# Comparative Analysis of Major Bioactive Compounds of Senna alexandrina Mill. as Potential Acetylcholinesterase (AChE) Inhibitors: an in-Silico Approach to Control Alzheimer's Disease

Mushfa Khatoon1 & Amita Dubey1
1- Genetic Engineering and Translational Biology Laboratory, Integral Centre of Excellence in Interdisciplinary Research (ICEIR-10), Department of Biosciences, Faculty of Science, Integral University, Kursi Road, Lucknow, 226026, India.
*Corresponding author- Dr. Amita Dubey, Email: amita@iul.ac.in

***Abstract*—** Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a significant reduction in acetylcholine levels, primarily resulting from the excessive activity of the enzyme acetylcholinesterase (AChE). Inhibiting AChE is a widely explored approach to manage AD symptoms. This study investigated the potential of four phytochemicals—Sennidin A, Sennidin B, Emodin, and Aloe-emodin—found in *Senna alexandrina* Mill. as AChE inhibitors using molecular docking studies. The binding interactions and affinities of these compounds were analyzed to assess their inhibitory potential against AChE. Docking results demonstrated that all tested compounds exhibited strong interactions with the enzyme's active site, with Sennidin A showing the highest binding affinity of -7.51, whereas Sennidin B, despite its structural similarity to Sennidin A, exhibited the lowest binding affinity of -5.55. The other compounds also showed significant binding affinities, with Emodin (-7.35), and Aloe-emodin (-6.97) exhibiting particularly notable interactions. These interactions, involving hydrogen bonding and hydrophobic forces, suggest their potential role in AChE inhibition. Therefore, the findings of this study highlight phytochemicals obtained from *S. alexandrina* as promising candidates for AD therapy, demanding further investigation through molecular dynamics simulations and experimental validation.

***Keywords*— Senna alexandrina, *Acetylcholinesterase, Alzheimer's disease, Molecular docking, Phytochemicals, In-silico study***

## I. INTRODUCTION

Neurodegenerative diseases, particularly Alzheimer's disease (AD), have become a major public health concern due to the increasing global life expectancy and aging population. AD is characterized by progressive neuronal degeneration, memory impairment, and cognitive decline. One of the main features of Alzheimer's disease is the loss of cholinergic signaling, mainly caused by the fast breakdown of acetylcholine (ACh) by the enzyme acetylcholinesterase (AChE; EC 3.1.1.7) in the synaptic cleft (Bartus et al., 1982). Enhancing cholinergic function through the inhibition of AChE has thus been recognized as an effective therapeutic approach to mitigate the cognitive symptoms associated with AD (Lane et al., 2006).

Although several AChE inhibitors such as donepezil, galantamine, and rivastigmine have been clinically approved, their therapeutic efficacy is often limited by adverse effects, high cost, and poor pharmacokinetic profiles (Anand and Singh, 2013). These limitations have intensified the search for new AChE inhibitors; especially those derived from natural sources, due to their structural diversity, multi-targeted actions, and typically lower toxicity profiles (Kennedy and Wightman, 2011). Phytochemicals from medicinal plants continue to provide a promising foundation for novel drug discovery, including treatments for neurodegenerative conditions.

Senna alexandrina Mill. (Synm: Cassia angustifolia), a member of the Fabaceae family, is an ancient medicinal plant traditionally used for its purgative effects due to the presence of anthraquinone glycosides such as sennosides (Sultana et al., 2009). Beyond its well-established role as a laxative, S. alexandrina is a rich source of diverse bioactive compounds with reported antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities (Dubey et al., 2022). Among these, anthraquinone derivatives such as Sennidin A, Sennidin B, Emodin, and Aloe-emodin have drawn scientific interest due to their neuroprotective, anti-inflammatory, and enzyme-modulating properties (Khattak et al., 2020; Malik and Müller, 2016).

