (lethal concentrations) of the isolated compound against the fourth stage larvae

of *Cx. quinquefasciatus* and *Ae. aegypti* are given in Table 2. Exposure of fourth stage larva of *Cx. quinquefasciatus* and *Ae. aegypti* to compound caused 100% mortality. Hence, the isolated compound was found to be the most effective, which showed LC_{50} and LC_{90} values of 3.99 ppm, 7.76 ppm against *Cx. quinquefasciatus* and 4.99 ppm, 10.78ppm against *Ae. aegypti* larvae, respectively (Table 2). Further, the larvicidal activity results were comparable to the positive control azadirachtin and temephos.

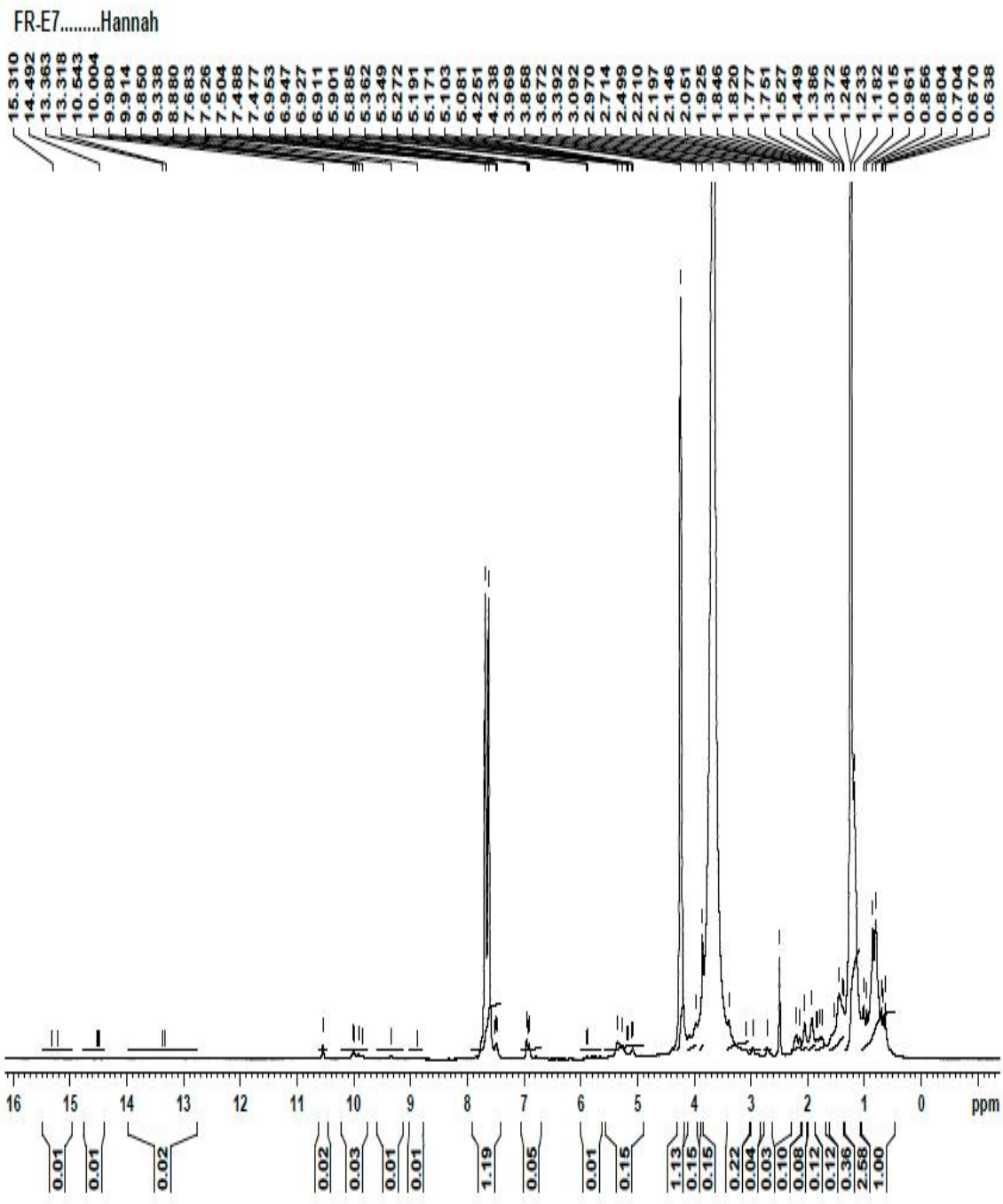


Fig. 1. ^{1}H NMR spectrum of the isolated compound

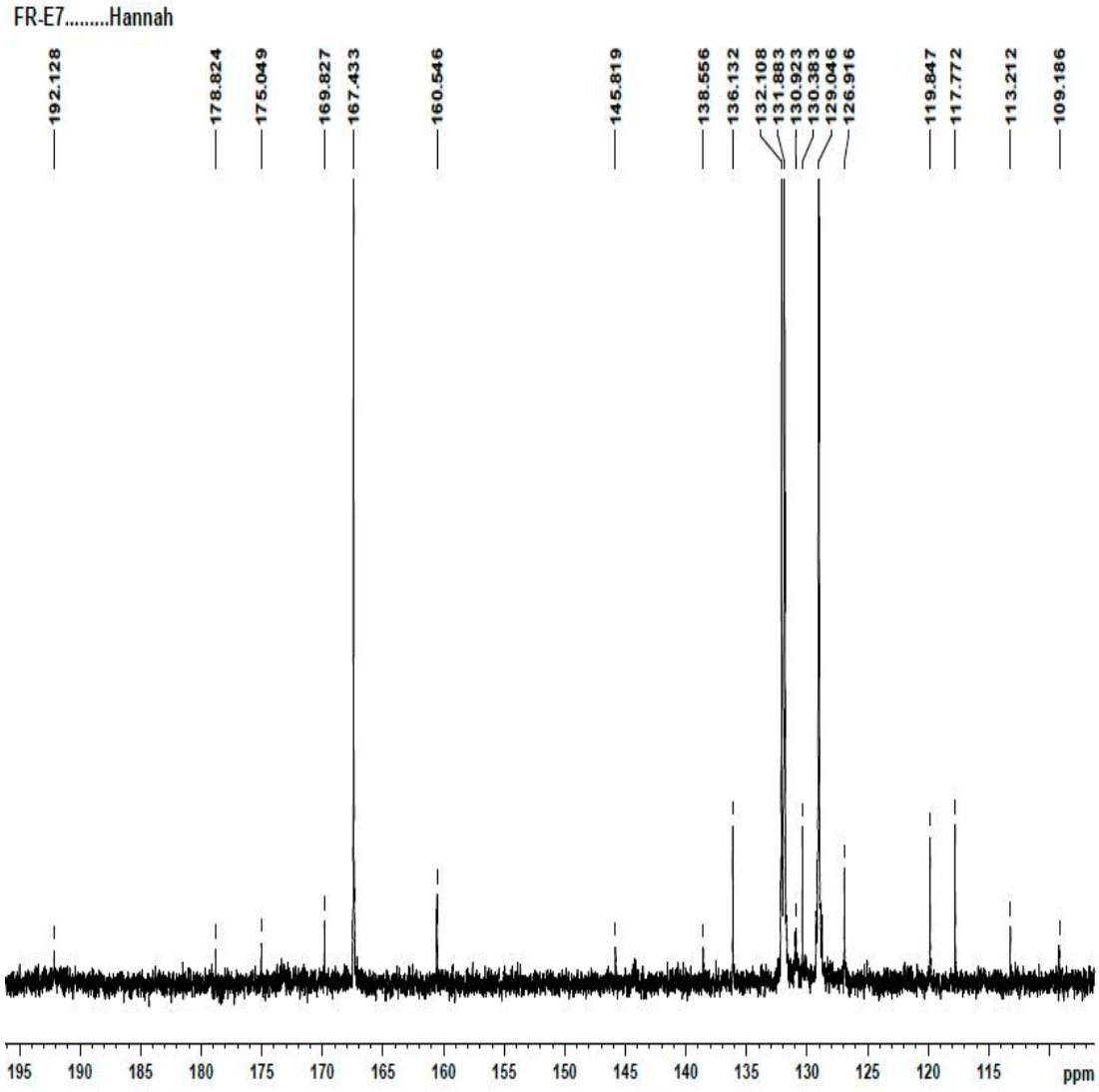


Fig. 2. ^{13}C NMR spectra of isolated compound (aromatic region only showed)

Table 1. Chemical shifts and interpretation of ^{1}H and ^{13}C NMR spectrum of the isolated compound

