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# Activity of *Artemisia pallens* leaf extracts against *Culex quinquefasciatus* larva and *in silico* docking of selected compounds against Acetylcholinesterase

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**Abstract**
Developed mosquitocidal resistance and associated health problems caused by synthetic insecticides, pave the way to control the mosquito population by using botanicals. *Artemisia pallens* / Davana, is renowned for its fragrance properties and thus well exploited in perfumery industries. The present study aimed to assess the larvicidal activity of *Artemisia pallens* leaf extracts against *Culex quinquefasciatus*. The preliminary qualitative analysis of the phytoconstituents and larvicidal activity against the fourth instar larvae of *Culex quinquefasciatus* was performed to analyse the efficacy of the chloroform, methanolic, and aqueous extracts of *Artemisia pallens* leaves. The chemical profiling of two extracts (Chloroform, Methanol) with better larvicidal activity was done by GC-MS. 3 compounds with insecticidal activity was docked against Acetylcholinesterase enzyme. All the extracts contain variable quantities of important secondary metabolites. The chloroform and methanolic extracts of *Artemisia pallens* leaves exhibit better larvicidal effect on *Culex quinquefasciatus* with a low $LC_{50}$ value. The GC-MS analysis revealed the presence of 21 and 36 phytocomponents in chloroform and methanolic extracts of *Artemisia pallens* leaves respectively. Amongst the chosen metabolites valproic acid, pseudo solasodine diacetate, and alpha-terpineol, pseudo solasodine diacetate binds with lowest energy (-9. 86 Kcal/Mol) against Acetylcholinesterase. Thus *Artemisia pallens* leaves could serve as a natural weapon against *Culex quinquefasciatus* in the larval stage and could also control the adult form by blocking their nervous activity. As plant-based mosquitocides, are safe to humans and environment, *Artemisia pallens* leaves could be used to control mosquito borne diseases.

**Keywords:** *Artemisia pallens, Culex quinquefasciatus,* fourth instar larvae, larvicidal bioassay, GC-MS analysis, *in silico* molecular docking

## Introduction
Though the earth is equally domicile for every living organism, some usually become unnerving to the others especially to the human community causing several ailments. Mosquitoes are blood-sucking insects belonging to the *Culicidae* family which are potent vectors for transmitting diseases such as malaria, dengue fever, yellow fever filariasis, etc. They cause a devastating impact on human health, especially in tropical and subtropical countries. *Culex quinquefasciatus* (Diptera: *Culicidae*) is the vector for the fastest spreading disease lymphatic filariasis affecting more than 146 million people in the tropics [1], tropical pulmonary eosinophilia in Pakistan [2] and transmitting malaria in birds [3]. *Culex quinquefasciatus* widely spread and breeds in stagnant water bodies like pools, marshes, wells, ponds, ditches, and river margins [4, 5, 6].
Mosquitoes were generally controlled by chemical insecticides like malathion, methoprene, DDT, carbamates, diflubenzuron, fenthion, and pyriproxyfen, [7]. Frequent, constant, and arbitrary use of these chemicals has affected human health, contaminated the environment, developed resistance to pests, and resulted in emergence of refractory vector behaviour [8]. Though several organisms are available to control mosquitoes like microbes, natural predators, etc. botanical insecticides obtained from plants were considered as eco-friendly as they are degradable, safe, have less or no toxic ill effects on humans and the environment, and also easily available at a low cost.

The secondary metabolites produced by plants are good insecticides, antifeedants, oviposition deterrents, repellents, juvenile hormone mimics, molting hormone mimics, and attractants [9]. The activity of many plant extracts was reported like essential oil of *Lippia alba* against *Cx. Quinquefasciatus* and *Aedes aegypti* [10], *Schinus terebinthifolia* essential oil against *Ae. Aegypti* [11], *Brassica nigra*, and *Aloe pirottae* extracts against *Anopheles arabiensis* [12] and *Rhinacanthus nasutus* leaves against *Cx. Quinquefasciatus* and *Ae. Aegypti* [13].

