

In-silico Assessment of the effects of *Hyptis suaveolens* as a natural source of Acetylcholine esterase (AChE)-targeting insecticide

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Short Report

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Abstract

Developing effective insecticides is crucial to the global fight against vector-borne diseases, epidemics, and pandemics. Acetylcholinesterase (AChE), a serine hydrolase responsible for regulating acetylcholine levels in various organisms, is one of the most common targets of synthetic insecticides such as organophosphates and carbamates. However, widespread exposure to these chemicals has led to a significant decline in insect sensitivity, necessitating higher effective doses and consequently increasing human exposure to their toxic effects. As a result, the search for safer and more effective insecticides derived from natural sources has gained significant attention. This study aimed to computationally evaluate the AChE inhibitory potential of bioactive compounds from *Hyptis suaveolens* (L.) Poit, a medicinal plant widely used in traditional medicine across tropical regions. Using *in silico* approaches, 39 bioactive compounds were assessed through molecular docking, ligand and protein preparation, and toxicity prediction, utilizing computational tools such as Lotus, Chimera, PyRx, and ProTox3. Docking analysis revealed that *Hyptis suaveolens* compounds exhibited binding affinities ranging from -10.5 to -3.8 kcal/mol against AChE. The standard reference ligand, 9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine (C₂₀H₂₀N₂), had a docking score of -9.3 kcal/mol. Among the tested compounds, LTS0222826 exhibited the strongest binding affinity (-10.5 kcal/mol), followed by LTS0163613 (-9.7 kcal/mol), while LTS0238624 showed the weakest binding affinity (-3.8 kcal/mol). Additionally, LTS0163613 and LTS0107905 exhibited the least predicted toxicological effects on human physiological systems. Notably, both compounds showed no adverse effect on the respiratory system and other key biological functions when administered orally. This suggests their potential safety as natural insecticides and insect repellents, supporting their further exploration as alternatives to synthetic chemical insecticides.

Background

Acetylcholine (ACh) is one of the major neurotransmitters involved in neurotransmission in both vertebrates and invertebrates [1] and the regulation of its activity by AChE-catalysed hydrolysis is essential for the normal functioning and/or survival of these animals. Acetylcholinesterase is a cholinergic enzyme that catalyses the breakdown of acetylcholine to acetic acid and choline, thereby terminating nerve transmission at the synapses [2]. Inhibition of AChE activity forms the basis for the action of many insecticides including organophosphates and carbamates [3].

Inhibition of AChE by these insecticides causes the accumulation of acetylcholine in the synapses, making acetylcholine receptors to be permanently open and resulting in some toxicological outcomes [2, 4]. The cholinergic stimulation induced by an AChE inhibitor (AChEI) may cause hyperactivity of excitable tissues, leading to fatal consequences like muscle paralysis, coma, and death. There is also, overwhelming production of oxygen/nitrogen derived free radicals leading to down-regulation of the antioxidant defense system [5] thereby exposing the insects to the toxic effects of the insecticides [5, 6].

However, several insects and pests are becoming increasingly resistance to several insecticides including AChE-targeting insecticides. This is attributed to extensive use of chemical insecticides [7–9]. This has reduced the efficacy of these chemicals and increased human exposure to the toxic adverse effects of the chemicals [10]. These and other factors, such as environmental pollution raises the need for natural eco- friendly alternatives. Insect repellent plants are considered safe and environmentally friendly alternatives for controlling insects, which are vectors to several infectious human diseases.

Some of these plants are sometimes used as ornamental plants around residential houses to repel insects and as indigenous traditional medicines in most of tropical areas [11] one of such plants is *Hyptis suaveolens*, commonly known as “wild hops”. It belongs to the family of Lamiaceae [12]. It is used traditionally in ethnomedicine for the treatment of various ailments including diabetes, fever, eczema, flatulence, cancers and headaches and there were no reports on adverse side effects on health. *H. suaveolens* (L) Poit has recently been shown to possess insecticidal and insect-repellent ability towards several insects, including mosquitoes [13], houseflies [14], sand flies [15] and fruit flies (*Drosophila melanogaster*) [16], hence, it might be a source of effective and non-toxic natural insecticides.

