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In Silico Molecular Docking Analysis of Potent Larvicidal Constituents from Ethyl Acetate Extract of *Sphaeranthus amaranthoides* Burm. f. on *Culex quinquefasciatus* Say, 1823 (Diptera:Culicidae)

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Abstract: Mosquitoes spread deadly illnesses to humans, leading to millions of fatalities each year, and the emergence of resistance to chemical-based insecticides is leading to rising vectorial potential. In order to combat insects and harmful microbes, plants release secondary metabolites called phytochemicals, which have the properties of poisons, growth regulators, feeding deterrents, and repellents. The use of botanicals as pesticide substitutes has gained traction because of their broad insecticidal effects, biodegradability and ecological flexibility. A logical method that includes a wide range of theoretical and computational techniques, computer-aided molecular design is frequently used in contemporary drug design. Because of recent developments in biochemistry and structural biology, it is now a crucial technique for lead screening, optimization, and the discovery of new, strong inhibitors, including insecticidal medicines. In the present study, *in silico* investigations were specifically centered on the enzyme acetylcholinesterase as the primary target for the compounds from ethyl acetate extract of *Sphaeranthus amaranthoides* on *Culex quinquefasciatus.* LigPlot analysis was performed on the lead hits to understand their intermolecular interactions better. Our findings indicated that compounds from ethyl acetate extract of *Sphaeranthus amaranthoides* exhibited promising insecticidal properties.

Keywords: *Culex quinquefasciatus*, *Sphaeranthus amaranthoides*, *In silico*, Acetylcholinesterase, Molecular Docking

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Introduction

Japanese Encephalitis (JE), filariasis, schistosomiasis, dengue disease, yellow fever, and malaria are all mostly spread by mosquitoes. With two to three million new cases each year, malaria is one of the leading causes of direct or indirect newborn, child, and adult mortality in India. *Culex* mosquitoes, often known to be common house mosquitoes, are known to cause medical and veterinary problems. They feed on humans, domesticated animals, and birds. There are

reports that *Culex* species can also spread the Zika virus. 51 million cases of lymphatic filariasis carried by *Culex* were reported in 2018 (WHO, 2021). Among the significant vectors of the public health burden *are Cx. quinquefasciatus, Cx. pipiens, Cx. vishnui, Cx. salinarius, Cx. tarsalis, Cx. perexiguus* and *Cx. modestus*. The probabilities for the annual incidence and mortality of JE worldwide are 30,000 to 50,000 and 10,000, respectively. The best way to avoid diseases spread by mosquitoes and lessen the impact on public health is to manage the mosquito population. Insecticides using synthetic chemicals are widely used to manage mosquito vectors. Chemical control of mosquitoes is comparatively the most popular approach since it is easy to apply and cost-effective for big mosquito breeding locations. As an alternative, biological mosquito control methods work well, especially when it comes to pesticides derived from plants (Hamed *et al*., 2022).

Asteraceae family includes *Sphaeranthus amaranthoides* Burm. f. which are widely found in tropical Asia, Africa, and Australia. According to ethnomedicine, native tribes utilize the leaves of *Sphaeranthus amaranthoides* as a paste to treat wounds, dermatitis, eczema, and acne. *Sphaeranthus amaranthoides* leaves, properly dried and powdered, can be used to treat jaundice, urethral discharges, and chronic skin problems. It has been observed that *Sphaeranthus amaranthoides* extract demonstrates outstanding antibacterial activity against both Gram-positive and Gram-negative bacteria. The plant's phytochemical research revealed that it contains flavonoids, tannins, glycosides, phenolic compounds, triterpenoids, and steroids. Studies have documented that the leaves of *Sphaeranthus amaranthoides* exhibit antibacterial, antioxidant and anti-mutagenic properties (Gayatri *et al*., 2103).