*Artemisia pallens* Wall ex. Dc (Sagebrush / Wormwood / Davanam) is a small, annual herb, an herbaceous plant widely distributed in the humid habitats of India. This plant species is widely cultivated in Sothern parts of India mainly for its medicinal values and aromaticity. Their beguiling fragrance properties have been exploited by several perfumeries and food industries. The essential oil from *Artemisia pallens* was found to possess antibacterial, antifungal, analgesic, anti-inflammatory, and antidiabetic activities making them significant in the medical industry [14]. The current study aimed to examine the larvicidal efficacy of the leaf extracts (aqueous, methanol, and chloroform) of the *Artemisia pallens* against *Culex quinquefasciatus* larva in the fourth instar stage, to analyze the chemical composition of the two promising extracts by GC-MS and prove the insecticidal potential of three compounds (among the best extract) against Acetylcholinesterase enzyme by *in-silico* docking method.

## 2.Materials and Methods

### 2.1 Collection of the plant material

The plant material *Artemisia pallens* was procured from Koyambedu Flower market, Chennai and was authenticated by Prof. P. Jayaraman (Director, Institute Of Herbal Science Plant Anatomy Research Centre) and a voucher specimen (No. PARC/2019/4132) was allotted for future references.

### 2.2 Extraction of *Artemisia pallens* & Collection of *Culex quinquefasciatus* larva

*Artemisia pallens* leaves were shade-dried, coarsely powdered and extracted with chloroform, methanol and water and the extracts were stored for further analysis. Preliminary qualitative phytochemical analysis of all the three extracts (aqueous, methanol and chloroform) of *Artemisia pallens* leaves was done [15, 16]. The fourth instar larva of *Culex quinquefasciatus* was collected from Broadway area, Chennai, Tamil Nadu.

### 2.3 Larvicidal Bioassay

Different concentrations (1000, 500, 250, 125 and 62.5 ppm) of aqueous, methanol and chloroform extracts of *Artemisia pallens* leaves were prepared. Larvicidal activity was performed by placing 25 fourth instar stage larvae of *Culex quinquefasciatus* in beaker and their motility were observed for 24 hours.

The beakers were kept in a room temperature with its mouth covered by muslin cloth and the larvae exposed to the water were considered as the control. The assay was replicated three times. No food was provided to the larva during the experiment. At the end of 24 hrs, the mortality rate was recorded.

### 2.4 GC-MS Analysis

With the results obtained from larvicidal assay, two promising extracts (methanol and chloroform) of *Artemisia pallens* leaves were analysed by GC-MS. Initially, the oven temperature was kept stable at 50 °C and slowly raised to 310ºC initially at the rate of 40ºC/min till 170ºC and then at the rate of rate of 10ºC/min in a HP 5 MS capillary column (Agilent, USA) (5% phenyl 95% poly methylsiloxane, 30m × 0.25µm ×0.25µm column). The temperatures of injector and detector were set at 280 ºC and 260 ºC, respectively and helium (99.999%) was used as carrier gas at a flow rate of 1 mL/min.

### 2.5 *In-silico* studies - Molecular docking

#### 2.5.1 Protein preparation

The sequence of *Culex quinquefasciatus* – Acetyl cholinesterase was retrieved from uniprot database (Q867X2) and as the structure of Acetyl cholinesterase was unavailable so it was modelled using Swiss model server using the template 5X61 – A Chain.

#### 2.5.2 Ligand Preparation and Optimization

The SMILES data of Valproic Acid, α-Terpineol and Pseudo solasodine diacetate were retrived from Pub Chem using Chemsketch Software. Its 3D structure was generated, optimised and saved as in a .mol file and it was converted by open BABEL molecular converter program and saved in PDB format [17].

#### 2.5.3 Docking analysis

For the calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors) Molinspiration tool was applied [18]. Docking of the ligands (Valproic Acid, α-Terpineol and Pseudo solasodine diacetate) against acetylcholinesterase was done using the Auto Dock software and visualised by Accelrys Discovery Studio Visualizer.

