

**STUDIES ON NATURAL INHIBITORY PHYTO-
METABOLITES TARGETING
ACETYLCHOLINESTERASE FOR ALZHEIMER'S
TREATMENT**

GOURAV CHOUDHIR



**CENTER FOR RURAL DEVELOPMENT AND TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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METABOLITES TARGETING
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by

GOURAV CHOUDHIR

Center for Rural Development and Technology

Submitted

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to the**



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*Dedicated to my
parents, friends, and
seniors.*

Certificate

This is to certify that the thesis entitled " **Studies on Natural Inhibitory Phyto-metabolites Targeting Acetylcholinesterase for Alzheimer's Treatment**" being submitted by **Mr. Gourav Choudhir** to the Indian Institute of Technology Delhi for the award of "Doctor of Philosophy" is a record of bonafide research work carried out by him. He has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of our knowledge, the results contained in this thesis have not been submitted in part or full to any other university or institute for the award of any degree or diploma.



(**Hariprasad P.**)

Associate Professor



(**Satyawati Sharma**)

Professor

Centre for Rural Development and Technology,
Indian Institute of Technology Delhi,
Hauz Khas, New Delhi

110016

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Abstract

More than 55 million people are suffering from dementia globally. According to estimates, 139 million people will be affected by 2050. Alzheimer's disease (AD) is a form of dementia that affects the brain's structure and function. It is a multi-factorial disease characterized by the depositions of β -amyloid that appear in the form of plaques and hyperphosphorylated tau protein that emerges as neurofibrillary tangles in the brain. Acetylcholinesterase is a vital enzyme of the CNS and PNS; it terminates nerve signals by the hydrolysis of acetylcholine. Reduction of acetylcholinesterase activity with the aid of inhibitors has been a reliable target for Alzheimer's therapy. Commonly used cholinesterase inhibitors mediated treatment of diseases has its limitations along with several side-effects. Therefore, the discovery of AD therapy is an urgent need and a significant area of interest. Natural compounds are comparatively safer than molecules of synthetic origin. The study aimed to identify some selected medicinal plants with acetylcholinesterase inhibitory potential using *in silico* and experimental analysis.

In objective I I have explored the inhibition potential of sixty-one samples of 58 plant species collected from different regions of India and subjected to the acetylcholinesterase inhibition assay. Among 366 extracts, 40 extracts belonging to 18 plant species were found to inhibit acetylcholinesterase activity by 50 % or more. *Cyperus rotundus* rhizome extract in acetone (0.5mg/ml) exhibited the lowest IC₅₀ values, followed by *Terminalia arjuna* bark extract in methanol (0.95 mg/ml), and *Acacia catechu* stems extract in water (0.95 mg/ml). This study does not provide information about the nature of inhibition by the PSMs (plant secondary metabolites).

In objective II *in-silico* analysis of plant secondary metabolites obtained from the positive plants. *in-silico* analysis revealed the drug-likeness of plant secondary metabolites and their binding affinity with acetylcholinesterase using molecular docking and molecular dynamic simulation. Plant secondary metabolites library was prepared using a literature survey and *in-silico* analysis of ADMET analysis 73 PSMs qualified the required parameters and were taken for further studies. These 73 with the four standard drug compounds were screened for site-specific docking by using AutoDock Vina. The top 12 compounds with binding energy less than -8.1 kcal/mol, standard drugs, and substrate were subjected to MD simulation for 50 ns. The molecular dynamics simulation studies revealed that RMSD of the backbone showed the stability of protein-PSMs, and protein-drug molecules manifested the stability throughout

the simulation time. Additionally, parameters such as RMSF, Rg, and SASA were also found to wave less during the simulation time. Hydrogen bond number, hydrogen bond distribution, and hydrogen bond occupancy showed that protein interacted with PSMs and drug molecules throughout the simulation time and occupied with crucial amino acid residues that play vital roles in the catalytic activity.

