

CAPITAL UNIVERSITY OF SCIENCE AND
TECHNOLOGY, ISLAMABAD



Identification of *Ziziphus nummularia*
Jujube Therapeutic Compounds to
Investigate their Effect Against
Amyotrophic Lateral Sclerosis through
Computational Tools

by

Fateh Sher

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

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CERTIFICATE OF APPROVAL

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Acknowledgement

All praise and gratitude to Allah Almighty, the Most Merciful and Beneficent, whose blessings and guidance have enabled me to complete this work. I send my humble salutations to the Holy Prophet Muhammad (PBUH), the epitome of guidance and knowledge.

“I extend my heartfelt gratitude to my esteemed supervisor, Dr. Rizwan Ur Rehman, for their invaluable guidance, expertise, and unwavering support throughout this journey.

My deepest gratitude goes to my family. Forever grateful to all my teachers, mentors, and well-wishers who have contributed to my growth and learning, I am forever grateful.”

(Fateh Sher)

Abstract

Amyotrophic Lateral Sclerosis (ALS), also known as Motor Neuron Disease (MND), is a progressive and fatal neurodegenerative disorder characterized by the degeneration of upper and lower motor neurons, leading to muscle weakness, paralysis, and respiratory failure. Despite extensive research, effective therapeutic options remain limited. Among the major molecular contributors to ALS pathogenesis are Superoxide Dismutase 1 (SOD1) and TAR DNA-binding Protein 43 (TDP-43), whose mutations, misfolding, and aggregation disrupt cellular homeostasis, induce oxidative stress, impair RNA metabolism, and promote neuronal death. Therefore, these proteins represent important therapeutic targets for drug discovery. The present study aimed to identify potential neuroprotective phytochemicals from *Ziziphus nummularia*, a medicinal plant known for its antioxidant, anti-inflammatory, and neuroprotective properties, against ALS-associated target proteins SOD1 and TDP-43 through computational approaches. An extensive literature review identified 79 phytochemicals, of which 13 bioactive compounds were selected based on their reported pharmacological activities. Structural information of the selected ligands was obtained from PubChem, while protein structures were retrieved from the Protein Data Bank. Drug-likeness and pharmacokinetic properties of the compounds were evaluated using Lipinski's Rule of Five and AD-MET analysis through the pkCSM platform. Active binding pockets of the target proteins were identified using DogSiteScorer, followed by molecular docking studies using PyRx and AutoDock Vina. Protein–ligand interactions were further analysed and visualized using BIOVIA Discovery Studio Visualizer. The study focused on identifying compounds with favourable binding affinities, stable interactions, and acceptable pharmacokinetic profiles. Particular attention was given to alkaloids, flavonoids, terpenoids, and phenolic compounds present in *Ziziphus nummularia*, including Amphibine-H, Nummularine derivatives, Betulin, Lupeol, Rutin, Ellagic Acid, and Taxifolin. The findings suggest that several phytochemicals exhibit promising interactions with SOD1 and TDP-43, indicating their potential to modulate protein aggregation, oxidative stress, and neurodegenerative pathways associated with ALS. These results highlight *Ziziphus nummularia*

as a valuable source of natural bioactive compounds for the development of novel therapeutic agents against ALS. Moreover, Nummularogenin could be possible MND drug. Further in vitro and in vivo studies are recommended to validate the computational findings and explore their clinical applicability.

Keywords: Nummularogenin , MND, anti-inflammatory, ADMET properties, neurodegenerative proteins, pharmacokinetics

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Abbreviations

AD	Alzheimer's Disease
ALS	Amyotrophic Lateral Sclerosis
AMPK	AMP-activated Protein Kinase
CTD	C-Terminal Domain
FTD	Frontotemporal Dementia
FTLD-TDP	Frontotemporal Lobar Degeneration with TDP-43
HDL	High-Density Lipoprotein
IL-6	Interleukin 6
LDL	Low-Density Lipoprotein
MND	Motor Neuron Disease
NaB	Sodium Butyrate
NaPB	Sodium Phenylbutyrate
NES	Nuclear Export Signal
NLS	Nuclear Localization Sequence
NTD	N-Terminal Domain
PEG	Polyethylene Glycol
RBD	RNA Binding Domain
RNA	Ribonucleic Acid
RRM	RNA Recognition Motif
SCFAs	Short Chain Fatty Acids
SOD1	Superoxide Dismutase 1
SOD2	Superoxide Dismutase 2
SOD3	Superoxide Dismutase 3
TARDBP	TAR DNA-binding Protein Gene

TC	Total Cholesterol
TDP-43	TAR DNA-binding Protein 43
TGL	Triglycerides
TNF-α	Tumor Necrosis Factor Alpha
VLDL	Very Low-Density Lipoprotein

Chapter 1

Introduction

Common neurodegenerative disorders include movement disorders and issues with cognition and behavior. Clinical manifestations can be used to generally group these disorders. Mixed clinical characteristics are more common among patients, and pure disorders are rare. The standard for definitive diagnosis comes from a neuropathological study of the brain post-mortem. Many neurodegenerative diseases, despite their characteristic specific anatomical and protein accumulations, share similar pathways of neuronal death and dysfunction that include stress to the proteome, and with that, impairment of ubiquitin and oxidative stress, neuroinflammation, autophagosomal/lysosomal and proteasomal systems, and programmed cell death. It is important to keep in mind that protein abnormalities and dysfunction may occur before the clinical signs of neurodegenerative illnesses [1]. Amyloidoses, tauopathies, asynucleinopathies, and transactivation response DNA-binding protein 43 (TDP-43) proteinopathies are the most prevalent neurodegenerative illnesses. The main histopathologic concerns for diagnosing these proteinopathies are aberrant protein conformations and their cellular and neuroanatomical distributions. Neuropathologic diagnosis may consider α -synuclein in Lewy bodies, tau in NFTs or Pick bodies, and TDP-43 in the cytoplasm and nuclei of neurons. Protein accumulations in astrocytes include tau in tufted astrocytes, astrocytic plaques, and thorn-shaped astrocytes. Tau in coiled bodies and α -synuclein in glial cytoplasmic inclusions are examples of protein accumulations

in oligodendroglia. [2]. Two prevalent and significant symptoms of neurological disorders that typically arise in many neurological conditions are depression and exhaustion. Many would contend that since they are treated symptomatically, they provide little insight into the underlying mechanisms of the diseases in which they arise. That may be the case, but the fact that fatigue appears to be more common in some conditions, like Parkinson's disease or multiple sclerosis, suggests that there may be a biological basis for these symptoms rather than just a reactive, psychological reaction [3]. Similarly, motor neuron disease (MND), often known as amyotrophic lateral sclerosis (ALS), is uncertain. Premature death is caused by progressive motor weakening and bulbar dysfunction, typically from respiratory failure. It may be challenging to confirm the diagnosis at first until all clinical symptoms are present. A professional neurological opinion should always be obtained because there is a substantial differential diagnosis to take into account for all manifestations of the disease, including disorders that can be treated. A small percentage of people with familial ALS have been shown to have clear genetic inheritance, but understanding the biochemical underpinnings of genetic subtypes is also yielding crucial information that could result in treatments for the disease's sporadic manifestations [4]. The TARDBP gene on chromosome 1 encodes TDP-43, contain 414 amino acids and weight of 43kDa. TDP-43 is synthesized in the cytoplasm and translocated to the nucleus to perform its biological functions. Like other hnRNPs, TDP-43 is involved in the control of gene expression and other processes that pertain to the splicing of RNA, the turnover of mRNA, the trafficking of RNA, and the synthesis of microRNA (miRNA). TDP-43 contains an N-terminal domain, a nuclear localization sequence (NLS), two RNA binding domains (RBD1 and RBD2), a nuclear export signal (NES), and a glycine-rich C-terminal domain. Like all the protein domains, the N-terminal domain and the glycine-rich C-terminal domain contain two alpha helices. The amyloidogenic core region of TDP-43 spans residues 311-360. In the context of TDP-43, the alpha helices transform to the amyloid-beta sheet structure. The NLS is required for the proper physiological functions of TDP-43, as missense mutations and deletions of the NLS cause TDP-43 to lose its proper localization and aggregate in an amyloid form. The NLS is needed for the proper physiological functions of TDP-43,

as missense mutations and deletions of the NLS cause TDP-43 to lose its proper localization and aggregate in an amyloid form. To enter the nucleus, TDP-43 must be bound to importin- α . To enter the nucleus, TDP-43 must be bound to importin- α . TDP-43 possesses exportin- α XPO1. TDP-43 is known to influence the clinical symptoms of dementia and its associated cognitive impairment and suggests the risk of dementia. The goal of the present study was to determine the prevalence of TDP-43 in dementia and the risk of cognitive impairment among the various subtypes of Alzheimer's disease.

They found that TDP-43 deposition was seen in the limbic dominated (67%) and typical AD (59%) more than in the hippocampus sparing subtype (21%). Even though the frequency of TDP-43 deposition in AD shows variation according to the pathological subtype, TDP-43 also has an effect on the aggravation of cognitive loss and on other clinical features irrespective of the pathological subtype of AD [5]. The first of the causal mutations in the superoxide dismutase 1 (SOD1) gene, which encodes a newly identified enzyme, were described in 1993. SOD1 has been suggested to resounsible for 20% and 3% of familial and sporadic ALS respectively, and since 1993 more than 155 mutations in SOD1 have been reported, although the pathogenicity has yet to be determined for the majority of these mutations [6]. SOD1 is a highly conserved gene across species. SOD1 is composed of five exons which code for 153 amino acids in the form of a metalloenzyme, which are ligated to Cu and Zn. It's located in the cytoplasm and lysosomes, as well as in the nucleus and the space lies between membranes of the mitochondria. SOD1 also forms a homodimer. SOD1 functions to detoxify superoxide by speed up the conversion of cell damaging superoxide to molecular O₂ and H₂O₂, that pose a far less threat to Oxygen. It has also been shown recently that SOD1 plays a role in the balancing of energy metabolism and respiration by coordinating responses and integrating superoxide, oxygen and glucose [5].

This function is independent of its action in oxidative stress [7]. Dismutase activity loss in ALS patients was the first evidence for loss of function, along with the detection of ALS-causing mutations throughout the SOD1 gene. However, loss of function of SOD1 was not supported in mutant SOD1 transgenic (tgSOD1) mice

studied later. The first of these, tgSOD1G93A, possesses a point mutation in ALS, whereby glycine (Gly) at residue 93 is substituted with alanine (Ala). Currently, there are over 12 different human mutant SOD1 transgenic strains. Most, including tgSOD1G93A, are reported to have high dismutase activity. These strains exhibit a clear degenerative phenotype of lower motor neuron loss and an adult-onset motor syndrome. The first direct causes of ALS, the mutations in TARDBP, were discovered (Sreedharan et al., 2008) after SOD1 mutations. Thus, SOD1 mutations have been studied comprehensively in numerous cellular and animal models. From these studies, several theories of gain of function were developed and studied extensively. In the past few years, however, several laboratories have focused on the neuromuscular and non-neuromuscular phenotypes of Sod1 knockout mice.

Loss of dismutase function may change the developing order of familial ALS disease. There is evidence based on research on SOD1 transgenic overexpressing mice [8]. SOD1 is known for its dismutase activity. In mammals there are two other dismutases. SOD2 (Mn-SOD) is a mitochondrial dismutase that is cofactored by manganese, and SOD3 (Fe-SOD) is a homotetramer that is of an extracellular origin. Measurement of SOD1 activity is likely to be complicated by the presence of three dismutases [9]. SOD1 activity may be defined in two ways. One way is when SOD1 is assessed in its “intrinsic” form, and the other is when SOD1 is assessed in its “extrinsic” form, that is, in a biological sample. When SOD1 is assessed in its “intrinsic” form, the “overall” SOD1 activity is likely to be supported by a range of modalities present in a biological sample. This form of activity is the one most often measured in patients with familial ALS.

Intrinsic SOD1 activity, although one of the many factors, influences overall activity [10]. The cause of many of the hereditary and nonhereditary forms of the fatal neurodegenerative disease motor neuron disease (MND), is still poorly understood [11]. It has been demonstrated that MND incidence and prevalence have been rising globally in recent decades. Although it is thought to be the main cause, the aging of the global population does not fully explain the rising incidence and prevalence. This suggests that the current increase in MND risk is caused by significant hereditary or perhaps avoidable environmental risk factors. Reported

the crippling in addition to highly care demanding nature of motor neuron diseases and its potential impact on health care services, it is critical to identify these risk factors as soon as possible [12]. While determining whether MND risk is rising may help with health service planning, it does not precisely pinpoint underlying risk factors. On the other side, by identifying particular illness clusters, geographic studies can promote etiologic study. Examples include the discovery of patient groups in Guam⁶ and the Kii Peninsula⁷ that were later connected to the ingestion of neurotoxic plants and the expansion of the C9orf72 repeat. However, methodologic problems like poor case assessment or spatial risk identification in a small area have frequently limited studies of trends with the passage of time or clusters [13]. A collection of clinically and genetically diverse neurodegenerative illnesses together referred to as HSP are defined by progressive lower limb weakness and stiffness brought on by the death of upper motor neurons. A tight genetic connection between HSP and other neurodegenerative illnesses is supported by the growing understanding of the genetic basis of these disorders, despite the fact that the clinical heterogeneity of HSP is linked to a considerable genetic heterogeneity. Numerous papers have employed zebrafish models of the illness to shed light on the pathogenic pathways that underlie it [14].

MNDs are characterized by progressive paralysis that results in severe impairment, despite the fact that their aetiologies vary greatly. The pathophysiology of MNDs is still poorly understood, and new therapeutic approaches are desperately needed to treat them. To guide the logical design of therapeutic options, a comprehensive understanding of disease pathophysiology is required. The degeneration of higher and/or lower motor neurons, as previously indicated, is a hallmark of MNDs and is caused by a malfunction of multiple cellular processes. Several pathogenic pathways are shared by at least two of these three illnesses despite the apparent variability in these MNDs [15].

Over the past ten years, there has been a revolution in the genetics underlying MNDs. A large portion of this can be ascribed to recent developments in sequencing technologies, which have significantly accelerated and decreased the

cost of genotyping and finding variations in disease-affected individuals. In human genomics, however, confirming the pathogenicity of discovered variations is still a difficult task for identifying causative mutations. Zebrafish have provided an affordable and quick substitute for the expensive and labor-intensive requirements associated with the creation of murine models of neurodegenerative disease, despite significant efforts being made to produce mammalian models of some of the described disease-causing mutations. This is especially true for several of the newly discovered genes linked to MNDs that cause the disease [16].

1.1 Problem Statement

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of motor neurons, resulting in severe disability and premature death. The accumulation and misfolding of proteins such as TDP-43 and SOD1 play a critical role in disease progression, yet effective treatments targeting these proteins remain limited. Natural products have emerged as promising sources of therapeutic agents due to their diverse bioactive compounds and lower toxicity. Therefore, this study investigates the potential of phytochemicals from *Ziziphus nummularia* to interact with and inhibit ALS-associated proteins (TDP-43 and SOD1) using in silico molecular docking and drug-likeness analyses to identify potential lead compounds for future drug development.

1.2 Aim and Objective

1.2.1 Aim of the Study

The primary aim of this research is to screen and analyze bioactive compounds from *Ziziphus nummularia* that may help in reducing or preventing the progression of Amyotrophic Lateral Sclerosis by interacting with disease-related proteins, especially TDP-43 and SOD1, through the use of computational techniques.

1.2.2 Objectives

The objectives of the present study are:

- i. To identify the bioactive compounds in *Ziziphus nummularia* against target proteins using bioinformatics tools.
- ii. To analyze the binding conformation between TDP-43 and SOD1 proteins with therapeutic compounds of *Ziziphus nummularia* possessing as an anti ALS potential.
- iii. To identify lead compound as an anti ALS drug candidate.

Chapter 2

Literature Review

2.1 Amyotrophic Lateral Sclerosis

Motor Neuron Diseases (MND) is a health disorder which includes a wide range of neuron degenerative complications affecting lower motor neurons (in the anterior horn or motor cranial nerve nuclei in the brain stem), upper motor neurons (corticospinal tract), or both. Muscle weakness, stiffness, and ultimately paralysis result from the slow degeneration of these motor neurons, which are found in the brain and spinal cord. As the disease progresses, patients lose the ability to move, speak, swallow, and breathe [17]. Despite these severe physical effects, cognitive functions often remain intact. The exact cause of ALS/MND is not fully understood, but it is believed to result from a combination of genetic mutations, protein misfolding (such as TDP-43 aggregation), oxidative stress, glutamate excitotoxicity, and inflammation within the nervous system. Although drugs like riluzole and edaravone may help reduce the disease's course and enhance quality of life, there is presently no cure for ALS/MND [18].

The disease does not usually affect cognitive or sensory functions, meaning that the patient remains mentally aware while gradually losing physical control. Early symptoms include muscle cramps, twitching, slurred speech, and weakness in the limbs, which worsen over time. As the disease progresses, patients may require

ventilator support due to respiratory failure, which is the most common cause of death in ALS/MND [19].

TABLE 2.1: Overview of ALS/MND [20].

Feature	Description
Full Name	Amyotrophic Lateral Sclerosis (ALS) / Motor Neuron Disease (MND)
Affected System	Central Nervous System (Brain and Spinal Cord)
Primary Target	Motor neurons controlling voluntary muscle movement
Main Symptoms	Muscle weakness, cramps, twitching, slurred speech, difficulty swallowing, breathing problems
Cause Type	10% Genetic (Familial), 90% Sporadic (Unknown cause)
Key Genes Involved	SOD1, C9orf72, TARDBP (TDP-43), FUS
Main Pathological Mechanisms	Protein misfolding (TDP-43), oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, neuroinflammation
Cognitive Function	Usually remains intact
Life Expectancy	Typically 3–5 years after diagnosis
Treatment Options	Riluzole (reduces glutamate toxicity), Edaravone (antioxidant), physiotherapy, speech therapy, respiratory support
Prognosis	Progressive and fatal, but supportive care can improve quality of life
Example Case	Stephen Hawking survived decades with ALS

2.2 Mechanism of Amyotrophic Lateral Sclerosis

The mechanism of Amyotrophic Lateral Sclerosis (ALS) involves a complex interaction of genetic, molecular, and environmental factors that lead to the gradual death of motor neurons in the brain and spinal cord. One of the main features of ALS is the accumulation of misfolded proteins, such as TDP-43 and SOD1 which disrupt normal cell processes [21]. These abnormal proteins interfere with RNA metabolism, mitochondrial function, axonal transport, and synaptic communication, causing energy imbalance and oxidative stress within neurons. Additionally, glutamate excitotoxicity a hyperactivity activation of glutamate receptors leads

to calcium overload and neuronal injury. Neuroinflammation, caused by overactive microglia and astrocytes, further damages motor neurons through the release of toxic molecules. Over time, these combined effects result in muscle weakness, paralysis, and ultimately respiratory failure. Having the knowledge of mechanisms is vital for formulating therapies that target protein misfolding, oxidative damage and inflammatory pathways to slow disease progression [22].

2.3 Symptoms of Amyotrophic Lateral Sclerosis

The symptoms of Amyotrophic Lateral Sclerosis (ALS) or Motor Neuron Disease (MND) develop gradually and vary from person to person depending on which motor neurons are first affected. In the early stages, the disease may present with subtle signs such as muscle weakness, cramps, twitching (fasciculation's) or stiffness, often starting in one part of the body commonly the hands, legs, or mouth and then spreading to other regions. As the condition progresses, the weakness becomes more pronounced, leading to difficulty in performing everyday tasks such as buttoning clothes, holding objects, walking, or climbing stairs. Speech and swallowing difficulties, known as dysarthria and dysphagia, are also common as the muscles that control the tongue, throat, and mouth become affected. This can cause slurred or slow speech, choking while eating or drinking, and unintentional weight loss due to reduced food intake [23].

2.3.1 Muscle Cramps

About one quarter ALS patients experience cramps as the main source of pain, especially those with spinal disease. This is due to instability in the motor units. Quinine sulphate, levetiracetam and mexiletine are some common treatments for muscle cramps. While mexiletine is effective in reducing muscle cramps in ALS patients, quinine is recommended against by the FDA to treat muscle cramps as it is associated with bradycardia, Q-T interval prolongation and cardiac arrhythmias. "Fig 2.1" [23].

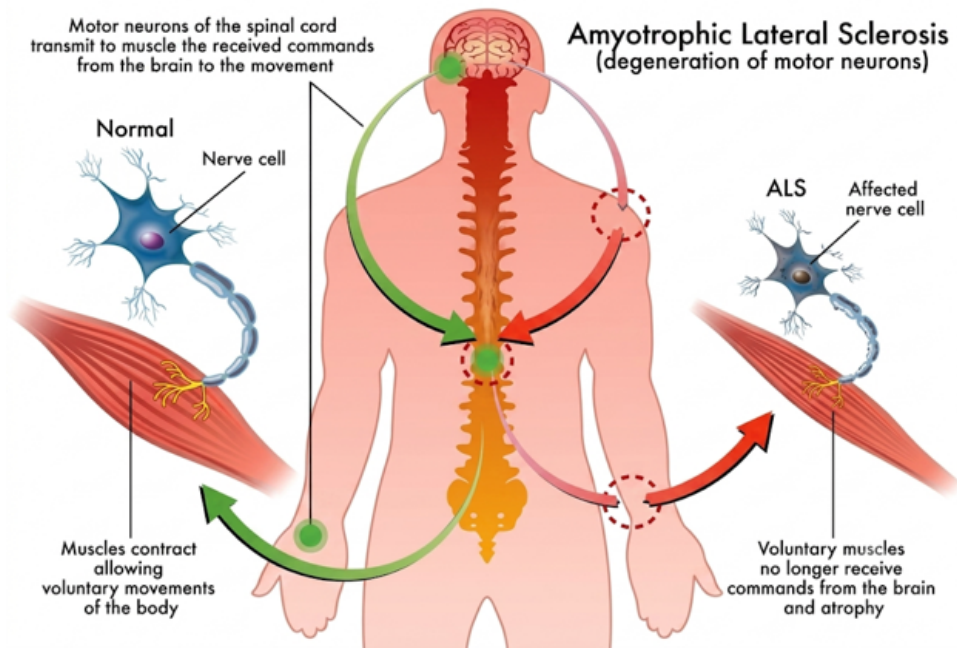


FIGURE 2.1: Muscle Cramps: This diagram illustrates how amyotrophic lateral sclerosis disrupts the vital neural pathways between the central nervous system and the musculoskeletal system [23].

2.3.2 Sialorrhoea

With ALS patients, hyper salivation, drooling and ample saliva in the mouth is one of the more alarming symptoms. It is especially more prevalent in bulbar-onset and late stage patients. Sialorrhoea is treated with anticholinergic drugs which are also associated with side effects of mouth drousal, vision blurriness and constipation. These drugs have contraindications to patients with heart conduction disturbances and with prostatic hypertrophy.

Emotional changes are also common and pseudobulbar affect, with episodes of uncontrollable laughter and crying is a result of damage of certain pathways of the brain. In late stage ALS, patients will have weakened intercostal and diaphragm muscles, and will have difficulty breathing in a normal manner especially in a resting position while lying down. Eventually ALS patients will rely on a machine to assist in breathing.

Most ALS patients maintain cognitive function despite extreme physical disability. This means patients remain aware and alert throughout the progression of the

disease. However, a small portion of patients may have frontotemporal dementia, which can alter their behavior and decision-making [24].

2.4 Global Prevalence and Demographic Patterns of Amyotrophic Lateral Sclerosis

The prevalence of Amyotrophic Lateral Sclerosis (ALS), also known as Motor Neuron Disease (MND), varies across different regions of the world due to genetic, environmental, and diagnostic factors. Globally, ALS is considered a rare disorder, affecting approximately 2 to 5 individuals per 100,000 people. Each year, about 1 to 2 new cases per 100,000 people are diagnosed worldwide. The disease is more common in men than women, and its onset typically occurs between the ages of 50 and 70 years. Higher prevalence rates have been reported in Europe, North America, and Japan, where advanced healthcare systems and improved diagnostic methods contribute to more accurate detection [25].

2.4.1 Geographical Variations and Diagnostic Criteria in ALS Epidemiology

ALS epidemiology studies were mostly conducted in North America (17 incidence studies, 16 prevalence studies) and in Europe (48 incidence studies, 29 prevalence studies). In the context of existing studies, and despite the absence of studies in a number of regions (e.g. Africa, Oceania and South America), there is variability when it comes to the reported incidence and prevalence of ALS. As of the current state of knowledge, the reasons for these discrepancies must be examined, but this requires contacting the authors of the studies and having them provide details of their methodology [26].

Differences in the ascertainment of cases, the target population coverage and representativeness, as well as genetic or environmental factors within and between

regions, may explain the variability. Since most studies apply the Original or Revised El Escorial criteria, it is unlikely that the differences that existed were on the basis of the diagnostic criteria for these studies because the El Escorial criteria state that there must be evidence of the degeneration of both the lower and upper motor neuron in order to have a diagnosis of Amyotrophic Lateral Sclerosis ”Fig 2.2” [27].

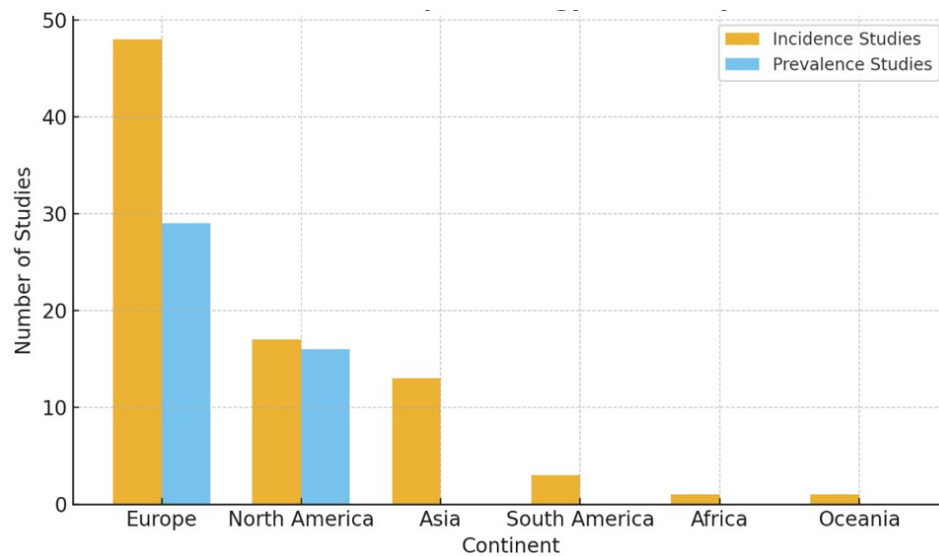


FIGURE 2.2: Distribution of ALS epidemiological studies by continent (most recent ALS incidence data per 100,000 person-years is reported by continent and city as applicable) [27]

2.4.2 Incidence of Amyotrophic Lateral Sclerosis Across Continents and Study Timeframes

Figure 2.2 displays results from studies that provide incidence per 100,000 person-years (for the most recent year reported), arranged by continent and, if applicable, by city. Thirteen research from Asia, seventeen from North America, and thirty-nine from Europe made up the 85 studies that provided information on the incidence of ALS. Additionally, three came from South America, one from Africa, and one from Oceania. Two research included estimations from several continents. The estimates of incidence of ALS were provided from the early 1960s to 2018.

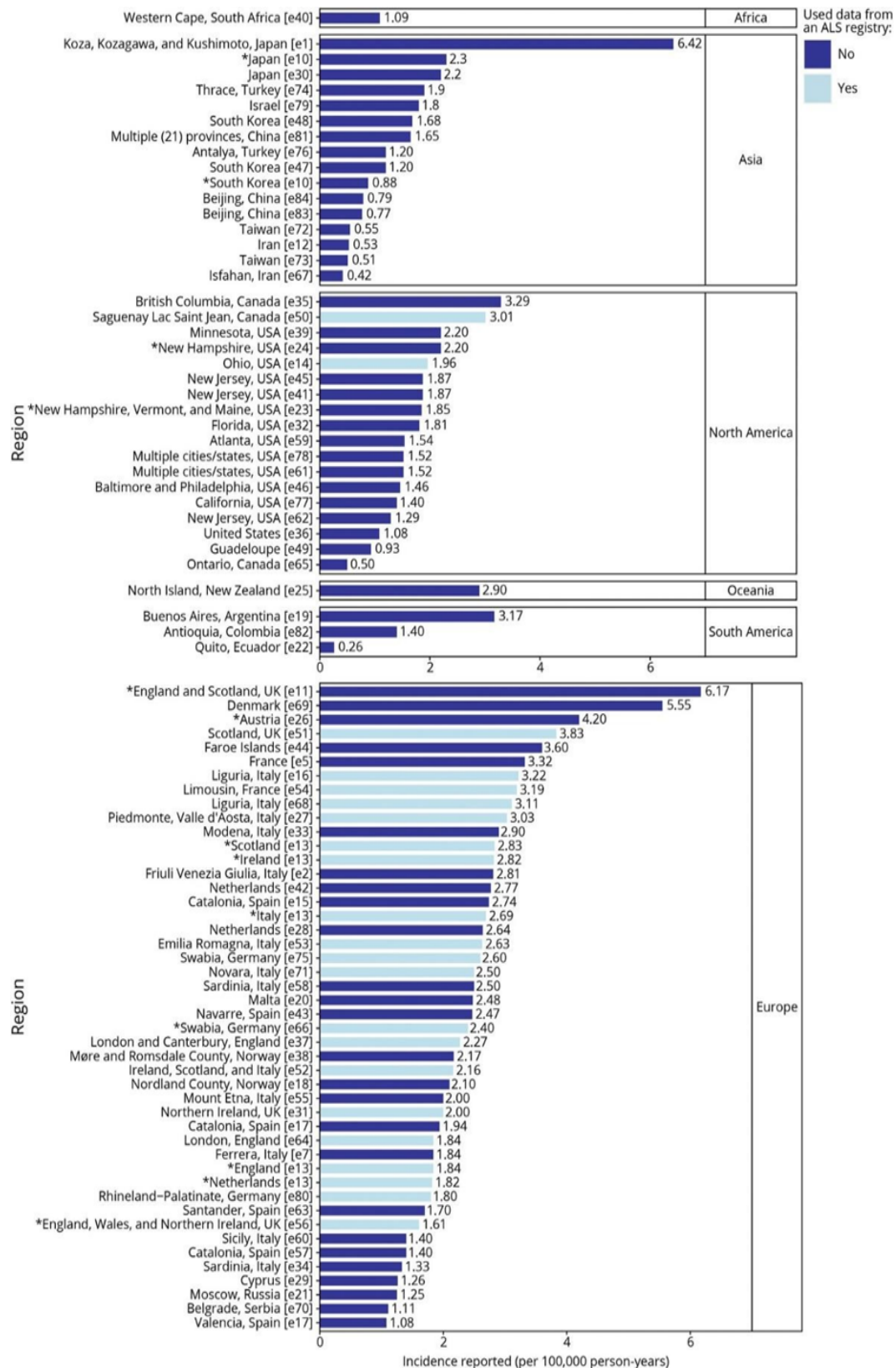


FIGURE 2.3: The Crude Incidence Reported per Study is stratified and represented by Continent (the Oshima, Japan, study describes incidence of ALS as 23.46 per 100,000 person-years. It was a continuation (extended study period, same methods) of previous studies. Thus, only the most recent study was included) [28]

Because the incidence of ALS in Australia was derived from mortality data, Figures 3 and 4 for Oceania were unable to display it. e10. A UK study used information from the UK Million Women Study. The incidence rate per 100,000 women-years (initially shown per 1,000) is estimated in Figure 2.3 (ALS = amyotrophic lateral sclerosis) [28].

