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Larvicidal and adulticidal activities of essential oil of *Helianthus annuus* (*L.*) seed and molecular docking of its GCMS constituents against *Aedes aegypti* acetylcholinesterase

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ABSTRACT

Essential oils (EOs) are known to possess lethal effects on various pests and insects and are ecologically safe. The larvicidal and adulticidal activities of *Helianthus annuus (L.)* seed EO (HASEO) against *Aedes aegypti* mosquitoes and *in silico* inhibitory action of its GCMS constituents against *Ae. aegypti* acetylcholinesterase (AChE) were investigated. Larvae and adult *Ae. aegypti* (320 of each) were grouped into 4 (A - D) and exposed to DMSO, 1500, 3000 and 4500 ppm HASEO in DMSO respectively. Mortality was recorded after 0, 15, 30 and 45 min. HASEO exhibited stronger larvicidal activity than adulticidal activity against *Ae. aegypti*. The larvicidal and adulticidal activities of HASEO increased with increasing exposure time, with the highest mortality recorded after 45 min of exposure (larvicidal activity LC_{50}- 2121.32 ppm; adulticidal activity LC_{50}- 2565.20 ppm). Increase in the concentration of HASEO resulted in an increase in the larvicidal and adulticidal activities of HASEO. After 45 min of exposure, 100 % larvae mortality was recorded at 3000 and 4500 ppm HASEO while 100 % adult mortality was recorded at 4500 ppm HASEO. GCMS analysis revealed the presence of 21 compounds in HASEO with 1-chlorononadecane (23.67 %) being the most abundant compound. Two compounds of HASEO; isopinocamphone and oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- demonstrated potential inhibitory activity against *Ae. aegypti* AChE with comparable binding affinity to standard ligand (1S)-2,2-difluoro-1-[1-(pentan-3-yl)-1H-pyrazol-4-yl]ethan-1-ol. The present findings reveal that HASEO may be useful as an effective biopesticide for the control of *Ae. aegypti* adult and larvae and may serve as an alternative to synthetic insecticides.

1. Introduction

Mosquitoes are disease-causing vectors that are major causative agents of human filariasis, malaria, and many other viral diseases like dengue, Japanese encephalitis, Zika virus, and West Nile virus [1]. *Ae. aegypti* mosquito is the primary transmitter of the four viruses that have had the greatest impact on human health, the viruses causing yellow fever, chikungunya, Zika fever, and dengue, one of the emerging fastest-growing global infectious with 100–400 million new infections a year [2]. Behind this rapid rise is the simple adaptation of *Ae. aegypti* to a new entomological niche carved out by human habitation [3]. Since *Ae. aegypti* is anthropophilic and coexists closely with people, it

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preferentially develops in urban and suburban areas in tropical and subtropical countries with easy access to human hosts [2,4]. Dengue fever is now a threat to over half of the world's population, having quadrupled in incidence since 1970. At the moment, vector control is the principal means of control as there is no dengue vaccine or effective medication available [5].

Mosquitoes continue to pose a serious infectious threat to modern society despite efforts to eradicate them, harming not only people but also other animal species. These insects are ideal disease vectors because of their extraordinary capacity for environmental adaptation, rapid and widespread reproduction, tolerance to some pesticides, and modification of eating patterns to withstand control measures [6].

Over time, synthetic insecticides like organophosphates, pyrethroids, and insect growth regulators have been employed to eliminate both the larvae and adult of *Ae. aegypti* mosquitoes [7]. Through the utilization of these insecticides, it is possible to achieve effective pest management. Nevertheless, the frequent application of these chemicals has posed a threat to the balance of biological ecosystems, leading to the extensive emergence of resistance and resurgence of mosquito-borne diseases [8]. Insecticides also have harmful effects on non-target populations as they are non-selective. This gives rise to adverse outcomes, such as harm to non-target organisms, as well as heightened worries regarding environmental and human well-being. The financial implication of chemical insecticides application is burdensome to both public health organisations engaged in mosquito control and individual homeowners [9]. This have necessitated the exploration for eco-friendly, simpler, safer, sustainable alternative means for mosquito control.

Lately, there has emerged a pressing requirement to employ eco-friendly botanical solutions for controlling vector mosquitoes. This involves utilizing natural products derived from plants through physical and chemical means as a biological control approach. The use of natural products is necessary in order to prevent further hazardous effects on humans and resources. Moreover, the cost-effectiveness of utilizing, producing, and applying plants as pesticidal agents is notably lower in comparison to the detrimental impacts of chemicals, pollution, and radiation [5]. Insecticides derived from plants hold great potential as viable substitutes to synthetic mosquito control agents, thanks to their limited toxicity towards mammals and swift degradation [9]. Particularly, EOs have gained considerable attention as potential bioinsecticides.

EOs are aromatic, liquid, and volatile compounds generated by various components of plants, including barks, buds, flowers, fruits, herbs, leaves, roots, seeds, twigs and woods. They consist of intricate natural blends of lipophilic substances, typically comprising around 20–60 different compounds [8]. The inherent characteristics of EOs, combined with their designation as GRAS (Generally Recognized As Safe) substances that pose minimal risks to both the environment and health, have sparked growing enthusiasm for exploring their potential applications in various domains, including agriculture [10]. EOs are environmentally safe and target specific, and lately, a multitude of EOs derived from plants have demonstrated insecticidal properties against a wide array of insects, mosquitoes [8,11]. From the perspective of insect resistance development, EOs offer greater advantages compared to traditional insecticides. Because EOs are naturally lipophilic, they can readily penetrate cell membranes, thereby disrupting a variety of biochemical, physiological, and behavioural processes within an organism [8,9]. Furthermore, because EOs consist of intricate combinations of bioactive components, they can operate through multiple mechanisms, thereby lowering the likelihood of mosquitoes developing resistance [9].

EOs have the potential to be fatal to both mature and young mosquitoes, and they can impede the growth of mosquito larvae by causing suffocation (obstructing the respiratory system), disturbing surface tension, inducing direct toxicity, or altering the chemosensory behaviour of gravid females [9]. EOs derived from plants are easily accessible in the majority of countries, cost-effective, and safe for human use. As a result, these products hold promise as mosquito insecticides to manage both young and mature mosquitoes in areas around households [9]. Even though plant EOs have been employed as pesticides for an extended period, and a few have found commercial application as repellents and pesticides for domestic and garden pests, their utilization as mosquito larvicides is restricted due to their comparatively modest levels of toxicity against mosquito larvae [11].

H. annuus (L.) (Sunflower) is an annual dicotyledonous plant that belongs to the family Asteraceae. It is an important oilseed crop native to South America and currently cultivated throughout the world [12,13]. In general, *H. annuus (L.)* is acknowledged for its nutritional and medicinal significance. Due to its positive impact on health, it has gained acknowledgment as a functional food or nutraceutical, although its potential is yet to be fully utilized [12]. Its roots, stems, leaves and seeds contain phenols, flavonoids and alkaloids [13]. *H. annuus (L.)* is cultivated basically for its seeds, which give the world's second most important source of edible oil [14]. The EOs from *H. annuus (L.)* are abundant in unsaturated fatty acids, including oleic and linoleic acids (omega-6), and are recognized as beneficial for human well-being [15]. *H. annuus (L.)* has been used in the formulation of bio-pesticide and carrier for agrochemicals [12]. Nevertheless, there has been no research conducted to assess the larvicidal and adulticidal effects of *H. annuus (L.)* EO on *Ae. aegypti* mosquito. Hence, this work evaluated the larvicidal and adulticidal effect of *H. annuus (L.)* seed EO on *Ae. aegypti* mosquitoes and also determined the potential inhibitory activity of *H. annuus (L.)* compounds on *Ae. aegypti* AChE activity using *in silico* approach.