In objective III, I have explored the permeability of acetylcholinesterase inhibitory drug and plant secondary metabolites showed promising binding affinity with the lipid bilayer membranes. Understanding of the permeability and interaction of PSMs and drugs with DOPC and POPC lipid bilayer using molecular dynamics simulation and meta-dynamics studies. These studies revealed that PSMs and drugs have differential permeability in the different membranes due to their structural diversity and DOPC and POPC membrane differences. Different analyses such as order parameters dipole movement, showed that the PSMs and drug molecules did not affect membrane integrity. The free energy barrier showed all PSMs, and drug molecules showed the free energy barrier below 100 kJ/mol expected acetylcholine. The free energy barrier analysis revealed high probability of drugs crossing the membrane. The structural diversity of drugs has a big impact on the drug permeability through the membrane. the *in-vitro* study can provide a more precise understanding of membrane permeability.

In objective IV based on the results of the above three objectives, rhizome extracts of *Cyperus rotundus* were selected for further studies. The LC-MS metabolomics of *Cyperus rotundus* PSMs based chemometrics analysis and their antioxidant activities. Chemometrics analysis and antioxidant activities showed that the acetone extract of *Cyperus rotundus* has more concentrated PSMs and is rich in antioxidant activity. Further chromatographic and enzyme kinetics experiments were performed to understand the enzyme inhibition and constant mechanism. The Acetone extract of *Cyperus rotundus* showed the un-competitive type of inhibition fractions 12th and 14th showed the mixed types of inhibitions. The 13th fraction showed the non-competitive type of inhibition. Fractions 15th, 16th, and 17th showed the un-competitive type of inhibition. Mechanisms of inhibition in mixtures of bioactive compounds were found due to the dominance of specific compounds. Further studies, such as *in vitro* and *in vivo* tests with purified bioactive compounds and toxicological studies, are needed to completely accept *Cyperus rotundus* as a source of natural drug molecules.

शोध सारांश

विश्व स्तर पर 55 मिलियन से अधिक लोग डिमेंशिया से पीड़ित हैं। अनुमान के मुताबिक, 2050 तक 139 मिलियन लोग प्रभावित होंगे। अल्जाइमर रोग (एडी) डिमेंशिया का एक रूप है जो मस्तिष्क की संरचना और कार्य दोनों को प्रभावित करता है। यह एक बहु-तथ्यात्मक रोग है जो डिस्क के रूप में प्रकट होने वाले β -amyloid के मैल और मस्तिष्क में न्यूरोफाइब्रिलरी टेंगल्स के रूप में उभरने वाले हाइपर-फॉस्फोराइलेटेड ताऊ प्रोटीन की विशेषता है। एसिटाइलकोलिनेस्टरेज़ सीएनएस और पीएनएस का एक महत्वपूर्ण एंजाइम है; यह एसिटाइलकोलाइन के हाइड्रोलिसिस द्वारा तंत्रिका संकेतों को समाप्त करता है। अवरोधकों की सहायता से एसिटाइलकोलिनेस्टरेज़ गतिविधि को कम करना अल्जाइमर के उपचार के लिए एक विश्वसनीय लक्ष्य रहा है। आमतौर पर इस्तेमाल किए जाने वाले कोलेलिनेस्टरेज़ इनहिबिटर्स की मध्यस्थता से रोगों के उपचार की अपनी सीमाएँ हैं, साथ ही कई दुष्प्रभाव भी हैं। इसलिए, एडी थेरेपी की खोज एक तत्काल आवश्यकता और रुचि का एक महत्वपूर्ण क्षेत्र है। सिंथेटिक उत्पत्ति के अणुओं की तुलना में प्राकृतिक यौगिक तुलनात्मक रूप से सुरक्षित हैं। अध्ययन का उद्देश्य सिलिको और प्रयोगात्मक विश्लेषण में एसिटाइलकोलिनेस्टरेज़ निरोधात्मक क्षमता वाले कुछ चयनित औषधीय पौधों की पहचान करना है।

