CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

In-Silico Analysis of Metabolites from *Withania somnifera* as Potential Theraputic Against Alzheimer's Disease

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences

Department of Bioinformatics and Biosciences

2026

CERTIFICATE OF APPROVAL

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Acknowledgement

All praise is due to Allah Almighty, the Most Gracious and Most Merciful, for granting me the strength and knowledge to complete this research. I am deeply grateful to my supervisor, **Dr. Muhammad Asad Anwar**, for his invaluable guidance, patience, and continuous support throughout this journey. I extend my sincere gratitude to the **Dean, Dr. Sahar Fazal, Head of Department, Dr. Syeda Marriam Bakhtiar,** and the faculty of the **Department of Bioinformatics and Biosciences, CUST, Islamabad**, for their academic support and conducive research environment, and I am also thankful to CUST, Islamabad, for providing excellent facilities and resources. **My deepest and most sincere gratitude goes to my family**, especially my **parents, siblings, brother Mr. Ameer Hamza,** and **uncle Mr. Yasir Rahim**, for their unwavering support, endless prayers, and constant encouragement. My thanks to my friends for their motivation, I am grateful to everyone who supported me directly or indirectly.

(Majida Rani)

Abstract

Cognitive decline is a hallmark of Alzheimer's disease, a complex neurological disorder. Neuroinflammation, oxidative stress, cholinergic dysfunction, amyloid-beta aggregation, and tau hyperphosphorylation are the hallmarks of this condition. As a result of Alzheimer's disease's intricate pathophysiology, multi-targeted treatment drugs are needed instead of single-target drugs. The current investigation aimed to identify multi-targeted phytochemicals from *Withania somnifera* that can simultaneously modify key Alzheimer's pathways, including protein aggregation, enzyme activity, neuroinflammation, and oxidative stress. Secondary metabolites of W. somnifera were used to screen for the three primary Alzheimer's targets in silico: tau protein, amyloid-beta peptide, and beta-secretase 1 BACE1.By examining a variety of compounds' ADMET characteristics and drug-likeness using the pkCSM web server.WithaferinA, withanolideA, cinnamic acid, anaferine,withanolide D, withasomnine, withanolide L, quercetin, chrysin, phenyl acetic acid, withanone, withanolide, B, isopelletierine and anthocyanins are among the substances we examined. We examined each compound's ADMET profile and determined if it complied with drug-likeness guidelines using pkCSM. We specifically identified the following important pharmacokinetic characteristics: toxicity, excretion, metabolism, distribution, and absorption. This enabled us to evaluate these compounds' potential as therapeutic options based on their anticipated safety profiles and bioavailability. The drug-likeness and ADMET properties of the chosen compounds were predicted using the pkCSM platform. WithaferinA, withanolideA, cinnamic acid, anaferine,withanolide D, withasomnine, withanolide L, quercetin, chrysin, phenyl acetic acid, withanone, withanolide, B, isopelletierine and anthocyanins were submitted for in silico analysis. We evaluated ADMET parameters and drug-likeness criteria compliance using pkCSM.Absorption, distribution, metabolism, excretion, and toxicity were the five main pharmacokinetic factors that were examined. By assessing the chemicals' bioavailability and toxicological safety, these projections shed light on their potential as therapeutic options. Molecular docking and interaction investigations showed that withaferin A, withanolide D, and withanolide L showed strong binding affinities like with tau

-8.3,-8.1 and -8.1 respectively,with amyloid beta -7.4,-7.6 and-7.5 respectively and with BACE1 -9.2 ,-9.1and -9.2 respectively ,long-lasting hydrogen bonds, and hydrophobic interactions with all three target proteins.Withaferin A was chosen as the smain lead molecule for additional in vivo and in vitro validation because it had the best docking score and interaction profile. Both withanolide D and withanolide L were kept as backup options for Alzheimer's treatment since they demonstrated similar multi-targeting capabilities. The findings imply that metabolites of *Withania somnifera*, particularly withaferin A, can behave as multifunctional agents by concurrently targeting oxidative stress pathways, BACE1-mediated amyloid formation, and protein misfolding. Compared to single-target tactics, this multi-targeted strategy may be more successful in halting the progression of Alzheimer's. To verify these compounds' therapeutic potential and blood-brain barrier permeability, more experimental validation is needed.Our lead chemical was compared to the commercially available anti-Alzheimer medication donepezil in order to assess its additional efficacy. Our lead compound's ADMET properties are similar to those of donepezil, according to a comparison of all drug-like characteristics. However, our lead compound's protein-ligand interaction profile is almost comparable and docking scores are noticeably superior to those of donepezil. These results suggest that our lead molecules may be a suitable anti-Alzheimer medication candidate for upcoming Alzheimer's disease treatment approaches.

Keywords: BACE1, tau protein, amyloid-beta, molecular docking, multiple targeting, Alzheimer's disease, Withania somnifera, withaferin A, and neuroinflammation

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Abbreviations

ADAD	A form of Alzheimer's disease that runs in families
ACh	Acetylcholine
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ARIA	Amyloid Related Imaging Abnormalities
Aβ	Amyloid beta
BADL	Basic activities of daily living
BBB	Brain-Blood Flow Block
CBC	Complete blood count
CDC	Centers for Disease Control
CMP	complete metabolic panel
CNS	Neurological System
CSF	Cerebrospinal Fluid
CT	Computed tomography
CVD	Cardiovascular Disorders
DTH	Delayed Type Hypersensitivity
EEGs	Electroencephalograms
FDA	Food and Drug Administration
FDG-PET	Fluorodeoxyglucose-PET
fMRI	functional MRI
FTD	Frontotemporal Dementia
GI	Glycaemic Index
GL	Glycaemic load
IADL	Instrumental activities of daily living

IPA	Inhibiting platelet-activating
LBD	Lewy Body Dementia
MAE	Microwave-Assisted Extraction
MCI	Moderate cognitive impairment
mhGAP	Mental Health Gap Action Programme.
MIBG	Metaiodobenzylguanidine
MMSE	Mini-mental Status Exam
MOCA	Montreal Cognitive Assessment Exam
MRI	Magnetic resonance imaging
ND	Neurodegenerative Disorders
NET	Neurofibrillary Tangles
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NMDA	N-methyl-D-aspartate
n-3 PUFA	Fatty acids with an omega-3 profile
PET	Positron Emission Tomography
PSG	Polysomnography
REM	Rapid Eye Movement Sleep Behavior Disorder
SAD	Acute dementia with sporadic symptoms
SE	Soxhlet's Extraction
SFE	Supercritical fluida Extraction
SPECT	Single-Photon Emission Computed Tomography
TSH	Thyroid-stimulating hormone
UAE	Ultrasound-AssistedExtraction
UASE	Ultrasound-assisted Extraction
VCI	Vascular Cognitive Impairment
WHO	World Health Organization

Chapter 1

Introduction

Changes in lifestyle and demography have led to an increase in the amount of people afflicted, and the psychological and monetary toll these conditions take on patients and caregivers, neurodegenerative disorders (NDs) have emerged as a pressing public health issue [1, 2]. Without adequate measures to prevent or treat these diseases, the already staggering number of individuals impacted by neurodegenerative illnessesmore than 50 millionis projected to rise to approximately 152 million by the year 2050. With 60-80% of all cases being Alzheimer's disease (AD), it is by far the most common neurodegenerative condition. Despite the fact that AD was initially documented in 1907, the precise reasons behind it are still unknown, and there is now no known cure. Autosomal-dominant Alzheimer's disease (ADAD) is a hereditary form of AD that typically manifests before the age of 65; the more prevalent sporadic form, on the other hand, typically manifests later in life [3].

The Food and Drug Administration classifies Alzheimer's disease as a degenerative brain disease that manifests over a long period of time. Misfiring and disconnection of neurons in the brain start early on. Immediate effects on cognition, memory, and decision-making result from interference with the brain's communication network. The exact reason for this cell damage is still a mystery to scientists. The severity of damage grows as the disease progresses to later stages. At this stage, the person has dementia, a significant deterioration in brain function that makes it challenging

to do daily tasks. Managing finances, keeping track of appointments, following discussions, and even identifying familiar locations become difficult. The brain's inability to process information normally causes changes in mood, behaviour, and personality in addition to memory loss [4].

The DSM-5 classifies Alzheimer's disease as a Major or Mild Neurocognitive Disorder owing to AD. It is brought on by beta-amyloid plaques and neurofibrillary tangles that harm cholinergic neurones. Behavioural symptoms and progressive memory loss result from the reduction of acetylcholine caused by that injury [5].

Damage to the CNS, both structural and functionalis a characteristic that happens at every stage of Alzheimer's disease (AD). Neuronal degeneration and abnormal protein accumulation are among these [6, 7]. Anatomical, neurophysiological, biological, and cognitive alterations occur slowly but steadily as Alzheimer's disease progresses [8]. In the early stages of the disease, soluble Aβ oligomers impair dendrites, synapses, and axonal circuits, leading to localized neuronal dysfunction. There has been increased focus on soluble Aβ oligomers (AβO) throughout the last decade because of their apparent higher toxicity and stronger correlation with disease development compared to other Aβ forms [9, 10].

Multiple environmental and genetic risk factors are associated with its development. The most important hereditary component is an allele of the apolipoprotein E gene, which is involved in lipid metabolism in humans. Some other risk factors include having a history of head trauma, severe depression, high blood pressure, or both. One hallmark of the progression of the disease is the creation of neurofibrillary tangles and amyloid plaques, which are aberrant protein deposits in the brain's cortex.As time goes on, these aggregation of misfolded proteins disrupt normal cell function, leading to neuronal degeneration and the eventual loss of synaptic connections in the brain.A probable diagnosis is reached by considering the patient's medical history, cognitive tests, imaging studies, and blood tests, all of which help to rule out other possible causes.Many people mistake the early symptoms for normal brain aging.It is believed that the first signs of Alzheimer's disease manifest as manifest before these oligomers ever exist [11, 12].

Clinical symptoms may not manifest at first, but brain lesions develop over time and cause neuronal death in certain areas [13–16]. The damage causes cognitive decline and memory loss [17]. In clinical trials, various methods for treating Alzheimer's disease have been investigated. However, current treatments primarily work to reduce symptoms rather than cure the underlying cause [18–20].

More and more, scientists are trying to figure out how to prevent issues from ever occurring. Multiple studies have shown that modifiable risk factors may be associated with over 30 of AD cases [21–23]. Because of these characteristics, there is a good chance that we can halt the progression of neurodegeneration and cognitive impairment by prophylactic measures. An extremely difficult aspect of Alzheimer's disease treatment is getting a diagnosis when the disease is still in its preclinical stages [24].

While certain treatments may momentarily alleviate symptoms, none can halt or reverse the disease's course. Social interaction, exercise, and a nutritious diet are usually good for ageing and may lower the risk of Alzheimer's and cognitive decline.[22].

Affected individuals become more dependent on others for support, which frequently puts a strain on carers. There may be social, psychological, physical, and financial demands.Exercise regimens may help with everyday living tasks and may lead to better results.Antipsychotics are occasionally used to address behavioural issues or dementia-related psychosis, but doing so increases the risk of dying young [25].

Brain health and cognitive function are significantly impacted by fatty nutritional deficiencies. The brain contains a high concentration of omega-3 polyunsaturated fatty acids (n-3 PUFA), which are part of the phospholipids that make up the membranes of neurons [26, 27]. Theoretically, these fatty acids facilitate synaptic plasticity and neuronal activity [28]. A better lipid balance in the central nervous system may be possible with dietary interventions, since food is a primary source of these nutrients [29]. Because of the CNS's protective barriers, it is difficult to transport therapeutic medicines to the brain, which is another obstacle in treating

AD [30, 31]. Toxins, toxic compounds, and germs are able to avoid reaching the brain because of the blood-brain barrier (BBB). But this same barrier prevents a lot of medicinal substances from reaching brain cells as well.

Large-scale funding for Alzheimer's research is due to the widespread effects of the disease, basic science and health funders in several nations have contributed. An example of this is the US National Institutes of Health's Alzheimer's research project, the National Plan to Address Alzheimer's Disease, which has a budget of US$3.98 billion for fiscal year 2026.[32] in Through the 2020 Horizon program, the European Union allocated more than €570 million to programs that combat dementia. Europe research program [25].

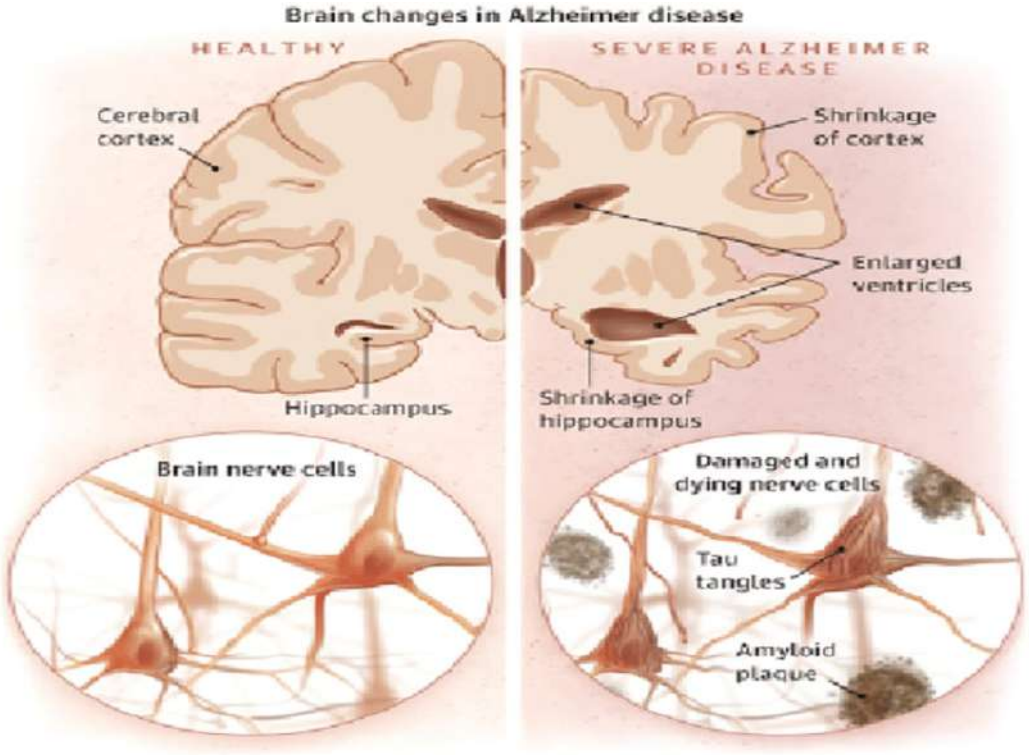


FIGURE 1.1: Comparison of Normal Brain vs Alzheimer's Brain.

This picture illustrates "Brain changes in Alzheimer disease" by contrasting the brains of people with severe Alzheimer's disease with those in good health. The hippocampus and cerebral cortex are full and normal in size in a healthy brain, and there are obvious connections between nerve cells. Severe Alzheimer's causes the hippocampus and cortex to shrink, the ventricles to grow as a result of tissue

loss, and damaged and dying nerve cells. Amyloid plaques accumulating outside of cells and tau tangles growing inside of cells are the causes of this damage, which impairs communication and results in memory loss and cognitive declin [33].

Researchers are currently showing a lot of interest in plant-based bioactives because of the health benefits they provide through the presence of beneficial secondary metabolites. The present state of global healthcare has forced more than 80% of the world's population, primarily in poor countries, to depend on phytomedicines for their therapeutic needs. Traditional medicinal systems such as Ayurveda, Greco-Arab (Unani-Tibb), and Chinese medicine still rely on phytomedicines due to their lower cost and less side effects. be successful [34].

There are over 4000 species and 90 genera in the Solanaceae family. The family is quite diversified, including perennial trees and herbaceous annual species that can be found in a variety range of terrestrial habitats, from tropical forests to arid wasteland. The wide variety of alkaloids found in the Solanaceae family is well-known. Unique bicyclic structures characterize these compounds, which comprise hyoscyamine, atropine, and scopolamine. When administered therapeutically, these anticholinergics block the action of the body's natural neurotransmitter acetylcholine, which is responsible for the transmission of nerve impulses.

The Solanaceae family contains a wide variety of bioactive compounds, including steroidal alkaloids, saponins, terpenes, flavonoids, and phenolics.Numerous pharmacological benefits, including hepatoprotective, cardioprotective, kidney-protective, anti-inflammatory, and anti-ulcerogenic actions, have been associated with this family. *Withania somnifera* (L.) Dunal is a member of the Solanaceae family and is used extensively in medicine worldwide [35].

Traditional Indian medicine (Ayurveda) is well-established as an effective treatment for neurological disorders. Around 450 medicinal plants, 56 of which are important species, and the active metabolites they contain are detailed in Ayurvedic medicine's treatment of neurological problems. There are many medicinal plants, but *Withania somnifera* is among the most famous. It is widely used in Ayurvedic therapies for neurological illnesses and has a long history of usage.

Ashwagandha, Indian ginseng, and Indian winter cherry are some of the names given to this member of the Solanaceae family of plants. The somnifera moniker is derived from the Sanskrit words for "horse" and "smell," alluding to the strong scent emanating from the plant's roots; the name "Ashwagandha" is derived from the same root word. [21].

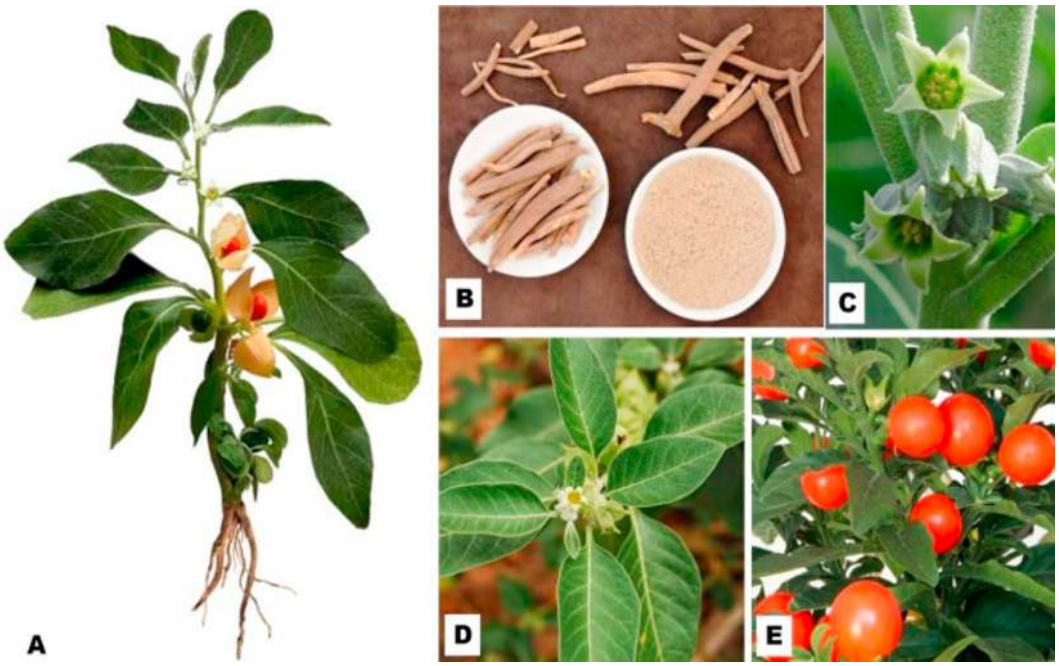


FIGURE 1.2: Morphological parts of *Withania somnifera*. (A) Whole plant with tuberous roots, (B) Dried roots and root powder, (C) Flower bud, (D) Leaves and flowers, (E) Mature berries [36].

It is considered a revitalizing herb that promotes bodily health, mental wellness, and lifespan in Ayurvedic medicine. There is some evidence that it may help with conditions including epilepsy, PD, AD, CS, and tardive dyskinesia [37]. *W. somnifera* has neuroprotective, anti-inflammatory, anti-diabetic, anti - hepatitis, anti-osteoporotic, and anti-neoplastic qualities. The treatment of insomnia is one of its traditional uses, thyroid issues, infections, and reproductive issues, as well as weight loss and other related conditions. The normal height of this evergreen shrub is half a meter to two meters. It has stems that are tuberous, leaves that are stalked, flowers that are greenish-red, and berries that are orange-red. Despite its widespread distribution in warm climatesincluding South Asia, the Mediterranean, the Middle East, and Africathe medicinal roots are mostly pursued in cultivation

on the Indian subcontinent. One of the main components of neurodegeneration is oxidative stress.

Neurons in the brain are particularly vulnerable to oxidative damage due to the high oxygen consumption and high concentration of polyunsaturated fatty acids. Lipid peroxidation and excessive reactive oxygen species formation lead to an imbalance between oxidants and antioxidants, which ultimately leads to neuronal death [37]. Research has shown that the active compounds and extracts of *W. somnifera* had strong antioxidant capacities. These compounds may mitigate AD-related neurodegeneration by controlling inflammation, reducing oxidative stress, and maintaining proper calcium homeostasis supporting aneurite development, and enhancing the elimination of beta-amyloid peptides [37].

Pharmaceutical options for Alzheimer's disease are very limited, despite decades of study. Although they are widely used, the most common medications, such as acetylcholinesterase inhibitors and NMDA receptor antagonists, only alleviate symptoms temporarily and do not halt the progression of the disease. In addition to the intended benefits, these medicines may bring about unwanted side effects including nausea, vomiting, and even heart problems. As a result, research for safer and more efficient alternatives that target AD's root causes is gaining momentum. A lot of people are interested in medicinal plants because of the neuroprotective, anti-inflammatory, and antioxidant effects they have. Ginkgo biloba, Bacopa monnieri, Curcuma longa, Rosmarinus officinalis, and Willow somnifera are some of the herbs that have demonstrated potential impacts on cognition and memory [38].

The bioactive components of Ashwagandha, which include withanolides, alkaloids, flavonoids, and phenolic compounds, are primarily responsible for its therapeutic effects. In various sections of the plant, the concentration of these compounds differs. As an example, the concentrations of withanolides and alkaloids vary among the plant's various parts, such as roots, leaves, and stems [32]. Ephedrine, coniine, lobeline, hyoscine, yohimbine, somniferine, withasomine, solanidine, and ashwagandha root are among the significant alkaloids found in this traditional Indian herb. These chemicals help with pain relief, inflammation, antioxidant

defense, and neuroprotection. The plant's other chemicals may have similar effects on mood, cardiovascular health, cognition, and relaxing of muscles [39].

Extracts from *Withania somnifera* exhibit high antibacterial properties. In agar diffusion tests, methanolic and n-hexane root/leaf extracts inhibited Salmonella typhimurium and E. coli, whereas aqueous leaf extract was more effective against Raoultella planticola. Oral aqueous extract demonstrated a bacteriostatic efficacy comparable to that of chloramphenicol, while the butanolic sub-fraction of methanolic extract demonstrated substantial activity against S. typhimurium. In tests for cytopathic impact reduction, root tuber glycoprotein also suppressed the bursal disease virus. Aspergillus flavus, Fusarium oxysporum, and F. verticilloides were all reduced in their ability to proliferate by monomeric glycoprotein from root tubers. Using disc diffusion and serial dilution techniques, leaf, root, and stem extracts showed modest fungistatic activity against Candida albicans and Candida flavus. Co-administration of hexane and methanolic extracts with "Tibrim" significantly boosted antibacterial activity, indicating potential for synergy [40].

A few review studies examining various characteristics of *Withania somnifera* have been published in the literature. Aiming to assess current knowledge regarding the miraculous plant's phytochemical profile, medicinal applications, and nutrapharmaceutical promise A comprehensive evaluation is still necessary for *Withania somnifera* [34].

To investigate *Withania somnifera*'s medicinal potential, it is necessary to extract its bioactive components. Some examples of traditional extraction methods are reflux, maceration, infusion, and Soxhlet. Isolating bioactive chemicals also makes use of modern technologies such ultrasonic extraction using solvents, extraction with the help of microwaves, extraction using supercritical fluids, and extraction with the help of ultrasounds [39, 41].

Existing research on Ashwagandha has many unsolved questions, despite its potential medicinal effects. Further research is necessary to have a better understanding of its exact mechanisms of action, the best dosage to use, its safety over the long term, and its effectiveness in different populations. Its impacts on fertility, stress,

cognitive function, and cardiometabolic health should be further investigated [42]. This review aims to explore the potential of Withaniasomnifera as an Alzheimer's disease treatment. What makes it bioactive and how it works pharmacological properties, and potential involvement in enhancing existing AD treatments are highlighted in particular.

In this study, we use computational techniques to model biological systems, predict molecular interactions, and identify potential drugs through in silico research.By producing high-quality forecasts, in silico methods have enhanced healthcare research throughout the past century. These techniques aid medical biotechnologists in creating vaccines, such as multi-epitope vaccines that employ immunoinformatics and reverse vaccinology. Numerous in vitro, in vivo, and clinical investigations have demonstrated encouraging outcomes.

These days, machine learning and artificial intelligence categorise data to create accurate forecasts and direct pandemic preparation. By studying biological systems using computer-based techniques, bioinformatics lowers laboratory expenses and the need for animals.

Additionally, in silico methods support ML-based servers for molecular sorting and classify proteins according to their structure and function. Natural ligand-receptor interactions are examined using molecular docking and other in silico techniques.They offer information about unidentified structures, like as enzymes and their ligands, which is useful for biotechnology's future genetic engineering [36].

1.1 Problem Statement

A key cause of dementia, Alzheimer's disease, currently has no effective disease-modifying medications, hence research into alternative therapeutic techniques is necessary. Because of the neuroprotective, anti-oxidant, and anti-inflammatory effects of the bioactive compounds in *Withania somnifera* (Ashwagandha), studies

investigating their potential to target key pathophysiological pathways in Alzheimer's disease are necessary.

1.2 Hypothesis

Withania somnifera bioactive compounds may improve cognitive function and reduce amyloid-beta aggregation in Alzheimer's disease.

1.3 Gap Analysis

Alzheimer's is a major cause of dementia-related mortality worldwide because current medicines only alleviate symptoms and have serious adverse effects. There are currently no therapeutics that target disease processes. Poor bioavailability, unknown blood-brain barrier permeability, and a focus on entire extracts rather than active components are the main limitations of ashwagandha research.Metabolites like withanolides can be found to target several pathways, such as tau protein, acetylcholinesterase, and BACE-1, using in-silico techniques including molecular docking, pharmacophore modelling, and ADMET prediction. These substances may protect neurones through various metabolic pathways since neurones are not injured completely at once and roughly 70% remain near-damaged. Because of its favourable safety profile and variety of metabolites.One promising option for creating novel Alzheimer's medications that target particular biochemical targets is *Withania somnifera*.

1.4 Aim and Objectives

Examining the potential of *Withania somnifera*'s bioactive components to inhibit acetylcholinesterase, amyloid-beta aggregation, neuroinflammation, and oxidative stress in slowing the progression of Alzheimer's disease is the primary goal of this research. This study has following objectives:

i. Investigate the potential of *Withania somnifera*'s bioactive components to prevent amyloid aggregation in Alzheimer's disease.

ii. Determine how well they protect neurons by means of antioxidant and anti-inflammatory mechanisms.

iii. Molecular docking experiments can be used to determine how the bioactive chemicals of *Withania somnifera* interact with Alzheimer's disease targets, including tau, acetylcholinesterase, and Aβ, and assess their potential to prevent pathogenic binding.

Chapter 2

Literature Review

Between 60% and 80% of dementia cases are caused by Alzheimer's disease, a terrible sickness that impacts thinking, being, and remembering. It is characterized by cognitive decline, neuronal death, tau tangles, and beta-amyloid plaques. Memantine, rivastigmine, and donepezil are effective medicines that aim to alleviate symptoms rather than stop the progression of the disease. The withanolides and withanosides that are bioactive in ashwagandha make it a potentially useful alternative medicine. The neuroprotective, anti-inflammatory, and antioxidant characteristics are included in these components. It is believed that these chemicals can reduce the symptoms of beta-amyloid plaques, tau tangles, and oxidative stress. The importance of in-silico research in creating novel Alzheimer's disease therapeutics and the bioactive chemicals obtained from *Withania somnifera* are both highlighted in this item.

2.1 Alzheimer's Disease

Alzheimer's disease, which disproportionately affects people aged 65 and up, is distinct from age-related memory loss. Slowly but surely, this permanent brain disorder will manifest itself in apathy, depression, and memory loss related to recent conversations or occurrences. The disease is associated with neuroinflammation, oxidative stress, mitochondrial dysfunction, genetic anomalies, and incorrect

protein folding. One of the major challenges in treating Alzheimer's is the lack of biomarkers that may be used for early detection, prevention, and treatment. A primary focus of the 2018 mhGAP Forum was the acceleration of mental health initiatives [43].

2.2 History and Physical

Diagnosing Alzheimer's disease requires a comprehensive review of medical records along with a physical examination. It is essential to get information from family members and caregivers, as some patients might not completely comprehend their ailment. The examination of functional abilities, which encompass both instrumental and fundamental activities of daily living, helps in determining the patient's cognitive and functional state. Instrumental activities, such as grocery shopping, necessitate higher-level cognitive ability., money management, cooking, and housework, which are especially helpful in detecting impairment.It is crucial to comprehend how symptoms, such as altered memory, behavior, cognition, and day-to-day functioning, start, develop, and evolve. A review of medication history is also necessary, particularly with regard to the usage of anticholinergic medications that may impair cognition. It is important to take into account social background, especially drug and alcohol usage, as these factors may affect cognitive function. To ascertain the stage of a comprehensive evaluation of mental health and neurological function is necessary to diagnose Alzheimer's disease and to rule out other possible diseases. For an accurate diagnosis, a comprehensive clinical evaluation is necessary [44].

In most cases, the Mini-Cog findings are unaffected by the patient's degree of education. Nervous system symptoms include primitive reflexes, apraxia, aphasia, and frontal discharge signals can be seen in patients with advanced Alzheimer's disease. As their condition progresses, they may lose the ability to move about on their own, become increasingly dependent on their carers, stop responding to spoken commands, and eventually enter a persistent vegetative state. It is important to measure the mental health of Alzheimer's patients across a number of cognitive

domains, including attention, focus, memory (both recent and distant), language, visual-spatial ability, practical skills, and executive function. Include these tests in your follow-up appointments to help monitor your disease and neuropsychiatric symptoms. determining the degree of cognitive impairment [44].

2.3 Etiology

A characteristic of Alzheimer's disease is the death of neurons, which often begins in the entorhinal cortex of the hippocampus. Trisomy 21 is linked to early-onset dementia, but there are other, non-genetic factors that can influence the condition as well. The main determinant of Alzheimer's disease risk is becoming older; the disease's incidence roughly doubles every five years after the age of 65. Neuroinflammation, beta-amyloid buildup, and cognitive impairment can be accelerated by modifiable risk factors such as cardiovascular disease, diabetes, and obesity.

Further risk factors include APOE e4 genotype, depression, smoking, traumatic brain injury, and a history of dementia in the family. Elevated homocysteine levels are another risk factor. If you have an immediate relative with Alzheimer's disease, your risk is 10-30% higher. If you have two or more affected relatives, your risk is about three times higher. Protective variables include having a bachelor's degree or above, using anti-inflammatory drugs as prescribed, eating well, exercising regularly, and enjoying hobbies like reading and music [45].

2.4 Epidemiology

The elderly make up the bulk of AD cases. From 20.3 million in 1990 to 43.8 million in 2016, there was a staggering 116% growth in the number of dementia cases globally. The prevalence of dementias, including Alzheimer's disease, increased by 160.84% between 1990 and 2019, whereas the incidence increased by 147.95%. An estimated 150 million individuals would be impacted by dementia in 2050, which is four times the current number.There is a 5% annual rise in the risk of having

Alzheimer's disease after 65. The age-specific incidence rate was 6% for people over the age of 85 and less than 1% for those under the age of 65.There is a 40% increase in the prevalence rates beyond the age of 65, up from 10% before. The incidence of Alzheimer's disease is slightly greater in females after the age of 85 [46].

2.5 Pathophysiology

Neuritic plaques, neurofibrillary tangles, and neuronal death are pathological characteristics of Alzheimer's disease [47]. This is particularly true of cholinergic neurons in the neocortex and basal forebrain. Deterioration of neurons in the Nuclei Basalis of Meynert causes acetylcholine levels to drop, which causes Alzheimer's disease, according to the cholinergic theory. In cholinergic dysfunction, beta-amyloid worsens things by boosting synapse loss and lowering acetylcholine release. According to the amyloid hypothesis, the amyloid precursor protein is cleaved by β- and γ-secretase enzymes, resulting in the production of amyloid beta peptides, specifically Aβ42. An rise in Aβ42 levels leads to the buildup of amyloid-β, a harmful protein that damages neurons and accelerates the progression of Alzheimer's disease. [48].

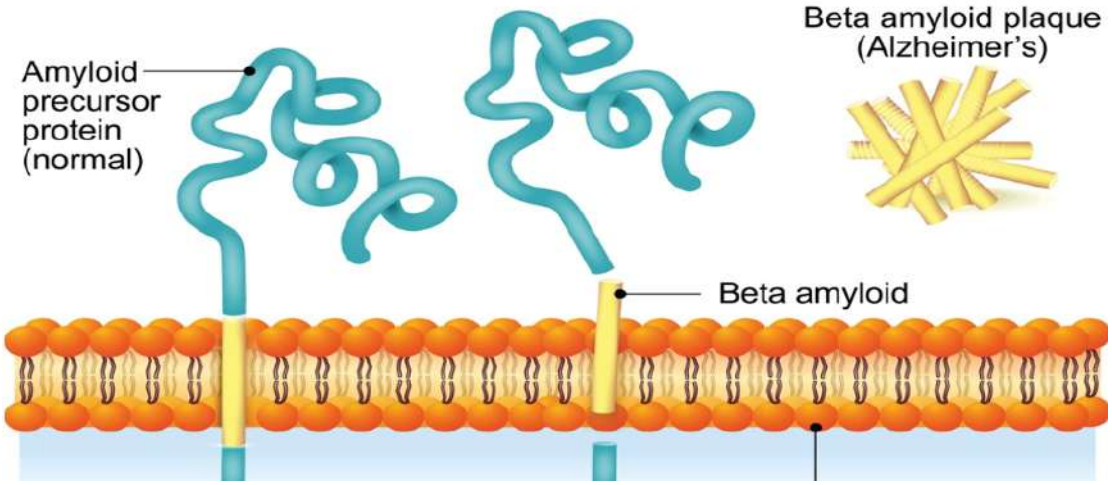


FIGURE 2.1: APP Processing and Beta Amyloid Plaque Formation in Alzheimer's Disease [49].

In Alzheimer's disease, tau pathology disrupts normal neuronal function; it typically begins in the entorhinal cortex, a region of the brain important for memory, and then spreads to other areas. Accumulated tau degeneration worsens brain shrinkage and cognitive impairment. Memory loss, disorientation, and difficulties with language and solving problems are common symptoms of Alzheimer's disease. [50].