2. Materials and methods

2.1. Plant material

H. annuus (L.) plants with flowers were harvested from Federal College of Forestry, Jos, Plateau State, and identified/authenticated at the Herbarium of Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos. The seeds used for this experiment were collected from the dried flowers.

2.2. Insect rearing

Ae. aegypti larvae were collected from different breeding sites (i.e. ponds, flower jars, suck away, abandon tires, stagnant water) within the premises of University of Jos. They were carefully transported to the insectary of the Department of Science Laboratory Technology without exposure to pathogens or insecticides for identification. The larvae were acclimated to fresh, non-chlorinated water from a well and given a diet of ground fish meal twice daily under controlled conditions of humidity (60 ± 10 %), temperature (26 ± 1 °C), and light cycle (12 h day/12 h night). They were permitted to mature into 4th instar larvae stage before being employed for the larvicidal investigation. Afterwards, the larvicidal bioassay was carried out using WHO protocols [16].

To obtain adult *Ae. aegypti*, the larvae were kept in a plastic container containing water and fed twice a day with a diet containing ground dog biscuits, yeast pills, and ground dried fish. The water was changed daily to maintain a clean environment. Once the larvae transformed into pupae and then into adult mosquitoes, which took about a week, the adult mosquitos were placed in a tray within a mosquito cage (30 x 30 × 30 inches) and fed with sucrose which they sucked from cotton wool presoaked in sucrose solution.

2.3. Extraction of essential oil

The extraction of EO from *H. annuus (L.)* seed was achieved by Soxhlet extraction using n-hexane as the solvent [17]. The Soxhlet extractor involved a solid-liquid (ground seeds and n-hexane) contact for the removal of the oil content by dissolution into a refluxing liquid

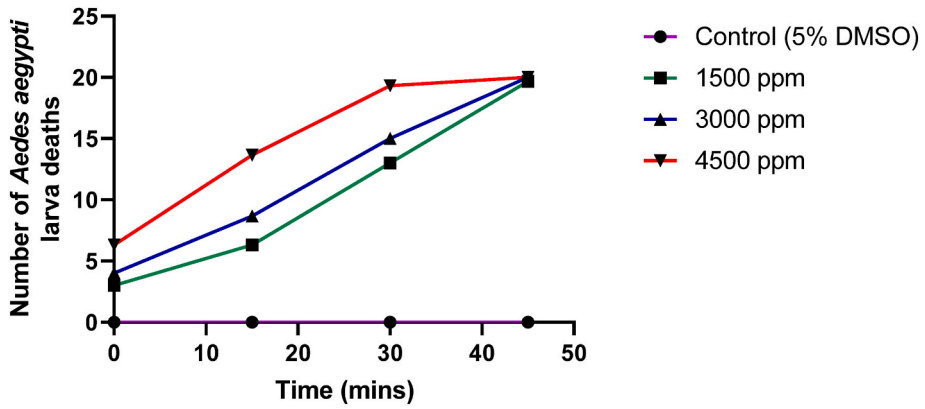


Fig. 1. Mortality rate of *Ae. aegypti* larva at different concentrations of *H. annuus (L.)* seed essential oil
Values represent mean of four replicates (each replicate has 20 larvae).

phase. *H. annuus (L.)* seed powder, pre-packaged within filter paper, was positioned within a cavity. The cavity was incrementally filled with the extracting liquid phase (n-hexane) through the condensation of vapours originating from a distillation flask. As the liquid reached a pre-determined level, a siphon drew the contents of the cavity back into the distillation flask, effectively transporting the extracted substances into a larger liquid volume. This cycle was iterated until near-complete extraction was attained.

2.4. Determination of the chemical constituents of H. annuus (L.) seed essential oil

Determination of the chemical constituents of *H. annuus (L.)* seed EO was carried out using gas chromatography combined with mass spectrometry (GCMS) as previously described (Ogunwande, Flamini [18].

2.5. Determination of insecticidal activity of H. annuus (L.) seed essential oil

2.5.1. Determination of larvicidal activity of H. annuus (L.) seed essential oil

The larvicidal activity of *H. annuus (L.)* seed EO was carried out according to the standard WHO procedure (WHO, 2005). A sum of 320 larvae were allocated into four groups (A-D) with four replicates per group, each containing 20 larvae. Group A was exposed to a solution of 1 mL of 5 % DMSO, while groups B, C, and D were exposed to different concentrations of HASEO (1500, 3000, and 4500 PPM respectively) in a 5 % DMSO solution for a maximum of 45 min. Mortality was recorded at 0, 15, 30 and 45 min. Any larvae that were immobile were considered dead and removed to prevent contamination of surviving larvae and were included in the mortality counts.

The percentage mortality was calculated thus:

$$\text{Percentage mortality} = \frac{\textit{Total number of dead larva}}{\textit{Total number of larva}} \text{ x } 100$$

The LC_{50} values were determined using the linear regression from the larvae mortality curves (concentration versus percentage mortality).

2.5.2. Determination of adulticidal activity of H. annuus (L.) seed essential oil

A total of 320 adult mosquitoes were divided into four groups (L-O) with similar groupings as previously described. Group L was exposed to 1 mL of 5 % DMSO, while groups M, N, and O were exposed to different concentrations of HASEO (1500, 3000, and 4500 PPM respectively) in a 5 % DMSO solution for a maximum of 45 min. Mortality was recorded at 0, 15, 30 and 45 min. The experiments were carried out in closed cylindrical plastic tubes (125 × 44 mm) which had one end covered with a 16-mesh wire gauze and the other end was opened and filter papers soaked with either DMSO or HASEO in 5 % DMSO were placed inside.

The percentage mortality was calculated thus:

$$\text{Percentage mortality} = \frac{\textit{Total number of dead mosquitoes}}{\textit{Total number of mosquitoes}} \text{ x } 100$$

The LC_{50} values were determined using the linear regression from the mosquito mortality curves (concentration versus percentage mortality).

2.6. In silico analysis

2.6.1. Homology modelling and protein grid generation

The amino sequence (in Fasta format) of *Ae. aegypti* AChE with Accession No: ABN09910 was retrieved from the National Centre for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/). *Anopheles gambiae* AChE (PDB ID: 6ARY) was chosen as the template for homology modelling of the 3D structure of *Ae. aegypti* AChE on the SWISS-MODEL web server (http://swissmodel.expasy.org). Using the protein preparation wizard pane of Glide (Schrödinger Suite 2020-3), the homology model structure was processed for docking. Site map was used to identify the binding pocket.

2.6.2. Receptor grid generation

The binding site of the newly modeled protein was idetified using Sitemap on Schrodinger Suite 2020-3. In order to produce an outline that will map the size and position of the binding site of *Ae. aegypti* AChE for ligand docking, a receptor grid was produced using the receptor grid generation tool within Schrödinger Maestro 12.5.

