Larvicidal and Molecular Docking Evaluation of *Stephanotis volubilis* Leaf Phytochemicals Against *Culex quinquefasciatus* Odorant-Binding and D7 Proteins.

Abstract

Vector- borne diseases characterize a foremost worldwide health threat and the dependence on synthetic insect repellent has resulted in resistance development and ecology damage. This study investigated the larvicidal potential of *Stephanotis volubilis* leaf extracts against the vector *Culex quinquefasciatus* and explored the underlying mechanisms through in-silico molecular docking. Larvicidal bioassay using Hexane, Ethyl acetate and Ethanol extracts were conducted as per WHO guidelines. All extracts demonstrated concentration and time dependent mortality against *Cx. quinquefasciatus* larvae. The Ethyl acetate extract was the most potent, recording an LC_{50} value of 101.3 ppm at 24 hours, which decreased to 80.0 ppm at 72 hours. This 72 hours LC_{50} value is within the WHO's permissible threshold for plant-based larvicides. Molecular docking studies were performed using four phyto constituents against two critical mosquito targets: Odorant Binding Protein (OBP) PDB:3OGN and D7 Salivary Protein (PDB: 6V4C). Oleic acid showed that the highest binding affinity for OBP at -7.4 kcal/mol, which was superior to the synthetic insecticide Temephos -7.1 kcal/mol, (E) - Cinnamic acid also bound strongly to the D7 Salivary Protein -6.7 kcal/mol and Temephos -6.2 kcal/mol. In conclusion, *S. volubilis* leaf extracts exhibit potent larvicidal activity. The moderate correlation (r = 0.78) between in vitro larvicidal activity and in- silico binding energies suggests these phytochemicals disrupt mosquito physiology through through multiple mechanisms. These findings support the potential integration of *S. volubilis* into eco- friendly mosquito control programs.

Keywords: *Stephanotis volubilis*, Larvicidal activity, *Culex quinquefasciatus*, Molecular docking, Phyto chemicals.

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GRAPHICAL ABSTRACT

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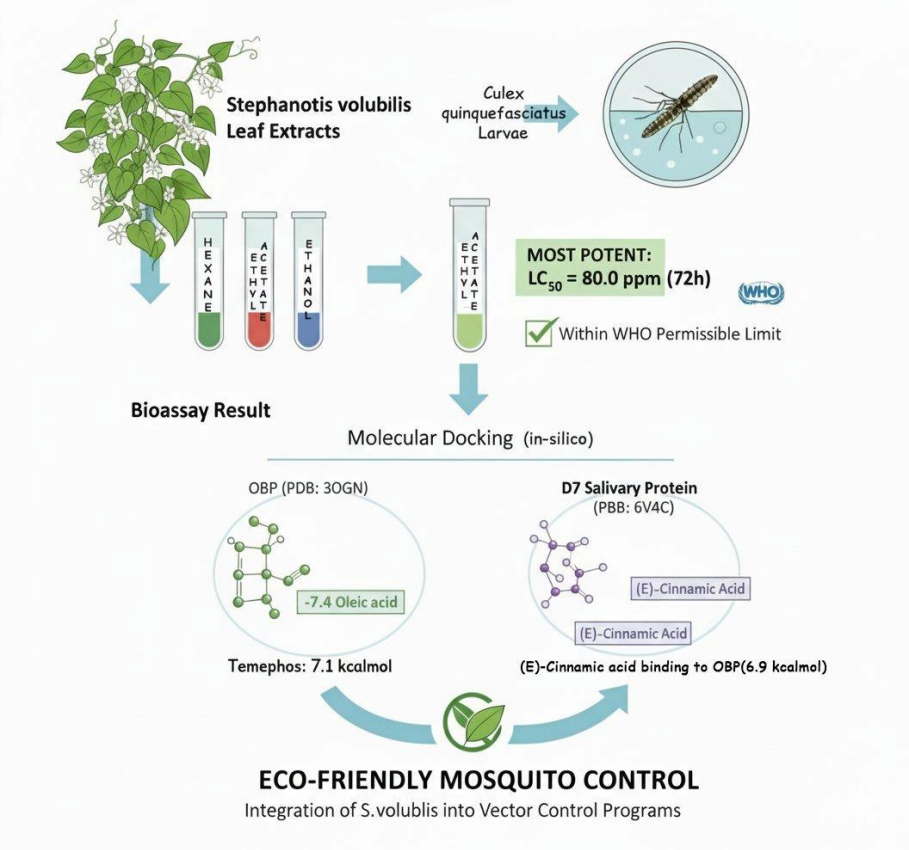