2.4.3 Prevalence and Reporting of ALS in Pakistan

In Pakistan, the exact prevalence of ALS is not well documented, primarily due to limited epidemiological studies and the lack of specialized neurological centers. However, based on available data and regional estimates, the occurrence of ALS in Pakistan is believed to be lower than in Western countries, possibly around 1 to 2 cases per 100,000 people. Underreporting, misdiagnosis, and limited access to neurodiagnostic facilities likely contribute to this lower recorded rate. Moreover, cultural and socioeconomic factors often delay medical consultations, resulting in late diagnosis and poor disease management [29].

Globally, the number of ALS cases is gradually increasing, partly due to longer life expectancy and improved awareness among clinicians. According to the World Health Organization (WHO) and recent studies, approximately 350,000 people are living with ALS worldwide, and around 100,000 new cases are diagnosed each year. The disease's degenerative nature and lack of a treatment place a heavy load on individuals, families, and healthcare systems [30].

2.4.4 Prevalence of Major Gene Mutations in Familial and Sporadic ALS

The graph compares the frequency of major gene mutations linked to familial (fALS) and sporadic (sALS) forms of Amyotrophic Lateral Sclerosis. It shows that C9ORF72 mutations are most common in familial cases, followed by SOD1, while TDP-43, FUS and VCP mutations occur less often. In sporadic cases, genetic mutations are much rarer, with only small percentages seen for C9ORF72

and SOD1. This indicates that inherited genetic changes are a key factor in familial ALS, whereas sporadic ALS usually arises from non-genetic or complex environmental causes [31].

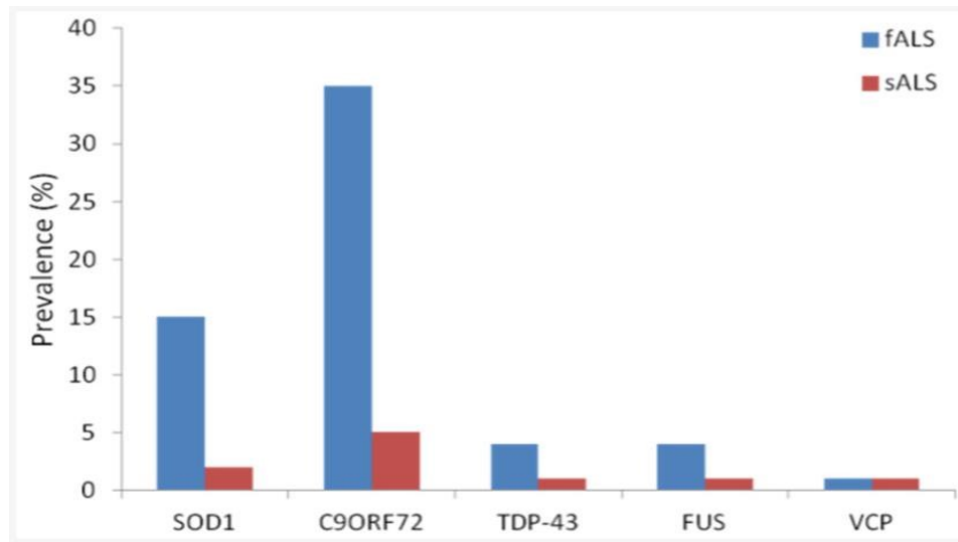


FIGURE 2.4: The prevalence (represented as a percentage) of mutations in the most significant ALS-related genes in familial (fALS) or sporadic (sALS) instances [32].

2.5 TAR DNA-binding Protein 43

TAR DNA-binding protein 43 (TDP-43) is a protein that plays an essential role in the regulation of RNA processing, including transcription, splicing, and transport. It is found on chromosome 1 produced by the TARDBP gene. Normally, TDP-43 stays inside the nucleus of the cell and helps control the activity of certain genes and the stability of messenger RNA (mRNA).

However, in neurodegenerative conditions such frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), the protein becomes abnormally modified and moves from the nucleus to the cytoplasm. This abnormal movement leads to the buildup of TDP-43 clumps, which interfere with normal cell functions and eventually cause nerve cell damage and death. Because of this, TDP-43 is widely recognized as a key factor in the development and progression of ALS and related disorders [33].

2.6 Structural Composition and Functional Domains of TDP-43

The 414 amino acids that make up TDP-43 include a NLS, two RNA-recognition motifs (RRM1, RRM2), and an intrinsically disordered C-terminal domain (CTD) with glutamine, asparagine, and glycine-rich areas [34]. The low projected Local Distance Difference Test (pLDDT) score of AlphaFold3, a protein structure prediction tool that measures confidence in protein structure predictions, indicates that the C-terminus lacks a well-defined native structure [35]. This low score is a result of the C-terminus's structural flexibility, which increases its mobility and malleability and permits interaction with a variety of molecular partners [36]. Because of its significant aggregation potential and the presence of several sporadic and familial ALS/FTLD-TDP-associated mutations, the C-terminus is also referred to as a prion-like domain [37]. One of the N-terminus's many functions is to stimulate TDP43 oligomerization and DNA binding affinity in both in vitro and in vivo settings [38].

2.7 Oligomerization Behavior and Pathological Aggregation of TDP-43 in ALS

The structure and oligomerization state of the full-length protein are significantly influenced by the NTD1–76. First of all, it is the first to be produced by the ribosome and to fold because it is the NTD of a lengthy protein [39]. Second, it is thought to be in charge of the dimerization and oligomerization of the whole full-length protein since it dimerizes and oligomerizes in vitro when separated from the rest of the protein [40]. TDP-43's function and tendency to aggregate the whole protein into pathogenic inclusions are modulated by its NTD-driven oligomerization state [41]. The gradual loss of upper and lower motor neurons in the brain and spinal cord is a hallmark of Motor Neuron Disease (MND), sometimes referred to as Amyotrophic Lateral Sclerosis (ALS), a deadly neurodegenerative illness. Muscle weakening, paralysis, and eventually respiratory failure are the outcomes of this

deterioration. The aberrant accumulation of TAR DNA-binding protein 43 (TDP-43) in ALS-affected neurons and glial cells is one of the disease's most recognizable clinical features [42]. TDP-43 is a nuclear RNA-binding protein that is generally involved in RNA transcription, alternative splicing, transport, and stability. It is encoded by the TARDBP gene on chromosome 1 [43]. TDP-43 is mostly found in the nucleus of healthy neurons, where it ensures that RNA metabolism and gene expression are properly regulated. Neuronal dysfunction and mortality result from this protein's mislocalization, aggregation, and molecular modification in ALS and kindred TDP-43 proteinopathies.

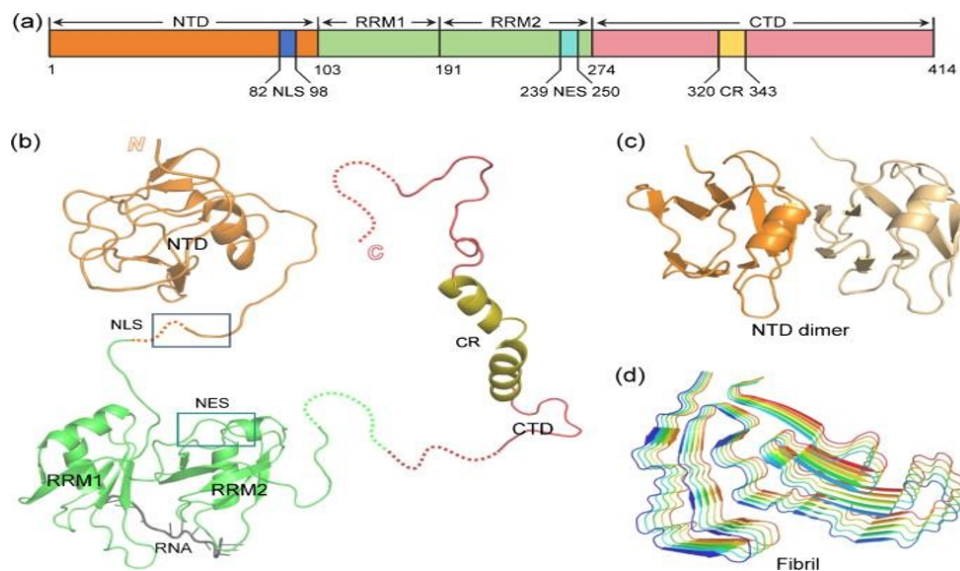


FIGURE 2.5: The Regulation of TDP-43 Structure: The domain architecture of the TDP-43 protein illustrating its physiological role in binding RNA via its RRM regions and its functional N-terminal dimerization. It highlights how its intrinsically disordered C-terminal tail can pathologically misfold and aggregate into structural amyloid fibrils linked to neurodegeneration ([42, 43]).

2.7.1 Pathogenic Role and Mislocalization Mechanism of TDP-43 in ALS

TDP-43 controls RNA processing, stress granule formation and protein turnover to preserve neuronal homeostasis under physiological settings. TDP-43 is mislocalized from the nucleus to the cytoplasm in ALS, where it experiences post-translational changes include proteolytic cleavage, ubiquitination and hyper phosphorylation [44]. These changes encourage the development of neurotoxic insoluble

cytoplasmic clumps [45]. The loss of normal nuclear function and the acquisition of harmful cytoplasmic function are the two main pathogenic processes caused by the mislocalization. TDP-43 nuclear depletion disrupts RNA regulation by affecting mRNA splicing, transcriptional control, and RNA transport. Cellular stress and degeneration result from the instability or improper processing of vital neuronal transcripts [46]. TDP-43 builds up in the cytoplasm and causes cellular damage in a number of ways. The autophagy and ubiquitin-proteasome systems, which are in charge of removing damaged proteins, are hampered by abnormal aggregates, which also sequester essential RNA-binding proteins [47].

The neuron's capacity to maintain proteostasis is further overwhelmed when these mechanisms malfunction, resulting in a vicious cycle of protein misfolding and aggregation. Mislocalized TDP-43 interferes with respiration and mitochondrial protein synthesis via interacting with mitochondrial RNA. This results in oxidative stress, energy depletion, and mitochondrial dysfunction all of which are important causes of motor neuron death [48].

Additionally, synaptic degeneration and muscle denervation are caused by cytoplasmic TDP-43 aggregates that interfere with axonal transport, affecting the passage of organelles and signaling molecules along the axon [49].

2.7.2 Normal Function of TDP-43

The first evidence of TDP-43's protein functions recognized TDP-43 as a 43-kDa protein that interacts with the TAR element of HIV and later as TAR DNA-binding protein. TDP-43 plays the role of a transcriptional repressor and associates with transcription-related proteins including MeCP2, which is mutated in patients with Rett syndrome. The role of TDP-43 on transcription regulation by direct binding to DNA is largely unexplored and therefore requires genome-wide efforts to discover target genes of TDP-43. TDP-43 further interacts with hnRNPs, miRNA-related proteins, and translation-related proteins through its role in RNA metabolism and plays a role in RNA granules (Figure 2.6).

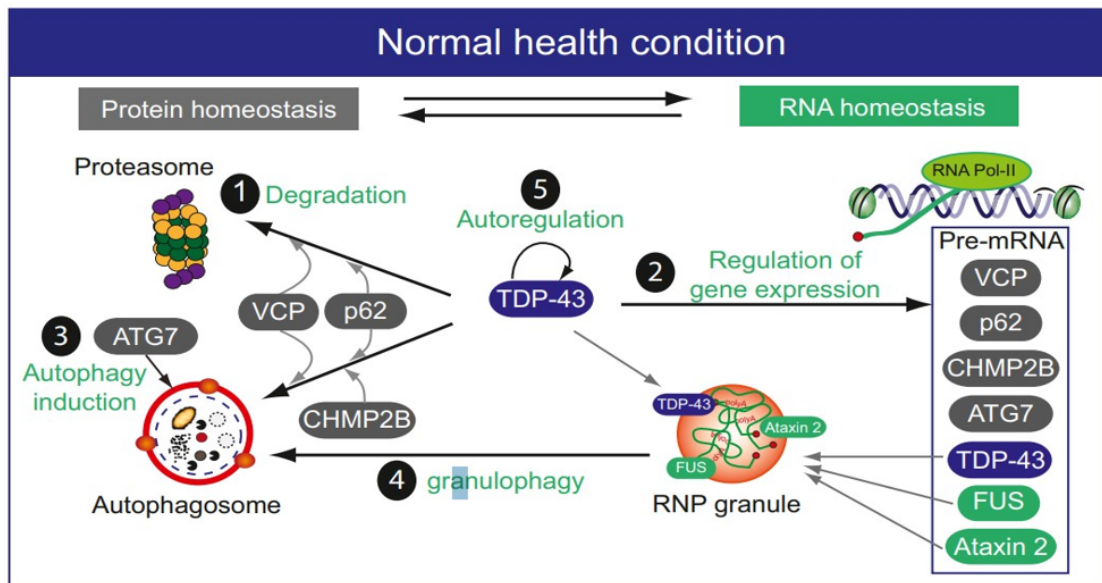


FIGURE 2.6: Normal Functioning of TDP-43

Additionally, an increasing amount of data points to the part neuroinflammation plays in TDP-43-mediated neurodegeneration. When misfolded TDP-43 aggregates activate microglia and astrocytes, inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) are released. In addition to aggravating neuronal damage, these inflammatory mediators foster a persistently inflamed milieu that encourages further neurodegeneration [50]. As a result, inflammation both contributes to and responds to TDP-43 disease. In addition to harming motor neurons, the persistent presence of inflammatory signals quickens the onset of ALS symptoms. A subgroup of familial ALS (fALS) patients, which make up roughly 3-5% of hereditary forms of the illness, are genetically associated with mutations in the TARDBP gene [51]. TDP-43 is more likely to misfold, collect and evade removal by cellular degradation processes as a result of these alterations. Nonetheless, abnormal TDP-43 inclusions are detected in more than 95% of patients of sporadic ALS (sALS), the most prevalent type of the disease, indicating that similar protein misfolding pathways may be caused by both hereditary and environmental causes [52]. Furthermore, TDP-43 aggregates have the ability to disseminate disease across interconnected brain networks via a "prion-like" mechanism in which misfolded TDP-43 causes normal TDP-43 in nearby cells to misfold as well [53].

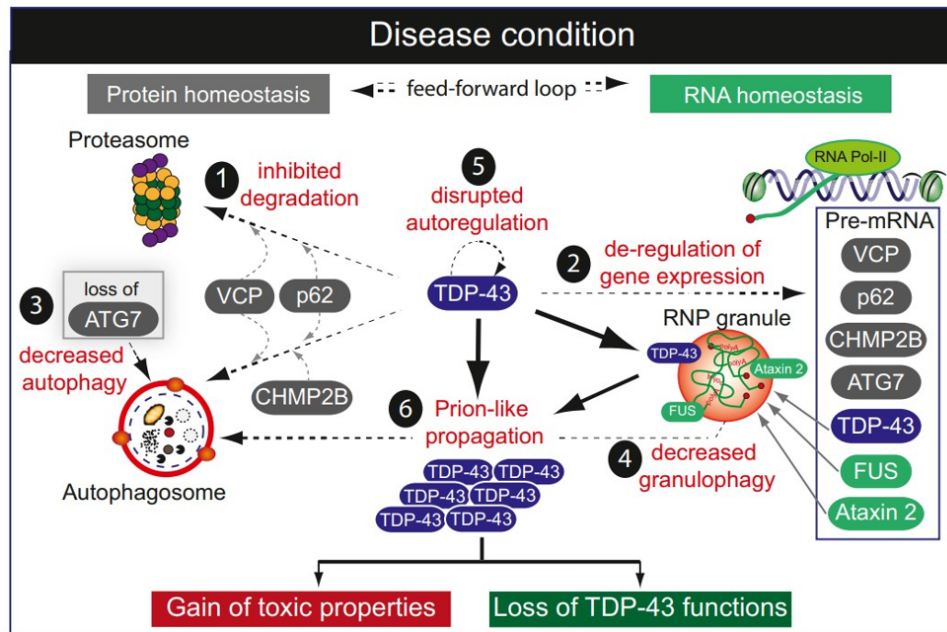


FIGURE 2.7: Disease Condition of TDP-43

TDP-43 disease has far-reaching and disastrous downstream effects on neuronal health. Motor neuron degeneration is caused by a confluence of cytoplasmic gain-of-function and nuclear loss-of-function, which results in oxidative stress, mitochondrial damage, axonal transport abnormalities, disruption of RNA processing, and chronic inflammation [54]. These processes clarify why TDP-43 disease is primary molecular cause of ALS and associated conditions [55].

2.8 Overview of the SOD1 Enzyme and Its Role in Cellular Protection

SOD1 (Superoxide Dismutase 1) is an enzyme found in nearly all human cells that helps protect them from damage caused by reactive oxygen molecules. It works by converting harmful superoxide radicals, which are by-products of normal cellular activity, into oxygen and hydrogen peroxide substances that are less toxic to the body. The enzyme plays a vital role in maintaining the balance between oxidation and antioxidation inside cells, especially in neurons. When the SOD1 gene is mutated, its protective function is lost, and the resulting protein can become unstable

and harmful, contributing to diseases such as Amyotrophic Lateral Sclerosis (ALS) [56].

2.9 Mechanism of SOD1 in ALS

Mutated SOD1 gene causes protein misfolding in Amyotrophic Lateral Sclerosis (ALS). In normal functioning SOD1, the superoxide radicals that carry a net positive charge and are considered unbound, are transformed and inactivated. However, in the case of sporadic amyotrophic lateral sclerosis, the enzyme SOD1 fails and becomes erratic.

This faulty version becomes an aggressor, forming trapped clumps of what used to be a protein that protects the cell. These clumps collide with the power plants of the cell, the mitochondria, and sabotage energy production. The clumps trap a multitude of superoxide radicals making them extremely toxic and worse because they are unbound.

The trapped unbound superoxide radicals are left to roam free and, in an uncontrollable way, damage the cell, creating a toxic environment for the other surrounding healthy cells and even the protein quality control systems. The inflammation from the deteriorating environment and oxidative stress traps the neurons and leads eventually to the muscle paralysis and atrophy that is characteristic of ALS [57].

2.10 *Ziziphus nummularia*

Ziziphus nummularia is a medicinal shrub from the Rhamnaceae family, commonly found in arid regions of South Asia. It is rich in phytochemistry and contains alkaloids, flavonoids, and saponins, which give it antioxidant, anti-inflammatory, and antimicrobial properties. Traditionally, from a long period of time it has been in

practice to treat fever, digestive issues, and skin diseases. Recent studies highlight its potential in protecting nerve cells and reducing oxidative stress, making it valuable for research on neurodegenerative diseases like ALS [58].

2.10.1 Taxonomic Classification of *Ziziphus nummularia*

TABLE 2.2: Classification of *Ziziphus Nummularian*

Taxonomic Rank	Classification
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida (Dicotyledons)
Order	Rosales
Family	Rhamnaceae
Genus	<i>Ziziphus</i> Mill.
Species	<i>Ziziphus nummularia</i>
Binomial Name	<i>Ziziphus nummularia</i> (Burm.fil.) Wight & Arn.

2.10.2 Botanical Description and Distribution of *Ziziphus nummularia*

Ziziphus nummularia, commonly known as Wild Jujube, Ber, or Jharber, is a spiny shrub belonging to the family Rhamnaceae. It is a tropical and subtropical plant widely distributed in South Asia, particularly in Pakistan, India, and parts of the Middle East, where it thrives in arid and semi-arid regions. The plant is highly valued for its drought resistance and medicinal properties. It bears small, round, edible fruits with a slightly sweet and tangy flavor. The yield of *Ziziphus nummularia* depends on climatic and soil conditions, but on average, a mature shrub can produce a significant quantity of fruits, which are used both for nutritional and therapeutic purposes [59].

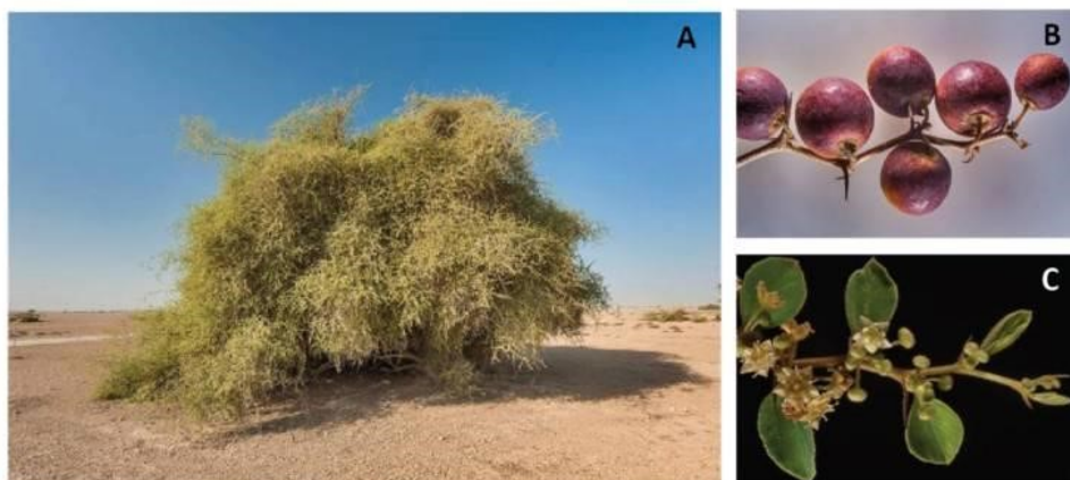


FIGURE 2.8: (A) *Ziziphus nummularia* plant, (B) *Ziziphus nummularia* fruit, and (C) *Ziziphus nummularia* leaves.

2.10.3 Phytochemical Properties of *Ziziphus nummularia*

Alkaloids, flavonoids, saponins, tannins, terpenoids, phenolic acids, and glycosides are just a few of the many bioactive substances that *Ziziphus nummularia* is rich in phytochemically. Its potent antioxidant, anti-inflammatory, antibacterial, hepatoprotective, and neuroprotective properties are partly due to the presence of these secondary metabolites.

Among its identified phytochemicals berberine and lycorine both alkaloids found in *Ziziphus* species have gained considerable scientific attention due to their potential neuroprotective roles [60].

Two new cyclopeptide alkaloids, nummularine M and nummularine N, were identified in a 1983 research that also found around a dozen more cyclopeptide alkaloids in the root bark [61].

Cyclopeptide alkaloids are macrocyclic chemicals that are mostly found in *Ziziphus* and *Rhamnaceae*. These compounds interact with the CNS and have sedative and analgesic effects. Antimicrobial properties as well as antidiabetic properties have also been noted in these compounds [62].

2.10.4 Neuroprotective Mechanisms of Berberine Against TDP-43 Toxicity

Berberine exhibits its mode of action by inhibiting TDP-43 misfolding and aggregation, thereby maintaining normal protein homeostasis within neurons. It also activates cellular defense mechanisms such as AMP-activated Protein Kinase (AMPK) pathways and reduces oxidative damage in motor neurons.

On the other hand, lycorine demonstrates strong anti-inflammatory and anti-apoptotic effects, reducing neuronal stress and preventing TDP-43-induced cytotoxicity.

Together, these compounds may help stabilize neuronal function, reduce protein aggregation, and enhance mitochondrial activity, offering a promising phytotherapeutic approach against TDP-43 associated neurodegeneration [63].

2.10.5 Neuroprotective Mechanisms of Lycorine Against TDP-43 Toxicity

Lycorine, a natural alkaloid found in plants such as *Ziziphus nummularia* and members of the Amaryllidaceae family, exerts a protective effect against TDP-43-induced neurotoxicity in Amyotrophic Lateral Sclerosis (ALS) [64].

It works by reducing oxidative stress and inflammation, promoting autophagic clearance of misfolded TDP-43 aggregates, and restoring normal nuclear TDP-43 function. Additionally, lycorine helps stabilize mitochondrial activity and prevents neuronal apoptosis by regulating key survival pathways.

Through these combined actions, lycorine helps protect motor neurons and may serve as a potential natural therapeutic agent for managing TDP-43 associated neurodegeneration in ALS and related disorders [65].

2.10.6 Phytochemical Composition and Bioactive Cyclopeptide Alkaloids in *Ziziphus nummularia*

Among the many substances found in *Zizyphus nummularia* are cyclopeptide alkaloids. Two new cyclopeptide alkaloids, nummularine M and nummularine N, were identified in a 1983 research that also found around a dozen more cyclopeptide alkaloids in the root bark. Cyclopeptide alkaloids are macrocyclic chemicals that are mostly found in *Ziziphus* and *Rhamnaceae*. These substances have sedative and analgesic properties and interact with the central nervous system. These chemicals have also been shown to have antimicrobial and antidiabetic effects.

2.10.7 Properties of *Ziziphus nummularia*

2.10.7.1 *Ziziphus nummularia*'s Ability to withstand Drought and Heat

Ziziphus trees and shrubs excel in extreme habitats. Here, we'll focus on their arid environment resettlement (Figure 2.1) [66]. Drought, one of the devastating effects of climate change, has become an important form of abiotic stress, affecting plant growth and decreasing the productivity and profits of agricultural systems. This form of stress will have an even greater effect in the future. This situation has triggered an increase in research in the field of stress resistance and tolerance of plants, like *Ziziphus nummularia*, which are naturally more tolerant to drought [67]. The objective is to create more tolerant and resilient crops to drought. Named physiologically and studied *Ziziphus nummularia*, which was under moisture stress. It was found that leaves of *Ziziphus nummularia* showed a decrease in chlorophyll a and b, and an increase in electrolyte leakage, which usually occurs in the stress response of plants [68]. Drought-responsive genes were extracted from *Ziziphus nummularia*, a plant that was stressed by applying 30% polyethylene glycol (PEG 6000) for drought, in an additional effort to comprehend the molecular underpinnings of stress tolerance, and were characterized in the context of development of drought resistant crops [69]. When drought tolerant

transgenic crops are developed, it is expected that ZnASR1 will be a candidate gene.

2.10.7.2 Antidiabetic and Hypolipidemic Potential of *Ziziphus nummularia*

Globally diabetes has become one of the most common chronic illnesses. The deregulation of blood sugar and chronic illness are prominent in developing countries. Diabetes is associated with higher morbidity and mortality than other chronic illnesses.

Diabetes will steadily worsen by damaging nerves and blood vessels, potentially causing vision loss, kidney dysfunction and heart disease if left to its own devices [70]. Diabetes has been treated with herbal medicine for centuries predating insulin and modern medicine. Patients prefer herbal medicine because they believe it has fewer side effects and are cheaper and easier to obtain [71].

In diabetes, prolonged hyperglycemia causes changes in the lipids and leads to atherosclerosis. In this regard, the antihyperglycemic and hypolipidemic activity of ethanolic and aqueous leaf extracts of *Ziziphus nummularia* were evaluated in alloxan induced diabetic rats [71]. Pancreatic damage was restored by both extracts with the ethanolic extract showing the most activity. Diabetic rats also showed a significant decrease in the blood levels of TGL, LDL, VLDL and TC with an increase in HDL [72]. The same was reported in [73] and also in a dexamethasone induced diabetic rat suggesting the potential of *Ziziphus nummularia* in developing anti-hyperglycemics and hypolipidemics for diabetes [70, 71]. The extracts of *Ziziphus nummularia* leaves have been reported to have potential against diabetes and inhibit α -amylase enzyme [74].

All extracts exhibited considerable inhibitory action. However, leaf extracts obtained via maceration displayed both the highest activity and the highest associated phenolic content [75].

2.11 Role of Short Chain Fatty Acids and Gut Microbiota in ALS Neuroprotection

Fatty acids with fewer than six carbon atoms are known as short chain fatty acids (SCFAs), these include butyrate, propionate, and acetate. Dysregulation of SCFA production may result from alterations in the profile and function of bacteria in ALS. SCFAs are well-known for their many uses, such as neuroprotection, colocyte energy, and properties against inflammation [76].

It has been illustrated that butyrate, one of the main SCFAs, has neuroprotective qualities in a number of neurodegenerative illnesses. It may lessen neuronal damage in ALS by promoting mitochondrial activity, lowering inflammation, and modifying gene expression [77]. Energy metabolism has been linked to SCFA produced in the colon.

One of the hallmarks of ALS is energy metabolism dysregulation [78]. Therefore, ALS patients have been found to have altered SCFAs. Sodium butyrate [79], Sodium Phenobutyrate (NaPB), and butyrate conjugated with cations are the primary topics of the present articles. Our groundbreaking study with sodium butyrate was favorably accepted by the ALS community [80].

”In the rotarod test, mice treated with sodium butyrate (NaB) or Sodium Phenobutyrate (NaPB) had a significantly longer latency to fall. “Generally speaking, these investigations are well-designed, and the findings are repeated by two different teams of researchers. ALS Untangled gives the TOE ”Pre-Clinical Models” grade of A in light of these research.

Some of the characteristics of ALS that cannot be explained by genetics alone may be influenced by the gut flora. Mechanistic research on the microbiome and metabolites in the therapy of ALS is still somewhat few, nevertheless. As far as we are aware, no research has yet been done on the involvement of propionate or acetate in ALS [81].

2.12 Remedies of Motor Neuron Disease

Motor Neuron Disease (MND), including Amyotrophic Lateral Sclerosis (ALS), currently has no permanent cure; however, several therapeutic strategies could slow down disease progression and improve quality of physical and mental health. Pharmacological treatments such as Riluzole reduce glutamate-induced neurotoxicity and prolong survival.

Another approved drug, Edaravone, acts as an antioxidant that decreases oxidative stress in motor neurons, thereby slowing functional decline in early-stage patients. Supportive interventions play a paramount of important role in managing symptoms and maintaining patient function [82].

Multidisciplinary care involving neurologists, physiotherapists, respiratory specialists, and nutritionists has been shown to extend survival and enhance quality of life.

Respiratory support through non-invasive ventilation improves both longevity and comfort in patients with respiratory muscle weakness. Nutritional management, including early use of percutaneous endoscopic gastrostomy (PEG), helps prevent malnutrition and aspiration.

Symptom management such as using baclofen or tizanidine for spasticity, anticholinergic drugs for drooling, and antidepressants for emotional lability further improves patient well-being.

In addition, gene therapy and stem cell research are emerging as promising future directions, aiming to repair or replace damaged motor neurons and target genetic mutations linked to MND.

Thus, while MND remains incurable, a combination of pharmacological, supportive, and emerging molecular therapies offers significant relief and prolonged survival for affected individuals [83].

2.13 Phytochemistry of *Ziziphus nummularia*

Alkaloids, saponins, fatty acids, triterpenoids, and flavonoids have all been found in *Ziziphus nummularia* as shown in table 2.3.

2.13.1 Alkaloids

A 13-membered formylcyclopeptide alkaloid Nummularine-T, was isolated from *Ziziphus nummularia* bark, and its structure was determined using chemical and spectroscopic techniques [84].

2.13.2 Cyclopeptide alkaloids

Along with the well-known alkaloid nummularine-B, two new peptide alkaloids from *Ziziphus nummularia* have been found and their structures elucidated: nummularine - M and nummularine-N.