2.6.3. Ligand preparation

The low energy 3D structures (having appropriate chiralities) of the 21 bioactive compounds of HASEO along with that of the reference ligand ((1S)-2,2-difluoro-1-[1-(pentan-3-yl)-1H-pyrazol-4-yl]ethan-1-ol) were prepared utilizing the Ligprep module within Maestro 12.5 (Schrödinger Suite 2020-3). The reference ligand is the co-crystallized ligand of the template (PDB ID: 6ARY). Under a physiological pH of 7.2 ± 0.2, the ionization state of each ligand's structure was determined, its stereoisomer computed by holding specific chiralities constant while allowing others to vary.

2.6.4. Molecular docking

The protein-ligand docking was performed through the Glide-Ligand Docking panel within Maestro 12.5 (Schrödinger Suite 2020-3). The prepared ligands were docked to the protein binding pocket employing the standard precision (SP) docking on Maestro workspace, while setting vdW radius scaling factor at 0.80, a partial charge cut-off at 0.15 for ligand atoms, and the ligand sampling method to be flexible. The

Table 1
Percentage mortality of larvae of *Ae. aegypti* mosquitoes exposed to *H. annuus (L.)* seed essential oil (HASEO) at different exposure durations.

Duration of exposure (Mins)	Concentration of HASEO (ppm)	Percentage mortality (%)
45	1500	98.35 ± 2.00[a]
	3000	100.00 ± 1.50[a]
	4500	100.00 ± 0.50[a]
30	1500	65.00 ± 2.50[a]
	3000	75.00 ± 1.50[b]
	4500	96.65 ± 1.50[c]
15	1500	31.65 ± 3.50[a]
	3000	43.35 ± 2.00[b]
	4500	68.30 ± 4.50[c]
0	1500	15.00 ± 3.00[a]
	3000	20.00 ± 3.50[a]
	4500	31.65 ± 2.50[b]

Each percentage mortality value represents a mean of four replicates (each replicate has 20 larvae) ± standard deviation. Values within the same duration of exposure group carrying different superscript alphabets are significantly different at $p < 0.05$.

induced-fit docking (IFD) of top-scoring compounds was carried out utilizing Maestro 12.5's induced fit docking panel.

2.7. Data analysis

Data were presented as means ± standard deviation (SD) or four replicates. The data were subjected to statistical analysis using the IBM® statistical package for social sciences (SPSS) software version 21. All significant differences were determined by one way analysis of variance (ANOVA). Post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$. Graphs were plotted using Microsoft excel while regression analysis/LC_{50} was determined using AAT Bioquest® calculator.

3. Results

3.1. Larvicidal activity of H. annuus (L.) seed essential oil

There were no larvae deaths recorded for the control group exposed to 5 % w/v DMSO in distilled water (Fig. 1). HASEO nevertheless caused larvae mortality at the various concentrations and exposure durations considered (Fig. 1 and Table 1). At 0 min, there was no significant difference ($p > 0.05$) in larvae deaths caused by exposure to 1500 and 300 ppm HASEO. However, the group exposed to 4500 ppm of HASEO showed a significant increase ($p < 0.05$) in larvae deaths when compared with the 1500 and 3000 ppm HASEO-exposed groups (Fig. 1 and Table 1). At 15 and 30 min exposure duration, larvae deaths significantly increased ($p < 0.05$) with increase in the concentration of HASEO while at 45 min exposure duration, no significant difference ($p > 0.05$) in larvae deaths among the groups exposed to the various concentrations of HASEO was recorded (Fig. 1 and Table 1). The mortality percentage also increased with exposure duration for all the concentrations studied. 100 % and 98.35 % *Ae. aegypti* larvae mortality was recorded after 45 min of exposure to 4500 and 3000 ppm, and 1500 ppm HASEO respectively (Table 1). The LC_{50} values decreased with increase in exposure time with the lowest LC_{50} value recorded at 45 min and the highest at 0 min (Fig. 3).

3.2. Adulticidal activity of H. annuus (L.) seed essential oil

There were no adult mosquito deaths recorded for the control group exposed to 5 % w/v DMSO in distilled water (Fig. 2). At 0 min exposure duration, there were no adult mosquito deaths for the various concentrations of HASEO tested; meaning there was no significant difference ($p > 0.05$) in adulticidal activity of the various HASEO concentrations and control. At 15, 30 and 45 min exposure duration, there was significant increase ($p < 0.05$) in adult mosquito deaths of the HASEO-exposed groups when compared with control. While the 1500 and 3000 ppm HASEO concentration produced similar mosquito deaths at 15 min, the 4500 ppm HASEO concentration produced a significantly higher ($p < 0.05$) mosquito death than the lower HASEO concentrations. Meanwhile at 30 and 45 min exposure duration, mosquito deaths significantly increased ($p < 0.05$) with increase in the concentration of HASEO (Fig. 2). The percentage mortality of *Ae. aegypti* mosquitoes also increased with increased duration of exposure of the different concentrations of HASEO as well as increase in concentration (Table 2).

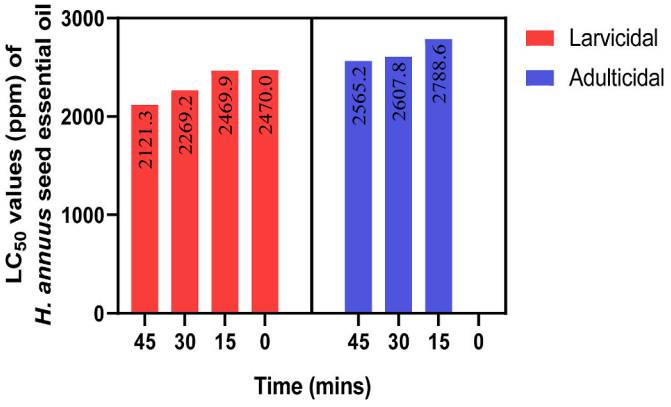


Fig. 3. Mean lethal concentrations (LD_{50}) of *H. annuus (L.)* seed essential oil (HASEO) against larvae and adult of *Ae. aegypti* mosquito.

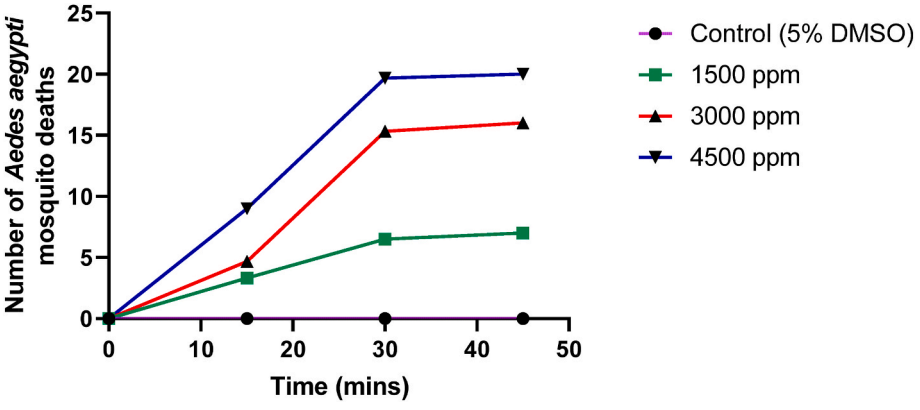


Fig. 2. Mortality rate of adult *Ae. aegypti* mosquitoes at different concentrations of *H. annuus (L.)* seed essential oil
Values represent mean of four replicates (each replicate has 20 larvae).