Emodin and Aloe-emodin, in particular, have been widely studied for their potential pharmacological activities, including antioxidant and neuroprotective effects (Srinivas et al., 2007). These compounds have demonstrated the ability to interfere with amyloid-β aggregation and to exert modulatory effects on key enzymes implicated in neurodegeneration, including acetylcholinesterase (Saha and Ahmad, 2024). Sennidin A and Sennidin B, although primarily associated with gastrointestinal activity, possess a similar anthraquinone scaffold and are hypothesized to share bioactivity profiles with emodin analogs. However, their potential as AChE inhibitors remains unexplored.

With the rapid development of computational tools in drug discovery, in silico methodologies such as molecular docking have become instrumental in predicting the interaction of bioactive compounds with their molecular targets (Kitchen et al., 2004).

Molecular docking allows the virtual screening of phytochemicals against target proteins, providing insights into their binding modes, affinities, and potential inhibitory mechanisms. These approaches not only accelerate the early stages of drug development but also reduce the cost and complexity associated with traditional experimental screening (Lionta et al., 2014).

This study aims to explore the potential of selected natural compounds from S. alexandrina—Sennidin A, Sennidin B, Emodin, and Aloe-emodin—as AChE inhibitors through a comprehensive in silico approach. Molecular docking study was conducted to evaluate their binding affinities and interactions with the active site of AChE.

By integrating ethnopharmacological knowledge with modern computational techniques, this study seeks to identify novel AChE inhibitors from S. alexandrina, potentially contributing to the development of safer and more effective therapeutic agents for the management of Alzheimer's disease. Moreover, the findings aim to expand the pharmacological relevance of this traditional medicinal plant beyond its conventional use.

## II. MATERIALS AND METHODS

### Selection of Ligands and Controls

In the present study, four natural anthraquinone derivatives—Sennidin A, Sennidin B, Emodin, and Aloe-emodin—derived from Senna alexandrina were selected based on their reported bioactivity and structural relevance. The 3D chemical structures of all compounds were retrieved in Simplified Molecular Input Line Entry System (SDF) format from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov; accessed on 20 January 2025) (Kim et al., 2016).

### Preparation of Target Protein (AChE)

The high-resolution crystal structure of human Acetylcholinesterase (AChE) complexed with an inhibitor (PDB ID: 7D9P) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org) (Burley et al., 2021). Protein structure preparation was conducted using PyMOL (version 2.5) (DeLano, 2002), which involved removing all crystallographic water molecules, co-factors, and non-essential ligands. Hydrogen atoms were added using AutoDockTools (MGTools) from the AutoDock suite (Trott and Olson, 2010) to ensure proper atom valency. The cleaned and hydrogenated protein was saved in PDB format for subsequent docking.

### Ligand Preparation

Ligands (including the co-crystallized inhibitor H0R) were converted from SDF to PDB format using OpenBabel (version 2.3.1) (O'Boyle et al., 2011). The converted ligand files were imported into PyRx (version 0.9.8) (Dallakyan and Olson, 2015), where hydrogen atoms were added, and energy minimization was performed using the Universal Force Field (UFF) via the conjugate-gradient algorithm. All ligands were then exported in PDBQT format, which is compatible with AutoDock Vina for docking simulations.

### Molecular Docking

Molecular docking was conducted using pyVSvina, a Python-based virtual screening tool integrated with AutoDock Vina (version 1.1.2) (Trott and Olson, 2010). The active site of the AChE receptor was defined by setting the grid box to center coordinates of X = 13.9, Y = 43.2, Z = 27.2, with grid dimensions of 23.1 × 16.3 × 21.0 Å for X, Y, and Z axes, respectively. During docking, ligand molecules were kept flexible while the receptor was treated as rigid. Docking results were evaluated based on binding affinity scores (in kcal/mol), and the best poses were selected for each compound based on the lowest docking energies.

### Visualization and Interaction Analysis

Protein-ligand complexes were visualized and analyzed using Discovery Studio Visualizer (BIOVIA, version 2021) (Biovia, 2021). Intermolecular interactions such as hydrogen bonds, π-π stacking, van der Waals forces, and hydrophobic interactions were examined to interpret the ligand binding conformation and interaction profile within the AChE active site.