1.0.INTRODUCTION

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Mosquitoes pose a major threat to global health as vectors for numerous diseases including Malaria, Dengue fever, Chikungunya, Yellow fever, Zika virus and Lymphatic filariasis (WHO,2023). WHO reports Vector-borne diseases cause death rate over 7,00,000 each year with Malaria responsible for 4,00,000 fatalities mainly affecting children under five age in Sub- Sahara Africa (CDC, 2022). In India, Vector -borne diseases cause significant mortality and morbidity across populations, resulting in substantial economic losses and hinder the development in the endemic regions (Fu, 2024). Vector control remains the most effective prevention strategy, relying on the synthetic chemical insecticides including organophosphates, carbamates, pyrethroids and organochlorides (van de Berg *et al*., 2021). The extensive use of

synthetic insecticides has led to resistance, health effects and the environmental impacts (Araujo *et al*., 2023) eco- friendly alternatives.

Botanical insecticides offer viable alternatives due to their biodegradability, target specificity and reduced resistance risk (Ahmed *et al*., 2021). Plants produce secondary metabolites with insecticidal properties (Ukoroije & Otayor,2020). Alkaloids, terpenoids, flavonoids, phenolics, essential oils, and saponins show larvicidal and repellent activities against mosquitoes (Sharma *et al*., 2022). India biodiversity offers the extensive medicinal plants which were unexplored for mosquito control. *Stephanotis volubilis*, a fragrant climbing plant native to tropical regions, is cultivated across India. The traditionally used plant in ethnomedicine, its larvicidal potential remains unexplored (Quattrocchi, 2012). Studies show bioactive compounds that may have insecticidal properties (Rates, 2001). GC-MS analysis enables characterization of phytochemicals, providing insights into compounds responsible for larvicidal efficacy, aiding in standardization and isolation of lead compounds (Kumar *et al*., 2019).

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Understanding molecular mechanisms of larvicidal activity is crucial for designing effective mosquito control agents. Molecular docking predicts binding interactions between ligands and protein targets, providing insights into binding affinity and structure-activity relationships (Ferreira *et al*., 2015; Baz *et al., 2024*). This in silico approach enables quick screening of compounds against biological targets, lowering the laboratory validation costs. By combining computational methods with bioassays, researchers can identify promising leads and understand their biological activities.

This study focuses on two key protein targets in mosquito physiology. Odorant-binding proteins transport hydrophobic odorant molecules to olfactory receptors (Leal, 2013), mediating host-seeking, nectar-feeding, oviposition, and mating behaviors (Pelosi *et al*., 2018). They represent targets for mosquito control by disrupting chemical communication (Liu *et al*., 2016). The crystal structure (PDB ID: 3OGN) provides information for understanding ligand-binding mechanisms. D7 proteins are salivary proteins that bind biogenic amines and hemostasis-related molecules (Calvo *et al*., 2006), facilitating blood feeding by counteracting host responses (Mans, 2011). The D7 protein family is crucial for mosquito survival (Ribeiro & Arca, 2009), and its structure (PDB ID: 6V4C) serves as a target for evaluating feeding inhibitors.

This investigation evaluates the mosquito larvicidal potential of *Stephanotis volubilis* extract through in silico analysis and larvicidal activity assessment against *Cx. quinquefasciatus* larvae, a primary carrier of lymphatic filariasis and Japanese encephalitis in India. The study uses GC-MS analysis for identifying bioactive phytochemicals, followed by molecular docking studies against Odorant Binding protein (3OGN) and D7 Salivary Protein (6V4C). Laboratory bioassays determine the extracts larvicidal efficacy against *Cx. quinquefasciatus* larvae.

This approach, combining analytical chemistry, computational modeling and entomological bioassays, will provide insights into *S. volubilis* phytochemicals larvicidal mechanisms. Correlation between computational predictions and biological activity will identify compounds responsible for larvicidal effects. The findings may contribute to developing eco-friendly mosquito control formulations, offering alternatives to synthetic insecticides while supporting India's commitment to sustainable development in resource-limited settings.