Table 2
Percentage mortality of adult *Ae. aegypti* mosquitoes exposed to *H. annuus (L.)* seed essential oil (HASEO) at different exposure durations.

Duration of exposure (Mins)	Concentration of HASEO (ppm)	Percentage mortality (%)
45	1500	35.00 ± 2.00^{a}
	3000	80.00 ± 5.00^{b}
	4500	100.00 ± 3.00^{c}
30	1500	32.50 ± 4.50^{a}
	3000	76.65 ± 2.50^{b}
	4500	98.35 ± 3.00^{c}
15	1500	16.65 ± 4.00^{a}
	3000	23.35 ± 5.50^{a}
	4500	45.00 ± 3.50^{b}
0	1500	0.00 ± 0.00^{a}
	3000	0.00 ± 0.00^{a}
	4500	0.00 ± 0.00^{a}

Each percentage mortality value represents a mean of four replicates (each replicate has 20 larvae) ± standard deviation. Values within the same duration of exposure group carrying different superscript alphabets are significantly different at $p < 0.05$.

Hundred percent *Ae. aegypti* mosquito mortality was observed after 45 min exposure of 4500 ppm HASEO. The LC_{50} values decreased with increased exposure time with 45 min having the least value and 15 min with the highest value (Fig. 3). LC_{50} at $t = 0$ was not determined because there was no *Ae. aegypti* mosquito death recorded.

3.3. Chemical constituents of H. annuus (L.) seed essential oil

The GCMS analysis revealed the presence of 21 compounds in HASEO (Table 3 & Fig. 4). According to the area percentage, 1-Chlorononadecane and 1,1-Cyclobutanedicarboxamide were the major compounds in HASEO.

3.4. In silico

3.4.1. Validation of homology model of Ae. aegypti AChE

The results of the quality assessment of the homology model of *Ae. aegypti* AChE are shown in Table 4 and Fig. 5. The model has a sequence identity of 92.58 % with the template, a GMQE of 0.74, and a global QMEANDisCo score of 0.92 ± 0.05. The QMEAN Z-score comparison plot (Fig. 5A) demonstrates that the model (red star) falls within the black dots representing experimental structures with a QMEAN score

Table 3
Compounds identified in the of essential oil of *H. annuus (L.)* by GC-MS.

Peak	Compound Name	Chemical Formula	Molecular Weight (g/mol)	Area (%)	Retention Time (Mins)
1	2-Butyl-1-Octanol	$C_{12}H_{26}O$	186.339	7.06	3.072
2	1-Nonadecanol	$C_{19}H_{40}O$	284.528	11.98	3.460
3	1,1-Cyclobutanedicarboxamide, 2-phenyl-N,N′-bis(1-phenylethyl)-	$C_{28}H_{30}N_2O_2$	426.5	13.66	3.822
4	1-Chlorononadecane	C19H40	268.529	23.67	4.249
5	1-Cyclohexylnonene	C15H28	208.389	8.85	4.635
6	(Z)-4-Decen-1-ol, pentafluoropropionate	$C_{13}H_{19}F_5O_2$	302.2808	10.83	5.106
7	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	$C_{24}H_{46}O_2$	366.6	7.31	5.388
8	Bicyclo[3.1.1] heptan-3-one, 2, 6, 6-trimethyl-,(1.alpha,2.beta,5.alpha.)-	$C_{10}H_{18}$	138.25	2.13	5.528
9	2,2-Dimethylbutane	C_6H_{14}	86.1754	6.78	5.623
10	Oxazol-5(4H)-one,4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)-	$C_{18}H_{12}F_3NO_2$	331.29	1.52	5.938
11	Tert-Butylbenzene	$C_{10}H_{14}$	134.2182	0.19	6.412
12	2,9-Dimethyldecane	$C_{12}H_{26}$	170.3348	1.68	7.066
13	1,3,5,2,4,6-Triazatriboriane	$B_3H_6N_3$	80.501	1.63	8.503
14	n-Octyliodide	$C_8H_{17}I$	240.1250	0.47	9.578
15	3,7-dimethylnonane	$C_{11}H_{24}$	156.3083	1.04	9.895
16	2,2-dimethylbutane	C_6H_{14}	86.1754	0.11	10.129
17	1,6-Heptadiene	C_7H_{12}	96.1702	0.25	18.496
18	2-cyclohexyl-2-(2-methylcyclohexyl) malononitrile	$C_{16}H_{24}N_2$	244.38	0.08	18.859
19	1-Oxetan-2-one,4-methyl-3-methylene-	$C_5H_6O_2$	98.10	0.29	20.740
20	Phthalic acid, dodecyl 2-isopropoxyphenyl ester	$C_{29}H_{40}O_5$	468.6249	0.43	21.275
21	1-benzylindole	$C_{15}H_{13}N$	207.27	0.05	21.658

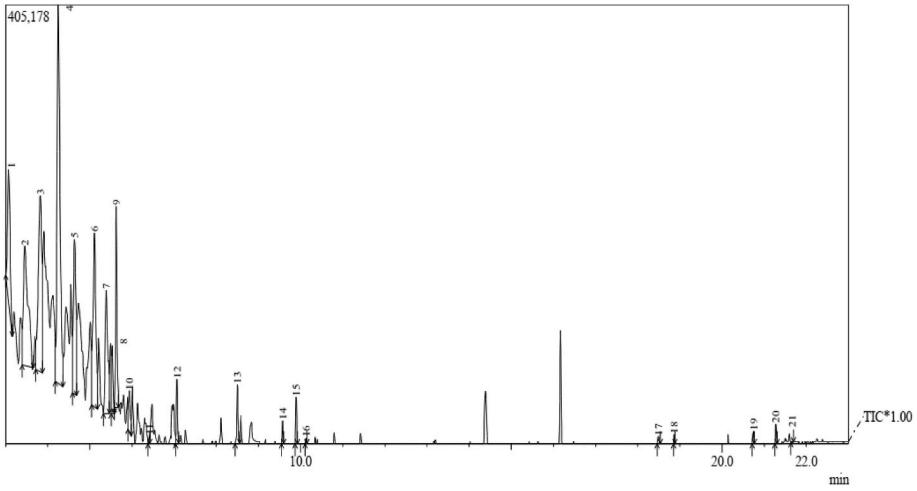


Fig. 4. GC chromatogram of *H. annuus (L.)* seed essential oil.

Table 4
Model evaluation scores for *Aedes aegypti* AChE.

Template	Model	Sequence Identity	GMQE	QMEANDisCo Global
6ary.1.A AChE. Crystal structure of an insecticide-resistant acetylcholinesterase mutant from the malaria vector *Anopheles gambiae* in complex with a difluoromethyl ketone inhibitor	*Ae. aegypti* AChE homology model	92.58 %	0.74	0.92 ± 0.05

within one standard deviation of the mean (Z-score between 0 and 1). The Local Quality plot in Fig. 5B shows the expected similarity to the native structure (y-axis) for each model residue (reported on the x-axis), with the majority of the residues scoring above 0.6.