While nummularine-N is a 13-membered cyclopeptide similar to nummularine-B, nummularine-M is a 14-membered cyclopeptide that is of the integerrinine type [85].

2.13.3 Glycosides

A new (25 S)-spirostane was found to be nummularogenin, (25 S)-3 alpha-hydroxy-5 alpha-spirostane-2, 12dione [86].

2.13.4 Glycosides or Saponin

β -Dglucopyranosyl-(1 $\rightarrow$ 2)-6-deoxy- α -L-talopyranosyl-(1 $\rightarrow$ 3) is the structure of Zizynummin, a novel dammarane saponin that was extracted from dried *Ziziphus nummularia* leaves.- α -Larabinopyranosyl (1 $\rightarrow$ 3)-jujubogenin [87].

2.13.5 Phenolic Compounds

GC-MS analysis of the leaf extract of *Ziziphus nummularia* led to the identification of several phenolic and fatty-acid-derived constituents, including ethyl alpha-D-glucopyranoside behenylbehenate, linoleic acid, stigmasterol, phytol, squalene, oleic acid, tricosane and tetradecane [88].

2.13.6 Terpenoids

GC-MS profiling of the leaves also revealed a range of terpenoid and related constituents, namely 1-eicosanol, betulin, campesterol, γ -sitosterol, geranylinalool isomer, linoleic acid, lupeol, palmitic acid, stearic acid, vitamin E and trans-geranylgeranyl oil [89].

2.13.7 Flavonoids

Pharmacological evaluation of *Ziziphus nummularia* leaf extracts for phytotoxic and molluscicidal activity led to the identification of the flavonoid constituents quercetin, daidzin, myricetin-3-O-galactoside and pyrogallol [90].

2.13.8 Phenols

Phytochemical investigation of the leaves and fruits of *Ziziphus nummularia* further identified the phenolic compounds morin, pyrogallol and rutin [91].

TABLE 2.3: List of phytocompounds reported in *Ziziphus nummularia*

Sr.No	Compound name	Phytochemical family	Reference
1	Nummularine-A	Alkaloids and Cy-clopeptides	[92–95]
2	Nummularine-B	Alkaloids	
3	Nummularine-C		
4	Nummularine-D		

Table 2.3 continued from previous page

Sr.No	Compound name	Phytochemical family	Reference
5	Nummularine-E		
6	Nummularine-F		
7	Nummularine-G		
8	Nummularine-K		
9	Nummularine-H		
10	Mucronin-D		
11	Jubanine-A		
12	Jubanine-B		
13	Mauritine-C		
14	Amphibinine-H		
15	Frangufoline		
16	Fubanine-B		
17	Nummularine-M		
18	Nummularine-N		
19	Nummularine-O		
20	Nummularine-P		
21	Nummularine-R		
22	Nummularine-S		
23	Nummularine-T		
24	Nummularine-U		
25	Mauritine-A		
26	Mauritine-F		
27	Mauritine-D		
28	Scutianine-C		
29	Nummularine N		
30	Nummularine P		
31	Nummularine R		
32	Zizynummin	Saponin	[96]
33	Ethylalpha-d-glucopyranoside	Phenolic Compounds	[88]
	Behenylbehenate		
34	Linoleicacid		
35	Stigmasterol		
36	Phytol		
37	Squalene		
38	Pleic acid		

Table 2.3 continued from previous page

Sr.No	Compound name	Phytochemical family	Reference
39	Tricosane	Terpenoids	[89]
40	Tetradecane		
41	1-eicosanol		
42	Betulin		
43	Campesterol		
44	Gamma sitosterol		
45	Geranyllinalool isomer		
46	Linoleic acid		
47	Lupeol		
48	Palmitic acid		
49	Stearic acid	Flavonoids	[90]
50	Vitamin E		
51	Trans-geranylgeran oil		
52	Quercetin		
53	Daidzin		
54	Myricetin-3-O-galactoside		
55	Pyrogallol		
56	Morin	Phenols	[91]
57	Pyrogallol		
58	Rutin		

2.14 Pharmacological Properties of *Ziziphus nummularia*

2.14.1 Anthelmintic Activity

The plant's crude extract in methanol solvent was tested for its in vitro anthelmintic effectiveness against *Haemonchus contortus* using the progression of larva, egg hatch, and adult motility assays. Sheep infected with nematodes of gastrointestinal tract were given escalating dosages of crude powder (CP) and CME (1.0-3.0g/kg) to assess in vivo anthelmintic efficacy [97].

2.14.2 Antitumor Activity

For the first time, lapatin was extracted from the *Ziziphus nummularia* plant. In female Swiss albino mice that were 6–8 weeks old and had sarcoma-180 (S-180) ascetic tumor cells, its anti-tumor properties were assessed both on their own and in conjunction with radiation.

S-180 viable ascetic cells were delivered intraperitoneally into 2x10⁵ female mice. It has been observed that naphthoquinones and their analogues exhibit anticancer properties against murphy sternum lymphosarcoma and rat tumor Walker 256 carcinosarcoma [98].

2.14.3 Antibacterial Activity

Plant aqueous and ethanol extracts formed an antibacterial zone that was evaluated against a few medically significant bacteria (*B. cereus*). Ten grams of each plant were used for ethanol and aqueous extraction after various plant parts were gathered, air dried, and ground into a powder in a homogenizer. The aqueous extraction was carried out for six hours at a low heat in distilled water. For the antimicrobial test, the extract was reduced to one-fifth of its initial volume. After being extracted in ethanol, the substance was kept on a rotating shaker for the whole night. The filtrate was centrifuged at 5000 rpm after it had been collected. For the antimicrobial assay, the extract was concentrated to one-fifth of its initial volume [99].

Nine strains of *Ziziphus nummularia* were inhibited in their development by the ethanol extracts. Compared to ethanol extracts, aqueous extracts exhibited lower activity. This might be due to two factors: either the same active ingredients were present in water extracts at low concentrations, or the active ingredients were soluble in organic solvents and consequently absent from water extracts. Plant extracts offer a lot of promise as antimicrobial substances, particularly when it comes to treating infectious disorders brought on by resistant microbes [100].

2.14.4 Antifertility and Abortifacient Activity

Abortion is induced by taking 3–5 g of *Ziziphus nummularia* root bark powder combined with candy sugar and milk twice a day. Of these, 35.13% are contraceptive, 56.75% are abortifacient, and 8.1% result in male sterility. While the aforementioned plant species have numerous different therapeutic applications, reports of their abortifacient and antifertility qualities have also come from other regions and nations [101]. Along with these properties Anti diarrheal, anti-inflammatory and anti-congestion activity are also proved by research [102].

Chapter 3

Databases and Tools

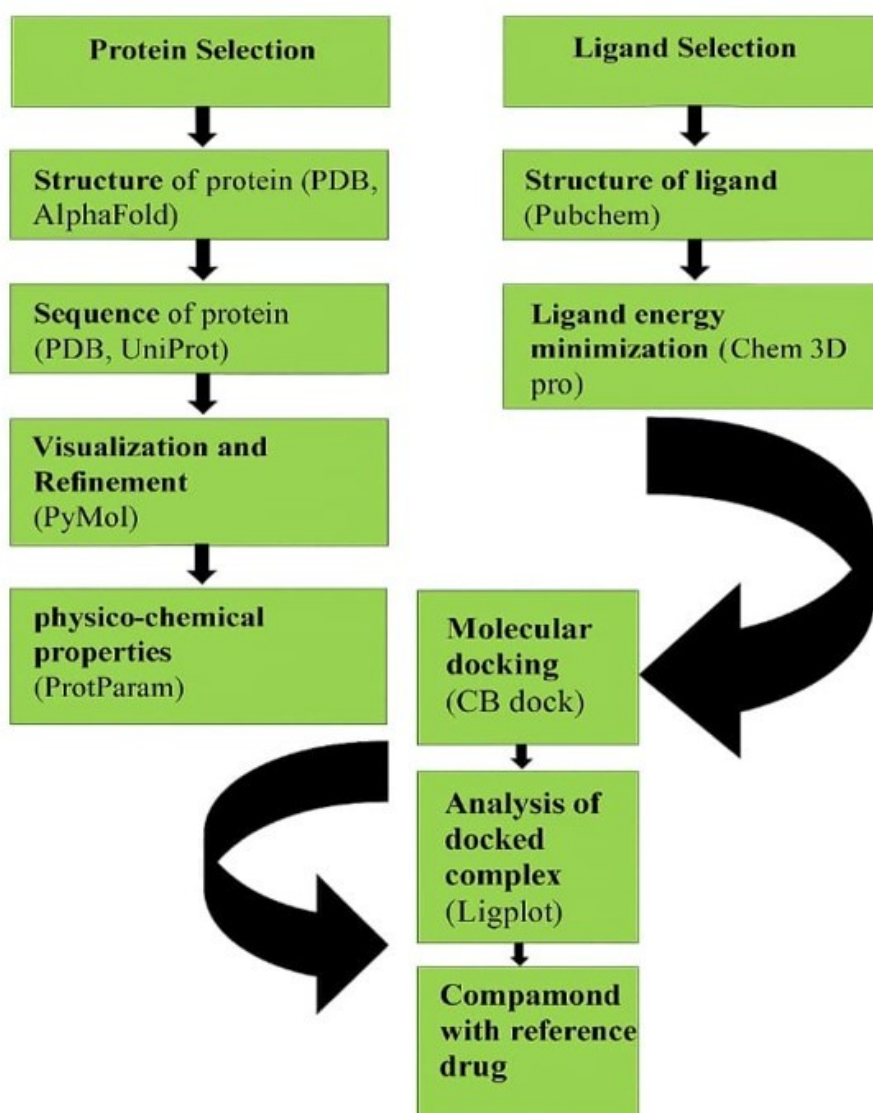


FIGURE 3.1: Workflow of in-silico methodology

3.1 Databases

The study used various databases for literature retrieval and analysis of data which includes following.

3.1.1 PubMed

The National Center for Biotechnology Information (NCBI) and the National Library of Medicine of the United States (NLM) oversee PubMed, a publicly available website at <https://pubmed.ncbi.nlm.nih.gov/>.

To locate peer-reviewed studies papers about SOD1 and TDP- 43 proteins, antimicrobial plant substances, motor neuron disease (MND) development and molecular docking studies, this research made use of PubMed as a source of literature retrieval resource. Boolean operators were used to apply certain keyword combinations, such as “Motor Neuron Disease and Superoxide Dismutase 1&TAR-DNA binding Protein 43” and “*Ziziphus nummularia* phytocompounds”. .

3.1.2 Google Scholar

Google Scholar, which is an educational search engine, also enabled the user to find academic articles, conference publications, thesis papers, and scholarly sources not included in the database of PubMed.

3.1.3 PubChem

PubChem was used to get the molecular details and chemical structures of a few selected phytocompounds from *Ziziphus nummularia*. Prior to docking and ADMET prediction analysis, Canonical SMILES, 2D structures, molecular weight, and physical and chemical characteristics were used to construct the ligand.

3.1.4 PkCSM

pkCSM was used as to assess the pharmacokinetic and toxicity properties of certain ligands. SMILES formats of each chemical have been supplied for the assessment of ADMET parameters. Results were examined for the sake of evaluate the safety profiles and as drug properties of possible compounds.

3.2 Tools

Following tools are used in this study.

3.2.1 DogSiteScorer

DogSiteScorer, is a computational tool for the detection and evaluation of ligand-binding pockets on protein surfaces. It is widely used in structure based drug discovery and molecular docking studies to predict potential active sites and assess their druggability.

The tool is available on the ProteinsPlus platform and can analyze three - dimensional protein structures from PDB files or from experimentally solved protein models <https://proteins.plus/help/dogsite> [103].

3.2.2 BIOVIA Discovery Studio Visualizer

This tool was used in the current investigation to analyze and visualize ligands and proteins <https://discover.3ds.com/discovery-studio-visualizer-download>.

BIOVIA Discovery Studio Visualizer was used to display and evaluate protein-ligand complexes after docking. It made it possible to investigate hydrogen bonds, hydrophobic interactions, binding conformations, and active-site residues. The tool was also used to generate high-resolution structural images and 2D interaction representations in order to assess and visualize the docking findings. [104].

3.2.3 PyRx

PyRx is a computerized drug development virtual testing tool that looks at a variety of compounds in connection to therapeutic targets. From data preparation to final analysis, it assists users at every step. It is a helpful tool for drug discovery research due to its user-friendly design. Because PyRx has spreadsheet-style functionality, docking tools, and strong presentation capabilities, it helps to carry out the structure-based drug design process.

The application is capable of doing molecular docking using multiple ligands and is able to show the necessary region for each ligand attachment to the protein as well. The results are evaluated quantitatively based on the ligand location evaluation and by examining the scoring algorithms. The highest binding energy is observed when docking is finished (<https://pyrx.sourceforge.io/>).

3.3 Selection of Disease

MND, progressive neurological disorders group that affect motor neurons, the specialized nerve cells that control voluntary muscle activities such as walking, talking, swallowing and breathing. In MND these neurons gradually deteriorate and collapse leading to muscle weakness, paralysis and loss of motor function. The motor cortex and spinal cord's upper and lower motor neurons are primarily affected by the illness.

The most prevalent type of MND is amyotrophic lateral sclerosis (ALS), although there are other types as well, such as progressive bulbar palsy, progressive muscle atrophy, and primary lateral sclerosis.

Although the exact cause of MND is still unknown, environmental variables, oxidative stress, protein aggregation, and genetic abnormalities are considered to contribute to the disease's progression [105]. Motor neuron disease is a relatively rare disease in the world with an estimated prevalence of about 4–6 cases per

100,000 individuals and an estimated incidence of 1–2 new cases per 100,000 populations per year.

The disease is more common in adults older than 50 years and occurs slightly more in males than females. MND may occur anywhere in the world, but differences in prevalence between populations and geographic areas have been reported. MND is a progressive and fatal disease and represents a major clinical, social and economic challenge underscoring the importance of early diagnosis and research into therapeutic interventions [106].

3.4 Selection of Protein

This *in silico* study was performed on Superoxide Dismutase 1 (SOD1) and TAR DNA-binding Protein 43 (TDP-43) due to their pivotal role in pathogenesis of the Motor Neuron Disease, especially Amyotrophic Lateral Sclerosis (ALS).

Both proteins have important roles in neuronal survival and cellular homeostasis, and their structural and functional aberrations are strongly associated with motor neuron degeneration. These proteins are considered important therapeutic targets in neurodegenerative disease research, considering their known part in disease progression [106].

Superoxide Dismutase 1 was selected, as mutations in the SOD1 gene are one of the major genetic causes of familial ALS. Under normal circumstances, SOD1 is an antioxidant enzyme that protects cells from oxidative stress by converting toxic superoxide radicals to oxygen and hydrogen peroxide.

Mutated or misfolded SOD1 proteins, however, lose their normal function and form toxic aggregates within motor neurons that cause oxidative damage, mitochondrial dysfunction, and neuronal cell death. SOD1's three-dimensional structure and active regions are well characterized and it is widely used in molecular docking and drug discovery studies in order to identify compounds that can stabilize the protein and reduce its toxic effects [107].

TDP-43 (TAR DNA-binding Protein 43) was similarly chosen because it is one of the most important pathological proteins associated with sporadic ALS cases. TDP-43 is usually involved in RNA processing, gene expression and mRNA transport in neurons. In disease conditions the protein is mislocalized from the nucleus to the cytoplasm, where it forms insoluble aggregates which disrupt normal cellular functions and contribute to neurodegeneration. TDP-43 aggregates are found in the vast majority of ALS patients and are considered a hallmark feature, thus representing an important target for therapeutic research.

3.5 Selection of Target Plant

Ziziphus nummularia was selected for this study as a medicinal plant with rich bioactive phytochemicals with proven antioxidant, anti-inflammatory and neuroprotective properties. These biological activities are of particular importance in Motor Neuron Disease where oxidative stress, protein misfolding and neuronal degeneration are major players in disease progression [108].

The plant contains important phytochemicals such as flavonoids, alkaloids, phenolics and terpenoids that can help to inhibit the toxic effects of disease-associated proteins such as Superoxide Dismutase 1 and TAR DNA-binding Protein 43. Moreover, natural compounds are widely assessed in the drug discovery process for their structural diversity and lower toxicity than synthetic drugs. Hence, *Ziziphus nummularia* was chosen as a probable natural source for identification of therapeutic compounds against motor neuron disease by *in silico* molecular docking studies.

3.6 Selection of Ligands

The therapeutic potential of 79 phytochemical substances found in *Z. nummularia* was investigated after a thorough examination of the literature. Out of these, 13 compounds were chosen for additional research based on their stated anti-oxidant,

anti-inflammatory, anti-excitotoxic, mitochondrial support, and autophagy regulation properties. The database PubChem, which offers chemical information in standard forms, is where the chosen chemicals were obtained [108]. The table below lists these chemicals' PubChem IDs and descriptions.

TABLE 3.1: Names of Phytochemicals with their families and PubChem IDs

Sr.	Compound Name	Phytochemical Family	PubChem IDs	Ref
1	Amphibine-H	Alkaloids	51029223	[109]
2	Nummularine-F	Alkaloids	5281594	[110]
3	Nummularine-R	Alkaloids	13946329	[110]
4	Mauritine-A	Alkaloids	11353668	[111]
5	Scutianine-C	Alkaloids	45268911	[111]
6	Betulin	Terpenoids	72326	[112]
7	Daidzin	Flavonoids	107971	[113]
8	Nummularine P	Alkaloids	101427782	[114]
9	Lupeol	Terpenoids	259846	[112]
10	Rutin	Flavonoids	5280805	[115]
11	Ellagic acid	Phenols	5281855	[116]
12	Nummularogenin	Steroids	21122661	[117]
13	Taxifolin	Flavonoids	439533	[116]

3.7 Virtual Screening

3.7.1 Lipinski Rule

Lipinski's rule of five was used to assess the phytochemicals' potential as drugs [118]. This recommendation aids in determining whether or not a substance is a good fit for oral medicine. This rule states that a prospective therapeutic molecule

should typically fulfill the specific requirements. Lipinski's rule of five states that an oral drug should not conflict with more than one of the following:

TABLE 3.2: Lipinski's parameters

Sr. No.	Parameters	Standards
1	Hydrogen bonds acceptors	≤ 10
2	Hydrogen bonds donors	≤ 5
3	Molecular weight	≤ 500 g/mol
4	LogP	< 5
5	Rotatable bonds	< 10

Compounds with acceptable absorption, appropriate distribution throughout the body, favorable metabolism, effective elimination, and minimal toxicity were identified thanks to this investigation. ADMET screening chemicals and Lipinski's guidelines were prioritized for further target protein interaction research.

3.7.2 ADMET Properties

To evaluate the pharmacokinetic and safety profiles of the selected ligands, The characteristics of ADMET were evaluated using the pkCSM server. The pkCSM program provides information related to drug design, blood-brain barrier permeability, and cytochrome p450 interactions used to estimate these properties [118].

3.8 Molecular Docking

After the SOD1 and TDP-43 proteins were prepared, their structures were kept in PDB format, which works with PyRx program. Additionally, PyRx was used to convert the SDF files of the chosen phytochemical ligands from *Z. nummularia* that were retrieved from PubChem into PDB format. The protein structure was submitted to PyRx and transformed into the pdbqt macromolecule file format needed for docking. All ligands were loaded, their energies were reduced, and

they were also transformed into pdbqt format using the Open Babel tool built into PyRx. The docking procedure was then built up using PyRx's Vina Wizard option. To make sure the protein was completely covered, the grid box size were changed using the maximum option once the protein and ligands were chosen.

Lastly, Autodock Vina inside PyRx was used to carry out molecular docking. With an emphasis on each ligand's binding affinities with the target protein, the docking data were documented. For further interaction analysis, the ligands with the highest scores were chosen.

3.9 Analysis and Visualization of Protein Ligand Interaction

Discovery Studios Visualizer were used to further investigate the molecular docking data. The precise interactions between SOD1, TDP-43, and the docked ligands were examined using this program. It gave precise details on the kinds of bonds that are formed, including hydrogen, hydrophobic, and electrostatic bonds, as well as the residues of certain amino acids that are involved in binding.

Discovery Studio generated excellent 2D and 3D visual representations of the protein-ligand complexes in addition to interaction details, which facilitated the interpretation and presentation of the docking data.

3.10 Lead Compound Identification

The most promising *Z. nummularia* compounds were selected as lead compounds based on the outcomes of the Lipinski'RO5 and ADMET screening. Lead compounds are those that meet every requirement.

Chapter 4

Results

4.1 The Selection and Preparation of Protein

4.1.1 The Structure of Protein

SOD1 and TDP-43 proteins were chosen as the study's focus because of their critical roles in motor neuron disorders. The 3D structures of these protein were downloaded from PDB with the unique IDs 4IUF and 2NAM respectively.

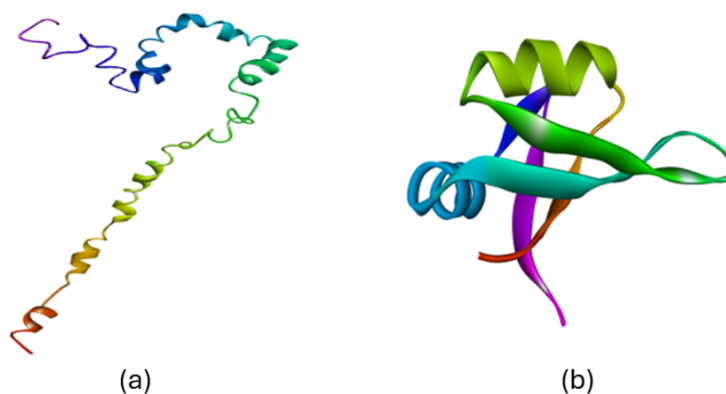


FIGURE 4.1: Structure of Proteins (a) SOD1 (b) TDP-43

The structures of proteins were revised using PyMol by eliminating any water molecules and associated ligands. In addition, polar hydrogens were inserted for the stable configuration, and other atoms were eliminated to prevent overlapping. The altered data were saved in PDB format.

4.1.2 Physicochemical Properties of Target Proteins

Using the web application ProtParam, a number of parameters about proteins were forecasted, including their molecular and structural attributes. The table below contain the physiochemical properties of selected protein which gave information about the proteins function, stability, interaction, biological activities and their behavior in experimental conditions

TABLE 4.1: The target proteins physicochemical attributes

Sr.	Target proteins	MW	PI	NR	PR	Ext. Co1.	Ext. Co2.	Instability Index	Aliphatic Index	GRAVY
1	SOD1	16815.64	6.13	21	15	5750	5500	20.35	75.03	-0.45
2	TDP43	8973.37	7.72	10	11	14105	13980	33.39	71.97	-0.416

SOD1 had a molecular weight of 16,815.64 Da, higher than TDP43 at 8,973.37 Da, indicating a larger protein structure. The theoretical isoelectric point (pI) of SOD1 was 6.13 indicating a slightly acidic nature while TDP43 was 7.72 indicating a slightly basic nature. This was also in line with the distribution of charged residues. SOD1 has more negatively charged residues (21) than positively charged residues (15), while TDP43 has a near balanced composition of negatively charged (10) and positively charged residues (11). TDP43 had higher extinction coefficients (14,105 and 13,980 M⁻¹ cm⁻¹) than SOD1 (5,750 and 5,500 M⁻¹ cm⁻¹), indicating that it has a higher potential for absorbance due to more aromatic amino acids. Both proteins had instability index values below 40, indicating that they are both stable in vitro, but SOD1 (20.35) seemed more stable than TDP43 (33.39). Similarly, the aliphatic index of SOD1 (75.03) was a little higher than that of TDP43 (71.97), indicating the relatively higher thermal stability. Both proteins had negative GRAVY values, SOD1 (-0.45) and TDP43 (-0.416), indicating hydrophilic nature and a favorable interaction with aqueous environments. Overall the analysis shows that SOD1 is relatively more stable and hydrophilic, TDP43 has better UV absorbance properties and is slightly basic [119].

4.1.3 Active Site Identification

The dogsitescorer software was used to determine number of pockets in each protein, which gave idea about surface area and volume that predict the active sites of the proteins.

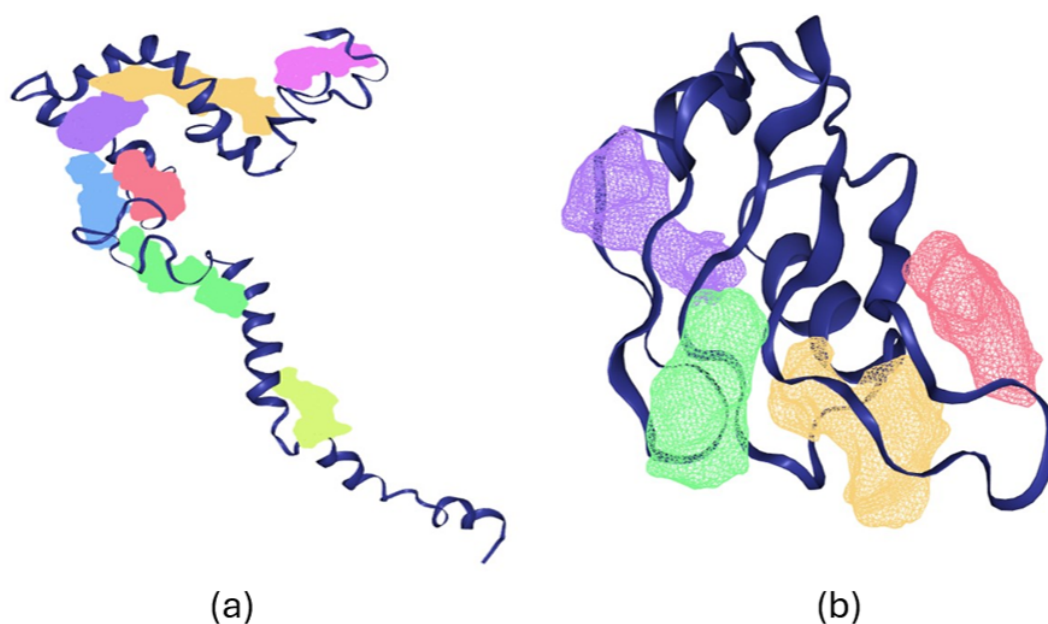


FIGURE 4.2: Active sites of refined target proteins (a) SOD1 (b) TDP-43

Dogsitescorer predict different number of pockets in each protein and these pockets have varying surface area and volume. TDP-43 has 07 active site for ligand binding while SOD1 has 04 pockets.

4.2 Ligands Chemical Structure Retrieval

A publicly available chemistry database, PubChem offers extensive details on the biological, physiochemical, and structural characteristics of tiny molecules. The National Institutes of Health's (NIH) website provides information about synthetic chemicals, natural products, and bioactive molecules relevant to medication research.

79 compounds were found in the literature review, and the structures of the 13 chosen compounds were retrieved in SDF format from PubChem.

TABLE 4.2: Chemical structures of ligands

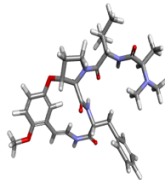
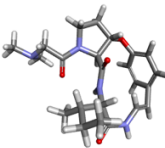
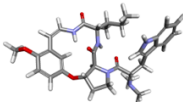
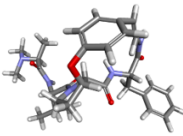
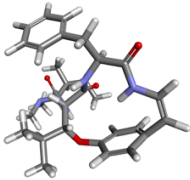
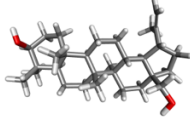
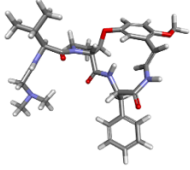
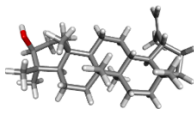
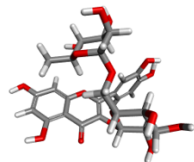
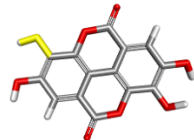
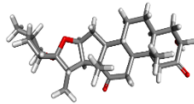
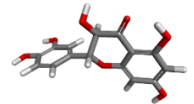
Sr.	Compound Name	Molecular Formula	Molecular Weight	Structure
1	Amphibine-H	$C_{33}H_{43}N_5O_6$	605.75	
2	Nummularine-F	$C_{23}H_{32}N_4O_4$	428.5	
3	Nummularine-R	$C_{33}H_{41}N_5O_5$	587.7	
4	Mauritine-A	$C_{32}H_{41}N_5O_5$	575.7	
5	Scutianine-C	$C_{31}H_{42}N_4O_4$	534.7	
6	Betulin	$C_{30}H_{50}O_2$	442.7	
7	Daidzin	$C_{21}H_{20}O_9$	416.4	
8	Nummularine P	$C_{32}H_{41}N_5O_6$	557.7	

Table 4.2 continued from previous page

Sr.	Compound Name	Molecular Formula	Molecular Weight	Structure
9	Lupeol	C ₃₀ H ₅₀ O	426.7	
10	Rutin	C ₂₇ H ₃₀ O ₁₆	610.5	
11	Ellagic acid	C ₁₄ H ₆ O ₈	302.19	
12	Nummularogenin	C ₂₇ H ₄₀ O ₅	444.6	
13	Taxifolin	C ₁₅ H ₁₂ O ₇	304.25	

4.3 Screening of Bioactive Compounds for Lead

4.3.1 Virtual Screening Through Lipinski's Rule of Five

This rule used as a first virtual screening filter for identify interesting ligands for more research. The rule states that if a molecule meets at least three of the following criteria, it is more likely to be orally bioavailable: molecular weight < 500 Da, a maximum of five hydrogen bond donors and ten hydrogen bonds acceptors, and octanol-water partition coefficient (LogP) < 5 [120].

Table 4.3 shows that the total number of ligands under investigation is 13 and that Lipinski's rule of five is applied for checking their drug likeness. The data indicate

that 05 ligands comply with all the Lipinski rules and are therefore presumed to be safe for oral administration; 07 ligands however, comply with all but one rule, which does not mean that they cannot be considered as molecules since one violation does not prohibit molecules from conforming.

On the other hand, just one of the thirteen ligands rutin violated three Lipinski criteria, making it inappropriate for direct pharmacological usage. Overall, these results imply that the majority of the chosen ligands have advantageous drug-likeness characteristics, with Lipinski's rule analysis excluding just one molecule. Docking studies were only conducted on ligands that satisfied these requirements.

TABLE 4.3: Virtual screening of ligands

Sr.	Ligands	Mol g/mol	Weight ≤500	LogP ≤5	Rotatable Bonds	HBA ≤10	HBD ≤5	Yes/No
1	Amphibine-H	591.7		1.9624	8	7	3	Yes
2	Nummularine-F	428.5		1.2279	4	5	2	Yes
3	Nummularine-R	587.7		3.3291	7	6	3	Yes
4	Mauritine-A	575.7		1.9538	7	6	3	Yes
5	Scutianine-C	534.7		3.3791	8	5	3	Yes
6	Betulin	442.7		6.9972	2	2	2	Yes
7	Daidzin	416.4		0.3443	4	9	5	Yes
8	Nummularine P	557.7		1.4236	8	7	4	Yes
9	Lupeol	426.7		8.0248	1	1	1	Yes
10	Rutin	610.5		-1.687	6	16	10	No
11	Ellagic acid	302.19		1.3128	0	8	4	Yes
12	Nummularogenin	444.6		4.1518	0	5	1	Yes
13	Taxifolin	304.25		1.1863	1	7	5	Yes

4.3.2 ADMET Properties

Pharmacokinetics (Pk), sometimes known as ADMET, describes how drug moves throughout the body over time, involving how much of it is absorbed, how it is distributed and metabolized, and how it is excreted. It also takes into account how the drug concentration in plasma and tissues is influenced by the formulation, the mode of administration, and the unique features of each patient. Determining a

dose plan that maximizes therapeutic effects while avoiding adverse consequences requires an understanding of PK [121].