उद्देश्य I मैंने भारत के विभिन्न क्षेत्रों से एकत्र किए गए 58 पौधों की प्रजातियों के इकसठ नमूनों की निरोधात्मक क्षमता का पता लगाया है और एसिटाइलकोलिनेस्टरेज़ निषेध परख के अधीन है। 366 अर्क में से, 18 पौधों की प्रजातियों से संबंधित 40 अर्क एसिटाइलकोलिनेस्टरेज़ गतिविधि को 50% या उससे अधिक बाधित करने के लिए पाए गए। एसीटोन (0.5mg/ml) में साइपरस रोटंडस राइजोम एक्सट्रैक्ट ने सबसे कम IC_{50} मान प्रदर्शित किया, इसके बाद मेथनॉल में टर्मिनलिया अर्जुन बार्क एक्सट्रैक्ट (0.95 mg/ml), और पानी में

बबूल केचु के तने का अर्क (0.95 mg/ml) था। यह अध्ययन PSMs (प्लांट सेकेंडरी मेटाबोलाइट्स) द्वारा निषेध की प्रकृति के बारे में जानकारी प्रदान नहीं करता है।

सकारात्मक पौधों से प्राप्त पादप द्वितीयक चयापचयों के वस्तुनिष्ठ II इन-सिलिको विश्लेषण में। इन-सिलिको विश्लेषण से पता चला है कि आणविक डॉकिंग और आणविक गतिशील सिमुलेशन का उपयोग करके एसिटाइलकोलिनेस्टरेज़ के साथ पौधे माध्यमिक मेटाबोलाइट्स की दवा संभावना और उनके बाध्यकारी संबंध। प्लांट सेकेंडरी मेटाबोलाइट्स लाइब्रेरी को एक साहित्य सर्वेक्षण और एडीएमईटी विश्लेषण के इन-सिलिको विश्लेषण का उपयोग करके तैयार किया गया था 73 पीएसएम ने आवश्यक मापदंडों को योग्य बनाया और आगे के अध्ययन के लिए लिया गया। चार मानक दवाओं के यौगिकों के साथ इन 73 को ऑटोडॉक वीना का उपयोग करके साइट-विशिष्ट डॉकिंग के लिए जांचा गया। -8.1 किलो कैलोरी/मोल से कम बाध्यकारी ऊर्जा वाले शीर्ष 12 यौगिकों, मानक दवाओं और सबस्ट्रेट को 50 एनएस के लिए एमडी सिमुलेशन के अधीन किया गया था। आणविक गतिशीलता सिमुलेशन अध्ययनों से पता चला है कि रीढ़ की हड्डी के आरएमएसडी ने प्रोटीन-पीएसएम की स्थिरता को दिखाया, और प्रोटीन-दवा के अणुओं ने पूरे सिमुलेशन समय में स्थिरता प्रकट की। इसके अतिरिक्त, सिमुलेशन समय के दौरान आरएमएसएफ, आरजी और एसएसए जैसे पैरामीटर भी कम तरंग पाए गए। हाइड्रोजन बॉन्ड नंबर, हाइड्रोजन बॉन्ड डिस्ट्रीब्यूशन और हाइड्रोजन बॉन्ड ऑक्यूपेंसी ने दिखाया कि प्रोटीन ने सिमुलेशन समय के दौरान पीएसएम और दवा के अणुओं के साथ बातचीत की और महत्वपूर्ण अमीनो एसिड अवशेषों के साथ कब्जा कर लिया जो उत्प्रेरक गतिविधि में महत्वपूर्ण भूमिका निभाते हैं।

ऑब्जेक्टिव III में, हमने एसिटाइलकोलिनेस्टरेज़ निरोधात्मक दवा की पारगम्यता का पता लगाया है और प्लांट सेकेंडरी मेटाबोलाइट्स ने लिपिड बाइलेयर मेम्ब्रेन के साथ आशाजनक बंधन संबंध दिखाया है। आणविक गतिशीलता सिमुलेशन और मेटा-गतिशील अध्ययनों का