3.4.2. Molecular docking

The standard precision docking scores of the GCMS-identified *H. annuus (L.)* compounds and the standard ligand against *Ae. aegypti* AChE are displayed on Table 5. Thirteen compounds and the standard ligand were found to occupy the binding pocket of the modeled structure. The standard ligand gave a docking score of −3.009 kcal/mol. Among the test compounds isopinocamphone scored highest with a binding energy of −2.853 kcal/mol. Next to it is oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- with a binding energy of −2.734 kcal/mol, while 2,2-dimethylbutane scored least with a binding energy of −0.274 kcal/mol. Table 6 shows the induced-fit docking scores of the ten best poses of the two top-scoring compounds and the standard against *Ae. aegypti* AChE. The standard ligand gave docking scores ranging from −6.13 and −5.618 kcal/mol, isopinocamphone scored between −6.173 and −5.161 kcal/mol, while Oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- gave the highest IFD scores of −6.805 to −6.401.

The molecular interactions of the two top-scoring compounds and the standard ligand are shown in Figs. 6 to 8. The standard ligand showed two hydrogen bond interactions with THR 357 and one with LYS 654 in addition to several hydrophobic interactions. Isopinocamphone displayed a hydrogen bond interaction with LYS 655, together with some polar and charged interactions. Oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- demonstrated hydrophobic interactions with several binding site amino acid residues and a hydrogen bond with TYR 525.

In the induced-fit docking analysis, the two highest-scoring compounds and the standard ligand showed improved molecular interactions with the binding site amino acid residues of the protein (Figs. 9 to 11). The standard ligand showed the ability to form hydrogen bonds with LEU 362, ALA 358, PRO 356 and GLN 432 (Fig. 9). Isopinocamphone maintained hydrogen bond interaction with LYS 655 (Fig. 10). Oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-

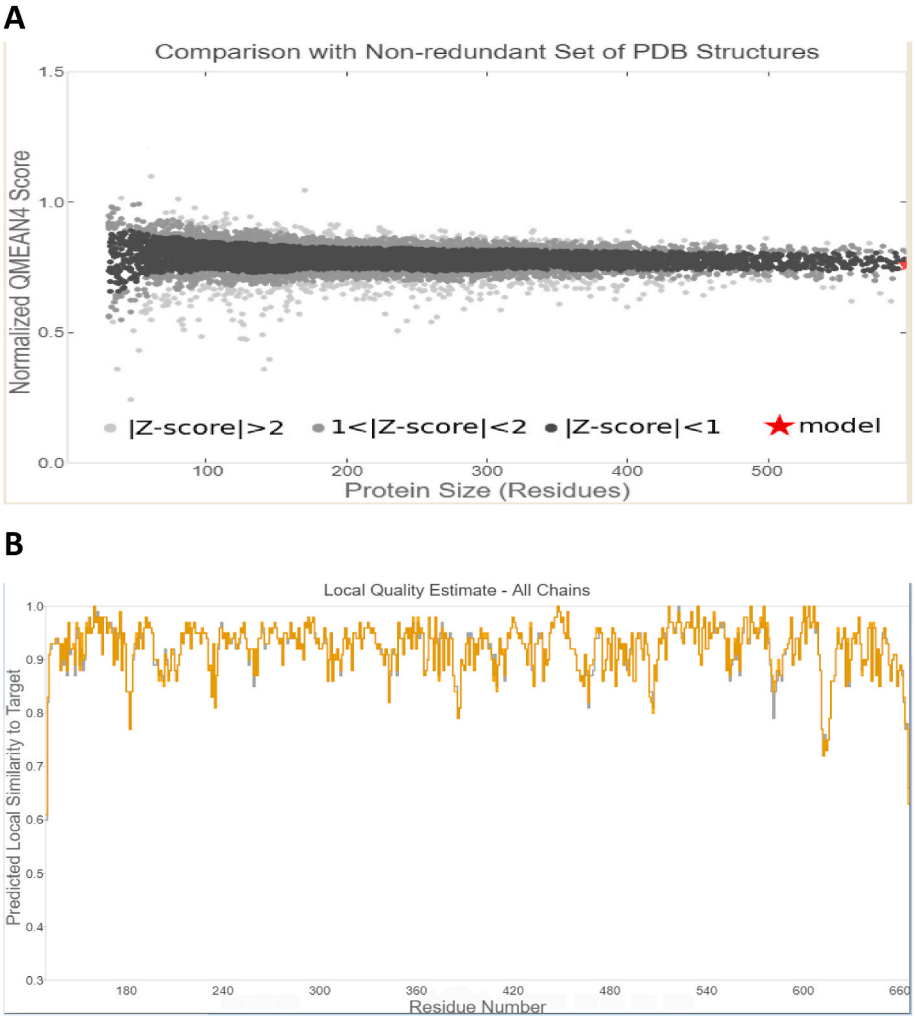


Fig. 5. *Aedes aegypti* AChE homology model validation. A: The QMEAN Z-score Comparison plot; B: The Local Quality plot.

Table 5
Docking scores (binding energies) of selected ligands against *Ae. aegypti* AChE.

S/No	Ligands	PubChem CID	Binding energy (Kcal/mol)
1	[a](1S)-2,2-difluoro-1-[1-(pentan-3-yl)-1H-pyrazol-4-yl]ethan-1-ol	350102473	−3.009
2	Isopinocamphone	84532	−2.853
3	Oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)-	5291348	−2.734
4	1-Benzylindole	96913	−2.572
5	Tert-Butylbenzene	7366	−2.485
6	2-Cyclohexyl-2-(2-methylcyclohexyl) malononitrile	544194	−2.394
7	2-Oxetanone, 4-methyl-3-methylene-	543106	−2.277
8	(Z)-4-Decen-1-ol, pentafluoropropionate	91692479	−2.190
9	1-Chlorononadecane	545634	−2.167
10	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	534597	−2.149
11	Phthalic acid, dodecyl 2-isopropoxyphenyl ester	6423457	−1.942
12	1,1-Cyclobutanedicarboxamide, 2-phenyl-N,N′-bis(1-phenylethyl)-	570689	−1.064
13	1-Nonadecanol	80281	−0.404
14	2,2-Dimethylbutane	6403	−0.274

[a] Standard ligand.

Table 6
Induced-fit docking scores of the ten best poses of the two top-scoring compounds and the standard against *Ae. aegypti* AChE.

Pose No	Binding energies (Kcal/mol)		
	A	B	C
1	−5.996	−6.096	−6.401
2	−5.967	−6.173	−6.805
3	−6.026	−5.595	−6.611
4	−5.693	−5.993	−6.789
5	−6.13	−5.793	−6.579
6	−6.096	−5.813	−6.650
7	−5.663	−5.461	−6.407
8	−5.618	−5.432	−6.448
9	−5.750	−5.406	−6.694
10	−5.746	−5.161	−6.465

A: Standard ligand ((1S)-2,2-difluoro-1-[1-(pentan-3-yl)-1H-pyrazol-4-yl]ethan-1-ol), B: Isopinocamphone, C: Oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)-.

methylphenyl)- showed hydrogen bond interactions with GLN 432 and LYS 654, Pi-cation with LYS 654 and Pi-pi stacking with TYR 525 (Fig. 11).

4. Discussion

The use of insecticides is a major control measure for eradicating *Ae. aegypti* mosquitoes. Similarly, a vital means to control the dengue fever epidemic is by killing both the larvae and adult *Ae. aegypti* mosquitoes using insecticides [5]. However, it is now well known insecticides are dangerous due to a number of reasons, such as the emergence of insecticide resistance, which causes pest resurgence, the disruption of ecosystems, concerns for human health and the environment, and their toxicity to non-target organisms [19]. As a result, considering their efficacy and safety, plant-based bioinsecticides have become more popular in recent years as alternatives. The use of isolated compounds, EOs inclusive, is beneficial owing to the possibility of their synthesis, enabling the possibility of obtaining them in large quantities necessary for practical use [20].