Therefore, using *Drosophila melanogaster* as a model insect, we investigated the insecticidal and AChE inhibitory potentials of *H. suaveolens* (L) Poit and its bioactive compounds through in silico approach. Previously characterized bioactive compounds of *H. suaveolens* (L) Poit were screened for inhibitory activity against DmAChE through molecular docking analysis to identify possible AChEIs among the compounds. We also investigated the possible toxic effects of these compounds on humans.

Materials and Methods

In silico analysis of the AChE inhibitory potentials of bioactive compounds of *Hyptis suaveolens* (L) Poit.

Ligand preparation

Thirty-one bioactive compounds of *Hyptis suaveolens* (L) Poit were obtained from previous reports and Ethnobotanical Database [10, 11][17]. The canonical SMILES of 10 compounds of *Hyptis suaveolens* (L) Poit from LOTUS database were converted to PDB format using Chimera 1.19 while the structure data file (SDF) format of standard ligand 9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine ($C_{20}H_{20}N_2$), was obtained from Protein Database. The SDF format of compounds and standard ligand were uploaded to PyRx software and converted to PDBQT format using the OpenBabel plugin. The output files were minimized to obtain the minimum energy for the ligand docking.

Protein preparation

The crystal structure of *Drosophila melanogaster* Acetylcholinesterase (DmAChE) with PDB ID: 6XYY was obtained from the Protein Data Bank (PDB) repository. Using UCSF Chimera 1.19, the protein structure obtained was prepared via the protein preparation wizard panel.

During the preparation processes, hydrogen atoms were added, bond orders were allocated, disulfide bonds were generated, the side chains and loops that are missing were replaced using prime. Water molecules located outside 3.0 Å of the heteroatoms were removed and the protein structure was minimized using OPLS3e and optimized using PROPKA [18, 19]. The prepared proteins and ligands were uploaded to the PyRx software for molecular docking analysis.

Molecular docking

Molecular docking of the prepared ligands and proteins were performed using AutoDock vina in the PyRx workspace. Using blind docking, the ligands were docked into the binding pocket of the target protein. Grid space was set by targeting important amino acid residues selected through literature [20] and from UniProtKB

Toxicity prediction

The highest-ranking compound based on binding affinity against the target protein was selected for toxicity testing. The toxicity potential of the highest-ranking compounds based on binding affinity against DmAChE were selected to analyze their potential toxicity on human physiological systems using ProTox 3- prediction of toxicity software.

Results

Table 1 shows the bioactive compounds of *Hyptis suaveolens* (L) Poit obtained from Ethnobotanical Database. Thirty-nine (39) bioactive compounds of *Hyptis suaveolens* (L) Poit were virtually extracted from the plant using LOTUS database.

Molecular docking analysis of bioactive compounds of *Hyptis suaveolens* (L) Poit against *Drosophila melanogaster* acetylcholine esterase (DmAChE)

Hyptis suaveolens (L) Poit compounds exhibited various levels of binding affinities with Gibbs free energy (ΔG kcal/mol) ranked from the best target (-10.5 kcal/mol) to worst (-3.8 kcal/mol). Five (5) compounds of *Hyptis suaveolens* (L) Poit with the best docking scores against DmAChE. were selected with Gibbs free energy ranging from -10.5 kcal/mol to -7.9 kcal/mol while the standard ligand (9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine ($C_{20}H_{20}N_2$)) showed a docking score of -9.3 kcal/mol. Among the *Hyptis suaveolens* (L) Poit compounds, LTS0222826 scored highest with docking score of -10.5 kcal/mol followed by LTS0163613 with a docking score of -9.7 kcal/mol while LTS0238624 scored lowest (-3.8 kcal/mol) (Table 1)

Toxicity potentials of some compounds of *Hyptis suaveolens* (L) Poit

Three compounds of *Hyptis suaveolens* (L) Poit (LTS0222826, LTS0107905 and LTS0163613) showed the least predicted toxicity effect on human physiological systems when consumed orally. LTS0107905 and LTS0163613 have no toxicity effect on the respiratory system (Table 2 and Figs. 2–4).

