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Full Length Research

Molecular docking and prediction of ADME/Drug-Likeness properties of potentially active neuroprotective compounds in mixed ethanolic extracts of *Rosmarinus officinalis* and *Curcuma longa*

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ABSTRACT: Alzheimer's disease (AD) and other neurodegenerative disorders are characterized by cholinergic dysfunction, making acetylcholinesterase (AChE) inhibition a therapeutic target. This study investigates the neuroprotective potential of phytoconstituents from *Rosmarinus officinalis* and *Curcuma longa*, aiming to identify and evaluate bioactive compounds as potential AChE inhibitors. Ethanolic extracts of *R. officinalis* and *C. longa* were analyzed using GC-MS and UHPLC-ESI-Q-TOF-MS/MS to identify key phytoconstituents. Molecular docking simulations were performed using PyRx to assess binding affinities between AChE (PDB ID: 1AMN) and the compounds, including Cycloeicosane, Octacosane, and cis-Vaccenic acid, alongside the standard substrate choline. Drug-likeness, physicochemical properties, lipophilicity, and solubility profiles were predicted using SwissADME, Open Babel for energy minimization, and Silicos-IT for specific solubility and lipophilicity predictions. The docking scores ranged from -6.3 to -7.8 kcal/mol for the identified compounds, significantly outperforming choline (-4.8 kcal/mol). Cycloeicosane exhibited the strongest binding (-7.8 kcal/mol), stabilized by hydrogen, π-sulfur, and multiple alkyl interactions. Physicochemical analysis revealed the hydrophobic nature of most compounds, while cis-Vaccenic acid demonstrated moderate solubility and balanced lipophilicity, enhancing its bioavailability potential. The findings indicate that compounds such as Cycloeicosane and cis-Vaccenic acid are promising AChE inhibitors, offering potential as therapeutic agents for AD. This suggests that the identified phytocompounds could be developed as a novel neuroprotective medication. While computational results are promising, *in vitro* validation, pharmacokinetic studies, and toxicity assessments are required to confirm their therapeutic efficacy and safety for clinical applications.

Keywords: Acetylcholinesterase (AChE) inhibition, *Curcuma longa*, drug-likeness prediction, molecular docking, neurodegenerative diseases, phytoconstituents, *Rosmarinus officinalis.*

INTRODUCTION

Neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease, are characterized by the progressive loss of structure or function of neurons, culminating in cognitive and motor dysfunctions (Choi *et al*., 2012). These conditions, which are linked to oxidative stress, neuroinflammation, and excitotoxicity, pose a significant burden on healthcare systems worldwide. Despite advances in understanding their pathogenesis, therapeutic interventions remain limited, highlighting the need for

novel, effective, and safer neuroprotective agents (Yang *et al*., 2019; Teleanu *et al.,* 2022).

Medicinal plants have emerged as an invaluable source of bioactive compounds with therapeutic potential against neurodegenerative diseases. Traditional remedies, often involving mixed plant extracts, capitalize on synergistic effects to enhance bioactivity (Tyler and Tyler, 2023). *Rosmarinus officinalis* (rosemary) and *Curcuma longa* (turmeric) are two such plants with a history of medicinal use. Rosemary, rich in polyphenols such as rosmarinic acid and carnosic acid, has demonstrated antioxidant and anti-inflammatory properties, which are critical in combating neurodegeneration (Oresanya and Orhan, 2024). Turmeric, on the other hand, contains curcumin, a compound known for its neuroprotective effects, including inhibition of amyloid-β aggregation, reduction of oxidative stress, and attenuation of neuroinflammation (Banji *et al*., 2021).

The integration of computational tools such as molecular docking and ADME (absorption, distribution, metabolism, and excretion) predictions offers a systematic approach to identifying promising neuroprotective compounds. Molecular docking simulates the interaction between a ligand and a receptor, providing insights into binding affinity and potential mechanisms of action (Morris and Lim-Wilby, 2008; Wu *et al*., 2020). ADME predictions evaluate a compound's drug-likeness, ensuring that potential therapeutic agents possess favourable pharmacokinetic properties, such as oral bioavailability and minimal toxicity (Xiao *et al*., 2022).

Although individual compounds from *R. officinalis* and *C. longa* have shown neuroprotective potential, studies exploring the synergistic effects of their mixed extracts remain scarce. A mixed ethanolic extract is hypothesized to enhance bioactivity by combining the therapeutic benefits of both plants, potentially targeting multiple neurodegenerative pathways simultaneously. Additionally, leveraging molecular docking and ADME prediction can streamline the discovery of drug-like neuroprotective agents from these extracts, providing a robust foundation for subsequent *in vitro* and *in vivo* evaluations.

This study focuses on the molecular docking of key bioactive compounds from the mixed ethanolic extracts of *R. officinalis* and *C. longa* against relevant neuroprotective targets. It also evaluates the ADME/drug-likeness properties of these compounds to identify those with the greatest therapeutic potential. The findings aim to contribute to the development of plant-based neuroprotective agents, offering a promising avenue for addressing the challenges posed by neurodegenerative diseases.

MATERIALS AND METHODS

Selection of potential neuroprotective compounds

The analyzed compounds were previously isolated from the leaves of *R. officinalis* and the roots of *C. longa*. The plants were sourced from a vegetable market in Port Harcourt Local Government Area, Rivers State, Nigeria. Detailed protocols for extraction, activity-guided isolation, and identification of these compounds were as described by Ibrahim *et al.* (2017), and Ibrahim *et al.* (2018). Thin-layer chromatography (TLC) was used to monitor the fractions and identify distinct bioactive constituents. Fractions demonstrating significant bioactivity were further analyzed using GC-MS and UHPLC-ESI-Q-TOF-MS/MS to identify key compounds such as phenolic constituents and terpenoids.

Software and tools

The following software tools were utilized for this study:

- Python Programming Language: Downloaded from www.python.com.
- MGL Tools (Molecular Graphics Laboratory): Retrieved from http://mgltools.scripps.edu.
- PyRx Version 0.8: Downloaded from https://pyrx.sourceforge.io/.
- BIOVIA Discovery Studio Visualizer (Version 2021): Obtained from http://accelrys.com.