Although there are numerous studies on the effectiveness of botanicals as possible sources of physiologically active toxins to mosquito larvae, only very few of them have been made commercially available and widely employed in mosquito control schemes. Insufficient quality assessment and an inability to identify the structure and percentage of active ingredients responsible for the larvae's mortality are the main reasons for this [21].

For the larvicidal and adulticidal assay, results showed that HASEO was lethal against *Ae. aegypti* larvae and adult mosquitoes. However, HASEO was more effective as a larvicide rather than as an adulticide against *Ae. aegypti* mosquitoes. This may be due to the variation in the body structure of each developmental stage. Hence, difference in the toxicity of HASEO. The recorded LC_{50} values of HASEO against larvae and adult *Ae. aegypti* imply that HASEO was more effective with increase in exposure duration. The increase in toxicity on larva and adult *Ae. aegypti* mosquitoes with increase in exposure time to HASEO corroborates previous studies [8,22]. The LC_{50} values obtained in this study was higher than the values previously reported for lemon, peppermint, and lavender EOs [5], but lower than the values reported by Sina and Shukri [23]. EOs from various plants have been reported to be lethal against *Ae. aegypti* larvae and adult mosquitoes [5,8,21]. From the study it was observed that the adulticidal and larvicidal activities of HASEO was dependent on the concentration as well as the duration of exposure to the EO.

The major constituents compounds present in HAESO, especially 1-Chlorononadecane, might be responsible for its larvicidal and adulticidal activity against *Ae. aegypti* mosquitoes. 1-Chlorononadecane is a chlorinated alkane, a class of compounds composed of hydrocarbons (alkanes) with one or more chlorine atoms substituted into the molecule. Chlorinated alkanes and various chlorinated hydrocarbons have been reported to possess larvicidal, insecticidal and pesticidal activities and have been found to be major constituents of some biopesticides (González-rizo et al., 2019; Monisha et al., 2020). The second major compound present in HASEO was 1, 1-Cyclobutanedicarboxamide, 2-phenyl-N, N′-bis(1-phenylethyl)-. 1, 1-Cyclobutanedicarboxamide, 2-phenyl-N, N′-bis (1-phenylethyl)- is a diamide compound and has been studied for its potential insecticidal activity. Amides and diamides have been reported to possess larvicidal/insecticidal activity against *Ae. aegypti* mosquitoes and other insects [24,25]. Diamides are a type of

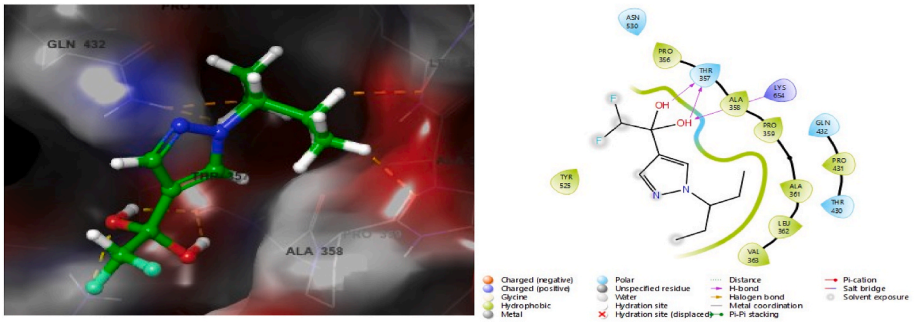


Fig. 6. Molecular interactions of (1S)-2,2-difluoro-1-[1-(pentan-3-yl)-1H-pyrazol-4-yl]ethan-1-ol with *Ae. aegypti* AChE.

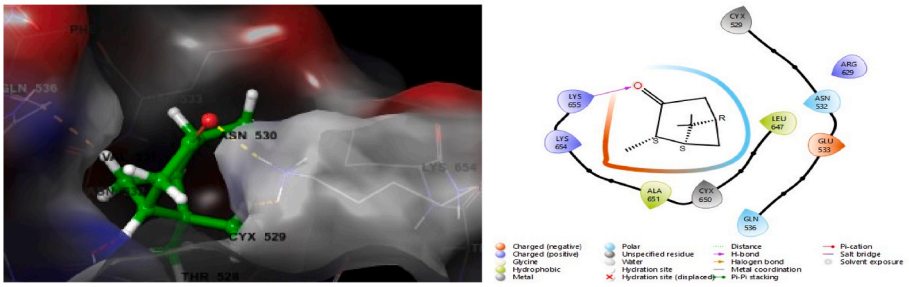


Fig. 7. Molecular interactions of isopinocamphone with *Ae. aegypti* AChE.

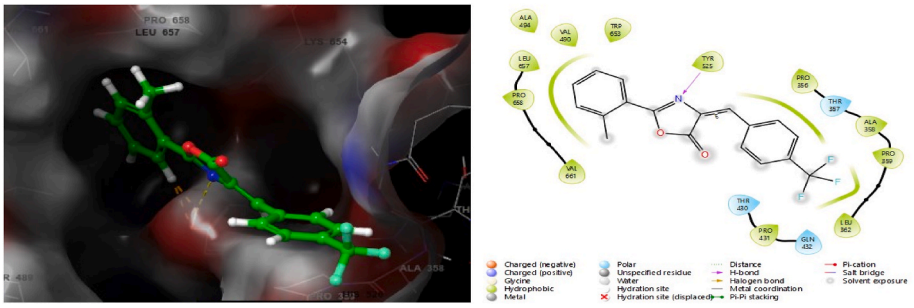


Fig. 8. Molecular interactions of Oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- with *Ae. aegypti* AChE.