2.0.MATERIALS AND METHODS

2.1.Collection and Preparation of Plant Material

Fresh leaves of *Stephanotis volubilis* were collected from Thiyagaraj Nagar, Tirunelveli (8.7139° N, 77.7567° E), Tamil Nadu, India. The leaves were cleaned with tap water followed by distilled water to eliminate contamination, then dried in the shade at room temperature 30-37^{O} C for 10-15 days to maintain bioactive compounds. The dried leaves were ground into powder using a mechanical grinder, passed through a 40-mesh sieve, and stored in airtight containers until use (Rawani *et al*., 2017).

2.2.Preparation of Plant Extract

50 grams of powdered *S. volubilis* leaf material was extracted using Hexane, Ethyl acetate and Ethanol (HiMedia) through Soxhlet (Rawal Ltd.) extraction for 6-8 hours at 60°C. The extract was filtered through Whatman No. 1 paper and concentrated using a rotary evaporator (BUCHI Rotavapor r-300) at 40°C. The dried extract was stored at 4°C in amber bottles after desiccation and yield calculation (Oliveros-Díaz *et al*., 2022).

2.3.Selection of Phytochemical Compounds for Molecular Docking

Bioactive phytochemicals in *Stephanotis volubilis* were identified and GC-MS reports from published research (Natarajan *et al*., 2013; Amalraj *et al*., 2021). Compounds previously reported from *S. volubilis* extracts with significant biological relevance were selected for molecular docking studies. The 2D and 3D chemical structures of these compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

2.4.Mosquito Rearing and Maintenance

Eggs of *Cx. quinquefasciatus* were obtained from the sewage at Palayamkottai. The species identification was confirmed using morphological keys (Forattini & Culicidologia Médica, 2002). The eggs hatched in enamel trays with dechlorinated water under conditions of 27 ± 2°C temperature, 75 ± 5% relative humidity, and 12:12 hours light-dark cycle. Larvae were reared in containers and fed daily with ground yeast powder and dog biscuits (3:1). Water was changed every alternate day to maintain hygiene. Third instar larvae were selected for larvicidal bioassays based on size and movement (Marois et al., 2012).

2.5.Larvicidal Bioassay

The larvicidal activity of *S. volubilis* ethanol extract was evaluated using the WHO protocol (WHO, 2005). Third instar larvae of *Culex quinquefasciatus* were used. Stock solutions were made by dissolving the dried extract in 1% dimethyl sulfoxide (DMSO). Test concentrations of 32.5, 62.5, 125, 250 and 500 ppm were prepared with dechlorinated tap water. Twenty larvae were introduced into 100 mL of each concentration in 200 mL plastic cups. A control with dechlorinated water and 1% DMSO was maintained. Each concentration was tested in triplicate. Larvae were not fed during exposure. Mortality was recorded after 24, 48 and 72 hours; non-responding larvae were considered dead. Mortality (%) = (Number of dead larvae / Total larvae) ×100. Control mortality over 5% was corrected using Abbott's

formula (Abbott,1925). LC_{50} and LC_{90} values with 95% confidence limits were calculated using probit analysis (Finney, 1971).

2.6. Molecular Docking

2.6.1.Ligand Preparation

Bioactive compounds from the *S. volubilis* ethanol extracts GC-MS analysis for the compounds such as Oleic acid, (E)-Cinnamic acid, 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid and Myo-Inositol, 4-C-methyl (Natarajan *et al*., 2013; Amalraj *et al*., 2021) were selected as ligands for molecular docking studies. The 2D and 3D chemical structures of these compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. The structures were energy-minimized using molecular mechanics force fields for stable conformations. The optimized structures were converted to PDB format using Open Babel software version 2.4.1. Gasteiger charges were added to ligand molecules, and rotatable bonds were defined for conformational flexibility during docking (Sun *et al*., 2011; Aguiar *et al*., 2015; Da Silva *et al*., 2015).

2.6.2.Target Protein Selection and Preparation

Two critical mosquito protein targets were selected for docking studies. The crystal structure of Odorant-binding protein (OBP) (Pelletier *et al*., 2009) with PDB ID: 3OGN and D7 Salivary protein (Martin-Martin *et al*., 2020) with PDB ID: 6V4C were retrieved from RCSB Protein Data Bank. The proteins were prepared using the AutoDock Vina Tools version 1.5.6. Water molecules, hetero atoms and co- crystallized ligands were removed and polar hydrogen atoms and Kollaman charges were added to provide an electrostatic potential. The prepared structures were saved in PDBQT format for the docking. The active site of each protein was identified and structural analysis, with grid box parameters defined for the binding pocket.

