CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Computational Analysis for Investigating the Efficacy of *Rhodiola Rosea* Phytoconstituents against Alzheimer's Disease

by

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

in the

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I dedicate this thesis to my loving and supportive family and friends who have fully helped me in achieving my life goals.

CERTIFICATE OF APPROVAL

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Abstract

Alzheimer's disease is among the leading causes of death within most parts of the world. Improved screening methods and therapies and treatments amplify, but the side effects are tremendous. Therefore, the preference for natural products over synthetic ones have considerably increased. The current study aims to identify effective phytochemicals from *Rhodiola rosea* against Alzheimer's disease by using computational approaches. Tau protein inhibitors for treatment of Alzheimer's disease work by inhibiting the formation of neurofibrillary tangles.10 phytochemicals from different classes of *Rhodiola rosea* were selected. Lipinski's Rule and ADMET properties were analyzed as primary and secondary filters and molecular docking as virtual screening was performed to identify their drug-likeness. By using PyMol and LigPlot+ the interactions were visualized and analyzed. Ferulic acid showed itself as a hit compound against target receptors. FDA approved drug Memantine was used as a standard drug for comparison. The lead compound Ferulic acid showed better results and was more active and less toxic than the approved anti-Alzheimer drug memantine. These compounds can be proved more effective as anti-Alzheimer's agents while giving least side effects through in vivo and in vitro analysis. Modern drug discovery techniques are a way in which new and potent drugs from natural sources can be discovered.

Keywords: *Rhodiola rosea*, Virtual screening, Medicinal Plants, Alzheimer's disease, Molecular Docking, Tau protein, Lead Compound, Ferulic acid, Memantine.

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Abbreviations

Aβ	Amyloid beta
AChE	Acetylcholinesterase enzyme
AD	Alzheimer's disease
ANS	Autonomic Nervous System
BBB	Blood-brain barrier
CNS	Central Nervous System
PDB	Protein Data Bank
R. rosea	*Rhodiola rosea*

Chapter 1

Introduction

Alzheimer's disease is the progressive destruction of brain tissue. This is the most common form of dementia. It is a gradually progressive neurodegenerative disorder marked by amyloid plaques and tau tangles due to amyloid beta peptide aggregation in the profoundly affected part of the brain, the cerebral cortex, and temporal lobe structures. Alois Alzheimer observed the accumulation of amyloid deposits and immense deprivation of neurons during the examination of the brain of his patient. His patient faced amnesia and personality transformations before death. Alois Alzheimer depicted his situation as a severe illness of the cerebral cortex. For the first time, Emil Kraepelin termed this illness Alzheimer's disease in his psychiatry handbook, 8th edition. The degeneration of cognitive skills can be induced by Alzheimer's disease and other aspects such as contagion and anomalies in the respiratory and cardiovascular systems, which trigger a decrease in circulation to the brain, tumors, and a deficiency of vitamin B12. Currently, there are about 50 million AD patients, and this value is estimated to multiply every half-decade and will expand to 150 million by 2050. There is no permanent treatment for AD; however, some drugs ameliorate signs [1]. The use of anti-Alzheimer drugs has different side effects, such as nausea, insomnia, diarrhea, and vomiting, so the concentration has been directed towards developing plant-based remedies for Alzheimer's disease [2]. Medicinal plants are nature's gift to treat incurable diseases. Medicinal plants grow naturally in many countries. Suppose a plant has potential importance in one country. Many scientists study and experiment

with such a compound to discover its toxicity and potency as well as to ascertain whether it has the potential to be used as medicine or not [3].

Medicinal herbs play a very primitive role in the treatment of Alzheimer's disease and related dementias. Traditional medicine is primarily defensive, alimentary, and therapeutic. Traditional medicines are risk-free and innocuous and medicate patients with minimal or no adverse effects. Medicinal plants originate in ancient cultures such as India and China. The nervous system control the conscious and unconscious states. The CNS and ANS are interlinked, and some drugs may affect the neurogenic processes associated with the ANS. Memory impairment is a major global health issue. Current therapies have several side effects, hence the dire need for alternative remedies for Alzheimer's disease and cognitive impairment. Many herbal medicines have been in place that can treat Alzheimer's disease [4].

Rhodiola rosea L. (family Crassulaceae) known also by the name "roseroot", has yellow-colored flowers. It thrives in elevated regions in desert soil, on coastal cliffs, and in the mountain clefts of the polar regions of Asia and Europe, as well as in the eastern maritime regions of North America. *R. rosea* is used as an herbal medicine that treats infections, depression, anemia, and nervous system disorders [5]. *R. rosea* has anti-stress, anti-cancer, and anti-aging properties etc. Salidroside, rosavins, tyrosol, gallic acid, and rhodionin are the phytochemicals of *R. rosea*. Salidroside is present in all the species of the genus Rhodiola. The specific components of *R. rosea* are rosavins [6]. Animal toxicology studies reveal the safety of *R. rosea* as a medicinal herb. *R. rosea* reveals neuroprotective action through attenuation of cellular oxidative stress and inflammation and delaying onset of brain disorders. This *R. rosea* has potential anti-inflammatory effects to reduce neuroinflammatory responses associated with AD and multiple sclerosis [7].

1.1 Problem Statement

Alzheimer's disease is a major global vigor issue due to its numerous linked health risks. People in poor countries face many challenges in approaching medications for Alzheimer's disease. There have also been a few side effects related to these

drugs. Hence, safety and easily accessible drugs need to be commercialized to treat Alzheimer's disease.

1.2 Hypothesis

The use of medicinal plants is a safer and more economical approach than commercially available drugs that are expensive or have adverse effects. Phytochemicals have always provided aid in drug discovery and development hence there is search of phytoconstituents for safe and effective treatment for Alzheimer's disease and also prevention of other diseases associated with it. In this context, effectiveness of phytoconstituents of *R. rosea* against Alzheimer's disease will be studied.

1.3 Aims and Objectives

The study aims to identify various plant-based anti-Alzheimer's phytochemicals through a computational approach. The objectives included:

- To determine whether *Rhodiola rosea* contains phytochemicals that can prevent aggregation of Tau protein.
- To determine drug-likeness and study the interaction pattern of these compounds with the target protein to find a lead compound for Alzheimer's disease treatment.
- To analyze the predicted anti-Alzheimer's compounds with the approved drug currently utilized for AD treatment.

Chapter 2

Literature Review

2.1 Alzheimer's Disease

Alzheimer's disease is currently responsible for dementia in nearly 70% of all patients, and it still remains as the most common cause of dementia globally. Moreover, the causative mechanism of AD has also partly been identified with two proteins: amyloid beta peptide and tau. In this disease, amyloid beta deposits manifest first, triggering hyper-phosphorylation of tau that results in tangles and neuronal damage [8]. The toxicity and amyloid beta accumulation occur in the brain, where there is an unusual interaction with cerebral metal ions such as Fe, Cu, and Zn. AD is high in females because of the increased constitutive activity of ZnT3 [9]. Neurological alterations consist of two types: positive lesions and negative lesions. In the positive lesions, there is an aggregation of the amyloid plaques, degenerative nerve fibers, and tau protein deposits present in the cerebrum of patients, while the negative lesions are due to the loss of neural tissues and synaptic connections [1].

People with AD face restlessness, unusual sleep duration, and inadequate overnight sleep quality. Such sleep problems consequently lessen the patient's standard of living. The more likelihood of mental deterioration is caused due to excessive daytime napping; the risk is very strong among the age groups. Aged people who do not take proper diet may suffer from Alzheimer's diseases; however, if

changes in diet are brought in then it will prevent age-related neurological stress and Alzheimer's disease in the coming years.

Various foods, such as eggs, green vegetables, vitamins B and E, fish, and olive oil, can reduce the risk of AD. There is a strong connection between hearing impairment and AD. Hearing impairment affects 40% of individuals over the age of 65 and up to 90% of those over the age of 90. It was discovered through an animal study that if feces from normal wild mice are transplanted into AD transgenic mice, amyloid-beta burden and tau tangles could potentially decrease, and memory impairment could also improve [10].

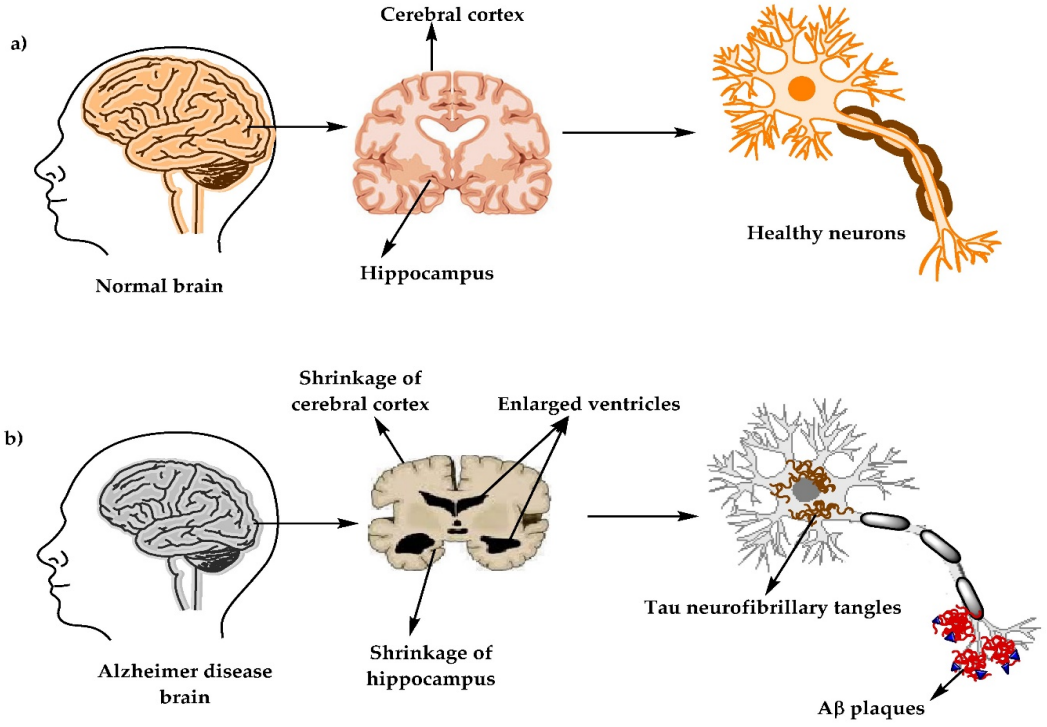


FIGURE 2.1: The anatomy of the brain and neurons in (a) healthy brain and (b) Alzheimer's disease (AD) brain [1].

2.2 Neuropsychiatric Symptoms

- Depression
- Anxiety

- Aggressiveness
- Apathy
- Psychosis [11]

2.3 Risk Factors of Alzheimer's Disease

Diet, aging, lifestyle, and genetics are the risk factors for AD [12]. Approximately 70% of disease development is associated with genetic factors. The early form of AD develops as the result of changes in the genes APP, PSEN1, and PSEN2. The late stage of AD is mainly associated with polymorphisms in the APOE gene due to $\epsilon 4$ allele forms.

Elevated blood pressure, obesity, and diabetes are other risk factors in AD. Smoking is considered an etiological factor that causes the process of development of AD. This is because it raises free radicals with high oxidation stress; thereby, it leads to inflammation in the immune system [13].

Physical inactivity is also a major factor in the contribution of AD. Exercise reduces the chances of getting AD. There is also a strong connection between allergies and AD. Allergy influences the cytokines and inflammatory agents that result in the enhancement of neuroinflammation [14].

2.4 Epidemiology

About 25 million people in the world are influenced by dementia. Approximately 5 million new cases of dementia are emerging annually. This number is expected to double each year [15].

People who are in the age group of 65–85 have a higher risk of getting AD. The prevalence rate of AD increased by about 0.17% at age 65 and by 0.71, 1.0, and 2.92% at 75, 80, and 85 per year. The occurrence rate of AD seems to be greater in the US as compared to Asia, Africa, and Europe [16].

2.5 Management of Alzheimer's Disease

The patients can manage this disease by open communication with physicians. This will help the physician to identify all symptoms and provide guidance for the management of this disease. Light, exercise, and cognitive behavioral therapies may aid in dealing with dementia. Involving in pleasurable activities also helps to manage AD [17].

2.6 Treatment for Alzheimer's Disease

The FDA has given approval to four medications; Donepezil and galantamine function as inhibitors of the acetylcholinesterase enzyme (known as AChEI) along with rivastigmine; meanwhile memantine serves as a receptor antagonist, for the molecule known as N-methyl-D-aspartate (NMDA). These medications only manage the symptoms of AD. They do not inhibit neuronal loss and degeneration of cognition [18]. Memantine can also be combined with AChE to treat patients with severe AD [17].

2.6.1 Donepezil

Donepezil is a drug that is used to address Alzheimer's disease. It is beneficial to treat the moderate, temperate, and extreme phases of dementia linked with Parkinson's disease and AD. Donepezil is assimilated gradually, but thoroughly from the stomach, attaining its highest levels in the blood within three to four hours. It can inhibit an enzyme called acetylcholinesterase that is associated with clinical benefits. About 96% of donepezil binds with plasma proteins. It has minimal interferences with other drugs. The 5 mg dose of donepezil is largely safe for patients with slight to intermediate liver and kidney issues. The side effects associated with donepezil are mild and short-lived because it mimics the acetylcholine neurotransmitter. Current research recommends that donepezil has other effects on the advancement of Alzheimer's disease [19].