Discussion

Insecticide development is a very important aspect of the global fight against epidemics and pandemics. Insect-borne diseases like malaria and dengue hemorrhagic fever are among the deadliest infections worldwide [21] accounting for over 700 000 deaths yearly [22] and one of the ways of controlling them is through the use of insecticides. Acetylcholinesterase (AChE) is one of the most common targets of synthetic insecticides and pesticides, such as organophosphates and carbamate [23]. This enzyme is a serine hydrolase and is responsible for regulating the levels of acetylcholine in a variety of organisms, from mammals to insects [24]. Due to its extensive “attack”, there has been drastic reduction in the sensitivity of insects to organophosphate and carbamate insecticides. This has elevated the effective doses of these chemicals, leading to increased human exposure to their toxic adverse effects [10]. Hence the search for new, safe and effective insecticides of natural origin is currently being promoted as a good alternative [25].

This study has not only provided a scientific basis for the alternative use of *Hyptis suaveolens* (L) Poit as an insecticidal/insect-repellent plant, but it has further revealed its potential as a source of AChE-targeting agents and possible toxicological effects when consumed orally by humans

In this study, during a search in the Protein Data Bank for Dm Acetylcholinesterase PDB_ID_6XYY, a structure a co-crystallized ligand (standard ligand (9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine (C₂₀ H₂₀ N₂) to the protein was found.

Also, to identify potential AChE inhibitors in *Hyptis suaveolens* (L) Poit, molecular docking analysis of some previously characterized bioactive compounds of the plant was conducted against DmAChE. The 39 compounds showed varying levels of binding affinities with docking scores ranging from – 10.5 to – 3.8 kcal/mol against the enzyme. The ten top-scoring *Hyptis suaveolens* (L) Poit, compounds were listed in Table 1. The compound, LTS0222826 scored highest with docking score of – 10.5 kcal/mol followed by LTS0163613 with a docking score of -9.7 kcal/mol. The 2D and 3D models of the protein-ligand interactions were not captured in this study. However, using PyRx virtual screening software showed that the 2 compounds were in close proximity or occupied the same binding pocket as the co-crystallized ligand (1qon) on the protein.

This is in line with the report by Gaddaguti, et al. (2012) [26]. These interactions, therefore, make them potential inhibitors of DmAChE and possible contributors to the insecticidal or repellent activities of *Hyptis suaveolens* (L) Poit. This present study, therefore, identifies the compounds- LTS0222826 and LTS0163613 as the probable AChE-targeting agents and potential insecticides in *Hyptis suaveolens* (L) Poit. Though, additional computational and experimental studies need to be performed to further optimize and develop this hypothesis.

Additionally, LTS0163613 and LTS0107905 exhibited the least predicted toxicological effects on human physiological systems. Notably, both compounds showed no adverse effect on the respiratory system and other key biological functions when administered orally. This suggests their potential safety as natural insecticides and insect repellents, supporting their further exploration as alternatives to synthetic chemical insecticides.

Conclusion

This present study, identifies LTS0222826 and LTS0163613 as the probable AChE-targeting agents and potential insecticides in *Hyptis suaveolens* (L) Poit. Also, LTS0163613 and LTS0107905 exhibited the least predicted toxicological effects on human physiological systems. Both showed no toxicological effect on the respiratory system among other systems and processes in the human body when consumed orally. This could affirm the safety of these compounds as natural insecticide and insect repellent, Notably, both compounds showed no adverse effect on the respiratory system and other key biological functions when administered orally. This suggests their potential safety as natural insecticides and insect repellents, supporting their further exploration as alternatives to synthetic chemical insecticides.

Limitation of the study

Additional computational and experimental studies are needed in order to further optimize and develop the hypothesis established in this study.

Ethics declarations

Not applicable

Data availability

Data will be made available on request.

Declarations

Ethics declarations

Not applicable

Data availability

Data will be made available on request.

Conflict of interest:

There was no conflict of interest.

Funding:

This study was funded by the authors.

Authors' Contributions

CAO conceived the study, participated in the in-silico analysis, interpretation and drafting of the original manuscript. TOJ mentored CAO and supervised the work, interpreted the results, reviewed and edited the manuscript.

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Tables

Tables 1 and 2 are available in the Supplementary Files section.