## III. RESULTS AND DISCUSSION

In Alzheimer's disease (AD), the degradation of acetylcholine (ACh) due to elevated acetylcholinesterase (AChE) activity disrupts cholinergic neurotransmission, contributing to cognitive decline. As a result, AChE inhibition has become a core strategy in AD treatment. In this study, molecular docking study was performed to assess the binding efficiency and interaction profiles of four natural compounds Sennidin A, Sennidin B, Emodin, and Aloe-emodin from *Senna alexandrina* against the AChE enzyme.

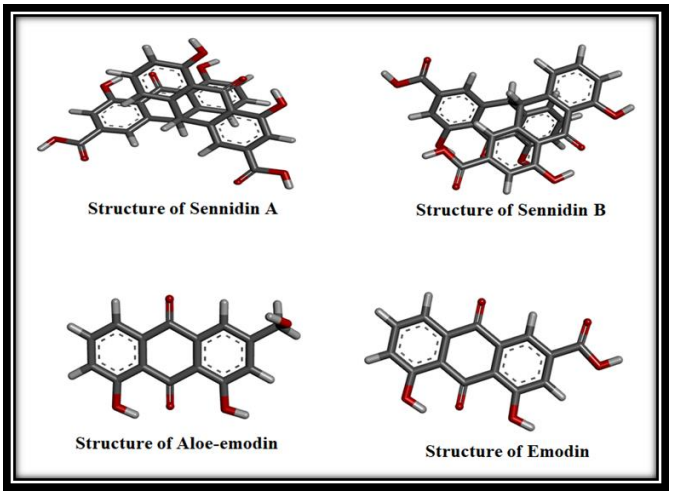


*Figure 1: 3- Dimensional structure of the compounds of S. alexandrina.*

Sennidin A exhibited the strongest binding affinity to AChE with a binding energy of −7.51 kcal/mol, suggesting a high potential to inhibit the enzyme. This molecule formed multiple interactions with catalytically important residues, such as ASP74, TYR70, ASP283, and HIS287, all of which are known to be involved in the hydrolysis of acetylcholine. The structural configuration of Sennidin A, with multiple hydroxyl and carbonyl groups and favors both hydrogen bonding, which likely contribute to its superior binding affinity. These results are consistent with previous reports indicating that anthraquinone derivatives can serve as effective AChE inhibitors due to their planar aromatic system, which allows them to insert into the enzyme's active site and interact with both the catalytic triad and the peripheral anionic site (PAS) (Ali et al., 2023; Rawat et al., 2022). Thus, Sennidin A emerges as a promising scaffold for designing novel anti-AChE agents.

Despite being structurally similar to Sennidin A, Sennidin B displayed the lowest binding energy of −5.55 kcal/mol, indicating a weaker interaction with AChE. It mainly interacted with peripheral residues like PRO432, GLN438, and ARG439, which are located away from the enzyme's catalytic core. The lower affinity may be attributed to subtle differences in the orientation or flexibility of functional groups, reducing its ability to form critical hydrogen bonds or π-π interactions with active site residues. This variation reinforces the idea that even small structural modifications can significantly alter binding efficacy. As previously reported, ligand geometry and the spatial arrangement of donor-acceptor groups are crucial for optimal binding within the AChE active site (Valasani et al., 2013). Therefore, while Sennidin B shares structural features with effective inhibitors, it lacks the precise interaction geometry required for robust inhibition.

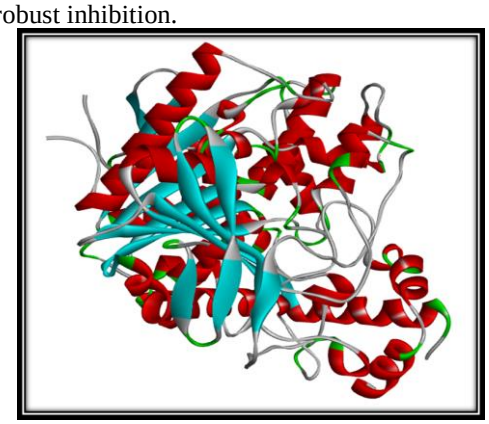

*Figure 2: 3- Dimensional structure of the Acetylcholinesterase (AChE).*

Emodin showed a favorable binding energy of −7.35 kcal/mol, closely following Sennidin A in terms of interaction strength. It formed interactions with key residues like TYR124, PHE338, ASP74, and TYR337. These residues are located in both the catalytic and peripheral sites, suggesting that Emodin may inhibit AChE through a dual-binding mechanism. Emodin is a well-known anthraquinone with reported neuroprotective and antioxidant properties. Prior studies have demonstrated its potential as a multi-target agent in neurodegenerative disorders due to its ability to inhibit AChE and reduce oxidative stress (Zhang et al., 2022).