उपयोग करके डीओपीसी और पीओपीसी लिपिड बाइलेयर के साथ पीएसएम और दवाओं की पारगम्यता और बातचीत की समझ। इन अध्ययनों से पता चला है कि पीएसएम और दवाओं की संरचनात्मक विविधता और डीओपीसी और पीओपीसी झिल्ली के अंतर के कारण विभिन्न झिल्लियों में अंतर पारगम्यता है। अलग-अलग विश्लेषण जैसे ऑर्डर पैरामीटर द्विध्रुवीय आंदोलन, ने दिखाया कि पीएसएम और दवा के अणुओं ने झिल्ली अखंडता को प्रभावित नहीं किया। मुक्त ऊर्जा बाधा ने सभी पीएसएम दिखाए, और दवा के अणुओं ने 100 kJ/mol अपेक्षित एसिटाइलकोलाइन के नीचे मुक्त ऊर्जा अवरोध दिखाया। निः शुल्क ऊर्जा बाधा विश्लेषण ने झिल्ली को पार करने वाली दवाओं की उच्च संभावना का खुलासा किया। झिल्ली के माध्यम से दवा की पारगम्यता पर दवाओं की संरचनात्मक विविधता का बड़ा प्रभाव पड़ता है। इन-विट्रो अध्ययन झिल्ली पारगम्यता की अधिक सटीक समझ प्रदान कर सकता है।

उद्देश्य में IV उपरोक्त तीन उद्देश्यों के परिणामों के आधार पर, आगे के अध्ययन के लिए साइपरस रोटंडस के प्रकंद अर्क का चयन किया गया। साइपरस रोटंडस पीएसएम आधारित केमोमेट्रिक्स विश्लेषण और उनकी एंटीऑक्सीडेंट गतिविधियों के एलसी-एमएस मेटाबोलॉमिक्स। केमोमेट्रिक्स विश्लेषण और एंटीऑक्सीडेंट गतिविधियों से पता चला है कि साइपरस रोटंडस के एसीटोन निकालने में अधिक केंद्रित पीएसएम हैं और एंटीऑक्सीडेंट गतिविधि में समृद्ध है। आगे क्रोमैटोग्राफिक और एंजाइम कैनेटीक्स प्रयोग एंजाइम निषेध और निरंतर तंत्र को समझने के लिए किए गए थे। साइपरस रोटंडस के एसीटोन के अर्क ने गैर-प्रतिस्पर्धी प्रकार के निषेध अंशों को दिखाया 12 वीं और 14 वीं ने मिश्रित प्रकार के अवरोधों को दिखाया। 13वें अंश ने गैर-प्रतिस्पर्धी प्रकार के अवरोध को दिखाया। 15वें, 16वें और 17वें अंशों ने अन-कॉम्प दिखाया।

Table of Contents

Certificate	i
Acknowledgments	ii-iii
Abstract	iv-viii
Table of Contents	ix-xii
List of Figures	xiii-xvi
List of Tables	xvii
Abbreviations	xviii-xix
Chapter 1: Introduction	1-12
<i>1.1 Alzheimer's Disease and its Pathogenesis</i>	
<i>1.2.1 Acetylcholine and its significant</i>	
<i>1.2.2 Acetylcholinesterase (AChE) (EC 3.1.1.7) Its functions and roles in Alzheimer's disease</i>	
<i>1.2.3 Catalytic Mechanism of AChE</i>	
<i>1.3 Butyrylcholinesterase (EC 3.1.1.8) and its relevance to Alzheimer's disease</i>	
<i>1.4 Choline acetyltransferase (ChAT) (2.3.1.6) and its relevance to Alzheimer's disease</i>	
<i>1.5 Three-dimensional structure of Acetylcholinesterase, Butylcholinesterase, and shared structural features</i>	
<i>1.6 Structural and functional appearance of the AChE meanwhile AD pathological events through the Amyloid pathway and P-Tau</i>	
<i>1.6.1 AChE interaction along with enzymes directly involved in β-Amyloid aggregation</i>	
<i>1.6.2 AChE arbitrate phosphorylation of the Tau protein</i>	
<i>1.6.3 AChE-associated morphological changes in CNS cell and neuronal cell loss</i>	
<i>1.7 Factors that affect the activity of Acetylcholinesterase</i>	
<i>1.7.1 Effect of Oxidative stress and inflammation on Acetylcholinesterase activity</i>	
<i>1.7.2 Metabolic disease and Acetylcholinesterase activity</i>	
<i>1.8 Clinical Approved drug for acetylcholinesterase inhibitor for Alzheimer's disease treatment</i>	
Chapter 2: Review of literature	13-28
<i>2.1 Diversity of plant secondary metabolites and their drug likeness</i>	
<i>2.2 Plants secondary metabolites reported as an inhibitor of AChE</i>	
<i>2.2.1 Alkaloids</i>	
<i>2.2.2 Terpenoids</i>	