### 2.6 Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating $LC_{50}$ and other statistics at 95% confidence limit with upper confidence limit and lower confidence limit, which were calculated using EPA probit analysis software version1.5.

## 3. Results and discussion

### 3.1 Preliminary analysis

The preliminary phytochemical screening of the three extracts (Aqueous, Methanol, and Chloroform) of *Artemisia pallens* leaves confirms the presence of phenols, tannins, flavonoids, reducing sugars, glycosides, quinones, proteins, and steroids in variable quantities (Table 1). Saponins are absent in chloroform extract of *Artemisia pallens* leaves. Many have reported that the secondary metabolites like phenols, alkaloids, saponins, tannins, terpenoids, and steroids are very effective in controlling mosquitoes by acting as repellents and oviposition deterrents [19], or by inhibiting their development [20], and metamorphosis [21]. Thus the extracts of *Artemisia pallens* leaves as a crude mixture of active phytoconstituents could act as an insecticide to reduce the mosquito population.

**Table 1:** Preliminary phytochemical analysis of aqueous, methanol and chloroform extracts of *Artemisia pallens*

| S.no | Phytochemicals | Aqueous | Methanol | Chloroform |
|---|---|---|---|---|
| 1. | Reducing sugar | +++ | +++ | +++ |
| 2. | Glycosides | + | ++ | ++ |
| 3. | Protein | ++ | +++ | +++ |
| 4. | Saponins | +++ | ++ | - |
| 5. | Phenol | ++ | +++ | +++ |
| 6. | Tannin | +++ | ++ | ++ |
| 7. | Alkaloids | ++ | +++ | + |
| 8. | Flavonoids | ++ | ++ | + |
| **9** | Terpenes | + | ++ | ++ |

+ - mild; ++ - moderate; +++ - more

### 3.2 Larvicidal Bioassay

From the results of probit regression analysis for different concentrations of aqueous, methanol, and chloroform extracts of *Artemisia pallens* leaves, the calculated $LD_{50}$ values are presented in Table 2. The $LD_{50}$ value of the methanol extract of *Artemisia pallens* leaves was better (489.13 ppm) when compared with chloroform (1015.34 ppm) and aqueous (1150.48 ppm) extracts. Studies by Deepak *et al.* [22] have stated that larvicidal activity of *Cassia occidentalis* (Linn.) whole plant extract was due to the presence of flavonoids, tannins, phenols, and glycosides. The extracts from different parts of the plants were proved to possess larvicidal potential as exhibited by leaf extract of *Mesua ferra* and *Typhonium trilobatum* [23, 24], flower extract of *Tiliacora acuminate and Tagetes erecta* [25, 26] *and* fruit extract of *Croton caudatus* [25] against *Culex quinquefasciatus.*

The larvicidal activities of the two potent extracts (methanol, chloroform) of *Artemisa pallens* leaves might be due to the presence of an appreciable amount of phytoconstituents as a complicated mixture of phenols, tannins, flavonoids, glycosides, alkaloids, terpenes and steroids when compared to aqueous extract. Thus methanol and chloroform extracts of *Artemisa pallens* leaves exhibit better larvicidal activity against the 4th instar stage larva of *Culex quinquefasciatus.* Hadjiakhoondia *et al.* [27] have also confirmed pronounced larvicidal activities of chloroform extract of *Tagetes minuta against Anopheles stephens*.