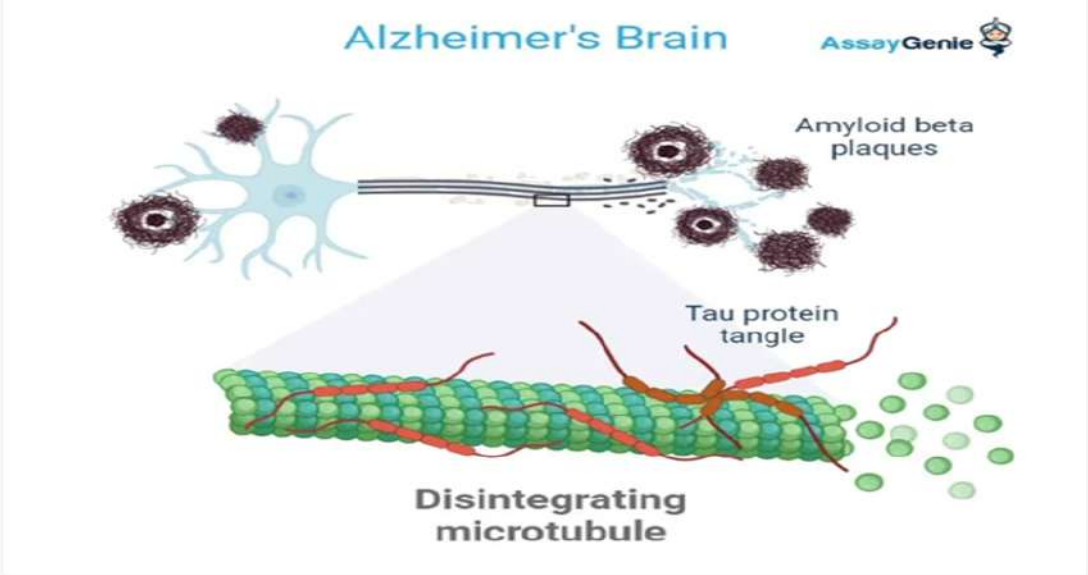


FIGURE 2.2: Amyloid Beta Plaques and Tau Tangles in Alzheimer's Brain Pathology [50].

2.6 Mechanism of Tau and Amyloid-beta Proteins in Alzheimer's Disease

Both Tau and APP are involved in the mechanism that cause neurodegeneration and cognitive loss in Alzheimer's disease. Mechanism 1:Tau protein typically aids in the stabilisation of microtubules within neurones, which are crucial for nerve cell transit and structure. Tau gets hyperphosphorylated, or has an excessive number of phosphate groups attached to it, in Alzheimer's disease. As a result, tau separates from microtubules, causing them to disintegrate. Neurofibrillary tangles are created when the free tau aggregates. Neurodegeneration and memory loss come from neurones losing their shape and dying [51].

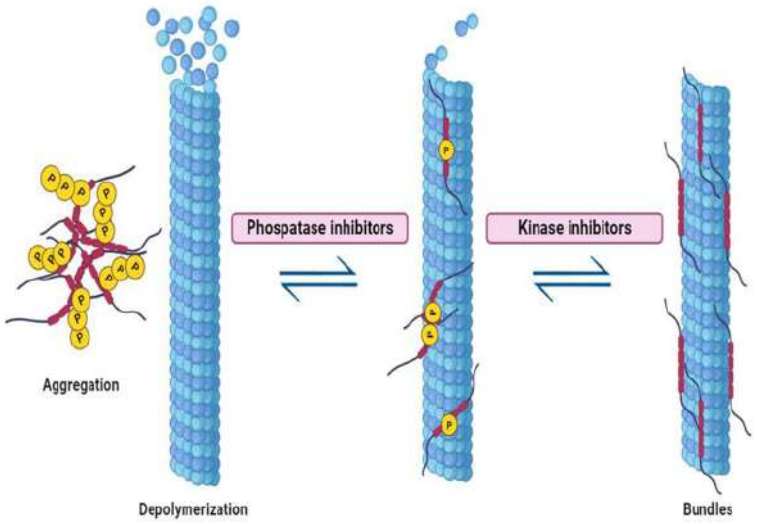


FIGURE 2.3: Tau hyperphosphorylation causes neurodegeneration by upsetting microtubules.

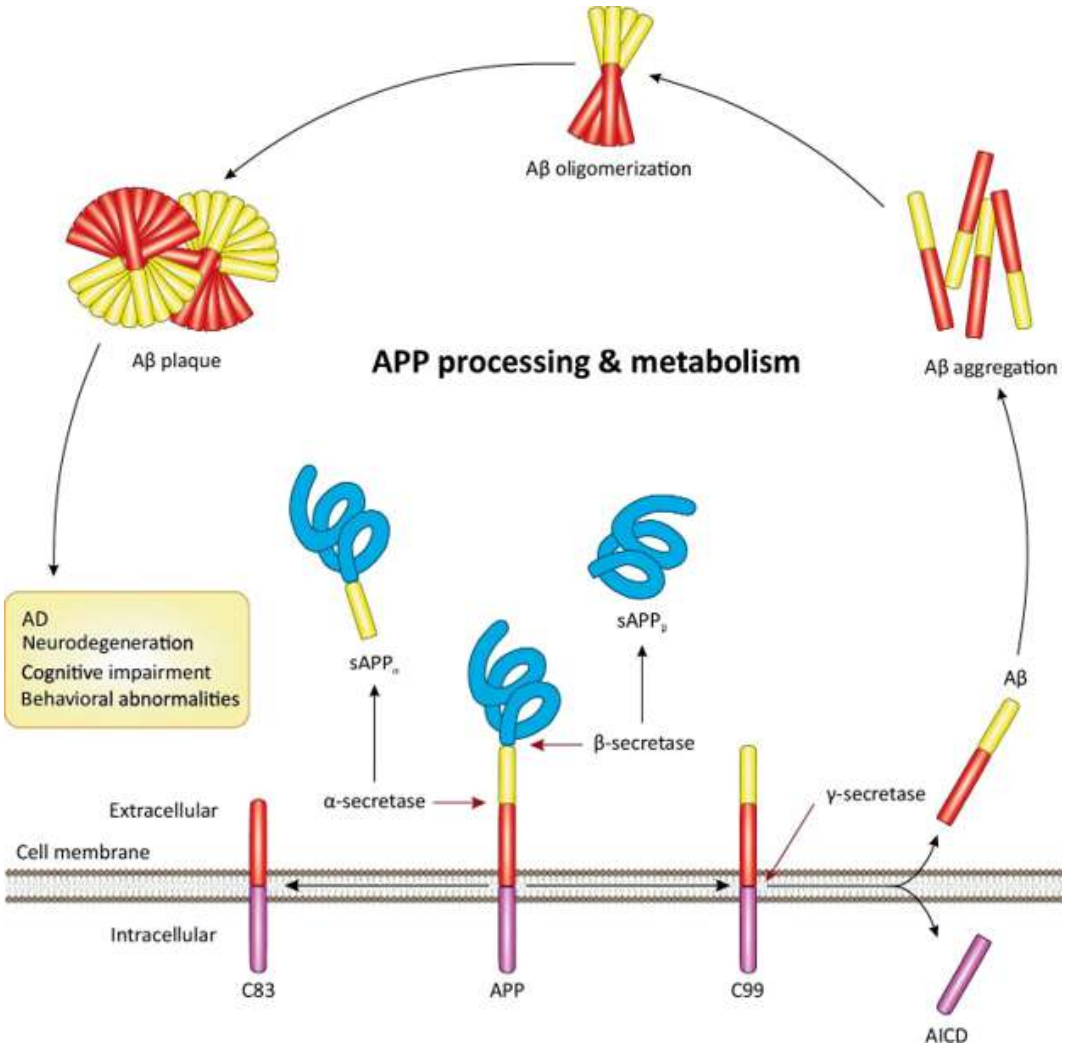


FIGURE 2.4: Neurotoxic plaques are produced by APP cleavage

Mechanism 2: There are two ways to cleave the membrane protein APP. In Alzheimer's disease, APP is broken down by β-secretase and then γ-secretase to create Aβ peptides. Outside of neurones, these Aβ peptides aggregate to form plaques. This plaque accumulation results in cognitive impairment, memory loss, and damage to neurones [51].

2.7 The Genetic Foundation of Alzheimer's

People with Down syndrome have an extra copy of chromosome 21, which is where the APP gene is located, hence the amyloid hypothesis and Down syndrome go hand in hand. An elevated risk of Alzheimer's disease is linked to an increase in the synthesis of amyloid-beta.

By the time they reach their 50s or 60s, the majority of individuals with Down syndrome will have neurofibrillary tangles and amyloid plaques. By that time, 40 to 80 percent will have clinical Alzheimer's disease. The APP, PSEN1, and PSEN2 genes are interconnected in inherited forms of Alzheimer's disease.

Mutations in PSEN1 and PSEN2 affect gamma-secretase activity, which supports amyloid buildup in the brain, in contrast to APP mutations that enhance amyloid-beta generation. The most prevalent of these mutations, known as PSEN1, is linked to early-onset dementia and accounts for 5-10% of Alzheimer's disease cases [52].

Three distinct variants, referred to as ε2, ε3, and ε4, exist within the APOE gene. The late-onset Alzheimer's disease is mainly caused by the ε4 allele, which is a significant genetic factor. The chances of acquiring the condition are around three times higher in individuals who carry one ε4 allele, and more than fifteen times higher in those who carry two ε4 alleles. In both familial and sporadic instances of Alzheimer's disease, some SORT1 subtypes have been associated. [53].

2.8 Histopathology

The amyloid hypothesis is closely related to Down syndrome because individuals with the disorder have an additional copy of chromosome 21, which contains the APP gene. Higher levels of amyloid-beta production are associated with an increased likelihood of developing Alzheimer's disease [54]. Most people with Down syndrome will develop neurofibrillary tangles and amyloid plaques by the time they are in their 50s or 60s. At that point, 40–80% will have developed Alzheimer's disease. The three different versions of the APOE gene are represented by variants $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$. [53].

2.8.1 Staging Braak and Braak

Neurofibrillary tangle progression in Alzheimer's disease is characterized by the Braak and Braak staging system, an aspect of conventional diagnostic criteria [55]. There is a stronger correlation between tau pathology and dementia severity than amyloid plaques, indicating that tau load is more closely linked to cognitive decline. The amyloid cascade theory postulates that amyloid acts as an initial trigger, and tau subsequently causes neurodegeneration and cognitive deterioration. The accumulation of Aβ is a hallmark of Alzheimer's disease, with Aβ40 being more common than Aβ42. Amyloid deposits in brain arteries can occasionally induce brain amyloid angiopathy (CAA), a condition linked to a faster rate of cognitive deterioration. Infarcts that do not reach the cortex and cerebrovascular illness both raise the risk of dementia and its progression [56].

2.9 Evaluation

In order to rule out treatable reasons, primary care physicians conducting an evaluation for suspected Alzheimer's disease will evaluate the patient's medical and family history, check for medications that can impair cognition, provide baseline

cognitive tests such as the Mini-Mental State Examination (MMSE) or the Mini-Cog Assessment (MCA), and request blood tests. Standard laboratory testing for cognitive impairment (complete blood count, CMP, thyroid stimulating hormone, vitamin B12) can help rule out other possible reasons, but they cannot diagnose Alzheimer's disease. Brain CT scans can show atrophy and enlarged third ventricles, but they don't tell you anything about the condition. When compared to CT, MRI is superior for structural examination and can detect entorhinal, hippocampal, and medial temporal lobe atrophy [57–59].

The use of volumetric magnetic resonance imaging (MRI) for the early detection of Alzheimer's disease is still uncertain, despite the fact that it can evaluate brain volume loss and identify patterns of other neurodegenerative disorders. Functional imaging techniques such as SPECT, PET, and fMRI can show patterns of brain dysfunction, but they cannot diagnose the condition definitively [60]. Since EEGs usually show normal or non-specific slowing, they are not useful for diagnosis. One of the best ways to detect moderate cognitive impairment (MCI) early on is via neuropsychological testing [61].

2.9.1 Biochemical and Imaging Indicators of AD

Improved early detection of Alzheimer's disease, especially in preclinical and early clinical phases, has been made possible by breakthroughs in neuroimaging and biochemical biomarkers, but these tools are primarily used in research settings at the moment [62].

The importance of accurate diagnosis before therapy has increased due to the rise of targeted medications such as anti-Aβ monoclonal antibodies. Functional imaging biomarker FDG-PET can detect metabolic abnormalities in brain areas including the hippocampus, which helps with early and differential diagnosis.

Amyloid is present in healthy older adults as well, however amyloid PET is a significant in vivo marker since it shows amyloid deposition, which is characteristic of

Alzheimer's disease. This is particularly useful when trying to distinguish between various proteinopathies and disorders associated to Aβ [45].

2.10 Therapy and Administration

2.10.1 AD Symptomatic Treatments

Since there is no cure for Alzheimer's disease at this time, the standard clinical strategy is to alleviate symptoms. Cholinesterase inhibitors and partial N-methyl D-aspartate (NMDA) blockers are the two main kinds of medicines that have historically been used to treat Alzheimer's disease [63].

2.10.2 Inhibitors of Cholinesterase

Three medications in this classdonepezil, rivastigmine, and galantaminehave been approved by the Food and Drug Administration to treat AD [64].

Nausea, vomiting, and diarrhea are some of the most common gastrointestinal side effects of cholinesterase inhibitors. By increasing vagal tone, these medications can cause bradycardia, syncope, and irregularities in cardiac conduction. Do not use them if the patient has a severe cardiac conduction issue.

2.10.3 Memantine and partial N - Methyl D - Aspartate

Through its action as a partial N-Methyl D-aspartate (NMDA) antagonist, memantine reduces the accumulation of calcium within cells. The FDA has approved it for the treatment of mild to severe AD. Anxieties, vertigo, headaches, and generalized body pains are some of the most prevalent side effects.

The combination of galantamine, donepezil, or rivastigmine with memantine may be beneficial for patients with moderate to severe AD.

2.10.4 AD Disease-Modifying Treatments

Symptom control has long been the main focus of Alzheimer's disease treatment. The deterioration of brain tissue has been going on for more than a decade when a patient is diagnosed with Alzheimer's disease. But now, even in people with mild cognitive impairment (MCI), we can detect the preclinical and presymptomatic stages of Alzheimer's disease because to developments in diagnostic tests that use imaging and biochemical markers and advances in our knowledge of the disease's biology. The FDA recently gave the go light to several novel, targeted disease-modifying treatments in development. Aducanemab and lecanemab were both approved by the FDA in 2020. It is also anticipated that donanemab will shortly be approved by the FDA. By eliminating brain amyloid, these monoclonal antibodies serve as an immunotherapy..

i. Acanemab was given accelerated approval by the FDA in June 2020. Evidence suggests it reduces amyloid-beta plaque in the brain. The primary aim of the phase III trial, which was an improvement in clinical outcomes, was never achieved.

ii. Lecanemab was granted accelerated approval by the FDA in January 2023. This reduced the amount of amyloid-beta in the brain. The rate of disease progression was shown to be 27% lower in the phase III trial. By 2023, donanemab should have received FDA approval. The amount of amyloid-beta in the brain was decreased, and cognitive decline was reduced by 35

2.10.5 Amyloid Related Imaging Abnormalities

ARIA occurs when the immune system reacts to treatments that target amyloid in the brain's vascular architecture, leading to bleeding in the cortical and leptomeningeal arteries as well as capillary leaking into perivascular regions. Radiology distinguishes between two forms of ARIA: oedema (ARIA-E) and hemorrhage (ARIA-H). As a side effect of amyloid immunotherapies, ARIA is well-documented. The most significant risk factors for developing ARIA in patients

undergoing amyloid immunotherapies include the apolipoprotein E4 genotype and cerebral amyloid angiopathy shown on brain MRIs. Between 26.1% and 26.7% of patients in the Aducanemab phase I/II studies had ARIA-E, while 26.1% to 30.5% of patients had ARIA-H. Notably, most of these individuals did not exhibit any symptoms.

2.10.6 Additional Techniques for AD Management

When Alzheimer's disease progresses into its later stages, it becomes critically important to treat the neuropsychiatric symptoms that often accompany it, including anxiety, depression, and psychosis. Because of their anticholinergic properties, tricyclic antidepressants should not be used since they might exacerbate cognitive impairment. If the patient's safety is in jeopardy owing to extreme agitation and no other treatments have worked, then antipsychotics may be prescribed. Cholinesterase inhibitors (such as donepezil) and selective serotonin reuptake inhibitors (SSRIs) (such as citalopram) are common first-line therapies. When necessary, second-generation antipsychotics should be used since they are less likely to have unwanted side effects outside of the central nervous system. The Food and Drug Administration (FDA) green-lighted brexpiprazole for the treatment of Alzheimer's disease agitation in May 2023.

2.11 Distinctive Diagnosis

Additional potential causes of Alzheimer's dementia include frontotemporal lobar degeneration, vascular dementia, Alzheimer's disease, and pseudodementia or depression. Other factors to consider and rule out include cognitive deterioration associated with aging, substance addiction, vitamin B12 insufficiency, those on dialysis, thyroid problems, and potential side effects of polypharmacy.

2.11.1 Lewy Body Dementia

The DSM-5 classifies 15% of all dementia cases as Neurocognitive Disorders with Lewy Bodies, which includes dementia with Lewy bodies. The density of cortical Lewy bodies, which are histologically related with dementia severity, can be used to identify the condition. The intracytoplasmic inclusions contain α-synuclein and ubiquitin within a thick eosinophilic core. Under medical supervision, it shows up as a mix of cognitive impairment, visual hallucinations, and tremors, which are signs of Parkinson's disease. While REM sleep without atonia on polysomnography and reduced 123-MIBG uptake on SPECT/PET in the basal ganglia are supportive biomarkers, REM sleep behavior disorder and excessive susceptibility to antipsychotics are suggestive features. We say that a diagnosis is likely when we see two core features or one core plus one suggestive feature; we say that it is feasible when we see one core or one suggestive characteristic.

2.11.2 Frontotemporal Dementia

Frontotemporal dementia is more common in men, makes about 5–10% of dementia cases, and usually manifests around age 53. Previously known as "Pick disease" because of Pick bodies, it shows as early behavioral and personality disorders, with or without language difficulty. Language variant and behavioral variant are its two primary forms. At least three characteristics disposition, indifference, loss of empathy, obsessive behaviors, hyperorality, or executive dysfunction are necessary for a behavioral variation. Progressive linguistic loss identifies the language variety. Genetic mutations or alterations in the frontal or temporal lobes on imaging match the diagnosis, and visuospatial abilities are typically unaffected.

2.11.3 Dialysis Dementia

Dementia caused by metabolic complications, complications with dialysis itself, or vascular causes (such as an increased risk of stroke) is a neurological complication

of long-term dialysis treatment. It was previously associated with aluminum toxicity, but its occurrence has decreased due to the use of dialysis materials that do not include aluminum. We still don't know the exact process. As people get older, the symptoms of Alzheimer's disease evolve. Memory loss is the first symptom that patients often notice; it impacts more than 90% of individuals aged 70–79 and 94% of those aged 79 and up. Nearly three-quarters of patients 60 exhibit memory loss, and about 25% also have early deficiencies in judgment and visuospatial skills [65].

2.12 Staging

2.12.1 Presymptomatic or Preclinical

It is possible for people to have clear pathological changes in preclinical Alzheimer's disease even when they do not show any symptoms. Early detection can be aided by biomarkers at this stage, even though they are not completely specific to Alzheimer's disease. Cerebrospinal fluid (CSF) levels that are higher in tau and lower in amyloid could be a sign of illness.

Predictors of mild cognitive impairment (MCI) include ApoE4 positivity, worse performance on cognitive tests (such as paired associates recall and digit symbol substitution tests), higher levels of tau in cerebrospinal fluid (CSF), and reduced MRI density and volume of the right entorhinal cortex and hippocampus [66].

2.12.2 Mild Cognitive Impairment

These individuals persist in working, interacting with others, and functioning autonomously, despite these cognitive oddities. There is an annual progression to Alzheimer's disease in about 10% of MCI cases. In addition to other known AD risk factors, the severity of impairments seen during evaluation is a risk variable for this progression [67].

2.12.3 Dementia

Along with severe memory loss, patients with intermediate to advanced Alzheimer's disease have substantial linguistic impairments such as alopecia, difficulties in speaking spontaneously, paraphasic mistakes, and circumlocution. Furthermore, visual-spatial dysfunction is the root cause of constructional apraxia and difficulties navigating familiar environments.

Visual hallucinations are more prevalent than auditory or olfactory ones, and 20-40% of patients experience delusions. There is a high prevalence of neuropsychiatric symptoms. Nearly 50% of patients display behaviors that are considered disruptive. There is an increased likelihood of patients becoming involved in car accidents., and sleep-wake cycle disruptions are common, leading to fragmented sleep [65].

2.13 Predictions

Alzheimer's disease progresses over time and cannot be reversed. The average post-diagnosis survival for patients 65 years of age and older is 4–8 years, even if some people might stay alive for twenty years after symptoms appear.

The decline in immune function, immobility, and swallowing abilities makes patients more vulnerable to infections as the disease advances. The leading cause of mortality in Alzheimer's disease is pneumonia [68].

2.14 Complexities

According to the CDC National Center for Health Statistics (2023), Alzheimer's disease accounts for around 120,000 fatalities each year, making it the sixth leading cause of death. The majority of its victims are 65 and older, and it has a profound effect on their emotional and physical well-being.

Physical and mental/behavioral issues are the two main types of Alzheimer's disease consequences [65].

2.15 Potential Advantages of Herbal Treatment for Alzheimer's Disorder and Bioactive Metabolites

Recent clinical and preclinical trials have shown that many herbal medicine solutions show promise as treatments for Alzheimer's disease. The medicinal properties of these plants are thought to manifest in many ways, including a decrease in oxidative stress and an inhibition of neuron inflammation., influencing neurotransmitter systems, and encouraging neurogenesis [69, 70].

Ginkgo biloba has is both historically grounded in traditional Chinese medicine and the subject of much research into its effects on cognitive function. The inclusion of flavonoids and terpenoids enhances its anti-inflammatory and antioxidant capabilities, which are the main methods by which it alleviates Alzheimer's disease symptoms. More blood flow to the brain and more oxygen delivered to neurons could be achieved if it suppresses platelet-activating factor (PAF). There have been small but noticeable improvements in memory, focus, and everyday functioning reported in certain clinical trials, others show no significant improvement at all. In general, its effects on cerebral circulation and neuroprotective potential are intriguing, but additional large-scale studies are required to confirm its therapeutic value [71].

Curcuma longa L(turmeric) contains the chemical curcumin, which has powerful antioxidant and anti-inflammatory effects on Alzheimer's sufferers. It has the potential to limit amyloid-beta formation, reduce oxidative stress, and regulate inflammatory and neuronal survival pathways such as NF-κB and MAPK. Its oral bioavailability restricts its therapeutic use, despite positive preclinical data

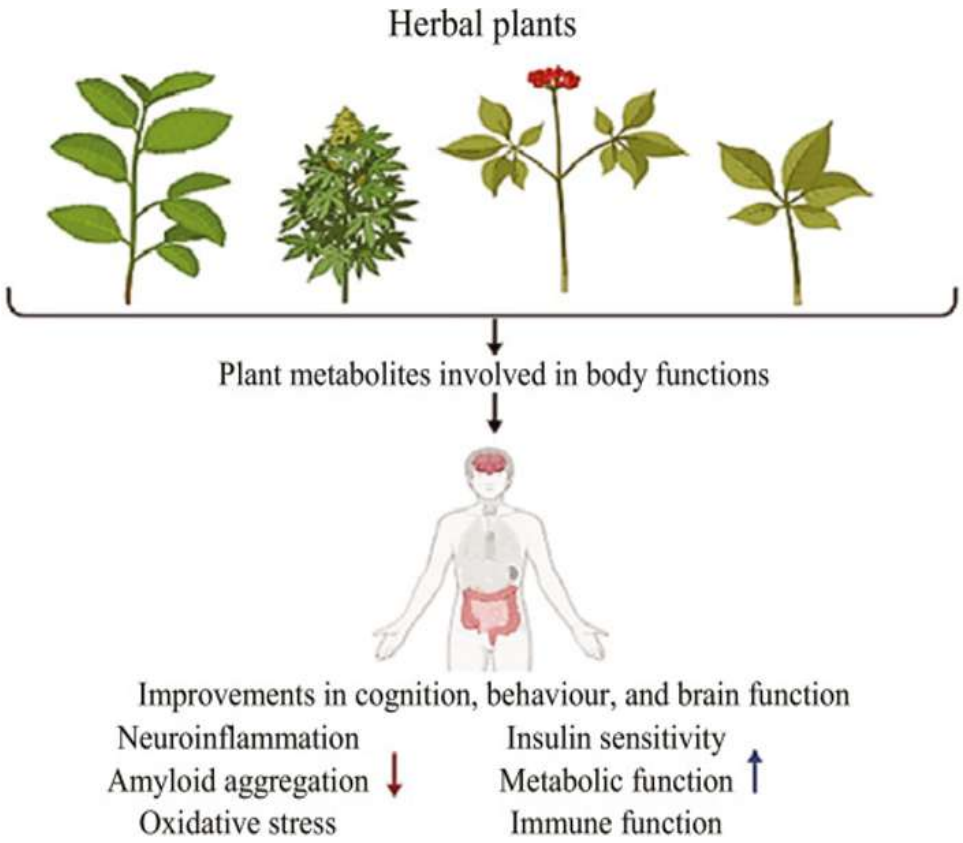


FIGURE 2.5: The effects of herbal metabolites on the brain, metabolism, and immune system. This diagram illustrate the herbal plants produce bioactive metabolites that influence human physiological systems. These metabolites lower oxidative stress, amyloid aggregation, and neuroinflammation while also improving behaviour, cognition, and general brain function. They also improve immunological responses, metabolic function, and insulin sensitivity, indicating therapeutic potential for metabolic and neurodegenerative diseases. [71].

showing reduced inflammation, increased neurogenesis, and fewer amyloid plaques [72].

Bacopa monnieri has a long history of use as a memory and learning aid. Bacosides, its active components, protect neurons from the neurodegenerative effects of Alzheimer's disease and enhance cholinergic signaling. Patients suffering from cognitive decline report less anxiety and improvements in memory, attention, and cognitive function, according to clinical studies. Additional evidence from preclinical studies supports its neuroprotective effects against oxidative stress [73].

An antioxidant and anti-inflammatory component of *Rosmarinus officinalis* (rosemary) called rosmarinic acid may protect neurons caused by oxidative stress in Alzheimer's disease. There is potential to enhance both cognitive performance

and cerebral blood flow. Preliminary research suggests that healthy adults who inhale the essential oil may see an enhancement in concentration and memory. Additional clinical evidence on Alzheimer's patients is still required [74].

Another traditional plant with memory-enhancing qualities is salvia officinalis, more often known as sage. Acetylcholinesterase is an enzyme that is low in Alzheimer's disease; inhibiting it raises acetylcholine levels. yet essential for learning and memory. Clinical studies have shown improvements in memory, cognition, and behavioral symptoms in Alzheimer's patients, lending credence to its possible role in disease controlmild to moderate cases [75].

2.16 *Withania somnifera*

Ayurveda uses ashwagandha, an adaptogenic plant, to promote general health and reduce stress. There is increasing interest in the herb's potential function in Alzheimer's disease. Its neuroprotective benefits are associated with cortisol modulation, which may aid in lowering the neuroinflammation associated with stress that contributes to the advancement of disease [76]. Additionally, it exhibits antioxidant activity, shields neurons from oxidative damage, and may encourage neurogenesis. According to preclinical research, ashwagandha slows cognitive decline, lessens stress-induced brain damage, and enhances memory. Although there is yet no clinical evidence for Alzheimer's disease, preliminary results point to possible advantages for cognitive performance in older persons [77].

2.16.1 Distribution and Habitat

The Old World-native Physalis is closely related to the *Withania somnifera* genus. From its native Mediterranean region, Withania has expanded across tropical Africa, South Africa, the Arabian Peninsula, the Middle East, and throughout Asia, particularly in Sri Lanka and China, where it flourishes in dry to relatively humid environments. Originally from warmer, drier climates in Europe, *Withania*

FIGURE 2.6: Ashwagandha plant morphology [78].

somnifera is now a common garden plant in parts of Australia, particularly in the states of South Australia and New South Wales. Grown mostly for agricultural purposes in India and other regions for its medicinal roots. Some parts of southern Africa, however, lack it. Under varied humidity and rainfall circumstances, it can grow in a variety of habitats, such as grasslands, savannas, scrublands, forests, coastal regions, riverbanks, and forest margins [79].

2.16.2 Habitat

Withania somnifera is found in Mediterranean regions like Sicily and Sardinia, as well as desert parts of Sri Lanka, Balochistan, and India, as well as the lower Himalayas (below 1600 m). The evergreen shrub can reach a height of 1 foot and is perpetual.30 to 150 cm and is frequently utilized for its roots, leaves, and seeds. Due to its therapeutic benefits, it is extensively grown throughout India and frequently grows as a weed in roadside areas and open wastelands.

Tomentum, or fine stellate hairs, covers the entire plant. Its ovate, simple, alternating leaves are 5–10 cm long, with reticulate venation and a cuneate or connate

base. In close proximity to the inflorescence, leaves may look opposite and are very hairy [80].

2.16.3 Morphological Traits

Withania somnifera is a small to medium-sized perennial undershrub (30–150 cm tall, occasionally up to 1-2 m) with an upright, branching, grayish habit and a potent, distinctive scent. The plant has woody, tuberous roots and is heavily covered in fine, stellate (star-shaped) grayish hairs. Brownish, upright stems frequently have fewer leaves in the bottom part. Simple, widely oval to oblong leaves (approximately 29–80 mm long and 21–50 mm wide) have thickly pubescent petioles and somewhat wavy margins. Small, greenish to yellow-green flowers with a 5–8 mm campanulate corolla are typically found alone or in few-flowered axillary cymes.The plant usually flowers from October to June and fruits from October to July; the fruit is a small spherical berry (5–8 mm), orange-red to scarlet when mature, surrounded by an enlarged papery calyx; the seeds are numerous, small (about 2.5 mm), kidney-shaped, and reticulate in texture [81]. The plant is also known for its potent green tomato-like scent.

Here is how ashwagandha is categorized by scientists:

TABLE 2.1: Taxonomic Classification of *Withania somnifera*

Taxonomic Rank	**Classification**
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Solanales
Family	Solanaceae
Genus	*Withania*
Species	*Withania somnifera*

2.16.4 The Ethnobotanical Significance of *Withania Somnifera*

Ethnobotany, the study of traditional plant knowledge for food, medicine, and shelter, makes extensive use of *Withania somnifera*. Drug discovery has benefited greatly from ethnobotanical research; many modern pharmaceuticals are sourced from botanical ingredients [82].

As a traditional medicine herb, ashwagandha has a long history of use in Africa and India. Powdered roots are commonly taken orally to treat conditions such as increased libido, diabetes, neurasthenia, rheumatism, and postpartum recovery; leaf juice is used to cure swelling and throat infections in India. Fruits are also used as a substitute for cooked vegetables and to alleviate gastrointestinal issues in certain regions [83–86]. Traditional Indian medicine makes use of this herb in a variety of ways, including a poultice to reduce swelling, a general health tonic, and remedies for conditions such as asthma, leucorrhea, and snakebite [87]. The presence of withanolides contributes to its medicinal effects, and it is traditionally used in Ethiopia for eye problems, typhoid prophylaxis, malaria, and as a fumigant for infection management [88, 89].

2.16.5 *Withania somnifera*'s Chemical Composition

Possess a diverse range of chemicals that make up *Withania somnifera*. Traditional medicine is just one of several biological consequences caused by these chemicals. food integration, and cosmetic drug compositions. The active biological chemical components of WS that give it its therapeutic properties are flavonoids (43.51 mg RE/g), alkaloids (0.13% and 0.31%), and withanolides (0.001%–1.5%) of a dry weight (DW) [90].

2.16.6 Shoots

Withania somnifera contains Discoveries made in its shoots have uncovered a number bioactive chemicals, especially withanolides. The withaferin A, withanolide A, withanolide B, and withanolide D families, somniwithanolide, withasomniferanolide, and somniferwithanolide, there are a number of compounds that are present at concentrations as low as 0.022 mg/g. Its many therapeutic and medical uses are mostly attributable to these substances.

2.16.7 Root

A number of bioactive chemicals with potential medical uses have been isolated from plant roots through rigorous scientific investigation. Significant compounds contained withanolide E, withanolide B, withanolide C, withanolide D (0.08%-0.11% in DW), withaferin A (2.36 mg/g DW), withanone (4.32 mg/g DW), withanolide E, 2,3-dehydrosmonifericin, 2,3-dihydro withaferin A, 27 - hydroxywithanolide A, and withanolide dimer sulphide were also included. Many studies have also demonstrated the existence of important compounds like 27-deoxy Withaferin A, Coagulin H, Withasomniferol A, Withanoside I (0.0020%), Withanoside II (0.012%), Withanoside III (0.0024%), Withanoside IV (0.048%), Withanoside V (0.017%), Withanoside VI (0.024%), Withanoside VII (0.0011%), 1,2-deoxywithastranolide (1.12 mg/g), withanine, β-sitosterol, and withanone, among others.

2.16.8 Fruits

The fruits of the WS plant contain a large number of bioactive chemicals, some of which have powerful medicinal properties. Asparaginase, sterols, tocopherols, fatty acids, linoleic acid (0.23%), elaidic acid (0.01%), and more are all components of this. Separate compounds such 4-deoxywithaperuvin, 14α, 17α - dihydroxywithanolide R, and Withanamides A, B, C, D, E, F, G, H, and I have also been found.

Other noteworthy compounds include squalene, tetracosanoic acid (0.880%), oleic acid (0.14%), 24,25-dihydrowithanolide VI, iso-withanone and 6α, 7α - epoxy - 1α, 3β, 5α-trihydroxy-witha-24-enolide.

2.16.9 Leaves

A variety of bioactive substances found in the leaves of the powerful plant WS may have therapeutic benefits. The primary components are withaferin A (1.35 mg/g), 17α-hydroxywithaferin A, withanone a (1.312%), withanolide A (350 μg/g), withanolide B (0.05 mg/g DW), withanolide C, and withanolide D (0.08-0.11% DW). Deoxywithastramonolide (0.18 mg/g DW), 12-deoxy with withastramonolide (0.07 +/- 0.00 mg/L), and Withanolide E, F, Withanoside VI (0.04 $\pm$ 0.01 mg/g) and Withanoside V (0.11 +/- 0.04 mg/g) are additional significant compounds. New studies have shown that WS leaves can benefit from other withanolides and withanosides that enhance their functional properties.

2.16.10 Bark

Many bioactive compounds, such as somniferanolide, somniwithanolide, withanolide, withasomnilide, and somniferawithanolide, are responsible for the pharmacological and therapeutic effects of bark from the *Withania somnifera* tree.

Traditional medicine practitioners have long relied on ashwagandha bark decoctions for the treatment of asthma and allergies, and localized applications have shown promise in the treatment of bed sores. These properties, mainly caused by its high withanolide content, support its usage in traditional medicine.

2.16.11 Alkaloids

The medicinal plant *Withania somnifera* has numerous pharmacological chemicals, including alkaloids. Among the several significant chemical classes present in WS, alkaloids stand out. A variety of alkaloids, which are manufactured within the

plant rather than brought in from outside, are present in WS, demonstrating its capacity to synthesize these molecules.

The therapeutic characteristics of the plant, particularly its anticancer actions, are supported by its alkaloids, which also contribute to its numerous bioactivities. The alkaloids in WS make it a great source of medicinal agents for many different diseases, since they have long been used in traditional medicine and have also contributed to modern medical research.

The presence of alkaloids in WS greatly enhances its therapeutic potential and pharmacological significance. Researchers dug deep within WS's roots to find the many substances with bioactive alkaloids that may have medicinal use.Solanidine, lobeline, theobromine, hyoscine, yohimbine, ephedrine, somniferinine, withasomine, and somniferine are the primary alkaloids identified in WS roots. Coniine, lobeline, theobromine, hyoscine, yohimbine, ephedrine, and somniferine make up the remaining alkaloids.

The analgesic, anti-inflammatory, and neuroprotective properties of these chemicals are well-known. Annaferin, isopelletierine, anahygrine, pseudotropine, withanine, scopoletin, nicotine, berberine, caffeine, and theophylline are some of the chemicals that improve the medicinal effect of the plant. Mood, cardiovascular health, cognitive function, and muscle relaxation are just a few of the many positive biological impacts of these alkaloids. These compounds have found their way into conventional medicine because of theirmany therapeutic characteristics.We investigated the alkaloid chemicals found in fruits that are active in WS. There is a wealth of withanamides in ashwagandha fruits, which are a special kind of compounds that make the plant more medicinal. A, B, C, D, E, F, G, H, and I are the primary withanamides identified in WS fruits.