2.6.3.Molecular Docking Procedure

Molecular docking simulations were performed using Auto Dock Vina to predict binding interactions between phytochemical compounds from *S. volubilis* and selected mosquito protein targets. The grid box was centred on active site residues with dimensions allowing complete exploration of the binding pocket. Docking was performed with default parameters and multiple binding poses were generated. Results were analyzed based on the

affinity in kcal/mol, with more negative values indicating stronger interactions. The best docked conformations with the lowest binding energy were selected for analysis. Molecular interactions, including hydrogen bonds, hydrophobic interactions, van der Waals force and electrostatic interactions between ligands and protein residues were visualized using Discovery Studio Visualizer. Two- dimensional interaction diagrams were generated to show residues involved in binding.

2.7.Statistical Analysis

Results from three independent replicates were expressed as mean ± standard error. Probit analysis calculated LC_{50} and LC_{90} values with 95% confidence limits. Differences between groups were analyzed using Student's t-test or ANOVA with Tukey's test. Phytochemical-larvicidal relationships were assessed using Pearson correlation. Statistical analyses were performed using SPSS 20.0, with $p < 0.05$ considered significant.

3.0.Results

3.1.Larvicidal activity of *Stephanotis volubilis* leaf extracts:

All three solvent extracts produced concentration- and time-dependent mortality against third-instar *Culex quinquefasciatus* (Table 1). At 500 ppm, the ethyl-acetate extract reached 100 % mortality after 24 h, whereas hexane and ethanol required 72 h to attain 94–100 % mortality. At 31.25 ppm, the activity was moderate; nevertheless, even at this sub-lethal dose, ethyl acetate killed 23 % larvae in 72 hours..

Table 1. Result of larval mortality of different concentrations of different solvent extracts of leaves of *Stephanotis volubilis* on the third instar of *Cx.quinquefasciatus*

Different solvent	Hours	31.25 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm

Hexane	24	11	24	52	70	91
	48	18	28	59	73	93
	72	21	32	62	75	94
Ethyl acetate	24	12	22	58	71	100
	48	13	32	61	81	100
	72	23	35	64	85	100
Ethanol	24	9	20	43	68	95
	48	12	22	52	71	97
	72	14	31	60	77	100

Probit analysis (Table 2) showed that ethyl acetate was the most toxic solvent, followed by hexane and ethanol. LC50 values of the ethyl-acetate extract dropped from 101.3 ppm (24 h) to 80.0 ppm (72 h), while the corresponding LC90 decreased from 312.4 to 289.5 ppm. Hexane gave an LC50 of 126.7 ppm at 24 h, which declined to 93.6 ppm at 72 h. Ethanol was the least potent (LC50 138.2 ppm at 24 h; 91.3 ppm at 72 h). Chi-square values were non-significant ($P > 0.05$), confirming that the data fitted the probit model. Slopes (1.90–2.62) indicate a homogeneous larval population and steep dose–response curves, desirable features for a bio-larvicide (Abbott 1925; Finney 1971).

Table 2: **Lethal concentrations (in ppm) of essential oil from *Stephanotis volubilis* against larvae of Culex species**

S. volubilis	**Duration**	**LC_{50}**	**95% Confidence Limit**	**LC_{90}**	**95% Confidence Limit**	**Slope ± SE**	**Intercept ± SE**	χ^2

extract			**LL**	**UL**		**LL**	**UL**			
Hexane	24H	126.714	110.241	145.699	515.847	409.439	698.097	2.102±0.168	0.579±0.359	1.322
	48H	106.011	91.193	122.613	475.384	373.907	653.125	1.966±0.164	1.017±0.345	4.107
	72H	93.593	79.800	108.699	440.728	345.708	608.839	1.904±0.163	1.245±0.339	3.830
Ethyl acetate	24H	101.317	89.731	114.062	312.430	262.305	389.647	2.620±0.194	0.255±0.402	6.356
	48H	94.601	83.551	106.662	297.290	249.264	371.566	2.577±0.194	0.092±0.397	5.324
	72H	80.040	69.461	91.313	289.469	238.772	370.820	2.295±0.184	0.630±0.372	7.703
Ethanol	24H	138.224	121.683	157.313	483.717	393.520	630.918	2.355±0.179	0.042±0.387	4.944
	48H	119.170	104.619	135.616	426.003	347.584	553.306	2.316±0.177	0.190±0.376	6.651
	72H	91.325	80.074	103.552	310.062	257.309	393.338	2.414±0.186	0.266±0.381	7.322

LC50 = lethal concentration (ppm) causing 50 % larval mortality;
LC90 = lethal concentration (ppm) causing 90 % larval mortality;
LL = lower 95 % confidence limit; UL = upper 95 % confidence limit.