4.3.2.1 Absorption

The process by which a medication moves from the site of administration to the systemic circulation is known as absorption, and it controls the amount and rate at which the medication becomes accessible in plasma. The pace and degree of medication absorption are influenced by variables such solubility, permeability, formulation, and physiological milieu. Recent studies have shown that improving absorption is crucial since inadequate absorption can lead even highly potent drugs to fail clinically [122].

All of the chemicals were divided into three groups based on the intestinal absorption values shown in table 4.4: high absorbers, which display absorption values greater than 80%, medium absorbers, which display absorption values between 30% and 79%, and low absorbers, which display absorption values less than 30%. The majority of compounds have good to excellent absorption (above 80%). Nummularogenin had the greatest absorption value (98%), while Nummularine-F and Daidzin had the lowest absorption rates (59%). The interpretation displays a scale from 0 (highly soluble) to -10 (insoluble) based on the water solubility measurements (Log S). The majority of medications claim less than -4. The majority of alkaloids and flavonoids, such as Taxifolin, Daidzin, and Nummularine-F, Mauritine-A, have values between -2 and -4. Lupeol and betulin, on the other hand, have extremely low solubility (more than -5).

TABLE 4.4: Absorption properties of selected ligands

Sr.	Ligands	Water Solubility	CaCO ₂ Permeability	Intestinal Absorption	Skin Permeability	P-glycoprotein Sub	P-glycoprotein I Inh	P-glycoprotein II Inh
1	Amphibine-H	-3.929	0.44	66.768	-2.734	Yes	Yes	Yes
2	Nummularine-F	-3.355	0.513	59.263	-2.807		No	No

Table 4.4 continued from previous page

Sr.	Ligands	Water Solubility	CaCO ₂ Permeability	Intestinal Absorption	Skin Permeability	P-glycoprotein Sub	P-glycoprotein I Inh	P-glycoprotein II Inh
3	Nummularine-R	-3.129	0.622	81.651	-2.735	Yes	Yes	Yes
4	Mauritine-A	-3.265	0.486	69.036	-2.732	Yes	Yes	Yes
5	Scutianine-C	-4.28	1.168	74.789	-2.731	Yes	Yes	Yes
6	Betulin	-5.446	1.191	94.539	-2.789	No	Yes	Yes
7	Daidzin	-2.784	0.24	59.319	-2.736	Yes	No	No
8	Nummularine P	-3.966	0.62	65.986	-2.768	Yes	No	No
9	Lupeol	-5.861	1.226	95.782	-2.744	No	Yes	Yes
10	Ellagic acid	-3.181	0.335	86.684	-2.735	Yes	No	No
11	Nummularogenin	-4.888	0.867	98.069	-3.99	Yes	Yes	Yes
12	Taxifolin	-3.042	0.924	64.709	-2.735	Yes	No	No

When examining the CaCO₂ permeability parameters, the intestinal wall permeability model's value should be higher than 0, but more than 0.90 is regarded as excessive. Lupeol, Betulin and Scutianine-C have higher values as compared to others, but overall all the ligands showed good CaCO₂ permeability, meanwhile Ellagic acid show the lowest penetration which is 0.335×10^{-6} cm/s. If, we talk about skin permeability the desired value is -2 and all ligand fulfill this value.

Finally, being P-glycoprotein substrate mean drug will be transported by these proteins all ligands are substrate except Betulin and Lupeol. Most of the ligands also act as P-glycoprotein inhibitors and they may affect the drug interaction with targets e.g Mauritine-A, Scutianine-C etc.

4.3.2.2 Distribution

After the substance enters the bloodstream, circulation distributes it throughout the body's tissues and organs. Blood flow, tissue permeability, the capacity of chemicals to attach to plasma proteins, and their lipophilicity all affect the pace of distribution [123]. Only the unbound portion of the medication may cross

the membranes and enter extravascular tissues, while medications with a strong affinity for proteins might stay in plasma and extend their effects.

Theoretical Distribution Volume VDss show how much medication is distributed from the blood to the tissues; a value greater than 0.45 L/kg indicates good tissue distribution [124]. Compounds with greater VDss, such as Taxifolin and Nummularine-R, may be better dispersed to tissues, whereas those with lower VDss, such as Nummularine-P and Scutianine-C, may remain in the systemic circulation rather than enter the tissues.

When analyzing BBB permeability, a mean of >-1.0 indicates high permeability and <-1.0 indicates low permeability. Lupeol has higher BBB penetrations, in contrast Ellagic acid and Daidzin has lower values of this parameter. For CNS permeability the values above -2 are preferred as they show the tendency to penetrate in CNS while talking about the selected ligands only Taxifolin, Ellagic acid, Nummularine-P and Diadzin has values lower than -2. It indicates most of the ligands has sufficient ability to penetrate CNS and BBB.

The percentage of medications that are not bound to plasma protein is known as the fraction unbound (Fu) value and free to perform their pharmacological activities, the > 0.1 is considered good. All the ligands have sufficient unbound fraction except Lupeol and Betulin which has $F_u=0$, indicate their absolute affinity with protein.

TABLE 4.5: Distribution properties of ligands

Sr.	Ligands	VDss (human)	Fraction bound (human)	un- BBB per- meability	CNS per- meability
1	Amphibine-H	0.495	0.149	-0.562	-3.426
2	Nummularine-F	0.435	0.374	-0.537	-2.849
3	Nummularine-R	0.782	0.307	-0.764	-2.702
4	Mauritine-A	0.028	0.224	-0.248	-2.887
5	Scutianine-C	-2.731	0.037	-0.498	-2.453
6	Betulin	-0.177	0	-0.295	-2.035
7	Daidzin	-0.166	0.199	-1.232	-3.584
8	Nummularine P	-2.768	0.355	-0.694	-3.313
9	Lupeol	0	0	0.726	-1.714

Table 4.6 continued from previous page

Sr.	Ligands	Substrates		Inhibitors				
		CYP2D6	CYP3A4	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
3	Nummularine-R	None	Yes	None	Yes	Yes	None	Yes
4	Mauritine-A	None	Yes	None	None	None	None	Yes
5	Scutianine-C	None	Yes	None	None	None	None	Yes
6	Betulin	None	Yes	None	None	None	None	None
7	Daidzin	None	None	None	None	None	None	None
8	Nummularine P	None	Yes	None	None	None	None	None
9	Lupeol	None	Yes	None	None	None	None	None
10	Ellagic acid	None	None	Yes	None	None	None	None
11	Nummularogenin	None	Yes	None	None	None	None	None
12	Taxifolin	None	None	None	None	None	None	None

4.3.2.4 Excretion

The crucial and last stage of pharmacokinetics is excretion, which is the removal of medications and their metabolites from the body. In addition to bile, feces, perspiration, and exhaled air, the kidneys play a significant role in the removal of these substances through urine.

The effectiveness of excretion is influenced by drug solubility, molecular size and renal or hepatic processes. Compounds that undergo substantial metabolism are removed as more hydrophilic conjugates, while less metabolized compounds are frequently eliminated unaltered. Excretion studies are especially crucial in the early stages of drug discovery since poor renal clearance can also have a substantial impact on drug exposure and even toxicity concerns [126, 127]

Faster clearance is indicated by a higher value. Taxifolin with -0.078 ml/min/kg is the lowest total clearance value indicate longer lasting effect and too much accumulation could cause toxicity. While talking about Renal Organic Cation Transporter 2 (OCT2) the ligand Nummularogenin only use this channel while other depends on other channels rather than kidney transporter for the excretion.

TABLE 4.7: Excretion properties of ligands

Sr. No.	Ligands	Total Clearance	Renal OCT2 substrate
1	Amphibine-H	0.271	No
2	Nummularine-F	0.474	None
3	Nummularine-R	0.409	None
4	Mauritine-A	0.418	None
5	Scutianine-C	0.297	None
6	Betulin	0.236	None
7	Daidzin	0.104	None
8	Nummularine P	0.228	None
9	Lupeol	0.153	None
10	Ellagic acid	0.537	None
11	Nummularogenin	0.264	Yes
12	Taxifolin	-0.078	None

4.3.2.5 Toxicity

Evaluation of toxicity is a crucial component of early drug discovery in addition to pharmacokinetic features since it aids in identifying compounds that may have harmful consequences. In actuality, toxicity refers to a substance's capacity to cause unfavorable outcomes including liver damage, genetic alterations, heart issues, and other systemic impacts [128].

By identifying chemical structures and comparing them with pre-existing hazardous information, computational techniques enable researchers to predict these characteristics quite effectively. In order to assess the safety profile of chosen ligands, factors This provides an overview of the properties of AMES mutagenicity, hepatotoxicity, maximum tolerated dose and hERG inhibition in detail.

The idea behind the predictive modeling is that it helps decrease the chance of failures at later stages and allows adding to the shortlist only the compounds that have enough safety margin. Rational medication design now relies heavily on in silico toxicity prediction tools, which offer a speedier and more affordable substitute for conventional lab testing [129].

AMES toxicity evaluation is done by predicting mutagenicity (DNA damage). A “Yes” is a critical failure. Analysis reveals that no one is mutagenic in the selected ligands.

Predicting mutagenicity (DNA damage) is how AMES toxicity is assessed. “Yes” is a grave mistake. Analysis shows that none of the chosen ligands are mutagenic. A maximum tolerable dosage of more than 0.477 log mg/kg/day is regarded as excessive and possibly harmful. Numerous substances are predicted to be within the safe range.

Analysis shows that none of the chosen ligands are mutagenic. A maximum tolerable dosage of more than 0.477 log mg/kg/day is regarded as excessive and possibly harmful. Numerous substances are predicted to be within the safe range.

A maximum tolerable dosage of more than 0.477 log mg/kg/day is regarded as excessive and possibly harmful. Numerous substances are predicted to be within the safe range.

However, Daidzin has slightly higher value which is 0.488 mg/kg/day. hERG inhibition predicts potential for cardiotoxicity, only Amphibine-H possess hERG I inhibition, while hERG II inhibition is shown by most of the compounds. While dealing with hepatotoxicity and skin sensitization reveals that no one is skin sensitive while most of the compounds are hepatotoxic for instance mostly Alkaloids.

Toxicity is shown by environmental toxicity models for *T. pyriforms* > -0.5 and Minnow toxicity > -0.3 . Results ranges from 0.285-0.318, since these all values are above -0.5 and all these compounds if released in the water will be toxic for protozoa [130]. Moreover, Lupeol and Nummularine P higher concentrations will be required to be toxic because of their negative values while slight amount of Taxifolin and Mauritine-A will be sufficient to kill half the population of that organism.

Moreover, Lupeol and Nummularine P higher concentrations will be required to be toxic because of their negative values while slight amount of Taxifolin and Mauritine-A will be sufficient to kill half the population of that organism.

TABLE 4.8: Toxicity attributes of ligands

Sr.	Ligands	AMES toxicity	Max. tol- erated dose (human)	hERG I inh	hERG II inh	Oral Acute Toxicity (LD50)	Rat Oral Chronic Toxicity (LOAEL)	Hepato toxicity	Skin Sensiti- sation	<i>T. Pyri- formis</i> toxicity	Minnow toxicity
1	Amphibine-H	None	-0.678	Yes	yes	2.862	2.438	Yes	None	0.285	3.823
2	Nummularine-F	None	-0.138	None	Yes	2.225	2.433	Yes	None	0.283	3.125
3	Nummularine-R	None	-0.534	None	Yes	2.788	2.457	Yes	None	0.285	1.541
4	Mauritine-A	None	-0.527	None	yes	2.932	2.727	Yes	None	0.285	5.366
5	Scutianine-C	None	-0.599	None	yes	2.633	1.268	Yes	None	0.285	1.239
6	Betulin	None	-0.794	None	yes	2.699	2.172	Yes	None	0.315	-1.118
7	Daidzin	None	0.488	None	yes	2.738	4.717	None	None	0.285	3.902
8	Nummularine P	None	-0.697	None	None	2.02	2.341	Yes	None	0.285	3.582
9	Lupeol	None	-0.502	None	yes	2.563	0.89	None	None	0.316	-1.696
10	Ellagic acid	None	0.476	None	None	2.399	2.698	None	None	0.295	2.11
11	Nummularogenin	None	-0.732	None	yes	2.083	1.792	None	None	0.318	1.561
12	Taxifolin	None	0.345	None	None	2.261	3.102	None	None	0.286	4.688

4.4 Molecular Docking

Molecular docking makes advantage of the three-dimensional structures of proteins and ligands. PyRx, an extremely high throughput program, is used to dock ligands and receptors. PyRX provides us with the optimal position of ligands that bind to protein target sites and can dock many ligands simultaneously. Molecular docking results may be quantitatively examined by tracking scoring algorithms and by determining the ligand location. The effective docking of the protein and ligand is closely correlated with binding energy [131].

TABLE 4.9: Docking scores of ligand-protein complexes

Sr.	Ligands	Target Proteins	
		SOD1	TDP-43
1	Amphibine-H	-6.8	-6.7
2	Nummularine-F	-5.7	-5.5
3	Nummularine-R	-6.9	-6.3
4	Mauritine-A	-7.2	-6
5	Scutianine-C	-6.2	-5.8
6	Betulin	-6.2	-6
7	Daidzin	-5.9	-6.1
8	Nummularine P	-5.8	-6.5
9	Lupeol	-6.3	-6.4
10	Ellagic acid	-5.3	-5.9
11	Nummularogenin	-7.1	-6.9
12	Taxifolin	-5.5	-5.5

The molecular docking analysis of selected phytochemicals against Superoxide dismutase 1 and TAR DNA-binding protein 43 showed moderate to strong binding affinities. Mauritine-A had the highest binding affinity for SOD1 (-7.2 kcal/mol), followed by Nummularogenin (-7.1 kcal/mol), Nummularine-R (-6.9 kcal/mol) and Amphibine-H (-6.8 kcal/mol).

Ellagic acid, on the other hand, showed the weakest interaction (-5.3 kcal/mol). The Nummularogenin showed the highest binding affinity to TDP-43 (-6.9 kcal /

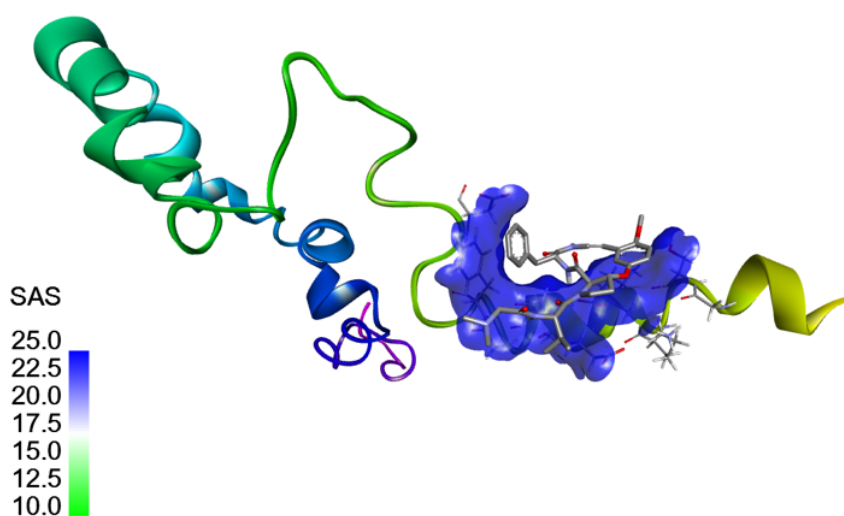
mol) followed by Amphibine-H (-6.7 kcal/mol), Nummularine P (-6.5 kcal/mol), and Lupeol (-6.4 kcal/mol).

Overall, Nummularogenin and Amphibine-H had relatively strong and balanced interactions with both target proteins, indicating their promise as potent bioactive compounds for further research in neurodegenerative diseases.

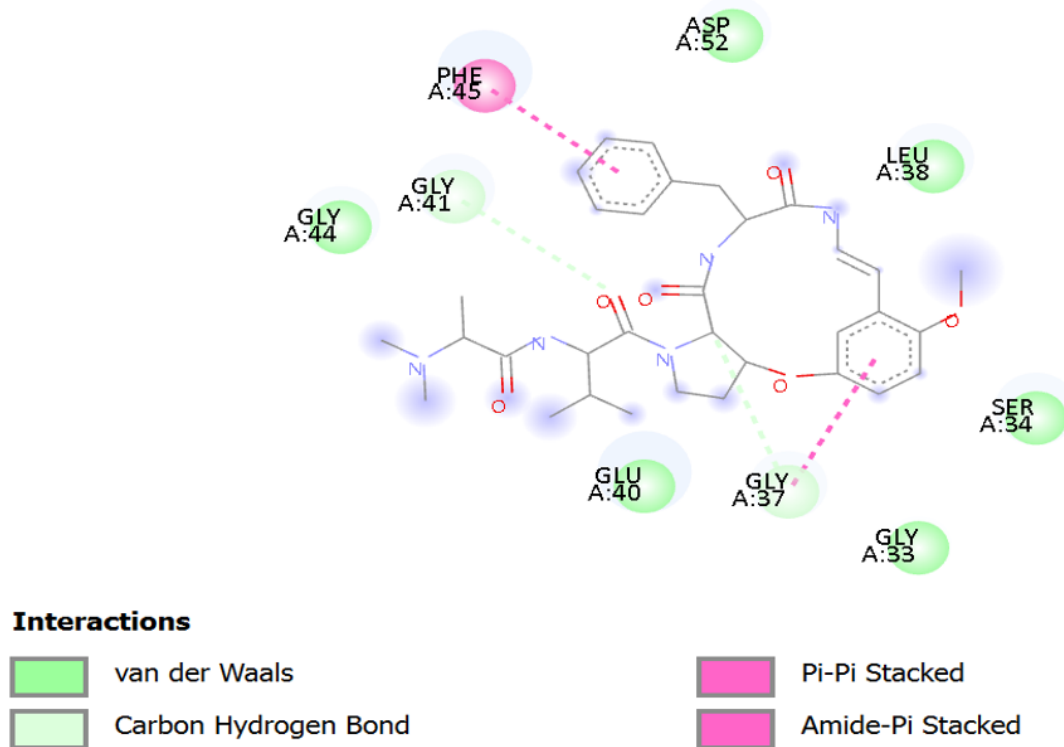
4.5 Analysis of Docked Complex via Discovery Studio

To understand docking results the interactions like hydrogen bonding, van der wall and alky interactions had been seen through Discovery Studio 2025 Client between proteins and ligands, the saved complex analyzed in detail. Numerous interactions had been seen which are discussed below between two proteins and twelve ligands.

Below are some images showcasing the docking complex and the ligand-receptor interactions. The first part of each of the images is dedicated to the docked complex while the second part explains the nature of the interactions.

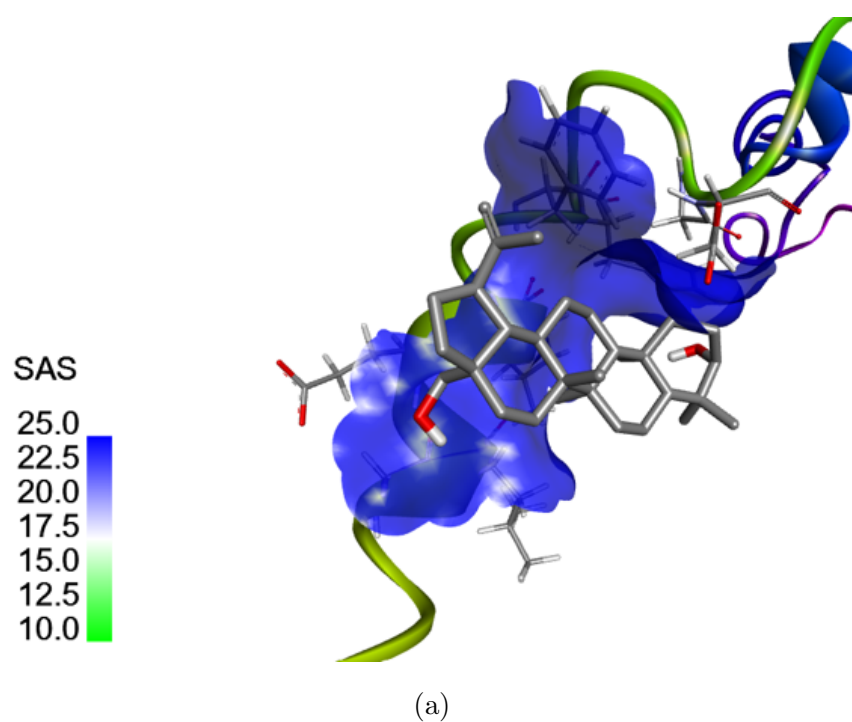


(a)



(b)

FIGURE 4.3: SOD1 protein and amphibine-H molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.



(a)

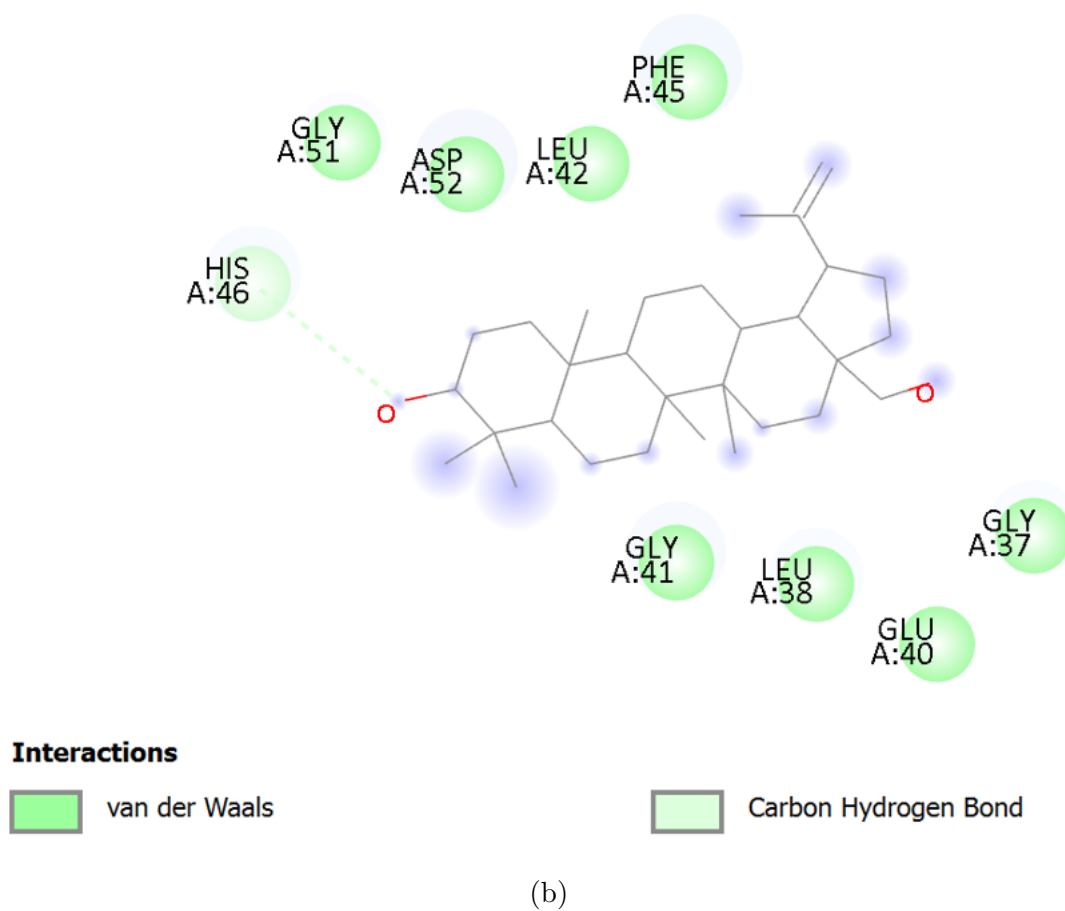
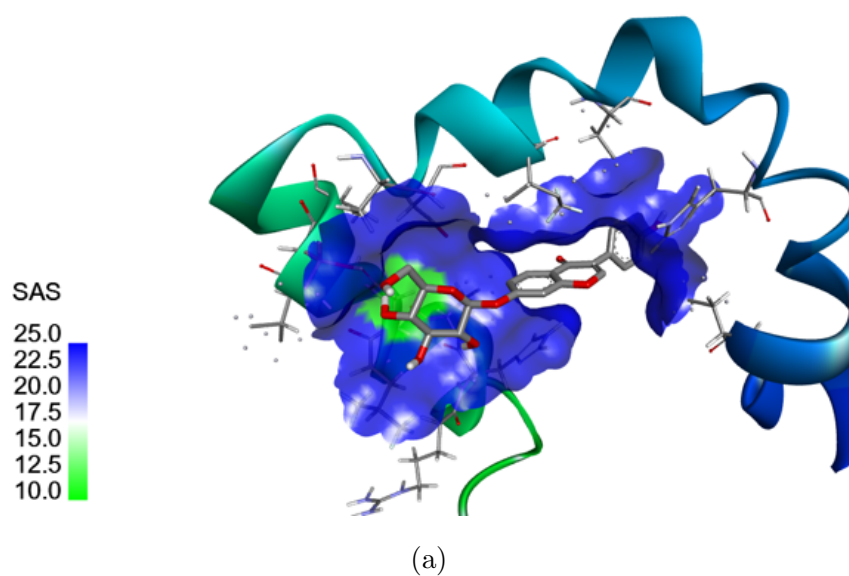


FIGURE 4.4: SOD1 protein and Betulin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.



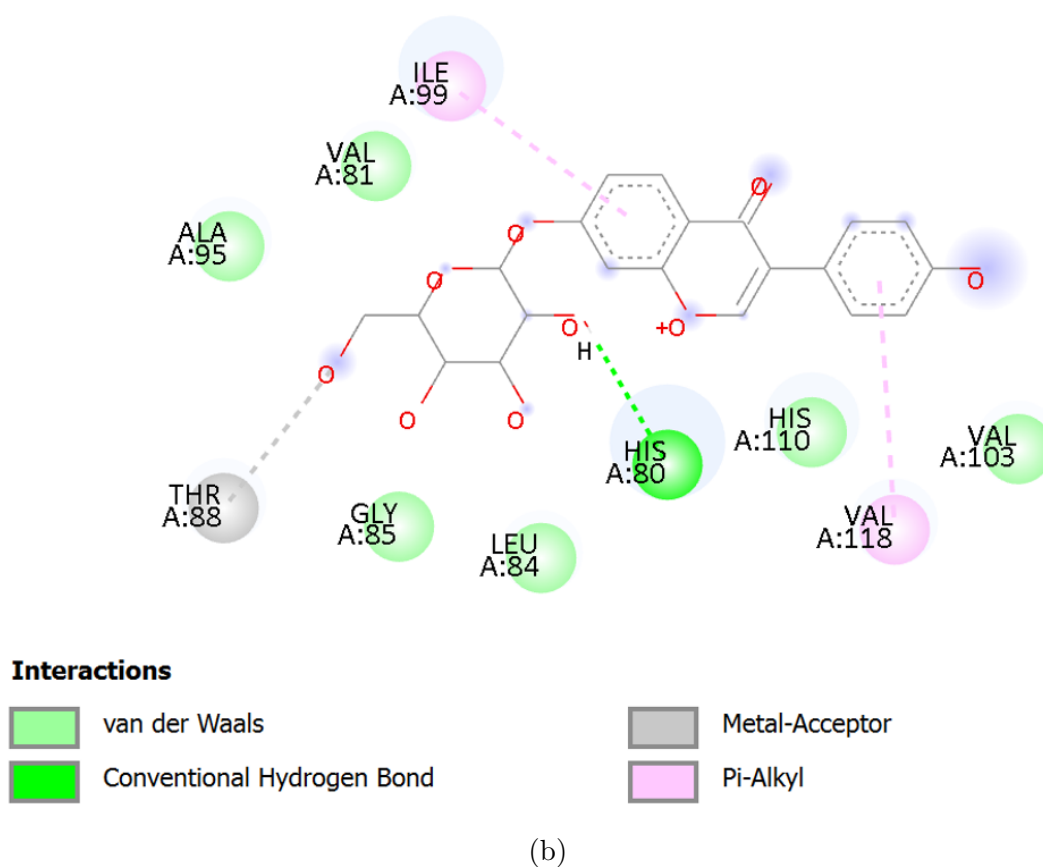
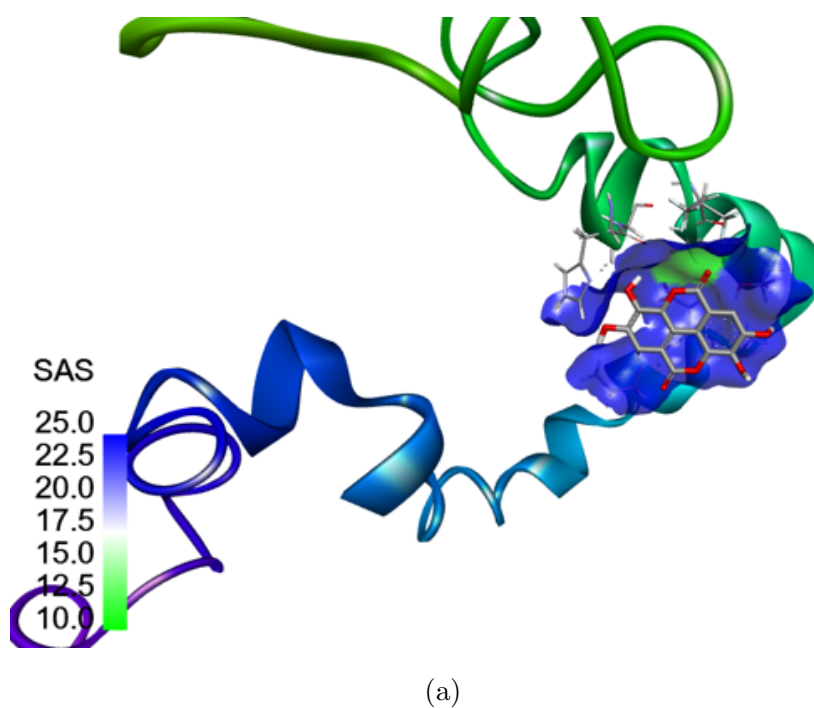


FIGURE 4.5: SOD1 protein and Daidzin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure



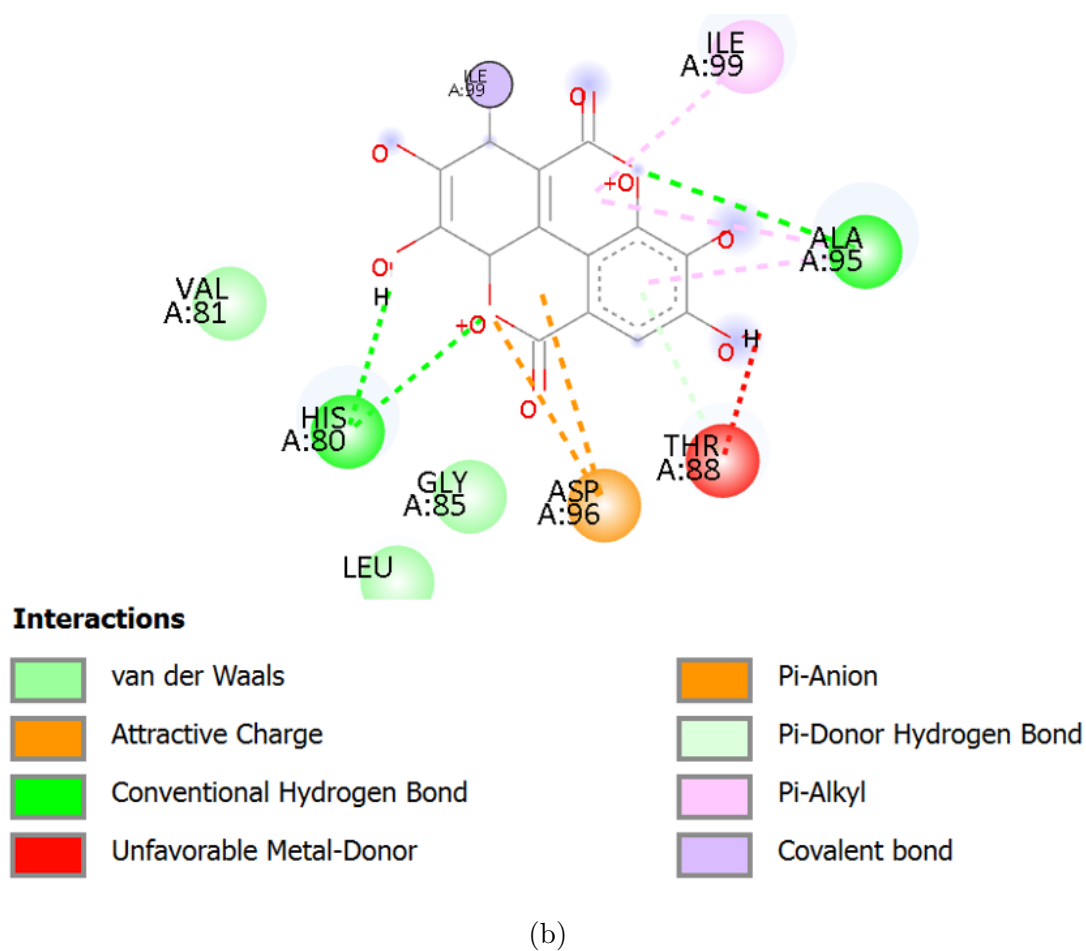
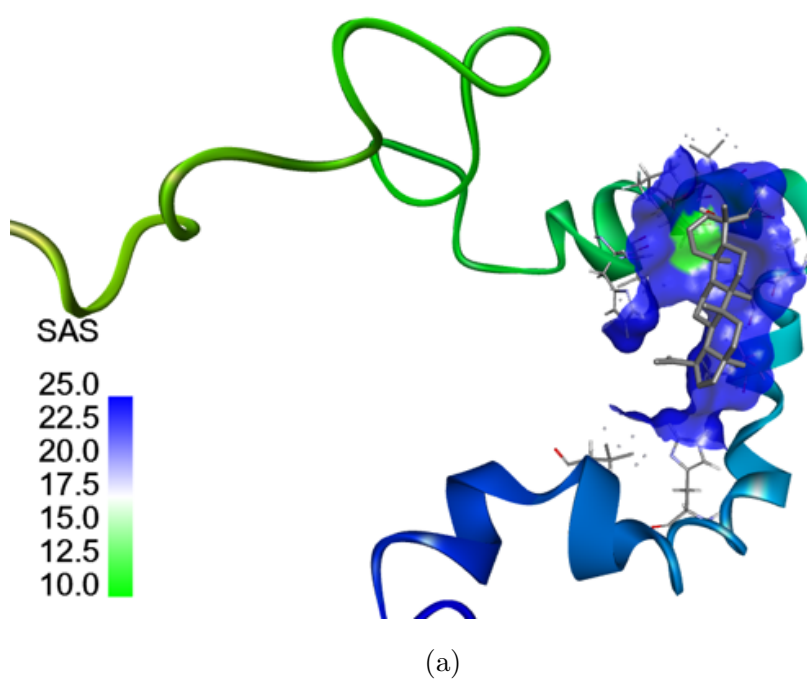


FIGURE 4.6: SOD1 protein and ellagic acid molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure



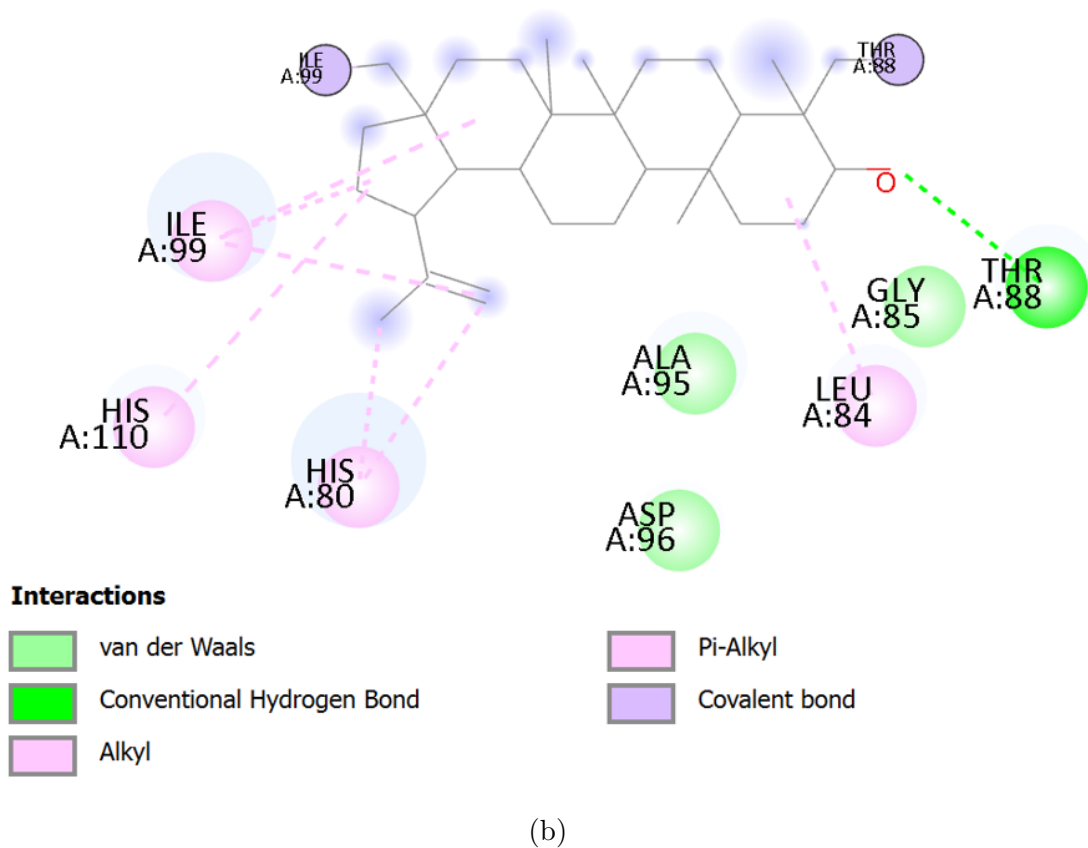
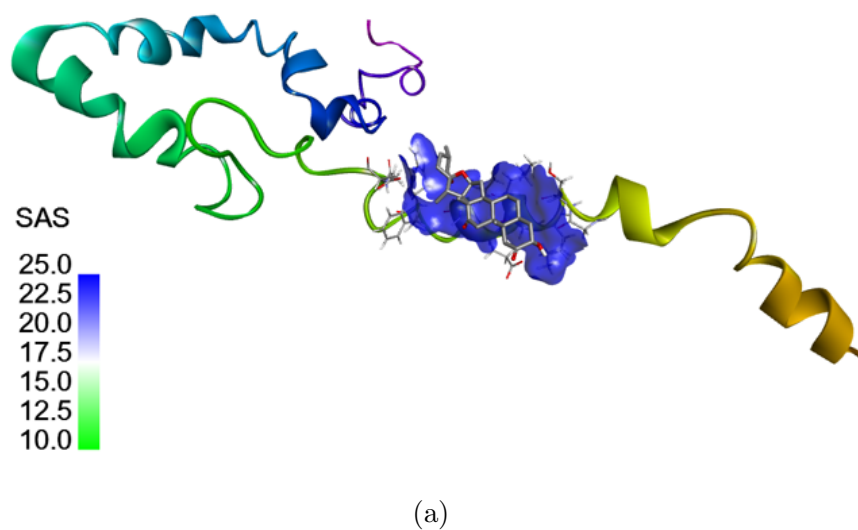
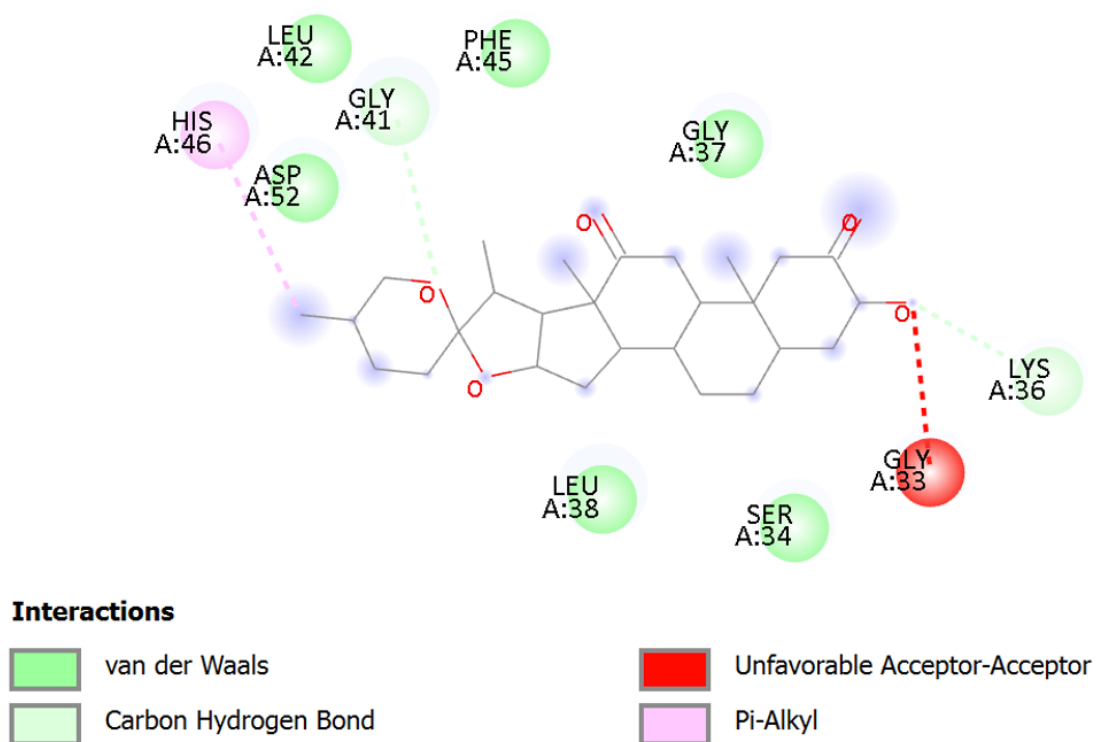


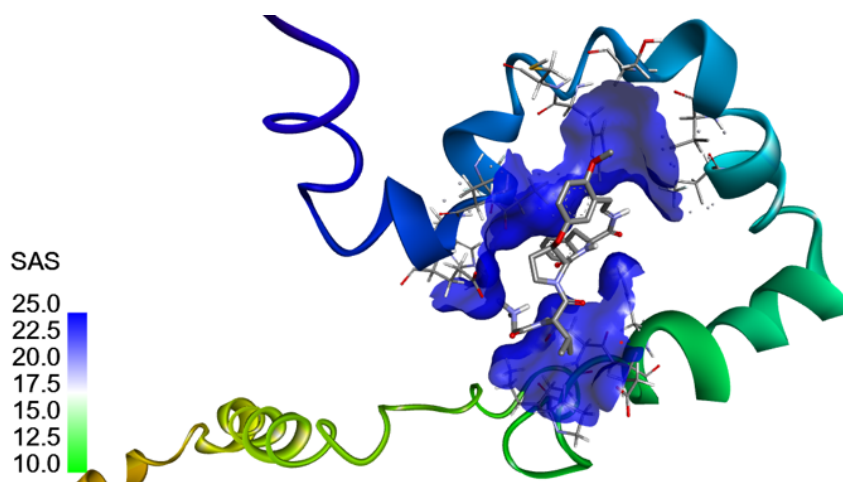
FIGURE 4.7: SOD1 protein and Lupeol molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.





(b)

FIGURE 4.8: SOD1 protein and Nummularogenin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure



(a)

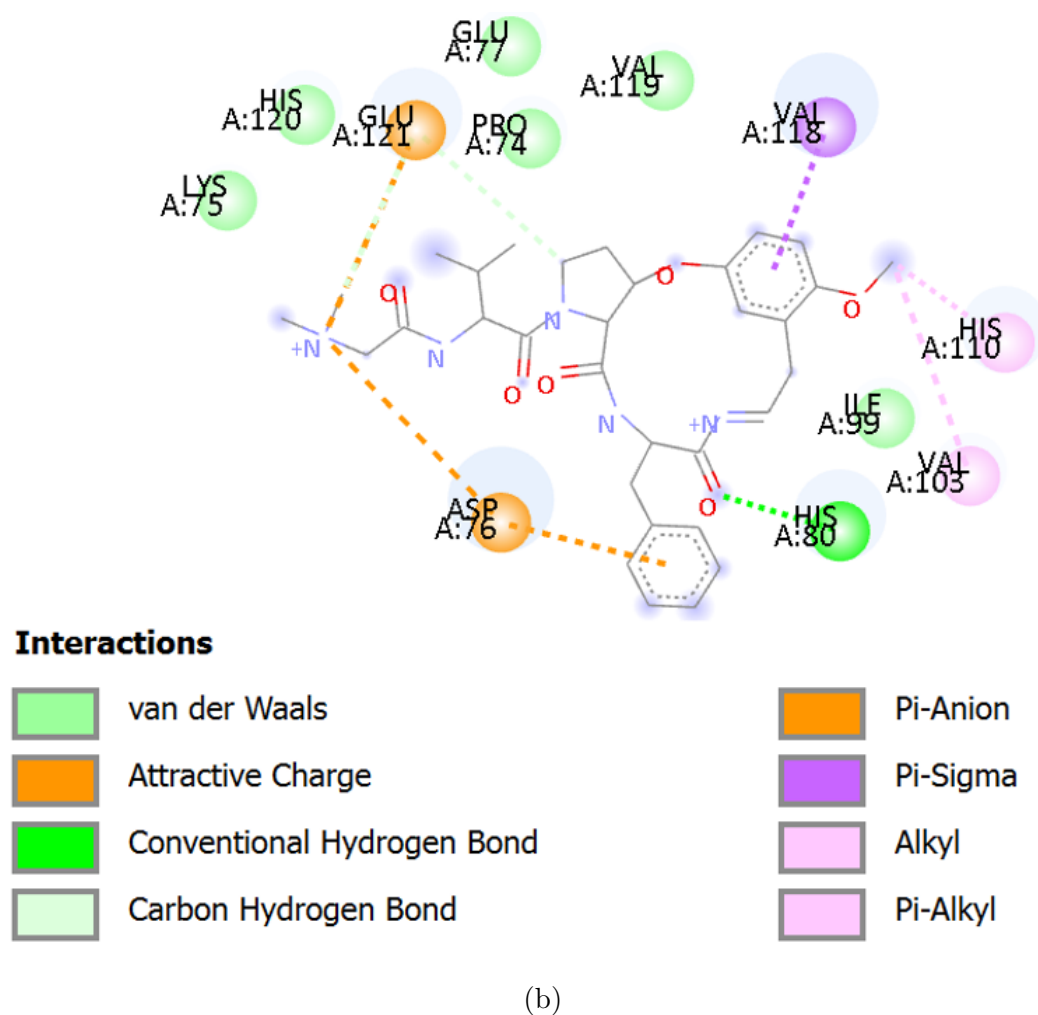
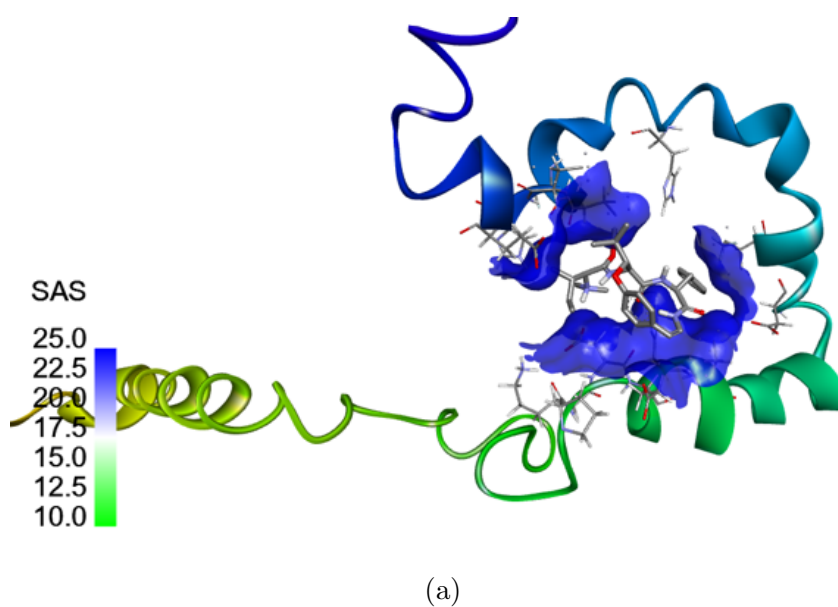
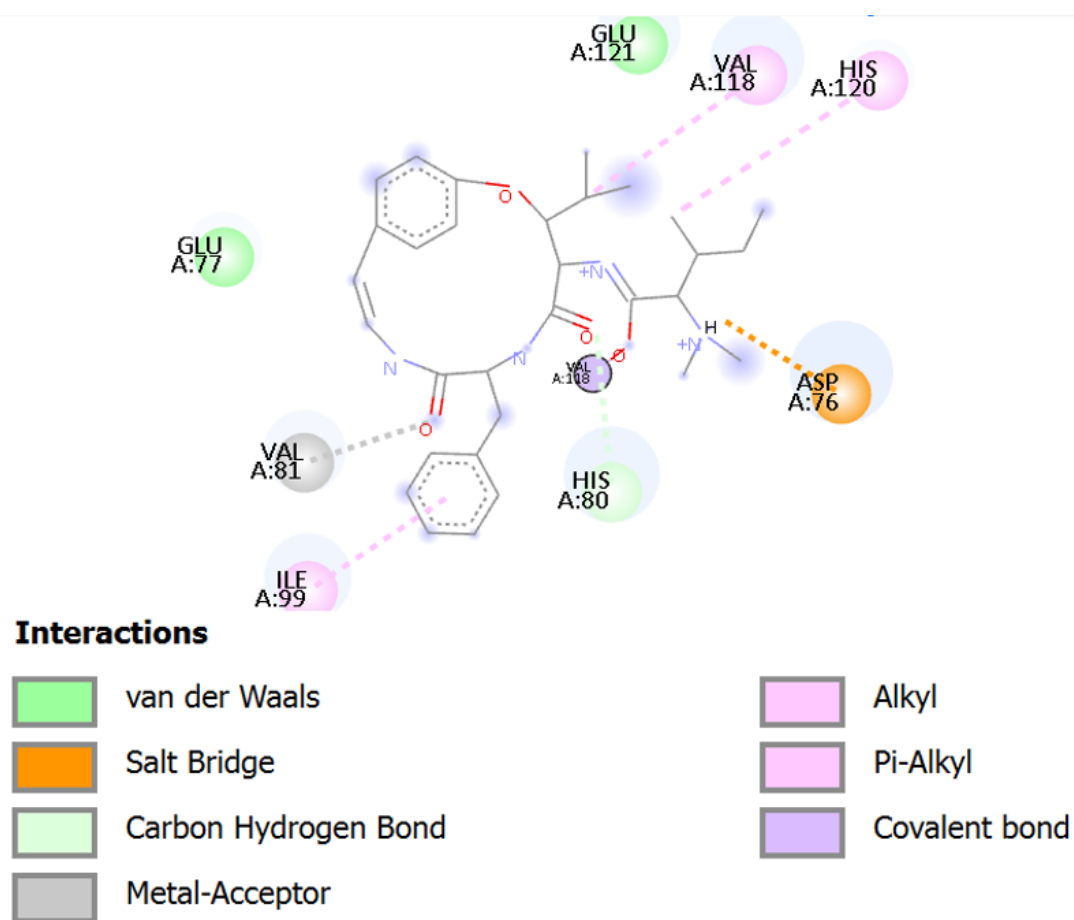


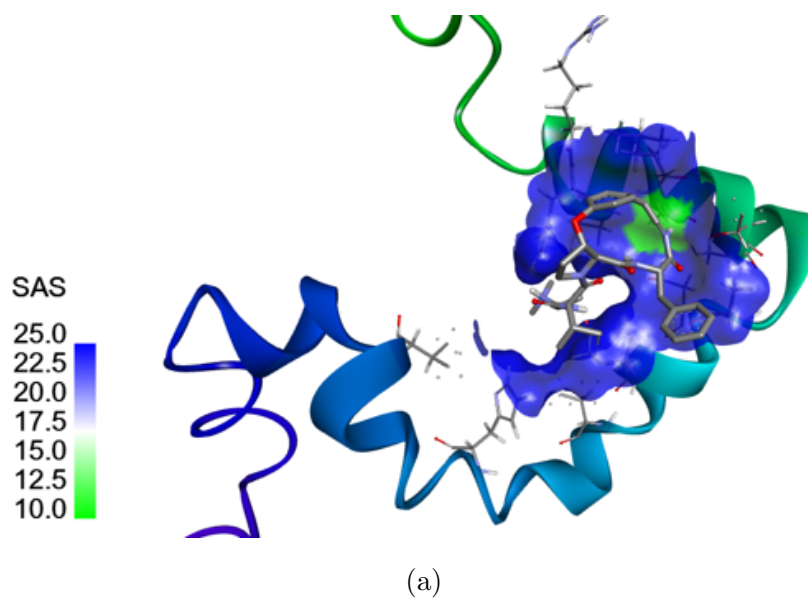
FIGURE 4.9: SOD1 protein and Nummularine P molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure

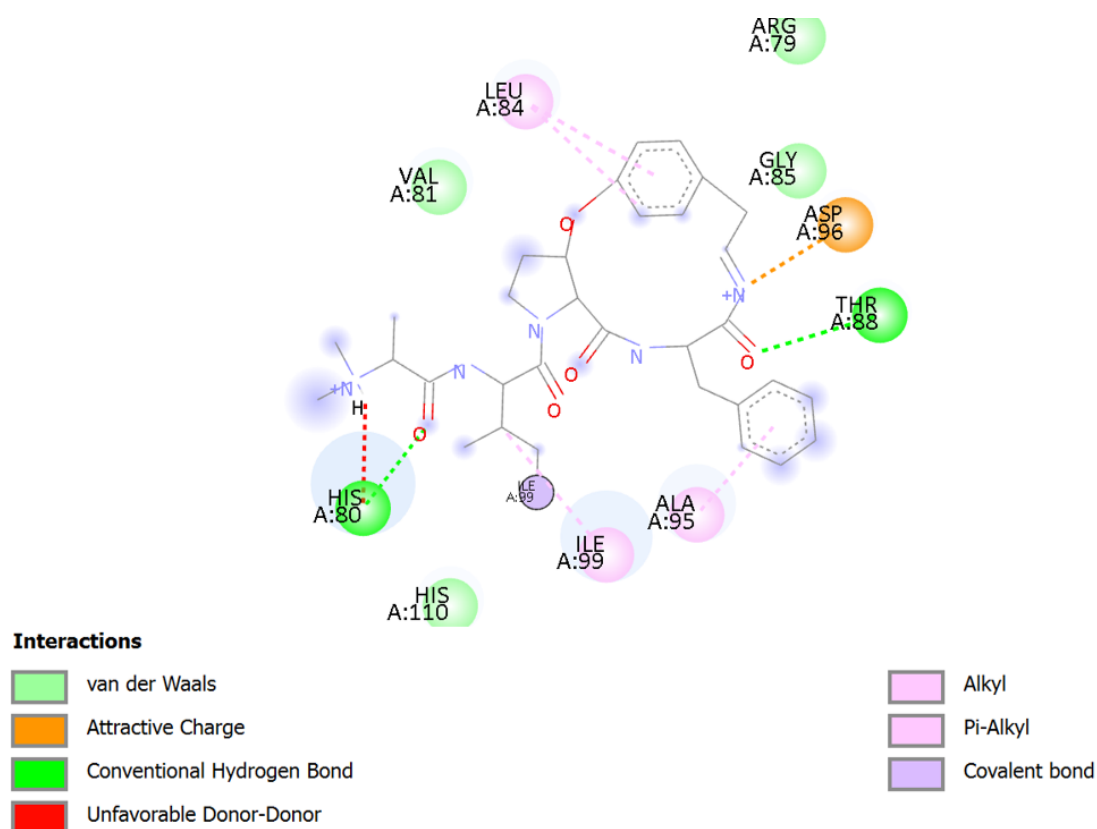




(b)

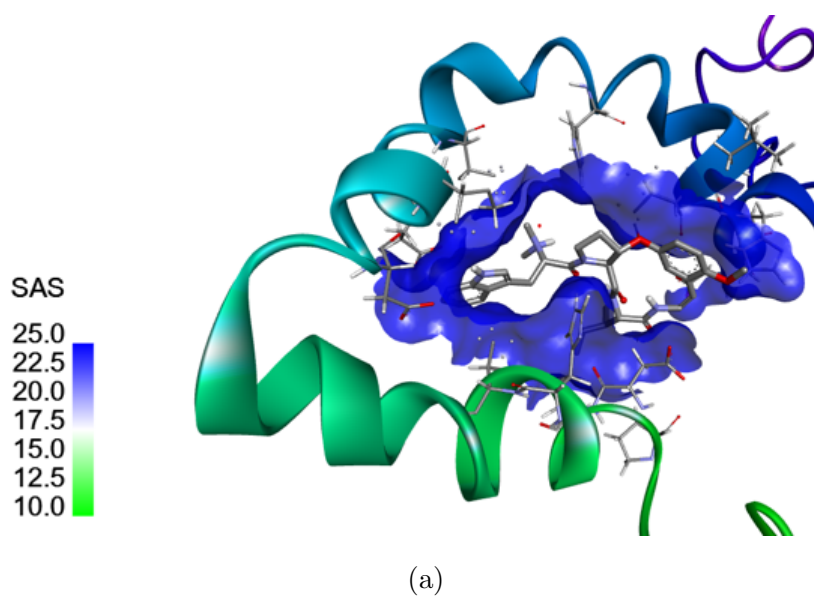
FIGURE 4.10: SOD1 protein and Scutianine-C molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.