2.3.3 Phenolic	
2.3.3.1 Phenylpropanoids	
2.3.3.2 Flavonoids	
2.3.3.3 Polyphenols	
2.3.3.4 Coumarins	
2.3.4 Glycosides	
Research Gaps	
Objectives	
Chapter 3: Screening of secondary metabolites from selected medicinal plants for AChE inhibition.	29-41
3.1 Introduction	
3.2 Materials and Methods	
3.2.1 Plant materials, collections, and identification	
3.2.2 Chemicals used in the studies	
3.2.3 Equipment used in studies	
3.2.4 Plant material processing and metabolites extractions	
3.2.5 In vitro Acetylcholinesterase inhibition assay	
3.3. Results and Discussions	
3.3.1 Acetylcholinesterase Inhibition Percentage Calculation	
3.3.2 IC ₅₀ calculated of plant extracts given Acetylcholinesterase 50 % inhibition	
3.4 Conclusion	
Chapter 4: Computational analysis of AChE inhibitory plant metabolites from the screened plants	42-92
4.1 Introduction	
4.2 Materials and Methods	
4.2.1 Virtual PSMs Library Preparation	
4.2.2. Physiochemical properties of metabolites	
4.2.3 Protein and ligand preparation	
4.2.4 Molecular docking of ligand and receptor	
4.2.5 Molecular Dynamics simulation analysis of protein and ligands	
4.2.6 Binding free energy of the interaction between AChE-ligand using MMPBSA	
4.3 Results and Discussions	
4.3.1 Virtual library preparation and Drug likeness of PSMs	
4.3.2 Active site of Acetylcholinesterase and docking protocol validation	

4.3.3. <i>Molecular docking results analysis</i>	
4.3.4 <i>Molecular dynamics simulation results analysis</i>	
4.3.4.1 <i>Root mean square deviation (RMSD) of protein-ligand during simulation</i>	
4.3.4.2 <i>Root mean deviation fluctuations (RMSF) of protein-ligand during simulation</i>	
4.3.4.3 <i>Radius of gyration (Rg) of protein-ligand during simulation</i>	
4.3.4.4 <i>Solvent accessible surface area (SASA) of protein-ligand during simulation</i>	
4.3.4.5 <i>Hydrogen bonding between ligand-protein during simulation</i>	
4.3.4.6 <i>Residue-wise energy contributions during the molecular dynamic simulation</i>	
4.4 <i>Conclusion</i>	
Chapter 5: Membrane permeability and interaction analysis of selected Plant secondary metabolites and drugs using computational methods	93-132
5.1 Introduction	
5.2 Materials and Methods	
5.2.1 <i>Molecules optimization and preparation</i>	
5.2.2 Membrane preparation and optimization	
5.2.2.1 <i>Membrane preparation and optimization</i>	
5.2.2.2 <i>Membrane and ligand simulation setup</i>	
5.2.2.3 <i>Well tempered meta dynamic for calculation free energy barrier</i>	
5.3 Results and discussions	
5.3.1 <i>Quantum mechanics calculations of PSMs and drug molecules</i>	
5.3.2 <i>Membrane properties, permeability, and interactions with PSMs and drug molecules</i>	
5.3.2.1 <i>Order parameter of membrane during PSMs and drug molecules simulation time</i>	
5.3.2.2 <i>Density profiles of lipids bilayer membrane with PSMs and drug molecules during the simulation time</i>	
5.3.2.3 <i>Lateral diffusion of the lipid's bilayer membrane during the simulation time with PSMs and drug molecules</i>	
5.3.3.4 <i>Dipole potential of lipid membrane during simulation time with PSMs and drug molecules</i>	
5.3.2.5 <i>Hydrogen bond between lipid membrane and PSMs and drugs molecules during simulation time</i>	
5.3.2.6 <i>Distance from centre of mass lipid membrane and PSMs and drug molecules during simulation time</i>	
5.3.2.7 <i>Trajectory Snapshot of lipid bilayer membrane different time frame during simulation time with PSMs and drug molecules</i>	
5.4 <i>Well-tempered Meta dynamics studies to explore free energy barrier of lipid membrane with PSMs and drug molecules</i>	