Protein preparation

The crystal structure of acetylcholinesterase bound to acetylcholine (PDB ID: 1amn) was retrieved from the Protein Data Bank (www.rcsb.org), and prepared by removing water molecules and ions to avoid interference, adding polar hydrogens using Biovia Discovery 2021 to optimize hydrogen bonding potential, and converting the structure to PDBQT format using PyRx virtual screening tool 35 for compatibility with docking simulations.

Ligand preparation

The selected phenolic compounds identified from GC-MS analysis of combined extract of *R. officinalis* and *C. longa* were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The ligands include: 1- Hexacosene (PubChem ID: 29303), cis-Vaccenic acid (PubChem ID: 5282761), Octacosane (PubChem ID:12408), Tricosene (PubChem ID:6385060), Cycloeicosane (PubChem ID:520444), while choline (PubChem ID: 305) was used as the positive control.

Docking protocol

The ligands, including 1-Hexacosene (PubChem ID: 29303), cis-Vaccenic acid (PubChem ID: 5282761), Octacosane (PubChem ID: 12408), Tricosene (PubChem

Table 1. Compound composition of combined ethanolic extract of *Rosmarinus officinalis* leaf and *Curcuma longa* rhizome.

S/N	Compound
1	1- Hexacosene
2	cis-Vaccenic acid
3	Octacosane
4	Tricosene
5	Cycloeicosane
6	Choline

Table 2. Docking scores (Kcal/mol) for compounds (ligands) from combined ethanolic extract of *Rosmarinus officinalis* leaf and *Curcuma longa* rhizome.

Compound	PubChem ID Number	Binding Energy (Kcal/mol)	RMSD (Á)
1- Hexacosene	29303	-6.4	0
cis-Vaccenic acid	5282761	-6.7	0
Octacosane	12408	-6.3	0
Tricosene	6385060	-6.6	0
Cycloeicosane	520444	-7.8	0
Choline	305	-4.8	0

ID: 6385060), Cycloeicosane (PubChem ID: 520444), and choline (PubChem ID: 305), were loaded into PyRx for processing. Their energies were minimized using Open Babel software (version 3.1.1) and converted into PDBQT format. The ligands selected for the second round of screening underwent further energy minimization and format conversion using PyRx. The binding conformations of the ligand-protein complexes were visualized with BIOVIA Discovery Studio 2021, and the residues involved in hydrogen bonding and hydrophobic interactions were analyzed and mapped using LigPlot+ software.

In-silico and ADME and drug-likeness prediction

The *in-silico* ADME screening and evaluation of drug-likeness properties for the identified compounds were conducted using the SwissADME web tool, developed by the Swiss Institute of Bioinformatics, which is freely available at https://www.swissadme.ch (Daina *et al*., 2017). A list of SMILES codes for the selected ligands from GC-MS identification of combined ethanolic extracts *R. officinalis* and *C. longa* were prepared and inputted into the software to compute drug-likeness. SwissADME was used to calculate the various drug-likeness parameters, including molecular weight, hydrogen bond donors, hydrogen bond acceptors, lipophilicity, and molecular refractivity. These calculated parameters were then compared against established drug-likeness criteria, such as Lipinski's rule of five, to assess the potential of each compound as a drug-like entity and its suitability for further pharmacological development.

RESULTS

Molecular docking

Table 1 presents the potential neuroprotective compound previously identified in the combined ethanolic *R. officinalis* and *C. longa* extracts that was used for this study. Table 2 presents the docking scores/interaction for the identified compounds: 1- Hexacosene, cis-Vaccenic acid, Octacosane, Tricosene, Cycloeicosane, choline and the protein target Acetylcholinesterase. The docking score ranged from -6.3 to -7.8 kcal/mol. The binding energies of all selected compounds differed significantly from that of the reference standard acetylcholine (-4.8 kcal/mol).

The 2D/3D interactions between acetylcholinesterase and the identified compounds

The 3D structure of acetylcholinesterase (Figure 1) provides a visual representation of the enzyme's active site, crucial for understanding the interaction dynamics of the identified compounds. This figure highlights the structural framework and the binding pocket, facilitating interpretation of the molecular docking results.

The 2D interactions of the identified compounds and the standard substrate, choline, with acetylcholinesterase (AChE) were analyzed and are illustrated in Figures 2–7. These interactions highlight the molecular binding profiles, which explain the docking scores and potential inhibitory activity of these compounds against AChE.

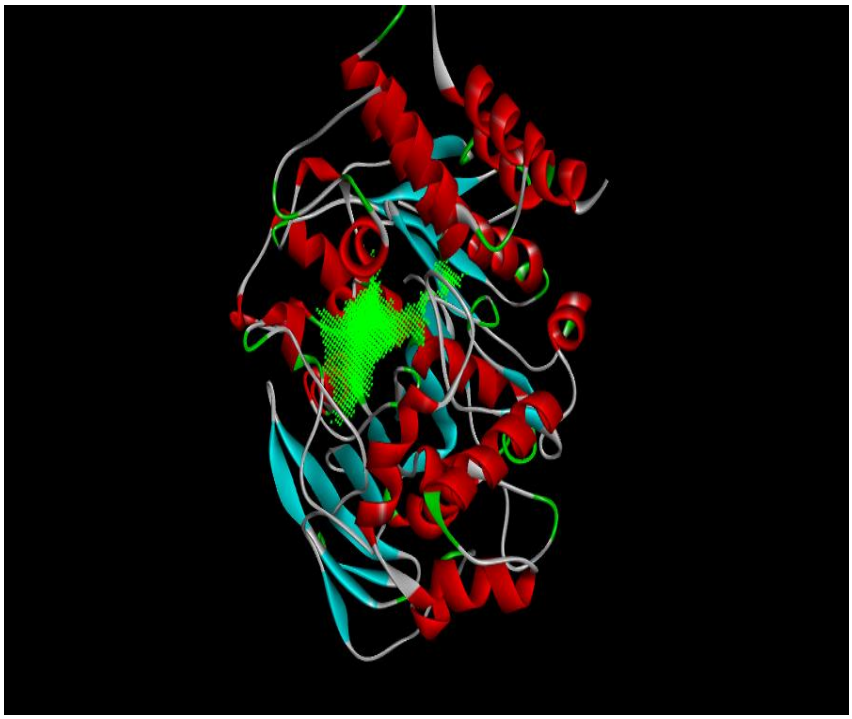

Figure 1. 3D Structure of Acetylcholinesterase (PDB ID: 1AMN) highlighting the active site.