(b)

FIGURE 4.11: SOD1 protein and Mauritine-A molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure



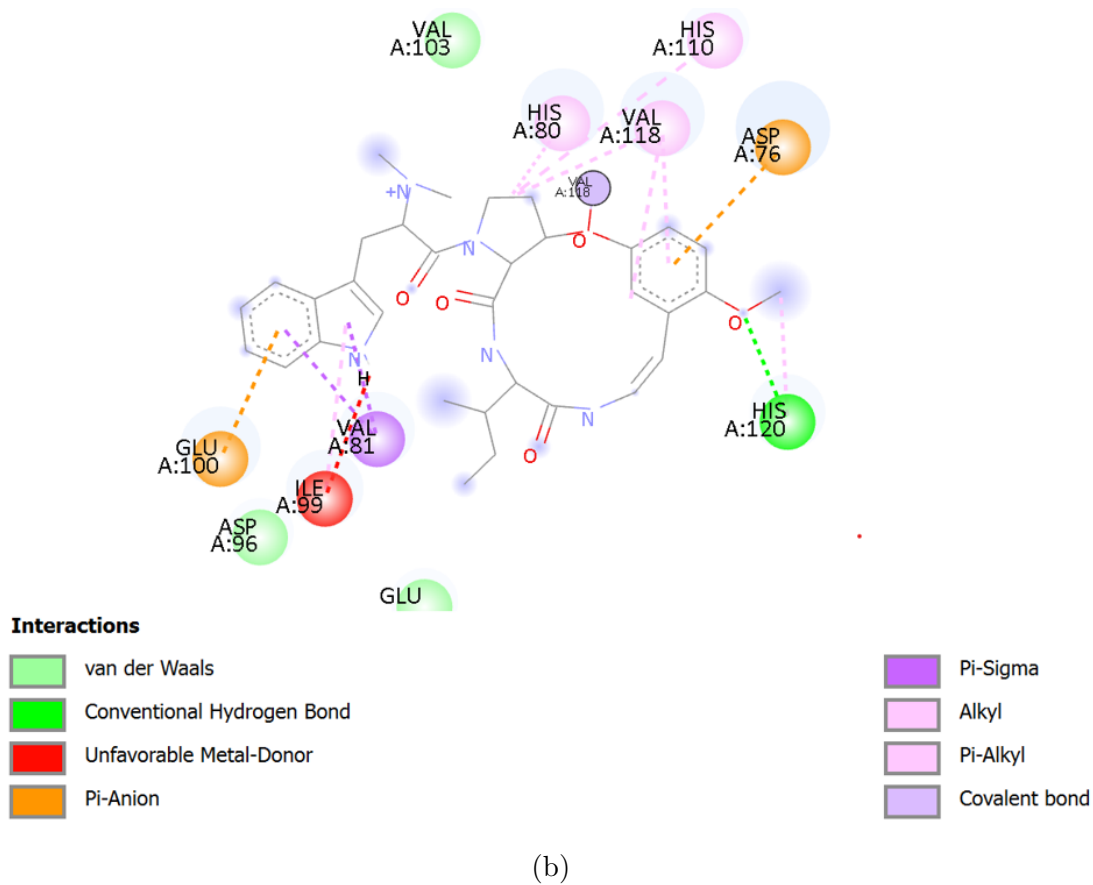
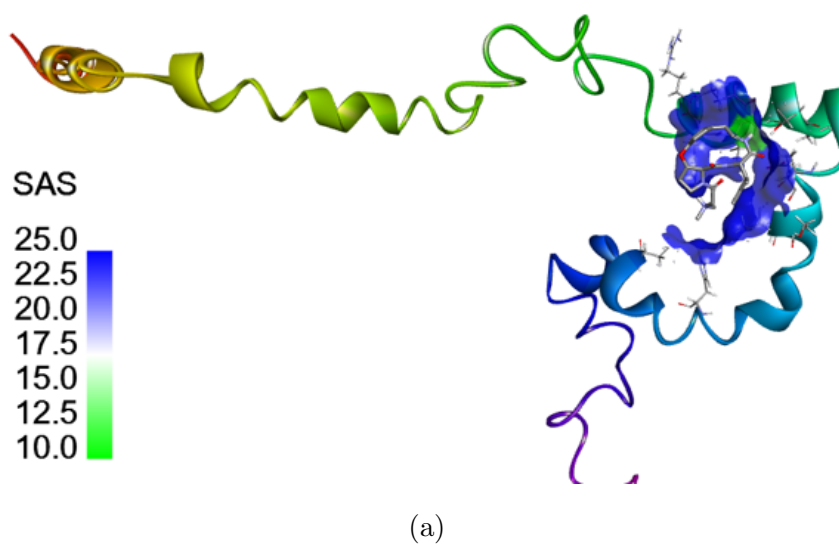
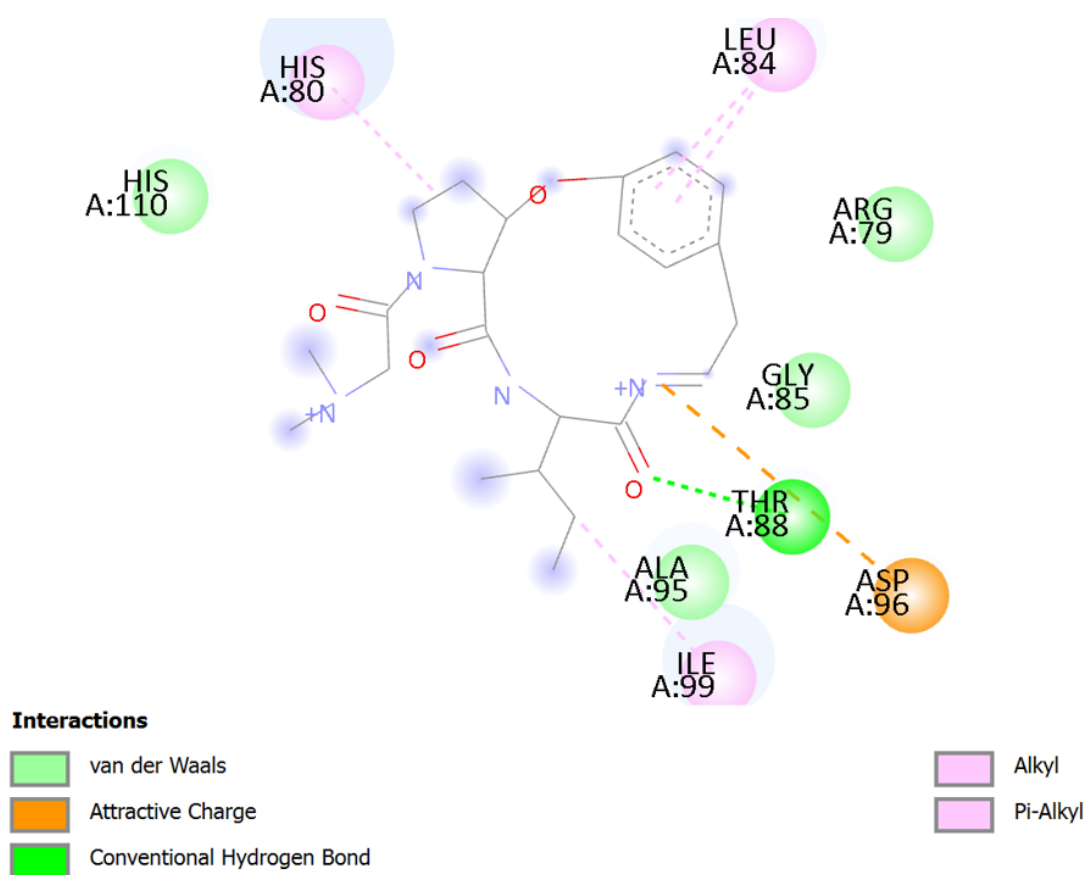


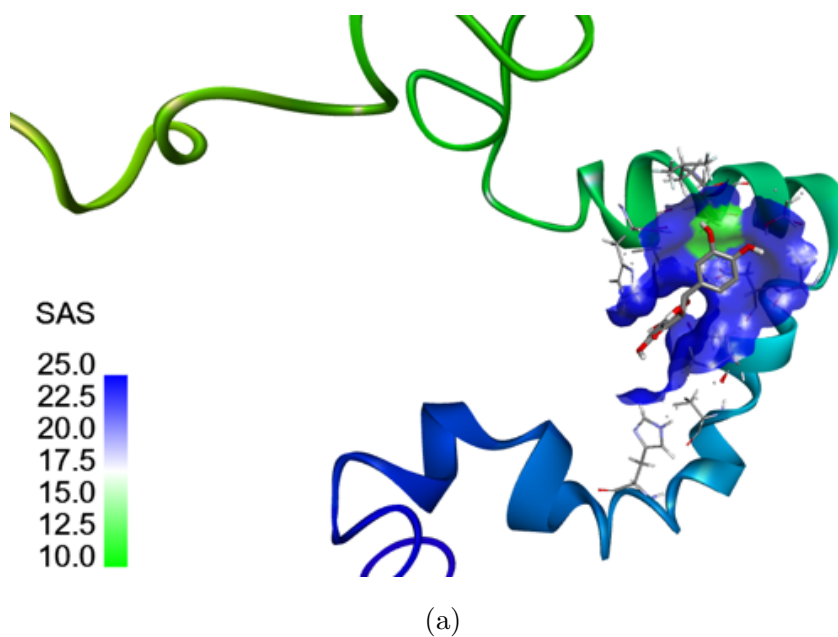
FIGURE 4.12: SOD1 protein and Nummularine-R molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.





(b)

FIGURE 4.13: SOD1 protein and Nummularine-F molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.



(a)

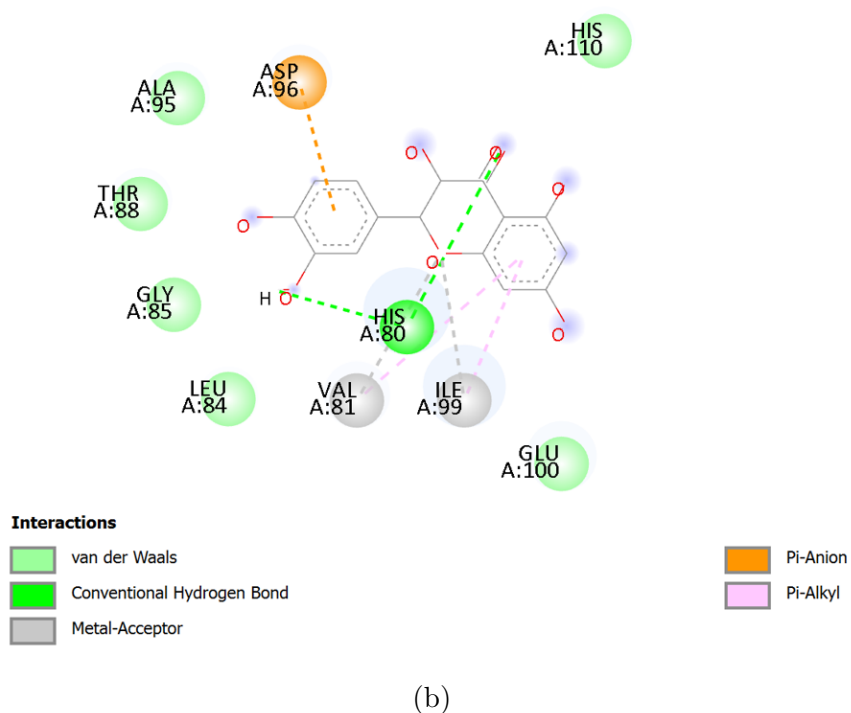


FIGURE 4.14: SOD1 protein and Taxifolin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

TABLE 4.10: Detail of interaction between SOD1 and 12 selected ligands from *Ziziphus nummularia*

Sr.	SOD1	Interactions				
		Vander waals	Hydrogen bonds	Alkyl	Unfavorable interactions	Pi Bonds
1	Amphibine-H	Six	Two C-H bonds	-	-	Two
2	Betulin	Eight	One C-H bonds	-	-	-
3	Daidzin	Six	One Conventional H-bond	Two	-	-
4	Ellagic Acid	Three	Three Conventional H-bonds	Three	Metal-donor	-
5	Lupeol	Three	One Conventional H-bond	-	-	Seven
6	Nummularogenin	Six	Two C-H bonds	One	Acceptor-Acceptor	-
7	Nummularine-P	Six	One Conventional H-bond & Two C-H bonds	Two	-	One
8	Scutianine-C	Three	One C-H bonds	Three		-

Table 4.10 continued from previous page

Sr. SOD1		Interactions					
		Vander waals	Hydrogen bonds		Alkyl	Unfavorable interactions	Pi Bonds
9	Nummularine-R	Three	One Conventional H-bond		Eight	Metal-donor	Four
10	Nummularine-F	Four	One Conventional H-bond		Four	-	-
11	Taxifolin	Six	Two Conventional H-bonds		-	-	One
12	Mauritine-A	Four	Two Conventional H-bonds		Four	Donor-Donor	-

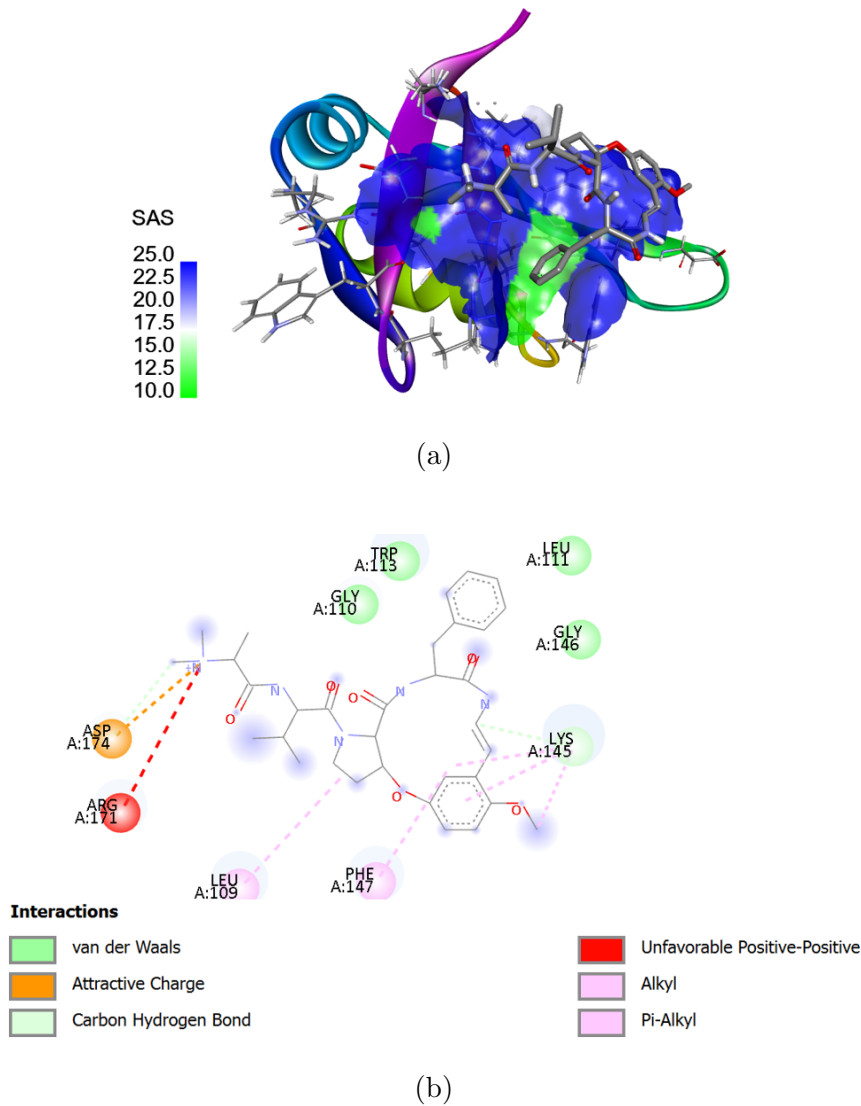


FIGURE 4.15: TDP-43 protein and Amphibine-H Molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

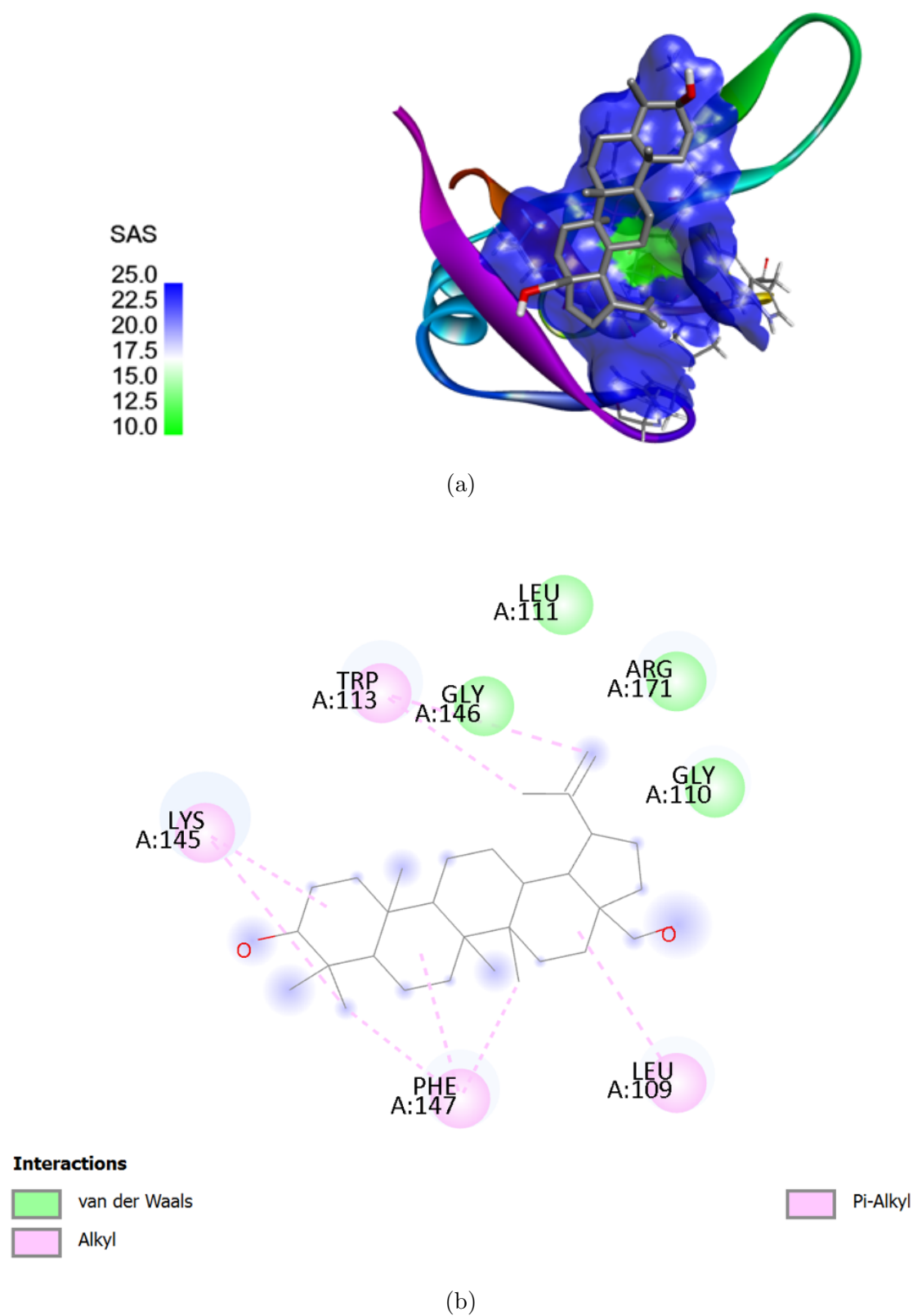


FIGURE 4.16: TDP-43 protein and betulin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

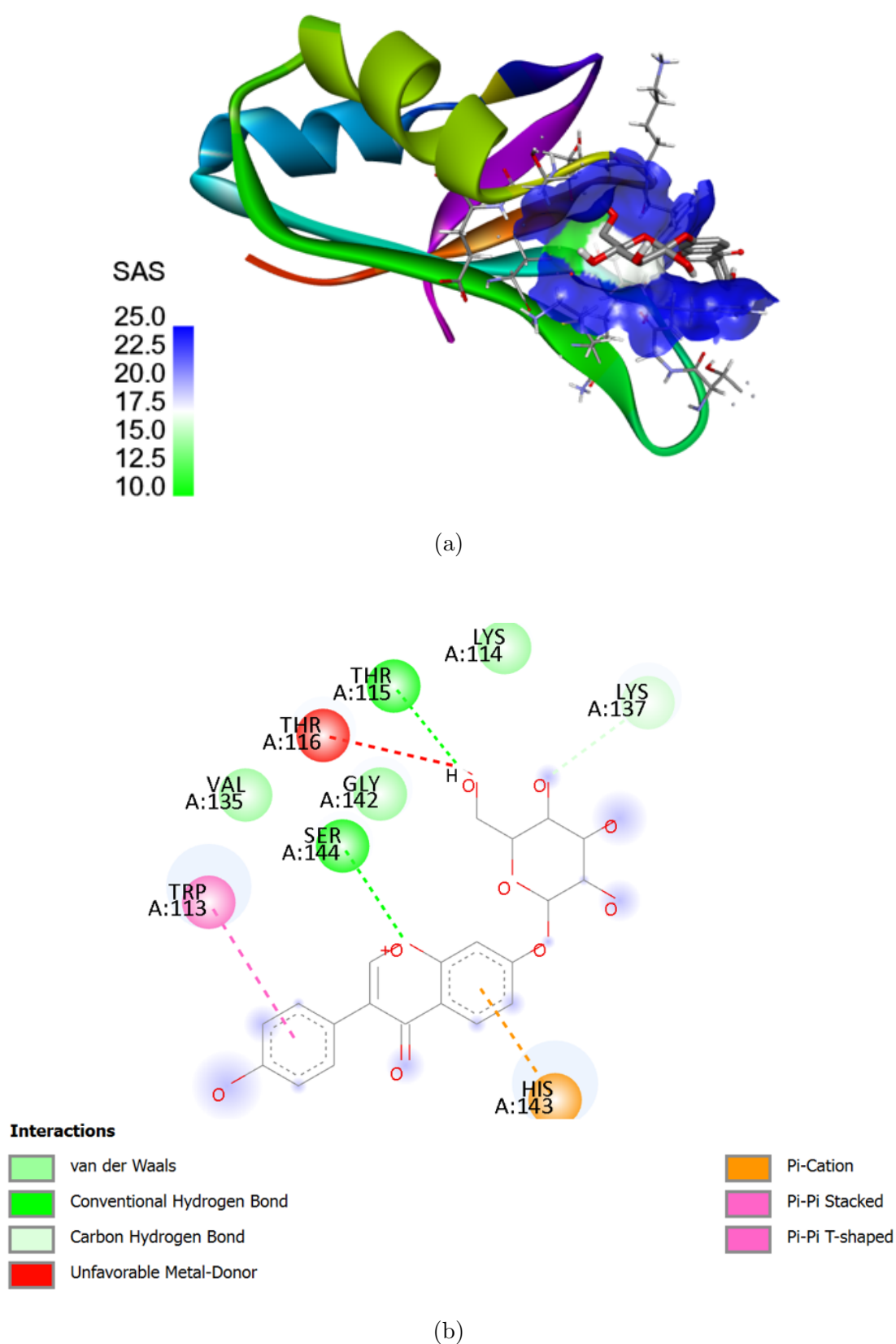


FIGURE 4.17: TDP-43 protein and Daidzin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

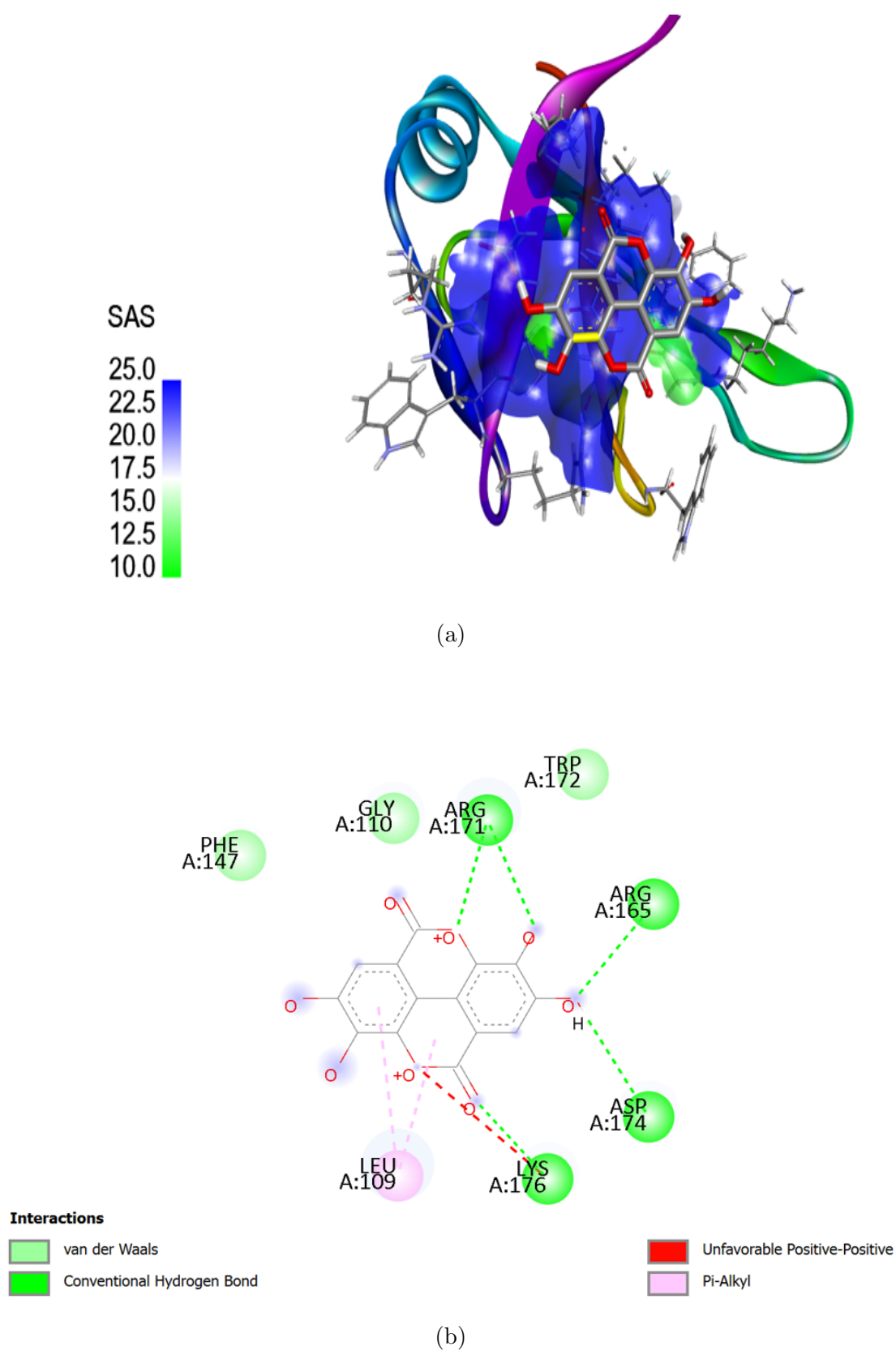


FIGURE 4.18: TDP-43 protein and ellagic acid molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

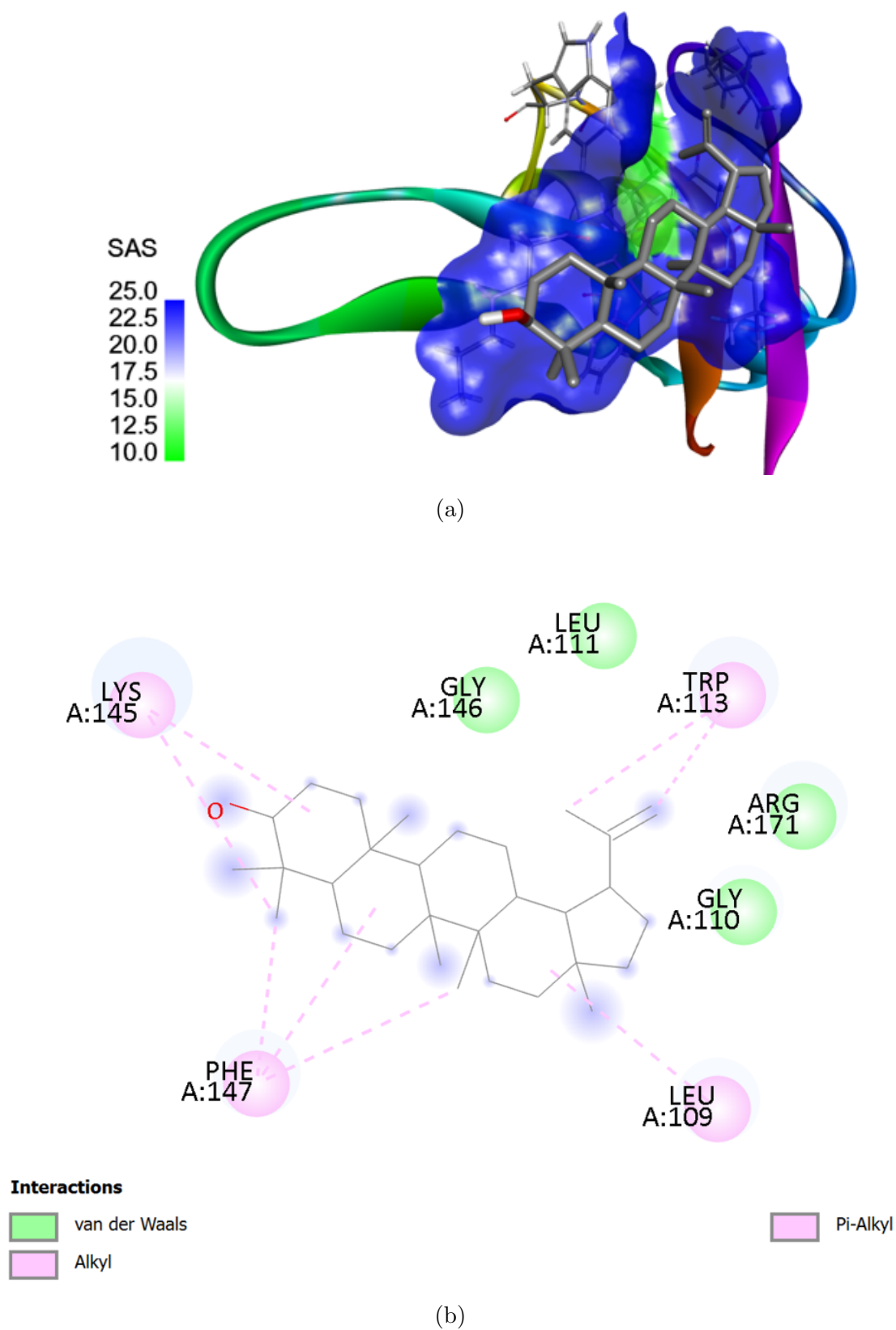


FIGURE 4.19: TDP-43 protein and Lupeol molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

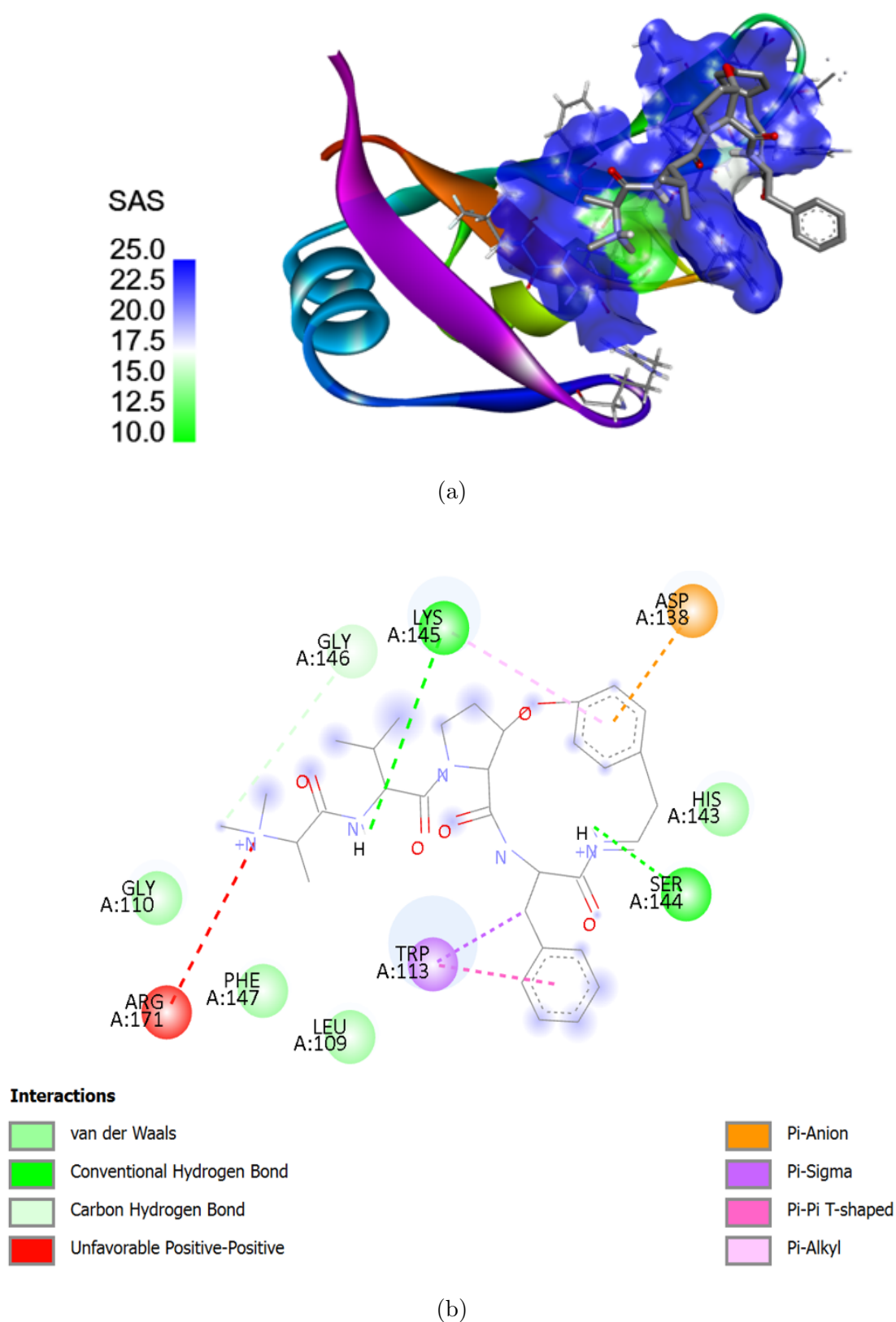


FIGURE 4.20: TDP-43 protein and Mauritine-A molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