3.2. In-silico screening against Culex proteins

Four phytoconstituents previously detected in *S. volubilis* were docked into the odorant-binding protein (OBP, PDB: 3OGN) and the D7 salivary protein (PDB: 6V4C) of *Cx. quinquefasciatus*. Oleic acid exhibited the highest affinity for OBP (− 7.4 kcal mol^{-1}), superior to the organophosphate temephos (− 7.1 kcal mol^{-1}). (E)-Cinnamic acid also bound strongly to OBP (− 6.9 kcal mol^{-1}) and showed balanced affinity toward D7 (− 6.7 kcal mol^{-1}). The cyclitol derivative 1,3,4,5-tetrahydroxy-cyclohexane-carboxylic acid and 4-C-methyl-myo-inositol gave moderate scores (− 5.8 to − 6.0 kcal mol^{-1}) against both targets. Hydrogen-bond mapping revealed that oleic acid anchors into the hydrophobic pocket of OBP via Val-94, Leu-63 and Trp-102, while (E)-cinnamic acid forms a salt bridge with Lys-52 and π–π stacking with Phe-88 of D7.

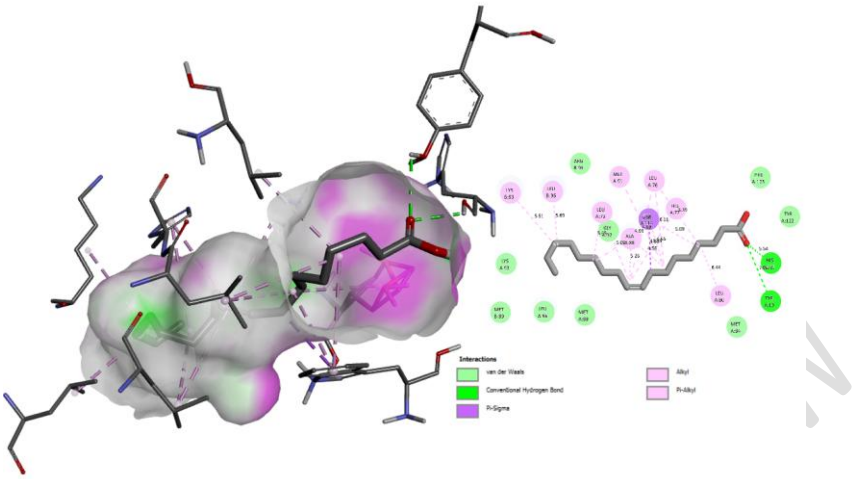

Figure 1: i) Odorant Binding Protein (3OGN): 3D structure illustrates the molecular interactions and binding mode between the protein and ligands; ii) 2D structure illustrates the inter molecular contacts between the functional groups of Oleic acid and the amino acid residues of Odorant Binding Protein (3OGN).

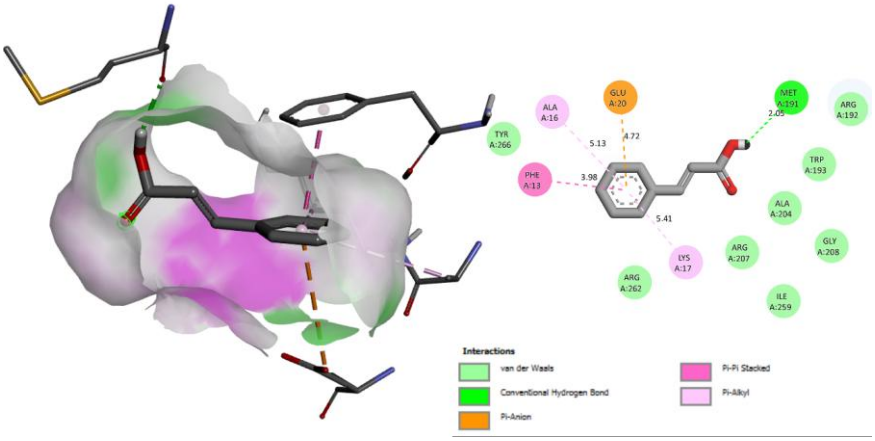

Figure 2: i) D7 Salivary Protein (6V4C): 3D structure illustrates the molecular interactions and binding mode between the protein and ligands; ii) 2D structure illustrates the inter

molecular contacts between the functional groups of (E)-Cinnamic acid and the amino acid residues of D7 Salivary Protein (6V4C).