Octacosane with Acetylcholinesterase

The interaction of Octacosane, a long-chain hydrocarbon, with acetylcholinesterase revealed the formation of five alkyl (π-alkyl) bonds. These bonds were mediated by key amino acid residues within the active site: Phe 331, Tyr 334, Trp 424, Phe 330, and His 440. The relatively high binding affinity of -6.3 kcal/mol indicates a stable interaction, suggesting that the lipophilic nature of Octacosane plays a significant role in anchoring the compound within the hydrophobic pocket of the enzyme. The presence of multiple π-alkyl bonds further stabilizes the compound at the enzyme's active site, potentially enhancing its inhibitory effect.

1-Hexacosene with Acetylcholinesterase

1-Hexacosene, another long-chain compound, demonstrated a binding interaction similar to that of Octacosane. It formed five alkyl (π-alkyl) bonds with residues Phe 331, Tyr 334, Trp 484, Phe 330, and His 440. The binding affinity was calculated to be -6.4 kcal/mol, slightly higher than Octacosane, suggesting a slightly enhanced stabilization due to subtle differences in molecular structure. These π-alkyl interactions likely improve the compound’s ability to occupy the active site, making it a potential inhibitor of acetylcholinesterase.

Cis-Vaccenic Acid with Acetylcholinesterase

The interaction of Cis-Vaccenic Acid, a monounsaturated fatty acid, with acetylcholinesterase was stabilized by multiple bond types, which included:

1. Hydrogen bonds involving the residues Gly 119 and Gly 118, facilitated by the oxygen moiety of the acid group.
2. A π-sigma bond with Phe 330.
3. An alkyl (π-alkyl) bond with Trp 184.

These diverse interactions suggest that the polar functional groups of Cis-Vaccenic Acid enhance binding stability within the enzyme's active site. The binding affinity of -6.6 kcal/mol indicates strong interactions, emphasizing the potential role of hydrogen bonding in anchoring this compound within the enzymatic cleft.

Tricosene with Acetylcholinesterase

The interaction of Tricosene, a hydrocarbon with three double bonds, was stabilized by seven alkyl (π-alkyl) bonds involving residues Tyr 121, Trp 84, Tyr 334, Leu 127, Phe 330, Tyr 130, and His 440. This compound exhibited a binding affinity of -6.6 kcal/mol, comparable to Cis-Vaccenic Acid. The extensive network of alkyl

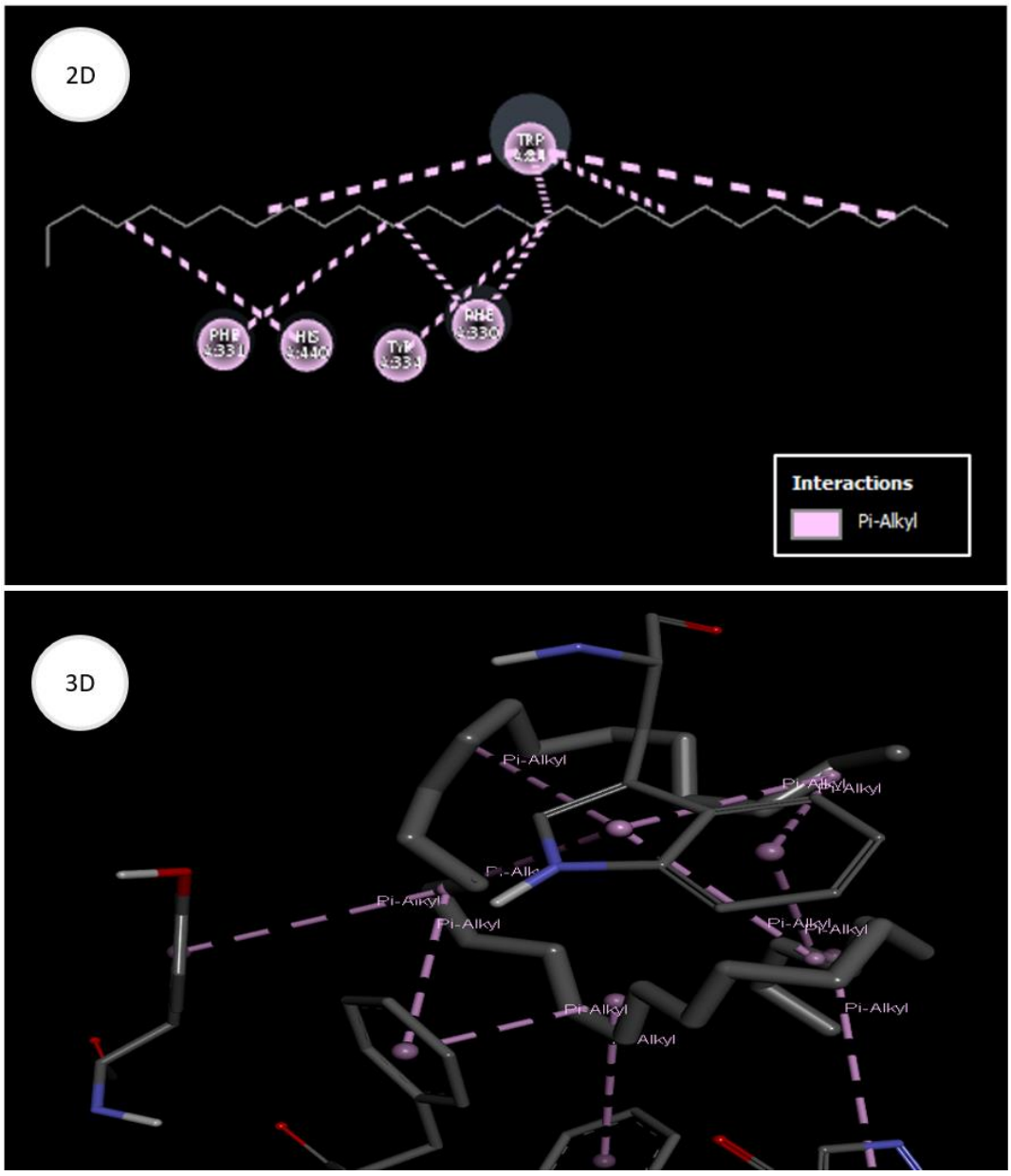

Figure 2. 2D/3D interaction between Acetylcholinesterase and Octacosane.

interactions demonstrates that Tricosene effectively occupies the hydrophobic binding pocket, contributing to its stability and potential inhibitory action. The increased number of bonds compared to Octacosane and 1-Hexacosene suggests a more extensive interaction network within the enzyme.