**Table 2:** Lethal dose ($LD_{50}$) of the extracts of *Artemisia pallens* leaves against fourth instar larvae of *Culex quinquefasciatus*.

| S.no | Extract of *Artemisia pallens* leaves | $LD_{50}$ (ppm) |
|---|---|---|
| 1. | Aqueous | 1150.48 |
| 2. | Methanol | 489.13 |
| 3. | Chloroform | 1015.34 |

### 3.3 GC-MS analysis

The chemical profiling of the two promising methanol and chloroform extracts of *Artemisa pallens* leaves by GC-MS has revealed 19 and 33 phytoconstituents respectively. Out of all the compounds analyzed, 8 compounds, Pseudo solasodine diacetate, Cyclohexene, 1-methyl-4-(1-methylethylidene)-, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-, α-Terpineol, Propanedioic acid, dipropyl-, diethyl ester, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid and phytol were found in both the extracts. As the study was focused on mosquitocidal properties, two constituents with insecticidal properties Valproic Acid (11. 81% in methanol extract) and α-Terpineol (2.09% in methanol extract and 1.46% in chloroform extract), and the third compound Pseudo solasodine diacetate which constituted the maximum peak area in both the extracts (28.36% in methanol extract and 11.69% in chloroform extract) were selected for the docking studies. These three phytoconstituents were docked against the acetylcholinesterase enzyme.

**Table 3:** Bioconstituents in methanol extract of *Artemisia pallens* leaves analysed by GC-MS.

| S.no | Retention time | Compound name | Peak area |
|---|---|---|---|
| 1. | 3.09 | Methyl 7,10,13,16,19-docosapentaenoate | 1.388% |
| 2. | 4.04 | Pseudo solasodine diacetate | 28.366% |
| 3. | 7.89 | Cyclohexene, 1-methyl-4-(1-methylethylidene)- | 1.669% |
| 4. | 8.33 | 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- | 1.284% |
| 5. | 9.11 | Valproic Acid | 11.817% |
| 6. | 10.21 | 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- | 1.689% |
| 7. | 10.41 | α-Terpineol | 1.459% |
| 8. | 11.08 | 1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate | 0.987% |
| 9. | 13.23 | Propanedioic acid, dipropyl-, diethyl ester | 0.824% |
| 10. | 13.64 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]- | 1.267% |
| 11. | 15.39 | β-D-Glucopyranose, 1,6-anhydro- | 2.309% |
| 12. | 16.95 | 12-Methyl-E,E-2,13-octadecadien-1-ol | 8.394% |
| 13. | 18.13, 18.38, 18.58 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 3.15% |
| 14. | 19.01 | Hexadecanoic acid, methyl ester | 0.990% |
| 15. | 19.46 | n-Hexadecanoic acid | 6.921% |
| 16. | 20.74 | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- | 3.491% |
| 17. | 20.85 | Phytol | 3.430% |
| 18. | 21.22 | 17-Octadecynoic acid | 14.190% |
| 19. | 21.29 | Eicosane | 6.372% |

**Table 4:** Phytocompounds identified by GC-MS in the chloroform extract of *Artemisia pallens* leaves

| S. No | Retention time | Compound name | Peak area |
|---|---|---|---|
| 1. | 3.09 | Methyl 18-fluoro-octadec-9-enoate | 0.270% |
| 2. | 4.04 | Pseudo solasodine diacetate | 11.692% |
| 3. | 6.48 | Bicyclo [3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)- | 3.272% |
| 4. | 7.13 | β-Phellandrene | 3.523% |
| 5. | 7.86 | Cyclohexene, 1-methyl-4-(1-methylethylidene)- | 4.700% |
| 6. | 8.45 | γ-Terpinene | 6.739% |
| 7. | 8.98 | Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis- | 13.131% |
| 8. | 10.23 | 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- | 2.922% |
| 9. | 10.42 | α-Terpineol | 2.092% |
| 10. | 11.08 | 1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate | 4.431% |
| 11. | 11.83 | 4-Terpinenyl acetate | 0.307% |
| 12. | 12.41 | Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)- | 1.081% |
| 13. | 12.90 | 2-Dodecene, (E)- | 0.571% |
| 14. | 13.21 | Propanedioic acid, dipropyl-, diethyl ester | 0.168% |
| 15. | 13.63 | Caryophyllene | 4.638% |
| 16. | 13.88 | 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene- | 0.616% |
| 17. | 14.07 | Humulene | 0.375% |
| 18. | 14.61 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 3.820% |
| 19. | 15.38 | Cetene | 1.881% |
| 20. | 15.64 | (-)-Spathulenol | 0.309% |
| 21. | 16.57 | 2-Propenoic acid, tridecyl ester | 0.412% |
| 22. | 17.61 | 3-Octadecene, (E)- | 2.320% |
| 23. | 18.11,18.37,18.56 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 3.43% |
| 24. | 18.99 | Pentadecanoic acid, 14-methyl-, methyl ester | 0.301% |
| 25. | 19.44 | n-Hexadecanoic acid | 6.666% |
| 26. | 19.53 | 5-Octadecene, (E)- | 3.650% |
| 27. | 20.73 | Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)- | 0.650% |
| 28. | 20.84 | Phytol | 4.337% |
| 29. | 21.20 | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | 5.877% |
| 30. | 21.49, 26.36 | 10-Heneicosene (c,t) | 3.33% |
| 31. | 23.30 | [1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)- | 0.684% |
| 32. | 23.57 | 3-Eicosene, (E)- | 1.150% |
| 33. | 23.89 | 1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1α,4aα,10aβ)]- | 0.650% |