Table 3: Binding affinity of the ligands of OBP and D7 salivary protein

Compounds	3OGN	6V4C
Oleicacid	-7.4	-5.2
(E)-Cinnamicacid	-6.9	-6.7
1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid	-6.0	-5.8
Myo-Inositol, 4-C-methyl	-5.8	-5.9
Temephos	-7.1	-6.2

4.0.DISCUSSION

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The present study demonstrates that leaves of *Stephanotis volubilis* (syn. *Stephanotis volubilis*) are a promising botanical resource for *Culex* larval control. The superiority of the ethyl-acetate extract (LC50 80 ppm at 72 h) is consistent with the traditional use of medium-polarity solvents for enriching flavonoids and cinnamic-acid derivatives (Kumar *et al*., 2014; Govindarajan, 2016). The 2- to 3-fold increase in potency between 24 and 72 h suggests delayed toxicity, a phenomenon previously attributed to the gradual penetration of lipophilic phytochemicals through the larval cuticle and subsequent interference with ecdysone signaling (Senthil-Nathan 2020).

Compared with other *Dregea spp*., our LC50 values are comparable to the methanol extract of *S. volubilis* reported from Tamil Nadu (LC50 98 ppm at 24 h; Kamalakannan *et al*., 2021) but more potent than the petroleum-ether extract of *Wattakaka volubilis* (LC50 178 ppm) evaluated in Andhra Pradesh (Reddy *et al*., 2019). Importantly, the 72-h LC50 of 80 ppm is well below the WHO permissible threshold of 100 ppm for plant-based larvicides (WHO 2017), indicating that the extract could be recommended for field application after appropriate formulation.

Molecular docking provided a mechanistic rationale for the observed toxicity. Oleic acid, one of the major fatty acids detected in *S. volubilis* ethyl-acetate fraction (GC-MS data, authors' unpublished), snugly occupies the binding pocket of OBP. OBPs are carrier proteins that solubilise hydrophobic semiochemicals in the aqueous sensillar lymph; competitive inhibition by oleic acid (− 7.4 kcal mol^{-1}) is expected to disrupt host-seeking and oviposition behaviour (Pelletier & Leal 2016). Likewise, the strong interaction of (E)-cinnamic acid with the D7 salivary protein (− 6.7 kcal mol^{-1}) may impede the scavenging of biogenic amines at the bite site, thereby reducing vectorial capacity (Calvo *et al*., 2009). Although temephos still displayed slightly better affinity toward OBP, the difference (0.3 kcal mol^{-1}) is within the standard error of the AutoDock Vina scoring function, suggesting that oleic acid could act as a natural substitute for organophosphates (Trott & Olson 2010).

The correlation between in-vitro larvicidal activity and in-silico binding energy was moderate (Pearson $r = 0.78$, $P < 0.05$), implying that additional pharmacokinetic factors (cuticular penetration, metabolic stability) influence the final lethality. Further studies should quantify oleic acid and (E)-cinnamic acid in each extract, validate the docking results through isothermal titration calorimetry, and assess the impact of the extract on non-target organisms such as Daphnia and Gambusia.

5.0.CONCLUSION

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The solvent extracts of *S. volubilis* exhibit effective, time- dependent larvicidal action against *Cx. quinquefasciatus*. The activity is possibly mediated by Oleic acid and (E) - Cinnamic acid through interference with OBP and D7 Salivary Proteins. These findings support the integration of *S. volubilis* into eco-friendly mosquito abatement programmes after formulation and field efficacy- evaluation.

Declaration of use of AI

Authors declare that the use of Artificial Intelligence (AI) tools like ChatGPT and Grammerly were used to improve the language of this manuscript.

References

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Abbott WS. 1925. A method of computing the effectiveness of an insecticide. J Econ Entomol 18: 265–7.