Figures

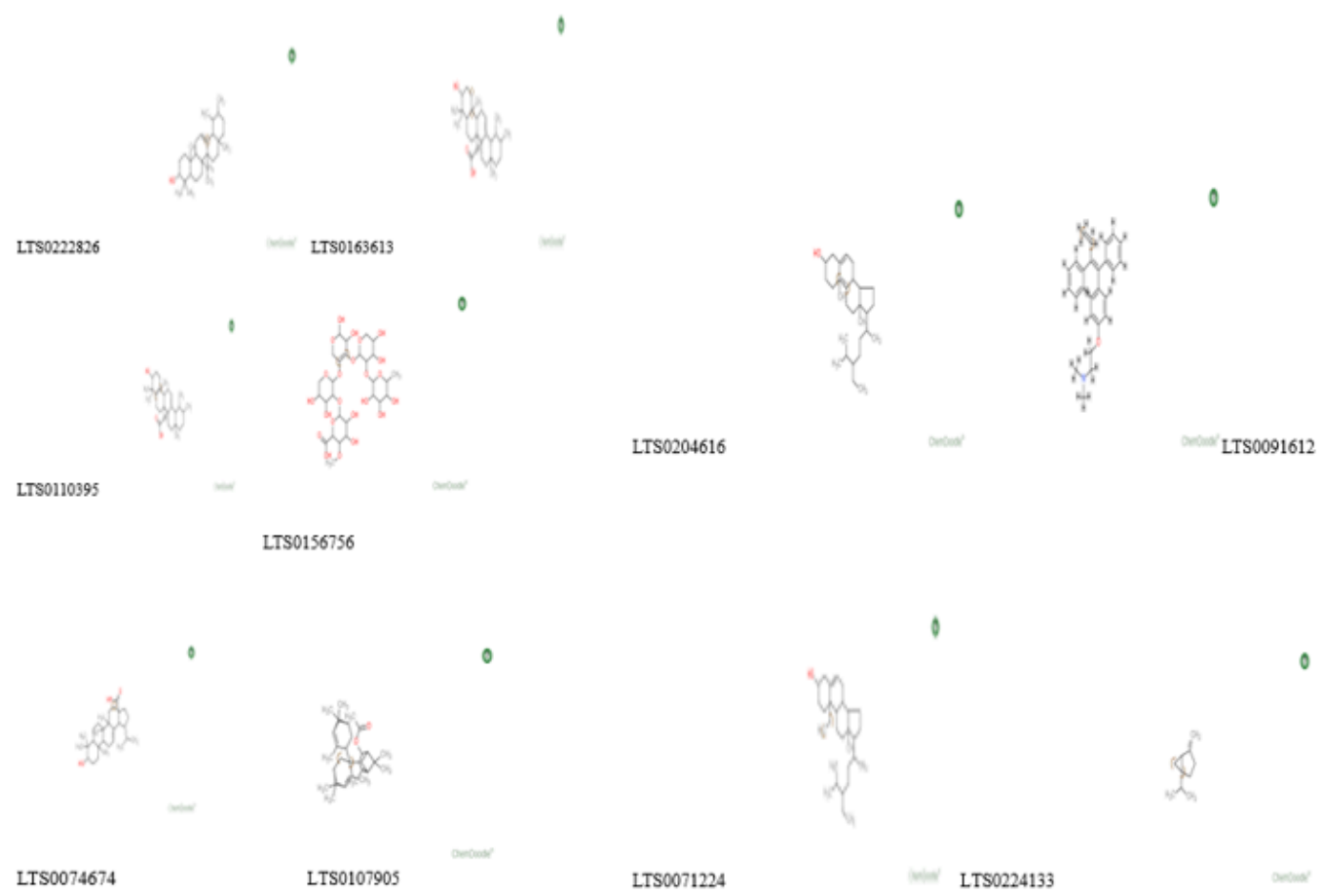


Figure 1

Structures of some compounds of *Hyptis suaveolens* (L.) Poit extracted from Lotus database



Figure 2

Toxicity radar chart of LTS0222826

* The toxicity radar chart is intended to quickly illustrate the confidence of positive toxicity results compared to the average of its class.



Figure 3

Toxicity radar chart of LTS0107905

* The toxicity radar chart is intended to quickly illustrate the confidence of positive toxicity results compared to the average of its class.



Figure 4

Toxicity radar chart of LTS0163613

* The toxicity radar chart is intended to quickly illustrate the confidence of positive toxicity results compared to the average of its class.

Supplementary Files

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