2.6.2 Galantamine

Galantamine originates, from the Galanthus nivalis plant. Received FDA approval in 2001 for treating mild to dementia. From researches it was indicated to have been utilized in addressing polio related myopathies and paralysis. With three centers and two aromatic protons on its tricyclic structure Galantamine exhibits potential benefits in neuromuscular blockade during surgeries and safeguarding blood vessels, in glaucoma. Additionally it possesses inflammatory and anti-diabetic properties. It is taken orally and primarily broken down by the enzymes CYP3A4 and CYP2D6. Galantamine works by blocking the action of acetylcholinesterase and adjusting the receptors to enhance memory and behavior functions in individuals. The typical adverse reactions involve feelings of nausea and vomiting along with cramps and low blood pressure seizures may also occur at times as, per reports. Meta-analysis studies confirm its effectiveness and consistency, in managing mild to cases of dementia.

2.6.3 Rivastigmine

Rivastigmine is a drug used to manage the early and moderate signs of AD. It interrupts the decomposition of acetylcholine neurotransmitters. Rivastigmine is an agonist that works to sustain the increased concentration of Rivastigmine in the brain, primarily relieving symptoms of Alzheimer's disease. Rivastigmine is taken orally and quickly absorbed by the body. Before reaching the brain, it undergoes metabolic changes in the liver. Rivastigmine serves to improve cognitive function. The 6-12mg dose is effective in enhancing the cognitive abilities [20].

2.6.4 Memantine

Memantine is the drug used to treat moderate and severe symptoms of AD. It is not useful for mild AD. It has a safe and well-tolerated profile. Memantine not only alleviates the symptoms of AD but also gives the ability to perform daily

activities. Memantine can sustain the cell's biochemical processes and membrane function by hindering glutamate hyperactivation [21]. The 10mg of memantine is advised to be administered twice daily. Various side effects associated with mementine are constipation, headache, vomiting, and urinary tract infections [22].

2.7 Medicinal Plants

Medicinal plants are also named medicinal herbs and are used to treat various diseases. Plants have different chemical substances with various functions such as defense against diseases, insects, and fungi. So far different types of phytochemicals have been identified. A single plant has different phytochemicals such plants are widely used for the synthesis of medicines. The potential world trade for medicinal plants was estimated at several hundred dollars in 2017. W.H.O. oversee the regulation of medicines production by focusing on the efficacy and quality of drugs as in many countries there are no proper regulatory authorities to supervise the production of medicines. On the other hand, medicinal plants face natural threats such as climate change, habitat destruction, erosion of soil, and acid rains may also affect the plants [23].

2.7.1 Significance of Medicinal Plants

Medicinal plants play a very significant role in disease prevention and control. For the identification of suitable medicinal plants, many conscious efforts are needed. In the field of medicinal plants, these approaches present emerging and interesting perspectives [24]. The annual sale of herbal medicine is rapidly approaching US $62 billion due to the increasing popularity of medicinal plants [25].

Medicinal plants are also popular due to their low cost as they are natural products with low toxicity and high efficacy to treat challenging diseases and it is easy to prepare and use herbal medicines. Medicinal plants include the preparation of naturally active biological substances that consist of herbal material. Certain animal plants, insects, and shells are used to manage several diseases [26].

Different reports are published on the use of medicinal plants to treat different diseases. It is also used to treat malaria [27]. On the other hand, it has also shown some cytotoxic and metabolic properties [28].

For various reasons, the attraction of herbal medicine will increase across the globe because of its efficacy, safe use, benefits, economic importance, fewer side effects, and quality control [26].

2.8 *Rhodiola rosea*

*Rhodiola rose*a L is an herb that goes by the common name roseroot or golden root. It belongs to Crassulaceae family. It grows in dry sandy soils and its habit is cold climate in Europe, Asia, and eastern North America.

It is over-the-counter medication and is mainly taken in Europe and Asia. It is used to treat fatigue, depression, digestive troubles, and nervous system issues. Advanced science categorizes *Rhodiola rosea* as an "adaptogen." [5].

Adaptogens are organic materials that make the body gain higher resistance against stress without interfering with normal functions. This concept started out as a result of the work of the Russian scientist Nikolai Lazarev in 1947. He recognized adaptogens like *Rhodiola rosea* as a potential herb that fights against stress in the body and also promotes internal balance [5].

Identifying both traditional practices and scientific data, the European Medicines Agency has authorized *Rhodiola rosea* as an adaptogen for the short-term relief of stress-triggered tiredness and frailty.

Worldwide investigations support its effectiveness in managing stress-related conditions such as depression, anxiety, fatigue, cardiovascular problems, sexual dysfunction, and nervous system disorders.

It is commonly available as a dietary supplement worldwide. *Rhodiola rosea* has acquired endorsements from the European Food Safety Authority regarding its advantages for mental and cognitive function [5].

FIGURE 2.2: *Rhodiola rosea* L plant and its rhizomes [7].

2.9 Phytochemistry of *Rhodiola rosea*

Scientists have identified a unique integration of six chemical groups in *Rhodiola rosea* roots. Among them are phenylpropanoids, rosavin, rosin, and osarian. The roots also harbor phenylethanol derivatives such as salidroside and tyrosol. Moreover, many flavonoids, including rodiolin, rodionin, rodiosin, acetylrodalgin, and tricin, enhance the root chemical profile. The rosiridol and rosaridin are the monoterpenes that augment the chemical profile of active components. Triterpenes, including daucosterol and beta-sitosterol, play a pivotal role in the phytochemical profile of *Rhodiola rosea*. Phenolic acids such as chlorogenic and hydroxycinnamic acids, along with gallic acid, complete the wide-ranging selection of compounds found within *R. rosea* root [29].

Supercritical CO2 extraction from *Rhodiola rosea* showed a rich variety of biologically active compounds. The most important constituents are acacetin, ferulic acid, luteolin, quercetin, and catechin. Other new metabolites like dihydroquercetin and eriodictyol-7-O-glucoside are also present in the extraction. Extraction has unveiled more about the diverse chemical profile and potential medicinal value of this plant [30]. These compounds have various pharmacological properties that are linked to this medicinal herb, extending from adaptogenic and antioxidant effects to potential therapeutic applications in different disease conditions [29].

2.10 Taxonomic Hierarchy

Following is the taxonomical Classification of *Rhodiola rosea* as shown in Table 2.1.

TABLE 2.1: Taxonomic Hierarchy of *Rhodiola rosea* [31].

Sr. No.	**Domain**	**Eukarya**
1	Kingdom	Plantae
2	Clade	Tracheophytes
3	Clade	Angiosperms
4	Clade	Eudicotes
5	Order	Saxifragales
6	Family	Crassulaceae
7	Genus	Rhodiola
8	Species	*R. rosea*

2.11 Medical significance of *Rhodiola rosea*

2.11.1 Neuroprotective Effects of *Rhodiola rosea*

Rhodiola rosea is a prominent herb for neuropathies. Studies reveal it may guard neurons, improve cognitive function, adjust emotional state, and mitigate swelling. A primary mechanism of *R. rosea* seems to be its proficiency to combat oxidative stress and inflammation. By alleviating oxidative stress and irritation, *R. rosea* may assist in decelerating the advancement of disease.

R. rosea enhance cognitive function by affecting neurotransmitters. These chemical messengers enable transmission between neurons. *R. rosea* seems to raise the availability of neurotransmitters, among them serotonin, dopamine, and norepinephrine the neurotra-nsmitters most important for mood, cognitive functions, and motor capabilities.

2.11.2 Cardioprotective Effects of *Rhodiola rosea*

R. rosea may have applications in enhancing cardiovascular health. Research shows that it can mitigate swelling and oxidative stress in the heart. Reports on animals have also indicated that salidroside, a chemical constituent of *R. rosea*, may prevent cardiac damage, improve circulation, and even reduce blood pressure [7].

2.12 Molecular Docking

For the past three decades, molecular docking has been used for designing drugs through computer assistance and discovering various structures in molecular biology. Docking is recommended while implementing virtual screening on the compounds present in the databases; results can be easily sorted with docking. It analyzes the ligand's interaction with the protein and locks it for optimizing the lead compounds for pharmaceutical research [32].

Many programs for docking simulation use one or several search algorithms for the anticipation of possible results of the receptor-ligand complex. This is the primary reason for molecular docking to become an essential tool for drug discovery [33].

The docking analysis provides a score for the interaction, and the fidelity of the scoring function makes docking more precise for ligand prediction, and the ligand's binding site can also be identified. It also estimates the binding affiliation, which in turn leads to identifying a potential lead drug in alliance with the target protein [33].

2.13 Tau Protein

Tau, the major MAP of mature neurons, binds and promotes tubulin polymerization and stabilizes the polymer. In Alzheimer's disease, tau protein becomes overly hyperphosphorylated, forming neurofibrillary tangles. The two major roles

of tau proteins in microtubule assembly and stabilization are lost in Alzheimer's disease, where 40% of hyperphosphorylated tau is free in the cytosol, inhibiting microtubule assembly and disrupting existing microtubules. This leads to AD P-tau which sequesters normal tau along with other key microtubule-associated proteins and dephosphorylation of tau can restore their normal activity [34].

2.14 Natural Compounds as Inhibitors of Tau Protein

In comparison with synthetic anti-tau substances, it is evident that natural products have an obvious advantage in that they can safely be fitted into a well-balanced diet and offer various other nutritional benefits. Indeed, polyphenols, including curcumin, resveratrol, and epigallocatechin-3-gallate, have already advanced clinical trials for the treatment of neurodegenerative diseases such as AD and PD. Indeed, polyphenols are useful for a variety of molecular scaffolds that could be exploited by rational drug design for the development of multifunctional anti-amyloid agents. Diverse functions like antioxidant, anti-inflammatory, and metal-chelating activities make the polyphenols exciting resources. The functional groups embedded into the polyphenol structure provide opportunities for the rational design of highly efficient drugs acting on multiple pathological pathways involved in Aβ and tau aggregation [35].

2.15 Inhibitors Against Tau Protein of Alzheimer's Disease in *Rhodiola rosea*

There are a large number of naturally occurring compounds that can serve as neuroprotective agents by inhibiting the aggregation of Tau protein. These compounds showed minimal side effects and low toxicity, and importantly, they are easily available to people. The plant *R. rosea* has been used since ancient times as medicine and herbs for anxiety, depression, fatigue, anemia, and headaches.

Chapter 3

Methodology

The following methodology was applied for the identification of the anti-Alzheimer compound from *Rhodiola rosea* through computational approaches (Figure 3.1).

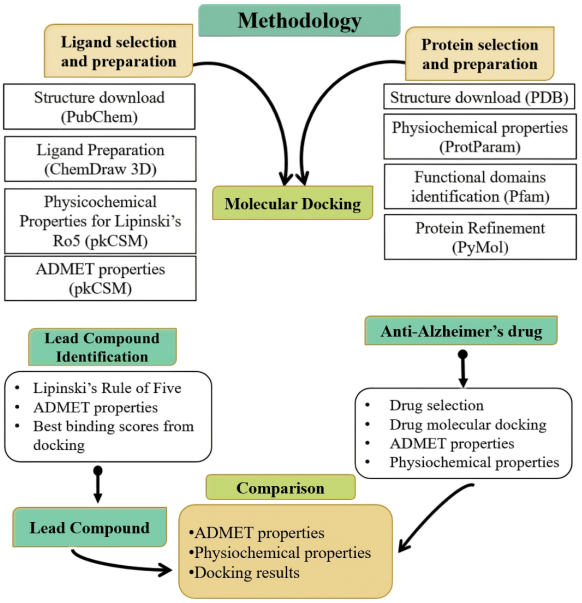


FIGURE 3.1: Flow chart of methodology.

3.1 Selection of Disease

Since there is no best drug treatment for Alzheimer's disease, neurodegenerative diseases related to Alzheimer's have become a big problem in the world. It happens to be the most severe form of dementia in human, characterized by memory,

thinking, and behavior. It is marked by the presence of amyloid plaques and neurofibrillary tangles in the brain. The conditions of Alzheimer's cause a great load on both individuals and families as well as healthcare systems. In addition to extensive research, developing effective treatments for the control of this disease's progression is very important. Despite all the time that has been spent, so much remains to be filled.

3.2 Selection and Preparation of Protein

3.2.1 Protein Selection

Initially, the target protein implicated with Alzheimer's disease was chosen. Because of its substantial role in the progression of disease, choosing a particular protein is essential. Its development is significantly influenced by the Tau protein. Alzheimer's disease can be effectively managed by inhibiting this protein.

3.2.2 Three-Dimensional Structure

A protein's distinctive form is determined by its tertiary, or three-dimensional, structure, which is essential for interactions with enzymes, substrates, and cell membrane receptors [36]. Protein Data Bank 3D protein structures were downloaded in.pdb format. Protein structural data can be found in the Protein Data Bank (https://www.rcsb.org/), a major database. Information is pulled from PDB by a number of secondary databases [37].

3.2.3 Determination of Physiochemical Properties

A protein's physical and chemical properties, which are essential to its stability and efficacy, define its structural and functional features. The ProtParam tool, which provided the protein sequence for the search query, was utilised to examine these Tau protein characteristics [38].

ProtParam provides information on a number of protein properties, such as molecular weight, aliphatic index, instability index, grand average of hydropathicity (GRAVY), atomic and amino acid composition, predicted half-life, extinction coefficient, and theoretical isoelectric point (pI) [39].

3.2.4 Determination of Functional Domains of Target Protein

The fundamental structural and functional units of proteins are called domains. These basic domains allow proteins to carry out their functions [40]. The amyloid beta protein's functional domains were found using the Pfam database. Protein domains are identified by Pfam (`http://pfam.xfam.org/`) using sequence alignments, hidden Markov models, and manual curation [41, 42].

3.2.5 Refinement of Proteins

The target protein must be prepared by eliminating the naturally occurring ligand molecules that are linked to it before molecular docking can begin. This procedure makes molecular docking more effective by removing protein binding sites from ligands. PyMol software was utilised to improve the protein by eliminating water molecules, oligosaccharides, and co-crystallized ligands.