The neurotransmitter acetylcholine is hydrolyzed by the enzyme acetylcholinesterase, which is vital to the neural system of insects. The juvenile hormone is also critical to the development and reproduction of insects. When essential oils inhibit AChE, the primary chemical allows nerve impulses to travel between cells through cholinergic synapses, super stimulating neurons, causing muscle spasms, convulsions, paralysis, and even death in insects. Secondary metabolites can impair the development cycle of *A. aegypti* by interfering with reproduction and reducing longevity, among other sublethal impacts on morphology, behavior, and physiological levels. They can also affect the hormone regulation of the insect (Lopez and Pascual-Villalobos, 2010; Alomar *et al*., 2021). Hence, in the present study *in silico* molecular docking study was performed by using Auto Dock tools against AChE of *Culex quinquifasciatus* using compounds from the ethyl acetate extract of leaves of *Sphaeranthus amaranthoides.*

Materials and Methods

Preparation of ligand structures of ethyl acetate extract of Sphaeranthus amaranthoides

The ligand structures of Temephos and N,N-Dimethylacetamide, 1,2,4,5-Benzenetetracarboxylic acid, tetramethyl ester, N,N-Dimethyldodecanamide and Dodecanamide, N-ethyl- were retrieved from Chemspider database (https://www.chemspider.com/) and three-dimensional structure was developed using ChemDraw Ultra $_{V}$12.0. Then the energy minimization and Geometry optimization were performed using PRODRG server (Van Der Spoel *et al*., 2005).

Homology model prediction

Modeller 9v10 software was used to model the Acetylcholinesterase (AChE1) of *Cx. quinquefasciatus* and the protein sequence of AChE1 was retrieved from UniProtKB (ID:Q867X2) EC: 3.1.1.7. Suitable template of AChE1 was analyzed by BLAST (blastP) by using BLOSUM62 matrix associated with PDB data bank. Structural qualities and stereochemical model were examined using PROCHECK (Ramachandran plot) analysis. The completed structure of the modelled AChE1 was visualized

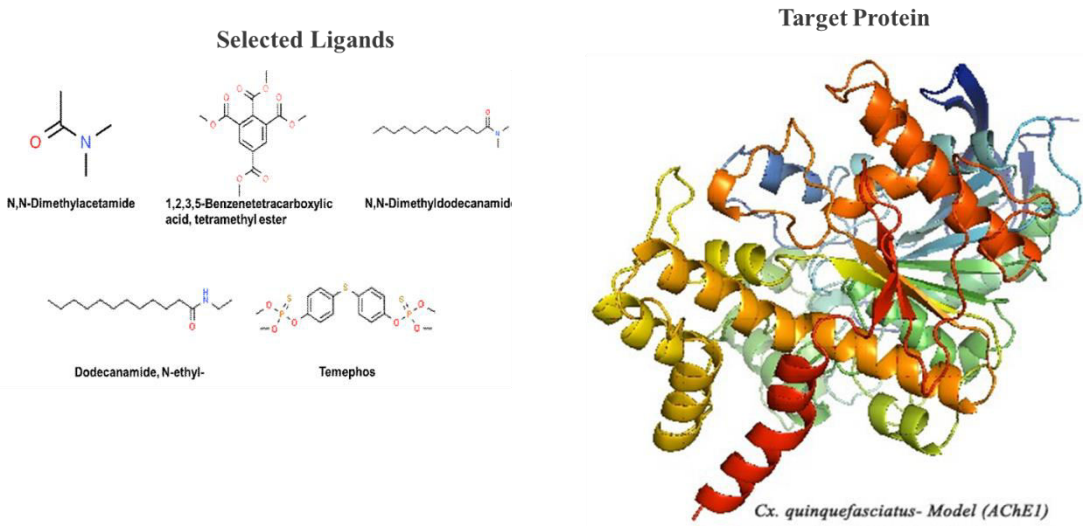

Fig. 1: Structures of Control and selected ligands from *S. amaranthoides* ethyl acetate extract and AChE1 of *Cx. quinquefasciatus.*