Cycloeicosane with Acetylcholinesterase

Cycloeicosane, a cyclic hydrocarbon, exhibited the most complex interaction profile among the tested compounds. Its binding was stabilized by:

1. A hydrogen bond with Tyr 121.
2. A π-sulfur bond with Tyr 70.
3. Five alkyl (π-alkyl) bonds involving residues His 440, Trp 84, Phe 330, Phe 290, and Phe 331.

The binding affinity of -7.8 kcal/mol was the highest recorded among the identified compounds, indicating exceptionally strong and stable binding interactions. The combination of hydrogen bonding, π-sulfur bonding, and multiple alkyl interactions suggests that Cycloeicosane

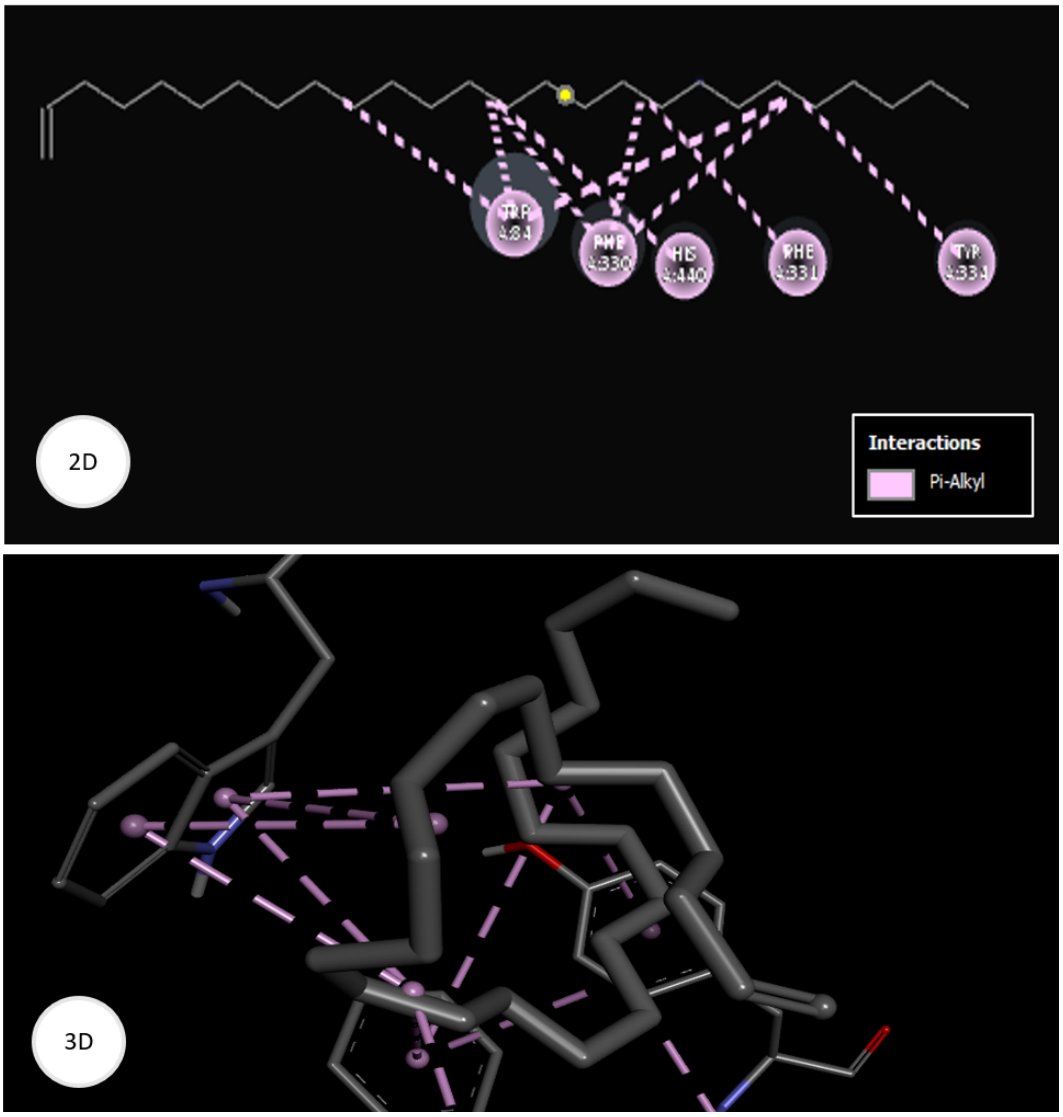

Figure 3. The 2D/3D interaction between acetylcholinesterase and 1- Hexacosene.

effectively locks into the active site, making it a highly promising candidate for acetylcholinesterase inhibition.

Interaction of Standard Substrate (Choline) with Acetylcholinesterase

The binding interaction of choline, the standard substrate, with acetylcholinesterase (PDB ID: 1AMN) was analyzed for comparison. Choline displayed a network of stabilizing interactions, including:

1. Hydrogen bonds with residues Gly 119 and Gly 118.
2. A carbon-hydrogen bond with Glu 119.
3. A π-carbon bond with Tyr 84 and Glu 199.
4. A π-sigma bond with Tyr 84.

The binding affinity of -4.8 kcal/mol, while sufficient for enzymatic interaction, was significantly lower compared to the identified compounds. This difference underscores the enhanced interaction strength and stability of the identified compounds relative to the standard substrate.