Position of protons (number of protons)	δ_H (ppm, 500 MHz)	Position of carbons	δ_c (ppm, 100 MHz)
H1 (1)	7.62-7.68 (J=30 Hz)	C1	145.8
-	-	C2	109.2
-	-	C3	167.4
-	-	C4	138.5
-	-	C5	117.8
-	-	C6	132.1
-	-	C7	160.5
-	-	C8	126.9
H9 (1)	7.47-7.50 (J = 15 Hz)	C9	119.8
H10 (3)	0.96	C10	22.5
H11 (3)	0.80	C11	40.8
H12 (3)	0.85	C12	35.5

Position of protons (number of protons)	δ_H (ppm, 500 MHz)	Position of carbons	δ_c (ppm, 100 MHz)
H13 (2)	4.23-4.25	C13	61.7
H14 (1)	5.08-5.10	C14	60.0
H16 (1)	3.85-3.96	C16	77.0
H17 (1)	3.85-3.96	C17	70.3
H18 (1)	5.08-5.10	C18	55.2
H19 (2)	4.23-4.25	C19	52.8
-	-	C20	173.7
H21 (2)	2.48-2.49	C21	41.9
H22	2.14-2.19	C22	36.8
H23	1.90-1.92	C23	31.7
H24-H26 (multiple)	1.23-1.24 (br)	C24-C26	29.4, 29.3, 28.9, 28.5
H27-H30 (multiple)	0.70-0.96 (br)	C27-C30	18.3, 17.2, 16.3, 15.5

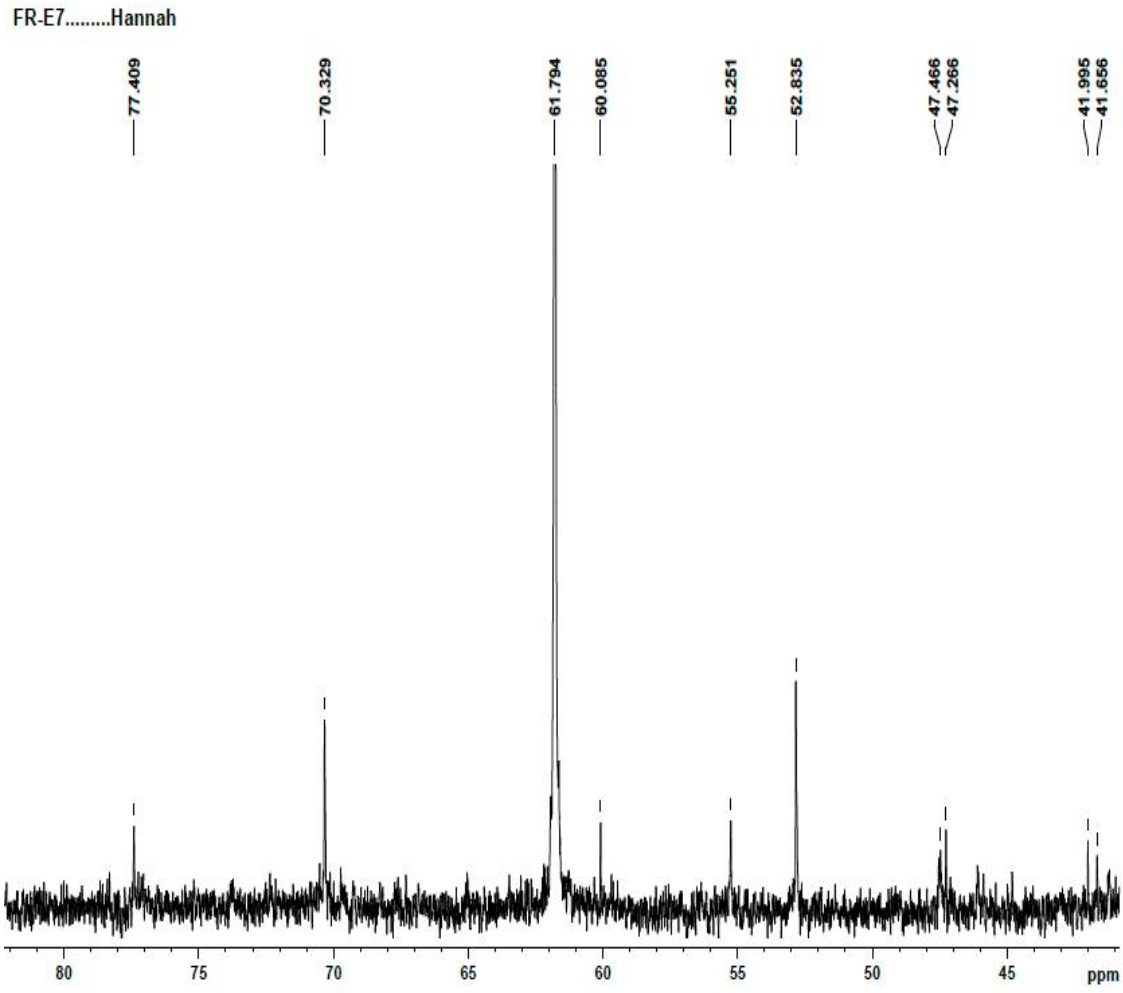


Fig. 3. ^{13}C NMR spectra of isolated compound (tetrahydro furan region only showed)