5.5 Conclusion	
Chapter 6: Metabolomic profiling of <i>Cyperus rotundus</i> and its chemometrics, chromatographic and kinetics studies	133-177
6.1 Introduction	
6.2 Materials and Methods	
6.2.1 Sample Chemicals reagents	
6.2.2 Total phenol and flavonoid content determination of <i>Cyperus rotundus</i> extracts	
6.2.3 Total antioxidant activity (phosphomolybdenum method) determination of <i>Cyperus rotundus</i> extracts	
6.2.4 DPPH free radical scavenging) determination of <i>Cyperus rotundus</i> extracts	
6.2.5 ABTS cation radical scavenging activity determination of <i>Cyperus rotundus</i> extracts	
6.2.6 Cupric ion reducing antioxidant capacity (CUPRAC) determination of <i>Cyperus rotundus</i> extracts.	
6.2.7 Ferric ion reducing antioxidant power (FRAP) determination of <i>Cyperus rotundus</i> extracts.	
6.2.8 HR-LCMS-based chemometrics analysis of <i>Cyperus rotundus</i> extracts.	
6.2.9 Bioassay guided fractionation of AChE inhibitory acetone extracts of <i>Cyperus rotundus</i>	
6.2.9.1 TLC bioautography and in-vitro AChE inhibition assay of column fractions	
6.2.9.2 Kinetics studies of selected column fractions of acetone extracts of <i>Cyperus rotundus</i>	
6.10 Statistical Analysis	
6. 3 Results and discussions	
6.3.1 Total phenol and flavonoids Antioxidant activity and Reducing power of <i>Cyperus rotundus</i>	
6.3.2 LC-MS-based chemometrics analysis of <i>Cyperus rotundus</i> extracts	
6.3.3 Thin layer chromatography and Column chromatography analysis of <i>Cyperus rotundus</i> acetone extract	
6.3.4 TLC bioautography and TLC profiling of column chromatography of acetone extracts of <i>Cyperus rotundus</i>	
6.3.5 Kinetics studies of acetone extracts of <i>Cyperus rotundus</i> and their selected fractions	
6.5.6 Determination of inhibition mechanism and calculation of K_m , V_{max} , and K_i of column fractions and acetone extract of <i>Cyperus rotundus</i>	
6.4 Conclusion	
Chapter 7: Summary, conclusion, and future prospects	178-182
References	183-215
Biodata	216-220

List of Figures

Figure no.	Figure title	Page no.
Figure 2.1	Classification of plant secondary metabolites based on chemical diversity of metabolites.	13
Figure 3.1	Plant Library and its coding	30
Figure 3.2	(a) Metabolites extraction procedure with soxhlet unit (b) Concentration of sample with rota vapor.	32
Figure 3.3	Heat map of percentage inhibition at different concentration	38
Figure 3.4	Acetylcholine esterase inhibitory activity of different plant extracts represented as IC ₅₀ values (mg/ml).	40
Figure 4.1	AChE coordinates with active site triad	63
Figure 4.2	Atomic alignment of the crystallographic ligand (Magenta) and the conformation obtained by re-docking (green) recorded RMSD of 1.323 Å with oxime reactivator RS-170B (4-carbamoyl -1-(3-{2- [(E)- (hydroxyimino) methyl] -1H-imidazol-1-yl} propyl) pyridine -1-ium).	64
Figure 4.3	(A) Physicochemical properties (B) 3D visualization (C) Visualization of different types of interactions between ligand and enzyme of selected PSMs and drugs. 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone, 13. Acetylcholine, 14. Donepezil, 15. Galantamine, 16. Rivastigmine, and 17. Tacrine.	66-74
Figure 4.4	RMSD of backbone during 50 ns simulation time 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	76
Figure 4.5	Ligand RMSD during 50 ns simulation time 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	77
Figure 4.6	Backbone RMSF during 50 ns simulation time 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	78