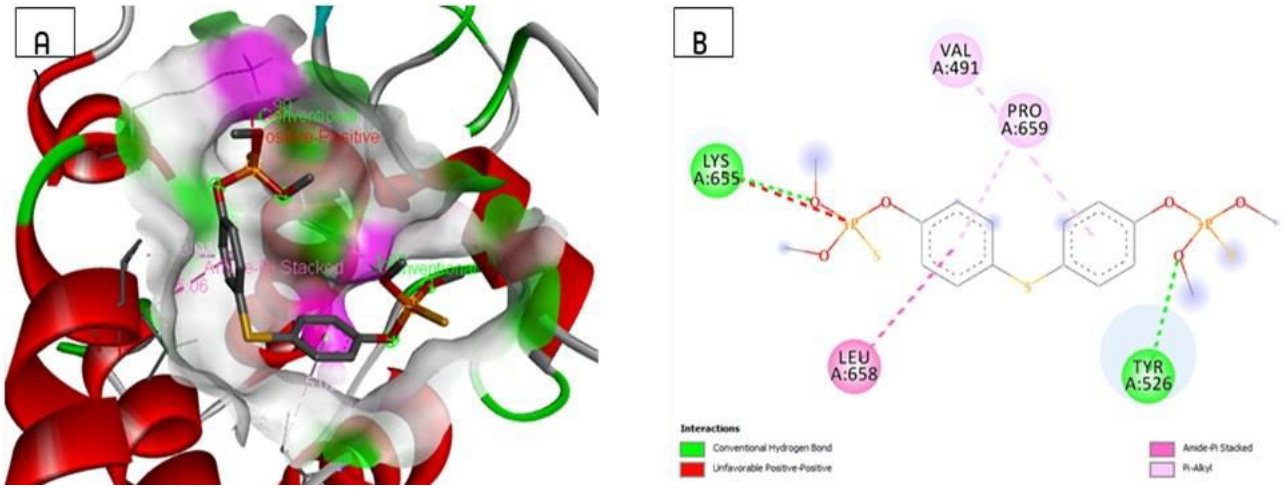

Fig. 2: Interaction of Temephos with *Cx. quinquefasciatus*- Model (AChE1). (A) Temephos with *Cx. quinquefasciatus* - Model (AChE1) complex. (B) 2D plot of the complex interaction generated by Discovery Studio Visualizer.

using PyMOL visualizing software (Laskowski *et al*., 1993).

Docking analysis

Computational docking analysis was performed using Auto Dock tools (ADT version 1.5.4 and 4.2 programs). Parameters like atomic solvation and Kollman charges were added. Nonpolar hydrogens were merged with the carbons and the polar hydrogen charges of the Gasteiger type were assigned with the carbons with the internal degrees of freedom and torsions were set. Compounds were docked with target protein model AChE1, the molecule considered as a rigid body and the ligand being flexible. The search was extended over the active sites of the receptor protein.

Results and Discussion

The present exemplified the anti-larvicidal activity of *Sphaeranthus amaranthoides* against AChE of *Culex quinquifasciatus* by using Auto

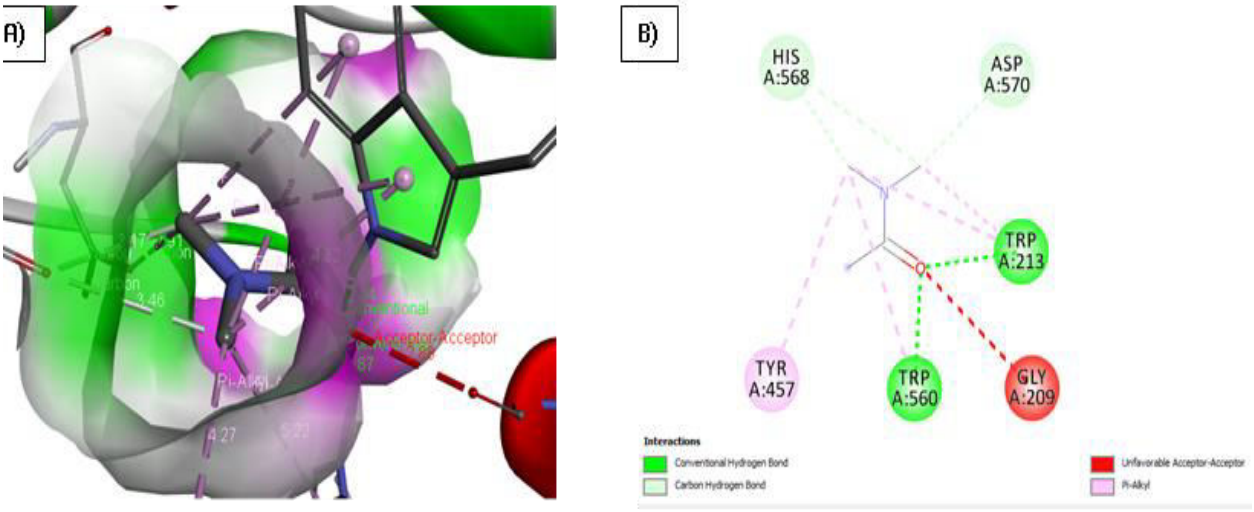

Fig. 3: Interaction of N,N-Dimethylacetamide with *Cx. quinquefasciatus*- Model (AChE1). (A) N,N-Dimethylacetamide with *Cx. quinquefasciatus*- Model (AChE1) complex. (B) 2D plot of the complex interaction generated by Discovery Studio Visualizer.