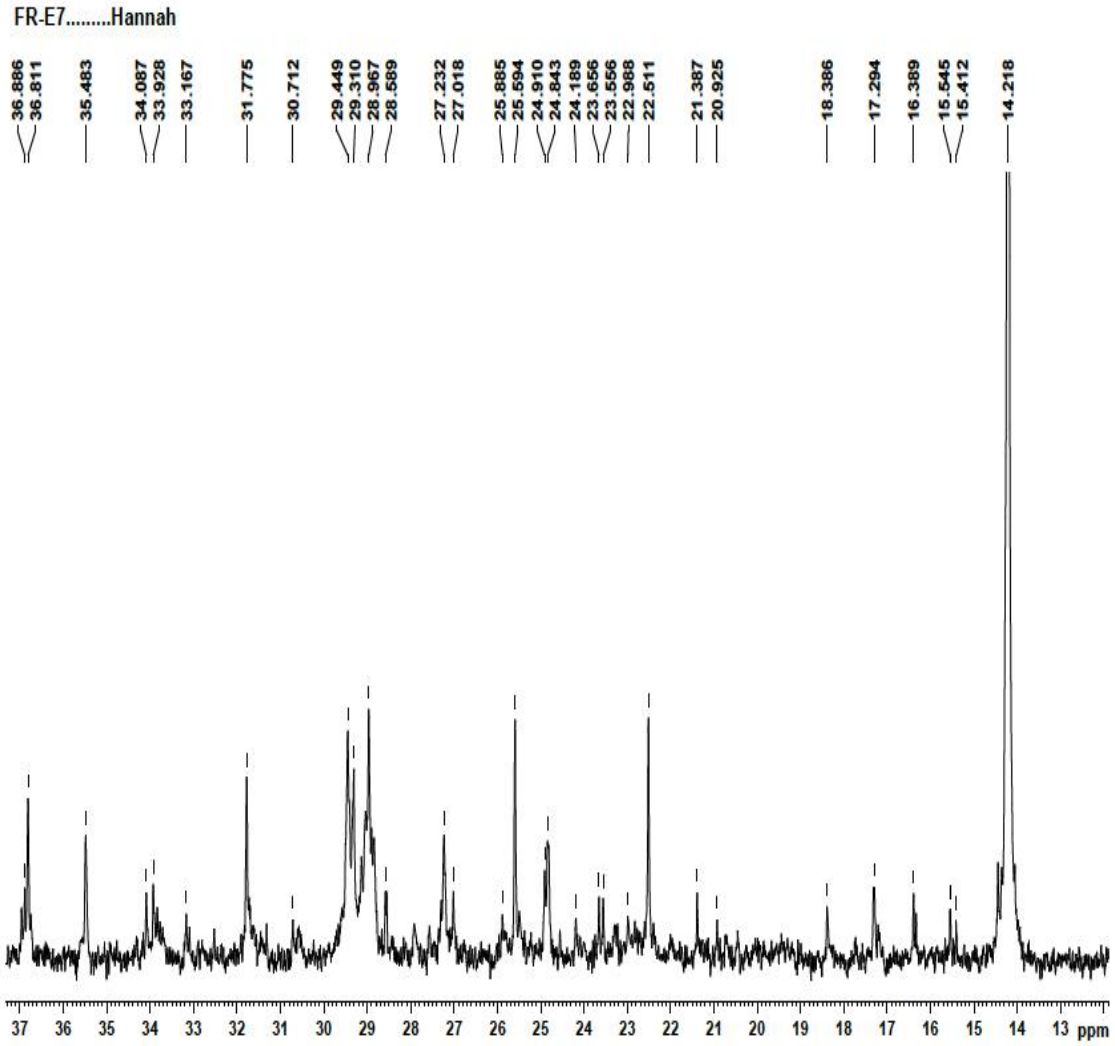


Fig. 4. ^{13}C NMR spectra of isolated compound (tetrahydro furan region only showed)

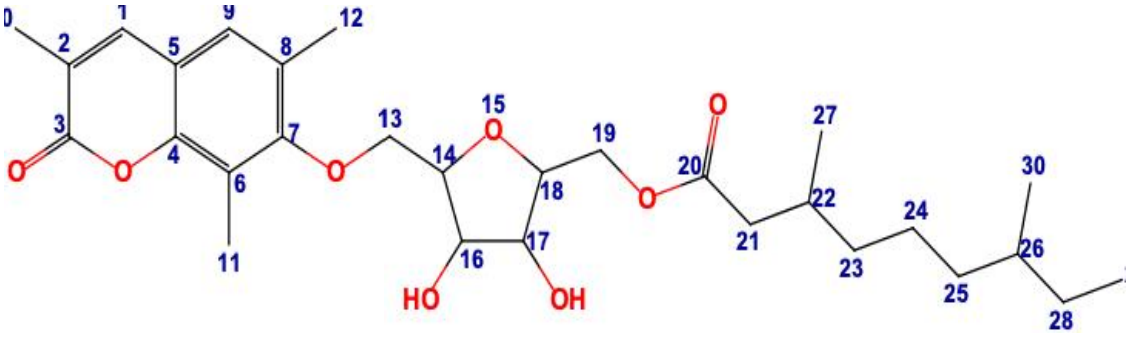


Fig. 5. Chemical structure of (3,4-dihydroxy-5-{[(3,6,8-trimethyl-2-oxo-2H-chromen-7-yl)oxy]methyl}oxolan-2-yl)methyl 3,7-dimethylnonanoate (Mol.weight: 518.6 g/mol; Mol. Form.: $C_{29}H_{42}O_8$)