2.16.12 Flavonoids

One class of beneficial chemicals found in abundance in WS is flavonoids. Flavonoids are present in the leaves at 43.51 ± 0.346 mg/g, the stem at 42.82 ± 1.189

mg/g, and the root at 39.13 ± 0.607 mg/g. Flavonoids offer numerous health benefits, according to research. They may help with insulin sensitivity, glucose metabolism, lipid metabolism, and even anxiety and depression.

2.16.13 Whole Plant

To identify and analyze the flavonoid components, a comprehensive analysis of the *Withania somnifera*plant was required. The primary flavonoids identified in the plant are rutin, naringin, and naringenin. These compounds improve the plant's entire medicinal potential and are well-known for their potential biological activity, including cardioprotective and antioxidant characteristics. With the added therapeutic effectiveness provided by flavonoids, WS is a multipurpose drug that finds application in both traditional and alternative medicine. All of these flavonoids are vital when it comes to medicine. One reason WS is useful as a medicine is because it contains a variety of flavonoids in its roots.

Among the several flavonoids found in WS, the most prominent ones are hyperoside, aesculentin, kaempferol, dihydroxykaempferol, catechinrhamnetin, quercetin, rutin, quercetin 3-O-robinobioside-7-O-glucoside, myricetin, and hyperoside. The potential biological benefits of these chemicals are acknowledged, as they enhance the medicinal value of the roots. Traditional and modern medicine have long recognized the medicinal benefits of *Withania somnifera*for a variety of reasons, including its antioxidant and cardioprotective properties, which are considerablyenhanced. There are a lot of flavonoid glycosides in the WS's aerial portions, which is why it has certain medicinal properties. Both quercetin-3-rutinoside-7-glucoside and kaempferol 3-robinobioside-7-glucoside were identified as major components in the chemical analysis.

These flavonoids, known for their possible biological characteristics including anti-inflammatory and anticancer effects, boost the entire medicinal potential of the plant's aerial sections. The medical industry likewise places a high value on these components.The *Withania somnifera* berry contains bioactive flavonoid molecules such as apigenin, catechin, rutin, kaempferol, and apigenin - 7 - O - β - D -

glucopyranoside. These compounds possess antioxidant and anti-inflammatory properties. These materials could have practical applications [90].

2.17 The Pharmacological Characteristics of Phytochemicals and Plant Extracts

2.17.1 Apoptotic and Anti-cancer Properties

Ashwagandha aqueous root extracts can change cell viability, increase antioxidant and caspase activity, and lower inflammatory markers. By inhibiting proteins necessary for cell survival, proliferation, and angiogenesis, it slows the growth of tumors. It also encourages cancer cells to undergo apoptosis through a number of mechanisms, such as the production of reactive oxygen species and disruption of mitochondria [91].

2.17.2 Anti-inflammatory Properties

Ashwagandha has anti-inflammatory qualities that include lowering inflammation in bowel disease and lupus, antioxidant capabilities similar to ascorbic acid and curcumin, and the ability to block oxidative stress markers (ROS, NO) and pro-inflammatory cytokines (IL-6, TNF-α). Additionally, it inhibits NFκB activation, EPCR shedding, and vascular barrier integrity with withaferin-A. [91].

2.17.3 Immunomodulatory Properties

Ashwagandha extracts have been shown to have immunomodulatory properties, increasing antibody responses, WBC counts, and platelet counts while lowering cyclophosphamide-induced immunosuppression. It enhances phagocytosis, reduces Delayed Type Hypersensitivity (DTH), and provides cytoprotection against

lead toxicity. Furthermore, in EAT-bearing mice, ashwagandha has dose-dependent effects on macrophage activity, spleen weight, and thymus weight [91].

2.17.4 Anti-diabetic Properties

Ashwagandha root powder (3g/day) stabilized human blood sugar levels without producing any adverse effects, much like the oral diabetic drug Daonil. In NIDDM rats, it increases insulin responsiveness while lowering blood sugar, HbA1c, and insulin levels. The leaf extract is more effective than the root extract at improving the absorption of glucose [91].

2.17.5 Antimicrobial Properties

Ashwagandha has amazing antimicrobial properties. It is effective against many bacteria, including MRSA, *E. Coli*, and *Salmonella typhi*. Leishmaniadonovani undergoes apoptosis when exposed to withanolides, however the leaf extract derived from methanol has a broad-spectrum effect. Ashwagandha also increases immunity and reduces the bacterial burden in infected mice when used with medications such as Tibrim [91].

2.17.6 Neuroprotective Properties

Ashwagandha's neuroprotective qualities are supported by preclinical and clinical studies that shows protection against scopolamine-induced toxicity, reduced oxidative stress, and improved brain health. Some of the primary benefits include decreased hippocampal degenerating cells, increased neurite outgrowth and synapse restoration, reduced oxidative stress and neuronal cell indicators, attenuated lead-mediated neurotoxicity in glial cells, and increased brain antioxidant function (SOD, catalase, GPx) [91].

Chapter 3

Methodology

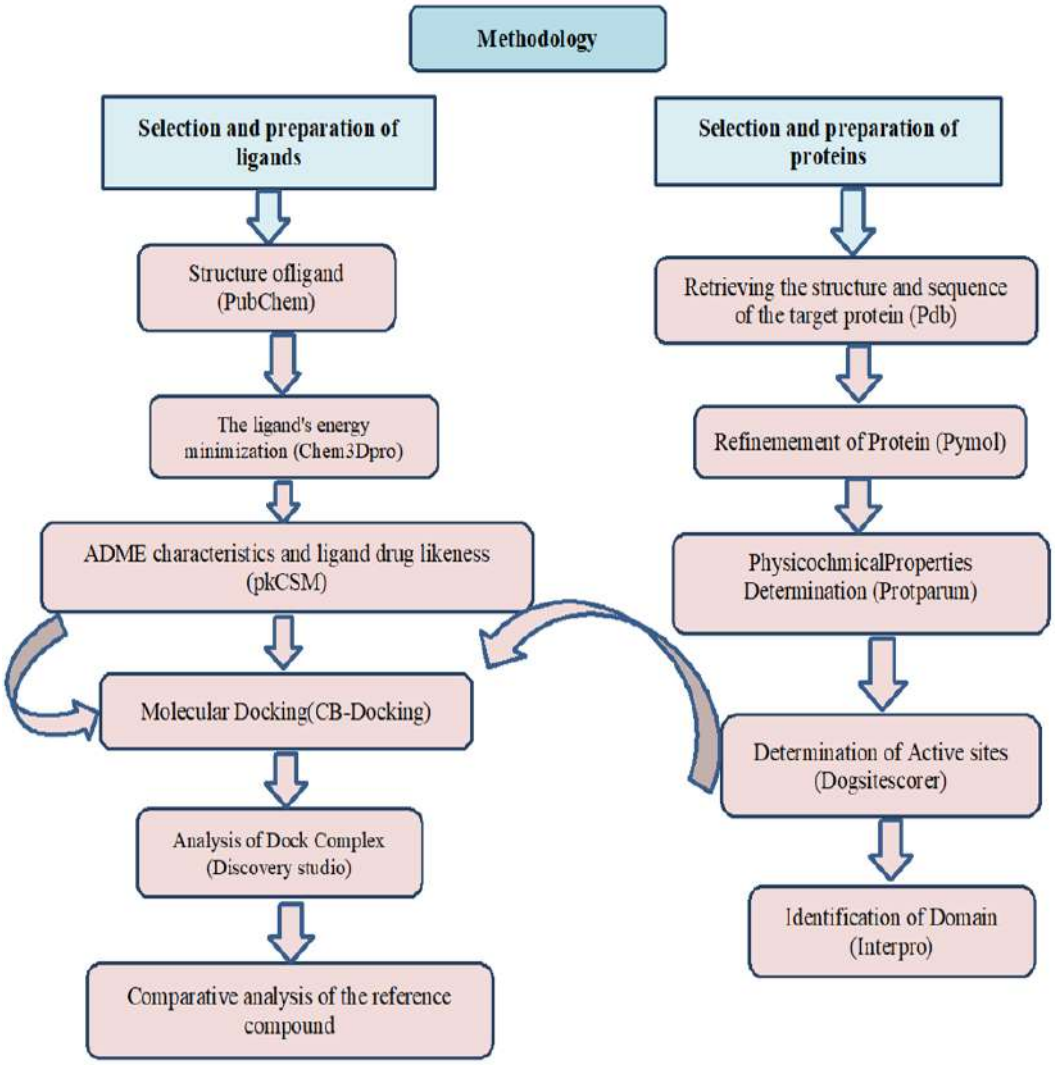


FIGURE 3.1: The Methodology Flow Chart

3.1 Problem Selection

Amyloid-β deposits, tau tangle formation, neuroinflammation, and synaptic damage are the hallmarks of Alzheimer's disease, a progressive neurodegenerative condition that causes memory loss and loss of independence [92]. Only approximately 30 percent of CA1 hippocampal neurones are destroyed in early AD, according to studies, implying that about 70 of these neurones are still alive but dysfunctional. Patients continue to deteriorate and have no cure because current FDA-approved treatments reduce clinical development but do not stop or reverse neurodegeneration. This indicates the necessity of employing multi-target techniques to safeguard the surviving neuronal reserve. Compounds produced from plants, such as withanolides from *Withania somnifera*, have the ability to reduce oxidative stress, tau phosphorylation, amyloid aggregation, and neuroinflammation through a variety of mechanisms.Therefore, the purpose of this study is to investigate if bioactive phytocompounds might target major AD pathways simultaneouslyin order to protect residual neurones, halt the progression of the illness, and maintain mental abilities beyond what is currently possible with single target medications [4].

3.2 Selection of Target Proteins

Target proteins for Alzheimer's disease are chosen based on their potential for therapeutic regulation and their crucial role in the disease's pathogenesis. Amyloid precursor amino acids is transformed to support synaptic plasticity in normal brain function, tau stabilises microtubules for axonal transportation, acetylcholinesterase hydrolyses acetylcholine to regulate memory, β-secretase BACE1 aids in myelination and APP processing, and GSK-3β regulates cell signalling and synaptic plasticity. These proteins are dysregulated in Alzheimer's disease. Increased neurotoxic Aβ42 generation from altered BACE1 activity results in plaques that damage synapses and cause inflammation. Tau hyperphosphorylation brought on by hyperactive GSK-3β disrupts the structure of neurones and forms

neurofibrillary tangles. Acetylcholine is diminished by cholinergic neurone death, whereas Aβ aggregation is encouraged by increased AChE .These proteins are proven therapeutic targets because they jointly produce inflammation, cholinergic deficiency, tau pathology, and amyloid deposition. A multi-target phytotherapeutic approach is supported by withanolides from species *Withania somnifera* that have inhibitory efficacy against Aβ accumulation, AChE, and GSK-3β [48].

3.2.1 FASTA Sequence Retrieval and Structure Prediction for Target Proteins

PDB (rcsb.org) can be used to forecast the 3D structures [3]. If certain structures are not available on PDB, another option is to use I-TASSER, which represents Interactive Threading Assembly Refinement (zhanglab.I-TASSER). The three-dimensional in nature function and structure of proteins are predicted using this software, which is accessible online. First, it determines the PDB's structural model using a variety of techniques, including as full-length atomic models that are constructed utilising simulations of the various threading sections [93]. The I-TASSER service also predicts the three-dimensional structure of proteins. It provides us with five protein 3D structures so that we can choose the best one based on the C-score [94]. Another trustworthy resource for predicting the three-dimensional structure of proteins is Alphafold (alphafold.com) [95]. The target proteins were obtained from the FASTA sequencing of the chosen protein sequence database. PDB. Alternatively, target protein sequences can be retrieved using the Uniprot (uniprot.org) or NCBI (www.ncbi.nlm.nihgov) databases [96].

3.2.2 Analysis of the Physicochemical Properties of Target Proteins

Proteins' physicochemical properties have a big impact on how they work [97]. These characteristics of the amyloid- peptide, tau protein, acetylcholinesterase,

β-secretase, and glycogen synthase kinase-3β were predicted using the ProtParam Expasy tool (web.expasy.org/protparam).

ProtParam was utilized to ascertain the following parameters: theoretical pI, molecular weight, aliphatic index, grand mean of hydropathicity, instability index, extinction coefficient (with Cys) and extinction coefficient (excluding Cys), number of negatively charged amino acid residues (Asp + Glu) and positively charged amino acid residues (Arg + Lys). [98].

3.2.3 Protein Structure Evaluation and Improvement Using PyMol

An open-sourced molecular graphics program called PyMOL (pymol.org) is widely used Allows to visualize and analyze a wide range of tiny molecules and proteins in three dimensions, including trajectories, densities of different electrons and surfaces, and amino acids. You may also use it to make videos and animations, change molecules, and even trace beams.

Drug targeting and design with PyMol software is made easier with the many plugin options available for this Python-based platform. The open-source PyMol tool was used to obtain the protein structure. Then, the protein's associated additional components had to be removed [99].

3.2.4 Identification of Functional Domains in Targeted Proteins

The database InterPro (www.interpro.com) identified the categories of functionality using proteins linked to Alzheimer's disease, including tau protein, amyloid-β peptide, acetylcholinesterase, β-secretase, & glycogen synthase kinase-3β. Evolutionary relationships, sequencing, and structure are all integrated into conserved domains [100].

3.2.5 Identification of Active Sites

Wherever the active site of the target protein is located on the protein is where the ligand and protein interact the most strongly. The creation of ligand-protein complexes relies heavily on amino acids. Protein binding pockets were found using the Dogsitescorer program (proteins.plus/help/dogsite) [101].

3.2.6 Choosing Active Metabolic Ligands

Bioactive substances from plant *Withania somnifera* (L.) Dunal that have been shown to have neuroprotective and anti-Alzheimer's qualities were chosen following a thorough assessment of the literature.

These comprise sominone, 12-deoxywithastramonolide, these compounds: withanolide A, withanolide B, withaferin A, and withanone. The withanosides IV, V, and sitoindoside-A X [102].

3.3 Retrieving the Ligands' Chemical Structure

The greatest collection of readily available chemical information databases in the world is PubChem (pubchem.ncbi.nlm.nih.gov) [103]. Therefore, The chemical compounds that were selected as potential ligands were obtained from the PubChem database using the SDF format. Next, we'll use PubChem to download canonical smiles, which we'll then input into Chem Draw to get the 3D structure if the chosen ligand structure is not accessible [104].

3.3.1 Ligand Energy Minimisation

Chem3D Ultra was used to minimise ligand energy. Prior to docking, the ligands must be refined otherwise, the docking scores will be unreliable [105] .

3.3.2 Ligand Virtual Screening Using the Lipinski Rule of Five

The Lipinski rule is a crucial criterion for assessing whether ligand are likely to be medicines. If a chemical complies with the Lipinski Rule of Five, it may be used as an active pharmaceutical in humans.

An online tool called pkCSM (omictools.com/pkcsm-tool) can be used to determine whether or not ligands follow the Lipinski Rule [106]. The guidelines are explained as follows:

i. A log P value tends to fall between five and six.

ii. There should be no more than ten H-bond acceptors.

iii. There should only be a maximum of five H-bond donors.

iv. Molecular weight must be less than 500 grams.

v. There should be no more than five rotatable bonds.

3.3.3 Ligands ADMET Evaluation

Predicting pharmacokinetic and toxicological parameters came next in the study after the ligands were filtered using the Lipinski method. The pharmacokinetic characteristics of particular ligands were predicted using pkCSM (omictools.com/pkcsm-tool).

During ADMET analysis, the drug's weak candidates would be discarded. The remaining contenders may be chosen as possible medications to combat the illness. The ADMET characteristics related to the human body were optimised using the target proteins [107].

3.4 The Molecular Docking

Cavity detector guided blind docking, or CB-dock, was employed to carry out molecular docking among the protein & the ligand. The docking sites are automatically found by CB-dock (clab.labshare.cn/blinddock). A ligand and protein docking technique called CB-Dock calculates the center, size, and bonding sites. Docking is carried out after the box area is modified based on the ligand. AutoDock Vina is used for the docking process. Because cavity binding is the primary focus of the docking procedure, its precision ratio is higher [108]. To do docking, we submitted the proteins and used the ligand's 3D form in SDF format and the three-dimensional structure in PDB format. Five distinct interaction poses would be the outcome of this docking. The lowest docking score, expressed in KJ/m-1, would be the optimum pose to choose. CBDock offers an interactive 3D visualisation of the data in five distinct stances .The ideal location was selected determined by the lowest vina score, expressed in (kJ/m1) [109].

3.4.1 Examining Docked Complexes using Discovery Studio

The chemical compounds that were selected as potential ligands were obtained from the PubChem database using the SDF format. Hydrophobic and hydrogen bonding interactions were among those studied. Using the Discovery Studio 2025 Client, the interactions between proteins and ligands were examined. The protein-ligand interaction diagrams for the selected ligand in the PDB file can be generated automatically by this program [110].

3.5 Identification of Lead Compounds

After a careful analysis of docking scores, pharmacokinetic tests, toxicity aspects, and ligand and protein interactions, the most effective inhibitor was identified. All of these criteria were met by our lead chemical.

3.5.1 Reference Selection for Alzheimer's Disease Targets

Finding the commercial medications now used to treat Alzheimer's disease is the aim of this phase. This was accomplished by using the DrugBank (go.drugbank .com) database, which offers information on medicines and their routes [111].

3.5.2 Lead Compound and Reference Drug Comparison

The reference anti-Alzheimer medication and the proposed lead compound were evaluated in terms of docking parameters, molecular interactions, & pharmacokinetic characteristics.

Chapter 4

Results

4.1 Predicting and Improving the 3D Structure of Specific Proteins

Target protein 3D structures for tau amyloid-beta, and BACE1 were obtained from the Protein Data Bank using PDB IDs 9PGO, 6SZF & 3VEUrespectively. All co-crystallized ligands, molecules of water, and heteroatoms were eliminated from the protein structures using PyMOL. Polar hydrogens were introduced to provide stable conformations, while alternative conformations were eliminated to avoid atomic overlaps. For use in later molecular docking investigations, the altered structures were stored in PDB format. Figure 4.1 displays the target proteins' refined structures.

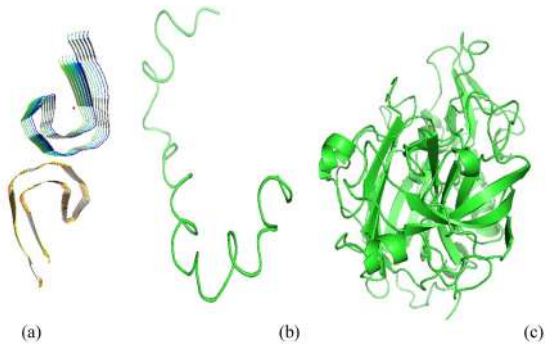


FIGURE 4.1: Structures of refined target proteins (a) Tauprotein (b) Amyloid-beta (c) BACE1.

4.1.1 Retrieving the Primary Sequence

We used PDB to get FASTA sequences. According to the image, the Tau amyloid-beta and BACE1 FASTA sequences were obtained from the Protein Data Bank (PDB) using the accession numbers 9PGO, 6SZF, and 3VEU. Figure 4.2.

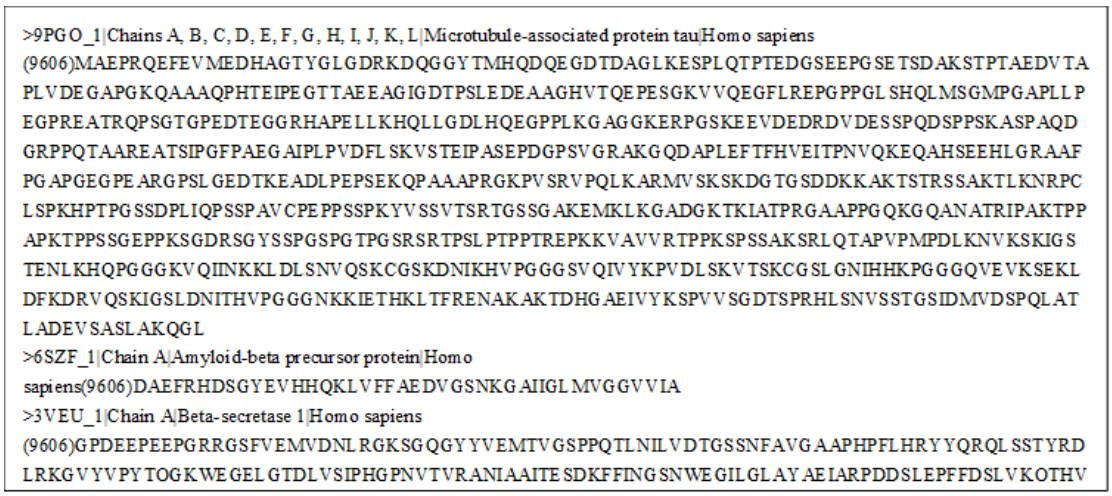


FIGURE 4.2: FASTA sequence of Tau protein, Amyloid-betaand BACE1.

4.1.2 Protein's Physicochemical Characterisation

We forecasted a number of criteria, including the chemical and structural characteristics of certain proteins, using an online program called ProtParam [112]. The physiochemical properties of tau,amyloid-beta, andBACE1are listed in Table 4.1.

TABLE 4.1: The physiochemical properties of tau protein, amyloid-beta and BACE1

Target Proteins	**MW**	**PI**	**PR**	**NR**	**Ext Co1**	**Ext Co2**	**Instability Index**	**Aliphatic Index**	**GRAVY**
Tau	78927.57	6.25	94	102	9190	8940	55.01	56.02	-0.882
Amyloid - beta	4514.10	5.31	3	6	1490	1490	18.17	97.38	0.205
BACE1	44736.57	5.02	34	48	67185	66810	43.31	81.92	-0.182

All proteins have molecular weights between roughly 44 and 78 kDa and isoelectric values between 5 and 6, indicating that they are probably soluble in physiological

settings. These The instability index values range from 43 to 55, suggesting that proteins may be unstable in vitro and may require optimal storage conditions. Hydrophilia is a property of these proteins that makes them well-suited to interact with water in biological systems, as stated by the negative GRAVY scores.

All things considered, these characteristics imply that the proteins are accessible but might require stabilisation for their biological functions. The tone is straightforward and formal.

4.1.3 Identifcation of Active Site

DogSiteScorer software, which estimates the number of possible binding pockets and provides information on the surface area and volume, was used to identify the proteins' active sites. The binding sites for the desired proteins Tau, amyloid beta, as well as BACE-1 are shown in Figure 4.3. Each protein's accessible active sites are shown by the coloured areas.

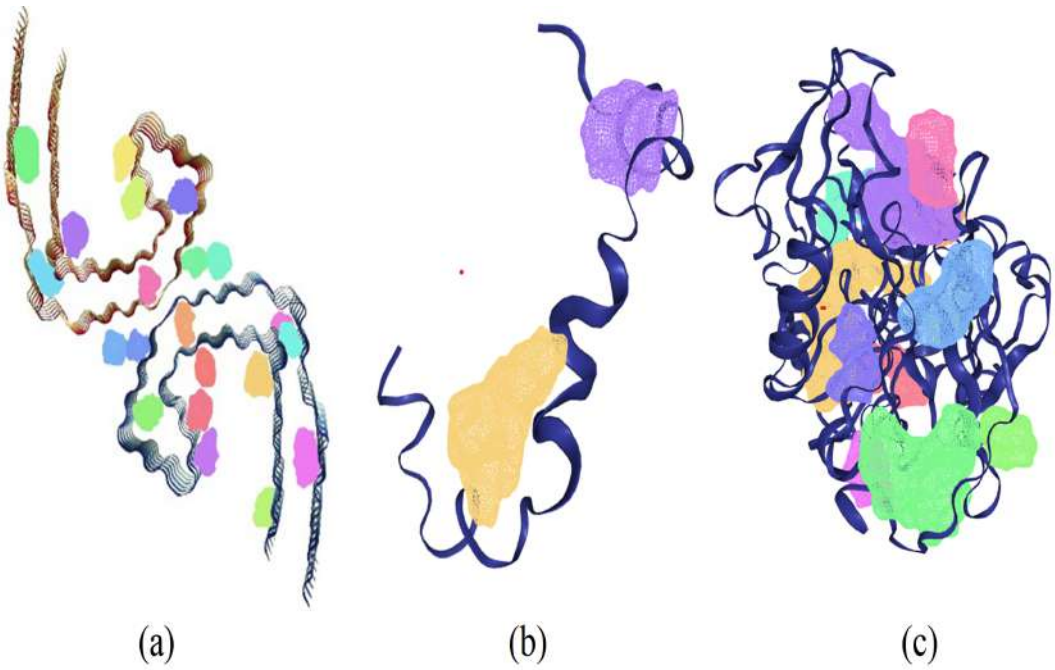


FIGURE 4.3: Active sites of (a) Tau protein (b) Amyloid-beta (c) BACE1

According to the DogSiteScorer data, each protein has a different number of binding pockets; tau protein has 21 pockets, amyloid beta has 2 pockets, and BACE-1

has 13 pockets. Table 4.2 below lists the total amount of pockets along with its surface area and volume

TABLE 4.2: Volume and area of binding pockets of Tau protein, Amyloid-beta and BACE1

Pocket	**Tau Vol ($Å^3$)**	**Tau Surf ($Å^2$)**	**Amyloid-β Vol ($Å^3$)**	**Amyloid-β Surf ($Å^2$)**	**BACE1 Vol ($Å^3$)**	**BACE1 Surf ($Å^2$)**
P0	495.28	904.76	21.18	933.41	934.72	921.64
P1	490.81	902.11	113.09	518.82	608.45	884.17
P2	439.19	497.09			521.92	714.33
P3	421.54	721.44			209.34	273.73
P4	412.23	584.96			180.03	297.18
P5	398.69	735.65			134.59	128.17
P6	388.42	523.62			132.29	311.35
P7	372.1	504.8			128.64	238.6
P8	366.42	475.36			118.59	186.74
P9	341.51	438.53			116.16	67.92
P10	332.2	389.73			112.19	239.84
P11	321.08	378.81			110.91	292.94
P12	317.46	451.19			102.85	253.46
P13	312.14	644.74				
P14	305.85	622.01				
P15	284.7	568.86				
P16	284.09	447.28				
P17	269.95	453.98				
P18	178.67	556.37				
P19	163.44	292.35				
P20	101.91	115.28				

4.1.4 Protein Functional Domain Identification

In order to identify the domains and functional sites of certain proteins, Interpro was employed. It is an effective technique for analyzing the functional aspects of protein sequences. Structure, sequencing, and links are all components of conserved domains.

Many different functional areas, each responsible for a certain task, can be found in proteins. The functional domain is the part of a protein responsible for binding and interacting with other substances.

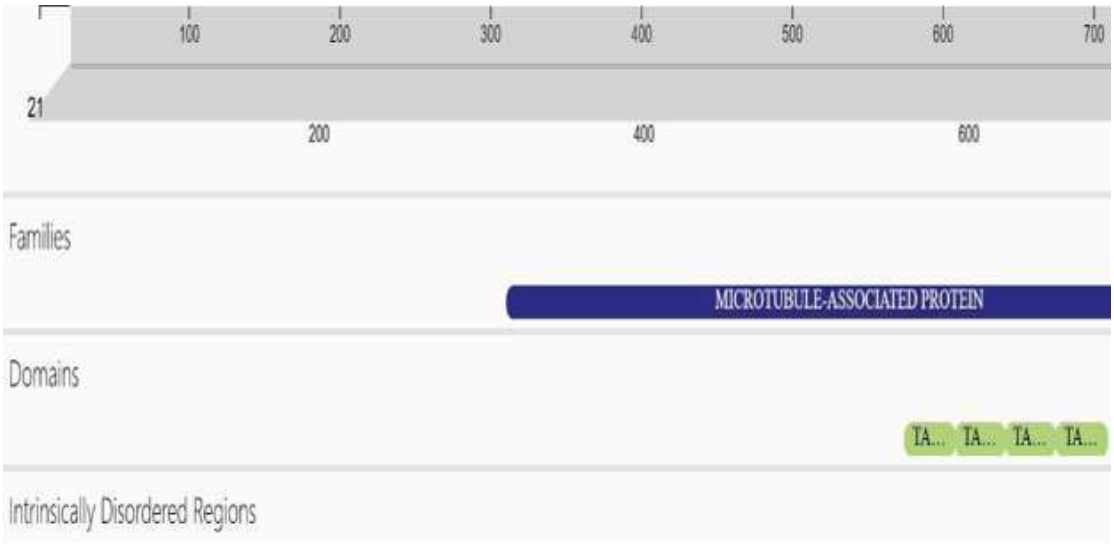


FIGURE 4.4: Domains of Tau proteins

Figure 4.4 shows MAP2/MAP4/Tau family membership is confirmed by InterPro analysis of the 758-aa Tau protein. Four T/TA domains, which are tubulin-binding repetitions that directly stabilise microtubules, are found in the C-terminal region.

This contact is reinforced by microtubule-binding motifs called M domains. Inter-repeat regions known as "I/IB domains" offer flexibility for the best possible binding. As a projection domain that controls microtubule positioning and protein interactions, the N-terminal two thirds are intrinsically disordered.

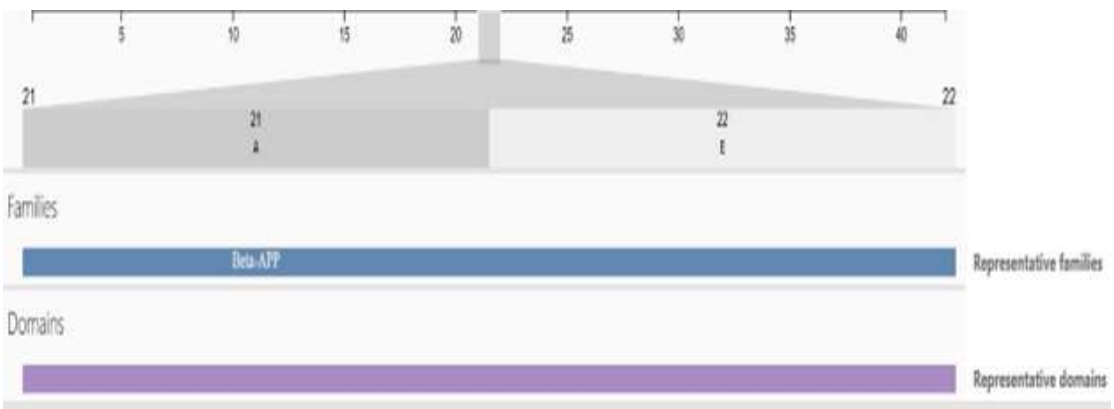


FIGURE 4.5: Domains of Amyloid-beta

Figure 4.5 show the 42-residue peptide's Beta-APP family membership is confirmed by InterPro analysis, which identifies it as amyloid-beta. Amyloidogenic signatures that start and encourage the development of fibrils are the Amyloid_glyco_Abeta domains.

Its pathogenic involvement in Alzheimer's disease is defined by the BETAAMYLOID motifs, which are core aggregation-prone areas that mediate β-sheet self-assembly into neurotoxic plaques.

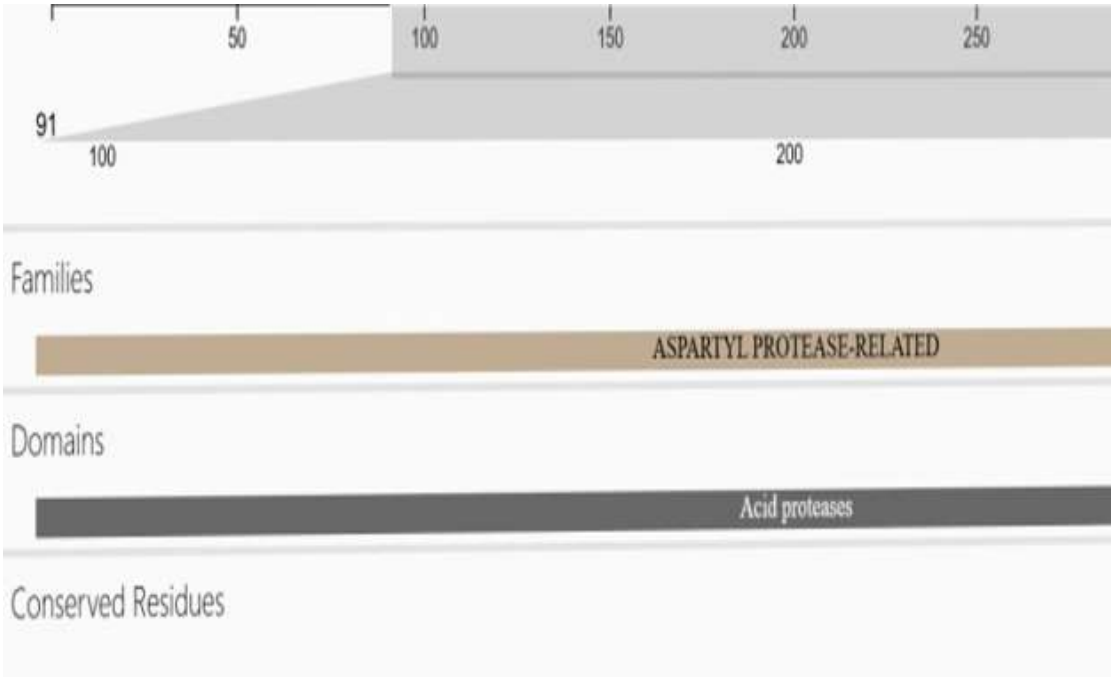


FIGURE 4.6: Domains of BACE1

Figure 4.6 show the 402-aa protein is identified as beta-secretase by InterPro analysis, which verifies its membership in the Aspartyl protease A1 and BACE1/BACE families. The catalytic unit that uses conserved aspartate residues to cleave APP at the β-site is the Aspartic peptidase A1 domain.

In endosomes, the Acid proteases domain permits enzymatic activity in acidic environments. The regulatory motif, active site flap, and inhibitory binding siteall crucial for substrate identification and processingare found in the beta secretase like conserved area. The function of BACE1 in starting the amyloidogenic process of Alzheimer's disease is defined by these features taken together.

4.2 Retrieving the Ligands' Chemical Structure

Based on their binding affinities and crystal-chemical class, the ligand to be chosen should be on the most effective solution structure. With that, the ligand's conformational choice is crucial. In this process of selection, A ligand binds more strongly to one conformer than the others, boosting the protein's strength relative to the population as a whole. We searched PubChem, the most comprehensive chemical database in the world, for ligands. To obtain the SDF formatted three-dimensional structures of these ligands, the PubChem database was queried. Every one of the selected ligands are displayed in Table 4.3 along with structural details.