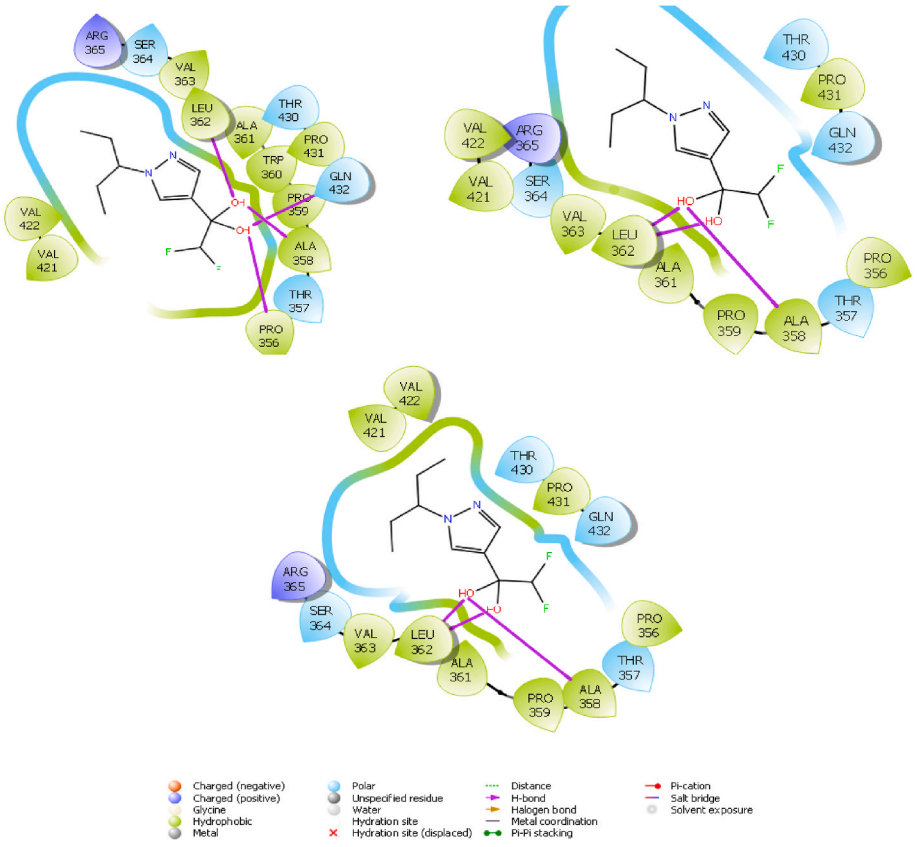


Fig. 9. Best three poses of (1S)-2,2-difluoro-1-[1-(pentan-3-yl)-1H-pyrazol-4-yl]ethan-1-ol in the binding site of *Ae. aegypti* AChE following the induced fit docking.

insecticide that work by binding to a specific receptor in insect muscle cells, causing the channels in the receptor to remain open for an extended period of time. This leads to overstimulation of the nervous system and paralysis of the insect. The calcium ion channel receptor, also known as Ryanodine receptor (RyR), presents a viable target for pesticide use. Diamides have shown to be highly effective in activating the RyR, which in turn disrupts the regular muscle contraction process by releasing stored calcium from the sarcoendoplasmic reticulum [26].

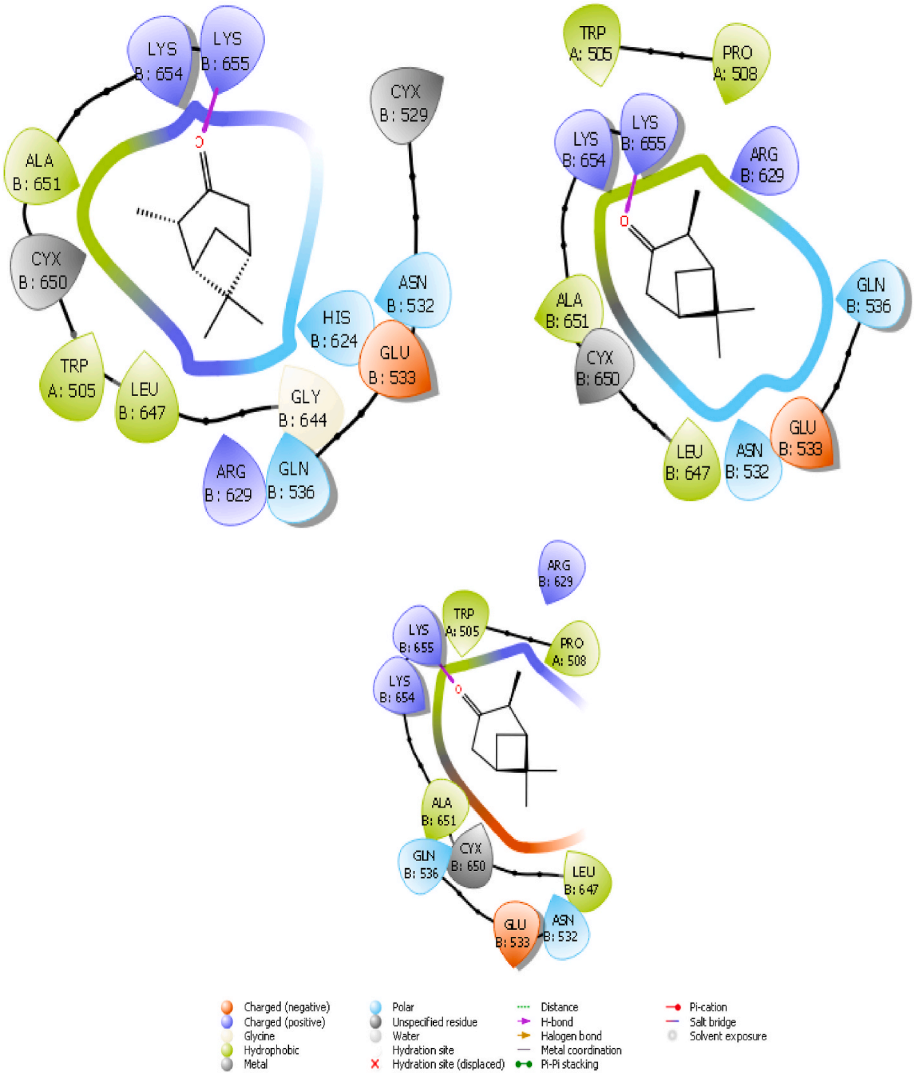


Fig. 10. Best three poses of isopinocamphone in the binding site of *Ae. aegypti* AChE following the induced fit docking.

Another prominent component of HASEO is 1-Nonadecanol. 1-Nonadecanol, a straight-chain fatty alcohol, has been reported to possess insect repellent properties [27], lethal against beetles (IARI, 2018), antifeedant effect on pea aphids [28,29]. Z)-4-Decen-1-ol, pentafluoropropionate, another prominent compound in HASEO is an ester derivative of (Z)-4-decen-1-ol, which is a fatty alcohol. Z)-4-Decen-1-ol, pentafluoropropionate may have insecticidal activity due to the presence of the pentafluoropropionate group, which can enhance the toxicity of the compound against insects. Some studies have investigated and reported the insecticidal properties of similar pentafluoropropionate containing compounds. Tea tree EO and *Azolla pinnata* extracts which contained a pentafluoropropionate containing compounds as a major constituent was reported to possess larvicidal activity against *Rhipicephalus annulatus* and *Ae. albopictus* respectively [30,31]. The insecticidal activities of pentafluoropropionate containing compounds have been reported by several authors [31–33].

A homology model of *Ae. aegypti* AChE was constructed using a template (i,e. the crystal structure of an insecticide-resistant AChE mutant from *Anopheles gambiae*). The developed model, which had a sequence identity of 92.58 %, a GMQE of 0.74, and a global QMEANDisCo score of 0.92 ± 0.05, resembled the template and therefore was considered of good quality for the molecular docking study. The GMQE and QMEANDisCo global scores are parameters used to assess the overall quality of the model and they comprise of values ranging between 0 and 1, with higher numbers indicating higher expected quality [34,35]. In addition to the high GMQE and QMEANDisCo global scores, the QMEAN score further validates the quality of the model. As illustrated in the QMEAN Z-score Comparison plot, the model falls within the experimental structures having a QMEAN score within one standard deviation of the mean, and this represents a nearly native structure [36]. In addition, the Local Quality plot illustrates the predicted similarity of each model residue to the native structure. Residues with a score below 0.6 are typically thought to be of low quality [34,35], and interestingly, the majority of the residues in the newly built *Ae. aegypti* AChE scored above 0.6. Hence the structure was used for the molecular docking analysis of HASEO compounds.