3.3 Selection and Preparation of Ligands

3.3.1 Downloading Structures of Phytochemicals

Rhodiola rosea secondary metabolites were chosen by reviewing the literature. A comprehensive database of chemical compound information is provided by PubChem. For tasks like toxicity and bioactivity prediction, virtual screening, and the identification of possible medicinal compounds, researchers use its vast amount of data. It helps with a variety of computational and experimental tasks in the fields

of chemistry and drug development by making it easier to retrieve SMILES notation and three-dimensional structures in.sdf format [43].

3.3.2 Refinement of Ligands

Ligands were subjected to energy minimization before molecular docking. This process optimizes the structure of ligands where they achieve a stable conformation with the lowest energy. This is necessary step as retrieved 2D structure of ligands is unstable [44]. The energy of ligand structures was minimized by MM2 method via Chem 3D ultra and the resulting compounds were saved in .pdb.

3.4 Molecular Docking

It is an approach to identify the major binding modes of a protein with a ligand. It is a key tool in silico drug design. The docking procedure identifies high-dimensional spaces and uses a scoring feature based on which the candidate dockings can be ranked [45]. For this purpose, the selected ligands were used against the target proteins. This process was done through CB-Dock [46].

The interaction of ligands and the active site of the protein were analyzed to interpret docking results after obtaining the docking complex. For this purpose, Ligplot+ was used to study hydrogen bonding and hydrophobic interactions. This tool generates a 2D representation of receptor-ligand interactions [47].

3.5 Virtual Screening

3.5.1 Lipinski's Rule of Five

It was applied to predict the pharmacological profile of selected compounds. The "Rule of Five" is used to analyze compounds during drug optimization and designing for their good permeability and solubility likelihood [48].

It eliminates lead compounds that have poor physicochemical properties for oral bioavailability [49]. It is called as "Rule of Five" because the parameter values are multiples of 5. According to this rule, only compounds are selected that fulfill three or more of these physicochemical properties [50]:

- Molecular masses < 500 Da
- H-bond donors ≤ 5
- H-bond acceptors ≤ 10
- Calculated Log P (CLogP) < 5

For calculation of Lipinski's Ro5, pkCSM tool was used. It provides information about physicochemical properties of a given compound that is applied in Ro5.

3.5.2 ADMET Properties

A medication's absorption, distribution, metabolism, excretion, and toxicity (ADMET) are all evaluated early in the drug development process. A molecule needs to have certain characteristics in order to work as a medication. When it reaches the target place, its concentration must be sufficient, and it must remain there for a specific amount of time in order to produce the necessary biological activities and events. The pkCSM tool was utilised to assess ADMET characteristics. The method predicted physiochemical and ADMET properties after chemical smiles were added as input. pkCSM predicts the pharmacokinetic and toxicological characteristics of chemical substances using graph-based signatures. By forecasting the safety, potency, and drug-likeness of chemical compounds, this technology aids in the creation of new drugs [51].

3.6 Lead Compound

Using Lipinski's Ro5 as a primary filter and ADMET characteristics as a secondary filter, the lead compound was chosen. The docking scores and protein-ligand

interaction were then analysed. The compound chosen as a lead compound was the one with the greatest binding scores and all necessary parameters met.

3.7 Drug Selection and Screening

3.7.1 Anti-Alzheimer Drug Identification

The medications that can be used to treat Alzheimer's disease were identified. The KEGG disease database was used for purpose. The analysis of diseases and the genes, pathways, diagnostic markers, and treatment medications linked to them is aided by KEGG disease [52].

3.7.2 Drug Selection

To choose a suitable medication, the identified medicines were filtered out. Analysis was done on parameters such the mechanism of action, side effects, physiochemical characteristics, and ADMET qualities. The detected medications' physiochemical characteristics, ADMET characteristics, side effects, and mechanism of action were ascertained using the PubChem, pkCSM, and KEGG databases, respectively.

3.7.3 Drug Docking

The chosen medication was then docked with the Tau protein to determine and assess how well it inhibited the protein. CB-Dock was utilized for this purpose.

3.8 Comparison with Standard Drug

The proposed anti-Alzheimer compound was compared with standard drugs through their ADMET, physiochemical properties, and docking results.

Chapter 4

Results and Discussion

4.1 Selection and Preparation of Protein

4.1.1 Three Dimensional Structure

The exact 3D and spatial arrangement of all atoms, the positions of all functional groups, and the secondary structural components of a protein make up its tertiary structure. A protein's three-dimensional structure can undergo conformational changes as a result of interactions with substrates, ligands, or other proteins, which can have significant implications [53]. PDB was used to download the 3D structure of Tau protein with PDB ID of 7UPG.The total structural weight was 463.78 kD. The accession No. was https://doi.org/10.2210/pdb7UPG/pdb.

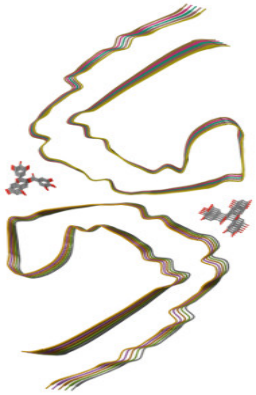


FIGURE 4.1: 3D structure of the Tau protein [54].

4.1.2 Physiochemical Properties

The molecular weight, atom count, and amino acid composition of a protein greatly influence its structure and function [39]. The pH level at which a protein carries no net charge is referred to as its isoelectric point (pI). A protein with a pI below 7 is considered acidic, while one with a pI above 7 is classified as basic [55]. Additionally, the instability index provides an estimate of a protein's stability in a test tube. Stable and unstable proteins differ greatly in the presence of particular dipeptides [56].

If a protein has an instability index above 40, it is considered unstable; if it is below 40, it is deemed stable. The aliphatic index refers to the volume taken up by the aliphatic amino acids—leucine, isoleucine, valine, and alanine—within a protein. Generally, the aliphatic index of a globular protein rises as its thermostability increases. The structure of a protein is influenced by the total of its positively and negatively charged residues.

Asparagine and glutamic acid are examples of negative residues (NR). Positively charged residues (PR) include arginine and lysine. The total quantity of negatively and positively charged residues is established because of their impact on the structure of the protein. GRAVY (Grand Average of Hydropathy) refers to the average hydropathy values of the amino acids in a protein [39]. An amino acid sidechain's hydrophilic and hydrophobic properties are indicated by a value in the hydropathy index [57]. Hydrophobic proteins are those with a positive GRAVY. The hydrophobicity increases as the number increases. Low GRAVY indicates better interaction with water molecules. Finding the protein GRAVY is essential for protein-ligand interaction in solution [58, 59].

The measurement of a protein's light absorption in water at 280 nm yields its extinction coefficient, sometimes referred to as molar absorptivity. This coefficient is influenced by the concentrations of tyrosine (Tyr), tryptophan (Trp), and cystine (a disulphide). Two values are calculated by ProtParam: one that accounts for each Cys residue from cystines and another that accounts for each Cys residue that is reduced. Half-life calculates the amount of time it will take for half of a cell's

protein synthesis to cease. ProtParam predicts a protein's half-life by analysing its N-terminal amino acids [39].

Results from table 4.1 showed that Tau protein was basic in nature with pI above 7. Tau protein was unstable. The protein was thermostable as the Aliphatic index showed higher value. Meanwhile these proteins had low GRAVY value indicating better interactions with water molecules. At 280nm the ranging extinction coefficients of tau protein were 7575 and 7450.

TABLE 4.1: Physicochemical Properties of Tau Protein (Protparam).

Sr. No.	**Properties**	**Tau Protein**
1	Molecular weight	45849.91
2	Number of amino acids	441
3	Isoelectric pH (pI)	8.24
4	Instability index (II)	47.59
5	Aliphatic index	57.30
6	Total number of atoms	6414
7	Formula	$C_{1959}H_{3197}N_{581}O_{669}S_8$
8	Positively charged residues (Arg + Lys)	58
9	Negatively charged residues (Asp + Glu)	56
10	Grand average of hydropathicity (GRAVY)	-0.868
11	Extinction coefficient (with all cys residues)	7575
12	Extinction coefficient (all cys residues reduced)	7450
13	Estimated half-life	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (*Escherichia coli*, in vivo)

4.1.3 Functional Domains

Proteins' structural and functional units are called domains. Proteins use unique, conserved domains to carry out their tasks. The active portion of a protein that interacts with other molecules is called the functional domain. Proteins acquire their domains and evolve through these domains to acquire new activities [40]. The protein's functional domains as predicted by the pfam tool are listed below.

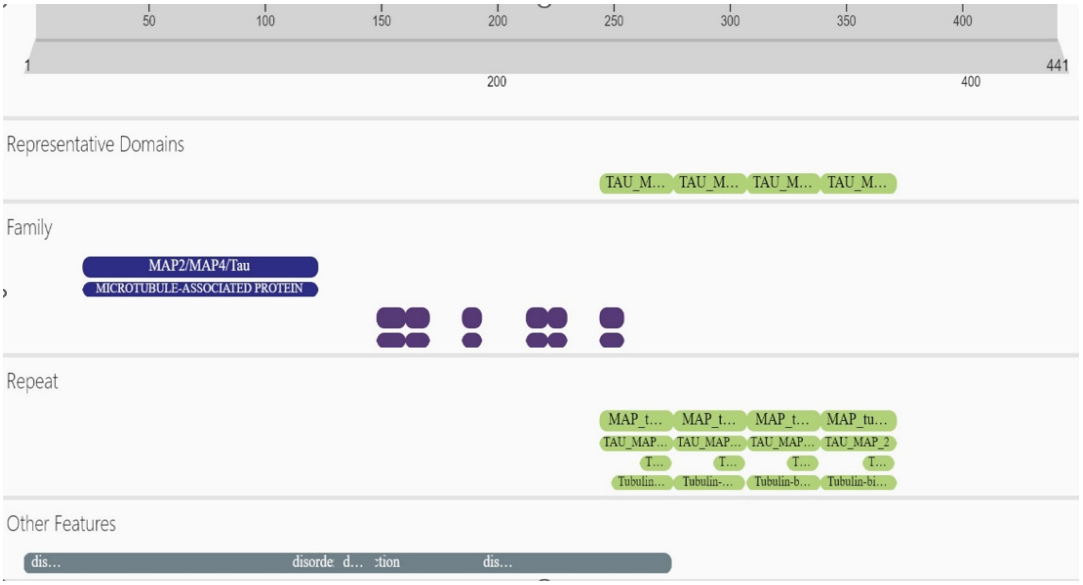


FIGURE 4.2: The functional domains of the protein to be targeted. It consisted of a 4 domains and chain with 441 residues.

4.1.4 Refinement of Protein

Pre-docking protein preparation was done by removing ligands, hetatoms and water molecules with pyMol software. Figure 4.3 showed the refined 3D structure.

FIGURE 4.3: Refined 3D structure of Tau protein.

4.2 Selection and Preparation of Ligands

4.2.1 Ligand Physicochemical Properties and Structure

The table 4.2 shows 3D structures and information of selected ligands downloaded from PubChem. These ligands were representing all the main classes of compounds like Salidroside, phenolic acids and flavonoids etc.

TABLE 4.2: Physicochemical properties and structure of ligands (PubChem)

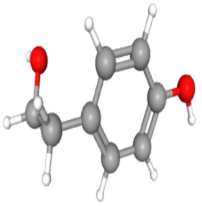
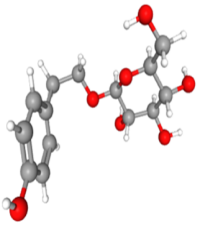
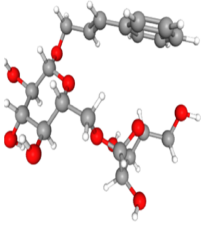
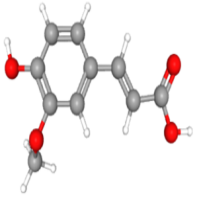
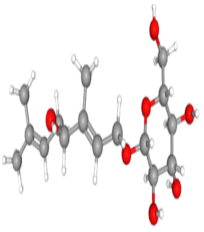
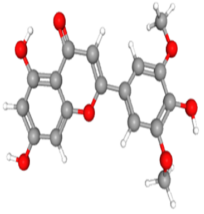
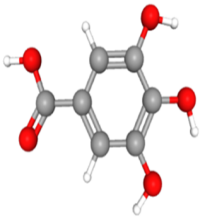
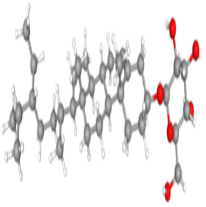
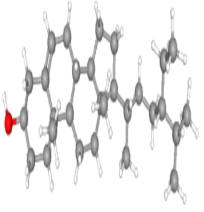
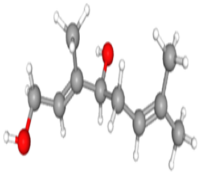
Sr #	**Ligands**	**PubChem CID**	**Formula**	**Mol. wt**	**Structure**
1	Tyrosol	10393	C8H10O2	138.16 g/mol	
2	Salidroside	159278	C14H20O7	300.30 g/mol	
3	Rosarin	10320370	C20H28O10	428.4 g/mol	
4	Ferulic acid	445858	C10H10O4	194.18 g/mol	

Table 4.2: Physicochemical properties and structure (Continued).

Sr #	**Ligands**	**PubChem CID**	**Formula**	**Mol. wt**	**Structure**
5	Rosiridin	25068281	C16H28O7	332.39 g/mol	
6	Tricin	5281702	C17H14O7	330.29 g/mol	
7	Gallic acid	370	C7H6O5	170.12 g/mol	
8	Daucosterol	5742590	C35H60O6	576.8 g/mol	
9	Beta Sitosterol	222284	C29H50O	414.7 g/mol	
10	Rosiridol	22323960	C10H18O2	170.25 g/mol	

4.2.2 Refinement of Ligands

The energy minimization method was used to prepare the pre-docking ligand. Through this method, ligands' structures are optimized to get the lowest energy and most stable conformation. Due to the energetic instability of the ligands' recovered 2D structure, this step is essential [44].