AChE1 of *Ae. aegypti*

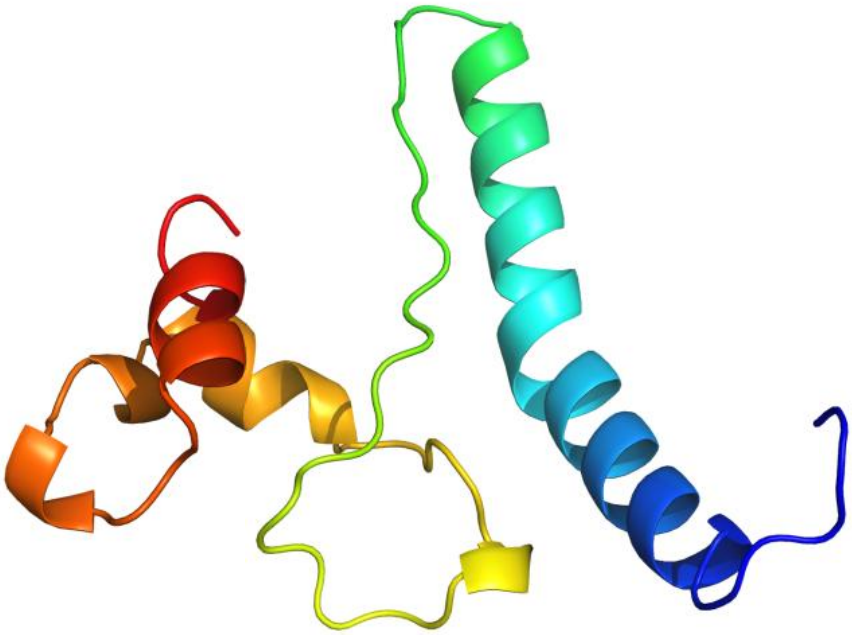


Fig. 6.Cartoon representation of the homology model of AChE1 from *Ae. aegypti* generated using PyMOL

AChE1 of *Cx. quinquefasciatus*

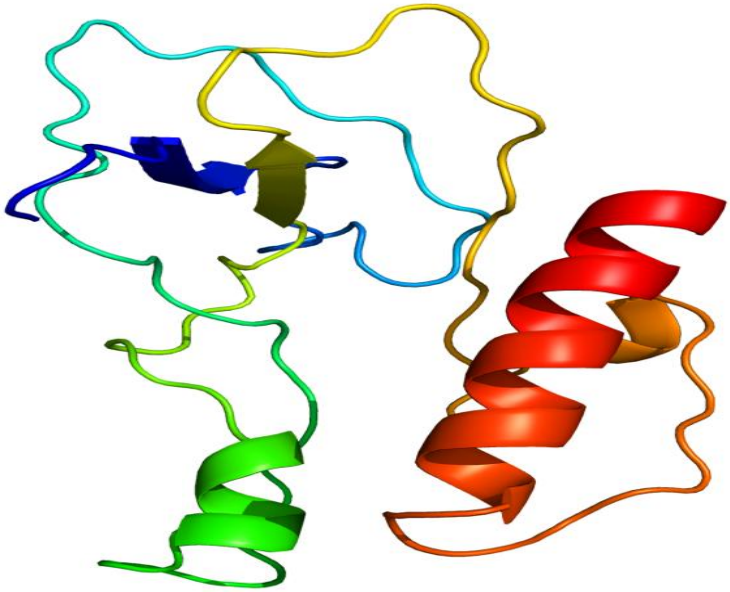
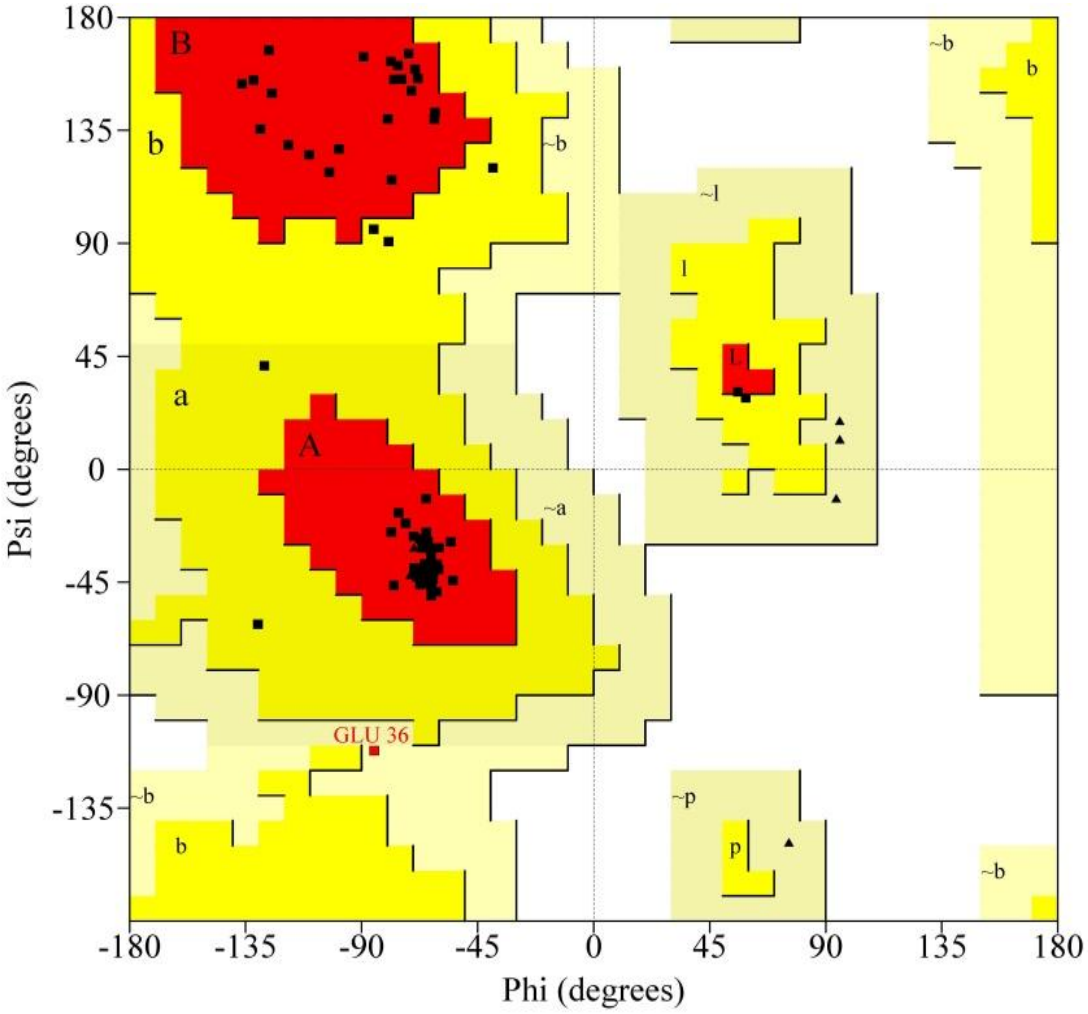


Fig. 7. Cartoon representation of the homology model of AChE1 from *Cx. quinquefasciatus* generated using PyMOL