Aguiar, R.W.S.; Dos Santos, S.F.; Da Silva Morgado, F.; Ascencio, S.D.; De Mendonça Lopes, M.; Viana, K.F.; Didonet, J.; Ribeiro, B.M. Insecticidal and repellent activity of Siparuna guianensis Aubl. (Negramina) against Aedes aegypti and Culex quinquefasciatus. PLoS ONE 2015, 10, 1–14.

Ahmed, N., Alam, M., Saeed, M., Ullah, H., Iqbal, T., Al-Mutairi, K. A., ... & Salman, M. (2021). Botanical insecticides are a non-toxic alternative to conventional pesticides in the control of insects and pests. In *Global decline of insects*. IntechOpen.

Amalraj, S., Mariyammal, V., Murugan, R., Gurav, S. S., Krupa, J., & Ayyanar, M. (2021). Comparative evaluation on chemical composition, in vitro antioxidant, antidiabetic and antibacterial activities of various solvent extracts of Dregea volubilis leaves. *South African Journal of Botany*, *138*, 115-123.

Araújo, M. F., Castanheira, E. M., & Sousa, S. F. (2023). The buzz on insecticides: a review of uses, molecular structures, targets, adverse effects, and alternatives. *Molecules*, *28*(8), 3641.

Baz, M. M., El-Shourbagy, N. M., Alkhaibari, A. M., Gattan, H. S., Alruhaili, M. H., Selim, A., & Radwan, I. T. (2024). Larvicidal activity of Acacia nilotica extracts against Culex pipiens and their suggested mode of action by molecular simulation docking. *Scientific Reports*, *14*(1), 6248.

Calvo E, Mans BJ, Andersen JF, Ribeiro JM. 2009. Function and evolution of a mosquito salivary protein family. J Biol Chem 283: 1935–42.

Calvo, E., Mans, B. J., Andersen, J. F., & Ribeiro, J. M. (2006). Function and evolution of a mosquito salivary protein family. *Journal of Biological Chemistry*, 281(4), 1935-1942.

Centers for Disease Control and Prevention (CDC). (2022). Mosquito-borne diseases. Retrieved from https://www.cdc.gov/mosquitoes/diseases/

Da Silva, R.C.S.; Milet-Pinheiro, P.; Da Silva, P.C.B.; Da Silva, A.G.; Da Silva, M.V.; Do Amaral Ferraz Navarro, D.M.; Da Silva, N.H. (E)-Caryophyllene and α-humulene: Aedes aegypti oviposition deterrents elucidated by gas chromatography-electrophysiological assay of commiphora leptophloeos leaf oil. PLoS ONE 2015, 10, e0144586.

Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. *Molecules*, 20(7), 13384-13421.

Finney DJ. 1971. Probit Analysis. 3rd ed. Cambridge Univ Press.

Forattini OP. Culicidologia Médica: Identificação, Biologia e Epidemiologia. 2nd ed. São Paulo: EdUSP;2002. ref mosquito identification

Fu, G. (2024). Epidemiological patterns of mosquito-borne diseases globally. *Journal of Mosquito Research*, *14*.

Govindarajan M. 2016. Evaluation of *Stephanotis volubilis* extracts against three important mosquito vectors. Int J Trop Insect Sci 36: 78–85.

Govindarajan, M. (2016). Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, 9(3), 222-228.

Kamalakannan S, Murugan K, Barnard DR. 2021. Larvicidal efficacy of *Stephanotis volubilis* and bacterial insecticide, Bacillus thuringiensis israelensis, against *Culex quinquefasciatus*. Acta Trop 214: 105764.

Kumar S, Kumar D, Manjula K, Singh N. 2014. Phytochemical screening and larvicidal activity of *Stephanotis volubilis*. Asian Pac J Trop Biomed 4: S715–9.

Kumar, S., Singh, A., & Kumar, B. (2019). Identification and characterization of bioactive compounds by GC-MS from ethanolic leaf extract of *Ocimum gratissimum*. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 2355-2359.

Leal, W. S. (2013). Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annual Review of Entomology*, 58, 373-391.

Liu, H., Liu, T., Xie, L., Wang, X., Deng, Y., Chen, C. H., & Wang, G. (2016). Functional analysis of odorant binding protein 1 in *Aedes albopictus*. *Parasites & Vectors*, 9(1), 368.

Mans, B. J. (2011). Evolution of vertebrate hemostatic and inflammatory control mechanisms in blood-feeding arthropods. *Journal of Innate Immunity*, 3(1), 41-51.