4.3 Molecular Docking

It is a technique that is used for the estimation of the strength between a ligand bonded to a receptor protein through the vina score function and for determining the correct structure of the ligand that binds to the binding site. The 3D structure of the ligands and the protein were taken to perform docking. For this purpose, CB dock an online blind auto docking tool was used. CB Dock predicted the binding sites of the protein and calculated the cavity sizes. After docking, CB Dock gave us the five best posses and receptor models. Among these five the best pose was selected depending on the vina score and the size of the cavity [46]. Results from Table 4.3 showed that binding scores of Tyrosol, Salidroside and Rosarin were -4.7,-6.6 and -7.5 respectively. Results from table 4.4 showed that binding scores of Ferulic acid, Rosiridin and Tricin were -5.8, -6.4 and -7.1 respectively.

TABLE 4.3: Docking results of Tyrosol, Salidroside and Rosarin with Tau protein (CB Dock).

Sr No	Compounds	Tyrosol	Salidroside	Rosarin
1	Binding score	-4.7	-6.6	-7.5
2	Cavity Volume	345	270	266
3	Center	0	2	13
4	Docking Size	17	26	21
5	HBD	2	5	6
6	HBA	2	7	10
7	Log P	0.927	-1.2488	-2.0204

Table 4.3: Docking results (Continued).

Sr No	Compounds	Tyrosol	Salidroside	Rosarin
8	Molecular weight g/mol	138.166	300.307	428.434
9	Rotatable bonds	2	5	8

TABLE 4.4: Docking results of Ferulic acid, Rosiridin and Tricin with Tau protein.

Sr No	Compounds	Ferulic acid	Rosiridin	Tricin
1	Binding score	-5.8	-6.4	-7.1
2	Cavity Volume	345	266	270
3	Center	2	13	2
4	Docking Size	27	24	21
5	HBD	2	5	3
6	HBA	3	7	7
7	LogP	1.4986	-0.5336	2.594
8	Molecular weight g/mol	194.186	332.393	330.292
9	Rotatable bonds	3	7	3

Results from table 4.5 showed that binding scores of Gallic acid, Daucosterol and Beta Sitosterol were -5.6, -7.9 and -7.5 respectively. Results from table 4.6 showed that binding score of Rosiridol was -5.2.

TABLE 4.5: Docking results of Gallic acid, Daucosterol and Beta Sitosterol with Tau protein (CB Dock).

Sr No	Compounds	Gallic acid	Daucosterol	Beta Sitosterol
1	Binding score	-5.6	-7.9	-7.5
2	Cavity Volume	270	266	373
3	Center	2	13	1
4	Docking Size	26	29	25

Table 4.5: Docking results (Continued).

Sr No	Compounds	Gallic acid	Daucosterol	Beta Sitosterol
5	HBD	4	4	1
6	HBA	4	6	1
7	LogP	0.5016	5.849	8.0248
8	Molecular weight g/mol	170.12	576.879	414.718
9	Rotatable bonds	1	9	6

TABLE 4.6: Docking result of Rosiridol with Tau protein (CB Dock).

Sr No	Compound	Rosiridol
1	Binding score	-5.2
2	Cavity Volume	270
3	Center	2
4	Docking Size	26
5	HBD	2
6	HBA	2
7	LogP	1.6422
8	Molecular weight g/mol	170.252
9	Rotatable bonds	4

4.4 Ligand-Protein Interactions

Interactions of ligands and target proteins were analyzed through Ligplot plus (v.1.4.5) that generates 2D structures from 3D coordinates. Results of Tau protein as described in table 4.7 showed that Ferulic acid, Tricin and Gallic acid made 02 hydrogen bonds, made hydrophobic interactions with 08, 10 and 06 residues respectively. Rosarin and Daucosterol made 06 hydrogen bonds, made hydrophobic interactions with 09, 09 residues respectively. Salidroside made 05 hydrogen

bonds and made 07 hydrophobic interactions with residues.Beta Sitosterol made 0 hydrogen bonds and made hydrophobic interactions with 11 residues. Tyrosol made 1 hydrogen bond and made hydrophobic interactions with 8 residues. Rosiridol made 2 hydrogen bonds and made hydrophobic interactions with 10 residues. Among all these ligands, Beta Sitosterol showed the most hydrophobic interactions. 2D diagrams of selected ligands interacting with Tau protein were shown from figure 4.4 to 4.13.

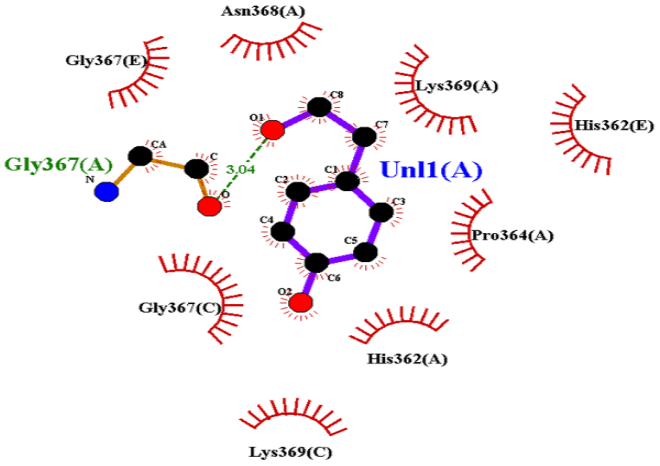


FIGURE 4.4: Interaction of Tyrosol with receptor protein.

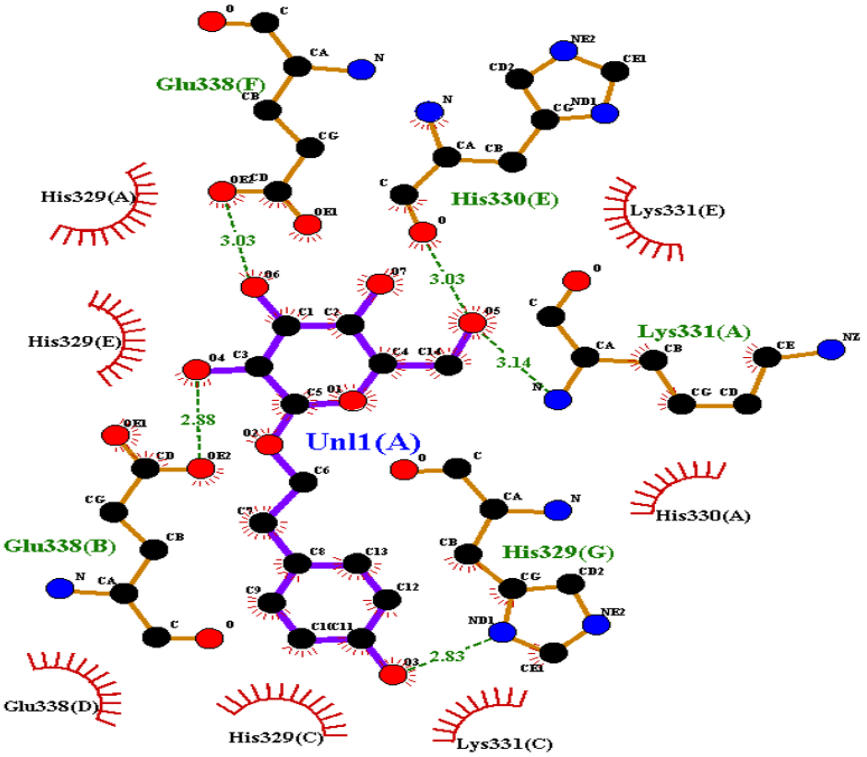


FIGURE 4.5: Interaction of Salidroside with receptor protein.

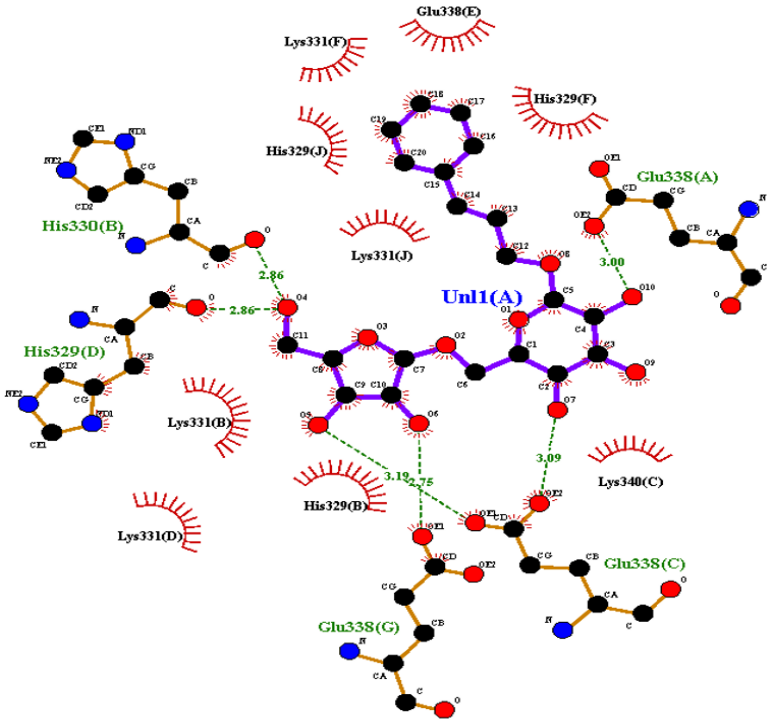


FIGURE 4.6: Interaction Rosarin with receptor protein.

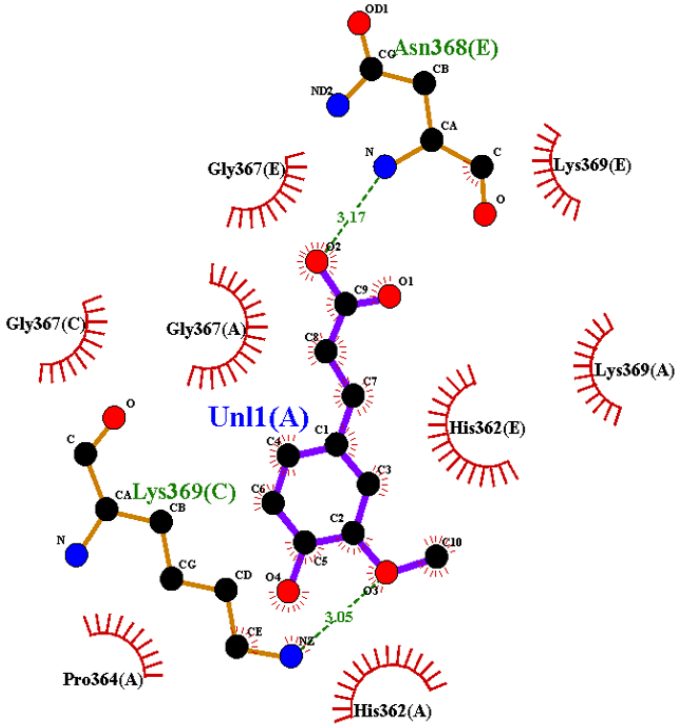


FIGURE 4.7: Interaction of Ferulic acid with receptor protein.

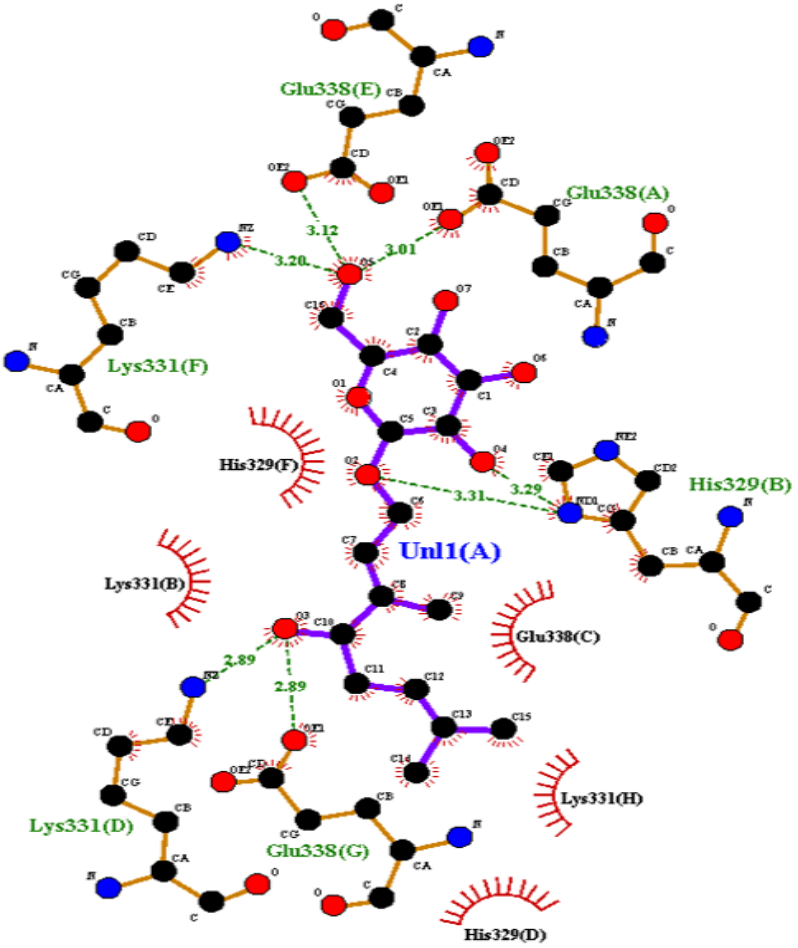


FIGURE 4.8: Interaction of Rosiridin with receptor protein.