Characterization, physicochemical properties, lipophilicity, and solubility profiles of phytoconstituents from mixed ethanol extracts of *R. officinalis* and *C. longa*

Table 3 outlines the general characteristics of the phytoconstituents identified in the mixed ethanol extract of *R. officinalis* and *C. longa*. The compounds listed, such as cycloeicosane, 1-trichosene, and cis-vaccinic acid, exhibit diverse molecular structures and compositions. Their

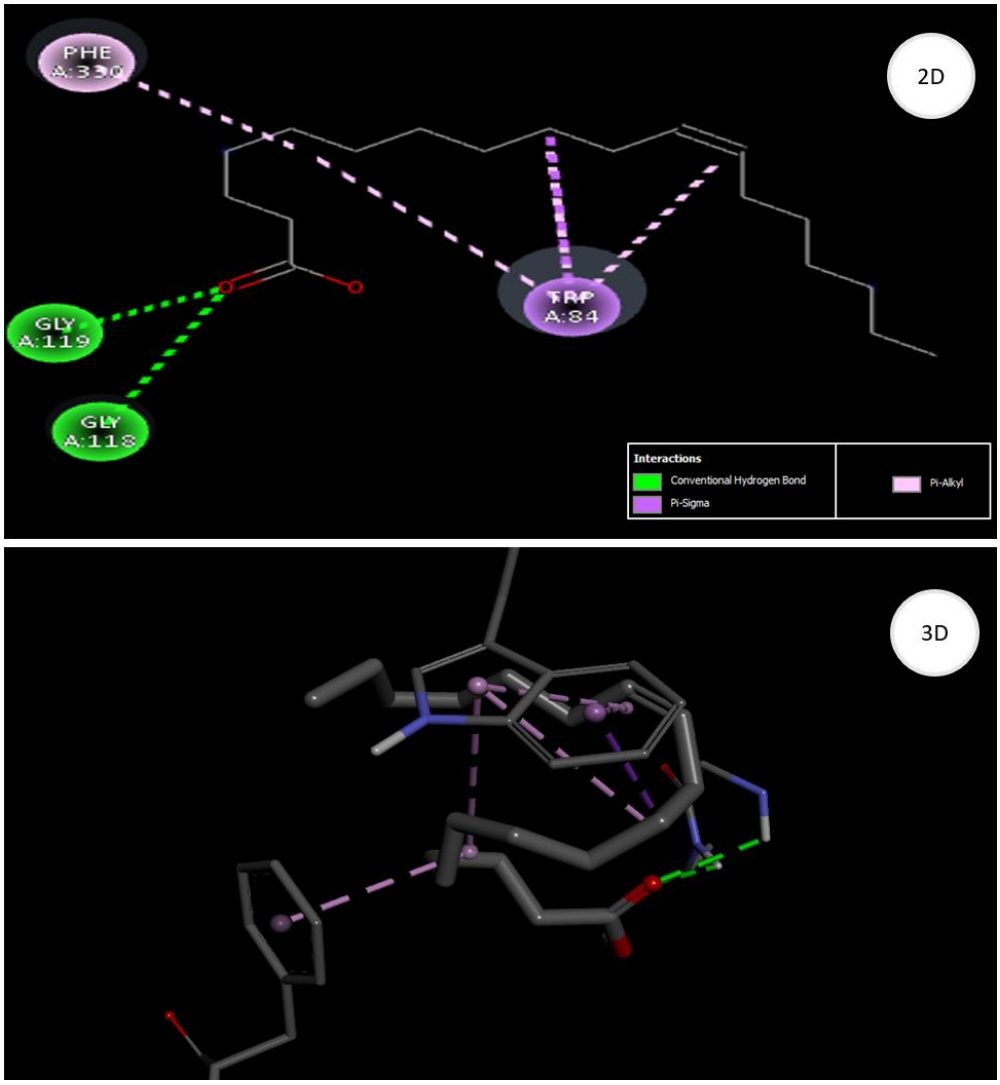

Figure 4. The 2D/3D interaction between acetylcholinesterase and cis-Vaccenic acid.

Table 3. General characteristics of the phytoconstituents of mixed ethanol extract of *Rosmarinus officinalis* and *Curcuma longa*.

S/N	Small molecule	Pubchem ID	Molecular formula	Canonical SMILES	Molecular weight (g/mol or D
1	cycloeicosane	520444	C20H40	C1CCCCCCCCCCCCCCCCCCC1	280.53
2	1- Trichosene	6385060	C23H46	CCCCCCCCCCCCC/C=C/CCCCCCCC	322.61
3	octacosane	12408	C28H58	CCCCCCCCCCCCCCCCCCCCCCCCCCCC	322.61
4	1-hexanecosane	29303	C26H52	CCCCCCCCCCCCCCCCCCCCCCCCCC=C	364.69
5	cis-Vaccnic acid	5282761	C18H34O2	CCCCCC/C=C\CCCCCCCCCC(=O)O	282.46
6	Choline	187		CC(=O)OCC[N+](C)(C)C	146,21

molecular weights range from 146.21 g/mol (choline) to 364.69 g/mol (1-hexanecosane), with varying molecular formulas indicating structural diversity. Canonical SMILES provides a detailed representation of their atomic connectivity, crucial for computational modeling and predicting biological activity. Table 4 expands on the physicochemical properties, showing that most compounds have a high fraction of sp3 hybridized carbons, indicative of saturated structures, and low hydrogen bond donor/acceptor counts, suggesting limited