TABLE 4.3: Ligand chemical structure

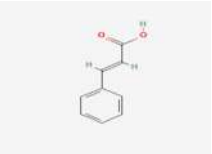
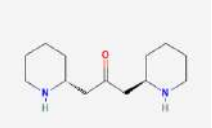
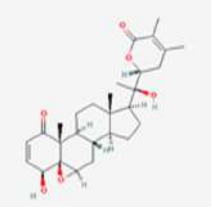
Secondary metabolites	**Molecular Weight**	**Molecular Formula**	**Smiles**	**Structures**
Withaferin-A	470.6 g/mol	$C_{28}H_{38}O_6$	CC1=C(C(=O)O[C@H](C1)[C@@H](C)[C@H]2CC[C@@H]3[C@@]2(CC[C@H]4[C@H]3C[C@@H]5[C@]6([C@@]4(C(=O)C=C[C@@H]6O)C)O5)C)CO	
Withanolide-A	470.6 g/mol	$C_{28}H_{38}O_6$	CC1=C(C(=O)O[C@H](C1)[C@@](C)([C@H]2CC[C@@H]3[C@@]2(CC[C@H]4[C@H]3[C@H]5[C@H](O5)[C@@]6([C@@]4(C(=O)C=CC6)C)O)C)O)C	
Cinnamic Acid	148.16 g/mol	$C_9H_8O_2$	C1=CC=C(C=C1)/C=C/C(=O)O	
Anaferine	224.34 g/mol	$C_{13}H_{24}N_2O$	C1CCN[C@H](C1)CC(=O)C[C@H]2CCCCN2	
Withanolide-D	470.6 g/mol	$C_{28}H_{38}O_6$	CC1=C(C(=O)O[C@H](C1)[C@@](C)([C@H]2CC[C@@H]3[C@@]2(CC[C@H]4[C@H]3[C@@H]5[C@]6([C@@]4(C(=O)C=C[C@@H]6O)C)O5)C)O)C	

Table 4.3 continued from previous page

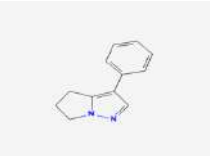
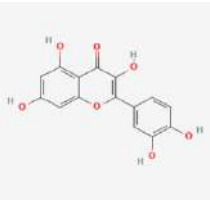
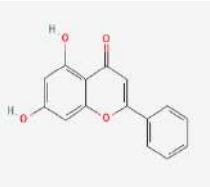
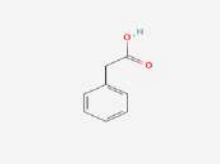
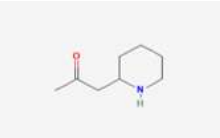
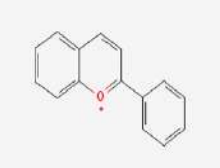
Secondary metabolites	Molecular Weight	Molecular Formula	Smiles	Structures
Withasomnine	184.24 g/mol	$C_{12}H_{12}N_2$	C1CC2=C(C=NN2C1)C3=CC=CC=C3	
Withanolide-L	452.6 g/mol	$C_{28}H_{36}O_5$	CC1=C(C(=O)O[C@H](C1)[C@@](C)([C@]2(CC=C3[C@@]2(CC[C@H]4[C@H]3CC=C5[C@@]4(C(=O)C=CC5)C)C)O)O)C	
Quercetin	302.23 g/mol	$C_{15}H_{10}O_7$	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	
Chrysin	254.24 g/mol	$C_{15}H_{10}O_4$	C1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O	
Phenyl Acetic Acid	136.15 g/mol	$C_8H_8O_2$	C1=CC=C(C=C1)CC(=O)O	
Withanone	470.6 g/mol	$C_{28}H_{38}O_6$	CC1=C(C(=O)O[C@H](C1)[C@@H](C)[C@]2(CC[C@@H]3[C@@]2(CC[C@H]4[C@H]3[C@H]5[C@H](O5)[C@@]6([C@@]4(C(=O)C=CC6)C)O)C)O)C	
Withanolide-B	454.6 g/mol	$C_{28}H_{38}O_5$	CC1=C(C(=O)O[C@H](C1)[C@@H](C)[C@H]2CC[C@@H]3[C@@]2(CC[C@H]4[C@H]3[C@H]5[C@H](O5)[C@@]6([C@@]4(C(=O)C=CC6)C)O)C)C	

Table 4.3 continued from previous page

Secondary metabolites	Molecular Weight	Molecular Formula	Smiles	Structures
Isopelletierine	141.21 g/mol	$C_8H_{15}NO$	CC(=O)CC1CCCCN1	
Anthocyanins	207.25 g/mol	$C_{15}H_{11}O^+$	C1=CC=C(C=C1)C2=[O+]C3=CC=CC=C3C=C2	

4.2.1 Ligand Energy Minimisation

Minimising the energy associated with these ligands was the next action taken after obtaining structures of the chosen ligands. This is a crucial step because Since the ligands are not stable and can affect the docking vina scores, we can't just use the structure that was downloaded. Figure 4.7 displays the ligand's revised forms following energy minimisation.

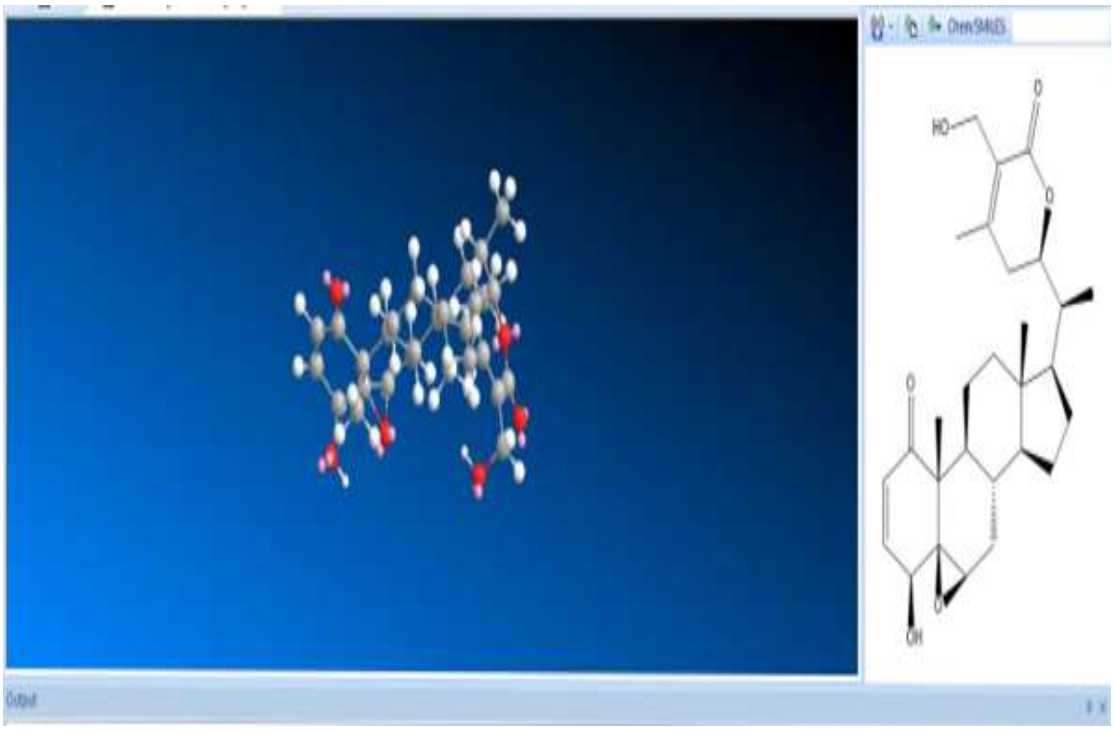


FIGURE 4.7: Energy Minimisation of Ligand

4.2.2 Virtual Ligand Screening

Pharmacokinetic characteristics and virtual screening are used to separate substances as the both drug-like and nondrug-such as. It is recommended that the molecular weight be between 500 and 5, the log P be between 5 and 10, the number of hydrogen bond donors be between 5 and 10, and the number of hydrogen bond acceptors be between 10 and 5, as per the Lipinski rule. All oral active compounds are required to adhere to these guidelines. The method of administration affects the pharmacological impact. If a chemical satisfies all three requirements, it is classified as a medicine. it doesn't meet in excess of two, it has poor absorption [113]. Table 4.4 displays the virtual screening of ligand.

TABLE 4.4: Virtual Ligand Screening

S.No	**Ligand name**	**Molecular weight**	**LogP Value**	**Rotatable bonds**	**H-bond acceptors**	**H-bond donars**
1.	Withaferin-A	470.606	3.3529	3	6	2
2.	Withanolide-A	470.606	3.4954	2	6	2
3.	Cinnamic Acid	148.161	1.7844	2	1	1
4.	Anaferine	-1.204	1.6199	4	3	2
5.	Withanolide-D	470.606	3.4954	6	2	6
6.	Withasomnine	184.242	2.4963	1	2	0
7.	Withanolide-L	452.591	4.3484	2	5	2
8.	Quercetin	302.238	1.988	1	7	5
9.	Chrysin	254.241	2.8712	1	4	2
10.	Phenyl Acetic Acid	136.15	1.3137	2	1	1
11.	Withanone	470.606	3.4954	2	6	2
12.	Withanolide-B	454.607	4.3805	2	5	1
13.	Isopelletierine	141.214	1.1076	2	2	1
14.	Anthocyanins	207.252	207.252	1	0	0

Table 4.4 shows that all ligands follow the Lipinski rule. Every ligand has a molecular weight of less than 500 and a log P value of less than5.Additionally, all ligands are with in range of the hydrogen bond's donor, receiver, and rotatable bonds.

4.3 ADMET Ligand Analysis

Another investigation was carried out to determine ligands' ADMET characteristics as a pharmacokinetic metric according to the Lipinski rule through the web application pkCSM. In pharmacology, the two overarching concepts are pharmacodynamics and pharmacokinetics.Medical drug safety is a branch of pharmacology that studies how drugs interact with the body. Pharmacokinetics is the study of the absorption, distribution, metabolism, and elimination of drugs [115].

4.3.1 Properties of Ligand Absorption

Important criteria for assessing a compound's bioavailability are revealed by the absorption measurements. Better solubility in water is indicated by readings larger than -2, which may improve absorption. A good result for Caco2 permeability is more than 0, while values greater than 1 indicate outstanding absorption potential and high permeability. When readings are higher than 50%, which indicate good absorption in people, intestinal absorption is considered favourable. Better skin permeability is indicated by values larger than -2, which is beneficial for topical applications. Finally, when it comes to P-glycoprotein substrates, a "Yes" means that the molecule can be actively carried by Pglycoprotein, which might or might not be advantageous depending on the situation. A "No" status is ideal for P-glycoprotein inhibitors in order to prevent possible medication interactions that can jeopardise treatment efficacy [114]. Table 4.5 lists the ligands' absorption characteristics.

TABLE 4.5: Values of ligand absorption

ADMET Properties	**Water solubility**	**Caco2 permeability**	**Intestinal absorption**	**Skin Permeability**	**P-glycoprotein sub**	**P-glycoprotein I inh**	**P-glycoprotein II inh**
Withaferin-A	-5.063	0.829	85.345	-3.202	Yes	Yes	Yes

Table 4.5 continued from previous page

ADMET Properties	**Water solubility**	**Caco2 permeability**	**Intestinal absorption**	**Skin Permeability**	**P-glycoprotein sub**	**P-glycoprotein I inh**	**P-glycoprotein II inh**
Withanolide-A	-5.096	0.846	100	-3.285	Yes	Yes	Yes
Cinnamic Acid	-2.608	1.717	94.833	-2.695	No	No	No
Anaferine	-1.204	1.336	93.738	-3.337	No	No	No
Withanolide-D	-5.127	0.831	99.2	-3.267	Yes	Yes	Yes
Withasomnine	-2.402	1.422	97.188	-2.031	Yes	No	No
Withanolide-L	-5.458	0.866	95.871	-3.969	Yes	Yes	Yes
Quercetin	-2.925	-0.229	77.207	-2.735	Yes	No	No
Chrysin	-3.538	0.945	93.761	-2.739	Yes	No	No
Phenyl Acetic Acid	-2.21	1.71	85.312	-2.703	No	No	No
Withanone	-5.127	0.849	100	-3.365	Yes	Yes	Yes
Withanolide-B	-5.355	0.765	97.465	-3.277	No	Yes	Yes
Isopelletierine	-0.426	1.363	97.349	-3.291	No	No	No
Anthocyanins	-4.852	1.631	96.182	-2.128	No	No	No

According to the ADMET statistics, the majority of chemicals can function well as oral tablets because their intestinal absorption is over 88%. Quercetin's bioavailability will be limited due to its low solubility and absorption, but cinnamic acid and anaferine have the maximum permeability. Withanone, Withaferin-A, Withanolide-D, and Withanolide-L all exhibit 85% to 100% absorption and Caco2 permeability above 0.8, indicating good intestinal absorption. Withanolide-D has a good balance between solubility and absorption, Withanolide-L has the best solubility of 5.458, and Withanone has 100% absorption.

The primary problem is that since all four are P-glycoprotein substrates, the body's P-gp pump may eliminate them and lessen their impact. The good news is that all four of these are P-gp inhibitors. They can prolong their duration in the body by blocking the pump.

4.3.2 Properties of Ligand Distribution

TABLE 4.6: Values of ligand distribution

ADMET Properties	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
Withaferin-A	-0.131	0.105	-0.03	-2.72
Withanolide-A	-0.097	0.096	-0.255	-2.681
Cinnamic Acid	-1.051	0.38	0.446	-1.834
Anaferine	0.908	0.754	0.112	-3.249
Withanolide-D	-0.048	0.093	-0.315	-2.696
Withasomnine	0.272	0.277	0.289	-1.699
Withanolide-L	0.141	0.086	-0.159	-1.887
Quercetin	1.559	0.206	-1.098	-3.065
Chrysin	0.403	0.136	0.047	-1.912
Phenyl Acetic Acid	-1.299	0.488	-0.157	-1.936
Withanone	0.081	0.154	-0.259	-2.719
Withanolide-B	-0.025	0.015	-0.095	-1.805
Isopelletierine	0.431	0.774	0.063	-2.997
Anthocyanins	0.24	0.147	0.147	-1.269

The ADMET distribution of different ligands is contrasted with typical pharmacokinetic limits in this table. Only quercetin and phenyl acetic acid exhibit higher VDss, indicating better tissue dispersion, while the majority of with anolides fall on the lower end, implying they remain mostly in plasma with minimal tissue diffusion. The typical range for VDss is 0.04–20 L/kg. While the majority of ligands are heavily plasma-bound, the fraction unbound typically ranges from 0 to 1. Anaferine and isopelletierine are on the upper side, meaning there is more free active medication accessible. When it comes to BBB permeability, values greater than 0.3 indicate CNS entranc, only quercetin clearly satisfies this criterion, while the rest are low. Quercetin meets the best parameters for tissue distribution and BBB crossing, Anaferine/Isopelletierine for free fraction, and most with anolides suit a systemic rather than CNS-targeted profile.

4.3.3 Properties of Ligand Metabolism

The liver's detoxification process is managed by the enzyme cytochrome P450.This enzyme deactivates a lot of medications, but it can also activate some. Inhibitors of this enzyme should not be utilised since they may have a direct impact on the drug's metabolism. Similarly, the metabolism of the medicines is carried out by CYP2D6 and CYP3A4. The pharmacokinetics of the medication in use are impacted when they are inhibited [115]. The prediction of ligand metabolism is displayed below. Table 4.7 presents the metabolic properties of ligands.

TABLE 4.7: Values of ligand metabolism

ADMET Properties	**CYP2D6 sub**	**CYP3A4 sub**	**CYP1A2 inh**	**CYP2C19 inh**	**CYP2C9 inh**	**CYP2D6 inh**	**CYP3A4 inh**
Withaferin-A	No	Yes	No	No	No	No	No
Withanolide-A	No	Yes	No	No	No	No	No
Cinnamic Acid	No	No	No	No	No	No	No
Anaferine	No	No	No	No	No	No	No
Withanolide-D	No	Yes	No	No	No	No	No
Withasomnine	No	No	Yes	No	No	No	No
Withanolide-L	No	Yes	No	No	No	No	No
Quercetin	No	No	Yes	No	No	No	No
Chrysin	No	No	Yes	Yes	Yes	No	No
Phenyl Acetic Acid	No	No	No	No	No	No	No
Withanone	No	Yes	No	No	No	No	No
Withanolide-B	No	Yes	No	No	No	No	No
Isopelletierine	No	No	No	No	No	No	No
Anthocyanins	No	Yes	Yes	Yes	No	No	No

This table provides an overview of the evaluated phytochemicals' metabolism and CYP-mediated interaction profile. There is little chance of CYP2D6/3A4-mediated drug-drug interactions because no ligand functions as a CYP2D6 substrate or inhibitor and there is no CYP3A4 inhibition throughout the set. While

the other ligands do not undergo primary metabolism via this isoenzyme, Withaferin - A, Withanolide-A, Withanolide-D, Withanolide-L, Withanone, Withanolide - B, and anthocyanins are anticipated to do so because they are CYP3A4 substrates. Chrysin is one of the inhibitors that broadly inhibits CYP1A2, CYP2C19, and CYP2C9. Quercetin, with anosomine, and anthocyanins inhibit CYP1A2, and anthocyanins also inhibit CYP2C19. Since the other ligands do not exhibit CYP inhibition, their potential for pharmacokinetic interactions is minimal. The majority of ligands have a low risk of DDI, which supports their potential as therapeutic candidates.

4.3.4 Properties of Ligand Excretion

The process of biliary drug excretion is carried out by the liver, whereas renal drug excretion is a kidney-dependent process. Two organs that help with drug excretion are the kidneys and the liver. The liver is engaged in biliary excretion and the kidneys in renal excretion. As an example, the lungs are involved in the excretion of gaseous or volatile substances, among other organs. Saliva, perspiration, and tears can also dissolve medications [116]. Table 4.8 displays the ligand excretion rates.

TABLE 4.8: Values of ligand excretion

ADMET Properties	Total Clearance (log ml/min/kg)	Renal OCT2 substrate
Withaferin-A	0.435	Yes
Withanolide-A	0.337	Yes
Cinnamic Acid	0.781	No
Anaferine	1.272	No
Withanolide-D	0.347	No
Withasomnine	0.340	No
Withanolide-L	0.438	Yes
Quercetin	0.407	No
Chrysin	0.405	No
Phenyl Acetic Acid	0.263	No
Withanone	0.385	Yes
Withanolide-B	0.354	Yes

Table 4.8 continued from previous page

ADMET Properties	Total Clearance (log ml/min/kg)	Renal OCT2 substrate
Isopelletierine	1.073	No
Anthocyanins	0.716	No

For every examined ligand, the excretion profile displays moderate to high clearance, indicating effective removal with minimal accumulating danger. Some OCT2 substrates, such as Withaferin-A, Withanolide-A, Withanolide-L, Withanone, and Withanolide-B, indicate active renal secretion, whereas others rely on glomerular filtration.

Overall, a safe pharmacokinetic profile for drug development is supported by the favourable clearance and low possibility for transporter interaction.

4.3.5 Properties of Ligand Toxicity

Using pkCSM, we determined how hazardous the ligands were. To determine if a chemical could cause mutations, the AMES toxicity test employs microorganisms. In the event of a favorable reaction, the ligand's carcinogenicity could be further amplified. Toxic effects on the protozoan *T. pyriformis* bacterium serve as the endpoint in the *T. pyriformis* toxicity method. Dangerous levels are defined as -0.5 log ug/L.

According to the predicted results of the Minnow toxic test, the concentration at which half of the minnows will die is demonstrated. Values below 0.5 mM are considered to cause acute toxicity [117].

In the oral rat chronic toxicity test, the expected log value of the lowest observed negative impact is linked to the drug concentration that requires a specific duration of therapy, represented as log mg/kg bw/day. To find out if a drug can affect liver function, scientists conduct hepatotoxicity tests. An increase in the maximum tolerable dose (MRTD) is an indication of improved safety [118].

Each ligand's toxicity values are listed in Table 4.9.

TABLE 4.9: Values of ligand toxicity

ADMET Properties	**AMES toxicity**	**Max. tolerated dose (human)**	**hERG I inhibitor**	**hERG II inhibitor**	**Oral Rat Acute Toxicity (LD50)**	**Oral Rat Chronic Toxicity (LOAEL)**	**Hepatotoxicity**	**Skin Sensitisation**	***T. pyriformis* toxicity**	**Minnow toxicity**
Withaferin-A	No	-0.695	No	No	2.779	0.918	No	No	0.299	0.738
Withanolide-A	No	-0.967	No	No	2.913	1.739	No	No	0.298	1.22
Cinnamic Acid	No	1.11	No	No	2.094	2.651	No	No	0.247	1.719
Anaferine	No	0.132	No	No	2.483	0.833	No	Yes	0.14	2.062
Withanolide-D	No	-0.867	No	No	2.831	1.776	No	No	0.305	1.178
Withasomnine	Yes	0.249	No	No	2.093	1.113	No	No	0.747	0.931
Withanolide-L	No	-0.952	No	No	2.13	1.899	No	No	0.36	0.444
Quercetin	No	0.499	No	No	2.471	2.612	No	No	0.288	3.721
Chrysin	No	0.016	No	No	2.289	0.955	No	No	0.535	1.746
Phenyl Acetic Acid	No	1.048	No	No	2.047	2.646	No	No	0.138	1.894
Withanone	No	-1.093	No	No	2.907	1.676	No	No	0.299	1.322
Withanolide-B	No	-0.593	No	No	2.474	0.132	No	No	0.319	0.191
Isopelletierine	No	0.576	No	No	2.249	1.312	Yes	Yes	-0.8	2.373
Anthocyanins	No	0.044	No	No	1.848	1.118	No	No	1.111	0.172

The toxicity profile of the studied phytochemicals is summarised in this table using important ADMET criteria. With the exception of Withasomnine, none of the ligands exhibited AMES toxicity, and none of the compounds exhibited hERG I/II inhibition, suggesting a low risk of mutagenicity and cardiotoxicity.

Only isopelletierine was anticipated to be hepatotoxic; anaferine and isopelletierine were identified as possible skin sensitisers; the other ligands were not. For the majority of substances, oral rat acute and chronic toxicity values indicate moderate safety margins; no high toxic liabilities were found. Overall, the toxicity results

show that most ligands have a good safety profile, which supports their potential as drug-like options with few toxicological issues.

4.4 Molecular Docking

We employ the three-dimensional protein and ligand geometries to carry out docking. In this case, CB dock, an online blind auto docking solution, is employed. Protein binding sites and cavity sizes are estimated by CB Dock. Using CB Dock, we can see the five best possess and receptor models after docking. From these five potential sites, the one with the best combination of cavity size and vina score was chosen [119].

TABLE 4.10: Docking score of protein-ligand complexes

Target Protein	**Tau**	**Amyloid-beta**	**BACE1**
Withaferin-A	-8.3	-7.4	-9.2
Withanolide-A	-6.5	-6.4	-8.8
Cinnamic Acid	-4.7	-5	-6.6
Anaferine	-5.3	-5	-6.2
Withanolide-D	-8.1	-7.6	-9.1
Withasomnine	-4.8	-5.2	-6.7
Withanolide-L	-8.1	-7.5	-9.2
Quercetin	-6.4	-5.7	-8.9
Chrysin	-6.5	-5.6	-8.6
Phenyl Acetic Acid	-4.4	-5.1	-5.4
Withanone	-7.4	-7.1	-9.1
Withanolide-B	-7	-7.7	-9.2
Isopelletierine	-4	-4.2	-4.9
Anthocyanins	-5.8	-5.9	-7.9

According to molecular docking studies, Withaferin-A, Withanolide-D, and Withanolide - L showed the highest binding affinities against Tau, Amyloid-β, and BACE1, the three targets linked to Alzheimer's disease. Withaferin-A showed the strongest overall multi-target inhibitory activity among them, especially against BACE1 (-9.2 kcal/mol) and Tau (-8.3 kcal/mol), indicating its potential as a

lead phytochemical for additional in vitro and in vivo testing against Alzheimer's disease. The therapeutic promise of chemicals produced from with ania as multi-purpose anti-Alzheimer's medicines is further supported by the significant binding affinities of Withanolide-D and Withanolide-L.

4.5 Docked Complex Analysis using Discovery Studio

In order to make sense of docking data, the ligand-protein interaction was approximated. Three main types of interactions were investigated: hydrogen bonding, alkyl, and van der Waals. Discover Studio 2025 The client was used to analyze these protein-ligand interactions. We looked at every molecule's stored conformation for the receptor-ligand pair. This program will automatically generate a schematic representation of the protein-ligand interactions for the specified ligands in the Protein Data Bank (PDB). The docked data, which were provided in PDB format [120], contained numerous interactions between the three types of target proteins and the fifteen ligands. We can see the docked complexes and ligand-receptor interactions in these diagrams.

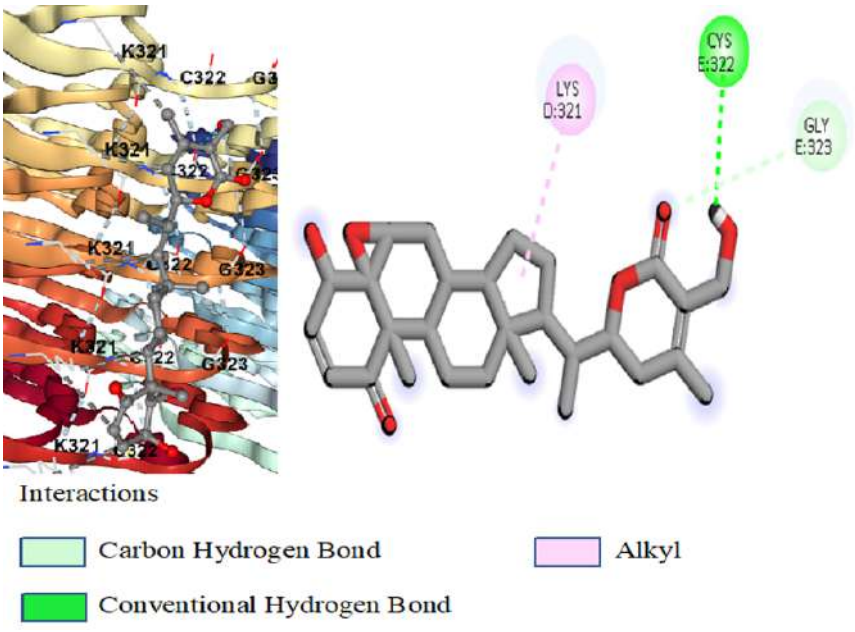


FIGURE 4.8: Docking analysis of Tau protein with Withaferin-A

Figure 4.8 shows the interactions of Tau protein with Withaferin-A. It Shows that there is one hydrogen bond with cysteine (322) and one alkyl bond with lysine (321).

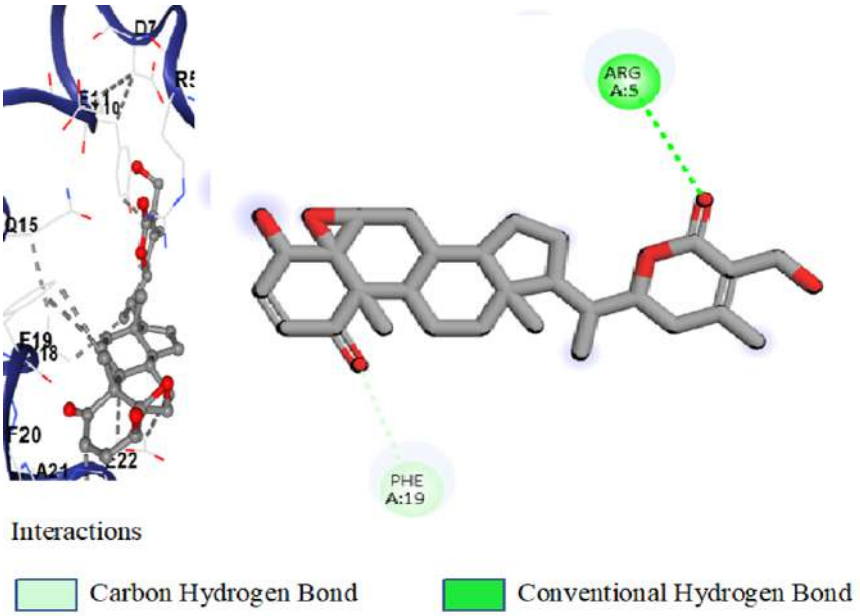


FIGURE 4.9: Docking analysis of Amyloid-beta with Withaferin-A

Figure 4.9 shows the interactions of Amyloid-beta with Withaferin-A.It shows that there is one hydrogen bond with arginine (5) and one carbon hydrogen bond with Phenylalanine (19).

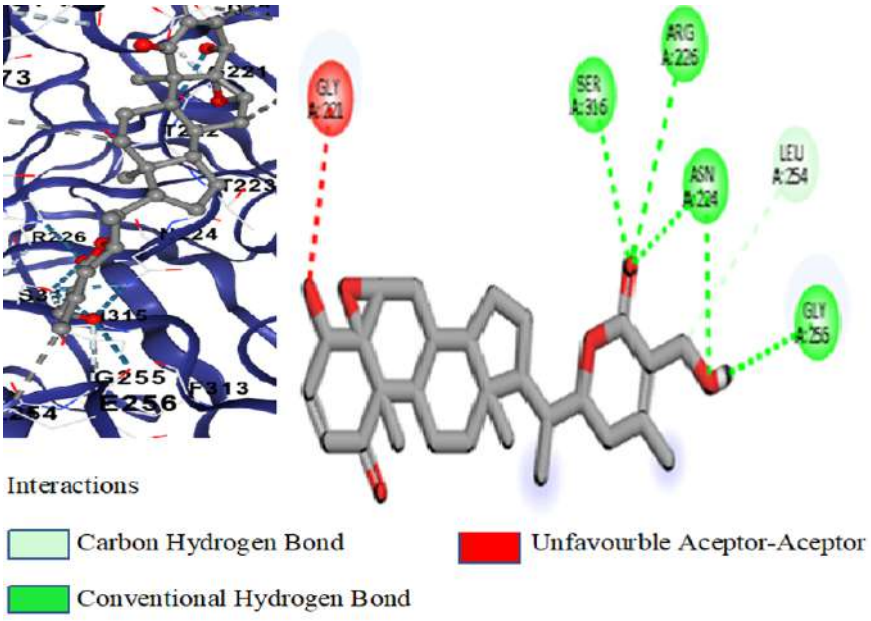


FIGURE 4.10: Docking analysis ofBACE1 with Withaferin-A

Figure 4.10 shows the interactions of BACE1 with Withaferin-A.It demonstrates that there three hydrogen bond and one carbon hydrogen bond.Moreover, there is one unfavourable acceptor-aceptor interation.

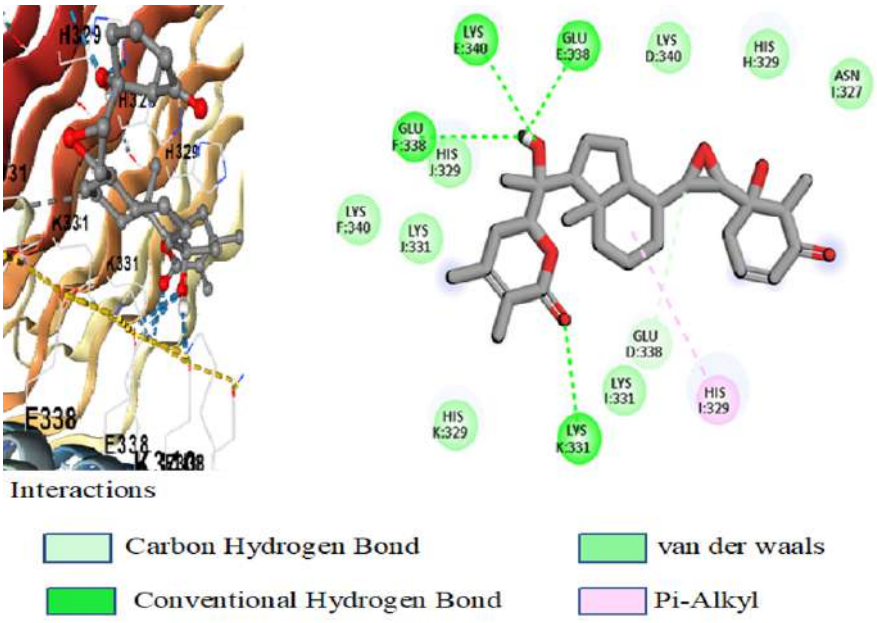


FIGURE 4.11: Docking analysis of Tau protein with Withanolide-A

Figure 4.11 shows the interactions of Tau protein with Withanolide-A. It shows eight van der waals interactions and four conventional hydrogen bonds.Moreover, there is also alkyl interactions.

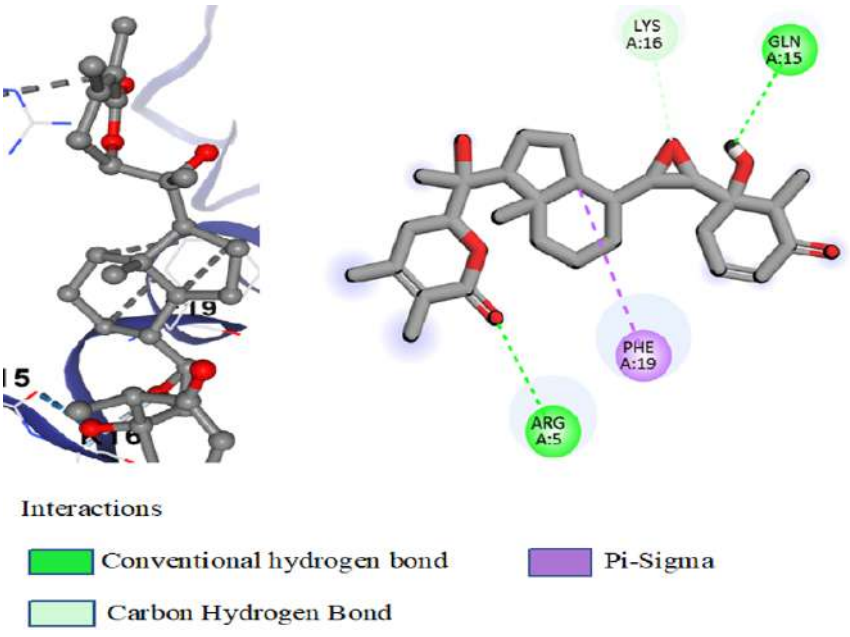


FIGURE 4.12: Docking analysis of Amyloid-beta with Withanolide-A

Figure 4.12 show the interactions of Amyloid-beta with Withanolide-A.It indicates that there is two hydrogen bonds with glycine (15) and arginine (5). Moreover, there is one carbon -hydrogen bond and one sigma bond is also present.

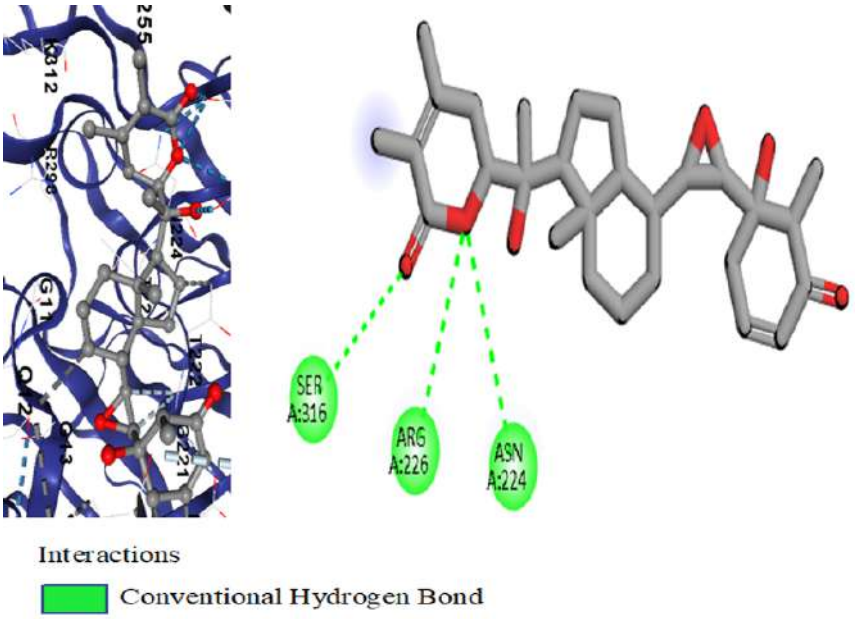


FIGURE 4.13: Docking analysis of BACE1 with Withanolide-A

Figure 4.13 indicates the binding of BACE1 with Withanolide-A. It shows that there is three hydrogen bonds with serine (316),arginine (226) and asparagine (224).