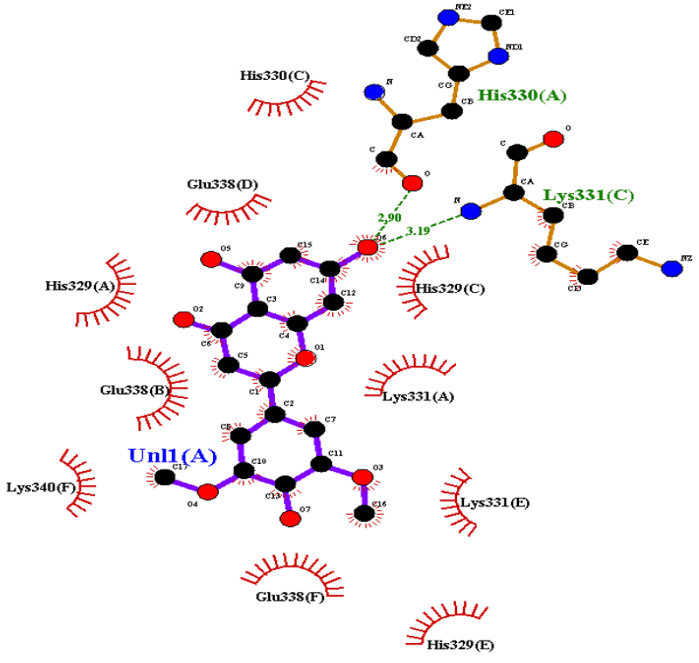


FIGURE 4.9: Interaction of Tricin with receptor protein.

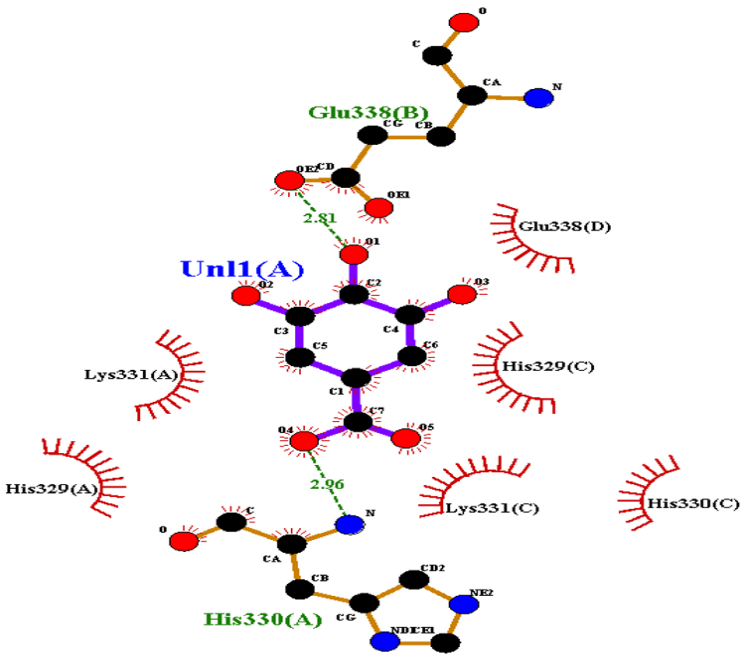


FIGURE 4.10: Interaction of Gallic acid with receptor protein.

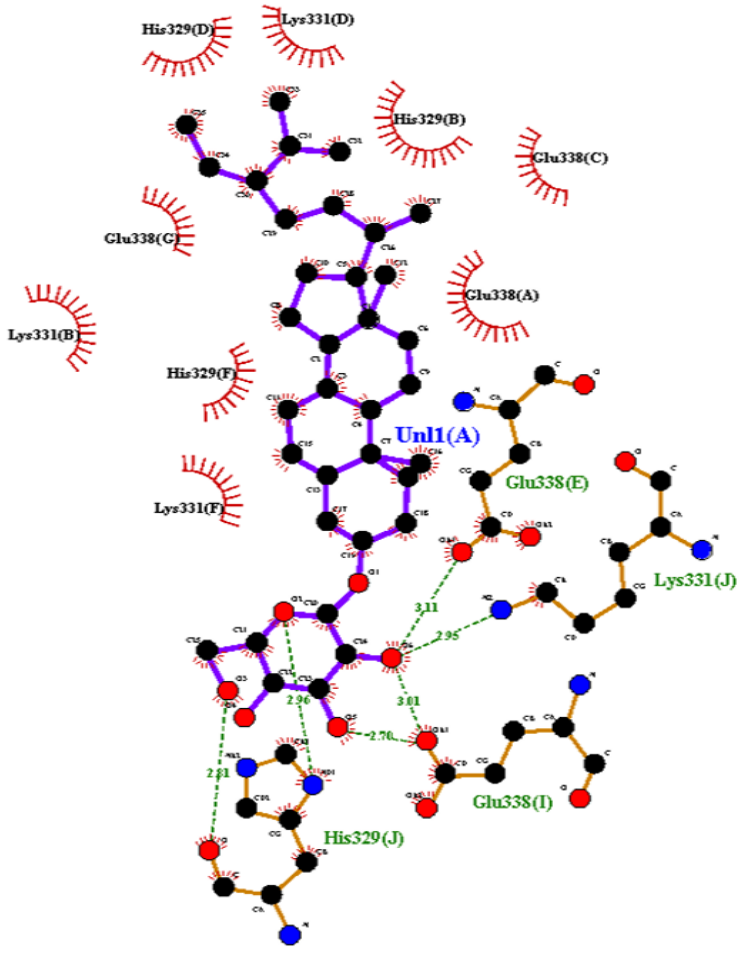


FIGURE 4.11: Interaction of Daucosterol with receptor protein.

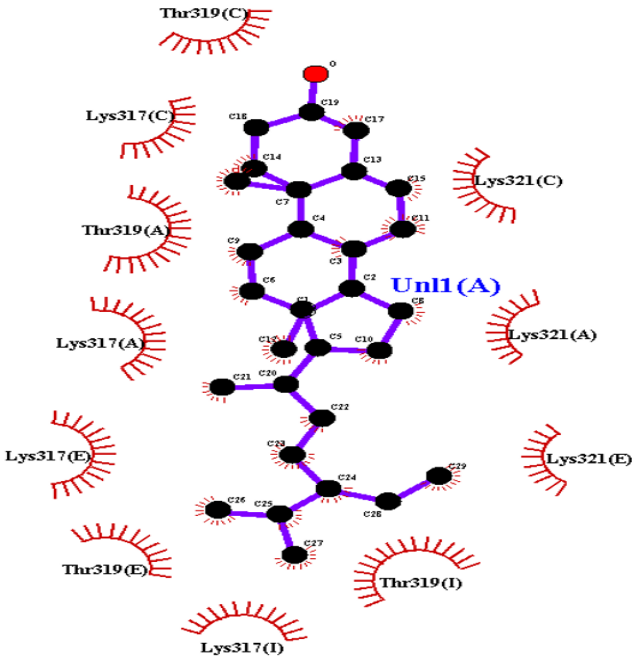


FIGURE 4.12: Interaction of Beta Sitosterol with receptor protein.

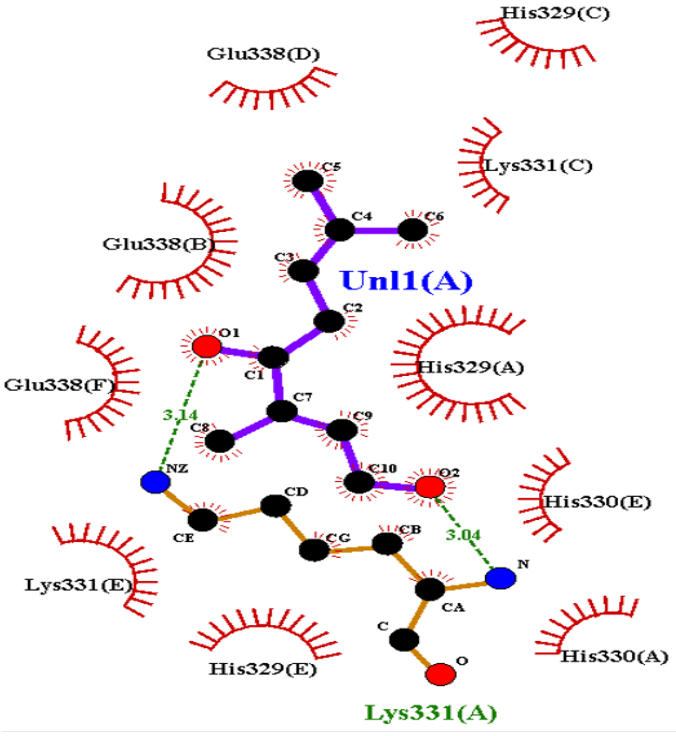


FIGURE 4.13: Interaction of Rosiridol with receptor protein.

TABLE 4.7: Interactions of Ligands with Tau protein (Ligplot)

Sr No	**Ligands**	**H-bonds**	**Interacting Amino Acids**	**HB distance (Å)**	**Hydrophobic Interactions**
1	Tyrosol	1	O-Gly367(A)-O1	3.04	Gly367(E), Asn368(A), Lys369(A), His362(A), Pro364(A), His362(A), Lys369(C), Gly367(C)
2	Salidroside	5	OE-Glu338(F)-O6, O-His330(E)-O5, O5-Lys331(A)-N, O4-Glu338(B)-OE1, O3-His329(G)-ND1	3.03, 3.03, 3.14, 2.88, 2.83	His329(A), His329(E), Glu338(D), His329(C), Lys331(C), His330(A), Lys331(E)
3	Rosarin	6	O-His330(B)-O4, 04-His329(D)-0, OE3-Glu338(A)-O10, OE3-Glu338(C)-O7, O5-Glu338(C)-OE1, O6-Glu338(G)-OE1	3.86, 3.86, 3.00, 3.09, 3.19, 2.75	His329(J), Lys331(F), Glu338(E), His329(F), Lys331(J), Lys331(B), Lys331(D), His329(B), Lys340(C)
4	Ferulic acid	2	O3-Lys369(C)-NZ, O2-Asn368(E)-N	3.05, 3.17	Gly367(C), Gly367(E), Gly367(A), Lys369(A), Lys369(E), His362(A), His362(E), Pro364(A)
5	Rosiridin	7	NZ-Lys331(F)-O5, OE3-Glu338(E)-O5, O5-Glu338(A)-OE1, O2-His329(B)-ND1, ND1-His329(B)-O4, O3-Glu338(G)-OE1, NZ-Lys331(D)-O3	3.20, 3.12, 3.01, 3.31, 3.29, 2.89, 2.89	Lys331(B), His329(F), Glu338(C), Lys331(H), His329(D)

Table 4.7: Interactions of Ligands (Continued).

Sr No	**Ligands**	**H-bonds**	**Interacting Amino Acids**	**HB distance (Å)**	**Hydrophobic Interactions**
6	Tricin	2	O-His330(A)-O6, O6-Lys331(C)-N	2.90, 3.19	His330(C), Glu338(D), His329(A), Glu338(B), Lys340(F), Glu338(F), His329(E), Lys331(E), Lys331(A), His329(C)
7	Gallic acid	2	OE2-Glu338(B)-O1, N-His330(A)-O4	2.81, 2.96	Lys331(A), His329(A), Glu338(D), His329(C), Lys331(C), His330(C)
8	Daucosterol	6	OK1-Glu338(E)-O6, M2-Lys331(J)-O6, O6-Glu338(I)-OK1, OK1-Glu338(I)-O5, O1-His329(J)-MD1, O-His329(J)-O3	3.11, 2.95, 3.01, 2.70, 2.96, 2.81	Lys331(B), His329(F), Lys331(F), Glu338(G), His329(D), Lys331(D), His329(B), Glu338(C), Glu338(A)
9	Beta Sitosterol	-	-	-	Thr319(E), Lys317(E), Lys317(A), Thr319(A), Lys317(C), Thr319(C), Lys321(C), Lys321(A), Lys321(E), Thr319(I), Lys319(I)
10	Rosiridol	2	N-Lys331(A)-O2, NZ-Lys331(A)-O1	3.04, 3.14	His330(E), His330(A), His329(A), Glu338(B), Glu338(F), Glu338(D), Lys331(E), His329(E), Lys331(C), His329(C)

4.5 Lipinski's Rule of Five

A compound's druggability is predicted by Lipinski's Rule of Five, which takes into account four physicochemical parameters: molecular weight < 500, log P<5, H-bond donors ≤5, and H-bond acceptors ≤ 10 [50]. A compound's route of absorption and mode of distribution are related to its term "drug-likeness" [60]. A substance is deemed drug-like if it complies with three or more criteria, and it is linked to poor body absorption if it complies with two or more [61]. According to the results in table 4.8, Tyrosol, salidroside, Ferulic acid, rosiridin, Tricin, Gallic acid and Rosiridol were obeying all rules. Rosarin, Beta Sitosterol was not obeying only one rule while Daucosterol was violating two rules.

TABLE 4.8: Lipinski's rule of five on selected ligands (PkCSM)

Sr No	**Ligands**	**Mol weight**	**Log p value**	**H-bond donor**	**H-bond acceptor**
1	Tyrosol	138.166	0.927	2	2
2	Salidroside	300.307	-1.2488	5	7
3	Rosarin	428.434	-2.0204	6	10
4	Ferulic acid	194.186	1.4986	2	3
5	Rosiridin	332.393	-0.5336	5	7
6	Tricin	330.292	2.594	3	7
7	Gallic acid	170.12	0.5016	4	4
8	Daucosterol	576.859	5.849	4	6
9	Beta Sitosterol	414.718	8.0248	1	1
10	Rosiridol	170.252	1.6422	2	2

4.6 ADMET Properties

When a drug is administrated into the body, it undergoes various systematic steps as it gets absorbed, distributed through the body, metabolized and then excreted (ADME) [62].

4.6.1 Absorption

The process by which the medication enters the body after being administered is known as absorption. The absorption characteristics of various administration routes vary, which impacts the drug's concentration and rate of delivery to the intended target site [63, 64].