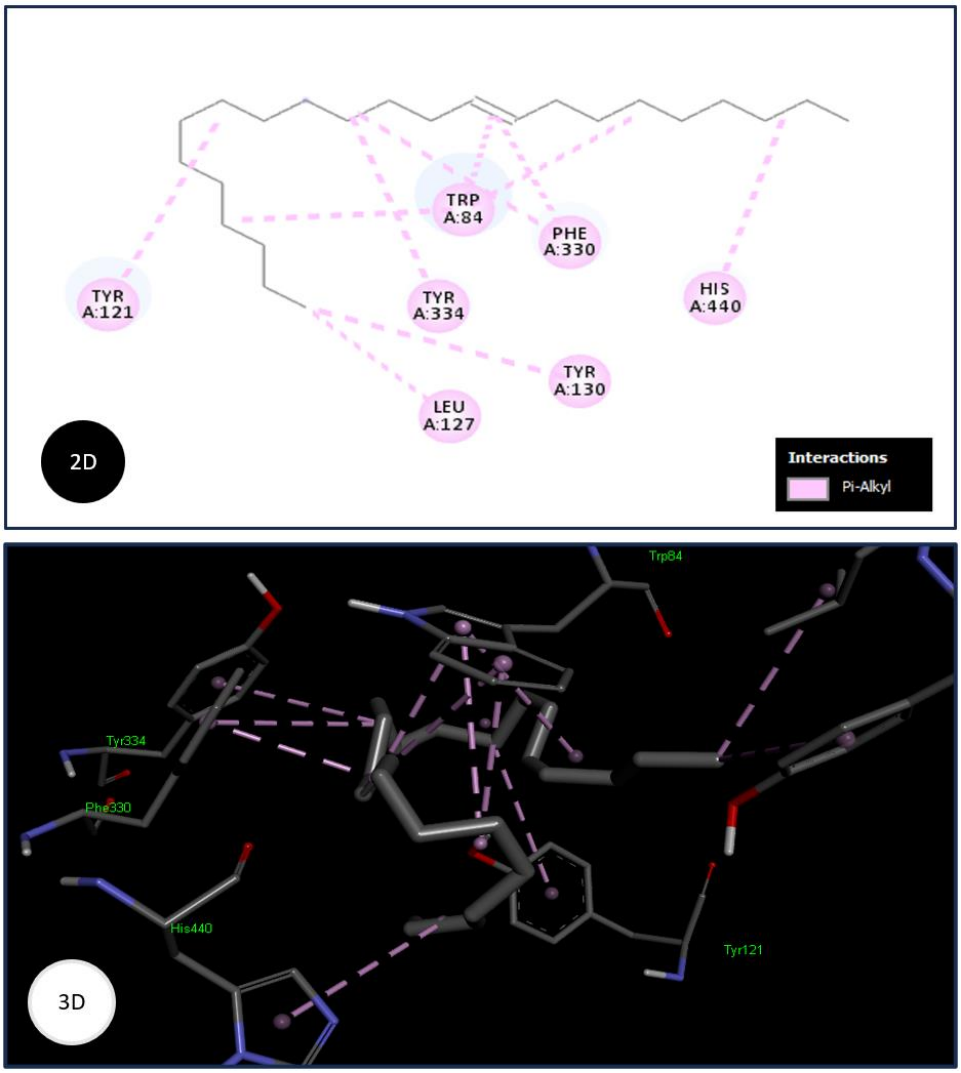

Figure 5. The 2D/3D interaction between acetylcholinesterase and Tricosene.

Table 4. Physicochemical properties the phytoconstituents of mixed ethanol extract of *Rosmarinus officinalis* and *Curcuma longa.*

S/N	Small molecule	NHA	NAHA	Fraction Csp3	NRB	NHbA	NHbD	Molar refractivity	TPSA
1	cycloeicosane	20	0	1	0	0	0	96.14	0
2	1- Trichosene	23	0	0.91	19	0	0	112.2	0
3	octacosane	28	0	1	25	0	0	136.71	0
4	1-hexanecosane	26	0	0.92	23	0	0	126.62	0
5	cis-Vaccnic acid	20	0	0.83	15	2	1	89.94	37.3
6	1-Octadecanesulfonyl chloride	22	0	1	17	2	0	102.4	42.52

NOTE: NHA: Number of Heavy Atoms; **NAHA:** Number of Aromatic Heavy Atoms; **NRB:** Number of Rotatable Bonds; **NHbA:** Number of H-bond Acceptors; **NHbD:** Number of H-bond Donors.

hydrophilicity. Cis-vaccinic acid and 1-octadecanesulfonyl chloride are exceptions, exhibiting moderate TPSA values (37.3 and 42.52, respectively), which influence their potential bioavailability and permeability.

Table 5 focuses on lipophilicity, a critical parameter for drug-likeness and absorption. Compounds like octacosane

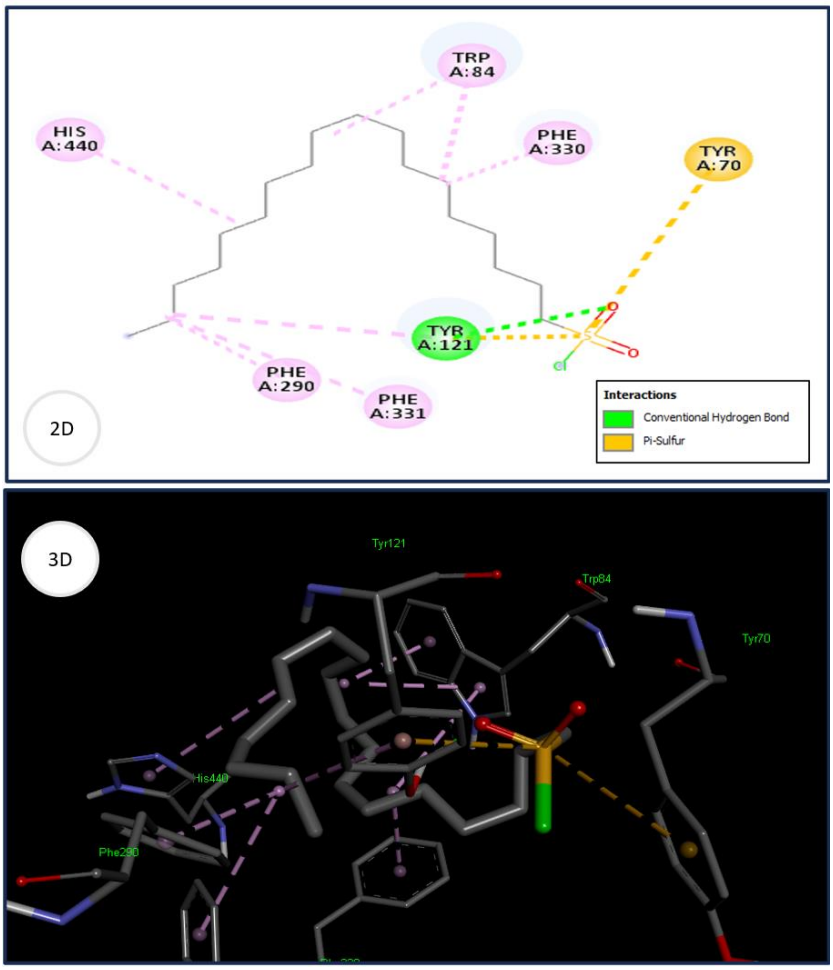

Figure 6. The interaction of cycloeicosane with acetylcholinesterase.

Table 5. Lipophilicity characteristics of the phytoconstituents of mixed ethanol extract of *Rosmarinus officinalis* and *Curcuma longa*.