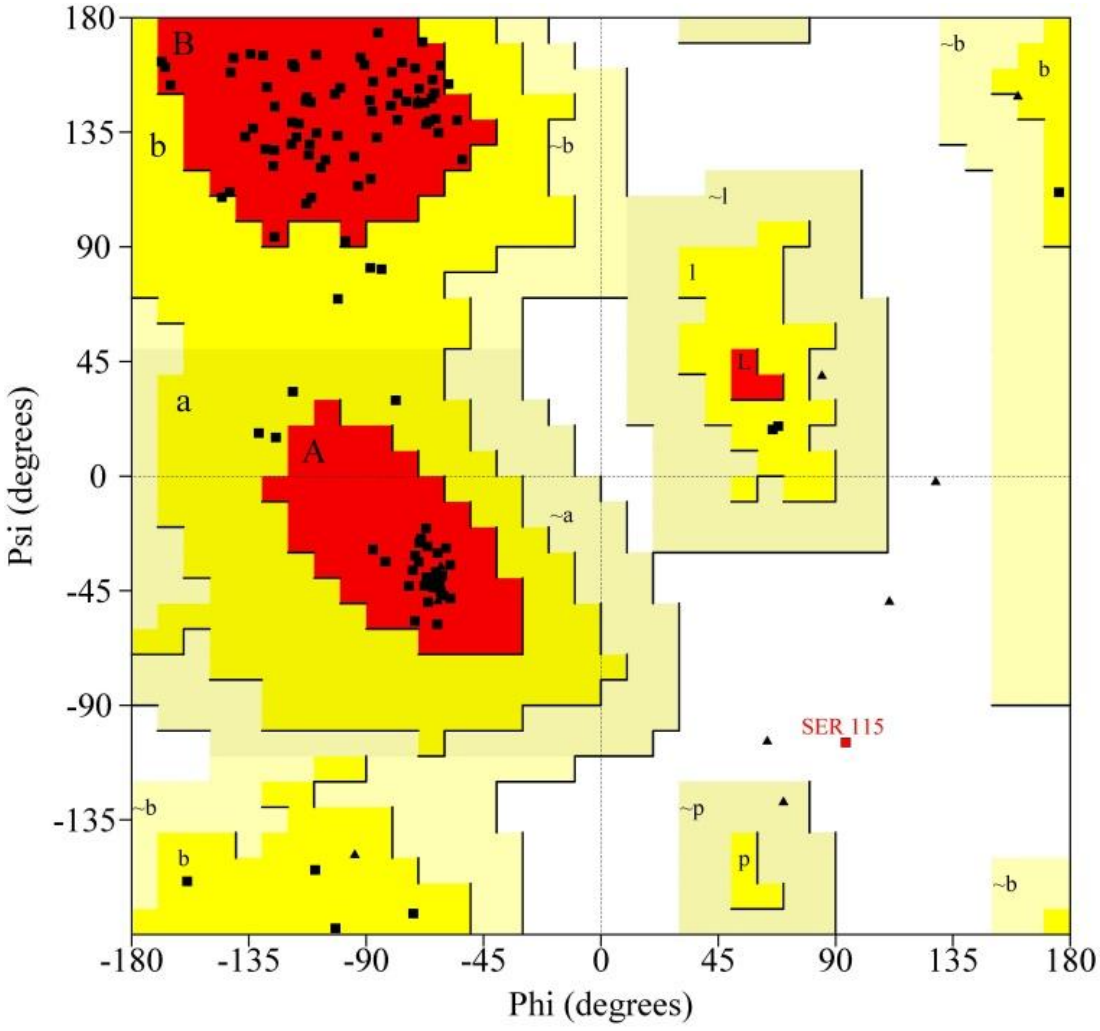


Plot statistics

Residues in most favoured regions [A,B,L]	70	90.9%
Residues in additional allowed regions [a,b,l,p]	6	7.8%
Residues in generously allowed regions [~a,~b,~l,~p]	1	1.3%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	77	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	7	
Number of proline residues	5	
Total number of residues	91	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Fig. 8. Ramachandran plot analysis of homology model of *Ae. aegypti* AChE1



Plot statistics

Residues in most favoured regions [A,B,L]	90	84.9%
Residues in additional allowed regions [a,b,l,p]	15	14.2%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	1	0.9%
Number of non-glycine and non-proline residues	106	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	11	
Number of proline residues	14	
Total number of residues	132	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Fig. 9. Ramachandran plot analysis of homology model of *Cx. quinquefasciatus* AChE1

Active sites AChE1 of*Ae. aegypti*

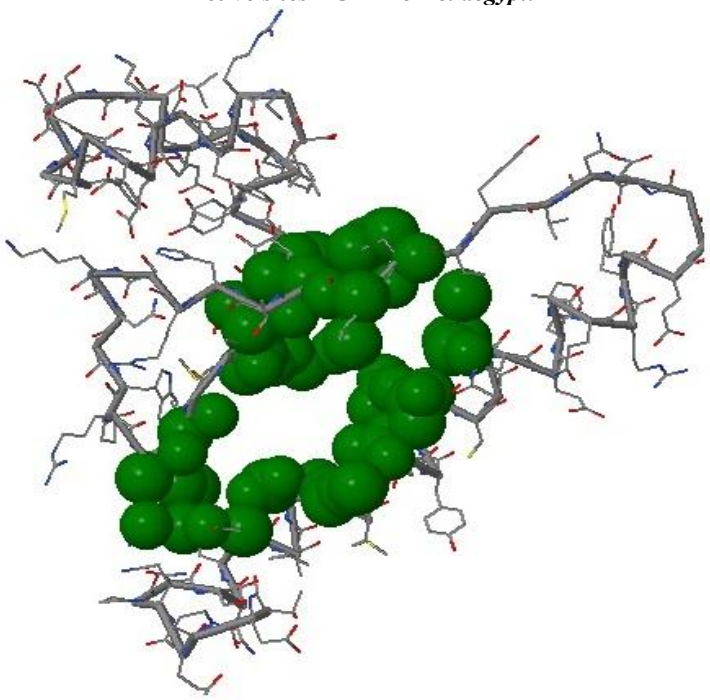


Fig. 10. Active amino acid cavities of *Ae.aegypti* AChE1 analyzed by the CASTp cavity prediction program