### 3.4 Molecular docking analysis

Molecular docking by *in silico* approach is a useful tool to confirm the theoretical hypothesis of the experts / validate folkloric literature studies by reducing time and resources. This bioinformatics tool will create a breakthrough in elucidating the action of insecticides. The central nervous system of the insects like acetylcholine receptor, acetylcholinesterase, sodium channels, chloride ion channels, acetylcholine receptor, ryanodine receptors, mineralocorticoid receptors, and octopamine receptors [28] were the primary target sites for the pest control agents to act.

The sequence of Acetylcholinesterase of *Culex quinquefasciatus* was retrieved from the UniProt database and its sequence ID was Q867X2. The structure of Acetylcholinesterase was not available in the PDB database. Hence it was modeled using a Swiss model server using the template 5X61 – A Chain. The modeled structure was found to be highly plausible as it had 93.94% sequence identity with that of the template in Fig 1. Moreover, the Ramachandran plot also showed 87.7% of residues in the most favored regions and no residues in the disallowed region.

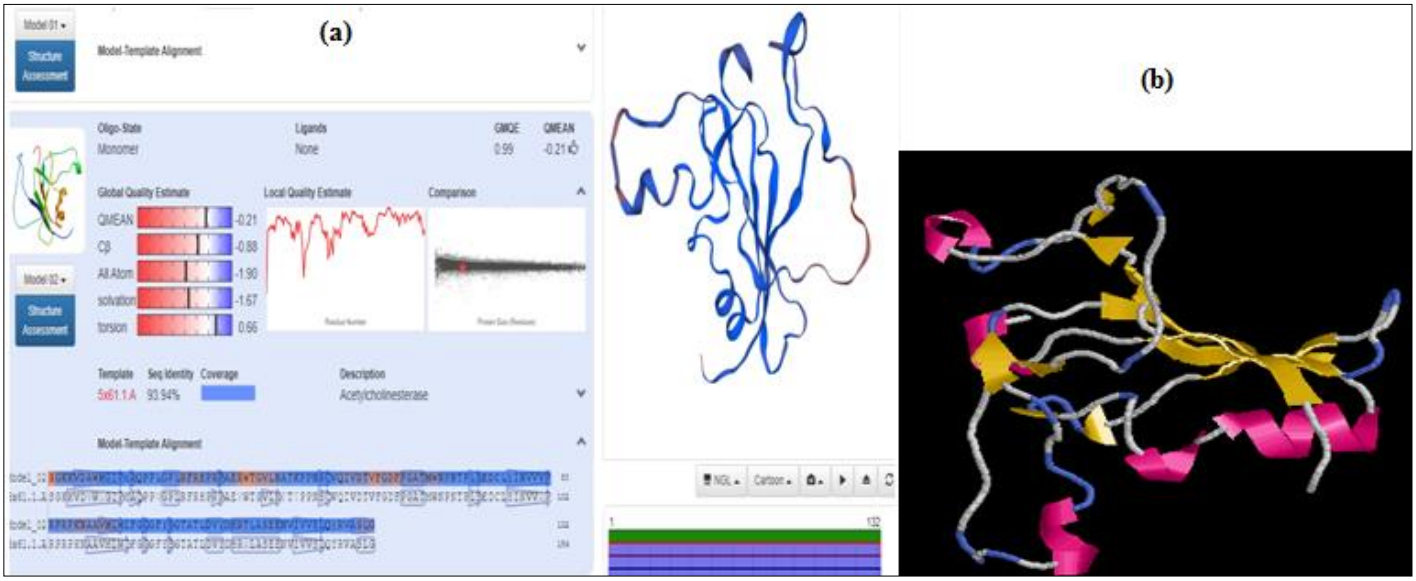


**Fig 1(a):** Acetylcholinesterase structure prediction by homology modelling using the Swiss-Model web server (b) 3D structure of Acetylcholinesterase

### 3.4.1 Lipinski rule predictions

The drug likeliness of compounds could be predicted by Lipinski's rule of five and Veber's rule. The "rule of five" states that if a molecule has a molecular weight less than or equal to 500 Da are likely to be orally active, log P (octanol-water Partition coefficient) ≤ 5 which tells whether the drug is hydrophilic or lipophilic, hydrogen bond donors less than or equal to 5, and acceptors less than or equal to 10 then it will have good permeability property [29]. Veber's rule states that TPSA should not exceed 140 Å for intestinal absorption.