S/N	Small molecule	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log Po/w
1	cycloeicosane	4.43	8.43	7.8	6.99	5.83	6.69
2	1- Trichosene	6.24	11.74	8.99	7.89	9.13	8.8
3	octacosane	7.53	14.78	11.17	9.06	11.53	10.81
4	1-hexanecosane	6.89	14.37	10.16	8.51	10.63	10.11
5	cis-Vaccnic acid	4.25	7.64	6.11	4.57	5.95	5.7
6	1-Octadecanesulfonyl chloride	4.84	9.22	7.9	4.8	6.63	6.68

Note: iLOGP: A computational model based on intrinsic molecular properties to estimate log Po/w. It integrates 3D molecular representations for prediction; **XLOGP3:** A fragment-based approach that calculates lipophilicity by summing the contributions of molecular fragments and correcting for specific effects; **WLOGP:** A fast and accurate algorithm that uses atom contributions derived from a large dataset to estimate log Po/w; **MLOGP:** A method based on molecular topology and electrotopological indices to estimate log Po/w; **SILICOS-IT:** A hybrid method combining fragmental and topological approaches for a balanced estimation of log Po/w; **Consensus Log Po/w:** An averaged value derived from multiple lipophilicity prediction methods (iLOGP, XLOGP3, WLOGP, etc.) to provide a more reliable estimate.

and 1-hexanecosane display exceptionally high consensus log P values (10.81 and 10.11, respectively), reflecting their strong hydrophobic nature. Cis-vaccinic acid, with a consensus log P of 5.7, is relatively less hydrophobic, suggesting a slightly better balance between lipophilicity and water solubility, which is crucial for

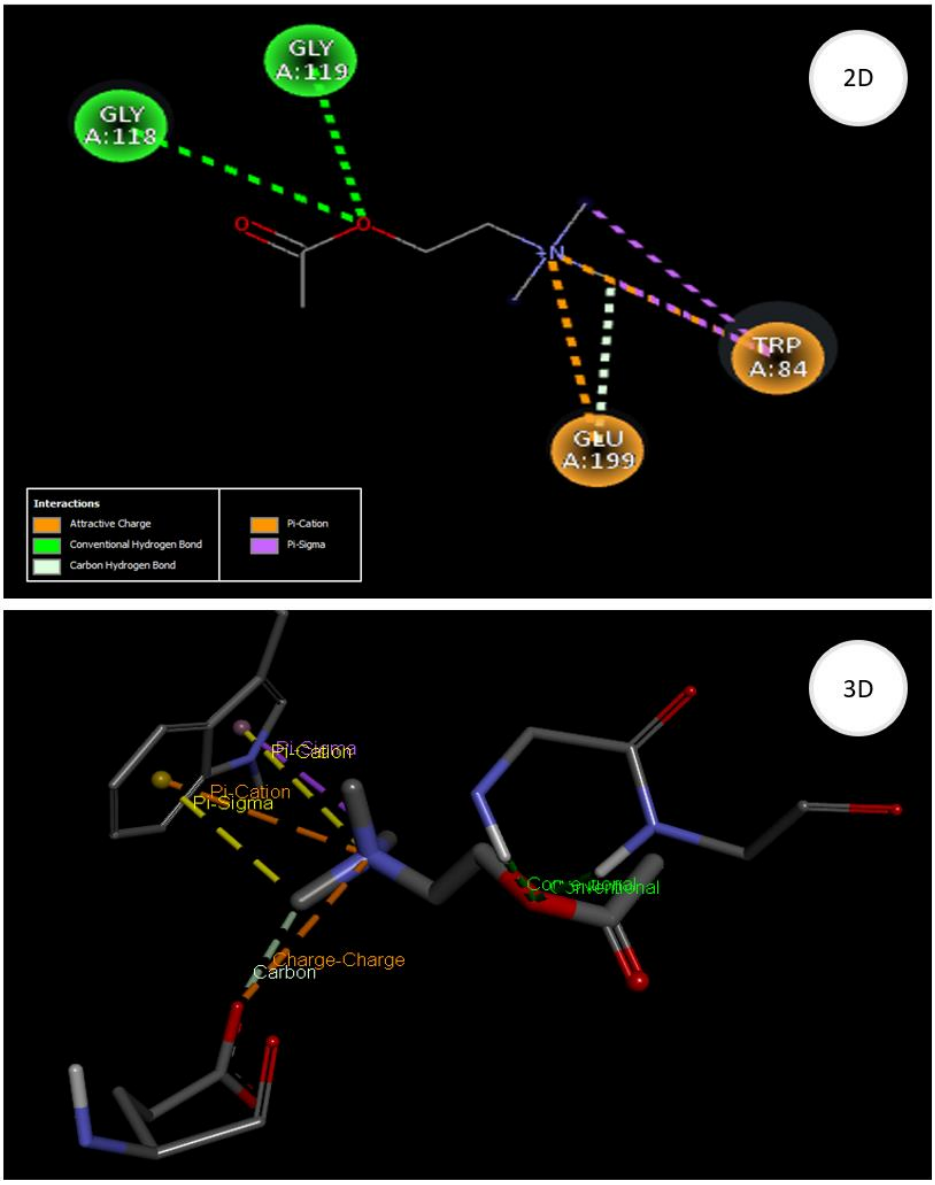

Figure 7. 2D/3D interaction between Acetylcholinesterase and Choline.

biological interactions.

Table 6 evaluates water solubility, highlighting challenges in formulation due to poor solubility for most compounds. Notably, cis-vaccinic acid exhibits moderate solubility in some models, which may enhance its potential for biological activity. The solubility classifications (poorly soluble, moderately soluble, or insoluble) provide insight into the feasibility of these compounds for pharmaceutical applications, as poor solubility often limits bioavailability.

Summarily, the phytoconstituents of *R. officinalis* and *C. longa* extracts are predominantly hydrophobic and poorly soluble, with some exceptions like cis-vaccinic acid showing moderate solubility and balanced lipophilicity.

DISCUSSION

The present study offers substantial insights into the potential of plant-derived compounds from *R. officinalis* (rosemary) and *C. longa* (turmeric) as acetylcholinesterase (AChE) inhibitors, focusing on their binding affinities and the nature of their interactions with the enzyme's active site. A comprehensive molecular docking analysis was employed to investigate the inhibitory potential of bioactive compounds present in these plants, and the results highlight the strong inhibitory potential of certain compounds, particularly Cycloeicosane and Cis-Vaccenic Acid.

Table 6. Water solubility characteristics of the phytoconstituents of mixed ethanol extract of *Rosmarinus officinalis* and *Curcuma longa*.