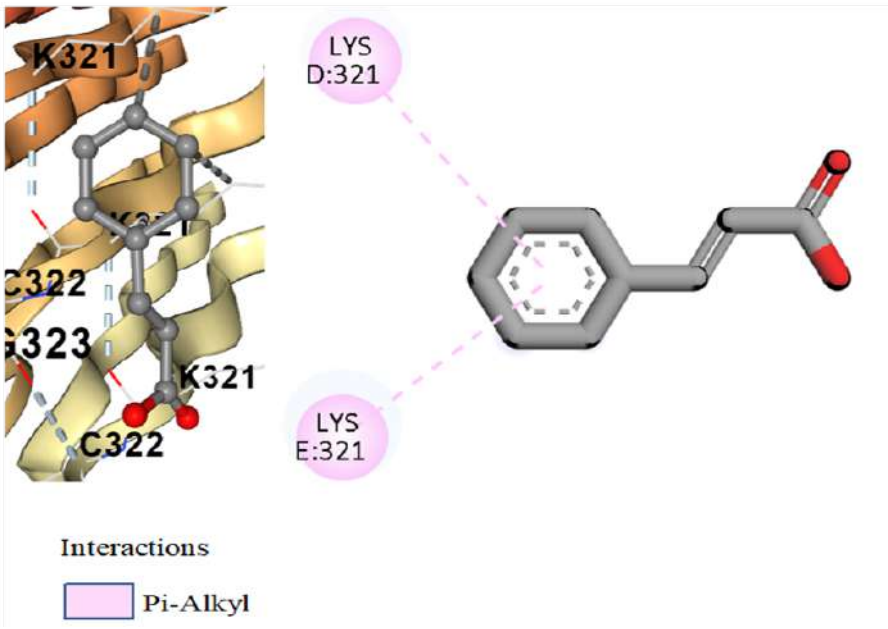


FIGURE 4.14: Docking analysis of Tau protein with cinnamic acid

Figure 4.14 shows the interface of Tau protein with cinnamic acid. It show that there is two alkyl interactions with the Lysine (321).

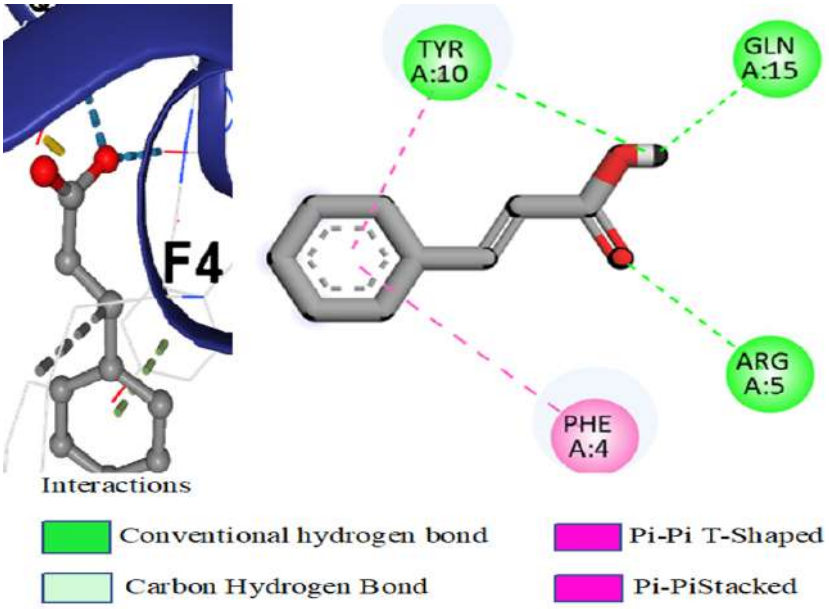


FIGURE 4.15: Docking analysis of Amyloid-beta with cinnamic acid

Figure 4.15 indicates the binding of Amyloid-beta with cinnamic acid It Shows the three hydrogen bonds, Pi-Pi stacked and Pi-Pi T-shaped.

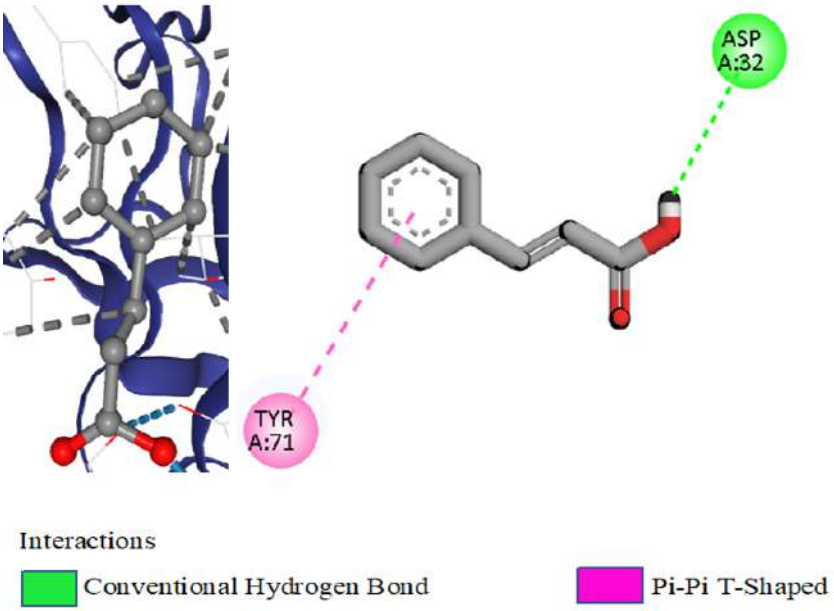


FIGURE 4.16: Docking analysis of BACE1cinnamic acid

Figure 4.16 shows the interface ofBACE1 with cinnamic acid.It shows that there is one hydrogen bond with aspartic acid (32) and Pi-Pi T-shaped binding.

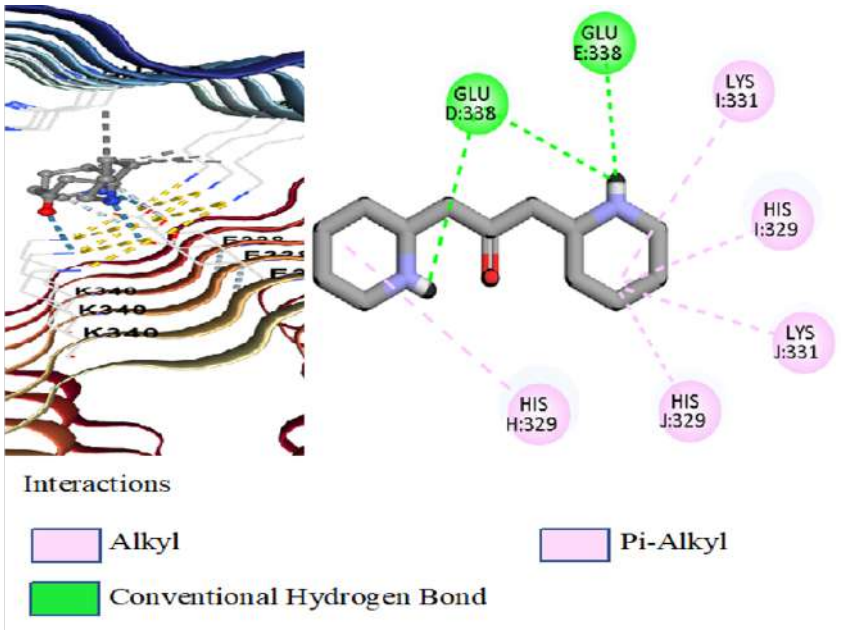


FIGURE 4.17: Docking analysis of Tau protein with anaferine

Figure 4.17 shows the binding ofTau protein anaferine. It indicates the two hydrogen bonds with glutamic acid (338) and five alkyl interactions.

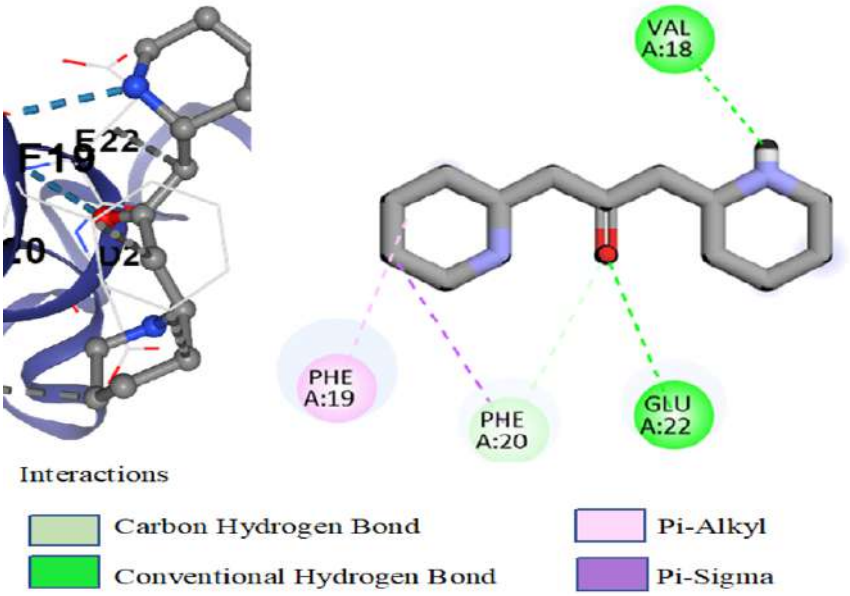


FIGURE 4.18: Docking analysis of Amyloid-beta with anafe

Figure 4.18 indicates the interaction of Amyloid-beta with anaferine.It shows that there is two hydrogen bonds with valine and glutamic acid and also shows the alkyl interaction with phenylalanine (20).

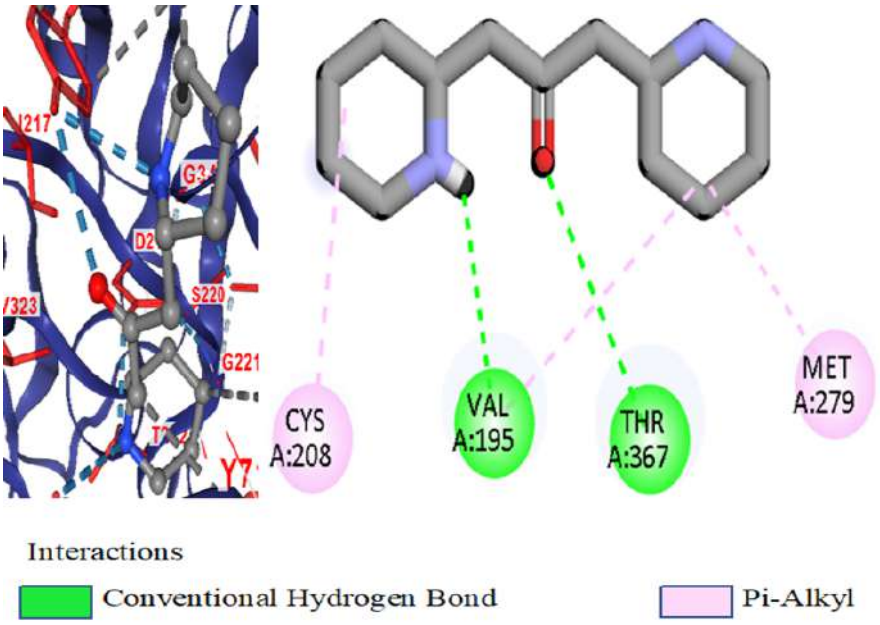


FIGURE 4.19: Docking analysis of BACE1 with anaferine

Figure 4.19 shows the binding of BACE1 with anaferine. It shows the two hydrogen bonds with valine and threonine and two alkyl interactions.

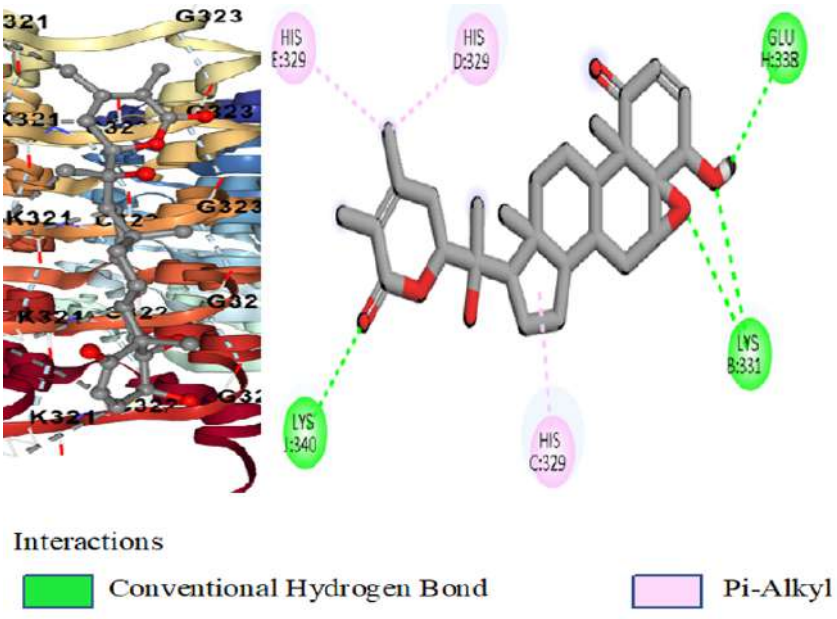


FIGURE 4.20: Docking analysis of Tau protein Withanolide-D

Figure 4.20 shows the interaction of Tau protein with Withanolide-D. It indicates three hydrogen bonds and three alkyl interactions.

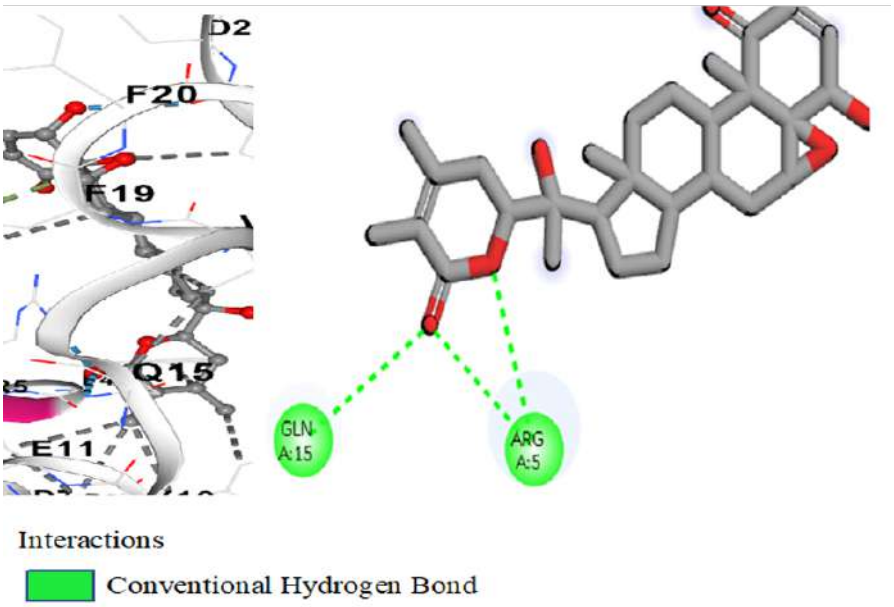


FIGURE 4.21: Docking analysis of Amyloid-beta with Withanolide-D

Figure 4.21 shows the binding of Amyloid-beta with Withanolide-D. It indicates two hydrogen bonds with Glutamine (15) and Arginine (5).

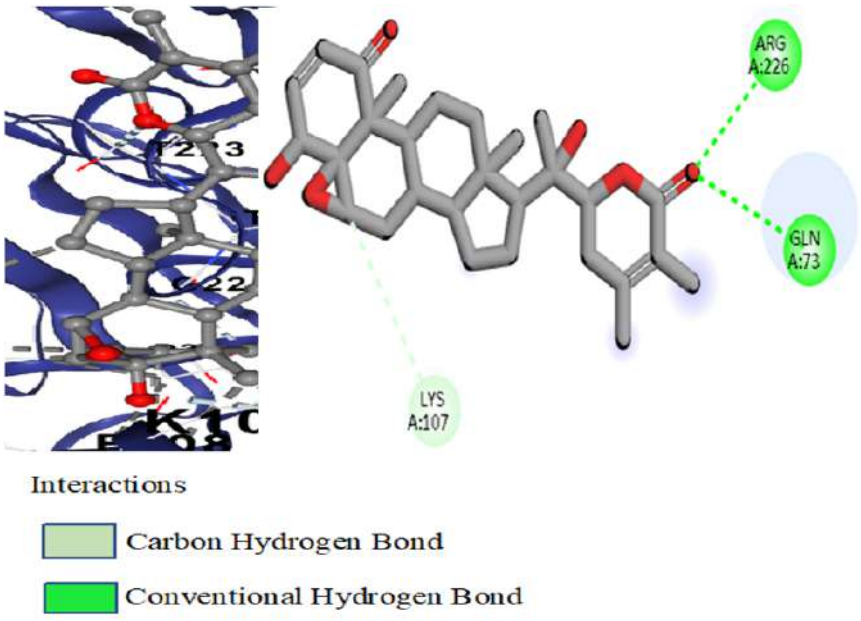


FIGURE 4.22: Docking analysis of BACE1 Withanolide-D

Figure 4.22 shows the interaction of BACE1 with Withanolide-D. It indicates the two hydrogen bonds with Arginine (226) and Glutamine (73).

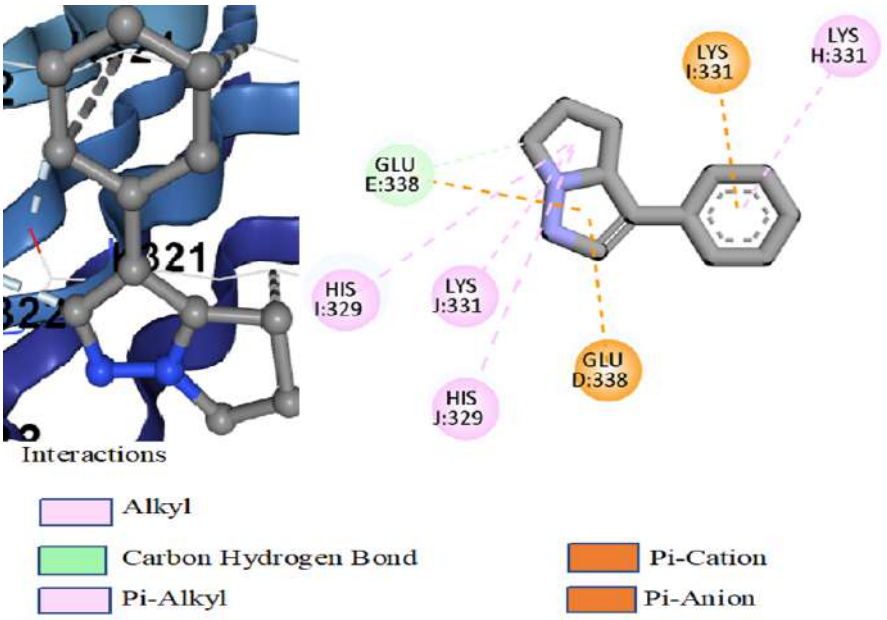


FIGURE 4.23: Docking analysis of Tau protein Withasomnine

Figure 4.23 shows the binding of Tau protein with Withasomnine. It shows one hydrogen bond with Glutamic acid (338) and four alkyl interactions.

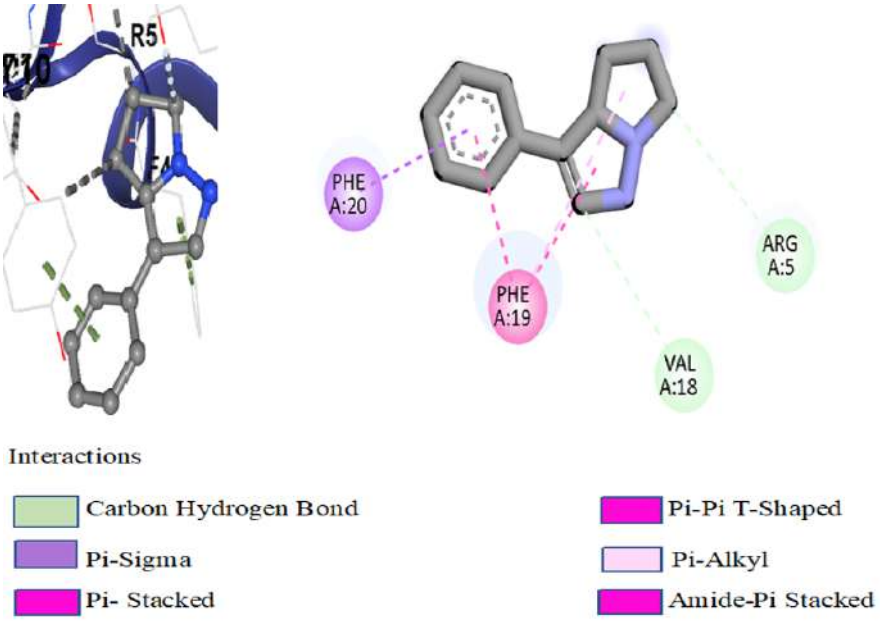


FIGURE 4.24: Docking analysis of Amyloid-beta Withasomnine

Figure 4.24 indicates the binding of Amyloid-beta with Withasomnine. It shows that there is two hydrogen bonds with valine (18) and Arginine (5) and one alkyl interaction with Phenylalanine (19).

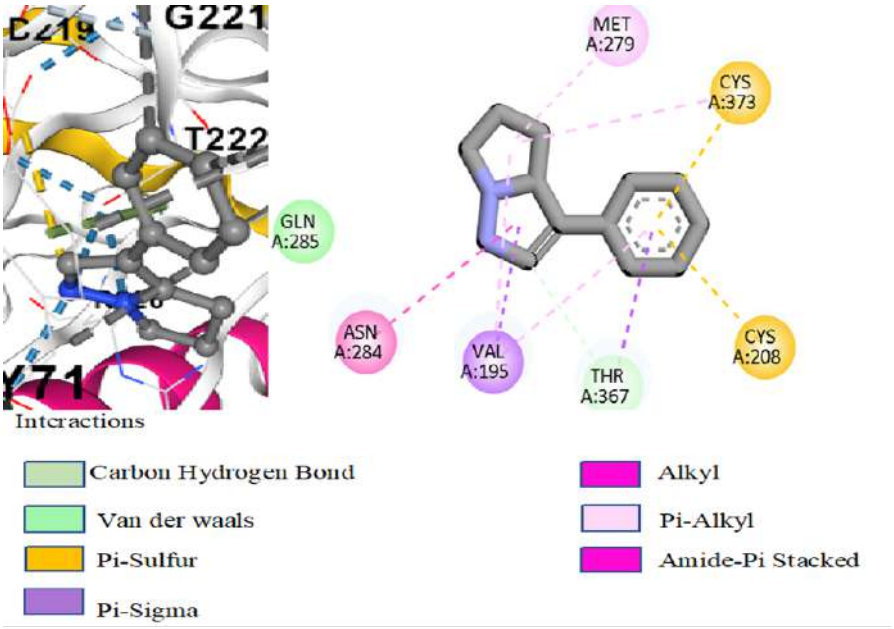


FIGURE 4.25: Docking analysis of BACE1 Withasomnine

Figure 4.25 shows the interaction of BACE1 Withasomnine. It shows one van der waal interactions with glutamine and two alkyl associations with valine (195).

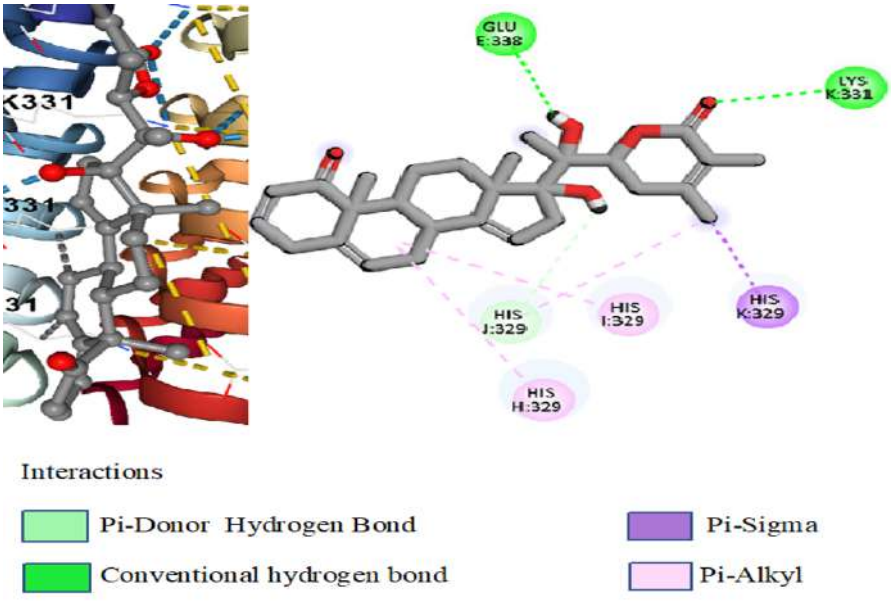


FIGURE 4.26: Docking analysis of Tau protein with Withanolide-L

Figure 4.26 shows the binding of Tau protein with Withanolide-L It indicates that there is two hydrogen bonds with glutamic acid and lysine and also two alkyl contacts with histidine. Moreover, there is one sigma bond also.

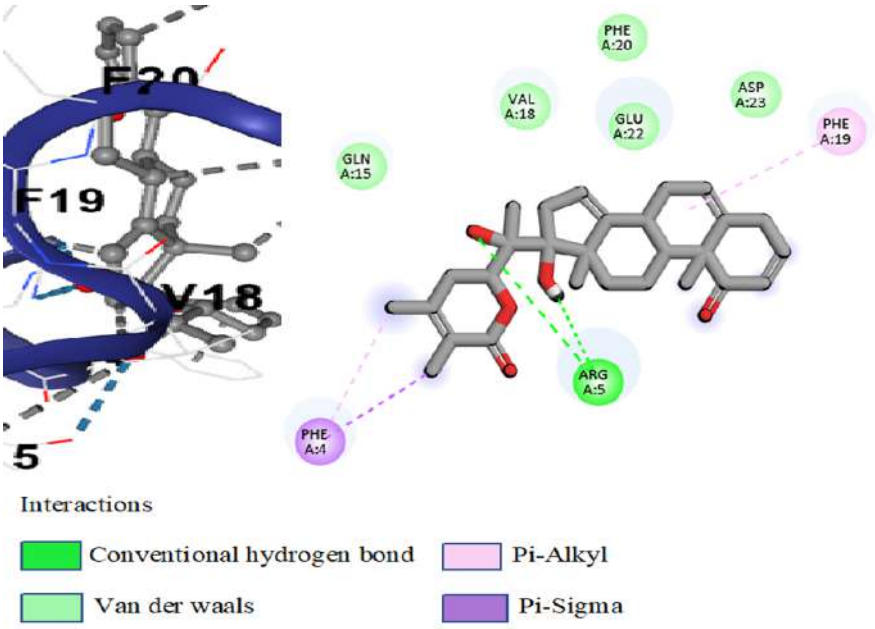


FIGURE 4.27: Docking analysis of Amyloid-beta Withanolide-L

Figure 4.27 shows the binding of Amyloid-beta Withanolide-L. It shows that there is one hydrogen bond with arginine (5), four van der waals interactions and one alkly contact with phenylalanine (4).

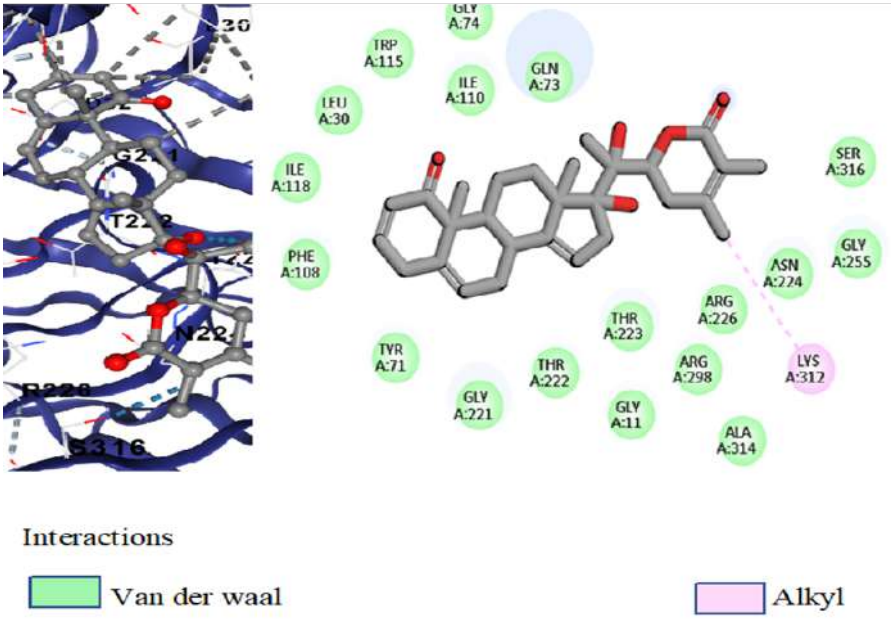


FIGURE 4.28: Docking analysis of BACE1 Withanolide-L

Figure 4.28 shows the interface of BACE1 Withanolide-L. It indicates the eighteen van der waals interactions and one alkly contact.

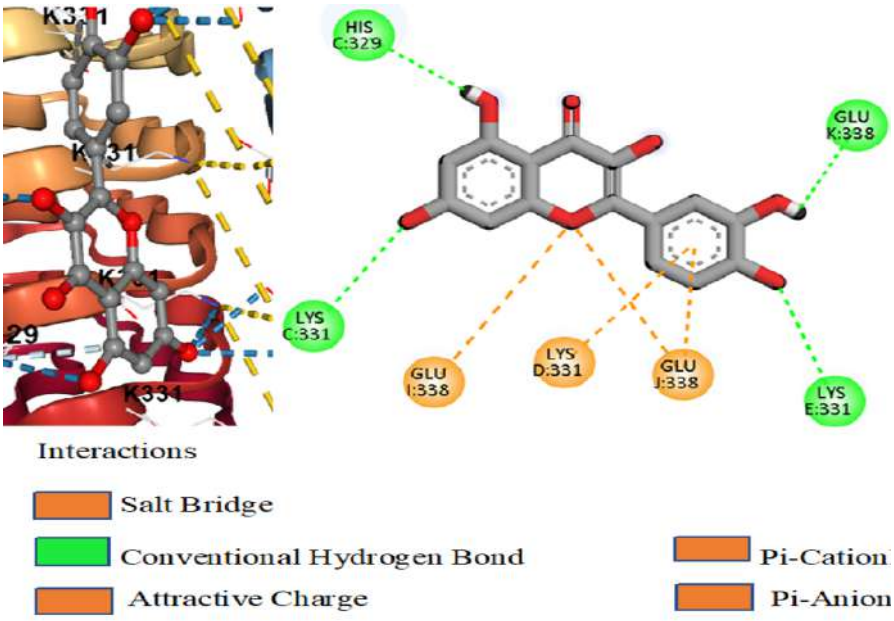


FIGURE 4.29: Docking analysis of Tau protein with quercetin

Figure 4.29 shows the binding of Tau protein with quercetin. It shows that there is four hydrogen bonds with two lysine one with histidine and one with glutamic acid, there is also salt bridge and attractive charges.

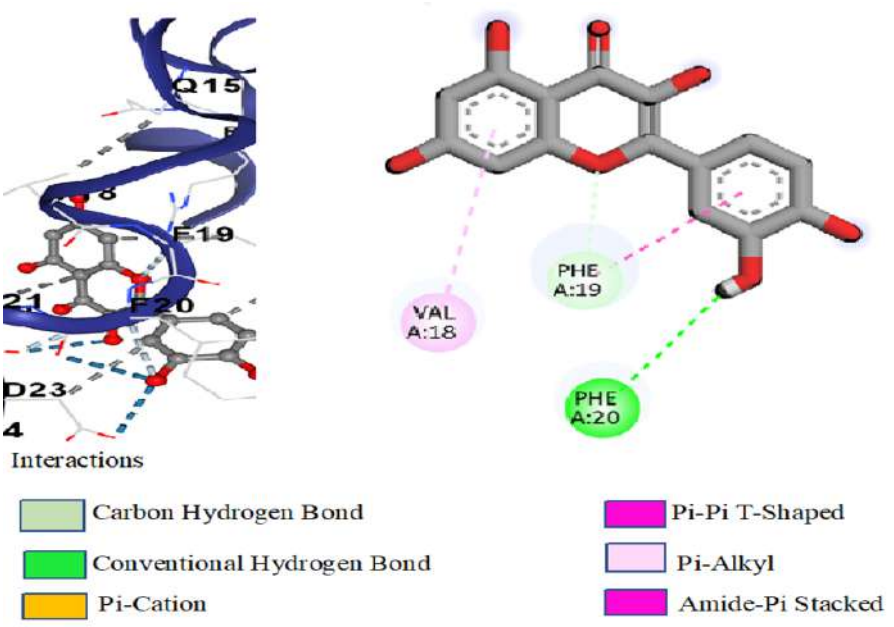


FIGURE 4.30: Docking analysis of Amyloid-beta with Quercetin

Figure 4.30 shows the interaction of Amyloid-beta with Quercetin. It shows that there is one hydrogen bond with phenylalanine (20), one carbon hydrogen bond with Phenylalanine (19) and one alkyl contact with valine(18).

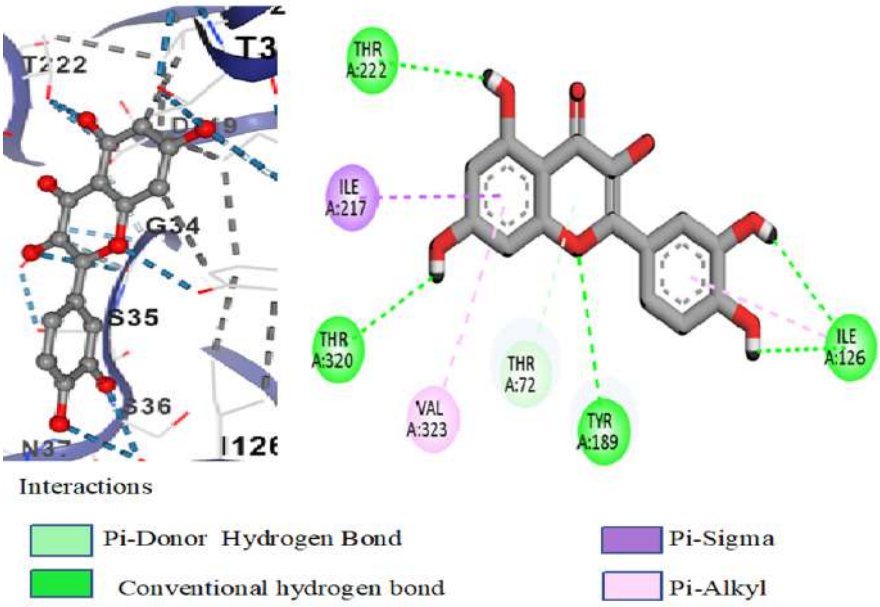


FIGURE 4.31: Docking analysis of BACE1 with quercetin

Figure 4.31 shows the interface of BACE1 with quercetin. It indicates that there is four hydrogen bond and one alkyl interaction. Moreover, there is also one sigma contact.