The properties of the three selected compounds (Valproic acid, α-Terpineol, and Pseudo solasodine diacetate) satisfied the drug-likeliness properties and Veber's rule (Table 5) with zero violations except Pseudo solasodine diacetate with 1 violation; studies have stated that if a drug is developed the molecular descriptors can be controlled to obtain a harmless drug [30]. Hence all three chosen compounds qualify as drug-like compounds.

**Table 5:** Drug likeliness properties of Valproic acid, α-Terpineol and Pseudo solasodine diacetate

| S.no | Compounds | Mol. wt (Da) | Hydrogen Bond Donor | Hydrogen Bond Acceptor | miLog P | Rotatable bonds | N Violations | TPSA (Å) |
|---|---|---|---|---|---|---|---|---|
| 1. | Valproic acid | 144 | 1 | 2 | 2.80 | 5 | 0 | 37.3 Å |
| 2. | Alpha-terpineol | 154 | 1 | 1 | 2.60 | 1 | 0 | 20.2Å |
| 3. | Pseudo solasodine diacetate | 499 | 1 | 2 | 5.88 | 7 | 1 | 64.6Å |

### 3.4.2 Docking results

Of the three compounds, Pseudo solasodine diacetate showed the highest docking energy of -9.86 kcal/mol followed by α-Terpineol at -5.7 kcal/mol and Valproic Acid at -4.79 kcal/mol (Table 6) towards acetylcholinesterase. The types of interactions and residues involved in binding are shown in Table 6, Fig 2 and 3. Valproic acid forms hydrogen bonds of bond length 2.91 Å and 1.94 Å with Gly2 and Lys3 respectively. Three hydrogen bonds are formed by Met9, Thr39, and Lys40 with a bond length of 2.29 Å, 2.39 Å, and 1.86 Å respectively. Pseudo solasodine diacetate forms hydrogen bonds of lengths 2.92 Å and 2.89 Å with Leu91 and Thr112 residues respectively.

Pseudo solasodine diacetate has better binding energy towards acetylcholinesterase when compared to Valproic acid and α-Terpineol. Acetylcholinesterase (AChE) is the key enzyme of the central nervous system responsible for terminating the nerve impulse transmission by cleaving acetylcholine. Inhibition of acetylcholinesterase will lead to acetylcholine accumulation at the synaptic cleft and causes cholinergic stress [31] and thus used as a target to control mosquitoes [32]. In the present study, the binding of Pseudo solasodine diacetate to acetylcholinesterase will competitively inhibit the enzyme, and the accumulation of acetylcholine creates cholinergic stress which might lead to the death of the insects.