Active sites AChE1 of *Cx. quinquefasciatus*

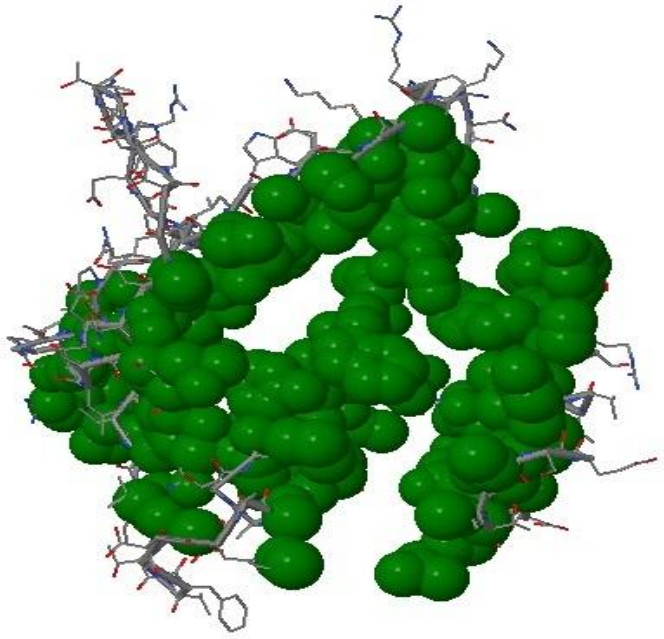
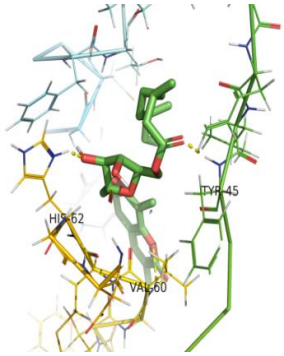


Fig. 11. Active amino acid cavities of *Cx. quinquefasciatus* AChE1 analyzed by the CASTp cavity prediction program

Isolated compound - AChE1*Ae. aegypti*
A



Hydrophobic interaction
B

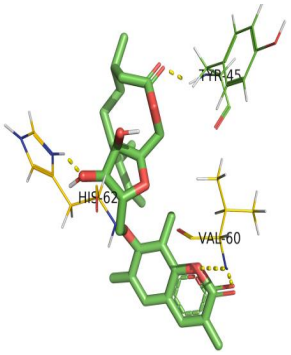
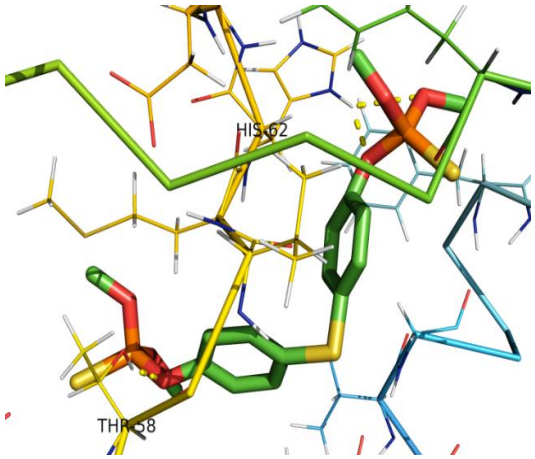


Fig. 12. Illustration of *Ae.aegypti* AChE1 binding pocket with the isolated compound (3D and 2D)

Temephos - AChE1*Ae. aegypti*
A



Hydrophobic interaction
B

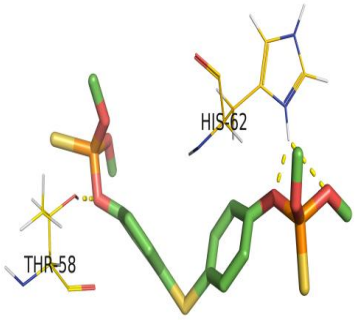
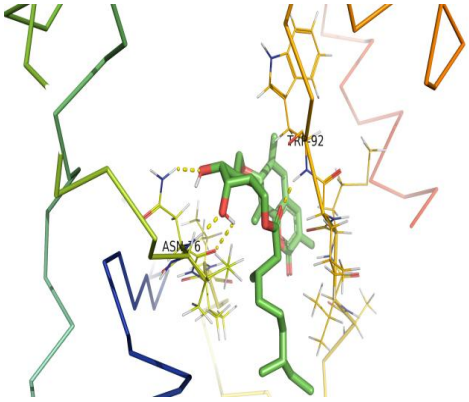


Fig. 13. Illustration of *Ae.aegypti* AChE1 binding pocket with Temephos

Isolated compound - *Cx. quinquefasciatus*
A



Hydrophobic interaction
B

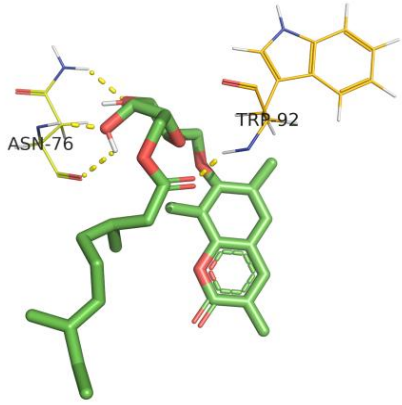


Fig. 14. Illustration of *Cx. quinquefasciatus* AChE1 binding pocket with isolated compound (3D and 2D)

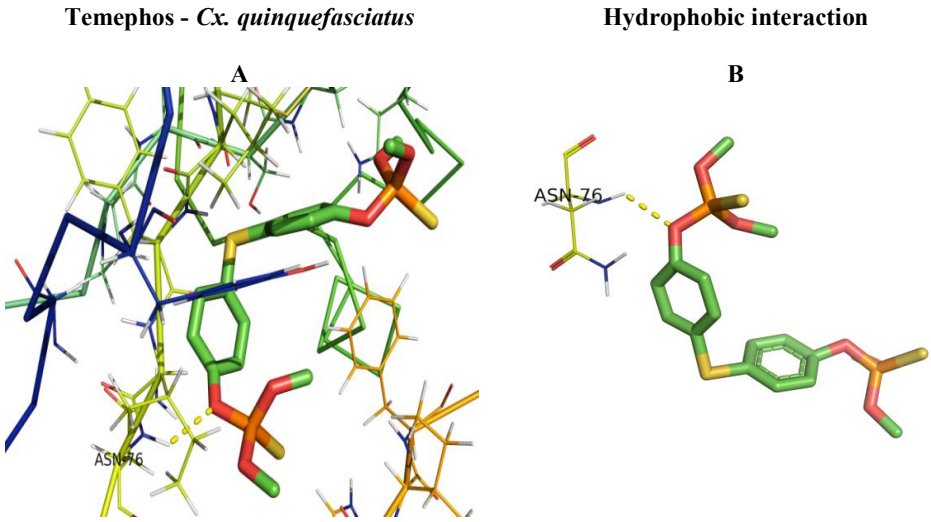


Fig. 15. Illustration of *Cx. quinquefasciatus* AChE1 binding pocket with temephos

The LC_{50} and LC_{90} values of positive control azadirachtin on *Cx. quinquefasciatus* larvae were 3.66ppm and 7.17 ppm, respectively (Table 2). On *Ae. aegypti* larvae, the LC_{50} and LC_{90} concentrations of azadirachtin were recorded as 3.81 ppm and 7.35 ppm, respectively (Table 2). Similarly, the LC_{50} and LC_{90} values of positive control temephos on *Cx. quinquefasciatus* larvae were 2.77 ppm and 5.20 ppm, respectively (Table 2). On *Ae. aegypti* larvae, the LC_{50} and LC_{90} concentrations of temephos were recorded as 2.94 ppm and 5.39 ppm, respectively (Table 2).