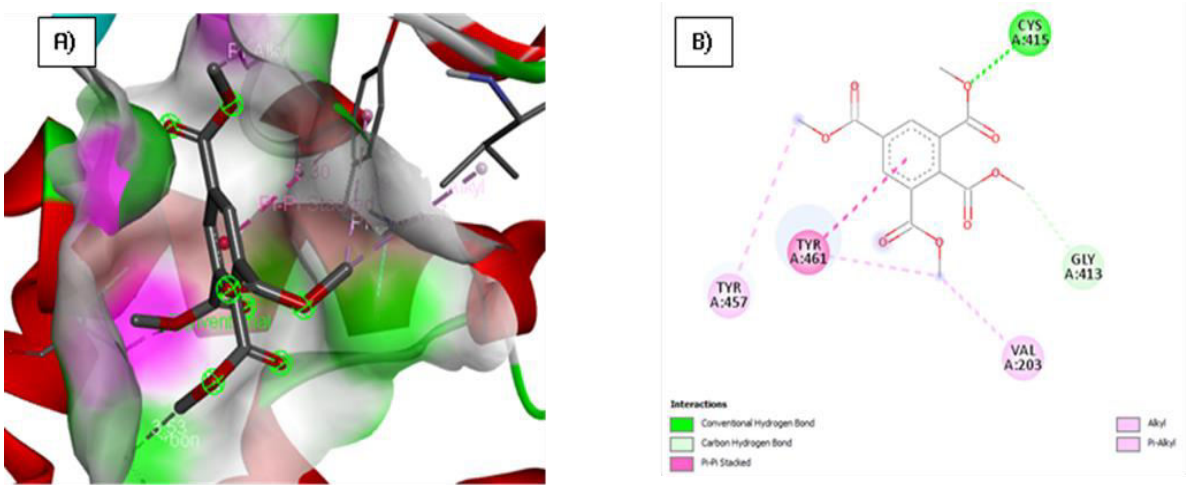

Fig. 4: Interaction of 1,2,3,5-Benzenetetracarboxylic acid, tetramethyl ester with *Cx. quinquefasciatus*- Model (AChE1). (A) 1,2,3,5-Benzenetetracarboxylic acid, tetramethyl ester with *Cx. quinquefasciatus*- Model (AChE1) complex. (B) 2D plot of the complex interaction generated by Discovery Studio Visualizer.

Dock tools.

Homology modeling and template identification

For AChE1, there is no three-dimensional structure available and it is essential to develop the protein structure to analyze the level of molecular activity and function of *Cx. quinquefasciatus.* In the present study, to build the homology model of AChE1, its protein sequence (GenBank: AHC03354.1) was used to identify the appropriate templates through the BLASTP analysis available with PDB database. The Crystal structure of *Anopheles gambiae* acetylcholinesterase (PDB ID: 5YDJ, Query cover: 84.71 % and Identity: 92.76 %) protein structure was identified as a template of *Cx. quinquefasciatus* AChE1 protein sequence (Reegan *et al*., 2016; Stalin *et al*., 2022).

The homology models were developed using Modeller 9v10 software and the three-dimensional image was created by PyMOL viewer. To check the quality of the predicted model, it