**Table 6:** Interactions of Valproic Acid, α-Terpineol and Pseudo solasodine diacetate towards Acetylcholinesterase residues.

| Ligand | Docking Energy (Kcal/Mol) | Type of Interaction | Residue Information |
|---|---|---|---|
| Valproic Acid | - 4.79 | Hydrogen bond | Gly2, Lys3 |
| | | Van Der Waals interactions | Ser1,Lys3,Lys4,Asp109,Asp112,Ser115 |
| | | Alkyl interactions | Val5, Arg111 |
| α-Terpineol | –5.7 | Hydrogen bond | Met9, Thr39,Lys40 |
| | | Van Der Waals interactions | Gly10,Leu36,Asn37,Ala30, Asn43 |
| | | Alkyl, π alkyl interactions | Pro42,Tyr74 |
| Pseudo solasodine diacetate | –9.86 | Hydrogen bond | Leu91, Thr112 |
| | | Van Der Waals interactions | Val29, Met90, Asp109, Arg111 |
| | | Alkyl, π alkyl interactions | Trp92, Tyr106,Leu113, Val121 |

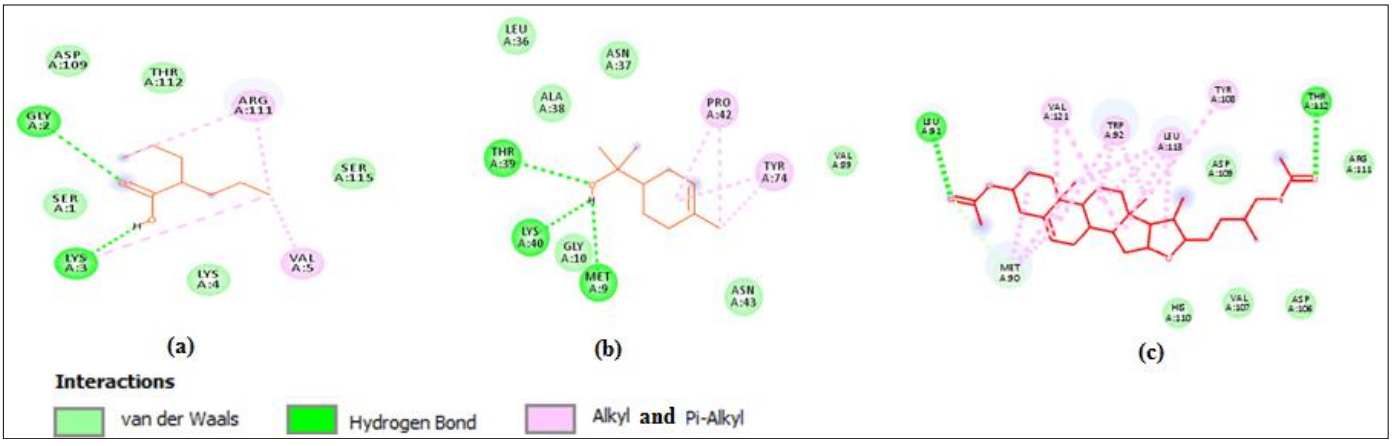


**Fig 2:** 2D interaction of Acetylcholinesterase with (a) Valproic Acid (b) α-Terpineol (c) Pseudo solasodine diacetate

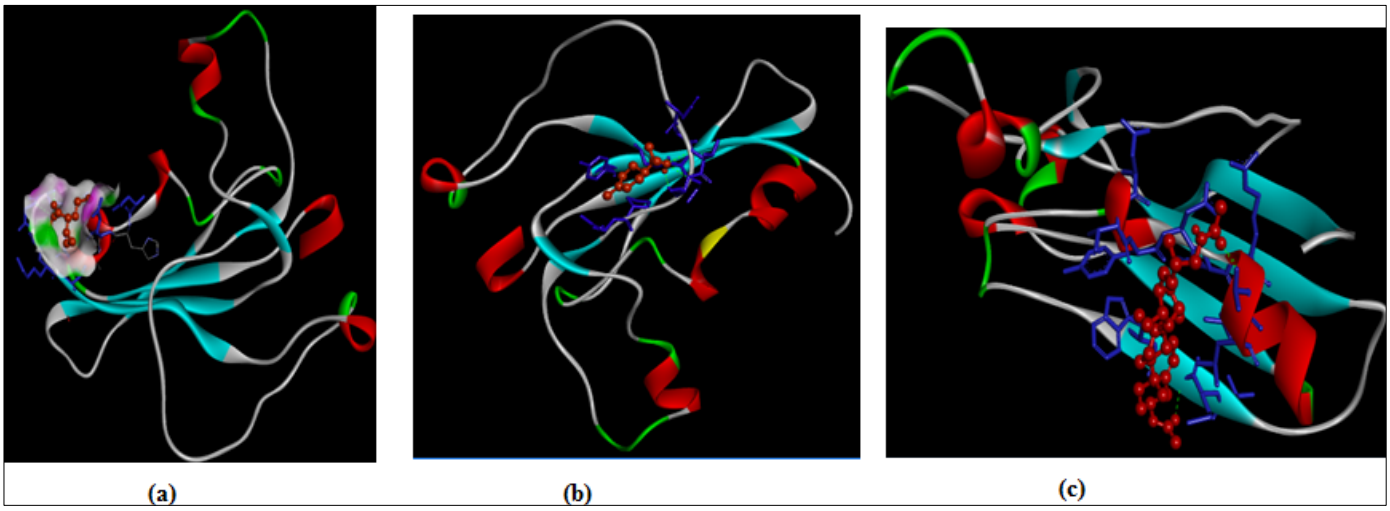


**Fig 3:** 3D molecular interaction of Acetylcholinesterase with (a) Valproic Acid (b) α-Terpineol (c) Pseudo solasodine diacetate

This was supported by the findings of Chaubey [33, 34] who reported that monoterpenes interfere with the activity of AChE. Thus the compound Pseudo solasodine diacetate could act as an insect repellent by blocking AChE and cause disturbance in neuro-transmission and has the potential to be included under insecticides. Based on the literature review, this is the first report on the effect of Pseudo solasodine diacetate, in