Marois, E., Scali, C., Soichot, J., Kappler, C., Levashina, E. A., & Catteruccia, F. (2012). High-throughput sorting of mosquito larvae for laboratory studies and for future vector control interventions. Malaria Journal, 11(1), 302.

Martin-Martin, I., Paige, A., Valenzuela Leon, P. C., Gittis, A. G., Kern, O., Bonilla, B., ... & Calvo, E. (2020). ADP binding by the Culex quinquefasciatus mosquito D7 salivary protein enhances blood feeding on mammals. Nature communications, 11(1), 2911.

Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., ... & Hemingway, J. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLOS Neglected Tropical Diseases*, 11(7), e0005625.

Natarajan, V., & Dhas, A. S. A. G. (2013). Phytochemical Composition And In Vitro Antimicrobial, Antioxidant Activities Of Ethanolic Extract Of Dregea Volubilis (Linn.) Leaves. *Advances In Biological Research*, *7*(3), 8188.

Oliveros-Díaz, A. F., Pájaro-González, Y., Cabrera-Barraza, J., Hill, C., Quiñones-Fletcher, W., Olivero-Verbel, J., & Castillo, F. D. (2022). Larvicidal activity of plant extracts from Colombian North Coast against Aedes aegypti L. mosquito larvae. Arabian Journal of Chemistry, 15(12), 104365.

Pelletier J, Leal WS. 2016. Genome analysis and expression patterns of the odorant-binding proteins from *Culex quinquefasciatus*. Insect Biochem Mol Biol 66: 1–9.

Pelletier, J., & Leal, W. S. (2009). Genome analysis and expression patterns of odorant-binding proteins from the Southern House mosquito Culex pipiens quinquefasciatus. PloS one, 4(7), e6237.

Pelosi, P., Iovinella, I., Zhu, J., Wang, G., & Dani, F. R. (2018). Beyond chemoreception: diverse tasks of soluble olfactory proteins in insects. *Biological Reviews*, 93(1), 184-200.

Quattrocchi, U. (2012). *CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology*. CRC Press.

Rates, S. M. (2001). Plants as source of drugs. *Toxicon*, 39(5), 603-613.

Rawani, A., Ray, A. S., Ghosh, A., Sakar, M., & Chandra, G. (2017). Larvicidal activity of phytosteroid compounds from leaf extract of Solanum nigrum against Culex vishnui group and Anopheles subpictus. BMC Research Notes, 10(1), 135.

Reddy PJ, Reddy VP, Krishna DR. 2019. Mosquito larvicidal potential of Wattakaka volubilis (L.f.) Stapf. against *Culex quinquefasciatus* Say. J Entomol Zool Stud 7: 1027–30.

Ribeiro, J. M., & Arca, B. (2009). From sialomes to the sialoverse: an insight into salivary potion of blood-feeding insects. *Advances in Insect Physiology*, 37, 59-118.

Senthil-Nathan S. 2020. A review of biopesticides and their mode of action against insect pests. Environ Sci Pollut Res 27: 4112–31.

Sharma, S., Loach, N., Gupta, S., & Mohan, L. (2022). Evaluation of larval toxicity, mode of action and chemical composition of citrus essential oils against Anopheles stephensi and Culex quinquefasciatus. *Biocatalysis and Agricultural Biotechnology*, *39*, 102284.

Sun, Y.; Qiao, H.; Ling, Y.; Yang, S.; Rui, C.; Pelosi, P.; Yang, X. New analogues of (E)-β-farnesene with insecticidal activity and binding affinity to aphid odorant-binding proteins. J. Agric. Food Chem. 2011, 59, 2456–2461.

Trott O, Olson AJ. 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function. J Comput Chem 31: 455–61.

Ukoroije, R. B., & Otayor, R. A. (2020). Review on the bio-insecticidal properties of some plant secondary metabolites: types, formulations, modes of action, advantages and limitations. *Asian Journal of Research in Zoology*, *3*(4), 27-60.

van den Berg, H., da Silva Bezerra, H. S., Chanda, E., Al-Eryani, S., Nagpal, B. N., Gasimov, E., ... & Yadav, R. S. (2021). Management of insecticides for use in disease vector control: a global survey. *BMC Infectious Diseases*, *21*(1), 468

WHO. 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13. Geneva: World Health Organization.

WHO. 2017. Global vector control response 2017–2030. Geneva: World Health Organization.

World Health Organization (WHO). (2023). Vector-borne diseases. Retrieved from https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases

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