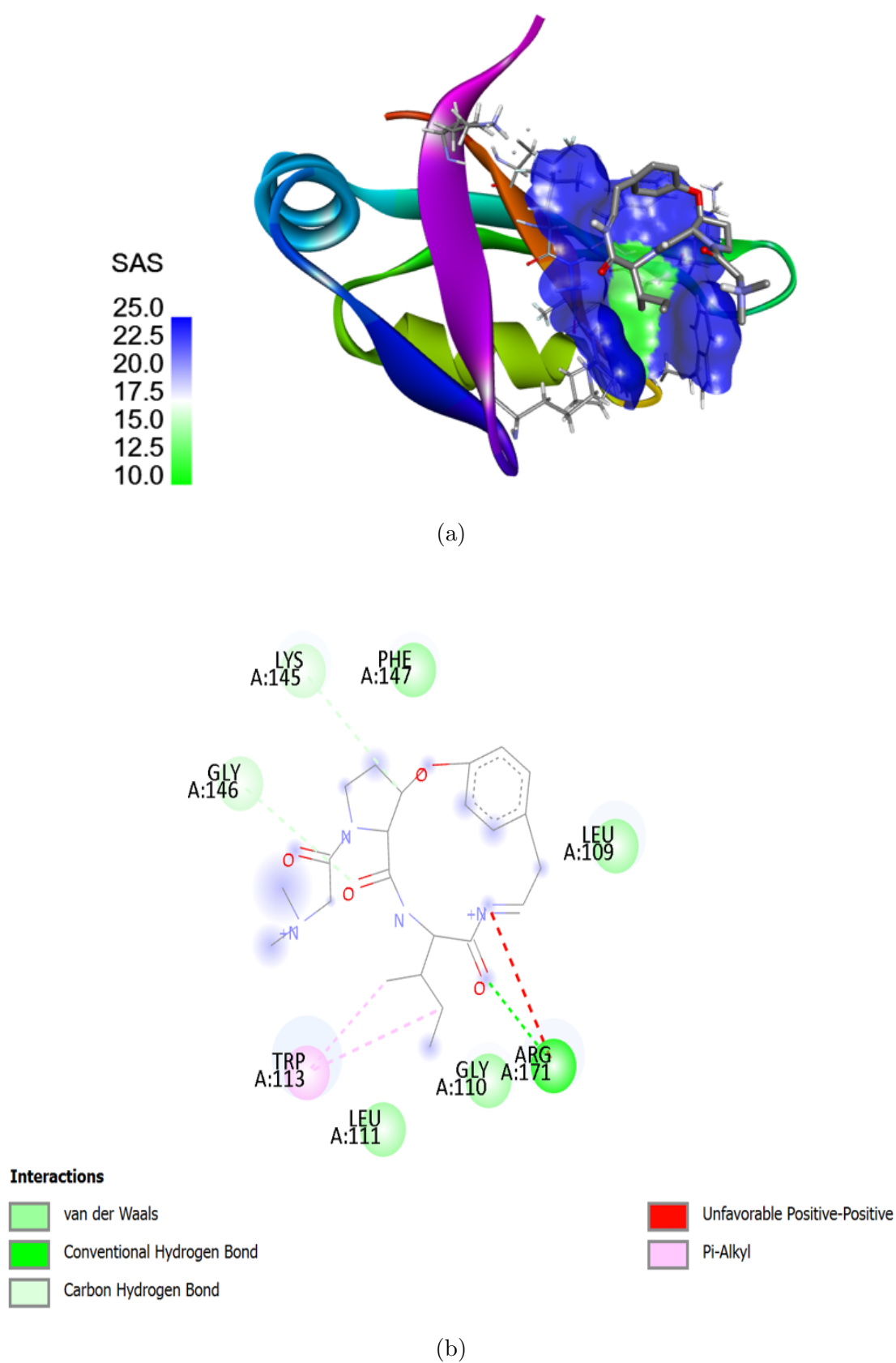


FIGURE 4.21: TDP-43 protein and Nummularine-F molecular docking analysis
(a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure

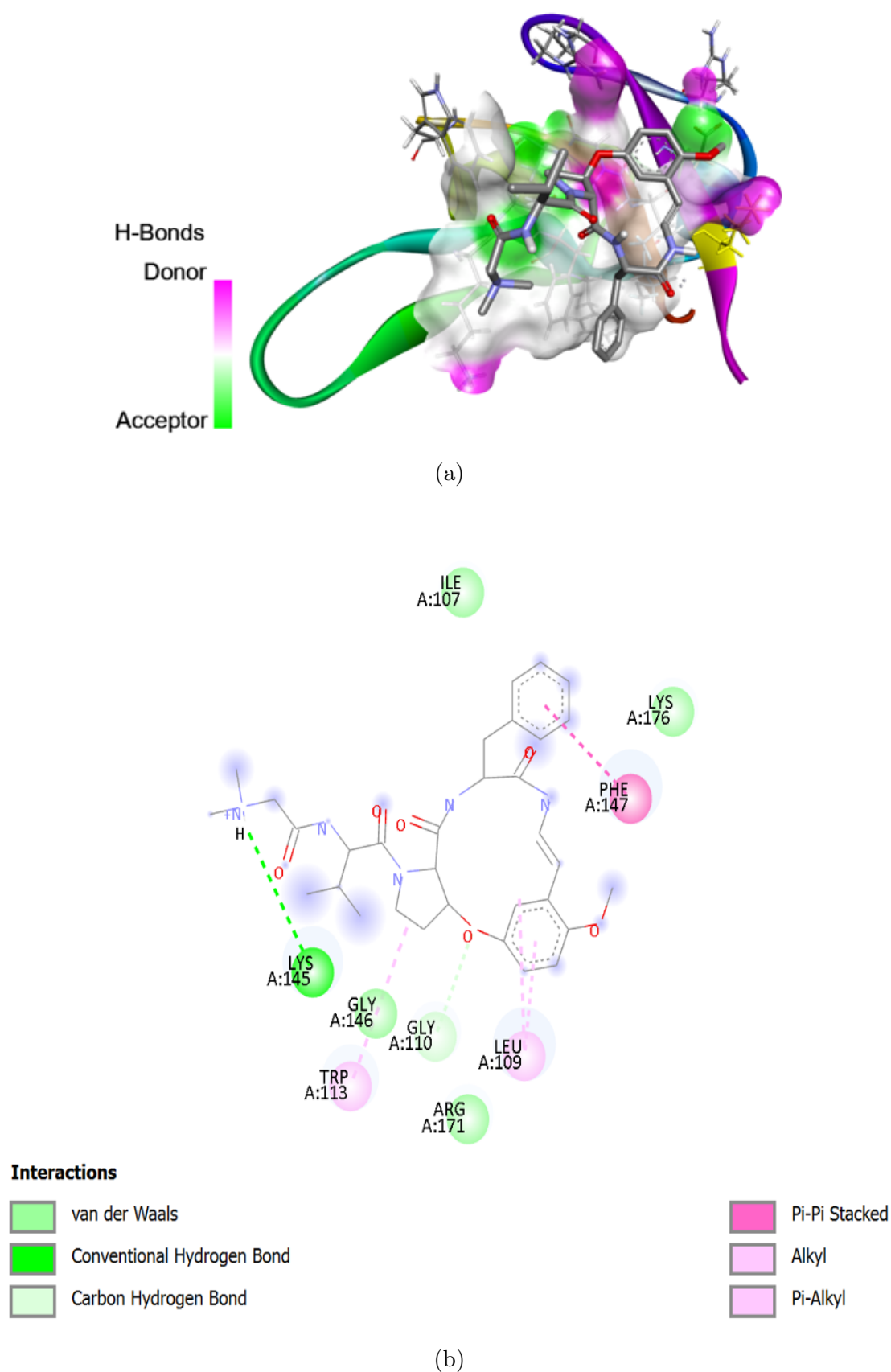


FIGURE 4.22: TDP-43 protein and Nummularine P molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure

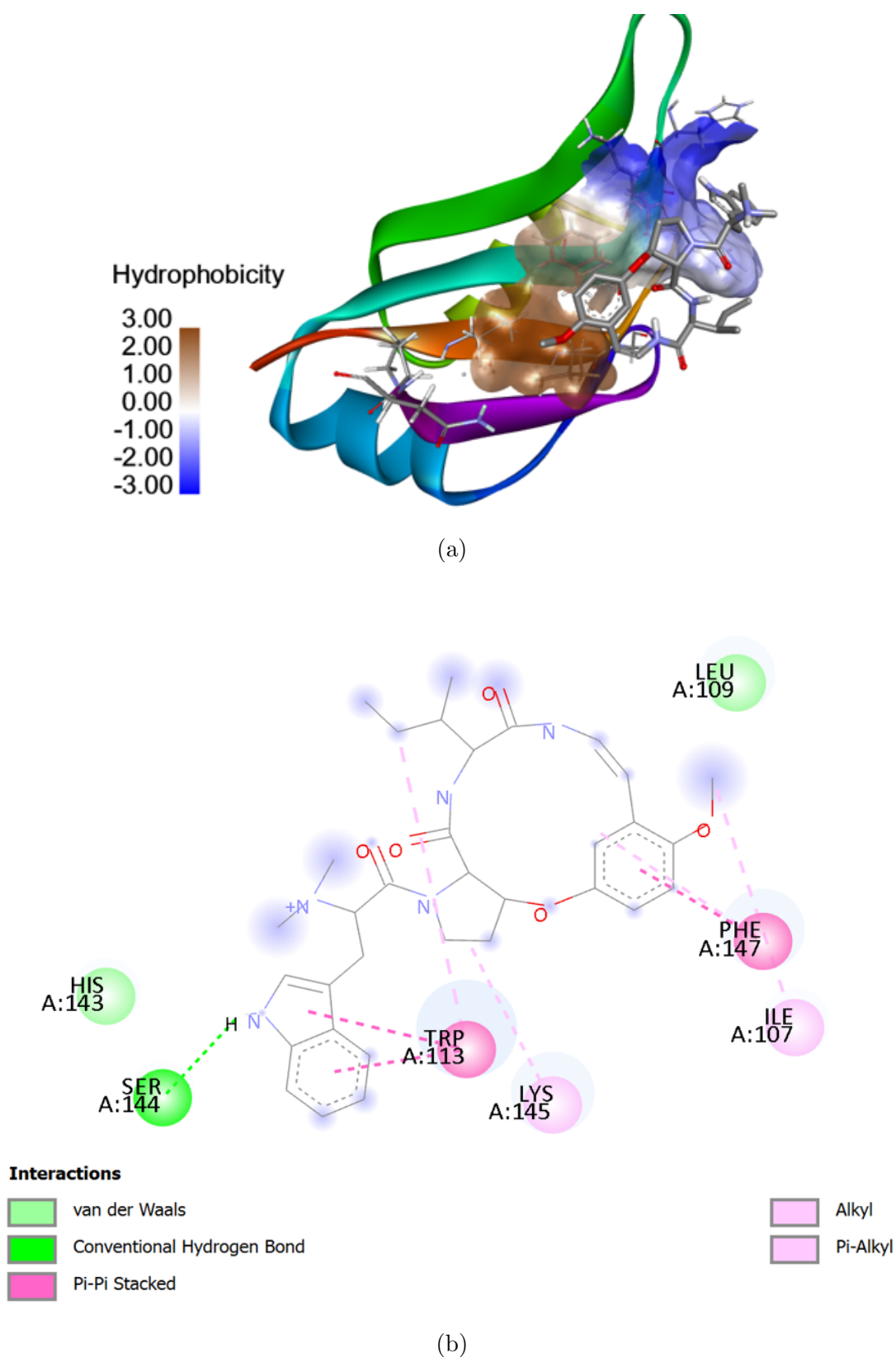


FIGURE 4.23: TDP-43 protein and Nummularine-R molecular docking analysis
(a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure

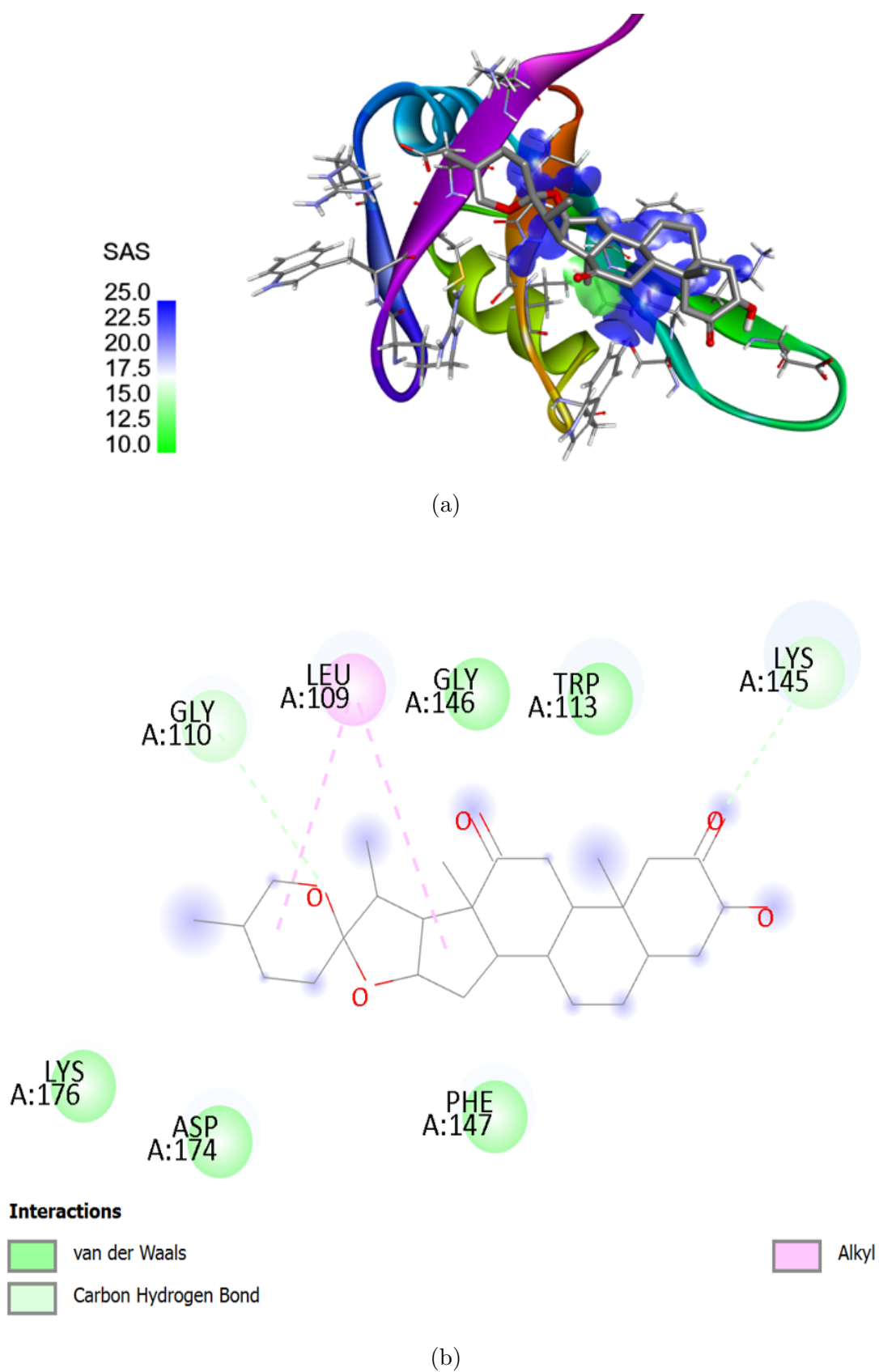


FIGURE 4.24: TDP-43 protein and Nummularogenin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

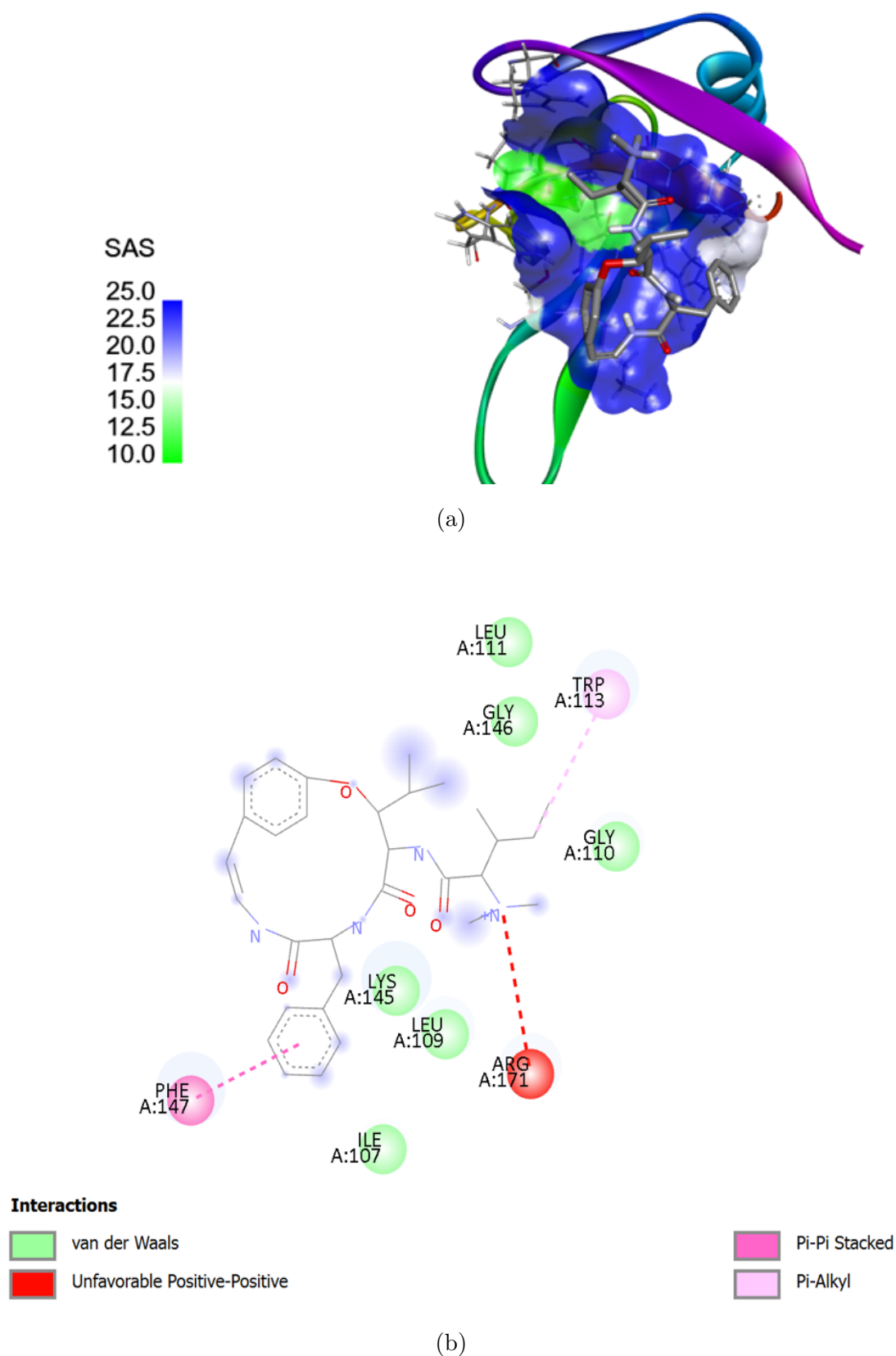
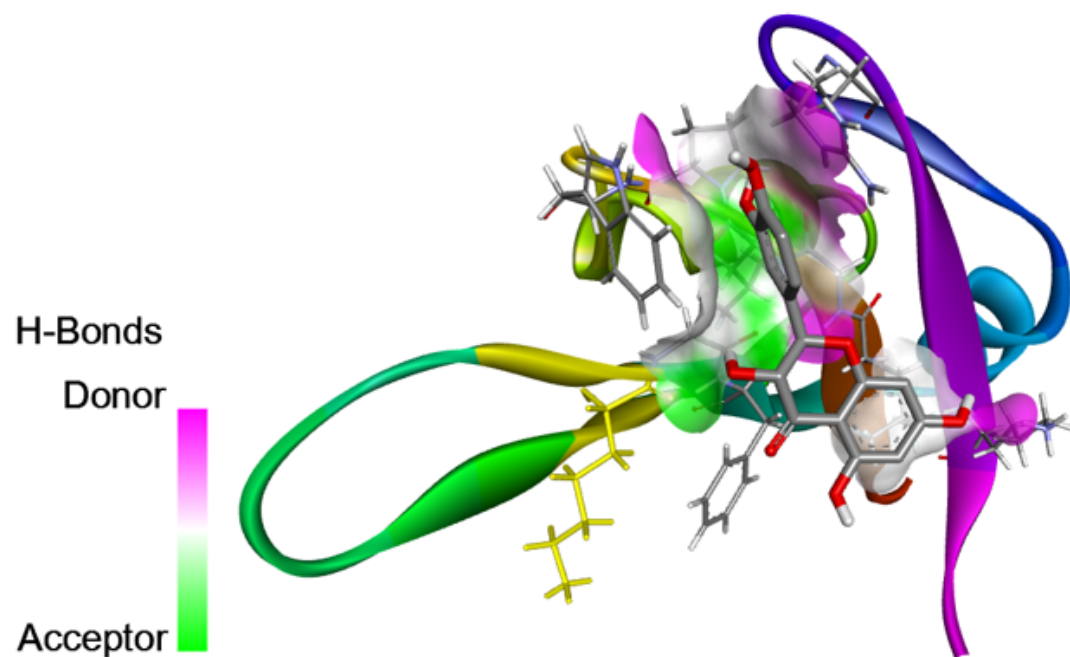
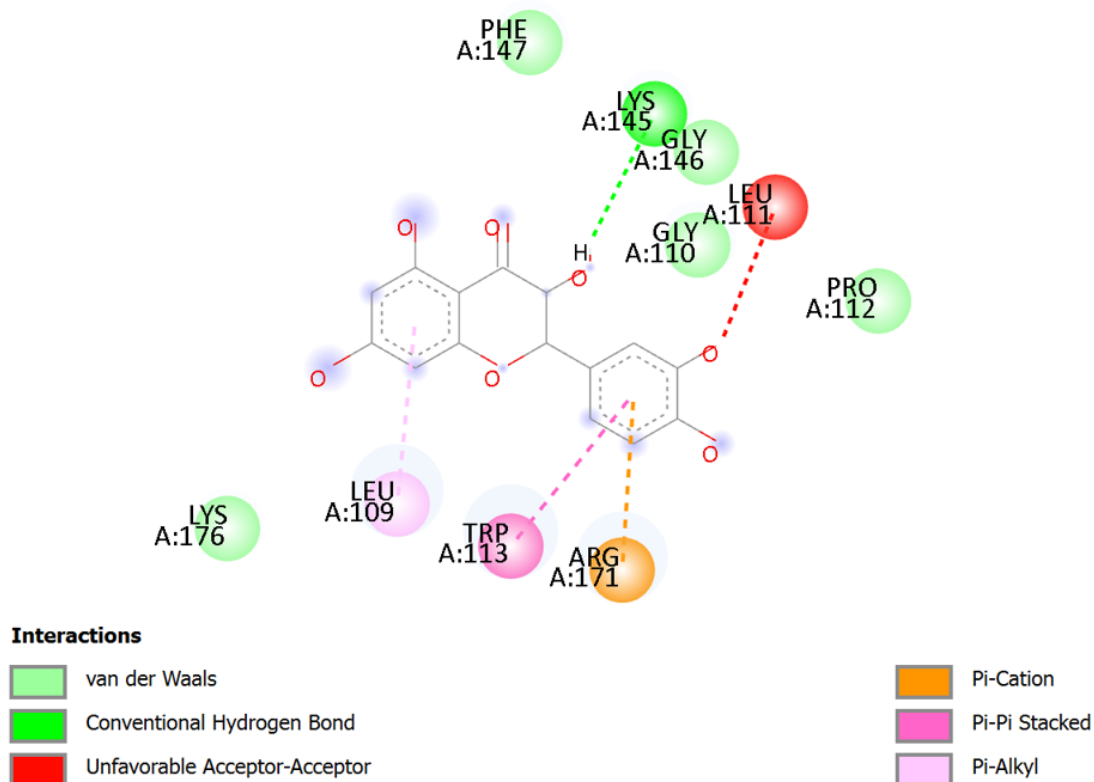


FIGURE 4.25: TDP-43 protein and Scutianine-C molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.



(a)



(b)

FIGURE 4.26: TDP-43 protein and Taxifolin molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure

TABLE 4.11: Detail of Interactions between TDP-43 protein and 12 Ligands of *Ziziphus nummularia*

Sr.	TDP-43	Interactions				
		Vander waals	Hydrogen bonds	Alkyl	Unfavorable interactions	Pi Bonds
1	Amphibine-H	Four	One C-H bonds	Five	Positive- Positive	-
2	Betulin	Four	-	Eight	-	-
3	Daidzin	Four	Two Conventional H-bonds&One C-H bond	-	Metal-donor	One
4	Ellagic Acid	Three	Five Conventional H-bonds	Two	Positive- Positive	-
5	Lupeol	Four	-	Eight	-	-
6	Nummularogenin	Five	Two C-H bonds	Two	-	-
7	Nummularine-P	Three	One Conventional H-bond&One C-H bonds	Three	-	One
8	Scutianine-C	Six	-	One	Positive- Positive	One
9	Nummularine-R	Two	One Conventional H-bond	Four	-	-
10	Nummularine-F	Four	One Conventional H-bond&Two C-H bonds	Two	Positive- Positive	-
11	Taxifolin	Five	One Conventional H-bonds	One	Acceptor-Acceptor	-
12	Mauritine-A	Five	Two Conventional H-bonds&One C-H bond	One	Positive- Positive	Two

4.6 Lead Compound Identification

The selection of Nummularogenin as the lead compound is the outcome of comprehensive analysis across docking scores, drug-likeness, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles. While several

ligands showed their strength in individual areas, Nummularogenin provided the most balanced and "drug-ready" profile for treating neurodegenerative conditions. The primary objective was to find a ligand with high affinity for both SOD1 and TDP-43, proteins central to the pathology of neurodegenerative diseases like ALS. Mauritine-A achieved the highest score for SOD1 -7.2, but its affinity for TDP-43 was relatively lower -6.0, on the other hand Nummularogenin maintained high affinity for both, with a SOD1 score of -7.1 and the highest overall TDP-43 score of -6.9. Although, Amphibine-H was a close competitor with a TDP-43 score of -6.7, but it falls short in safety and size.

A lead compound must not only bind well but also be "druggable." Like Nummularogenin 444.60g/mol is well within the ideal range ≤ 500 g/mol. In contrast, many competitors like Amphibine-H (591.70), Nummularine-R (587.70) and Mauritine-A (575.70) exceed the 500 g/mol threshold, potentially limiting their oral absorption and cellular uptake. Nummularogenin has a LogP of 4.15, which is ideal for crossing cell membranes while remaining soluble enough for blood transport. While Lupeol and Betulin has respectively 8.02 and 6.99 have high docking scores, their extreme lipophilicity violates Lipinski's Rule and often leads to poor metabolic stability and "sticky" non-specific binding. While talking about Safety most high-affinity alkaloids in the study (Amphibine-H, Mauritine-A, Nummularine-R, and Nummularine-F) are predicted to be hepatotoxic. Nummularogenin is non-hepatotoxic, making it a significantly safer candidate for long-term therapeutic use. Like the other candidates, it is non-mutagenic, ensuring it will not damage DNA.

Moreover, Nummularogenin has the highest intestinal absorption which is 98.07% among all selected phytochemicals and with logBB -0.073 Nummularogenin has the ability to penetrate into the central nervous system. With all these considerations it was selected as a lead compound and predicted that it could be the possible candidate use to ameliorate motor neuron diseases.

4.7 Reference Drug Identification Against Motor Neuron Diseases

Riluzole is commonly used against motor neuron disease and act through reducing glutamate toxicity progression because excess of glutamate overstimulates neurons which produce excitotoxicity, that ultimately cause motor neuron death. When riluzole act it slow down the process of degradation of neuron. Besides glutamate pathway, it also affects sodium channel [132].

In case of ALS, SOD1 protein produce oxidative damage and this medicine indirectly help to reduce reactive oxygen species (ROS), oxidative stress and mitochondrial injury. As it is known that ROS damage lipids, proteins, nucleic acid and cellular membranes resultantly it activates apoptosis and neurodegenerative pathways in motor neurons [133]. Like SOD1, riluzole also have some effect on TDP-43 through reducing neural stress which help in the removal of TDP-43 aggregates [134].

4.7.1 Riluzole and Lead Compound Comparison

Riluzole effective mechanism of action, acknowledgment and wide use against MND make it an ideal candidate for comparative studies in that particular disease. So, it provides a solid and important foundation for evaluating new potential active pharmaceutical ingredients, such as Nummularogenin in the context of SOD1 and TDP-43.

4.7.2 Riluzole Structure Retrieval

The first step in the comparative study was the structure retrieval of the standard drug, for that the SDF file was downloaded from PubChem with ID 5070. Riluzole is a benzothiazole derivative and contain a benzene ring, a thiazole ring (containing sulfur and nitrogen), an amino group and a trifluoromethoxy group. These group

make this drug lipophilic, blood brain barrier penetrator, and interactor with neural ion channels and receptors. The molecular formula of riluzole is $C_8H_5F_3N_2OS$, and its structure is given below.

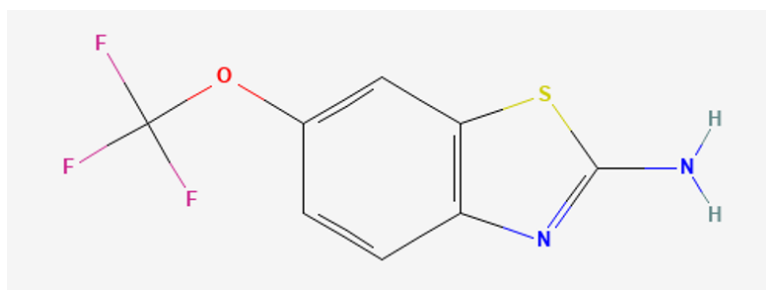


FIGURE 4.27: Structure of Riluzole

4.7.3 Lipinski Rule of Five

The riluzole and nummularogenin were compared to observe their response to the Lipinski rule to evaluate their oral availability in humans. It will help to compare the absorption, permeability and drug-likeness. The comparison is given in table 4.12.

TABLE 4.12: The Lipinski rule of five comparison

Sr.	Ligands	Mol Weight	LogP	Rotatable Bonds	HBA	HBD	Yes/No
		g/mol ≤ 500	≤ 5		≤ 10	≤ 5	
1	Riluzole	234.2	2.7771	1	4	1	
2	Nummularogenin	444.6	4.1518	0	5	1	

Both reference drug and lead compounds are following all the rules, although the values are slightly different but in range.

4.7.4 ADMET Properties Comparison

The ADMET qualities are used to evaluate the drugs and the lead chemicals ADMET to identify a better drug candidate.

4.7.4.1 Absorption Properties Comparison

A comparison between riluzole and nummularogenin to check absorbance parameters in given table 4.13.

TABLE 4.13: Absorption properties Comparison

Sr.	Ligands	Water Solubility	CaCO ₂ Permeability	Intestinal Absorption	Skin Permeability	P-glycoprotein Sub	P-glycoprotein I Inh	P-glycoprotein II Inh
1	Riluzole	-3.558	1.316	89.074	-3.003	No	No	No
2	Nummularogenin	-4.888	0.867	98.069	-3.99	Yes	Yes	Yes

According to the above data lead compound and standard drug had comparable absorption properties, nummularogenin has high intestinal absorption but it also p-glycoprotein substrate as well as inhibitor which could affect its absorption.

4.7.4.2 Distribution Properties Comparison

Distribution indicate the transport and dispersion of drug in the body after absorption, the comparison between distribution properties of ligand and leads compound is present in table 4.14.