3.4 Physiochemical Properties of the Compounds

The isolated compound and temephos were drawn using Chem Draw Ultra, 12.0, and chemical properties were analyzed. For isolated compound, Mass is 518.6390, Exact mass is 518.2879, Formula is $C_{29}H_{42}O_8$, IUPAC name is (3,4-dihydroxy-5-(((3,6,8-trimethyl-2-oxo-2H-chromen-7-yl)oxy)methyl) tetrahydrofuran-2-yl)methyl 3,7-dimethylnonanoate, clogP is 4.651, clogS is -5.578 Hydrogen bond acceptor is 8, Hydrogen bond donor is 2.

3.5 Template Identification and Homology Modelling

The 3D (three-dimensional) structures of acetylcholinesterase (AChE1) for *Ae.aegypti*and *Cx. quinquefasciatus* is not available in the protein data bank (PDB). Hence, the homology model was developed to analyze the molecular drug interaction with compounds. The both target protein sequences of AChE1 were retrieved and the BLASTP [18] and used to classify appropriate templates in the PDB database. Racemic vx was used to inhibit the human butyrylcholinesterase, which has an x-ray crystal structure (pdb id:2XQF) and the human butyrylcholinesterase inhibited by tabun analogue TA1 (PDB id: 2WID) were identified as a best-fit template for AChE1 of *Ae. Aegypti* and *Cx. quinquefasciatus*, respectively. The clustal W program was used to analyze the multiple sequence alignment of both AChE1 sequences with its templates. The homology modelling software Modeller v9.19 [19] and the Swiss model server was used to develop the homology model of AchE1 protein. PyMol visualized the modelled image; it is shown in Figs. 6 and 7. The SAVES server was used to examine the stereo-chemical assessment of the models by the Ramachandran plot (PROCHECK) analysis [15].

The structural assessment of AChE1 of *Ae. aegypti* and *Cx. quinquefasciatus* models interpreted the placement of amino acid residues in the Ramachandran plot. The plot describes the residual regions as the most favoured region, additional allowed regions, generously allowed regions, and disallowed regions (Figs. 8 and 9). The percentage of amino acid occupancy is mentioned in the figures. The active sites of both AchE1 structures identified by the CastP tool were shown in figures (Figs. 10 and 11).

3.6 Protein-Ligand Interaction

The results of the docking studies of the isolated compound with AChE1 in the case of *Ae. aegypti* and *Cx. quinquefasciatus* are given below.

Table 2. Lethal concentrations of compound (in ppm) against larvae of *Cx. quinquefasciatus*

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ^2
			LL	UL		LL	UL			
Culexquinquefasciatus	Coumarin	3.99	2.16	7.55	7.76	4.96	68.85	2.3± 0.4	4.4±0.7	9.1*
	Azadirachtin	3.66	3.35	4.01	7.17	6.33	8.40	2.5± 0.2	± 0.3	5.4*
	Temephos	2.77	2.53	3.02	5.20	4.62	6.06	2.9±0.1	4.6±0.3	1.6
Aedesaegypti	Coumarin	4.99	4.53	5.52	10.78	9.28	13.12	2.3± 0.2	3.8±0.3	4.8*
	Azadirachtin	3.81	2.45	5.97	7.35	4.99	25.96	2.3± 0.4	4.4± 0.6	6.5*
	Temephos	2.94	2.70	3.20	5.39	4.80	6.25	2.7± 0.2	4.8± 0.4	0.6*

$p \leq 0.05$, level of significance of chi-square values

Table 3. Protein- Ligand interaction result of two mosquito model

Ligand	Protein	Binding Residues	Binding Energy (kcal/mol)	Vdw_hb_desolv_energy (kcal/mol)	Inhibition Constant	RMSD (Å)	Ligand efficiency
Coumarin	Culex - model	ASN`76/O/1HD2/HN, TRP`92/HN	-5.26	-9.7	139.72 (uM)	62.26	0.14
Temephos	”	ASN`76/HN	-5.28	-6.7	134.68 (uM)	40.3	0.20
Coumarin	Aedes - model	TYR`45/HN, VAL`60/HN, HIS`62/HD1	-5.06	-9.48	195.01 (uM)	61.24	0.14
Temephos	”	THR`58/HG1, HIS`62/HD1	-4.75	-7.6	328.46	34.74	0.18

For *Ae. aegypti*, the carbonyl and oxygen atom present in the coumarin showed hydrogen bonding with VAL60. The hydroxyl group present in the C18 of tetrahydrofuran showed a hydrogen bond with HIS62. Another carbonyl group linked with an aliphatic chain showed a hydrogen bond with TYR 45 with the least binding energy of -5.06 kcal/mol (Fig. 12a and 12b). For temephos (Standard drug), the methoxy and phenoxy oxygen atom showed a hydrogen bond with HIS 62 and the oxygen atom present in the phenoxy substituent showed a hydrogen bond with THR 58 of least binding energy with -4.75 kcal/mol (Fig. 13a and 13b).

The hydrogen bond interactions of the isolated compound with the *Cx. quinquefasciatus* target protein is: the carbonyl group linked with the aliphatic chain showed a hydrogen bond with TRP 92, and both hydroxyl groups present in the C18 and C19 of tetrahydrofuran showed hydrogen bond with ASN76 with the least binding energy of -5.26 kcal/mol (Fig. 14a). For temephos, the phenoxy oxygen along one side showed binding with one polar amino acid ASN 76. In contrast, the sulphur atom in the same residue showed binding with one nonpolar amino acid TRP 92 with the binding energy value of -5.28 kcal/mol (Fig. 15a). The above results are shown in Table 3. Figs. 14b and 15b show the hydrophobic interactions of the isolated compound and the chemical compound temephos (Standard drug) on the AchE1 of *Ae. aegypti Cx. quinquefasciatus*, respectively.

4. DISCUSSION

Chemical insecticides are been used on large scale in tropical and sub-tropical countries including India against a wide range of insect pests including disease-causing insects [19]. The application of a high amount of insecticides created many problems for humans. Therefore, plant isolated compounds would be a perfect alternative to chemical insecticides. Further, plant isolated compounds are safe, effective and widely accepted as it will not pollute the environment [20]. In the present study, the isolated compound was an active compound on the fourth stage larva of *Cx. Quinquefasciatus* and *Ae. aegypti*.