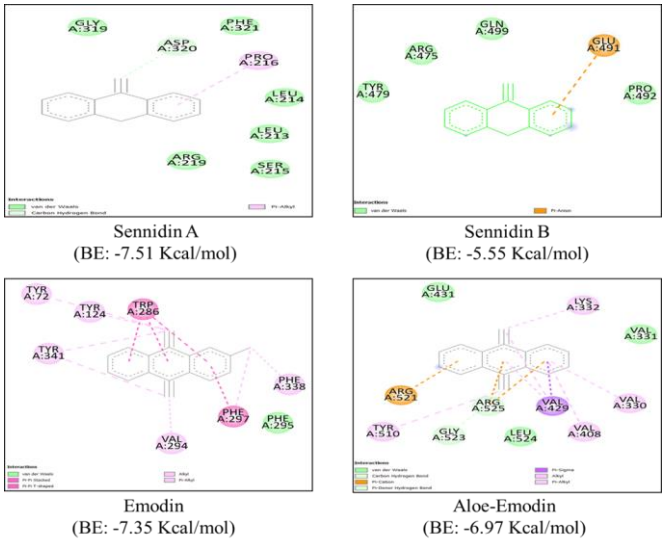


*Figure 3: Protein-ligand interactions illustrating hydrogen bonds and hydrophobic interactions between AChE and compounds of S. alexandrina.*

Aloe-emodin exhibited a binding affinity of −6.97 kcal/mol, forming interactions with several key residues, including TYR72, TYR124, GLY121, and GLU202. These residues are known to be crucial for ligand stabilization and enzymatic inhibition. Aloe-emodin's binding pattern suggests it can engage with both catalytic and peripheral domains of AChE, potentially interfering with substrate entry or turnover. Similar to Emodin, Aloe-emodin belongs to the anthraquinone family and has previously been reported for its AChE-inhibitory and antioxidant activities (Chen et al., 2021).

Overall, Sennidin A and Emodin emerged as the most promising inhibitors of AChE among the tested compounds. Their favourable binding energies and rich interaction networks with catalytically relevant residues suggest their potential as lead compounds for further development. These results provide a scientific rationale for the traditional use of Senna species in cognitive health and encourage further exploration of its bioactive constituents.

However, it is essential to acknowledge that docking studies are predictive and based on static models. Therefore, to verify these findings, further molecular dynamics (MD) simulations, in vitro enzyme inhibition assays, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) studies are recommended.

## CONCLUSION

This study presents in silico analyses and molecular docking of four natural compounds Sennidin A, Sennidin B, Emodin, and Aloe-emodin derived from Senna alexandrina against acetylcholinesterase (AChE). The compounds were evaluated for their binding affinities, interactions, and potential as AChE inhibitors. Results highlight that Sennidin A and Emodin exhibit the strongest binding affinities, suggesting their potential as therapeutic agents for Alzheimer's disease. These findings underscore the significance of natural phytochemicals in drug design and offer valuable insights into the molecular mechanisms of AChE inhibition.

## ACKNOWLEDGMENT

The authors acknowledge the support and resources provided by Integral University, Lucknow, in writing this manuscript.