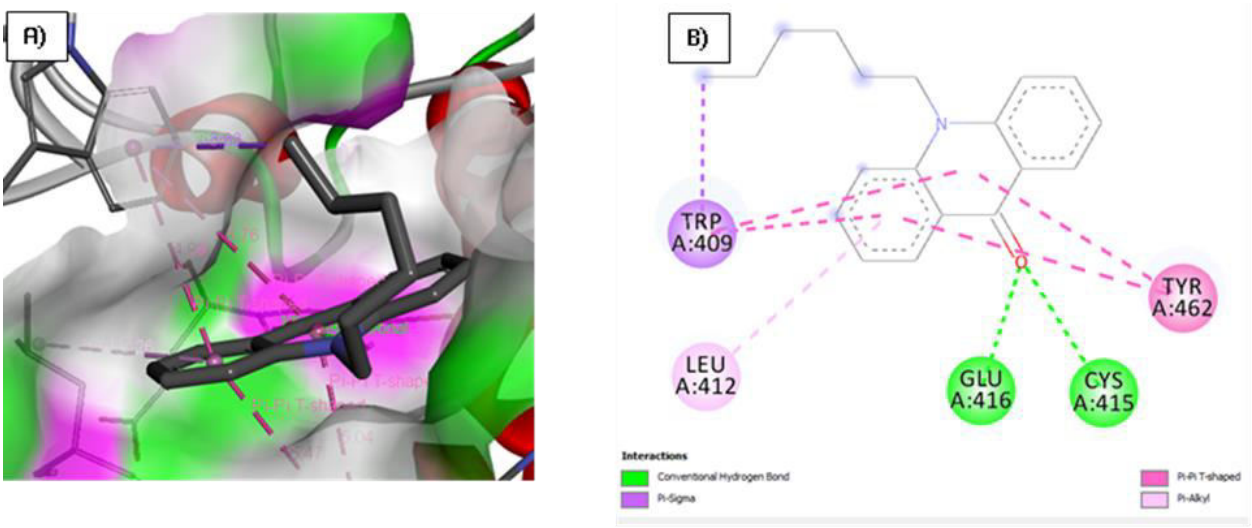

Fig. 5: Interaction of N,N-Dimethyldodecanamide with *Cx. quinquefasciatus*- Model (AChE1). (A) N,N-Dimethyldodecanamide with *Cx. quinquefasciatus*- Model (AChE1) complex. (B) 2D plot of the complex interaction generated by Discovery Studio Visualizer.

was examined by PROCHECK analysis based on Ramachandran plot on SAVES server (Aguayo-Ortiz *et al*., 2013). AChE1 model of *Cx. quinquefasciatus* showed the amino acids residues of 98.686% at most favored region, 1.314% at additional allowed regions and 1.3% at no additional allowed regions and 0 % at disallowed regions.

Researchers have used *in silico* molecular docking technologies to try to find a new insecticidal chemical (Niraj *et al*., 2015). N, N-Dimethyldodecanamide interacted with Asn-76 and carbonyl oxygen interacted with the hydrogen bonds TRP-213 and TRP-560, as well as the carbon hydrogen bonds HIS-568 and ASP-570. 1, 2, 3, 5-Benzenetetracarboxylic acid, tetramethyl ester interacts with CYS-415 via hydrogen bonds and has a carbon hydrogen bond with GLY-413. The compounds had high binding energies of -7.35 and -5.13 kcal/mol, respectively (Figs. 3-5) The ligand efficiency was 0.35 and 0.23, respectively. The oxygen atom of the phenoxy substituent on one side of temephos demonstrated interaction with LYS-695 and 6 with a binding energy of -4.96 kcal/mol (Fig. 2). Similarly, Moralev *et al.* (2001) also reported that the essential oils of *A. triplinervis* can also inhibit the acetylcholinesterase enzyme of *A. aegypti*. Based on the outcomes of molecular docking, it was determined that AChE, an enzyme that belongs to the carboxylesterase (hydrolase) class and hydrolyzes substituted cinnamic esters, could be the enzyme responsible for the enzymatic hydrolysis of these esters. AChE is responsible for hydrolyzing the neurotransmitter acetylcholine into acetic acid and choline, which is essential for the development of various living organisms, particularly vectors like *Cx. quinquefasciatus*. On the other hand, when it is suppressed, more acetylcholine builds up, which can result in convulsions, respiratory arrest, synaptic collapse, and loss of muscle control. The present study reveals that the *S. amaranthoides* possess the potential larvicidal activity against the *Culex quinquifasciatus* species.

Conclusion

This study highlights the urgent need for creative ways to halt the spread of diseases carried by mosquitoes around the world. The research focuses on the potential of *S. amaranthoides* Burm. f. leaf extracts, as a viable biopesticide for effectively managing *Culex quinquefasciatus* larvae. According to an *in silico*

docking study *S. amaranthoides* phytoconstituents possessed larvicidal activity as the common medication. Additional *in vivo* molecular pharmacodynamic research could be beneficial in elucidating the mechanism of action of this plant's active ingredients, which have a larvicidal impact on *Culex quinquefasciatus* species and may be exploited for commercial purposes in the near future.

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