Figure 4.7	Radius of gyration of backbone during 50 ns simulation time 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	79
Figure 4.8	Solvent accessible surface area of backbone during 50 ns simulation time 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	80
Figure 4.9	Hydrogen bond number of AChE-PSMs and AChE-standard 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	81
Figure 4.10	Hydrogen bond distribution of PSMs-AChE and AChE-standard 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	82
Figure 4.11	Heat map showing amino acid residues forming hydrogen bonding with PSMs-AChE and AChE-standard 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	83
Figure 4.12	Residue wise energy contribution of amino acid residues with AChE-PSMs and AChE-standard 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5- hydroxy-3-(trifluoromethyl)- 4H- pyrazol-1-yl] -2- (5-methyl -2- propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	92
Figure 5.1	2D structure of selected plant secondary metabolites used in studies.	95
Figure 5.2	2D diagram (A) POPC (B) DOPC (C)Cholesterol (D) Membrane system with water.	97
Figure 5.3	(A) HOMO and (B) LUMO energy diagram of selected molecules 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-	99-103

	propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone 13. Acetylcholine 14. Donepezeil 15. Galantamine 16. Rivastigmine 17. Tacrine	
Figure 5.4	Order parameter of lipid bilayer (A) POPC sn1 chain (B) DOPC sn1 chain 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	106
Figure 5.5	Order parameter of the lipid bilayer (A) POPC sn2 chain (B) DOPC sn2 chain 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4 Beta-cyperone, 5. (1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin, 11. Rotundenol, 12. Nootkatone.	107
Figure 5.6	Density profile of membrane and its environment (A) POPC (B) DOPC 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone	109-113
Figure 5.7	Lateral diffusion of lipid of bilayer of the membrane (A) POPC (B) DOPC 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone	114-115
Figure 5.8	Dipole potential of lipid membrane of lipid of bilayer of membrane (A) POPC (B) DOPC 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone	116-117
Figure 5.9	Hydrogen bond between lipid membrane and molecules (A) POPC (B) DOPC 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone	118
Figure 5.10	Distance traveled of center of mass of molecules and bilayer of lipid of membrane (A) POPC (B) DOPC 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-	119-120

	yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone	
Figure 5.11	Trajectory snapshots illustrating the interaction of free PSMs and drug molecules (A) POPC (B) DOPC 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone 13. ACh 14. Donepezil 15. Galantamine 16. Rivastigmine 17. Tacrine	121-129
Figure 5.12	Free energy profiling of molecules transport through lipid bilayer (A) POPC (B) DOPC 1. Sugiol, 2. Margolone, 3. 7-Hydroxy-3',4'-(Methylenedioxy) flavan, 4. Beta-cyperone, 5. 1-[5-Tert-butyl-5-hydroxy-3-(trifluoromethyl)-4H-pyrazol-1-yl]-2-(5-methyl-2-propan-2-ylphenoxy) ethanone, 6. Isomargolonone, 7. Serpentine, 8. Cryptolepine, 9. Rotundone, 10. Strictamin 11. Rotundenol, 12. Nootkatone	131
Figure 6.1	Various properties of ethyl acetate, acetone, and methanol extract of <i>Cyperus rotundus</i> rhizome (A) Total phenolic content (B) Total flavonoid content (C) FRAP (D) CUPRAC (E) DPPH (F) ABTs (G) Total antioxidant activity.	142-143
Figure 6.2	Total Ion Chromatogram (HR-LCMS) <i>Cyperus rotundus</i> (A) Positive mode (B) Negative mode 1. Ethyl acetate 2. Acetone 3. Methanol	145-147
Figure 6.3	Multivariate metabolomics data analyses from three extracts of <i>Cyperus rotundus</i> (A) 2D PCA score plot, (B) 2D PLS-DA score plot, (C) Identified important biomarker metabolites from PLS-DA using VIP scores (metabolites with VIP > 1 are displayed), (D) PLS-DA model efficiency in the form of accuracy, the goodness of fit (R ²) and prediction ability (Q ²) with distinct principle component numbers (E) Heatmap analysis changed by the extraction process	155-158
Figure 6.4	Important metabolites based on VIP score (A) Name of metabolites (B) LC-MS spectra (C) 2D diagram of metabolites.	159-161
Figure 6.5	Multidimensional scaling of LC-MS metabolites of three extracts of <i>Cyperus rotundus</i>	161
Figure 6.6	Optimized TLC of acetone extracts of <i>Cyperus rotundus</i> under different light (A) Long wavelength, (B) Short wavelength, (C) Bright light.	163
Figure 6.7	Inhibition percentage of different column fractions at 1 mg/ml concentration after 15 min	164
Figure 6.8	TLC under different light (A) Long wavelength, (B) Short wavelength, (C) Bright light (D) Bioautography of selected fractions of column chromatography	165
Figure 6.9	Column loaded with silica mesh size 100-200 with plant extracts.	166
Figure 6.10	Acetylcholinesterase inhibition mechanism (A) Michaelis–Menton and (B) Lineweaver–Burk plots of acetone extract of <i>Cyperus rotundus</i> (1) Acetone extracts (2) fraction 12 (3) fraction 13 (4) fraction 14 (5) fraction 15 (6) fraction 16 (7) fraction 17.	168-174