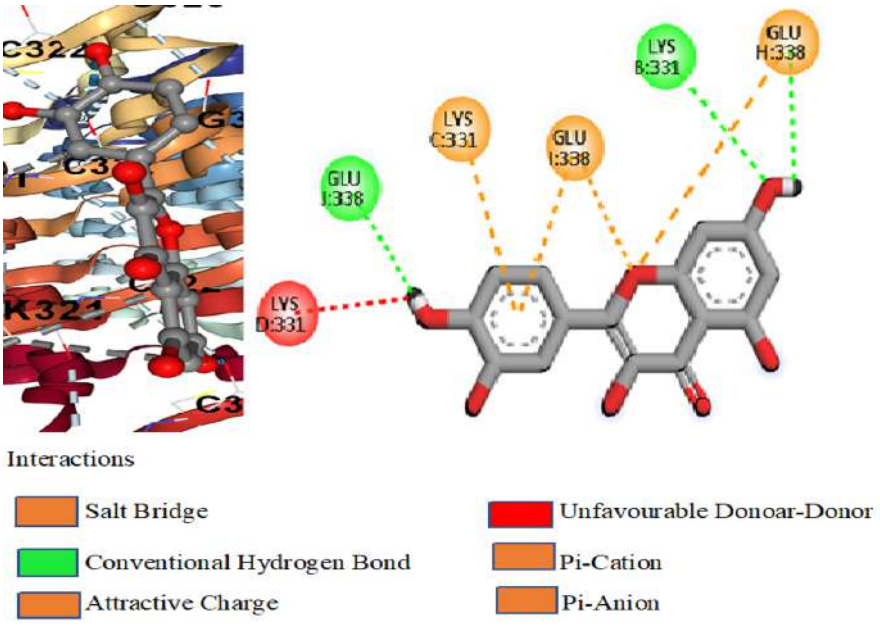


FIGURE 4.32: Docking analysis of Tauproteim with chrysin

Figure 4.32 shows the interaction of Tau proteim with chrysin. It shows that there is two hydrogen bond with glutamic acid (338) and lysine (331).There is also salt bridge and attractive charge.

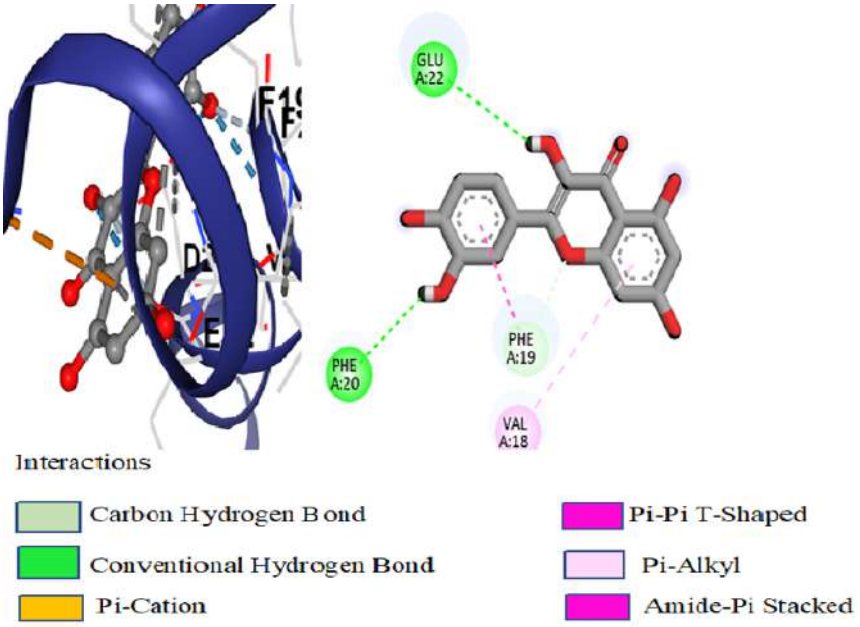


FIGURE 4.33: Docking analysis of Amyloid-beta with chrysin

Figure 4.33 shows the binding of Amyloid-beta with chrysin. It indicated that there is two hydrogen bonds with phenylalanine (20) and glutamic acid (22). Moreover, there is also one alkly contact and one carbon-hydrogen bond.

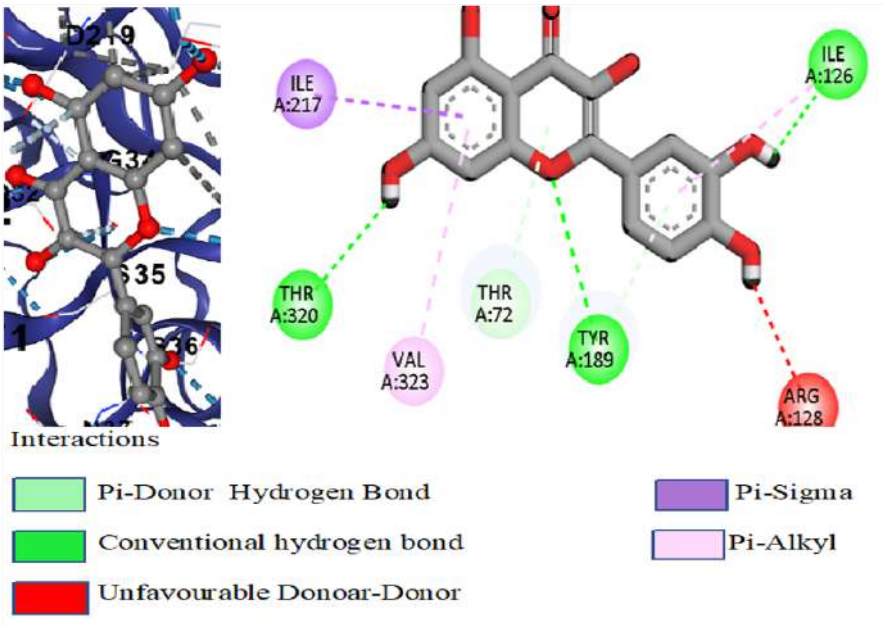


FIGURE 4.34: Docking analysis of BACE1 with chrysin

Figure 4.34 shows the interaction of BACE1 with chrysin. It indicates that there is three hydrogen bonds, one pi donor hydrogen bond and one sigma contact. Moreover, there is also one alkly interaction.

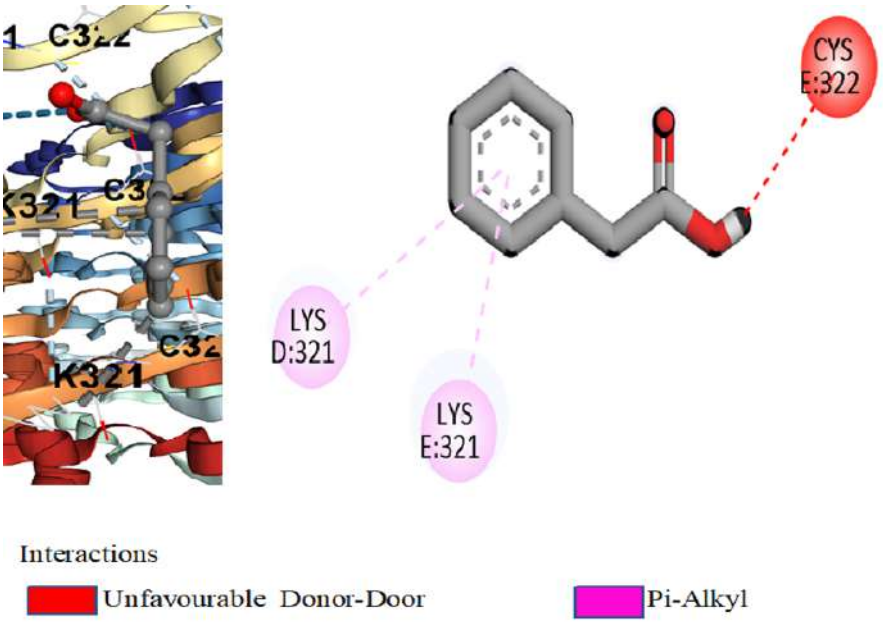


FIGURE 4.35: Docking of Tau protein with phenyl acetic acid

Figure 4.35 shows the binding of Tau protein with phenyl acetic acid. It shows that there is two alkyl interaction with lysine (321) and also have unfavourable donar donar- contact with cysteine.

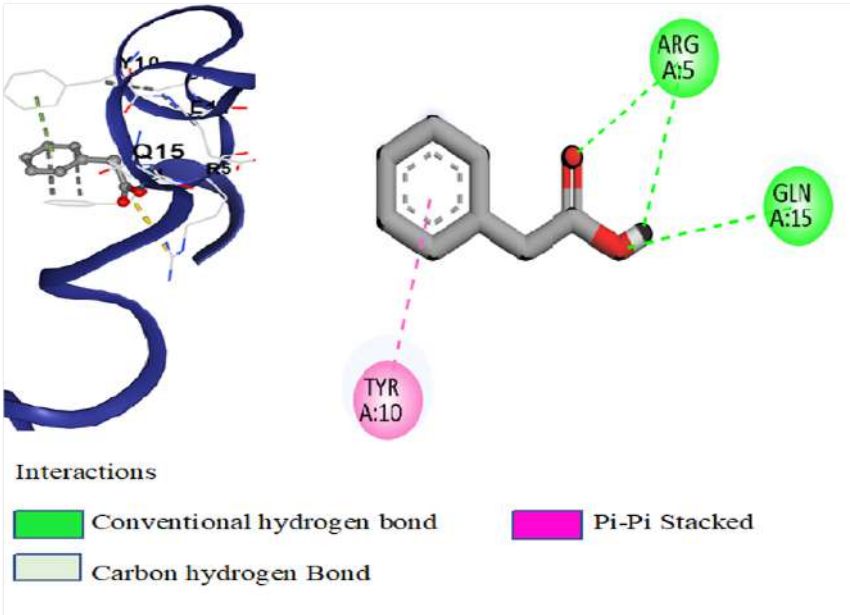


FIGURE 4.36: Docking analysis of Amyloid-beta with phenyl acetic acid

Figure 4.36 indicates the binding of Amyloid-beta with phenyl acetic acid. It shows that there is two hydrogen bond arginina and glutamine.

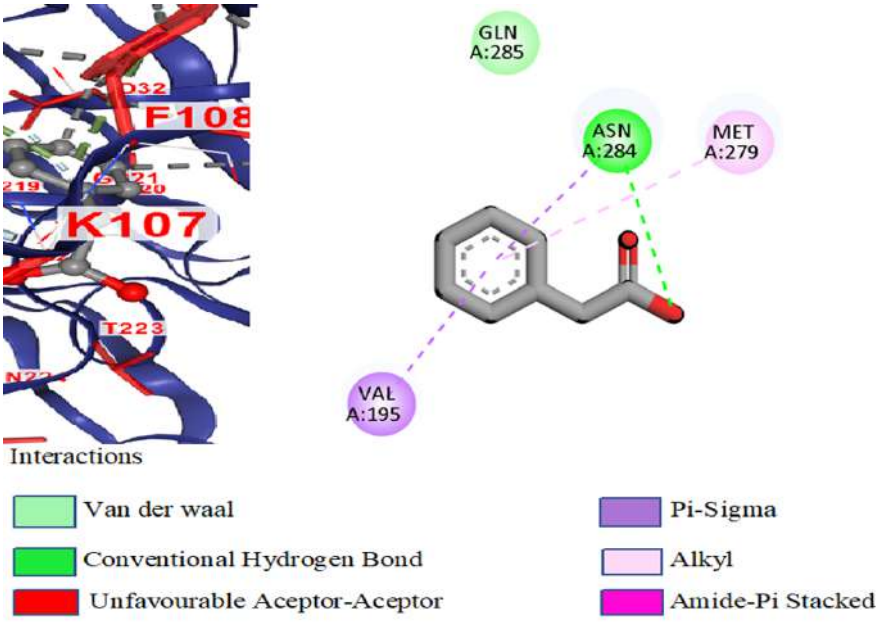


FIGURE 4.37: Docking analysis of BACE1 with phenyl Acetic Acid

Figure 4.37 shows the binding of BACE1 with phenyl Acetic Acid. It shows that there is one van der waal interaction , one hydrogen bond and one sigma bond. Moreover, there is also one alkyl contact.

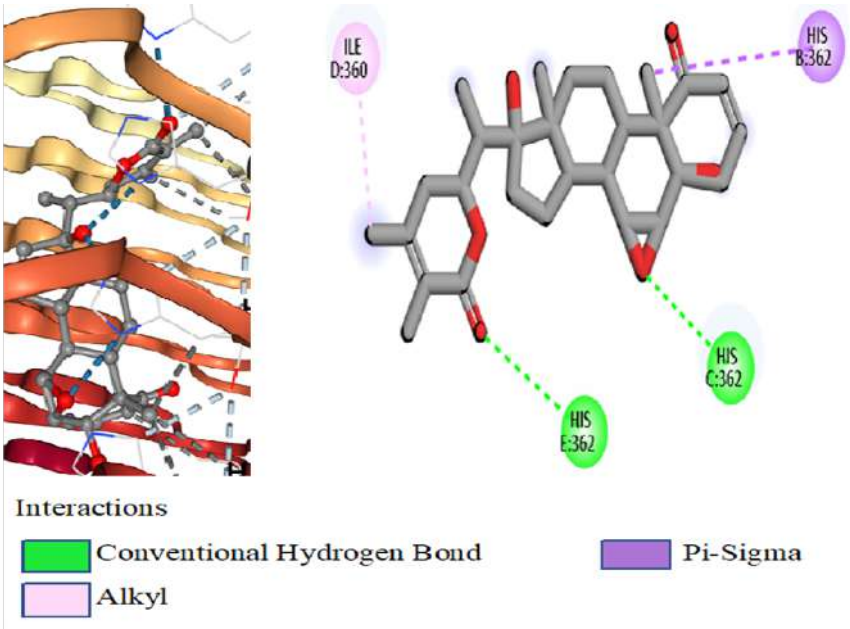


FIGURE 4.38: Docking analysis of Tau protein Withanone

Figure 4.38 shows the interaction of Tau protein Withanone. It indicates that there is two hydrogen bonds with histidine (362) and one alkyl contact. Moreover, there is also one sigma bond.

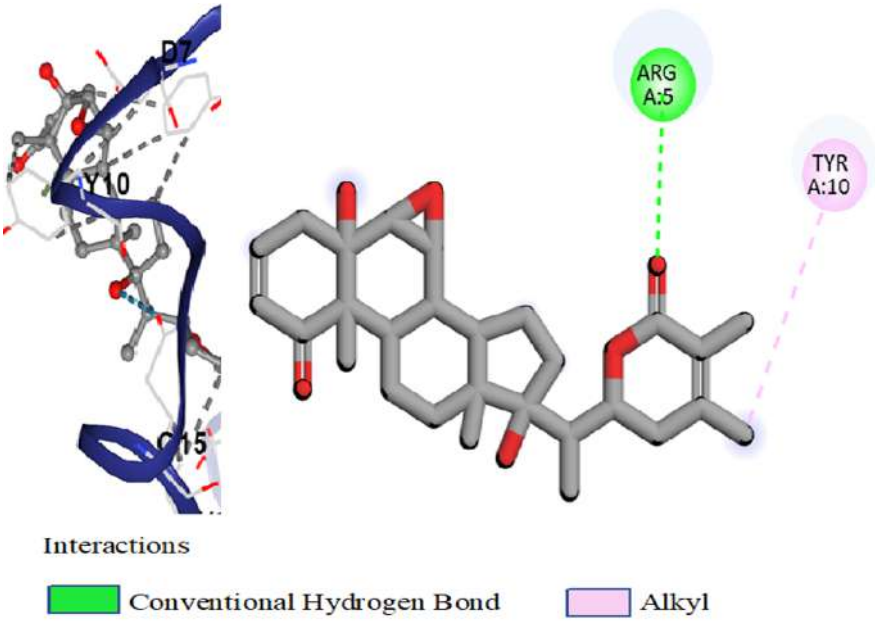


FIGURE 4.39: Docking analysis of Amyloid-beta Withanone

Figure 4.39 shows the binding of Amyloid-beta Withanone. It shows that there is one hydrogen bond with arginine and one alkyl contact with tyrosine.

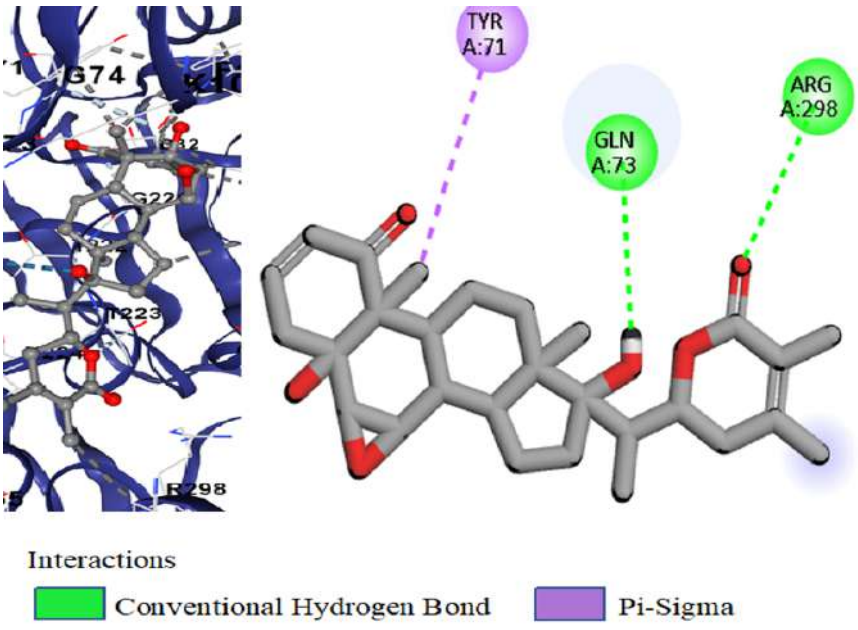


FIGURE 4.40: Docking analysis of BACE1 Withanone

Figure 4.40 shows the interface of BACE1 Withanone. It indicates that there is two hydrogen bonds with glutamine and arginine it also has, one sigma bond.

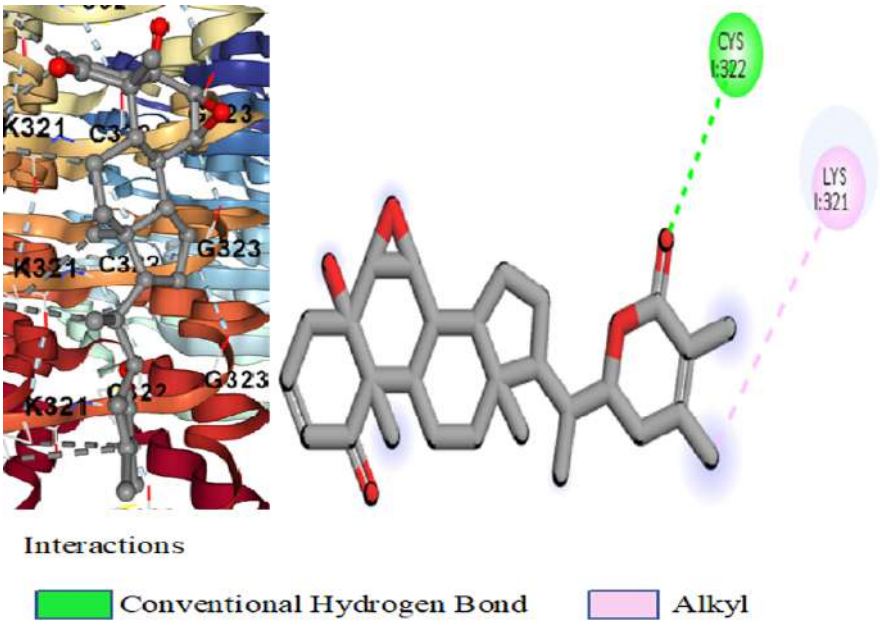


FIGURE 4.41: Docking analysis of Tau protein Withanolide-B

Figure 4.41 shows the binding of Tau protein with with anolidwit.It shows that there is one hydrogen bond with cysteine and one alkyl contact with lysine.

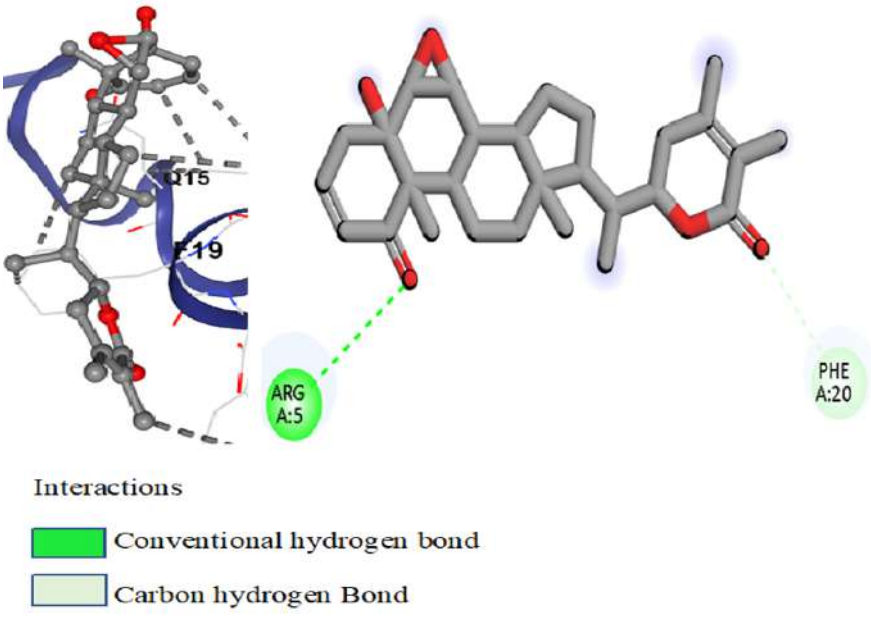


FIGURE 4.42: Docking analysis of Amyloid-beta Withanolide-B

Figure 4.42 shows the interaction of Amyloid-beta Withanolide-B. It shows that there is one hydrogen bond with Arginine and carbon hydrogen bond with Phenylalanine.

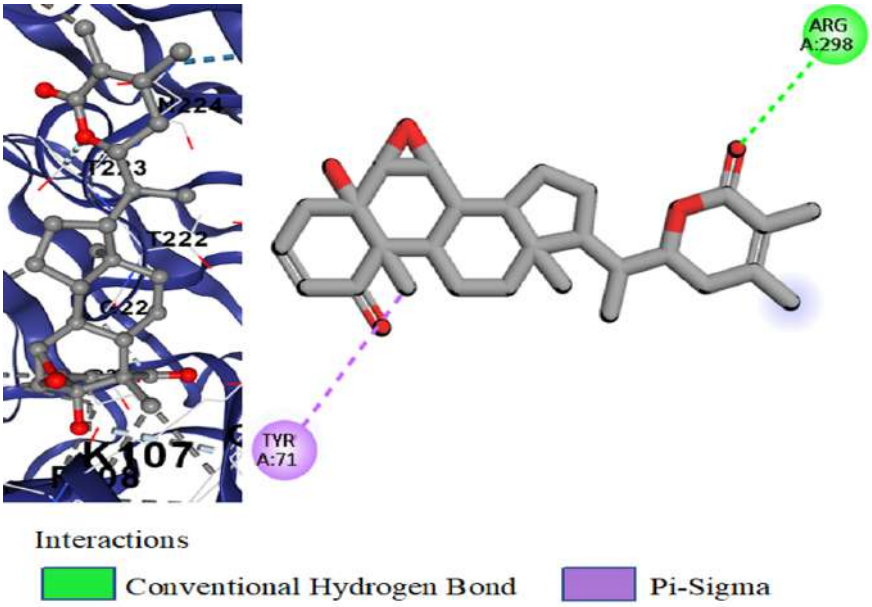


FIGURE 4.43: Docking analysis of BACE1 Withanolide-B

Figure 4.43 shows the interactions of BACE1 Withanolide-B. It indicates that there is one hydrogen bond with the Arginine and one sigma bond with Tyrosine.

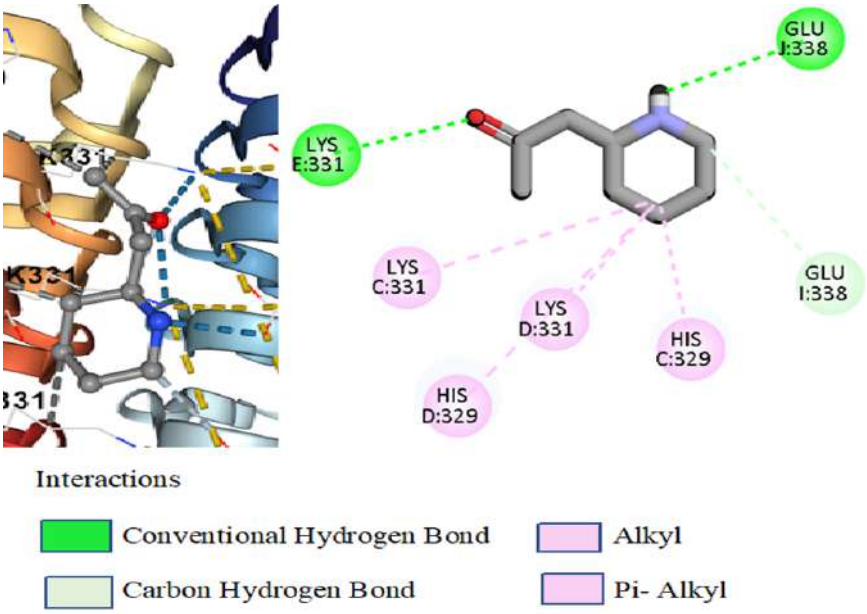


FIGURE 4.44: Docking analysis of Tau protein with Isopelletierine

Figure 4.44 shows the interaction of Tau protein with Isopelletierine. It shows that there is two hydrogen bonds with Lysine and glutamic acid. It also shows four alkyl interactions. Moreover, there is carbon hydrogen bond with glutamic acid.

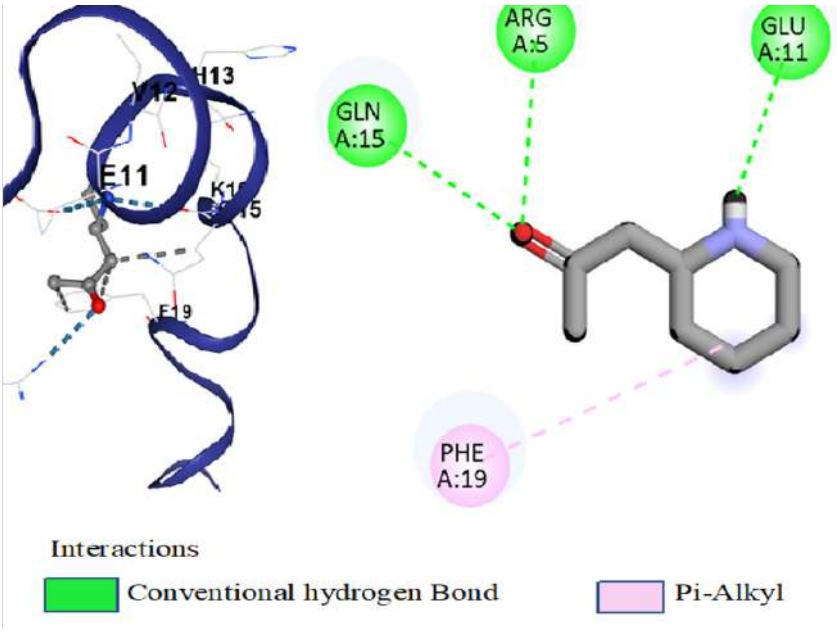


FIGURE 4.45: Docking analysis of Amyloid-beta with Isopelletierine

Figure 4.45 shows the interactions of Amyloid-beta with Isopelletierine. It shows that there is three hydrogen bonds and one alkyl interaction.

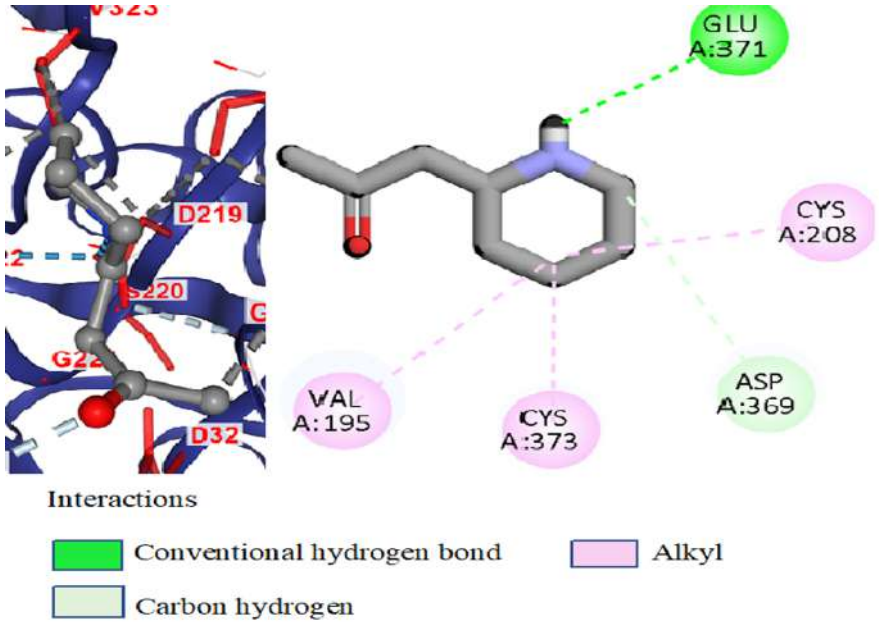


FIGURE 4.46: Docking analysis of BACE1 with Isopelletierine

Figure 4.46 shows the interaction of BACE1 with Isopelletierine. It indicates that there is one hydrogen bond with glutamic acid anand one carbon hydrogen bond with aspartic acid. Moreover, there are three alkyl interactions.

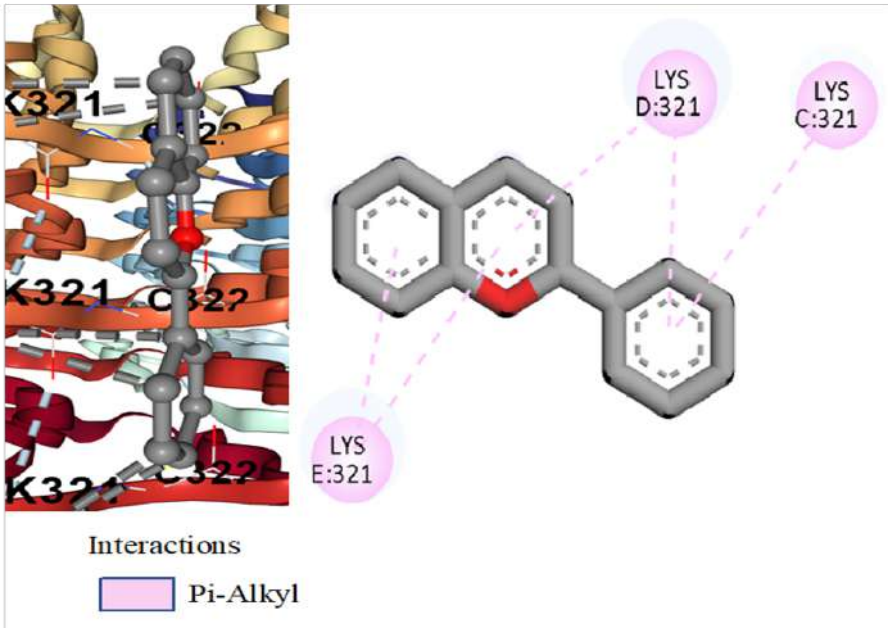


FIGURE 4.47: Docking Analysis of Tau protein with Anthocyanins

Figure 4.47 shows the interactions of Tau protein with Anthocyanins. It indicates that there are three alkyl interactions with Lysine.

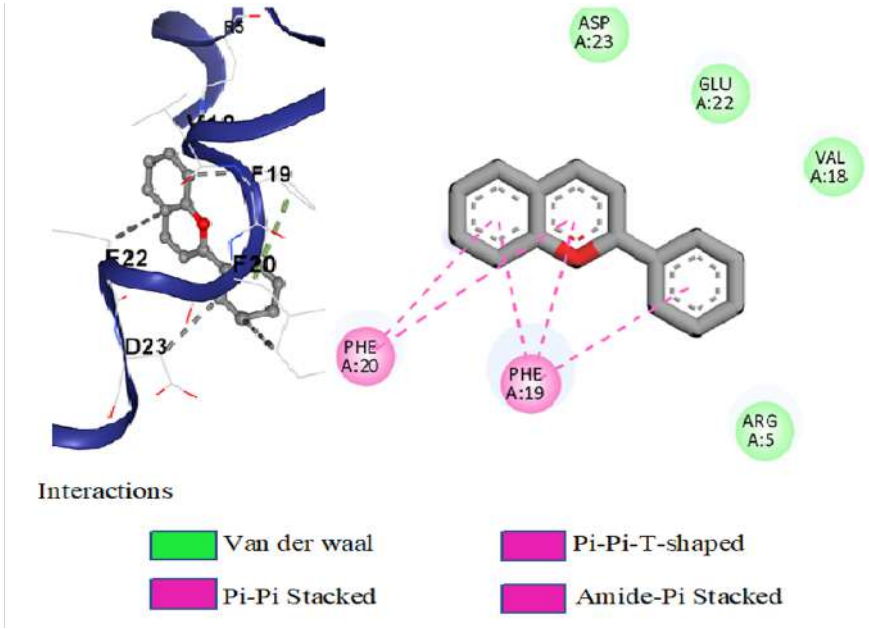


FIGURE 4.48: Docking Analysis of Amyloid-beta with Anthocyanins

Figure 4.48 shows the binding of Amyloid-beta with Anthocyanins. It shows that there is four van der waal interactions with the aspartic acid ,glutamic acid ,valine and arginine.

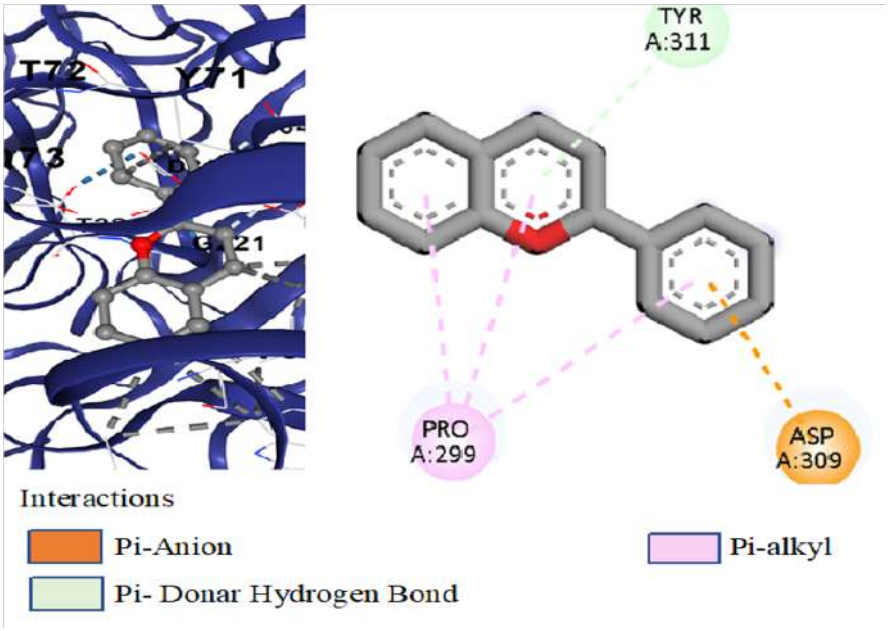


FIGURE 4.49: Docking Analysis of BACE1 with Anthocyanins

Figure 4.49 shows the interaction of BACE1 with Anthocyanins. It shows that there is one donar hydrogen bond with Tyrosine and one Pi-alkyl interaction with Proline.