binding to Acetylcholinesterase with high binding energy (-9.86 kcal/mol) than the normal substrate acetylcholine (-6.4 kcal/mol) [35]. Commercial insecticide organophosphates and carbamates also work by inhibiting Acetylcholinesterase [36].

## 4. Conclusion

Currently, the daunting problem faced by us is controlling the mosquito vector population. In the current study, methanol and chloroform extracts of *Artemisia pallens* leaves revealed potent larvicidal activity. GC-MS analysis of methanol and chloroform extracts of *Artemisia pallens* revealed the presence of active phytoconstituents. By *in-silico* docking studies, the bioactive compounds Pseudo solasodine diacetate, α-Terpineol, and Valproic acid showed strong inhibitory activity against the Acetylcholine esterase enzyme and thus provide evidence for the mechanism of action and insect behavior. Thus *Artemisia pallens* chloroform/methanol extract or the compounds Pseudo solasodine diacetate, α-Terpineol, and Valproic acid could be used as a botanical insecticide to reduce the *Culex quinquefasciatus* population and the diseases caused by them. In addition, this is the first study to report the Acetylcholinesterase inhibition by Pseudo solasodine diacetate in *Artemisia pallens* leaves. So, *Artemisia pallens* leaves could be added to stagnant water bodies/breeding sites or used as repellents to control *Culex quinquefasciatus* population and this could pave way for developing eco-friendly pest control agents.

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