In ^{1}H NMR spectrum, protons of coumarinalkenyl proton at C1 (Ph-CH=C(CH_3)-) attached to C5 appeared as a doublet at 7.62-7.68 ppm with a coupling constant value of 30 Hz. The value of coupling constant, 30 Hz indicates 1,3-coupling with methyl (C10). IN the ^{13}C NMR spectrum, C1 was observed in 145.8 ppm, suggests the presence of extended conjugation in the coumarin group. Whereas, protons of aromatic carbon (C9) group of coumarin moiety have appeared as a doublet at 7.47 - 7.50 ppm with 15 Hz coupling constant. The coupling constant value indicates that aromatic proton has 1,3couping with C1 proton. The C9 carbon appeared at 119.8 ppm. The aromatic carbons of coumarin at C2, C4, C5, C6, C7 and C8 appeared at 109.2, 138.5, 117.8, 132.1, 160.5 and 126.9 respectively. The ester carbonyl compound of the coumarin showed at 167.4 ppm. The presence of eight carbons indicates that a benzene ring is connected by a cyclic ester group. Besides, the presence of the ester carbonyl is also confirmed. Both ^{1}H and ^{13}C NMR indicate that the presence of coumarin group attached with three methyl groups C10, C11 and C12 attached at C2, C6 and C8 respectively. The methyl groups of C10, C11, and C12, have appeared at 0.96, 0.80 and 0.85 ppm as a broad singlet in the ^{1}H NMR spectrum. Whereas C10, C11 and C12 carbons have appeared at 22.5, 40.8 and 35.5 ppm respectively in the ^{13}C NMR spectrum.

In the ^{1}H NMR spectrum, the methylene protons of C13 and C19 showed peaks at 4.23-4.25 ppm and 4.23-4.25 ppm, respectively as a doublet. The doublets revealed that the C13 and C19 are attached to oxygen atoms. Further, the carbon atoms C13 and C19 observed at 61.7 and 52.8 ppm respectively in ^{13}C NMR. The presence of methine proton attached to C14, C16, C17 and C18 are appeared multiplets at 5.08-5.10, 3.85-3.96, 3.85-3.96 and 5.08-5.10 ppm respectively in ^{1}H NMR spectrum. In the ^{13}C NMR spectrum, the carbons of C14, C16, C17 and C18 are observed at 60.0, 77.0, 70.3 and 55.2 ppm. Thus, with the presence of two methylene protons, four methine protons and six carbons in the polyol region, the isolated compound may contain tetrahydrofuranoyl group substituted with four oxygen atoms.

The presence of ester carbonyl (C20) groups was identified from ^{13}C peak appeared at 173.7 ppm. The two carbonyl peaks showed that the compound contains a coumarin carbonyl as well as an ester group. The protons of α--methylene group of C21 and the b-methylene group of C22 attached to methylene CH_2- (C23) and ester carbon (C20) (-$\underline{CH_2}$-C=O) appeared as a multiplet at 2.48-2.49 and 2.14-2.19 ppm. On the other hand, in the ^{13}C NMR spectrum, both C21 appeared at 41.9 ppm and C22 appeared at 36.8 ppm in ^{13}C NMR. The aliphatic proton of C23 appeared a multiplet 1.90-1.92 ppm in the ^{1}H NMR spectrum. At the same time, C23 has appeared at 31.7 ppm in the ^{13}C NMR spectrum. The aliphatic protons of C24-C26 appeared as a broad singlet 1.90-1.23-1.24 ppm in the ^{1}H NMR spectrum. Simultaneously, carbons of C24-C26 have appeared at 29.4, 28.9, and 28.5 ppm in the ^{13}C NMR spectrum. The aliphatic

protons of C27-C30 appeared as a broad singlet at 0.70 - 0.96 ppm in the ^{1}H NMR spectrum. In contrast, carbons of C27 – C30 have appeared at 18.3, 17.2, 16.3 and 15.5 ppm in the ^{13}C NMR spectrum.

Both ^{1}H and ^{13}C NMR showed the presence of three methyl group substituted coumarin containing 3,4-dihydroxy tetrahydrofuron esterified with 3,7-dimethyl nonanoic acid (Fig. 5). Thus, based on the ^{1}H and ^{13}C NMR, the isolated compound was identified to be a coumarin based compound linked with tetrahydrofuran substituted with 3,7-dimethyl nonanoic acid. The molecular formula of the compound is $C_{29}H_{42}O_{8,}$ and the molecular weight is 518.6 g/mol as shown in Fig. 5.

Similar to our study, Raja et al., [21] have isolated a compound named musizin from the ethyl acetate extract of the plant *Rhamnuswightii* and tested against the third stage larvae of *Cx. quinquefasciatus*. Their results showed that the isolated compound was effective with LC_{50} and LC_{90} values of 1.62ppm and 4.51ppm, respectively against the third stage larvae of *Cx. quinquefasciatus*. Similarly, Sandhanam et al., [22] isolated a compound called tiliamosine from the plant *Tiliacora acuminate* and tested against the third stage larvae of *Cx. quinquefasciatus*. Their results showed that the isolated compound was effective with LC_{50} and LC_{90} values of 1.13 ppm and 2.85 ppm, respectively against the third stage larvae of *Cx. quinquefasciatus*. Similarly, two compounds, namely, ecbolin-A and ecbolin-B were isolated from the ethyl acetate extract of the plant *Ecboliumviride* and tested for larvicidal activity against the larvae and pupae of *Cx. quinquefasciatus* mosquito [23]. Their results showed that the ecbolin-B was very effective than ecbolin-A and the LC_{50} and LC_{90} values of ecbolin B on *Cx. quinquefasciatus* larvae were 1.36 and 2.76 ppm, and on pupae, these were 1.54 and 3.51 ppm, respectively [23].

5. CONCLUSION

In this study, a coumarin compound (3,4-dihydroxy-5-{[(3,6,8-trimethyl-2-oxo-2H-chromen -7-yl)oxy]methyl}oxolan-2-yl)methyl 3,7-dimethylnonanoate (Mol.weight: 518.6 g/mol; Mol. Form.: $C_{29}H_{42}O_{8}$) was isolated from the ethanol extract of *Eucalyptus deglupta*leaves. The bioassay results showed that the compound was very effective and the results were analogous to azadirachtin and temephos. Similarly, docking studies recorded effective binding to the two-mosquito docking model. Based on the result, the isolated compound is effective and could be used in *Ae. aegypti* and *Cx. quinquefasciatus* mosquito control program.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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