To identify potential AChE inhibitors from the HASEO compounds, molecular docking analysis of the GCMS-identified compounds was conducted against *Ae. aegypti* AChE. The cholinergic enzyme, AChE, facilitates the degradation of acetylcholine into acetic acid and choline, thus ending nerve transmission at the synapse [34]. Acetylcholine is one of the most abundant neurotransmitters within the central nervous system (CNS) of both vertebrates and invertebrates, and the activity of acetylcholine is tightly regulated by AChE-catalyzed hydrolysis. Numerous insecticides, such as organophosphates and carbamates, function as specific inhibitors of insect AChE [35,36]. Since AChE is responsible for terminating impulse transmission in insects, the inhibition caused by these insecticides leads to an accumulation of

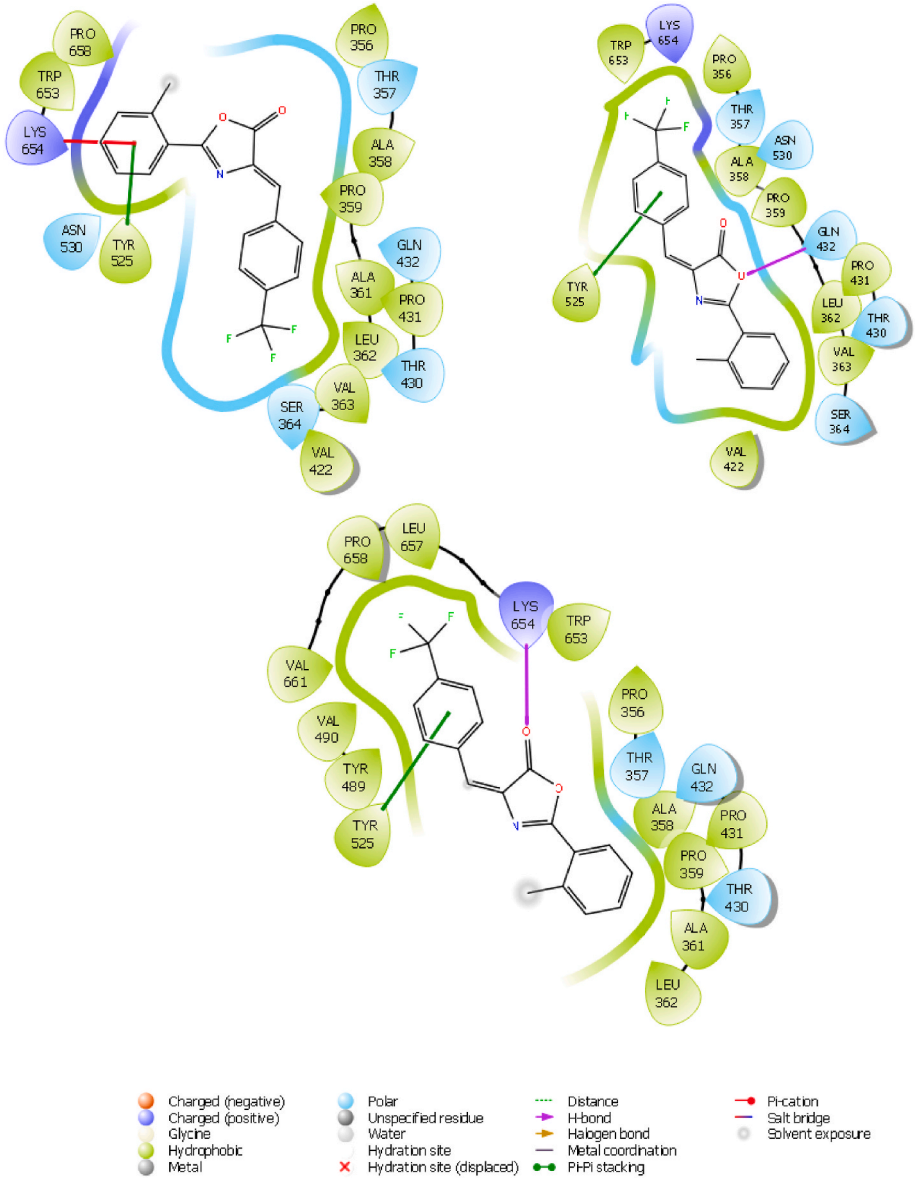


Fig. 11. Best three poses of Oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- in the binding site of *Ae. aegypti* AChE following the induced fit docking.

acetylcholine in synapses. This, in turn, keeps acetylcholine receptors constantly activated, giving rise to various toxicological consequences [34,36]. In the molecular docking analysis, 13 *H. annuus (L.)* compounds were found to bind to the AChE binding site with varying affinities. Isopinocamphone demonstrated the highest binding affinity as shown by its lower binding energy and it was followed by oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)-. The induced-fit docking analysis of these two compounds showed even higher docking scores and improved molecular interactions. Induced-fit docking (IFD) is a method used to simulate the protein structural changes triggered by the binding of a ligand [37]. In typical docking investigations, the receptor remains inflexible while the ligand exhibits mobility. Nevertheless, in reality, numerous proteins undergo structural alterations when interacting with. Upon the binding of a ligand to its receptor, the receptor can adapt its binding site to more effectively match the structure and binding mechanism of the ligand [38]. Therefore, the IFD was utilized in this research to predict more realistic binding interactions between the selected compounds and the *Ae. aegypti* AChE.

Apart from giving higher docking scores, more important interactions were generated from the IFD, most especially with oxazol-5(4H)-one, 4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- displaying hydrogen bond interactions with GLN 432 and LYS 654, pi-cation with LYS 654 and pi-pi stacking with TYR 525. The involvement of aromatic interactions (pi-cation and pi-pi stacking) in addition to hydrogen bond formation, in the interaction of this compound with the enzyme, might have contributed to the higher binding affinity of the compound. The presence of hydrogen bonds is a significant predictor of a molecule's inhibitory capacity against a protein target. Aromatic interactions are also an essential feature for several protein–ligand and protein–protein interactions [39,40]. These interactions are thought to be crucial for protein stability and molecular recognition. Therefore, the ability of the selected *H. annuus (L.)* to exhibit these crucial molecular interactions with *Ae. aegypti* AChE is indicative of their inhibitory potential against the protein and their potential insecticidal activity.

5. Conclusion

It can be concluded based on the outcomes of this investigation that

HASEO possesses larvicidal and adulticidal activities on *Ae. aegypti* mosquitoes, the dengue fever virus vector. The compounds in HASEO, especially isopinocamphone and oxazol-5(4H)-one,4-(4-trifluoromethylbenzylidene)-2-(2-methylphenyl)- demonstrated potential inhibitory activity against *Ae. aegypti* AChE. Therefore, HASEO may be useful as an effective biopesticide for the control of *Ae. aegypti* adult and larva and may serve as an alternative to synthetic insecticides.

CRediT authorship contribution statement

Babasoji Percy Omoniwa: Conceptualization, Supervision, Validation, Writing – review & editing. **Titilayo Omolara Johnson:** Software, Validation, Writing – review & editing. **Marvellous Amarachi Acho:** Writing – original draft. **Charles Obiora Nwonuma:** Writing – original draft, Writing – review & editing. **Omolola Soji-Omoniwa:** Data curation, Formal analysis. **Adekemi Grace Oluwafemi:** Writing – original draft. **Blessing Eshionimi Tomo:** Investigation. **Kayode Odejobi:** Investigation. **Omokolade Alejolowo:** Writing – original draft. **Emmanuel Olorunleke Oludipe:** Writing – original draft.

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.

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