4.6 Identification of Lead Compound

Their pharmacokinetic and physiochemical properties dictate whether the ligands will be drug or non-drug molecules. Two filters are used for this identification: Lipinski's rule and pharmacokinetics. All of the ligands were selected for docking because they were shown to be in compliance with the Lipinski Rule of Five. After that, there are two further elimination steps: pharmacokinetic screening and docking score. Withaferin-A, Withanolide-L, and Withanolide-D were selected as the lead compounds in this screening due to their favorable docking and ADMET profiles. A binding affinity of -8.3 and -9.2 kcal/mol, respectively, for BACE1 and tau, was most consistently and strongly exhibited by Withaferin-A. There is promise as multi-target inhibitors for Withaferin-A and Withanolide-L, as they demonstrated consistent binding across Tau and BACE1 with scores ranging from -8.1 to -9.2 kcal/mol. There was moderate systemic diffusion and mild toxicity

across the three compounds. Important active site residues with which they formed numerous hydrogen bonds and van der Waals interactions further contributed to their stability with in the binding pockets. These compounds still have room for improvement in terms of BBB and CNS permeability, but they are still good candidates for further study.

4.7 Identification of Ant-Alzheimer Drug

Due to its proven effectiveness as an acetylcholinesterase inhibitor approved for the treatment of Alzheimer's disease, donepezil was chosen as the reference drug for comparison with the lead compounds. Through interactions with important residues like Tyr155 and Trp317, it demonstrates a significant binding affinity to the AChE active site, creating hydrophobic contacts and hydrogen bonds that stabilise the ligand-enzyme complex.

Donepezil is a standard in molecular docking investigations, with reported binding affinities of about -8.1 kcal/mol. In addition to inhibiting AChE, donepezil has demonstrated action against BACE1 and Aβ42, lowering their levels in vivo by 57.5% and 0.5%, respectively. The binding modalities, interaction patterns, and anticipated potency of the newly screened compounds can be directly compared by using donepezil as a reference [112].

4.8 Donepezil and Lead Compounds Comparison

The reference medicine donepezil was compared to four lead compounds Withaferin-A, Withanolide-L, and Withanolide-D to find the best bioactive metabolite for controlling disease-related pathways and the best treatment for the illness. Some of the metrics utilized for comparison were ADMET features, Lipinski's rule of five, and molecular docking analysis.

4.8.1 Donepezil Structure Prediction

The donepezil structure was initially obtained from PubChem in SDF format. Afterwards, the refined structure was obtained by minimizing its energy using chem3D pro. Donepezil has the molecular formula $C_{24}H_{29}NO_3$ and its crystal structure is shown in Figure 4.50.

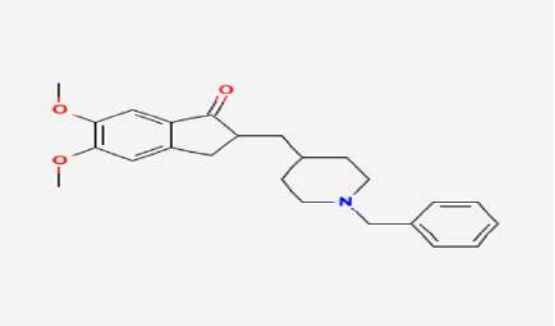


FIGURE 4.50: Structure of donepezil

4.8.2 The Way Donepezil Works in Alzheimer's

Aβ plaques in Alzheimer's disease contribute to neuroinflammation and the release of TNF-α and IL-1β, which trigger apoptosis through cleaved caspase-3 and PARP, resulting in the death of neurones and cognitive impairment.Donepezil decreases inflammatory cytokines, boosts microcurrent and GSK-3β Ser9 phosphorylation, and inhibits AChE. This improves cognitive function and reduces apoptosis.

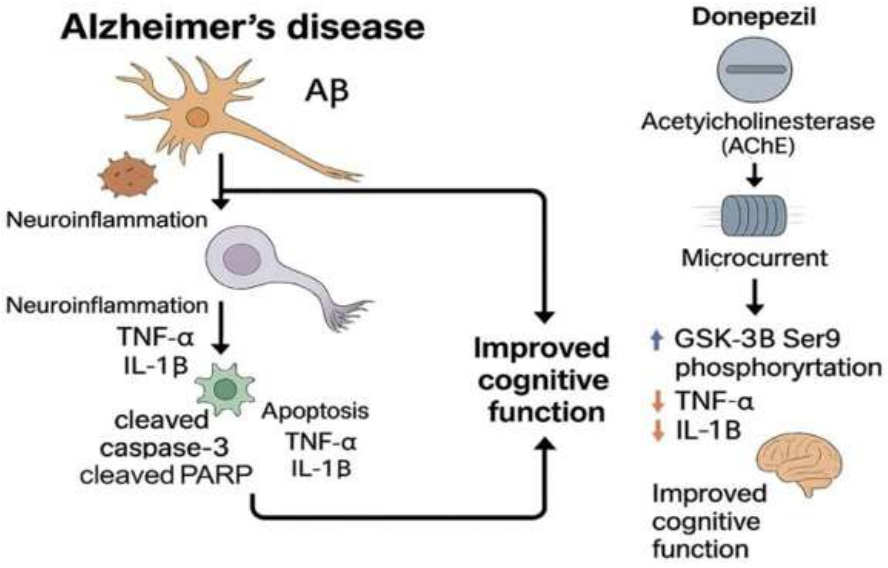


FIGURE 4.51: This image illustrated how Donepezil lowers apoptosis and neuroinflammation in Alzheimer's patients [121].

4.9 Lipinski Rule Comparison

In order to compare donepezil and Withaferin-A, Withanolide-L, and Withanolide-D, we looked at their pharmacokinetic characteristics, bioavailability, safety, effectiveness, and drug-likeness, as well as their reaction to the lipinski rule. There is a comparison provided in table 4.11.

TABLE 4.11: Lipinski Rule Comparison

Ligands	**Log P Value**	**Molecular Weight**	**H-bond Donor**	**H-bond Acceptor**	**Rotatable Bonds**
Donepezil	4.7829	415.961	0	4	6
Withaferin-A	3.3529	470.606	2	6	3
Withanolide-L	4.3484	452.591	2	5	2
Withanolide-D	3.4954	470.606	6	2	6

4.10 Comparison of ADMET Properties

By evaluating the medication's and the lead chemical's ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties, a superior pharmacological candidate could be identified.

4.10.1 The Comparison of Absorption Properties

The table 4.12 compares donepezil to Withaferin-A, Withanolide-L and Withanolide - D in order to verify absorbance models.

TABLE 4.12: Comparison of Absorption Properties

ADMET Properties		**Donepezil**	**Withaferin-A**	**Withanolide-L**	**Withanolide-D**
Absorption	Water solubility	-5.133	-5.063	-5.458	-5.127
	Caco2 permeability	1.27	0.829	0.866	0.831
	Intestinal absorption (human)	92.669	85.345	95.871	99.2

Table 4.12 continued from previous page

ADMET Properties	Donepezil	Withaferin-A	Withanolide-L	Withanolide-D
Skin Permeability	-2.597	-3.202	-3.969	-3.267
P-glycoprotein substrate	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor	Yes	Yes	Yes	Yes
P-glycoprotein II inhibitor	Yes	Yes	Yes	Yes

Table 4.12 shows that absorption values for bothdonepeziland Withaferin-A, Withanolide - L, and Withanolide-D arein range.The three with anolides Withaferin-A, Withanolide-L, and Withanolide-Dhave absorption patterns that are similar to, and occasionally superior to donepezil, according to the ADMET absorption data. Notably, donepezil's 92.6% anticipated human intestine absorption is outperformed by Withanolide-D, which show 99.2% predicted absorption. In addition to having the same P-glycoprotein substrate and inhibitor profiles as donepezil, which suggests comparable efflux and interaction behaviour, all ligands have moderate Caco2 permeability in the 0.82–0.87 range, which supports their intestinal absorption. The greater intestinal absorption of Withanolide-D and Withanolide-Lindicates that they are prospective oral medication candidates with absorption characteristics suited for further development as donepezil-like medicines, despite donepezil's increased water solubility and skin permeability.

4.10.2 Comparison of Distribution Properties

The table 4.13 compares donepezil to Withaferin-A, Withanolide-L, and Withanolide - D in order to verify distribution models

TABLE 4.13: Comparison of Distribution Properties

ADMET Properties		Donepezil	Withaferin - A	Withanolide - L	Withanolide - D
Distribution	VDss (human)	1.242	-0.131	0.141	-0.048
	Fraction unbound (human)	0	0.105	0.086	0.093
	BBB permeability	0.153	-0.03	-0.159	-0.315

Table 4.13 continued from previous page

ADMET Properties	Donepezil	Withaferin-A	Withanolide-L	Withanolide-D
CNS permeability	-1.451	-2.72	-1.887	-2.696

The distribution characteristics of donepezil and three with anolides from with ania somnifera are contrasted in the table 4.13. Donepezil's significant CNS penetration is explained by its increased volume of distribution and positive BBB permeability. While the VDss and BBB permeability values of the with anolides are lower, their proportion unbound in plasma ranges from 0.086 to 0.105, which is significantly greater than donepezil's 0, indicating that more of the molecule is free and pharmacologically active in circulation. Crucially, although Withaferin-A, Withanolide-L, and Withanolide-D do not directly match donepezil, their BBB permeability values fall on the borderline, suggesting that these drugs can still be taken into consideration for CNS activity. Withanolide-L is the best option for CNS-targeted action in this series since it has the lowest negative CNS permeability score.These with anolides exhibit favourable plasma availability and dispersion characteristics, making them attractive candidates for additional CNS delivery optimisation.

4.10.3 Comparison of Metabolism Properties

The table 4.14 compares donepezil to Withaferin-A, Withanolide-L, and Withanolide - D in order to verify metabolism models.

TABLE 4.14: Comparison of Metabolism Properties

ADMET Properties		Donepezil	Withaferin - A	Withanolide - L	Withanolide - D
Metabolism	CYP2D6 substrate	Yes	No	No	No
	CYP3A4 substrate	Yes	YES	Yes	Yes
	CYP1A2 inhibitior	No	No	No	No
	CYP2C19 inhibitior	No	No	No	No
	CYP2C9 inhibitior	No	No	No	No

Table 4.14 continued from previous page

ADMET Properties	Donepezil	Withaferin-A	Withanolide-L	Withanolide-D
CYP2D6 inhibitior	Yes	No	No	No
CYP3A4 inhibitior	Yes	No	No	No

Drug-drug interactions are more likely since donepezil is both a substrate and an inhibitor of CYP2D6 and CYP3A4, according to the metabolism data. The three with anolides, on the other hand, Withaferin-A, Withanolide-L, and Withanolide-D, are substrates of CYP3A4 but do not inhibit any of the major CYP enzymes examined, such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Despite depending on CYP3A4 for clearance, this indicates that the with anolides have a reduced risk of metabolic interactions than donepezil, making them more advantageous from a means of protection and drug-interaction perspective.

4.10.4 Comparison of Excretion Properties

The table 4.15 compares donepezil to Withaferin-A, Withanolide-L, and Withanolide - D in order to verify excretionmodels.

Table 4.15: Comparison of Excretion Properties

ADMET Properties		Donepezil	Withaferin - A	Withanolide - L	Withanolide - D
Excretion	Total Clearance	1.235	0.435	0.438	0.347
	Renal OCT2 substrate	Yes	Yes	Yes	No

In comparison to the three with anolides, which vary from 0.347 to 0.438, donepezil had the highest total clearance (1.235), indicating quicker elimination, according to the excretion data. While Withanolide-D is not an OCT2 substrate and has the lowest clearance at 0.347, indicating a larger dependence on non-renal routes and thus a longer half-life, donepezil, Withaferin-A, and Withanolide-L are expected OCT2 substrates and hence undergo active renal secretion. Withanolide-D is the

best option among the with anolides because of its minimal renal OCT2 involvement. Overall, the with anolides are better for excretion than donepezil, with lesser clearance and a decreased likelihood of kidney-related medication interactions.

4.10.5 Comparison of Toxicity Properties

The table 4.16 compares donepezil to Withaferin-A, Withanolide-L, and Withanolide - D in order to verifytoxicity models.

TABLE 4.16: Comparison ofToxicity Properties

ADMET Properties		**Donepezil**	**Withaferin - A**	**Withanolide - L**	**Withanolide - D**
Toxicity	AMES toxicity	No	No	No	No
	Max. tolerated dose (human)	-0.236	-0.695	-0.952	-0.867
	hERG I inhibitor	No	No	No	No
	hERG II inhibitor	Yes	No	No	No
	Oral Rat Acute Toxicity (LD50)	2.888	2.779	2.13	2.831
	Oral Rat Chronic Toxicity (LOAEL)	1.989	0.918	1.899	1.776
	Hepatotoxicity	Yes	No	No	No
	Skin Sensitisation	No	No	No	No
	T. pyriformis toxicity	0.78	0.299	0.36	0.305
	Minnow toxicity	-2.329	0.738	0.444	1.178

Three with anolides have a better safety profile than donepezil, according to the toxicity data. While donepezil is a hERG II inhibitor and a hepotoxicity, both of which raise concerns about cardiotoxicity and allergic reactions, all other drugs, including donepezil, are negative for AMES toxicity and and hERG I inhibition. On the other hand, none of the with anolides exhibit cutaneous sensitisation or hERG II inhibition. The with anolides exhibit similar or slightly higher LD50 and LOAEL values than donepezil for both acute and chronic oral toxicity, indicating

equivalent or lower acute toxicity. Furthermore, Withanolide-L and Withaferin-A exhibit significantly reduced toxicity against *T. pyriformis* and minnow, suggesting improved environmental safety. With anolides are more desirable for continued development since they are safer than donepezil overall across important toxicity endpoints.

4.11 Comparison of Docking Scores

We determined the binding value by docking the lead and reference compounds with the target proteins Tau, amyloid-beta, and BACE1. An analysis of the docking scores between the lead compound Withaferin-A, Withanolide-L, and Withanolide-D and the standard medicine donepezil is shown in table 4.17

Table 4.17: Comparison of Docking Scores

Target Proteins	**Ligands Withaferin-A**	**Ligands Withanolide-L**	**Ligands Withanolide-D**	**Donepezil**
Tau	-8.3	-8.1	-8.1	-6.9
Amyloid-beta	-7.4	-7.5	-7.6	-6.7
BACE1	-9.2	-9.2	-9.1	-3.7

All three of the with anolide-based ligands have better binding affinities than the reference medication donepezil across the three target proteins, according to the docking analysis. Withaferin-A exhibits the greatest binding to Tau protein at -8.3 kcal/mol, closely followed by Withanolide-L and Withanolide-D at -8.1 kcal/mol, and donepezil at -6.9 kcal/mol. Withanolide-D exhibits the highest affinity against amyloid-beta at -7.6 kcal/mol, while Withanolide-L and Withaferin-A both outperform donepezil at -7.5 and -7.4 kcal/mol, respectively, as opposed to donepezil's -6.7 kcal/mol . Withaferin-A and Withanolide-L also score -9.2 kcal/mol.Overall, the findings show that these Withanolide-Derivativesespecially Withaferin-A for Tau BACE1 have better projected binding potential than donepezil, indicating that they would be viable lead molecules for more research.

4.12 Comparison of Docking Analysis

Discovery Studio assessed the docking data based on the degree of hydrogen, alkyl, and van der Waals bonding. The docking analysis of donepezil is displayed in the following figures.

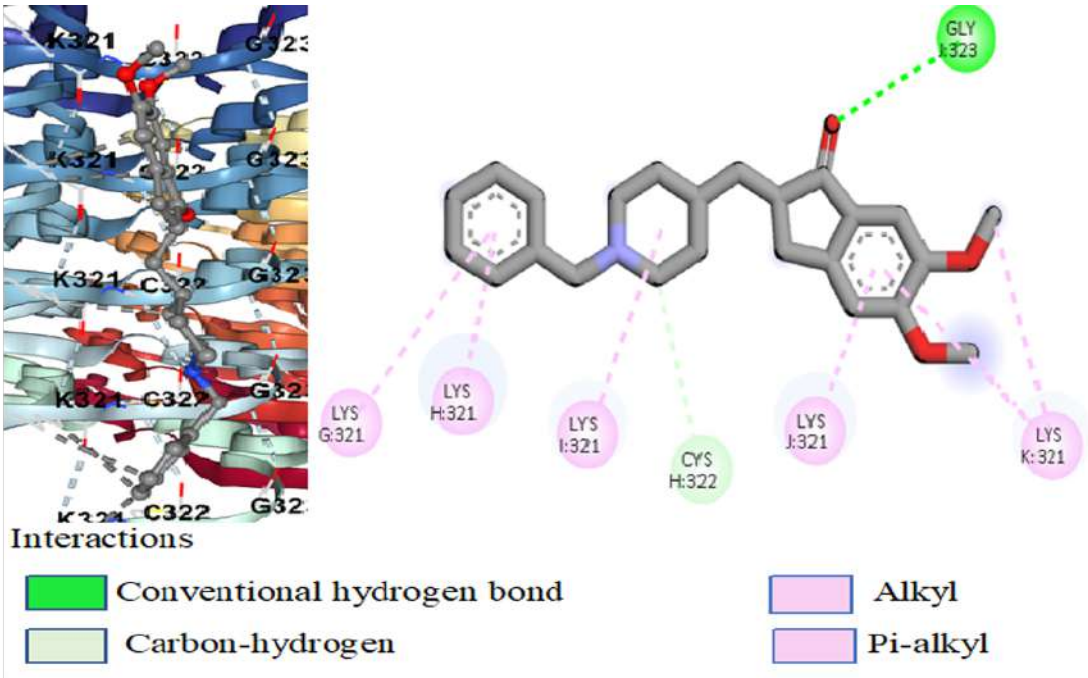


FIGURE 4.52: Docking Analysis of Tau protein with donepezil

Alkyl interactions, hydrogen bonds, and carbon-hydrogen bonds were discovered when the reference chemical donepezil was docked with the Tau protein. Different patterns of interaction were seen when compared with lead compounds. Alkyl connections, Pi-sigma interactions, and conventional hydrogen bonds were all created by Withanone. Like donepezil, Withaferin-A exhibited alkyl interactions, carbon-hydrogen bonds, and typical hydrogen bonds together with extra hydrophobic contacts. Withanolide-D only showed two interactions: the Pi-alkyl interaction and the typical hydrogen bond. On the other hand, with four interactions a normal hydrogen bond, a Pi-donor hydrogen bond, a Pi-sigma bond, and a Pi-alkyl interaction Withanolide-L showed the most varied binding profile. Overall, lead compounds, especially Withanolide-L, exhibit broader and more diverse interactions, indicating greater and more stable binding potential with target protein, whereas donepezil mostly relies on hydrogen and alkyl connections.

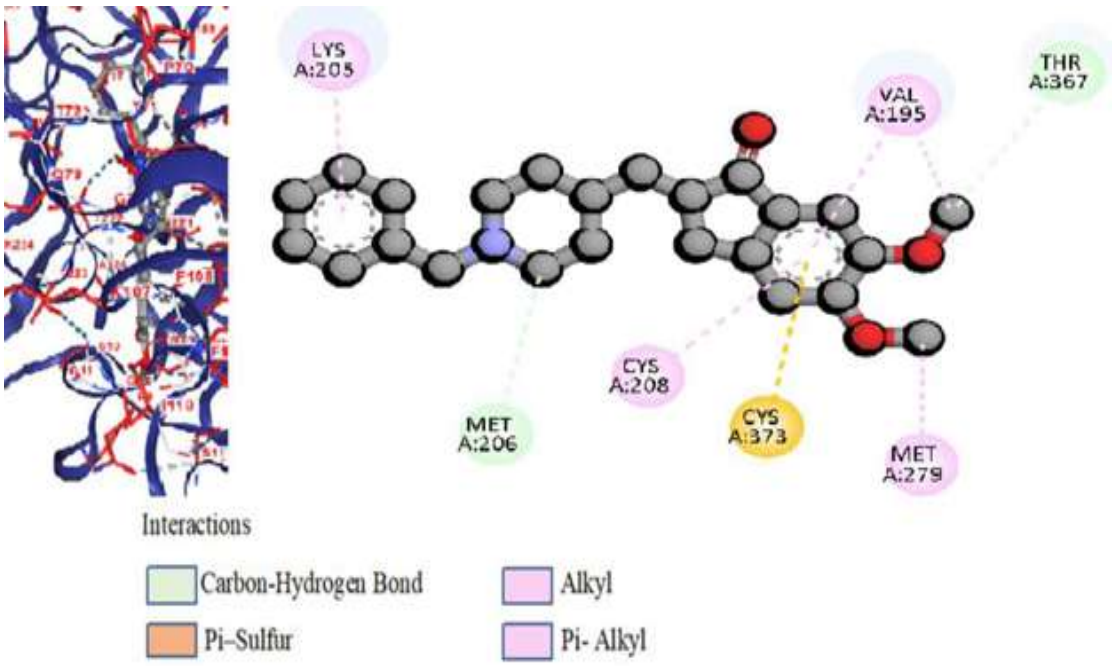


FIGURE 4.53: Docking Analysis of Amyloid-beta with donepezil

Conventional hydrogen bonds, carbon-hydrogen bonds, alkyl interactions, and pi-pi stacked interactions were all observed in the interactions of the reference chemical donepezil with amyloid beta. Three interactions were seen in Withaferin-A: an alkyl interaction, a carbon-hydrogen link, and a typical hydrogen bond. Withanolide-L demonstrated the most varied binding pattern with a conventional hydrogen bond, a pi-sigma bond, and a pi-alkyl interaction, whereas Withanolide-D displayed only one conventional hydrogen bond interaction. All things considered, the lead compound sparticularly Withanolide-L display similar or wider interaction patterns in comparison to the reference drug, indicating advantageous binding with the target.

The reference chemical donepezil's docking with BACE1 revealed an alkyl interaction, a pi–pi stacking interaction, a carbon–hydrogen link, and a typical hydrogen bond at the active site. Van der Waals and alkyl contacts were the two interactions that Withanolide-L also displayed. Withanolide-D showed both a carbon-hydrogen bond and a traditional hydrogen bond. Three interactions were shown by Withaferin-A: an unfavourable acceptor-acceptor contact, a carbon-hydrogen link, and a typical hydrogen bond. Overall, the lead compounds displayed similar

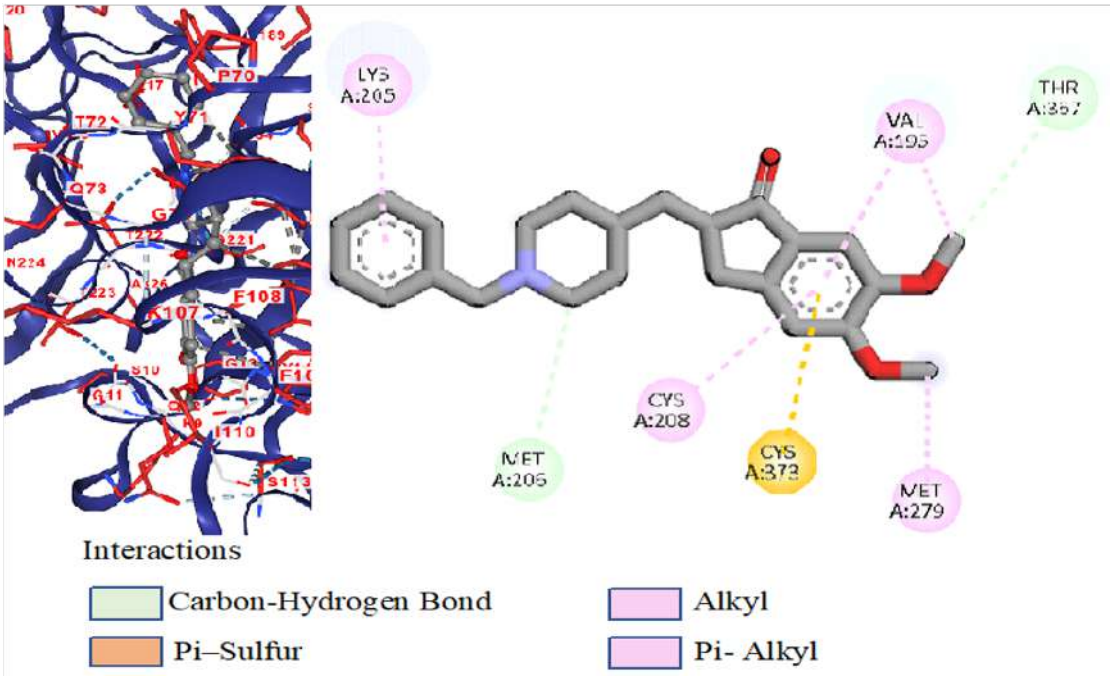


FIGURE 4.54: Docking Analysis of BACE1 with donepezil

hydrogen bonding and hydrophobic contacts, with aferin-A having the most varied interaction profile despite one unfavourable contact, whereas donepezil had a wider range of interactions, including pi–pi stacking.

Chapter 5

Discussion

The current study uses an in-silico method that combines molecular docking with ADMET profiling to examine the inhibitory potential of *Withania somnifera* metabolites, particularly withanolides, against Alzheimer's disease targets. Because of increasing neuronal loss, amyloid-β aggregation, tau hyperphosphorylation, oxidative stress, and neuroinflammation, Alzheimer's disease is the primary cause of dementia globally and continues to be a significant public health concern. Its complex aetiology includes epigenetic modifications, long-term inflammation, environmental factors, and genetic mutations in the APP , PSEN1, and PSEN2 genes, which result in early-onset autosomal dominant AD. The biggest genetic risk factor for late-onset AD is the APOE gene, where the ε4 allele increases risk and the ε2 allele protect [122].

The need for safe, economical, and efficient substitutes has increased due to the drawbacks of traditional treatments, such as high toxicity, single-target methods, and little clinical alleviation. Plant-derived bioactives have special benefits in this situation, such as the capacity to pass the blood-brain barrier, decreased side-effect profiles, and multi-target efficacy.Withanolides and withanosides, which have been linked to neuroprotection and cognitive improvement, are abundant in *Withania somnifera* (Ashwagandha). The antioxidant, anti-inflammatory, and anti-amyloid properties of *W. somnifera* phytochemicals, specifically withanolide A, withaferin

A, withanone, and withanoside IV, have been reported in earlier studies. For example, in APP/PS1 transgenic mice, withanolide A and withanoside IV increase neurite outgrowth and decrease Aβ burden. It has been demonstrated that withaferin A and withanone prevent BACE1 and Aβ aggregation, two crucial stages in amyloid[123].Similarly, extracts from *W. somnifera* reduce oxidative stress and Aβ plaque deposition in the hippocampus by upregulating Nrf2 and NAD(P)H quinone oxidoreductase 1 [124].

Beyond amyloid disease, withanolides influence neuroinflammation by inhibiting NF-κB, mitochondrial biogenesis via BDNF/SIRT1 signalling, and tau-related pathways. Additionally, they increase the production of liver LRP, which aids in the peripheral removal of Aβ and corrects behavioural abnormalities in AD animal models. The intricate pathophysiology of AD, in which oxidative stress, neuroinflammation, and protein aggregation work in concert, is consistent with these multi-target processes [125].

Withanolide-based ligands are therefore attractive multi-target options for the treatment of AD. Their potential as disease-modifying drugs is supported by their capacity to concurrently address oxidative damage, tau phosphorylation, Aβ buildup, and neuroinflammation, which calls for additional preclinical and clinical confirmation.The ability of *Withania somnifera* metabolites to bind to three targets associated with Alzheimer's disease tau protein, amyloid-beta, and BACE1 was assessed using molecular docking analysis. Strong binding affinities were shown by withaferin A, withanolide D, and withanolide L among the ligands examined. Withanone showed the highest binding score and the most favourable interaction with BACE1, suggesting substantial inhibitory potential against this important enzyme involved in amyloid-beta formation. These findings are in line with earlier research demonstrating that withanolides from *Withania somnifera* decrease amyloid-beta aggregation and inhibit BACE1 activity in both in vitro and transgenic AD mice models [125]. All three proteins had significant binding energies for the other compounds, withaferin A, withanolide D, and withanolide L, indicating their potential to inhibit multiple targets.These results are consistent with reports that by improving Aβ clearance and lowering oxidative stress, *W.*

somnifera extracts correct behavioural impairments and plaque formation. All of these findings point to the potential of withanolides produced from *Withania somnifera* as neuroprotective treatments for Alzheimer's disease, which calls for additional in vitro and in vivo validation.

Significantly, this study's ADMET analysis showed that a number of withanolides from *Withania somnifera* have advantageous pharmacokinetic characteristics, especially withanolide D, withanolide L, and withaferin-A. These compounds had a better docking profile against BACE1, Tau protein and amyloid-beta but their drug-likeness was similar to that of the typical anti-Alzheimer's agent employed as a reference compound. This is consistent with observations that withanolides have good liver permeability and strong neuroprotective and anti-amyloid action against Alzheimer's disease models in vitro. Furthermore, the idea of "polypharmacology" in neurodegeneration where simultaneous regulation of many pathways might improve outcomes and slow the progression of the disease is supported by the multi-target nature of these drugs [126].

The calculated water solubility logS of *Withania somnifera* is approximately -5, which is less than the generally accepted limit for acceptable drug-like solubility, which is logS $>$ -2. Withanolides are C28 steroidal lactones with a large, rigid, extremely lipophilic ergostane skeleton and very few polar functional groups in comparison to their molecular weight of 450-500 Da. This is the primary cause of their low solubility. Because withanolides have strong intermolecular packing and a restricted capacity for hydrogen bonding, their lattice energy is high and their hydration energy is low. Aqueous solubility depends on the balance between the energy needed to break the crystal lattice and the energy acquired from hydrating molecules.

According to a quantitative structure-property model, aqueous solubility decreases dramatically with increasing logP and molecular weight because these factors decrease interactions with water and raise the entropic cost of creating a cavity in the solvent. Withanolides precisely suit this trend.According to Lipinski's Rule of Five research, substances with logS ¡ -4 typically have limited absorption due

to solubility; hence, a value of -5 implies low dissolving rate, unpredictable oral bioavailability, and possible formulation difficulties [127].

Since each unit decrease in logP usually improves logS by about 0.8 log units, you can either modify the scaffold medicinally by adding hydrogen bond donors or acceptors like hydroxyl, carboxylic acid, sulfonamide, or ionisable amine groups, lowering the number of aromatic rings, and introducing sp3 character to disrupt planarity.

If structural changes impair activity, you can also employ formulation strategies. Bypassing the crystalline lattice energy barrier, amorphous solid dispersions, nanosuspensions, cyclodextrin inclusion complexes, self-emulsifying drug delivery systems, and phospholipid-based phytosomes are proven methods that can boost apparent solubility and dissolution rate by 10 to 1000 times. Numerous studies on withanolide nanoemulsions and herbosomes have already shown improved bioavailability.

Because of the lipophilic steroidal core with strong crystal packing, your -5 value is lower than -2 both mathematically and biopharmaceutically. The practical solution is to either increase the polarity of the molecule or use sophisticated delivery systems to improve dissolution and absorption.

Promising multi-target leads for Alzheimer's disease, Withaferin-A, Withanolide D, and Withanolide L from *Withania somnifera* demonstrated good docking scores against tau protein, amyloid-β, and BACE1. Nevertheless, all three drugs had low brain penetration values according to in silico ADMET projections. Withaferin-A, Withanolide D, and Withanolide L were predicted to have blood-brain barrier scores of -0.03, -0.315, and -0.159, respectively. Withaferin-A, Withanolide D, and Withanolide L had even lower CNS penetration scores of -2.72, -2.696, and -1.887, respectively. Compounds are projected to remain largely in blood and not reach brain targets in relevant levels in these models because negative BBB and CNS values indicate limited ability to pass into the brain.There are multiple overlapping barriers that contribute to this inadequate brain exposure, but each one also offers ways to enhance compound [128, 129].

The first challenge is the size and stiffness of molecules. Withanolides are C28 steroidal lactones that have molecular weights between 470 and 490 Da, which is near the Lipinski limit of 500 Da. Because brain blood vessels have tight connections, the blood-brain barrier is far more stringent than stomach absorption. Passive diffusion decreases dramatically above around 450 Da unless the molecule is extremely lipophilic. Additionally, the molecule finds it difficult to get through the fatty membrane due to the inflexible fused-ring arrangement, which increases the amount of energy required. Prodrug design can be used to address this. The molecule becomes more lipophilic and can more readily cross the blood-brain barrier by temporarily hiding the OH groups at C4 and C27 with ester or carbamate groups. After entering the brain, additional groups are eliminated by brain enzymes, releasing the original active withanolide. For other polar natural compounds with low BBB penetration, this "Trojan horse" strategy has been effective [128, 130].

High polarity is the second barrier. Because Withaferin-A, Withanolide D, and Withanolide L contain a large number of OH and carbonyl groups, their polar surface area exceeds 90-110 Å^2. PSA below 70-90 Å^2 and $\geq$3 hydrogen bond donors are often required for brain medicines that cross via passive diffusion. A molecule with a high PSA retains water firmly and requires more energy to get across the membrane of a fatty brain cell. Because of this, the CNS scores are quite negative: -2.72 for Withaferin-A, -2.696 for Withanolide D, and -1.887 for Withanolide L. Small structural adjustments can help correct this. Small oily groups like methyl or fluorine can be substituted for extra OH groups that are not required for binding to tau or BACE1. This enhances brain entry while maintaining activity, decreases PSA, and moves logP into the 2-4 range [129, 131].

Donepezil was the reference medication used in my research. The comparisons and profiles of all other ADMET properties were good, just hepatotoxicity and cardiotoxicity were predicted. We evaluated our lead chemical with the FDA-approved medication donepezil. Computer-based forecasts used in in-silico research are not entirely reliable. For true verification, we also need clinical data and an FDA label because they occasionally overlook uncommon adverse effects.

Even at the 23 mg dosage, donepezil did not raise liver enzymes or result in significant cardiac issues as compared to a placebo in clinical trials [121].

Active efflux by P-glycoprotein pumps on brain blood arteries is the third main obstacle. P-gp substrates are frequently steroidal lactones with a large number of H-bond acceptors. P-gp pumps Withaferin-A, Withanolide D, or Withanolide L back into the bloodstream even if some of them get into the brain vascular wall. This keeps brain levels extremely low by creating a "revolving door." The main cause of BBB scores being negative even after minor chemical modifications is this outflow. Formulation-based delivery is the answer. Withanolides can be concealed from P-gp by encasing them in lipid nanoparticles, PLGA nanoparticles, or exosomes. Receptor-mediated transport across the blood-brain barrier is made possible by adding brain-targeting ligands to the surface of nanoparticles, such as transferrin, lactoferrin, or Angiopep-2 peptide. In animal models, research using Angiopep-2 nanoparticles enhanced medication delivery to the brain by more than ten times.The brain-to-plasma ratio can be improved by avoiding P-gp while using the same technique for withanolides [132, 133].

Fast metabolism is the fourth obstacle. Glutathione in the liver and blood reacts rapidly with the α,β-unsaturated ketone in ring A of withanolides. There is also relatively little free medication to penetrate the blood-brain barrier due to high plasma protein binding. This quick clearance keeps CNS scores negative and reduces brain exposure more. Compounds can be shielded from degradation by nanoparticle encapsulation. The reactive portion of a prodrug can be concealed until the drug enters the brain.Ligand-targeted nanoparticles are safer for long-term Alzheimer's treatment than repeatedly breaching the blood-brain barrier using focused ultrasound, which can have negative side effects. Strong binding of Withaferin-A, Withanolide D, and Withanolide L can reach actual amounts in the brain by combining tiny chemical modifications with clever delivery mechanisms. Future research should evaluate real brain and blood levels in animals and examine P-gp interaction in MDCK-MDR1 cells [130, 134].

The current work highlights the value of computational methods for Alzheimer's

disease early-stage medication discovery. Before resource-intensive laboratory research, natural compounds can be quickly and affordably screened against important target proteins via in-silico docking. This technique has been effectively used to find promising neuroprotective leads from plant sources, including as inhibitors of Tau protein, Acetylcholinesterase, and Beta-secretase 1. By identifying withaferin A, withanolide D, and withanolide L Aas strong binding candidates and recommending them for additional in vitro and in vivo validation, our results add to this body of evidence [36].