For possible drug absorption within the body, parameters such water solubility, Caco2 permeability, skin permeability, intestinal absorption, P-glycoprotein I and II inhibitors, and P-glycoprotein substrate are anticipated. Water solubility is the ability of a molecule to dissolve in water at normal temperature. The hydrophobic or hydrophilic character of a medication determines its absorption and passage through cell membranes; hydrophilic medicines exhibit superior absorption. A compound's water solubility is indicated by a more positive value [51]. When a medicine is taken orally, its permeability across the intestinal mucosa is predicted using monolayer Caco-2 cells. Human epithelial cells from colorectal adenocarcinoma are the source of these cell lines.

If a compound's Papp value is greater than 8×10-6 cm/s (pkCSM: 0.90), it is considered to have higher Caco-2 permeability [65]. Intestinal absorption predicts how much medication may be absorbed in the small intestine. Poor absorption is defined as less than 30%. Skin permeability is an important factor for drugs that are applied topically. If it is less than -2.5 logkp, it is regarded as having poor skin permeability. P-glycoprotein serves as a barrier in biology. Toxic and xenobiotic substances are released from the cell by this ATP-binding cassette transporter [51]. P-glycoprotein can be inhibited or induced by a substance that is its substrate. In contrast to P-glycoprotein inhibitors, which decrease its activity and hence increase absorption and bioavailability, P-glycoprotein inducers result in low oral absorption and bioavailability [66].

According to table 4.9, all these ligands showed low water solubility, low skin permeability. The tyrosol showed highest Caco-2 permeability and Beta Sitosterol exhibited highest intestinal absorption. Moreover, they gave negative results for P-glycoprotein model except Daucosterol and Beta Sitosterol.

Table 4.9: Absorption properties of Ligands (pkCSM).

S #	Ligands	Water solubility (log mol/L)	Caco2 permeability (logPapp in 10-6 cm/s)	Intestinal absorption (human) (%)	Skin Permeability (log Kp)	P-gp substrate (Yes/No)	P-gp I inhibitor (Yes/No)	P-gp II inhibitor (Yes/No)
1	Tyrosol	-1.146	1.691	85.255	-2.796	No	No	No
2	Salidroside	-1.776	0.46	45.49	-2.796	No	No	No
3	Rosarin	-2.307	0.262	38.619	-2.738	Yes	No	No
4	Ferulic acid	-2.817	0.176	93.685	-2.72	No	No	No
5	Rosiridin	-1.971	-0.132	41.835	-2.99	No	No	No
6	Tricin	-3.276	0.12	89.713	-2.735	Yes	No	No
7	Gallic acid	-2.56	-0.081	43.374	-2.735	No	No	No
8	Daucosterol	-4.741	0.472	79.677	-2.748	Yes	Yes	Yes
9	Beta Sitosterol	-6.773	1.201	94.464	-2.783	No	Yes	Yes
10	Rosiridol	-1.542	1.585	93.643	-2.962	No	No	No

4.6.2 Distribution

The process by which the medication passes throughout the body and reaches the target receptor site at an effective concentration is known as distribution. There are two key components to an efficient medicine delivery strategy. First one is the plasma-protein bound/unbound ratio, and the other is the volume of distribution, which is its assigned compartmental destination [63].

The distribution of a substance is assessed using models such the human fraction unbound, the volume of distribution, the permeability of the blood-brain barrier, and the permeability of the central nervous system.

Volume of distribution (VDss) is calculated by amount of drug in body compartments divided by drug concentration in plasma. logL/ kg value above 2.81 is considered high, whereas value below 0.71 is considered low.

Drugs generally bind with serum proteins whereas only free drug (fraction unbound) can act at the target sites (receptors), move through compartments or be excreted. High concentration of free drug (FU) at receptor site produces high effect [67].

Blood-brain barrier protects brain from exogenic compounds. This is an important factor to consider in drug designing in order to reduce toxicity and side-effects of compounds that can cross this barrier. This model calculates ratio of drug concentration in brain to drug concentration in plasma. Value greater than 0.3 predicts the ability of a compound to cross the barrier while value less than -1 shows inability of drug to cross the barrier [68].

CNS permeability is calculated by Blood-brain permeability into surface area. Value less than -3 shows inability of a compound to cross CNS while value above -2 shows ability of a compound to cross CNS [51].

According to table 4.10, Tricin exhibited highest volume of distribution. All the ligands had high BBB permeability. All the ligands demonstrated low CNS permeability except Tyrosol, Ferulic acid, Beta Sitosteriol and Rosiridol.

TABLE 4.10: Distribution properties of Ligands (pkCSM)

Sr no	**Ligands**	**Volume of distribution (VDss) (human) (log L/kg)**	**Fraction unbound (FU) (human)**	**BBB permeability (log BB)**	**CNS permeability (log PS)**
1	Tyrosol	-0.114	0.485	-0.218	-2.109
2	Salidroside	-0.043	0.577	-0.872	-3.761
3	Rosarin	-0.053	0.55	-1.013	-4.272
4	Ferulic acid	-1.367	0.343	-0.239	-2.612
5	Rosiridin	-0.193	0.654	-0.907	-3.623
6	Tricin	0.798	0.084	-1.115	-3.411
7	Gallic acid	-1.855	0.617	-1.102	-3.74
8	Daucosterol	-1.163	0.078	-0.785	-3.021
9	Beta Sitosterol	0.193	0	0.781	-1.705
10	Rosiridol	-0.033	0.559	0.24	-2.591

4.6.3 Metabolism

Metabolism is the process through which a medication is converted into other molecules. Although it mostly involves phase I and phase II reactions in the liver, metabolism can occasionally also take place in other organs such the kidney, lungs, skin, etc. [63]. In the case of prodrug administration, such as codeine, it transforms the drug into its active form or enhances its water solubility for proper clearance [69]. Cytochrome P450 is a family of enzymes that metabolizes various drugs (xenobiotics). These enzymes either activate or deactivate the drugs. PkCSM tool examines the metabolism of a compound on basis of Cytochrome P450 enzymes as their substrate or inhibitor. More than 90% of the drugs are metabolized by CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4/5 [70].

If a compound is potential inhibitor of a CYP450 enzyme, then it can reduce the activity of drug. On the other hand, if it acts as a substrate of a CYP450 enzyme, it can enhance drug activity [51]. The inhibitory effect of a compound on these enzymes can result in various harmful reactions, especially for drugs with narrow therapeutic index [71]. Table 4.11 showed that none of the above ligands was substrates or inhibitors for any of the listed cytochrome P450 (CYP) enzymes. According to table 4.12 Ferulic acid and Rosiridin was not an inhibitor or substrate of any of CYP450 enzymes while Tricin was a inhibitor of CYP2C19 and CYP1A2 enzymes.

TABLE 4.11: Metabolism properties of Tyrosol, Salidroside and Rosarin.

Sr. No	**Ligands**	**Tyrosol**	**Salidroside**	**Rosarin**
1	CYP2D6 substrate	No	No	No
2	CYP3A4 substrate	No	No	No
3	CYP1A2 inhibitor	No	No	No
4	CYP2C19 inhibitor	No	No	No
5	CYP2C9 inhibitor	No	No	No
6	CYP2D6 inhibitor	No	No	No
7	CYP3A4 inhibitor	No	No	No

TABLE 4.12: Metabolism properties of Ferulic acid, Rosiridin and Tricin (pkCSM).

Sr. No	**Ligands**	**Ferulic acid**	**Rosiridin**	**Tricin**
1	CYP2D6 substrate	No	No	No
2	CYP3A4 substrate	No	No	No
3	CYP1A2 inhibitor	No	No	Yes
4	CYP2C19 inhibitor	No	No	Yes
5	CYP2C9 inhibitor	No	No	No
6	CYP2D6 inhibitor	No	No	No
7	CYP3A4 inhibitor	No	No	No

According to the table 4.13, Daucosterol and Beta Sitosterol were substrates of CYP3A4. Gallic acid was not an inhibitor or substrate of any of CYP450 enzymes. According to the table 4.14, Rosiridol was not an inhibitor or substrate of CYP450 enzymes.

TABLE 4.13: Metabolism properties of Gallic acid, Daucosterol and Beta Sitosterol (pkCSM)..

S. No	**Ligands**	**Gallic acid**	**Daucosterol**	**Beta Sitosterol**
1	CYP2D6 substrate	No	No	No
2	CYP3A4 substrate	No	Yes	Yes
3	CYP1A2 inhibitor	No	No	No
4	CYP2C19 inhibitor	No	No	No
5	CYP2C9 inhibitor	No	No	No
6	CYP2D6 inhibitor	No	No	No
7	CYP3A4 inhibitor	No	No	No

TABLE 4.14: Metabolism properties of Rosiridol (PkCSM).

S. No	**Ligands**	**Rosiridol**
1	CYP2D6 substrate	No
2	CYP3A4 substrate	No
3	CYP1A2 inhibitor	No
4	CYP2C19 inhibitor	No
5	CYP2C9 inhibitor	No
6	CYP2D6 inhibitor	No
7	CYP3A4 inhibitor	No

4.6.4 Excretion

Excretion is the process by which a substance is removed from the body. Although the kidneys are most frequently involved, some medications can also be eliminated

by the lungs, gastrointestinal system, skin, etc. Two crucial elements taken into account in drug excretion are drug half-life and clearance (elimination ratio) [63].

The PkCSM tool forecasts a compound's excretion based on its OCT2 substrate and overall clearance rate. The rate at which a medication is eliminated relative to its plasma concentration is used to assess clearance. A low clearance rate results in prolonged medication exposure in the systemic circulation, which might trigger negative effects. Furthermore, a high clearance rate causes early excretion, which reduces the drug's body's ability to retain it and its effectiveness [72]. A transport protein called renal organic cation transporter 2 (OCT2) aids in the kidneys' removal of drugs [73]. If a compound is OCT2 substrate, it aids in excretion process. Table 4.15, showed that the total clearance values of above ligands, ranging from 0.187 to 1.672 log ml/min/kg. None of the ligands interacted with the renal OCT2 transporter.

TABLE 4.15: Excretion properties of all ligands (pkCSM).

Sr No	**Ligands**	**Total Clearance (log ml/min/kg)**	**Renal OCT2 substrate**
1	Tyrosol	0.283	No
2	Salidroside	0.187	No
3	Rosarin	1.196	No
4	Ferulic acid	0.623	No
5	Rosiridin	1.672	No
6	Tricin	0.62	No
7	Gallic acid	0.518	No
8	Daucosterol	0.689	No
9	Beta Sitosterol	0.628	No
10	Rosiridol	0.473	No

4.6.5 Toxicity

Drug toxicity screening is a crucial step in the development of new medications and aids in the early identification of those that may have major side effects. More than half of those compounds are attrited due to undesirable effects that drug candidates create [74].

Using its toxicity models, PkCSM tools forecast possible harmful substances. AMES toxicity tests a medication candidate's mutagenesis potential using microorganisms. A positive result indicates that the chemical is carcinogenic and mutagenic. The maximum tolerable dose of a medicine that does not result in toxicity is predicted by max.tolerated dose. This aids in the early recommendation of a suitable dosage amount. The maximum tolerable dose is high when the value is greater than 0.477 log (mg/kg/day), and vice versa [51]. Potassium channel activation is mediated by the hERG I and II genes. When these genes are inhibited, channels are blocked, which causes QT syndrome and cardiotoxicity [75].

When 50% of rats die after receiving a particular dose all at once, the lethal dose (LD50) of the substance is measured via oral rat acute toxicity. Conversely, chronic toxicity quantifies the LOAEL, or lowest observed adverse effect. Hepatotoxicity models forecast medications that harm and malfunction the liver. Skin sensitisation tests a drug's ability to trigger a skin allergy. *T. pyriformis* is a protozoa bacterium that causes poisoning. This test evaluates a substance's capacity to prevent bacterial development in order to estimate the possible harm it may cause. A value greater than -0.5 log ug/L suggests that a substance is hazardous. Minnow toxicity measures a compound's lethal concentration (LC50) at which 50% of bait fish (Flathead Minnows) are killed. Value below 0.5 mM indicated high acute toxicity of a compound [51]. Table 4.16, showed that Tyrosol exhibited no AMES toxicity but had potential for skin sensitization. Both compounds were predicted to had similar maximal tolerated doses and no hepatotoxicity or hERG inhibition. Table 4.17 showed that Rosarin exhibited AMES toxicity while Ferulic acid was non-toxic. Both were non-hepatotoxic and had no potential for skin sensitization.

TABLE 4.16: Toxicity properties of Tyrosol and Salidroside (pkCSM).

Sr No	**Model Name**	**Predicted Value of Tyrosol**	**Predicted Value of Salidroside**
1	AMES toxicity	No	Yes
2	Max. tolerated dose (human)	1.396	1.533
3	hERG I inhibitor	No	No
4	hERG II inhibitor	No	No
5	Oral Rat Acute Toxicity (LD50)	1.861	1.924
6	Oral Rat Chronic Toxicity (LOAEL)	2.331	4.228
7	Hepatotoxicity	No	No
8	Skin Sensitisation	Yes	No
9	*T.Pyriformis* toxicity	-0.244	0.285
10	Minnow toxicity	2.207	4.495

TABLE 4.17: Toxicity properties of Rosarin and Ferulic acid.

Sr No	**Model Name**	**Predicted Value of Rosarin**	**Predicted Value of Ferulic Acid**
1	AMES toxicity	Yes	No
2	Max. tolerated dose (human)	0.648	1.082
3	hERG I inhibitor	No	No
4	hERG II inhibitor	No	No
5	Oral Rat Acute Toxicity (LD50)	2.246	2.282
6	Oral Rat Chronic Toxicity (LOAEL)	3.858	2.065
7	Hepatotoxicity	No	No

Table 4.17: Toxicity properties (Continued).