## REFERENCES

1. Bartus RT, Dean RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science. 1982;217(4558):408–14. doi:10.1126/science.7046051
2. Lane RM, Potkin SG, Enz A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. Int J Neuropsychopharmacol. 2006;9(1):101–24. doi:10.1017/S1461145705006535
3. Anand P, Singh B. A review on cholinesterase inhibitors for Alzheimer's disease. Arch Pharm Res. 2013;36(4):375–99. doi:10.1007/s12272-013-0036-3
4. Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Adv Nutr. 2011;2(1):32–50. doi:10.3945/an.110.000117
5. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009;14(6):2167–80. doi:10.3390/molecules14062167
6. Dubey, A., Gupta, S., Khatoon, M., & Gupta, A. K. (2022). *Saussurea costus* (Kust) and *Senna alexandrina* (Senna). In Herbs, shrubs, and trees of potential medicinal benefits (pp. 261-290). CRC Press.
7. Khattak, A. K., Syeda, M. H., & Shahzad, S. M. (2020). General overview of phytochemistry and pharmacological potential of Rheum palmatum (Chinese rhubarb). Innovare Journal of Ayurvedic Sciences, 8(6), 1-5.
8. Malik, E. M., & Müller, C. E. (2016). Anthraquinones as pharmacological tools and drugs. Medicinal research reviews, 36(4), 705-748.

9. Srinivas, G., Babykutty, S., Sathiadevan, P. P., & Srinivas, P. (2007). Molecular mechanism of emodin action: transition from laxative ingredient to an antitumor agent. Medicinal research reviews, 27(5), 591-608.
10. Saha, P., & Ahmad, F. (2024). Neuroprotective, Anti-Inflammatory and Antifibrillogenic Offerings by Emodin against Alzheimer's Dementia: A Systematic Review. *ACS omega*, *9*(7), 7296-7309.
11. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov. 2004;3(11):935–49. doi:10.1038/nrd1549
12. Lionta E, Spyrou G, Vassilatis DK, Cournia Z. Structure-based virtual screening for drug discovery: principles, applications and recent advances. Curr Top Med Chem. 2014;14(16):1923–38. doi:10.2174/1568026614666140929124445
13. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem Substance and Compound databases. *Nucleic Acids Res.* 2016;44(D1):D1202–13. doi:10.1093/nar/gkv951
14. Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, et al. RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education. *Nucleic Acids Res*. 2021;49(D1):D437–51. doi:10.1093/nar/gkaa1038
15. DeLano WL. PyMOL: An open-source molecular graphics tool. *Delano Scientific, San Carlos, CA*. 2002. Available from: http://www.pymol.org
16. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31(2):455–61. doi:10.1002/jcc.21334
17. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. *J Cheminform*. 2011;3:33. doi:10.1186/1758-2946-3-33
18. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In: Hempel JE, Williams CH, Hong CC, editors. *Chemical Biology*. New York, NY: Humana Press; 2015. p. 243–50. doi:10.1007/978-1-4939-2269-7_19
19. BIOVIA, Dassault Systèmes. Discovery Studio Visualizer, v21.1.0. San Diego: Dassault Systèmes; 2021. Available from: https://discover.3ds.com/discovery-studio-visualizer-download
20. Rawat[1], K., Tewari, D., Bisht, A., & Chandra[1], S. Exploring [1, 1'-bianthracene]-9, 9', 10, 10'-tetrone from Senna hirsuta as a potential acetylcholinesterase inhibitor for alzheimer's disease treatment: An In-Silico investigation.
21. Ali, A., Mir, G. J., Ayaz, A., Maqbool, I., Ahmad, S. B., Mushtaq, S., ... & Rehman, M. U. (2023). In silico analysis and molecular docking studies of natural compounds of Withania somnifera against bovine NLRP9. Journal of Molecular Modeling, 29(6), 171.
22. Valasani, K. R., Chaney, M. O., Day, V. W., & ShiDu Yan, S. (2013). Acetylcholinesterase inhibitors: structure based design, synthesis, pharmacophore modeling, and virtual screening. Journal of chemical information and modeling, 53(8), 2033-2046.
23. Zhang, H., Jiang, X., Ma, L., Wei, W., Li, Z., Chang, S., ... & Li, H. (2022). Role of Aβ in Alzheimer's-related synaptic dysfunction. *Frontiers in Cell and Developmental Biology*, *10*, 964075.
24. Chen, X., Drew, J., Berney, W., & Lei, W. (2021). Neuroprotective natural products for Alzheimer's disease. *Cells*, *10*(6), 1309.