Despite the fact that this in silico study provides insightful computational information, a number of limitations need to be noted. First, the correctness of publicly accessible databases determines the outcomes, which are solely based on computational forecasts. Results may be impacted by inconsistencies, out-of-date records, or structural errors in databases like Protein Data Bank or PubChem.

Second, the intricacy of biological systems, including important pharmacokinetic elements like absorption, distribution, metabolism, and excretion, cannot be replicated by in silico models, nor can they take environmental fluctuations in phytochemical content into account. Thirdly, the predicted chemicals' bioactivity is yet theoretical. Their safety profile and therapeutic potential cannot be definitively determined without experimental validation through in vitro or in vivo investigations. Thus, additional laboratory and clinical research is necessary to validate these computational results [135].

Although encouraging, these findings should be viewed cautiously. Despite being predictive, docking studies are unable to fully account for the complexity of real systems, such as immunological regulation, tissue distribution, and metabolic changes. Thus, in vitro cytotoxicity tests, apoptosis detection, and mechanistic pathway analysis should be part of future study. These should be followed by in vivo studies in Alzheimer's disease models that focus on the proteins Tau, amyloid beta, and BACE1. Additionally, as certain natural substances have been demonstrated to improve treatment efficacy while reducing adverse effects, synergistic effects between withaferin A, withanolide D, and withanolide L and conventional

therapeutic drugs should be investigated. This study provides computational evidence for these metabolites' robust interactions with Alzheimer's disease-related targets and favourable drug-likeness, confirming and expanding earlier findings on their therapeutic potential.The emergence of withaferin A, withanolide D, and withanolide L as supplemental or alternative treatments for Alzheimer's disease is encouraged by these findings.

Chapter 6

Conclusion and Future Work

The therapeutic value of *Withania somnifera* derived phytochemicals against Alzheimer's disease is highlighted by this in-silico investigation. The capacity of three lead compounds Withaferin A, Withanolide D, and Withanolide L to inhibit amyloid aggregation, β-secretase activity, and tau hyperphosphorylation is suggested by their significant binding affinity with important pathogenic targets Amyloid-beta, BACE1, and tau protein. Their drug-likeness is further supported by ADMET profiling, which shows favourable pharmacokinetic characteristics and little toxicity risks. All things considered, these multi-target interactions show that substances can alter several characteristics of Alzheimer's disease. Although computational results are promising, neuroprotective effects must be confirmed by in-vitro and in-vivo validation.Future research should concentrate on formulation development to improve bioavailability, pharmacokinetic optimisation, and blood-brain barrier permeability. Investigating these withanolides' synergistic combinations may also offer a safer plant-based treatment approach for controlling the advancement of Alzheimer's disease.

It is crucial to confirm these in-silico results with thorough in-vitro and in-vivo research. Confirming the safety and neuroprotective effectiveness of *Withania somnifera* metabolites will require experimental validation employing neuronal cell lines like SH-SY5Y and transgenic animal models of Alzheimer's disease. Deeper understanding of Withanone, Withaferin A, Withanolide D, and Withanolide L's

therapeutic potential will come from understanding the precise molecular processes by which they regulate tau hyperphosphorylation, BACE1-mediated β-secretase activity, and amyloid-beta aggregation. In addition to bolstering the computational predictions, this confirmation will open the door for the creation of focused multi-pathway treatments for Alzheimer's [136].

Additionally, examining how *W. somnifera* withanolideswork in concert with other well-known neuroprotective substances may greatly increase their anti-Alzheimer's action. Amyloid-beta clearance and tau stabilisation may benefit from the combination of withanone and withaferin A with substances that target oxidative stress or neuroinflammation. By concurrently treating several characteristics of Alzheimer's pathology, such as protein misfolding, synaptic dysfunction, and neuronal death, this combinatorial strategy may maximise therapeutic benefits. Investigating such synergism may increase the range of plant-based medicines and lessen the toxicity linked to dosage in single-agent therapies [137].

The pharmacokinetics, bioavailability, safety profile, and cognitive efficacy of metabolites of *Withania somnifera* in human individuals with mild cognitive impairment and early-stage Alzheimer's disease must yet be assessed through clinical trials. Standardised formulations with enhanced blood-brain barrier permeability will be necessary to translate these preclinical and in-silico findings into clinical practice. Prior to incorporating these withanolides into normal treatment procedures, it will be crucial to establish dose-response correlations and long-term safety data. A natural, multi-target therapeutic option that enhances current cholinesterase inhibitors and NMDA receptor antagonists for the treatment of Alzheimer's disease may be provided by successful clinical validation [138].

Additionally, the bioavailability and targeted distribution of *Withania somnifera* withanolides to the central nervous system may be enhanced by the development of sophisticated drug delivery systems. The effectiveness of therapy of withanone, withaferin A, withanolide D, and withanolide L against Alzheimer's targets may be improved by methods including liposomal carriers, controlled-release formulations, and nanoencapsulation. These methods can guarantee that bioactive metabolites

reach amyloid-beta, BACE1, and tau protein at efficient concentrations by optimising delivery across the blood-brain barrier. By maximising neuroprotective advantages and minimising systemic side effects, such approach would increase the clinical viability of *W. somnifera* based therapy for Alzheimer's patients.

Bibliography

[1] P. Scheltens, K. Blennow, M. M. Breteler, B. de Strooper, G. B. Frisoni, S. Salloway, and R. Vandenberghe, "Alzheimer's disease," *Lancet*, vol. 388, pp. 505–517, 2016.

[2] M. J. Prince, *World Alzheimer Report 2015: The Global Impact of Dementia*. Alzheimer's Disease International, 2015.

[3] Alzheimer's Association, "2018 alzheimer's disease facts and figures," *Alzheimer's Dement.*, vol. 14, pp. 367–429, 2018.

[4] U.S. Food and Drug Administration, "Alzheimer's disease fact sheet," 2024.

[5] D. Porter. (2026) Major or mild neurocognitive disorder due to Alzheimer's disease DSM-5 331.0 (G30.9). [Online]. Available: https://www.theravive.com

[6] D. R. Thal, U. Rüb, M. Orantes, and H. Braak, "Phases of aβ-deposition in the human brain and its relevance for the development of ad," *Neurology*, vol. 58, pp. 1791–1800, 2002.

[7] H. Braak and E. Braak, "Neuropathological stageing of alzheimer-related changes," *Acta Neuropathol.*, vol. 82, pp. 239–259, 1991.

[8] D. S. Knopman, H. Amieva, R. C. Petersen, G. Chételat, D. M. Holtzman, B. T. Hyman, M. Tanaka, G. Soto-Osuba, A. E. Guimaraes, and P. J. Visser, "Alzheimer disease," *Nat. Rev. Dis. Primers*, vol. 7, p. 33, 2021.

[9] Z.-X. Wang, L. Tan, J. Liu, and J.-T. Yu, "The essential role of soluble aβ oligomers in alzheimer's disease," *Mol. Neurobiol.*, vol. 53, pp. 1905–1924, 2016.

[10] U. Sengupta, A. N. Nilson, and R. Kayed, "The role of amyloid-β oligomers in toxicity, propagation, and immunotherapy," *EBioMedicine*, vol. 6, pp. 42–49, 2016.

[11] M. Tolar, S. Abushakra, J. A. Hey, A. Porsteinsson, and M. Sabbagh, "Neurotoxic soluble amyloid oligomers drive alzheimer's pathogenesis and represent a clinically validated target for slowing disease progression," *Int. J. Mol. Sci.*, vol. 22, p. 6355, 2021.

[12] E. Y. Hayden and D. B. Teplow, "Amyloid β-protein oligomers and alzheimer's disease," *Alzheimer's Res. Ther.*, vol. 5, p. 60, 2013.

[13] L. Mucke, "Neuroscience: Alzheimer's disease," *Nature*, vol. 461, pp. 895–897, 2009.

[14] M. P. Mattson, "Pathways towards and away from alzheimer's disease," *Nature*, vol. 430, pp. 631–639, 2004.

[15] J. C. Vickers, S. Mitew, A. Woodhouse, C. M. Fernandez-Martos, M. T. Kirkcaldie, A. J. Canty, G. H. McCormack, and A. E. King, "Defining the earliest pathological changes of alzheimer's disease," *Curr. Alzheimer Res.*, vol. 13, pp. 281–287, 2016.

[16] V. L. Villemagne, S. Burnham, P. Bourgeat, B. Brown, K. A. Ellis, O. Salvado, C. Szoeke, S. L. Macaulay, R. Martins, P. Maruff, D. Ames, C. C. Rowe, and C. L. Masters, "Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic alzheimer's disease: A prospective cohort study," *Lancet Neurol.*, vol. 12, pp. 357–367, 2013.

[17] American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*, 5th ed. Washington, DC, USA: American Psychiatric Publishing, 2013.

[18] P. V. Fish, D. Steadman, P. Whiting, and S. E. Ward, "New approaches for the treatment of alzheimer's disease," *Bioorg. Med. Chem. Lett.*, vol. 29, pp. 125–133, 2019.

[19] J. Cummings and N. Fox, "Defining disease modifying therapy for alzheimer's disease," *J. Prev. Alzheimer's Dis.*, vol. 4, p. 109, 2017.

[20] S. Andrieu, N. Coley, S. Lovestone, P. S. Aisen, and B. Vellas, "Prevention of sporadic alzheimer's disease: Lessons learned from clinical trials and future directions," *Lancet Neurol.*, vol. 14, pp. 926–944, 2015.

[21] G. Livingston, J. Huntley, A. Sommerlad, D. Ames, C. Ballard, S. Banerjee, C. Brayne, A. Burns, J. Cohen-Mansfield, C. Cooper, S. G. Costafreda, A. Dias, N. Fox, L. N. Gitlin, R. Howard, H. C. Kales, M. Kivipelto, E. B. Larson, A. Ogunniyi, V. Orgeta, K. Ritchie, K. Rockwood, E. L. Sampson, Q. Samus, L. S. Schneider, G. Selbæk, L. Teri, and N. Mukadam, "Dementia prevention, intervention, and care: 2020 report of the lancet commission," *Lancet*, vol. 396, pp. 413–446, 2020.

[22] S. Norton, F. E. Matthews, D. E. Barnes, K. Yaffe, and C. Brayne, "Potential for primary prevention of alzheimer's disease: An analysis of population-based data," *Lancet Neurol.*, vol. 13, pp. 788–794, 2014.

[23] G. Livingston, A. Sommerlad, V. Orgeta, S. G. Costafreda, J. Huntley, D. Ames, C. Ballard, S. Banerjee, A. Burns, J. Cohen-Mansfield, C. Cooper, N. Fox, L. N. Gitlin, R. Howard, H. C. Kales, E. B. Larson, K. Ritchie, K. Rockwood, E. L. Sampson, Q. Samus, L. S. Schneider, G. Selbæk, L. Teri, and N. Mukadam, "Dementia prevention, intervention, and care," *Lancet*, vol. 390, pp. 2673–2734, 2017.

[24] B. DuBois, H. Hampel, H. H. Feldman, P. Scheltens, P. Aisen, S. Andrieu, H. Bakardjian, H. Benali, L. Bertram, K. Blennow, K. Broich, S. Cappa, S. Crutch, J.-F. Dartigues, B. de Schutter, L. C. de Souza, A. Fagan, C. Franceschi, G. B. Frisoni, P. Friedrich, S. Galluzzi, M. Grundman, M. Ingelsson, K. Johnson, Z. Khachaturian, M. Kilenky, M. Kivipelto, W. Klunk,

J. L. Liss, R. Martins, I. McKeith, V. Mok, J. L. Molinuevo, J. E. Müller, C. Phillips, S. Reisa, C. Rowe, S. Salloway, L. S. Schneider, R. Sperling, M. Teichmann, M. C. Carrillo, C. R. Jack, and Y. Stern, "Preclinical alzheimer's disease: Definition, natural history, and diagnostic criteria," *Alzheimer's Dement.*, vol. 12, pp. 292–323, 2016.

[25] J. M. Long and D. M. Holtzman, "Alzheimer disease: An update on pathobiology and treatment strategies," *Cell*, vol. 179, no. 2, pp. 312–339, 2019.

[26] A. A. Spector, "Essentiality of fatty acids," *Lipids*, vol. 34, no. 1, pp. S1–S3, 1999.

[27] S. M. Innis, "Essential fatty acids in growth and development," *Prog. Lipid Res.*, vol. 30, no. 1-2, pp. 39–103, 1991.

[28] E. Kawakita, M. Hashimoto, and O. Shido, "Docosahexaenoic acid promotes neurogenesis in vitro and in vivo," *Neuroscience*, vol. 139, no. 3, pp. 991–997, 2006.

[29] L. A. Horrocks and A. A. Farooqui, "Docosahexaenoic acid in the diet: Its importance in maintenance and restoration of neural membrane function," *Prostaglandins Leukot. Essent. Fatty Acids*, vol. 70, no. 3, pp. 361–372, 2004.

[30] C.-T. Lu, Y.-Z. Zhao, H.-L. Wong, S.-Z. Hou, C.-Y. Xu, F.-X. Cao, and X.-B. Zhang, "Current approaches to enhance cns delivery of drugs across the brain barriers," *Int. J. Nanomedicine*, vol. 9, p. 2241, 2014.

[31] R. I. Teleanu, C. Chircov, A. M. Grumezescu, and D. M. Teleanu, "Current strategies to enhance delivery of drugs across the blood–brain barrier," *Pharmaceutics*, vol. 14, p. 987, 2022.

[32] H. G. and et al., "Biodiversity, biochemical profiling, and pharmacocommercial applications of *Withania somnifera*: A review," *Molecules*, vol. 28, no. 3, p. 1208, 2023.

[33] "Normal and alzheimer brain," researchgate.net.

[34] S. Saleem and et al., "*Withania somnifera* l.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective," *Iran. J. Basic Med. Sci.*, vol. 23, no. 12, pp. 1501–1526, 2020.

[35] M. S. Tambe and et al., "Network pharmacology and in-silico studies for molecular mechanisms of analgesic, anti-inflammatory and anti-arthritic effects of *Withania somnifera* (l.) dunal phytoconstituents," *J. Ayurveda Integr. Med.*, vol. 16, no. 4, p. 101088, 2025.

[36] M. Moradi and et al., "A contemporary review on the important role of in silico approaches for managing different aspects of covid-19 crisis," *Informatics Med. Unlocked*, vol. 28, p. 100862, 2022.

[37] V. Lerose, S. De Vita, A. Scarselli, G. Carpinelli, and M. Gualtieri, "*Withania somnifera* (l.) dunal, a potential source of phytochemicals for treating neurodegenerative diseases: A systematic review," *Plants*, vol. 13, no. 6, p. 771, 2024.

[38] D. Kirubakaran, "Alzheimer's disease: neuroprotective mechanisms and cognitive enhancement potential," *J. Drug Clin. Med.*, 2025.

[39] S. K. Singirala, P. K. Dubey, and S. Roy, "Extraction of bioactive compounds from *Withania somnifera*: The biological activities and potential application in the food industry: A review," *Int. J. Food Sci.*, vol. 2025, no. 9922626, 2025.

[40] M. Pant, T. Ambwani, and V. Umapathi, "Antiviral activity of ashwagandha extract on infectious bursal disease virus replication," *Ind. J. Sci. Tech.*, vol. 5, pp. 2750–2751, 2012.

[41] S. Shinde and et al., "Recent advancements in extraction techniques of ashwagandha (*Withania somnifera*) with insights on phytochemicals, structural significance, pharmacology, and current trends in food applications," *ACS Omega*, vol. 8, no. 44, pp. 40 982–41 003, 2023.

[42] M. Sprengel, R. Laskowski, and Z. Jost, "*Withania somnifera* (ashwagandha) supplementation: a review of its mechanisms, health benefits, and role in sports performance," *Nutr. Metab.*, vol. 22, no. 9, 2025.

[43] A. M. Beera, S. M. Seethamraju, and L. P. Nori, "Alzheimer's disease: Perspective on therapeutic options and recent hallmarks in clinical research," *Int. J. Pharm. Res. Allied Sci.*, vol. 10, no. 4, pp. 110–120, 2021.

[44] X. Jia and et al., "A comparison of the mini-mental state examination (mmse) with the montreal cognitive assessment (moca) for mild cognitive impairment screening in chinese middle-aged and older population: a cross-sectional study," *BMC Psychiatry*, vol. 21, no. 1, p. 485, 2021.

[45] I. Anjum, M. Fayyaz, A. Wajid, W. Sohail, and A. Ali, "Does obesity increase the risk of dementia: A literature review," *Cureus*, vol. 10, no. 5, p. e2660, 2018.

[46] C. Qiu, M. Kivipelto, and E. von Strauss, "Epidemiology of alzheimer's disease: occurrence, determinants, and strategies toward intervention," *Dialogues Clin. Neurosci.*, vol. 11, no. 2, pp. 111–128, 2009.

[47] Z. Breijyeh and R. Karaman, "Comprehensive review on alzheimer's disease: Causes and treatment," *Molecules*, vol. 25, no. 24, 2020.

[48] G. Paroni, P. Bisceglia, and D. Seripa, "Understanding the amyloid hypothesis in alzheimer's disease," *J. Alzheimers Dis.*, vol. 68, no. 2, pp. 493–510, 2019.

[49] V. Marchione, "New research may explain the reasons behind normal aging and alzheimer's disease," *Brain Function*, 2017.

[50] Assay Genie, "A guide to tau proteins & tauopathies," 2024.

[51] A. A. Abdulkhalig, B. Kim, and D. Pratico, "Amyloid-β and tau in Alzheimer's disease: pathogenesis, mechanisms, and interplay," *Cell Death & Disease*, vol. 17, no. 21, p. 21, 2026.

[52] A. Salehi, J. W. Ashford, and E. J. Mufson, "The link between alzheimer's disease and down syndrome. a historical perspective," *Curr. Alzheimer Res.*, vol. 13, no. 1, pp. 2–6, 2016.

[53] G. Nicolas and et al., "Somatic variants in autosomal dominant genes are a rare cause of sporadic alzheimer's disease," *Alzheimers Dement.*, vol. 14, no. 12, pp. 1632–1639, 2018.

[54] A. Serrano-Pozo, M. P. Frosch, E. Masliah, and B. T. Hyman, "Neuropathological alterations in alzheimer disease," *Cold Spring Harb. Perspect. Med.*, vol. 1, no. 1, p. a006189, 2011.

[55] H. Braak, D. R. Thal, E. Ghebremedhin, and K. Del Tredici, "Stages of the pathologic process in alzheimer disease: age categories from 1 to 100 years," *J. Neuropathol. Exp. Neurol.*, vol. 70, no. 11, pp. 960–969, 2011.

[56] S. M. Greenberg and et al., "Cerebral amyloid angiopathy and alzheimer disease—one peptide, two pathways," *Nat. Rev. Neurol.*, vol. 16, no. 1, pp. 30–42, 2020.

[57] A. Haapasalo and M. Hiltunen, "A report from the 8th kuopio alzheimer symposium," *Neurodegener. Dis. Manage.*, vol. 8, no. 5, pp. 289–299, 2018.

[58] H. Kim, "Detection of severity in alzheimer's disease (ad) using computational modeling," *Bioinformation*, vol. 14, no. 5, pp. 259–264, 2018.

[59] R. C. Petersen, "How early can we diagnose alzheimer disease (and is it sufficient)? the 2017 wartenberg lecture," *Neurology*, vol. 91, no. 9, pp. 395–402, 2018.

[60] M. Sobański and et al., "A review of neuroimaging in rare neurodegenerative diseases," *Dement. Geriatr. Cogn. Disord.*, vol. 49, no. 6, pp. 544–556, 2020.

[61] C. M. Bottino and et al., "Volumetric mri measurements can differentiate alzheimer's disease, mild cognitive impairment, and normal aging," *Int. Psychogeriatr.*, vol. 14, no. 1, pp. 59–72, 2002.

[62] G. B. Frisoni and et al., "Strategic roadmap for an early diagnosis of alzheimer's disease based on biomarkers," *Lancet Neurol.*, vol. 16, no. 8, pp. 661–676, 2017.

[63] K. Mirkovic and et al., "Sodium restriction potentiates the renoprotective effects of combined vitamin d receptor activation and angiotensin-converting enzyme inhibition in established proteinuric nephropathy," *Nephrol. Dial. Transplant.*, vol. 32, no. 8, pp. 1293–1301, 2017.

[64] D. Cattran and P. Brenchley, "Membranous nephropathy: thinking through the therapeutic options," *Nephrol. Dial. Transplant.*, vol. 32, no. suppl 1, pp. i22–i29, 2017.

[65] L. Forshing and J. W. Tsao, *Alzheimer Disease*. Treasure Island, FL: StatPearls Publishing, 2026.

[66] K. A. Johnson, N. C. Fox, R. A. Sperling, and W. E. Klunk, "Brain imaging in alzheimer disease," *Cold Spring Harb. Perspect. Med.*, vol. 2, no. 4, p. a006213, 2012.

[67] Alzheimer's Association, "2022 alzheimer's disease facts and figures," *Alzheimer's Dementia*, vol. 18, no. 4, pp. 700–789, 2022.

[68] K. Mirkovic and et al., "Sodium restriction potentiates the renoprotective effects of combined vitamin d receptor activation and angiotensin-converting enzyme inhibition in established proteinuric nephropathy," *Nephrol. Dial. Transplant.*, vol. 32, no. 8, pp. 1293–1301, 2017.

[69] A. Behera and M. K. Dharmalingam Jothinathan, "Artificial intelligence transforms the future of oncology care," *J. Stomatol. Oral Maxillofac. Surg.*, vol. 125, no. 4, p. 101915, 2024.

[70] S. E. Fanibunda and et al., "*Withania somnifera* regulates mitochondrial biogenesis and energetics in rat cortical neurons: Role of bdnf and sirt1," *Mol. Neurobiol.*, vol. 62, no. 8, pp. 10 277–10 295, 2025.

[71] M. Sanjivkumar, T. Silambarsan, and K. Nagajothi, "Translation research and herbal drug development," in *Translational Research in Biomedical Sciences: Recent Progress and Future Prospects*. Singapore: Springer Nature Singapore, 2024, pp. 263–274.

[72] H. Xue and et al., "Repetitive transcranial magnetic stimulation for alzheimer's disease: an overview of systematic reviews and meta-analysis," *Front. Aging Neurosci.*, vol. 16, p. 1383278, 2024.

[73] S. Ahamad and U. Dhuldhaj, "Potential plasma biomarkers for diagnosis of alzheimer's disease: an overview," *Prog. Chem. Biochem. Res.*, vol. 7, no. 2, pp. 114–128, 2024.

[74] P. B. Gonçalves, A. C. R. Sodero, and Y. Cordeiro, "Natural products targeting amyloid-β oligomer neurotoxicity in alzheimer's disease," *Eur. J. Med. Chem.*, vol. 276, p. 116684, 2024.

[75] M. A. Shabbir and et al., "Therapeutic application of natural products in alzheimer's disease using computational methods," in *Computational and Experimental Studies in Alzheimer's Disease*. Boca Raton: CRC Press, 2024, pp. 138–154.

[76] P. K. Mukherjee, S. Banerjee, S. Biswas, B. Das, A. Kar, and C. K. Katiyar, "*Withania somnifera* (l.) dunal – modern perspectives of an ancient rasayana from ayurveda," *J. Ethnopharmacol.*, vol. 264, p. 113157, 2021.

[77] Y. Y. Huang and et al., "Depression in alzheimer's disease: epidemiology, mechanisms, and treatment," *Biol. Psychiatry*, vol. 95, no. 11, pp. 992–1005, 2024.

[78] "Amazon product listing," product Reference Code: 07WTjgp8.

[79] B. Govindaraju and et al., "High frequency plant regeneration in ashwagandha (*Withania somnifera* (l.) dunal): An important medicinal plant," *Plant Cell Biotechnol. Mol. Biol.*, vol. 4, pp. 49–56, 2003.

[80] L. D. Kapoor, *Handbook of Ayurvedic Medicinal Plants*. Boca Raton: CRC Press, 1990.

[81] M. H. Mirjalili and et al., "Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine," *Molecules*, vol. 14, no. 7, pp. 2373–2393, 2009.

[82] J. I. Okogun, "Drug discovery through ethnobotany in nigeria: some results," in *Advances in Phytomedicine*, M. M. Iwu and J. C. Wootton, Eds. Elsevier, 2002, vol. 1, pp. 145–154.

[83] R. Silambarasan and M. Ayyanar, "An ethnobotanical study of medicinal plants in palamalai region of eastern ghats, india," *J. Ethnopharmacol.*, vol. 172, pp. 162–178, 2015.

[84] T. Thirumalai and et al., "Ethnobotanical study of anti-diabetic medicinal plants used by the local people in javadhu hills tamilnadu, india," *Asian Pac. J. Trop. Biomed.*, vol. 2, no. 2, pp. S910–S913, 2012.

[85] S. Prabhu and et al., "An ethnobotanical study of medicinal plants used in pachamalai hills of tamil nadu, india," *J. Herb. Med.*, vol. 25, p. 100400, 2021.

[86] K. Poonam and G. S. Singh, "Ethnobotanical study of medicinal plants used by the taungya community in terai arc landscape, india," *J. Ethnopharmacol.*, vol. 123, no. 1, pp. 167–176, 2009.

[87] S. Sarkhel, "Ethnobotanical survey of folklore plants used in treatment of snakebite in paschim medinipur district, west bengal," *Asian Pac. J. Trop. Biomed.*, vol. 4, no. 5, pp. 416–420, 2014.

[88] P. Mahishi, B. H. Srinivasa, and M. B. Shivanna, "Medicinal plant wealth of local communities in some villages in shimoga district of karnataka, india," *J. Ethnopharmacol.*, vol. 98, no. 3, pp. 307–312, 2005.

[89] P. Pathak and et al., "Standardized root extract of *Withania somnifera* and withanolide a exert moderate vasorelaxant effect in the rat aortic rings by

enhancing nitric oxide generation," *J. Ethnopharmacol.*, vol. 278, p. 114296, 2021.

[90] S. K. Singirala and et al., "Extraction of bioactive compounds from *Withania somnifera*: The biological activities and potential application in the food industry a review," *Int. J. Food Sci.*, vol. 2025, p. 9922626, 2025.

[91] S. Paul and et al., "*Withania somnifera* (l.) dunal (ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects," *Biomed. Pharmacother.*, vol. 143, p. 112175, 2021.

[92] J. Hardy and D. J. Selkoe, "The amyloid hypothesis of alzheimer's disease: progress and problems on the road to therapeutics," *Science*, vol. 297, no. 5580, pp. 353–356, 2002.

[93] S. K. Burley and et al., "Protein data bank (pdb): The single global macromolecular structure archive," in *Protein Crystallography: Methods and Protocols*. New York, NY: Springer, 2017, pp. 627–641.

[94] A. Roy, A. Kucukural, and Y. Zhang, "I-tasser: A unified platform for automated protein structure and function prediction," *Nat. Protoc.*, vol. 5, no. 4, pp. 725–738, 2010.

[95] R. Evans and et al., "Protein complex prediction with alphafold-multimer," *bioRxiv*, p. 2021.10.04.463034, 2021.

[96] S. Pundir, M. Martin, and C. O'Donovan, "Uniprot protein knowledgebase," in *Protein Bioinformatics: From Protein Modifications and Networks to Proteomics*. New York, NY: Springer, 2017, pp. 41–55.

[97] E. Gasteiger and et al., "Protein identification and analysis tools on the expasy server," in *The Proteomics Protocols Handbook*, J. M. Walker, Ed. Humana Press, 2005, pp. 571–607.

[98] R. Azimi and et al., "Bioinformatic analysis of small humanin like peptides using alphafold-2 and expasy protparam," *Invest. Ophthalmol. Visual Sci.*, vol. 65, no. 7, p. 1320, 2024.

[99] W. L. DeLano, "Pymol: An open-source molecular graphics tool," *CCP4 Newsl. Protein Crystallogr.*, vol. 40, no. 1, pp. 82–92, 2002.

[100] M. Punta and et al., "The pfam protein families database," *Nucleic Acids Res.*, vol. 40, no. D1, pp. D290–D301, 2012.

[101] A. Volkamer and et al., "Dogsitescorer: A web server for automatic binding site prediction, analysis and druggability assessment," *Bioinformatics*, vol. 28, no. 15, pp. 2074–2075, 2012.

[102] H. Ghorab and et al., "Proteomic analysis of sweet algerian apricot kernels (prunus armeniaca l.) by combinatorial peptide ligand libraries and lc-ms/ms," *Food Chem.*, vol. 239, pp. 935–945, 2018.

[103] M. Butkiewicz and et al., "Benchmarking ligand-based virtual high-throughput screening with the pubchem database," *Molecules*, vol. 18, no. 1, pp. 735–756, 2013.

[104] V. K. Narayanaswamy, M. Rissdörfer, and B. Odhav, "Review on cambridgesoft chembiodraw ultra 13.0 v," *Int. J. Theor. Appl. Sci.*, vol. 5, pp. 45–49, 2013.

[105] J. J. Irwin, "Software review: Chemoffice 2005 pro by cambridgesoft," *J. Chem. Inf. Model.*, vol. 45, no. 5, pp. 1461–1462, 2005.

[106] C. A. Lipinski, "Lead- and drug-like compounds: The rule-of-five revolution," *Drug Discov. Today Technol.*, vol. 1, no. 4, pp. 337–341, 2004.

[107] J. C. Dearden, "In silico prediction of admet properties: How far have we come?" *Expert Opin. Drug Metab. Toxicol.*, vol. 3, no. 5, pp. 635–639, 2007.

[108] N. S. Pagadala, K. Syed, and J. Tuszynski, "Software for molecular docking: A review," *Biophys. Rev.*, vol. 9, no. 2, pp. 91–102, 2017.

[109] D. A. Gschwend, A. C. Good, and I. D. Kuntz, "Molecular docking towards drug discovery," *J. Mol. Recognit.*, vol. 9, no. 2, pp. 175–186, 1996.

[110] S. S. Pawar and S. H. Rohane, "Review on discovery studio: An important tool for molecular docking," *Asian J. Res. Chem.*, vol. 14, no. 1, pp. 81–84, 2021.

[111] D. S. Wishart and et al., "Drugbank: A comprehensive resource for in silico drug discovery and exploration," *Nucleic Acids Res.*, vol. 34, no. suppl 1, pp. D668–D672, 2006.

[112] H. Ogura and et al., "Donepezil, a centrally acting acetylcholinesterase inhibitor, alleviates learning deficits in hypocholinergic models in rats," *Methods Find. Exp. Clin. Pharmacol.*, vol. 22, no. 2, pp. 89–95, 2000.

[113] J. Tibbitts and et al., "Key factors influencing adme properties of therapeutic proteins: A need for adme characterization in drug discovery and development," *mAbs*, vol. 8, no. 2, pp. 229–245, 2016.

[114] E. Sjögren, H. Thorn, and C. Tannergren, "In silico modeling of gastrointestinal drug absorption: Predictive performance of three physiologically based absorption models," *Mol. Pharmaceutics*, vol. 13, no. 6, pp. 1763–1778, 2016.

[115] S. R. Kazmi, R. Jun, M.-S. Yu, C. Jung, and D. Na, "In silico approaches and tools for the prediction of drug metabolism and fate: A review," *Comput. Biol. Med.*, vol. 106, pp. 54–64, 2019.

[116] G. Luo and et al., "In silico prediction of biliary excretion of drugs in rats based on physicochemical properties," *Drug Metab. Dispos.*, vol. 38, no. 3, pp. 422–430, 2010.

[117] M. D. Segall and C. Barber, "Addressing toxicity risk when designing and selecting compounds in early drug discovery," *Drug Discov. Today*, vol. 19, no. 5, pp. 688–693, 2014.

[118] A. Roncaglioni, A. A. Toropov, A. P. Toropova, and E. Benfenati, "In silico methods to predict drug toxicity," *Curr. Opin. Pharmacol.*, vol. 13, no. 5, pp. 802–806, 2013.

[119] S. Durdagi, M. G. Papadopoulos, D. P. Papahatjis, and T. Mavromoustakos, "Combined 3d qsar and molecular docking studies to reveal novel cannabinoid ligands with optimum binding activity," *Bioorg. Med. Chem. Lett.*, vol. 17, no. 24, pp. 6754–6763, 2007.

[120] U. Baroroh, M. Biotek, Z. S. Muscifa, W. Destiarani, F. G. Rohmatullah, and M. Yusuf, "Molecular interaction analysis and visualization of protein-ligand docking using biovia discovery studio visualizer," *Indones. J. Comput. Biol.*, vol. 2, no. 1, pp. 22–30, 2023.

[121] National Institute of Diabetes and Digestive and Kidney Diseases. Donepezil. Bethesda, MD. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK548197/

[122] H. H. Won and et al., "Polymorphic markers associated with severe oxaliplatin-induced, chronic peripheral neuropathy in colon cancer patients," *Cancer*, vol. 118, no. 11, pp. 2828–2836, 2012.

[123] D. Petit and et al., "Aβ profiles generated by alzheimer's disease causing psen1 variants determine the pathogenicity of the mutation and predict age at disease onset," *Mol. Psychiatry*, vol. 27, no. 8, pp. 3259–3269, 2022.

[124] S. Pavlov and et al., "Advances in bioactive compounds from plants and their applications in alzheimer's disease," *Biomolecules*, vol. 16, no. 1, p. 7, 2025.

[125] N. Sehgal and et al., "*Withania somnifera* reverses alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver," *PLoS One*, vol. 7, no. 3, p. e32009, 2012.

[126] I. Ulkü and et al., "Inhibition of bace1 affected both its aβ producing and degrading activities and increased aβ42 and aβ40 levels at high-level bace1 expression," *J. Biol. Chem.*, vol. 300, no. 8, p. 107510, 2024.

[127] W. L. Jorgensen and E. M. Duffy, "Prediction of drug solubility from structure," *Adv. Drug Delivery Rev.*, vol. 54, no. 3, pp. 355–366, 2002.

[128] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings," *Adv. Drug Delivery Rev.*, vol. 46, pp. 3–26, 2001.

[129] H. Pajouhesh and G. R. Lenz, "Medicinal chemical properties of successful central nervous system drugs," *NeuroRx*, vol. 2, no. 4, pp. 541–553, 2005.

[130] J. Rautio and et al., "The expanding role of prodrugs in contemporary drug design and development," *Nat. Rev. Drug Discov.*, vol. 17, no. 8, pp. 559–587, 2018.

[131] S. A. Hitchcock, "Structural modifications that alter the p-glycoprotein efflux properties of compounds," *J. Med. Chem.*, vol. 55, no. 11, pp. 4877–4895, 2012.

[132] D. E. Masri and et al., "The role of blood-brain barrier in brain cancer: From pathophysiology to therapeutic approaches," *Cancer Control*, vol. 33, 2026.

[133] K. B. Johnsen and et al., "Targeting the transferrin receptor for brain drug delivery," *Prog. Neurobiol.*, vol. 181, p. 101665, 2019.

[134] T. T. Wager and et al., "Moving beyond rules: the development of a central nervous system multiparameter optimization (cns mpo) approach to enable alignment of druglike properties," *ACS Chem. Neurosci.*, vol. 1, no. 6, pp. 435–449, 2010.

[135] Proventa International, "A critical evaluation of the advantages and limitations of in silico methods in clinical research," 2024.

[136] T. Kuboyama, C. Tohda, and K. Komatsu, "Withanoside iv and its active metabolite, sominone, attenuate aβ(25-35)-induced neurodegeneration," *Eur. J. Neurosci.*, vol. 23, no. 6, pp. 1417–1426, 2006.

[137] M. H. Mirjalili and et al., "Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine," *Molecules*, vol. 14, pp. 2373–2393, 2009.

[138] K. Chandrasekhar, J. Kapoor, and S. Anishetty, "A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults," *Indian J. Psychol. Med.*, vol. 34, no. 3, pp. 255–262, 2012.