Sr No	**Model Name**	**Predicted Value of Rosarin**	**Predicted Value of Ferulic Acid**
8	Skin Sensitisation	No	No
9	*T.Pyriformis* toxicity	0.285	0.271
10	Minnow toxicity	6.529	1.825

Table 4.18 showed that both Rosiridin and Tricin were non-toxic .Both exhibited no hepatotoxicity and hERG inhibition. Table 4.19 showed that Gallic acid and Daucosterol both were non-toxic. Both were non-hepatotoxic and had no potential for skin sensitization. Table 4.20 showed that Both Beta Sitosterol and Rosiridol were non-toxic. Beta Sitosterol was hERGII. Both were non-hepatotoxic and but Rosiridol had potential for skin sensitization.

TABLE 4.18: Toxicity properties of Rosiridin and Tricin.

Sr No	**Model Name**	**Predicted Value of Rosiridin**	**Predicted Value of Tricin**
1	AMES toxicity	No	No
2	Max. tolerated dose (human)	1.395	0.351
3	hERG I inhibitor	No	No
4	hERG II inhibitor	No	No
5	Oral Rat Acute Toxicity (LD50)	1.834	2.229
6	Oral Rat Chronic Toxicity (LOAEL)	3.109	1.82
7	Hepatotoxicity	No	No
8	Skin Sensitisation	No	No
9	*T.Pyriformis* toxicity	0.285	0.329
10	Minnow toxicity	3.878	1.754

Table 4.19: Toxicity properties of Gallic acid and Daucosterol.

Sr No	**Model Name**	**Predicted Value of Gallic Acid**	**Predicted Value of Daucosterol**
1	AMES toxicity	No	No
2	Max. tolerated dose (human)	0.7	-0.887
3	hERG I inhibitor	No	No
4	hERG II inhibitor	No	No
5	Oral Rat Acute Toxicity (LD50)	2.218	2.571
6	Oral Rat Chronic Toxicity (LOAEL)	3.06	3.293
7	Hepatotoxicity	No	No
8	Skin Sensitisation	No	No
9	*T.Pyriformis* toxicity	0.285	0.285
10	Minnow toxicity	3.188	-0.811

Table 4.20: Toxicity properties of Beta Sitosterol and Rosiridol.

Sr No	**Model Name**	**Predicted Value of Beta Sitosterol**	**Predicted Value of Rosiridol**
1	AMES toxicity	No	No
2	Max. tolerated dose (human)	-0.621	1.022
3	hERG I inhibitor	No	No
4	hERG II inhibitor	Yes	No
5	Oral Rat Acute Toxicity (LD50)	2.552	1.379
6	Oral Rat Chronic Toxicity (LOAEL)	0.855	2.756

Table 4.20: Toxicity properties (Continued).

Sr No	**Model Name**	**Predicted Value of Beta Sitosterol**	**Predicted Value of Rosiridol**
7	Hepatotoxicity	No	No
8	Skin Sensitisation	No	Yes
9	*T.Pyriformis* toxicity	0.43	-0.337
10	Minnow toxicity	-1.802	1.873

4.7 Lead Compound Identification

Lipisnki's Ro5 and ADMET properties as primary filter while binding scores as secondary filter was applied to identify the lead compound. Parameters were applied such as violating not more than one Lipisnki's Ro5, complying with BBB and CNS permeability models of distribution, AMES toxicity, hepatotoxicity, skin sensitization and hERG I and II inhibitor models for toxicity and high binding scores. Daucosterol with highest binding score -7.9 was knocked out for violating the two rules of Lipinski while rest of the ligands were following the rules. In secondary filter screening ADMET properties were observed. Rosiridin, Tricin, Gallic acid were knocked out for violating parameter of BBB and CNS permeability. Salidroside and Rosarin were removed for causing AMES toxicity while Tyrosol and Rosiridol for Skin sensitization. Beta Sitosterol was knocked out for being hERG II inhibitor. After primary and secondary filter screening remaining compound Ferulic acid was chosen as a Lead compound.

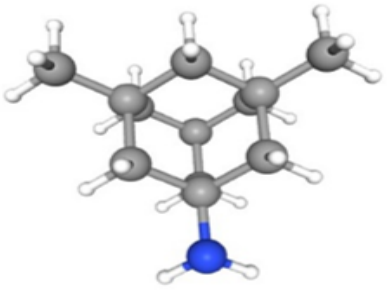
4.8 Drug Selection

KEGG disease database (https://www.genome.jp/kegg/disease/) was used for identification and selection of anti-Alzheimer's drug.

4.8.1 Memantine

Memantine is a prescription drug widely used for the treatment of Alzheimer's disease. It works by inhibiting tau protein. Chemical and structural information shown in table 4.21, was retrieved through PubChem.

TABLE 4.21: Chemical and structural information of Memantine (PubChem).

Property	**Value**
Drug	Memantine
PubChem CID	4054
Rotatable Bonds	0
Formula	$C_{12}H_{21}N$
Structure	

4.8.2 Mechanism of Action

Memantine is characterized as a low-to-medium affinity, uncompetitive, voltage-dependent channel blocker that exhibits partial trapping properties. Its mechanism of action primarily involves the antagonism of the NMDA receptor. Research indicates that memantine serves as a superior neuroprotective agent and NMDA-induced current inhibitor in vivo when compared to another channel blocker, MK-801. A significant advantage of memantine is its ability to maintain near-physiological NMDA activity in the brain, even in the presence of elevated glutamate levels, thereby ensuring minimal organ function impairment. Additionally, memantine's rapid-response kinetics and comparatively lower incidence of side effects enhance its therapeutic profile against NMDA neurotoxicity, distinguishing it from traditional open-channel blockers. Furthermore, memantine has been shown

to inhibit ADDL-induced reactive oxygen species (ROS) formation, which helps to reduce increases in calcium ions (Ca2+) and oxidative stress associated with ADDLs. Conversely, memantine's inhibition of internal ribosome entry site (IRES) activity may also prevent the expression of amyloid precursor protein and tau protein, potentially reducing the availability of proteins that exacerbate Alzheimer's symptoms [76].

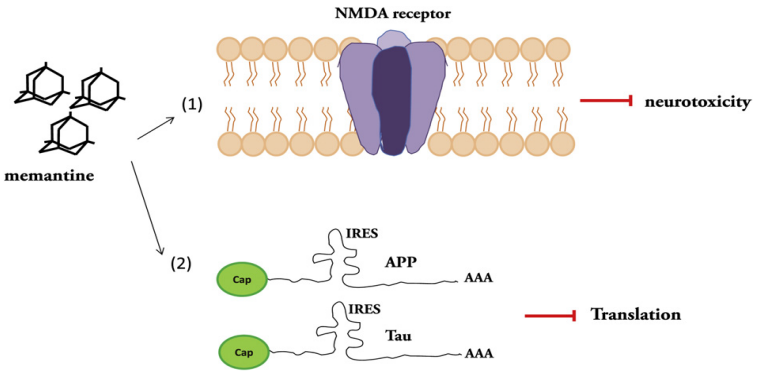


FIGURE 4.14: Memantine has a "dual" mechanism of action in the treatment of Alzheimer's disease. On One hand, it can block NMDA receptors, thus preventing neurotoxicity caused by glutamate. On the Other hand, memantine might have an inhibitory effect on translation of Tau and APP proteins Through interference with IRES activity in the mRNA of the latter [76].

4.8.3 Effects on Body

Some of the frequent side effects that have been recognized in the clinical trials of memantine dizziness, are headache, confusion, diarrhea, and constipation. It also causes other symptoms like fatigue, pain, hypertension, weight gain, hallucinations, aggressive behavior, vomiting, abdominal discomfort, and urinary incontinence. Some of the more severe side effects have been reported only infrequently through post-marketing surveillance, clinical trials or case reports. The neurological side effects include akathisia, dystonia, hyperkinesia, involuntary movements, neuroleptic malignant syndrome, opisthotonos, akinesia, akathisia, choreoathetosis, and tardive dyskinesia.

Cardiovascular side effects could be bradyarrhythmia, congestive heart failure, myocardial infarction, peripheral oedema, syncope, and tachycardia. In this respect, the major system affected is the endocrine system, which could lead to changes

in weight, with other gastrointestinal symptoms resulting from anorexia and nausea. The hematologic system mentions anemia, in addition to hepatic disorders including hepatitis and hepatic failure. The complications of the renal system can be acute renal failure and urinary tract infections.

The complaints of the respiratory system are bronchitis, pneumonia and upper respiratory tract infections. Dermatological reactions can be as simple as a rash to as severe as Stevens-Johnson syndrome. Arthralgia is also among the symptoms reported. Other adverse events include falls with injuries, influenza like symptoms and neuroleptic malignant syndrome [77].

4.9 Physicochemical Properties

Information related to drug was retrieved through, pkCSM tool (http://biosig.unimelb.edu.au/pkcsm/). Four of these properties are used for calculation of Lipinski's Rule of Five as shown in table 4.22.

TABLE 4.22: Physicochemical Properties (Lipinski's Ro5) of Memantine .

Sr no	Physicochemical Properties	Memantine
1	Molecular Weight	179.307
2	LogP value	2.6941
3	Hydrogen bond acceptors	1
4	Hydrogen bond donors	1
5	Rotatable bonds	0

4.10 ADMET Properties

When a drug is administrated into the body, it undergoes various systematic steps as it gets absorbed, distributed through the body, metabolized and then excreted (ADME) [62]. pkCSM tool (http://biosig.unimelb.edu.au/pkcsm/) was used for ADMET properties of drug.

4.10.1 Absorption

According to the results shown in table 4.23, water solubility and skin permeability of Memantine was low. The Caco2 permeability and intestinal absorption was high while it gave negative results for P-glycoprotein substrate and inhibitor.

Table 4.23: Absorption properties of Memantine (PkCSM).

Sr No	Model Name	Predicted Value
1	Water solubility (log mol/L)	-2.317
2	Caco2 permeability (log Papp in 10-6 cm/s)	1.329
3	Intestinal absorption (human) (% Absorbed)	91.234
4	Skin Permeability (log Kp)	-2.436
5	P-gp substrate (Yes/No)	No
6	P-gp I inhibitor (Yes/No)	No
7	P-gp II inhibitor (Yes/No)	No

4.10.2 Distribution

According to the results in table 4.24, volume of distribution was high. The BBB and CNS permeability of memantine were 0.603 and -2.478.

Table 4.24: Distribution Properties of Memantine (PkCSM).

Sr No	Model Name	Predicted Value
1	Volume of distribution (VDss) (human)	0.988
2	Fraction unbound (FU) (human)	0.601
3	BBB permeability	0.603
4	CNS permeability	-2.478

4.10.3 Metabolism

Memantine was a substrate of CYP3A4 enzyme as shown in table 4.25.

Table 4.25: Metabolic properties of Memantine (PkCSM).

Sr No	Model Name	Predicted Value
1	CYP2D6 substrate	No
2	CYP3A4 substrate	Yes
3	CYP1A2 inhibitor	No
4	CYP2C19 inhibitor	No
5	CYP2C9 inhibitor	No
6	CYP2D6 inhibitor	No
7	CYP3A4 inhibitor	No

4.10.4 Excretion

The results in table 4.26 showed positive total clearance of Memantine. Moreover, the drug was not a substrate of renal OCT2.

Table 4.26: Excretion properties of Memantine (PkCSM).

Sr No	Model Name	Predicted Value
1	Total Clearance (log ml/min/kg)	0.548
2	Renal OCT2 substrate	No

4.10.5 Toxicity

According to table 4.27, AMES toxicity, minnow toxicity, hepatotoxicity and hERG inhibitor results of memantine were negative. It showed the drug was not toxic for *T. pyriformis* and had a slightly low maximum tolerated dose. It exhibited positive results for skin sensitization.

TABLE 4.27: Toxicity properties of Memantine (PkCSM).

Sr No	Model Name	Predicted Value
1	AMES toxicity	No
2	Max. tolerated dose (human) (log mg/kg/day)	0.322
3	hERG I inhibitor	No
4	hERG II inhibitor	No
5	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.673
6	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg$_b w$/*day*)	1.25
7	Hepatotoxicity	No
8	Skin Sensitisation	Yes
9	*T. pyriformis* toxicity (log ug/L)	0.468
10	Minnow toxicity (log mM)	1.426

4.11 Molecular Docking

Following results were obtained through docking done by CB Dock tool as shown in table 4.28.

TABLE 4.28: Molecular docking of Drug with Tau protein.

Sr No	Drug	Binding Score (kcal/mol)	Cavity Volume (A3)	Center	Docking Size
1	Memantine	-4.5	373	1	23

4.12 Drug-Protein Interactions

Interactions of Memantine with Tau protein as analyzed through Ligplot plus (v.1.4.5) are given in figure 4.15. According to the results in table 4.29, Memantine

made 1 hydrogen bond and 05 hydrophobic interactions with Tau protein.

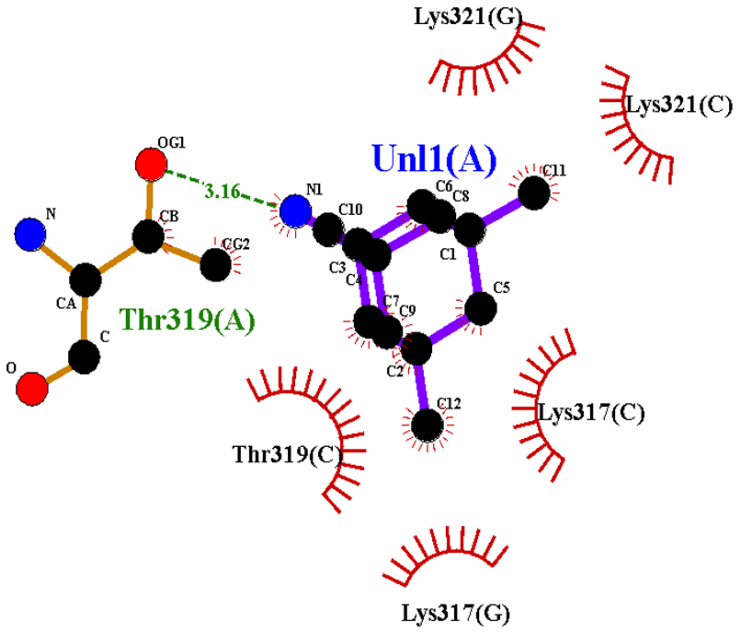


FIGURE 4.15: Interaction of Memantine with Tau protein.