List of Tables

Table no.	Table title	Page no.
Table 1.1	Types of dementia, its effects, and symptoms	1-3
Table 1.2	Clinically approved AChE inhibitory drugs	12
Table 3.1	Details of the plant used in the present study	34-37
Table 4.1	List of metabolites and their details	48-60
Table 4.2	ADME prediction using the Swiss ADME and pkCSM server of selected molecules.	61-62
Table 4.3	Lowest binding energy obtained from the docking study of PSMs against AChE	75
Table 4.4	Binding energies of AChE-Ligand complexes calculated by Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) method.	85
Table 5.1	Comparison of HOMO, LUMO, and energy difference of molecules	104
Table 6.1	LC-MS metabolites their peak height and retention time	148-154
Table 6.2	Values of Vmax, Km inhibition constant, and IC ₅₀	167
Table 6.3	Lineweaver Burk plot for substrates and enzymes without inhibitors	175
Table 6.4	Lineweaver Burk plot for acetone extracts and enzymes	175
Table 6.5	Lineweaver Burk plot for fraction 12 and enzyme	175
Table 6.6	Lineweaver Burk plot for fraction 13 and enzyme	175
Table 6.7	Lineweaver Burk plot for fraction 14 and enzyme	176
Table 6.8	Lineweaver Burk plot for fraction 15 and enzyme	176
Table 6.9	Lineweaver Burk plot for fraction 16 and enzyme	176
Table 6.10	Lineweaver Burk plot for fraction 17 and enzyme	176

Abbreviations

AD	Alzheimer's disease
AChE	Acetylcholinesterase
BChE	Butyl cholinesterase
ACh	Acetylcholine
FDA	Food and Drug Administration
IC	Inhibitory concentration
MIC	Minimum inhibitory concentration
VWE	Van der Waals energy
EE	Electrostatic energy
PSE	Polar solvation energy
MD	Molecular dynamic
Kcal	Kilocalorie
KJ	Kilo joule
ADME/T	Absorption Distribution Metabolism Excretion Toxicity
μg	Microgram
ml	Microliter
PSM	Plant secondary metabolite
MMPBSA	Molecular mechanics Poisson–Boltzmann surface area
SDF	Structure Data File
PDB	Protein databank file
2D	2 dimension
3D	3 dimension
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
Rg	Radius of gyration
SASA	Solvent accessible surface area
HBO	Hydrogen bond occupancy
ns	nano second
nm	nano meter

BAS	Bioavailability score
LUMO	Lower unoccupied molecular orbital
HOMO	Highest occupied molecular orbital
QM	Quantum mechanics
DFT	Density functional theory
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
DOPC	1,2-Dioleoyl-sn-glycero-3-phosphocholine
PME	Particle Mesh Ewald
NVT	Constant Number of atoms, Volume, and Temperature
NPT	Constant Number of atoms, Pressure, and Temperature