S/N	Small molecule	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)	ESOL Class	Ali Log S	Ali Solubility (mg/ml)	Ali Solubility (mol/l)	Ali Class	Silicos-IT LogSw	Silicos-IT Solubility (mg/ml)	Silicos-IT Solubility (mol/l)	Silicos-IT class
1	cycloeicosane	-6.89	3.61E-05	1.29E-07	PS	-8.3	1.41E-06	5.02E-09	PS	-5.45	9.95E-04	3.55E-06	MS
2	1- Trichosene	-7.98	3.36E-06	1.04E-08	PS	-11.73	5.95E-10	1.85E-12	I	-8.41	1.25E-06	3.87E-09	PS
3	octacosane	-9.95	4.44E-08	1.12E-10	PS	-14.89	5.10E-13	1.29E-15	I	-11.11	3.10E-09	7.85E-12	I
4	1-hexanecosane	-9.64	8.43E-08	2.31E-10	PS	-14.46	1.26E-12	3.44E-15	I	-9.97	3.88E-08	1.06E-10	PS
5	cis-Vaccnic acid	-5.41	1.09E-03	3.85E-06	PS	-8.26	1.54E-06	5.46E-09	PS	-5.39	1.14E-03	4.04E-06	MS
6	1-Octadecanesulfonyl chloride	-6.72	6.80E-05	1.93E-07	PS	-10.01	3.44E-08	9.73E-11	I	-7.65	7.94E-06	2.25E-08	PS

NOTE: I-Insoluble, PS- Poorly soluble, MS- Moderately soluble.

The binding affinities observed in this study, ranging from -6.3 kcal/mol for Octacosane to -7.8 kcal/mol for Cycloeicosane, suggest a significant interaction between these compounds and the AChE enzyme. These values indicate that, when compared to the standard AChE substrate choline (with a binding affinity of -4.8 kcal/mol), the identified compounds show a notably stronger interaction, which is critical for their potential role as competitive inhibitors. The stronger binding affinities are indicative of the ability of these compounds to occupy the active site of AChE and prevent acetylcholine from binding, a key mechanism in the management of Alzheimer's disease and other neurodegenerative disorders (Cortes *et al.,* 2015; Walczak-Nowicka and Herbet, 2021; Moreira *et al*., 2022). Specifically, Cycloeicosane stood out due to its higher affinity, which may contribute to its potency as an AChE inhibitor.

From a molecular interaction perspective, the analysis of 2D and 3D interactions revealed that Cycloeicosane forms multiple types of bonds—hydrogen bonds, π-alkyl interactions, and π-sulfur interactions—with crucial active site residues such as His 440, Trp 84, and Tyr 334. These interactions play a pivotal role in stabilizing the ligand-enzyme complex, thus facilitating stronger binding (Colović *et al*., 2013; Smyrska-Wieleba and Mroczek, 2023). Cis-Vaccenic Acid, though less binding-affinitive than Cycloeicosane, still demonstrated promising interactions with the enzyme, exhibiting favourable lipophilicity and moderate polar surface area, which is essential for effective bioavailability. The bioavailability of these compounds is of particular importance in therapeutic settings, as it directly influences the compound's efficacy in clinical applications (Gagliardi *et al*., 2018; Chainoglou and Hadjipavlou-Litina, 2020; Lo Cascio *et al.,* 2021). On the other hand, Octacosane, with its more hydrophobic nature, may face challenges in absorption due to its lower binding affinity and larger molecular size, a common issue observed in lipophilic compounds (Asgharian *et al.,* 2020).

These results align with prior studies suggesting that plant-based compounds with high AChE inhibitory potential could offer alternative or adjunctive therapies for Alzheimer's disease (Walczak-Nowicka and Herbet, 2021; Moreira *et al.,* 2022). For instance, compounds derived from *C. longa*, such as curcumin, have previously been investigated for their AChE inhibitory properties (Chainoglou and Hadjipavlou-Litina, 2020). The current study extends this knowledge by evaluating compounds from *R. officinalis*, which has also been traditionally used for cognitive enhancement and neuroprotective effects. These compounds could offer a dual advantage in neurodegenerative disease management, targeting not only AChE but also other pathophysiological mechanisms such as oxidative stress and inflammation (Walczak-Nowicka and Herbet, 2021; Moreira *et al.,* 2022).

Despite the promising molecular docking results, *in vitro* experimental validation remains a crucial next step. *In silico* findings alone do not guarantee physiological efficacy, as factors such as enzymatic activity, compound stability, and interaction with other cellular pathways must be confirmed experimentally. Several studies have demonstrated that compounds with strong docking affinities do not always translate into high inhibitory activity in biological systems due to factors such as poor solubility, metabolic instability, or non-specific interactions with other proteins (Walczak-Nowicka and Herbet, 2021). Therefore, *in vitro* AChE

inhibition assays using recombinant AChE or brain tissue extracts would be essential to validate the findings of this study and further assess the potency of the identified compounds.

Furthermore, the pharmacokinetic profile of the identified compounds warrants further investigation. The bioavailability, absorption, distribution, metabolism, and excretion (ADME) properties of these compounds need to be evaluated to determine their suitability for therapeuticm use. Previous studies on curcumin, for instance, have shown that its clinical use is limited due to its low bioavailability, which can be improved with formulations that enhance solubility (Gagliardi *et al*., 2018; Chainoglou and Hadjipavlou-Litina, 2020). Similarly, toxicity studies should be conducted to assess the safety of these compounds in long-term use, as their potential to inhibit AChE could also lead to adverse effects if the dosage is not carefully controlled.

Conclusion

The results of this study offer valuable insights into the potential of compounds derived from *R. officinalis* and *C. longa* as potent acetylcholinesterase inhibitors, presenting a promising approach for developing novel therapies for neurodegenerative diseases, especially Alzheimer's disease. The molecular docking results reveal strong binding affinities, with compounds such as Cycloeicosane and Cis-Vaccenic Acid exhibiting interactions that suggest they may serve as effective inhibitors, potentially surpassing the standard substrate choline. These findings highlight the need to explore plant-based compounds as viable alternatives to synthetic drugs. However, further validation through *in vitro* testing and pharmacokinetic studies is necessary to fully assess their therapeutic potential and safety. As research advances, these compounds could play a key role in developing new, effective treatments for conditions related to acetylcholinesterase dysfunction.

CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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