TABLE 4.29: Interaction of Memantine with Tau protein.

Sr No	**Drug**	**Binding Score (kcal/mol)**	**Cavity Volume (A3)**	**Center**	**Docking Size**
1	Memantine	1	OG1-Thr319(A)-N1	3.16	Lys321(G), Lys321(C), Lys317(C), Lys317(G), Thr19(C)

4.13 Comparison of Drug and Lead Compound

Comparison between Memantine and Ferulic acid helps to identify the better treatment for Alzheimer's disease. Comparison is being performed through parameters like: Physiochemical properties, ADMET properties and docking score of Memantine and Ferulic acid.

4.13.1 Comparison of Physicochemical Properties

Results in table 4.30 show that the drug and Ferulic acid followed the required rules.

TABLE 4.30: Comparison of Physicochemical Properties (Lipinski's Ro5) of Memantine and Ferulic acid.

Sr No.	Properties	Memantine	Ferulic Acid
1	Molecular Weight	179.309	194.186
2	LogP value	2.6941	1.4986
3	Hydrogen bond acceptors	1	3
4	Hydrogen bond donors	1	2
5	Rotatable bonds	0	3

4.13.2 Comparison of ADMET Properties

ADMET properties help to determine the safety and efficacy of drug. According to the results in table 4.31, water solubility and Caco2 permeability of memantine was higher than Ferulic acid while intestinal absorption of Ferulic acid was higher than drug. Both gave negative results for P-glycoprotein substrate and inhibitor.

TABLE 4.31: Comparison of predicted values of Absorption of Memantine and Ferulic acid.

Sr No.	Model Name	Predicted Values of Memantine	Predicted Values of Ferulic Acid
1	Water solubility (log mol/L)	-2.317	-2.817
2	Caco2 permeability (log Papp in 10^{-6} cm/s)	1.329	0.176
3	Intestinal absorption (human) (% Absorbed)	91.234	93.685
4	Skin Permeability (log Kp)	-2.436	-2.72

Table 4.31: (Continued).

Sr No.	**Model Name**	**Predicted Values of Memantine**	**Predicted Values of Ferulic Acid**
5	P-gp substrate (Yes/No)	No	No
6	P-gp I inhibitor (Yes/No)	No	No
7	P-gp II inhibitor (Yes/No)	No	No

According to the results volume of distribution in table 4.32, BBB permeability and volume of distribution of Memantine was higher than Ferulic acid while CNS permeability of Memantine was slightly higher than Ferulic acid.

TABLE 4.32: Comparison of predicted values of Distribution of Memantine and Ferulic acid.

Sr No.	**Model Name**	**Predicted Values of Memantine**	**Predicted Values of Ferulic Acid**
1	Volume of distribution (VDss) (human)	0.988	-1.367
2	Fraction unbound (FU) (human)	0.601	0.343
3	BBB permeability	0.603	-0.239
4	CNS permeability	-2.478	-2.612

Memantine was a substrate of CYP3A4 while Ferulic acid was not an inhibitor or substrate of any of CY450 enzymes as shown in table 4.33.

TABLE 4.33: Comparison of predicted values of Metabolism of Memantine and Ferulic acid.

Sr No.	**Model Name**	**Predicted Values of Memantine**	**Predicted Values of Ferulic Acid**
1	CYP2D6 substrate	No	No

Table 4.33: (Continued).

Sr No.	**Model Name**	**Predicted Values of Memantine**	**Predicted Values of Ferulic Acid**
2	CYP3A4 substrate	Yes	No
3	CYP1A2 inhibitor	No	No
4	CYP2C19 inhibitor	No	No
5	CYP2C9 inhibitor	No	No
6	CYP2D6 inhibitor	No	No
7	CYP3A4 inhibitor	No	No

The results in table 4.34 showed positive total clearance of Memantine and Ferulic acid while that of Ferulic acid was higher than Memantine. Moreover, both these compounds were not a substrate of renal OCT2.

TABLE 4.34: Comparison of predicted values of Excretion of Memantine and Ferulic acid.

Sr No.	**Model Name**	**Predicted Values of Memantine**	**Predicted Values of Ferulic Acid**
1	Total Clearance (log ml/min/kg)	0.548	0.623
2	Renal OCT2 substrate	No	No

According to the results in table 4.35, Both Memantine and Ferulic acid were non-mutagenic. Ferulic acid had higher maximum tolerated dose. Both were hERG inhibitors.

Memantine had higher LD50 value, it was less acutely toxic compared to Ferulic acid. Memantine had a lower LOAEL, it have had higher chronic toxicity compared to ferulic acid. Both were non-hepatotoxic.

Memantine was skin sensitive. Memantine was more toxic to *T. pyriformis* than ferulic acid. Memantine had a lower minnow toxicity value, it was more toxic to minnows as compared to ferulic acid.

TABLE 4.35: Comparison of predicted values of Toxicity of Memantine and Ferulic acid.

Sr No.	**Model Name**	**Predicted Values of Memantine**	**Predicted Values of Ferulic Acid**
1	AMES toxicity	No	No
2	Max. tolerated dose (human) (log mg/kg/day)	0.322	1.082
3	hERG I inhibitor	No	No
4	hERG II inhibitor	No	No
5	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.673	2.282
6	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	1.25	2.065
7	Hepatotoxicity	No	No
8	Skin Sensitisation	Yes	No
9	*T.pyriformis* toxicity (log ug/L)	0.463	0.271
10	Minnow toxicity (log mM)	1.426	1.825

4.13.3 Comparison of Docking Interactions

Given below is comparison of docking scores, hydrogen bond and hydrophobic interactions of Memantine and Lead with Tau protein (Table 4.36) along with their 2D and 3D interaction figures 4.16 and 4.17.

TABLE 4.36: Comparison of docking interactions of Memantine and Ferulic acid against Tau protein.

Sr No.	**Compounds**	**Binding Scores (kcal/mol)**	**Hydrogen Bonds**	**Hydrophobic Interactions**
1	Memantine	-4.5	1	5
2	Ferulic acid	-5.8	2	8

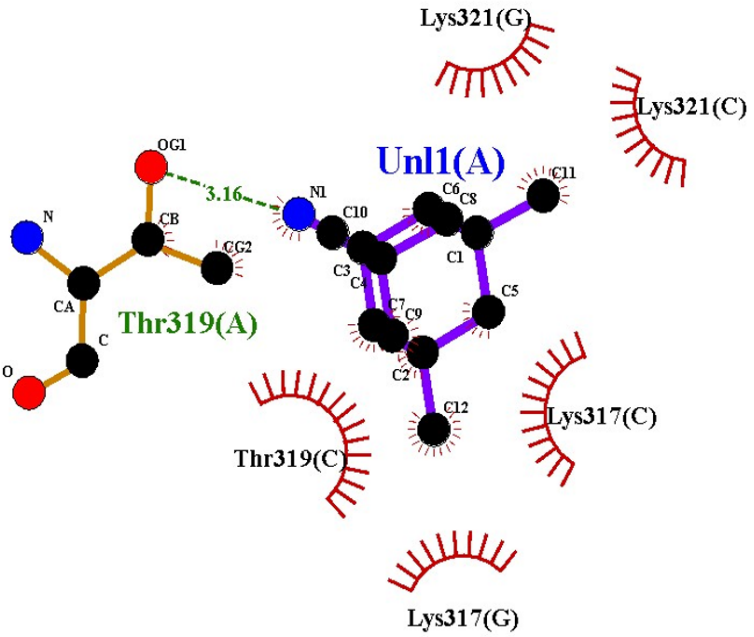


FIGURE 4.16: Secondary structure interaction of Memantine with Tau protein.

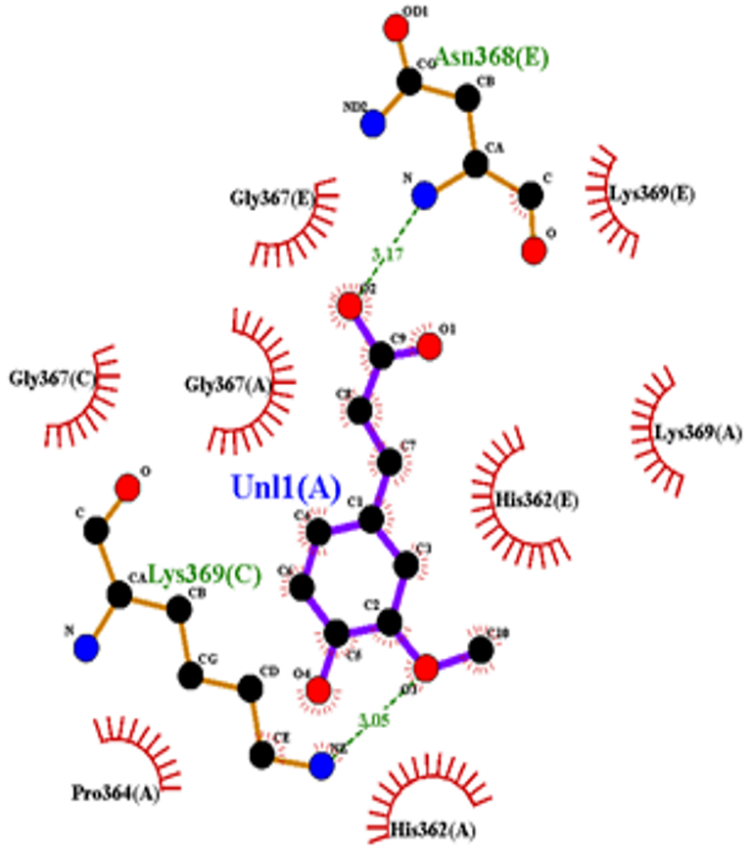


FIGURE 4.17: Secondary structure interaction Ferulic acid of with Tau protein.

According to the results in table 4.36, Memantine made 01 hydrogen bond and 05 hydrophobic interactions with Tau protein while Ferulic acid made 02 hydrogen bonds and 08 hydrophobic interactions. Ferulic acid has more interactions with Tau protein as compared to Memantine.

Bioactive compounds have been of interest in research for the last few years in connection with the treatment of neurodegenerative diseases and neuroprotection for brain health. One of the researches very recently done was concerned with therapeutic ligands against Tau protein, which has a relationship with Alzheimer's disease. The compounds selected for studies were Ferulic acid, Memantine, and many others based on drug-likeness, ADMET, and affinity to Tau protein ligand. Among these Ferulic acid was promising with good pharmacokinetic profile and low toxicity, most importantly safer and interacting with Tau better than Memantine. Although Memantine was toxic, it is a neuroprotective agent whose efficacy had already been proved. These compounds, for example Ferulic acid, had immense promise for the treatment of Alzheimer's disease, but would require further in vivo studies for confirmation.

Rhodiola rosea, an adaptogenic plant with known benefits for lowering stress, was the target of some of the investigations. It was believed that rosavin and salidroside were among the compounds responsible for its medicinal action. With a focus on their capacity to penetrate the blood-brain barrier (BBB) and reach the brain, these compounds' bioavailability and adherence to Lipinski's Rule of Five were evaluated. Compounds that had a small TPSA were more likely to cross the BBB, making them good candidates to target neurological pathways. Some of the *Rhodiola rosea* compounds, such as salidroside, rosavin, and caffeic acid, had TPSA within the optimal range for passing the BBB, thus allowing it to exert activity in the brain. Being drug-like along with these candidates, they have been considered promising for neurotherapeutic applications and especially in addressing cognitive disorders [78].

Both lines of research emphasized the importance of evaluating pharmacokinetic properties, such as the ability to cross the BBB, as well as adherence to Lipinski's guidelines. Although compounds targeting Tau, such as Ferulic acid, gave

insight into the therapeutic strategies against Alzheimer's, compounds from *Rhodiola rosea* offered added potential for stress management and neuroprotection. It is only a matter of time before such compounds can be effectively translated into treatments if their bioavailability allows them to interact with specific targets in the brain. These studies constitute part of research advancing the uses of natural compounds as potential future treatments for neurodegenerative diseases.

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

The goal of the current study was to identify active phytoconstituents from *R. rosea* that could be used as a medication to treat Alzheimer's disease. Ten ligands were chosen for this purpose using data mining investigations on literature databases. These ligands were subsequently docked against Tau protein, a receptor protein implicated in Alzheimer's disease. Protein and ligand structures were obtained using PDB and PubChem, respectively. Using data obtained from the PkCSM tool, primary and secondary filter screening (Lipinski's Rule of 5 and ADMET Properties) was used to perform drug similarity analysis. The CB Dock tool was used for the docking process, and LigPlot Plus was used for result visualization and analysis.

Ferulic acid was chosen as a lead drug against Tau protein receptors after a careful examination of the ligands' physicochemical characteristics, ADMET characteristics, and binding energy score. These characteristics of lead were contrasted with those of the FDA-approved medication memantine. The findings demonstrated that the chosen lead compound is less toxic than the typical medication and has a higher binding affinity to the target protein.

5.2 Recommendations

The process of developing new treatments is greatly aided by virtual drug design and discovery. All of the software and tools used in this study are genuine, and the outcomes are trustworthy. These results can be further supported by in vitro and in vivo experiments. Given the results of this investigation, the lead molecule should be looked into as a potential drug candidate for the treatment of Alzheimer's disease.

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