TABLE 4.14: Distribution properties comparison

Sr.	Ligands	VDss (human)	Fraction bound (human)	un- BBB per- meability	CNS per- meability
1	Riluzole	-0.105	0.396	0.126	-2.375
2	Nummularogenin	0.042	0.055	-0.073	-1.687

By analyzing distribution properties, it indicates that nummularogenin has higher VDss and CNS permeability while Riluzole demonstrate better fraction unbound and BBB. So, both these compounds had comparable and overall equal distribution properties.

4.7.4.3 Comparative Evaluation of Metabolism Parameters

The Comparative Evaluation of Metabolism Parameters of the lead compound and drug are given in table 4.15.

TABLE 4.15: Metabolism properties of ligands

Sr.	Ligands	Substrates		Inhibitors				
		CYP2D6	CYP3A4	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
1	Riluzole	None	None	Yes	None	None	None	None
2	Nummularogenin	None	Yes	None	None	None	None	None

Riluzole has increased metabolic stability as it is not a substrate for major CYP enzymes but may lead to CYP1A2-mediated drug interactions. In contrast, Nummularogenin had a safer CYP inhibition profile but relies on CYP3A4 for metabolism, which may affect its bioavailability and metabolic stability.

4.7.4.4 Excretion Properties Comparison

Comparison between the excretion properties of riluzole and nummularogenin are given in the table 4.16.

TABLE 4.16: Excretion properties of ligands

Sr. No.	Ligands	Total Clearance	Renal OCT2 substrate
1	Riluzole	0.258	None
2	Nummularogenin	0.264	Yes

Regarding the clearance properties, there was only a minor difference between Riluzole and Nummularogenin, where the clearance of Nummularogenin (0.264) was slightly higher than Riluzole (0.258). It indicates that similar rates of clearance of both compounds from the body may exist. Riluzole was predicted not to be a substrate for the renal OCT2, suggesting a lower potential for transporter-mediated renal interactions.

In contrast, Nummularogenin was identified as an OCT2 substrate, indicating that its renal excretion might be mediated by this transporter and may be affected by drugs that modulate OCT2 activity.

In general, Riluzole has a relatively safer profile for renal interaction, while Nummularogenin might have a higher potential for renal transporter related interaction, although with similar clearance rate.

4.7.4.5 Toxicity Properties Comparison

Toxicity profile comparison between the lead compound and standard drug is given below in table 4.17.

TABLE 4.17: Toxicity properties comparison

Ligands	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	<i>T. Pyriformis</i> toxicity	Minnow toxicity
Riluzole	-	0.345	-	-	2.517	1.229	Yes	-	0.923	1.784
Nummularogenin	-	-0.732	-	yes	2.083	1.792	-	-	0.318	1.561

Toxicity studies demonstrated that Riluzole and Nummularogenin were non - mutagenic and non-sensitizing to skin. Riluzole was predicted to be hepatotoxic but showed better human tolerability and lower risk of cardiotoxicity as it did not inhibit hERG channels. On the other hand, Nummularogenin did not show hepatotoxicity but showed hERG II inhibition indicating potential cardiotoxicity issues. In brief, Riluzole appears to be safer in cardiac and acute toxicity profiles whereas Nummularogenin shows safer liver toxicity profile.

4.7.5 Docking Score Comparison

The standard drug for motor neuron disease riluzole and lead phytocompound nummularogenin from *Ziziphus nummularia* docked against target proteins and their binding scores were obtained-which are given below in table 4.18.

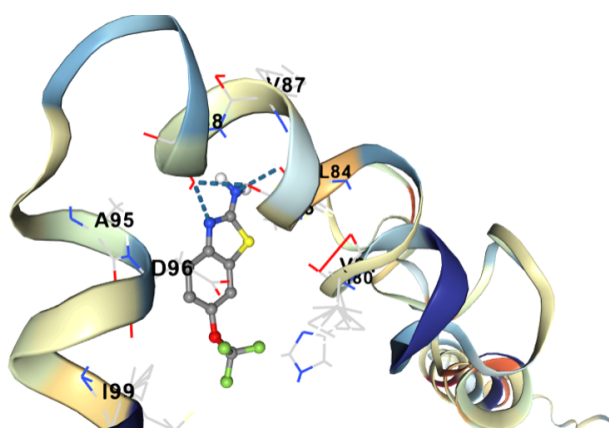
TABLE 4.18: Docking scores of ligand-protein complexes

Sr.	Ligands	Target Proteins	
		SOD1	TDP-43
1	Riluzole	-4.7	-4.6
2	Nummularogenin	-7.1	-6.9

As indicated in the table 4.18 the docking scores of lead compound nummularogenin are higher than those of the standard drug riluzole. The docking scores of riluzole against target protein SOD1 and TDP-43 are -4.7 and -4.6 respectively, on the other hand these scores for nummularogenin are -7.1 and -6.9 for SOD1 and TDP-43 respectively.

4.7.6 Docking Analysis Comparison

The interactions between protein and ligand were evaluated through Discovery Studio and given below.



(a)

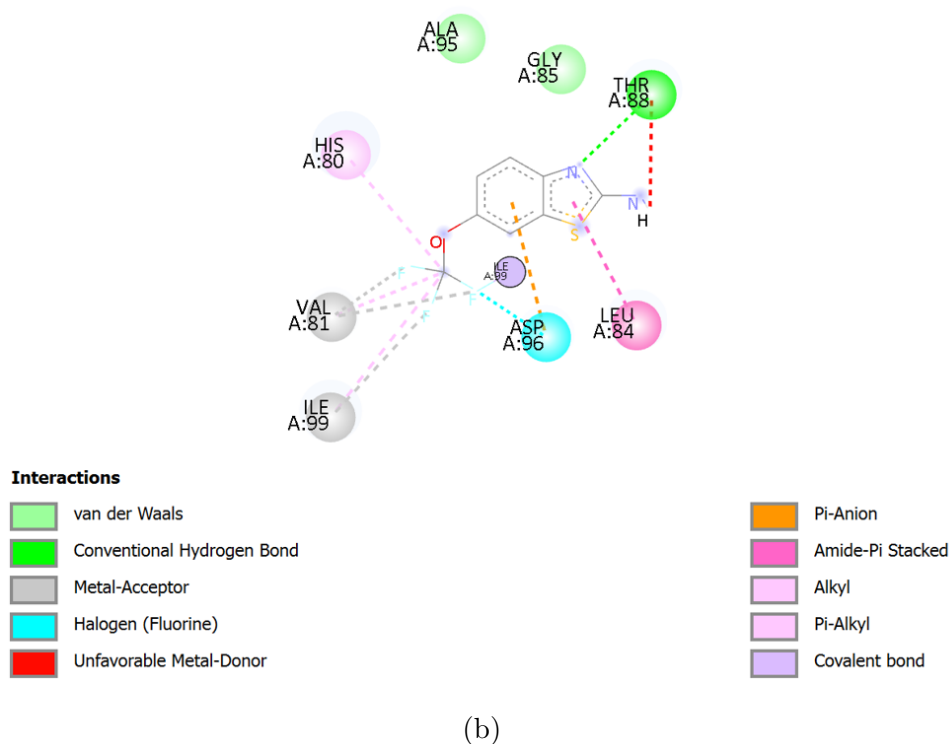
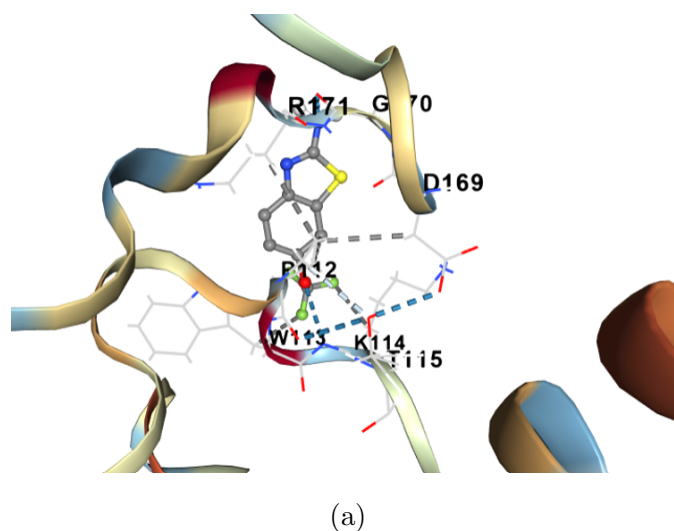


FIGURE 4.28: SOD1 protein and Riluzole molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure.

Figure 4.28 shows that there is conventional hydrogen bond, two van der waals, three alkyl bonds, one covalent bond, three metal acceptors, one halogen and an unfavorable metal-donor bond between SOD1 and riluzole, while nummularogenin has eight van der waals, two carbon hydrogen, one alkyl and one unfavorable acceptor-acceptor bonds. The data demonstrate that both nummularogenin and riluzole share a robust and comparable interaction profiles.



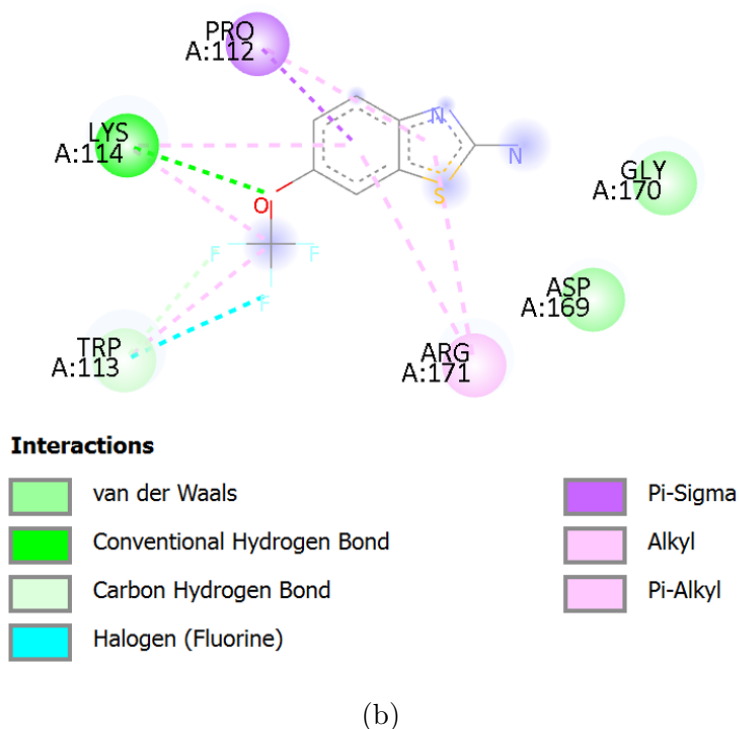


FIGURE 4.29: TDP-43 protein and Riluzole molecular docking analysis (a) Protein-ligand interaction's solvent-accessible surface (b) the ligand and amino acid active site's two-dimensional structure

Figure 4.29 indicate the nature and number of interaction between protein TDP-43 and riluzole. They are interacted through three van der waals, one conventional, one carbon hydrogen, six alkyls, one Pi and single halogen (Fluorine) bonds, while nummularogenin has seven van der waals, two carbon hydrogen and one alkyl bond which indicate that in case of TDP-43 riluzole has better interactions as compare to lead compound while the docking score is greater for nummularogenin which is -6.9 as compared to -4.6 of riluzole.

Overall, the comparison of riluzole and nummularogenin shows that both contain comparable Lipinski rule of five, ADMET analysis, but the docking scores are higher for the lead compound while receptor-ligand interactions are almost same in the case of SOD1 but in case of TDP-43 riluzole somehow has better interactions as compared to nummularogenin.

Chapter 5

Discussion

In the present *in silico* study, we have identified some potential phytochemicals from *Ziziphus nummularia* that can exhibit neuroprotective properties against two important proteins involved in the progression of Motor Neuron Disease (MND), namely Amyotrophic Lateral Sclerosis (ALS) and Superoxide Dismutase-1 (SOD1) [135]. The hallmarks of a neurodegenerative disorder, like ALS, are progressive loss of motor neurons, oxidative stress, mitochondrial dysfunction, neuroinflammation and abnormal protein aggregation [136]. The currently available treatments, such as Riluzole, only briefly relieve symptoms and offer only small benefits in survival; hence, new drugs derived from plants that have a broad therapeutic effect on several pathological pathways of interest have become more popular [137]. The drug-likeness and therapeutic potential of the selected phytochemicals of *Ziziphus nummularia* were explored by evaluating their drug-likeness using Lipinski's Rule of Five, ADMET profiling, molecular docking and interaction analysis with proteins associated with ALS. The study results highlight the pharmaceutical relevance of *Ziziphus nummularia* and indicate that a number of phytochemicals exhibit a good binding affinity and favorable pharmacokinetic characteristics in relation to the SOD1 and TDP-43 proteins.

The molecular targets of SOD1 and TDP-43 were chosen as they are both essential in the pathogenesis of ALS and neuronal degeneration. SOD1 mutations

are believed to be among the primary causes of familial ALS and TDP-43 cytoplasmic aggregation is thought to be a common feature of sporadic ALS [138]. SOD1 is normally an antioxidant enzyme that catalyzes the transformation of toxic superoxide radicals to oxygen and hydrogen peroxide. But SOD1 mutations or misfolding causes aggregation, oxidative stress, mitochondrial dysfunction and neuronal apoptosis [139]. Likewise, TDP-43 is an RNA binding protein that plays a role in transcriptional regulation, RNA splicing and maintenance of neuronal homeostasis. In ALS, TDP-43 moves away from its normal location in the nucleus into the cytoplasm, where it aggregates to become toxic and disrupt RNA metabolism, mitochondrial function, axonal transport, and cell signaling pathways [140]. Thus, a simultaneous approach towards the inhibition of both SOD1 and TDP-43 could be a more effective therapeutic approach for ALS progression.

ProtParam analysis revealed that both proteins showed good physicochemical characteristics that are ideal for molecular docking studies [141]. SOD1 was more structurally stable than TDP-43 determined by instability index. The proteins were found both to be stable under in vitro conditions with their instability index values being below 40. The positive value of aliphatic index obtained for SOD1 signified higher thermal stability while the negative values of GRAVY for both the proteins confirmed their hydrophilic nature and their preference to an aqueous environment. In general, hydrophilic proteins can interact with ligands through hydrogen-bonding and electrostatic interactions [141]. Also, the increased extinction coefficient of TDP-43 indicated the presence of aromatic amino acid residues which could be involved in the binding of ligands. The results coincide with a previous structural study that concluded SOD1 has a relatively stable β -barrel structure while TDP-43 has intrinsically disordered regions that enhance its aggregation tendency [142].

Based on the pharmaceutical properties and oral bioavailability evaluated by Lipinski's Rule of 5, the majority of the phytonutrients that were chosen had sufficient drug-like properties. Of the selected compounds, several ligands did not violate any of Lipinski's rules, and a few compounds violated just one of Lipinski's rules, which is generally acceptable in natural products research. There

were compounds with suitable molecular weight, hydrogen bond donor and acceptor values and lipophilicity profile, namely compounds Nummularogenin, Daidzin, Taxifolin, Nummularine-F and Betulin were identified. Amphibine-H, Mauritine-A and Nummularine-R were below the threshold for MW, but were just a bit over the threshold for drug-likeness as only one parameter was exceeded. These results corroborate with earlier studies where it was reported that a lot of natural compounds with biological activity have, in general, lower molecular weight, but still exhibit therapeutic activity [143]. Multiple violations were found for only rutin, which had a relatively poor suitability for oral therapeutic application.

The information obtained from the ADMET analysis was useful for understanding the pharmacokinetic and toxicity properties of the selected phytochemicals [144]. Absorption analysis showed that the majority of the compounds had a good intestinal absorption and decent permeability parameter. The intestinal absorption of nummularogenin was found to be very high and therefore it is recommended for oral administration. It was also determined to be a substrate and an inhibitor of P-glycoprotein, and thus it may interact with efflux transporters. Riluzole and Nummularogenin showed somewhat similar absorption characteristics with the exception that riluzole was more water soluble. However, Nummularogenin is not as soluble but its high absorption in the intestine may overcome this drawback.

Analysis of distribution showed that some of the compounds had the ability to cross into the blood-brain barrier and central nervous system, a crucial requirement for medicines that are used to treat neurodegenerative disorders [145]. Nummularogenin exhibited good BBB permeability and penetration and Lupeol exhibited particularly high BBB penetration capability, indicating high neuropharmacological potential. The VDss was higher for Taxifolin and Nummularine-R, suggesting higher tissue distribution. These compounds are very important for crossing the BBB as ALS pathology is mainly found in CNS neurons. Most compounds seemed to be highly accessible in their free form in that they were not highly bound, as suggested by the fraction unbound values. Betulin and Lupeol demonstrated high plasma protein binding that could increase their plasma time but decrease their immediate bioavailability [146].

There were different interactions between phytochemicals and cytochrome P450 enzymes, revealed by metabolism analysis [147]. Compounds like Nummularine-F, Taxifolin and Daidzin showed a less disturbed metabolism profile with less CYP interactions indicating decreased risk of drug-drug interactions. Some alkaloid compounds showed multiple CYP enzyme interaction potentially affecting metabolic stability and retention time. Most ligands had acceptable excretion profiles when analyzed via excretion. The moderate clearance of nummularogenin was predicted as a Renal OCT2 substrate, indicating renal excretion mechanisms. Taxifolin had lower clearance values, which could be related to a longer half-life in the body [148]. The toxicity analysis revealed good safety data for most compounds as all did not appear to be AMES mutagenic or skin sensitizing. Some alkaloid compounds, however, were predicted to be hepatotoxic and hERG inhibitors, suggesting a potential cardiac or hepatic toxicity at higher concentrations. In particular, Amphibine-H showed the ability to inhibit hERG-I, a possible cardiotoxicity issue. Nevertheless, Nummularogenin showed relatively balanced toxicity property, and hence was selected as a more promising lead candidate.

The results of the molecular docking showed moderate to strong interactions between the selected phytochemicals with the target proteins SOD1 and TDP-43. Mauritine-A showed the highest binding affinity towards SOD1 followed by Nummularogenin, Nummularine-R, and Amphibine-H. Mauritine-A is strongly bound to SOD1, indicating that it could have potential interactions with amino acid residues that play a role in the stability and aggregation of the enzyme. The structures of the interaction revealed multiple H-bonds and alkyl interactions that helped to stabilize the ligand in the binding pocket. Additionally, the strong interaction between nummularogenin and SOD1 was observed, which was supported by van der Waals forces, alkyl interactions, and carbon-hydrogen bonds. One poor interaction with an acceptor was seen, the overall docking profile was still favorable. Multiple hydrophobic and π -bond interactions were identified in the active site of Nummularine-R which might participate in stabilizing the active site. The lower inhibitory potential was shown by ellagic acid, which was the least interacted with SOD1.

Of the four, Nummularogenin had the highest binding affinity for TDP-43, with Amphibine-H, Nummularine-P, and Lupeol being in order. The tight binding of Nummularogenin to TDP-43 is especially noteworthy, since one of the most significant pathological features of ALS disease progression is the aggregation of TDP-43 [149]. The interaction analysis revealed that multiple van der Waals interactions and carbon-hydrogen bonds were formed between Nummularogenin and TDP-43, that helped to stabilize the complex of the proteins with the ligand. Amphibine-H showed good affinity for TDP-43, but was not a good lead candidate due to its higher molecular weight and potential toxicity concerns. The lipophilic characteristics and high BBB permeability of Lupeol may be correlated to its strong hydrophobic interactions with TDP-43 [150].

Discovery Studio Analysis of the interactions showed that hydrogen bonds, van der Waals forces, alkyl interactions, π -bonds and electrostatic interactions all played a role in the stabilization of the ligands within the protein binding pocket. Hydrogen bonding is especially significant since it increases the specificity and stability of the complexes between proteins and ligands [151]. The multiple hydrogen bonds formed between the compounds and the target proteins suggested favorable binding orientation, as in the case of Mauritine-A, Taxifolin, Ellagic acid, and Nummularogenin. The hydrophobic interactions such as alkyl interaction and π -alkyl interaction have also been found extensively and played an important role in binding affinity and stabilization in the hydrophobic interior of proteins [152].

Among the different phytocompounds tested, Nummularogenin has been identified as a balanced lead compound as it performed well in all docking, drug-likeness, and ADMET calculation. Mauritine-A showed the highest binding affinity against SOD1, but the relatively low binding affinity toward TDP-43 and high molecular weight decreased its suitability. Amphibine-H also performed well in docking but exhibited some issues with safety (hERG inhibition). For comparison, Nummularogenin showed robust SOD1 and TDP-43 binding properties and had a favorable PK profile. The molecular weight was in an acceptable range for oral drug candidates and the BBB permeability and CNS penetration properties also favor its neuroprotective potential.

The increase in docking affinity of Nummularogenin compared to Riluzole was confirmed, as it was found to have a higher docking affinity for both SOD1 and TDP-43, particularly for TDP-43 in which it was seen to have a much higher docking affinity than riluzole. While forming multiple hydrogen bonds, alkyl interactions, halogen bonds and metal acceptor interactions, Nummularogenin showed a more stable interaction profile with favorable hydrophobic and van der Waals interactions. These results indicate that Nummularogenin has a higher direct interaction potential with proteins related to ALS. But, molecular docking cannot replace experiments to prove therapeutic efficacy.

This study gives a strong indication of the medicinal value of *Ziziphus nummularia*, demonstrating it as a valuable medicinal source of neuroprotective phytochemicals. Some previous studies have reported the presence of alkaloids, flavonoids, terpenoids, glycosides, and phenolic compounds [153, 154], which have antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and neuroprotective properties in this plant [155, 156]. The present results further expand this knowledge by showing that a number of phytocompounds in *Ziziphus nummularia* have potential interactions with ALS associated proteins. These compounds might provide neuroprotection through a variety of mechanisms including, reduction of oxidative stress [157], inhibition of protein aggregation [158], protection of mitochondria, anti-inflammatory effects, and modulation of cellular signaling pathways [159]. This multi-target activity is especially significant in neurodegenerative diseases, where multiple pathological processes are taking place at the same time.

Chapter 6

Conclusion and Future Recommendation

6.1 Conclusion

In the present in silico study, phytochemicals from *Ziziphus nummularia* were shown to have potential therapeutic effect on ALS associated proteins SOD1 and TDP-43. Based on Lipinski's Rule of Five, ADMET analysis, molecular docking and interaction studies several compounds were found to possess good drug-like properties and good binding affinity to the target proteins. Of all the compounds, Nummularogenin was found to be the most promising lead compound with highest docking affinity, favorable pharmacokinetic profile, blood brain barrier permeability and comparatively safe toxicity profile. Hydrogen bonding and hydrophobic interactions were also found to be the major factors that accounted for the stability of the protein-ligand complexes in the active site, as revealed by the interaction analysis. Comparative analysis was done with Riluzole, indicating that Nummularogenin might have a better interaction with the chosen targets. Overall, the study reveals the medicinal value of *Ziziphus nummularia* and offers a computational rationale for further development of plant-based therapeutics for Motor Neuron Disease and Amyotrophic Lateral Sclerosis.

6.2 Future Recommendations

Experimental validation of the identified lead compounds in in vitro and in vivo investigation specially Nummularogenin should be further explored in future studies. Neuroprotective activity, toxicity, pharmacokinetics and blood-brain barrier penetration needs to be assessed in cell culture and animal model environments under physiological conditions. Molecular dynamics simulations can also be carried out to investigate the stability of the complex of the ligand bound to the protein over time. The mechanisms of action of the other phytochemicals present in *Ziziphus nummularia* should be studied and potential synergic action with conventional treatments like Riluzole should be investigated. The structural modification and optimization of lead compounds can also be helpful for the future development of therapeutic agents for ALS and other neurodegenerative diseases, with the aim of enhancing their efficacy and safety profiles.

Bibliography

- [1] B. N. Dugger and D. W. Dickson, “Pathology of neurodegenerative diseases,” *Cold Spring Harbor Perspectives in Biology*, vol. 9, no. 7, p. a028035, 2017.
- [2] I. Alafuzoff and P. Hartikainen, “Alpha-synucleinopathies,” in *Handbook of Clinical Neurology*. Elsevier, 2018, vol. 145, pp. 339–353.
- [3] M. Husain, “Transdiagnostic neurology: Neuropsychiatric symptoms in neurodegenerative diseases,” *Brain*, vol. 140, no. 6, pp. 1535–1536, 2017.
- [4] K. Talbot, “Motor neurone disease,” *Postgraduate Medical Journal*, vol. 78, no. 923, pp. 513–519, 2002.
- [5] K. A. Josephs, J. L. Whitwell, N. Tosakulwong, S. D. Weigand, M. E. Murray, A. M. Liesinger, L. Petrucelli, M. L. Senjem, D. S. Knopman, B. F. Boeve, R. J. Ivnik, G. E. Smith, C. R. Jack, J. E. Parisi, R. C. Petersen, and D. W. Dickson, “TAR DNA-Binding Protein 43 and pathological subtype of Alzheimer’s disease impact clinical features,” *Annals of Neurology*, vol. 78, no. 5, pp. 697–709, 2015.
- [6] Z. Osking, J. I. Ayers, R. Hildebrandt, K. Skrubber, H. Brown, D. Ryu, J. Yang, E. A. Burton, and E. A. Vitriol, “ALS-linked SOD1 mutants enhance neurite outgrowth and branching in adult motor neurons,” *iScience*, vol. 11, pp. 294–304, 2019.
- [7] A. R. Reddi and V. C. Culotta, “SOD1 integrates signals from oxygen and glucose to repress respiration,” *Cell*, vol. 152, no. 1–2, pp. 224–235, 2013.

- [8] H. Ilieva, M. Polymenidou, and D. W. Cleveland, “Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond,” *Journal of Cell Biology*, vol. 187, no. 6, pp. 761–772, 2009.
- [9] H. Debbarh, N. Louanjli, S. Aboulmaouahib, M. Jamil, L. Ahbbas, I. Kaarouch, O. Sefrioui, and R. Cadi, “Antioxidant activities and lipid peroxidation status in human follicular fluid: Age-dependent change,” *Zygote*, vol. 29, no. 6, pp. 490–494, 2021.
- [10] K. C. Wilcox, “The mechanism of Cu,Zn-Superoxide Dismutase aggregation in familial amyotrophic lateral sclerosis,” Ph.D. dissertation, University of North Carolina at Chapel Hill, 2009.
- [11] A. Al-Chalabi, F. Fang, M. F. Hanby, P. N. Leigh, C. E. Shaw, W. Ye, and F. Rijdsdijk, “An estimate of amyotrophic lateral sclerosis heritability using twin data,” *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 81, no. 12, pp. 1324–1326, 2010.
- [12] A. Moore, C. A. Young, and D. A. Hughes, “Health utilities and costs for motor neurone disease,” *Value in Health*, vol. 22, no. 11, pp. 1257–1265, 2019.
- [13] S. F. Olsen, M. Martuzzi, and P. Elliott, “Cluster analysis and disease mapping—why, when, and how? a step by step guide,” *BMJ*, vol. 313, no. 7061, pp. 863–866, 1996.
- [14] G. Novarino, A. G. Fenstermaker, M. S. Zaki, M. Hofree, J. L. Silhavy, A. D. Heiberg, M. Abdellateef, B. Rosti, E. Scott, L. Mansour, A. Masri, H. Kayserili, J. Y. Al-Aama, G. M. H. Abdel-Salam, A. Karminejad, M. Kara, T. Ben-Omran, T. Al-Shidi, F. Al-Jasmi, and J. G. Gleeson, “Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders,” *Science*, vol. 343, no. 6170, pp. 506–511, 2014.
- [15] L. W. Thompson, K. D. Morrison, S. L. Shirran, E. J. N. Groen, T. H. Gillingwater, C. H. Botting, and J. E. Sleeman, “Neurochondrin interacts with the SMN protein suggesting a novel mechanism for spinal muscular

- atrophy pathology,” *Journal of Cell Science*, vol. 131, no. 8, p. jcs211482, 2018.
- [16] C. Blackstone, “Hereditary spastic paraplegia,” in *Handbook of Clinical Neurology*. Elsevier, 2018, vol. 148, pp. 633–652.
- [17] P. Masrori and P. Van Damme, “Amyotrophic lateral sclerosis: A clinical review,” *European Journal of Neurology*, vol. 27, no. 10, pp. 1918–1929, 2020.
- [18] M. A. van Es, O. Hardiman, A. Chio, A. Al-Chalabi, R. J. Pasterkamp, J. H. Veldink, and L. H. van den Berg, “Amyotrophic lateral sclerosis,” *The Lancet*, vol. 390, no. 10107, pp. 2084–2098, 2017.
- [19] J. Morris, “Amyotrophic lateral sclerosis (ALS) and related motor neuron diseases: An overview,” *The Neurodiagnostic Journal*, vol. 55, no. 3, pp. 180–194, 2015.
- [20] S. Zarei, K. Carr, L. Reiley, K. Diaz, O. Guerra, P. F. Altamirano, W. Pagan, G. Lodin, David Orozco, and A. Chinea, “A comprehensive review of amyotrophic lateral sclerosis,” *Surgical Neurology International*, vol. 6, p. 171, 2015.
- [21] O. Hardiman, A. Al-Chalabi, A. Chio, E. M. Corr, G. Logroscino, W. Robberecht, P. J. Shaw, Z. Simmons, and L. H. van den Berg, “Amyotrophic lateral sclerosis,” *Nature Reviews Disease Primers*, vol. 3, p. 17071, 2017.
- [22] J. P. Taylor, J. Brown, Robert H., and D. W. Cleveland, “Decoding ALS: From genes to mechanism,” *Nature*, vol. 539, no. 7628, pp. 197–206, 2016.
- [23] O. Hardiman, A. Al-Chalabi, A. Chio, E. M. Corr, G. Logroscino, W. Robberecht, P. J. Shaw, Z. Simmons, and L. H. van den Berg, “Amyotrophic lateral sclerosis,” *Nature Reviews Disease Primers*, vol. 3, no. 1, p. 17071, 2017.
- [24] E. V. Hobson and C. J. McDermott, “Supportive and symptomatic management of amyotrophic lateral sclerosis,” *Nature Reviews Neurology*, vol. 12, no. 9, pp. 526–538, 2016.

- [25] E. O. Talbott, A. M. Malek, and D. Lacomis, “The epidemiology of amyotrophic lateral sclerosis,” in *Handbook of Clinical Neurology*. Elsevier, 2016, vol. 138, pp. 225–238.
- [26] C. Wolfson, S. Kilborn, M. Oskoui, and A. Genge, “Incidence and prevalence of amyotrophic lateral sclerosis in canada: A systematic review of the literature,” *Neuroepidemiology*, vol. 33, no. 2, pp. 79–88, 2009.
- [27] National Institute of Neurological Disorders and Stroke, “Motor neuron diseases fact sheet,” <https://www.ninds.nih.gov/health-information/disorders/motor-neuron-diseases>, 2021, accessed 14 June 2021.
- [28] A. Wang, A. E. Conicella, H. B. Schmidt, E. W. Martin, S. N. Rhoads, A. N. Reeb, A. Nourse, D. Ramirez Montero, V. H. Ryan, R. Rohatgi, F. Shewmaker, M. T. Naik, and N. L. Fawzi, “A single N-terminal phosphomimic disrupts TDP-43 polymerization, phase separation, and RNA splicing,” *The EMBO Journal*, vol. 37, no. 5, p. e97452, 2018.
- [29] P.-H. Kuo, L. G. Doudeva, Y.-T. Wang, C.-K. Shen, and H. S. Yuan, “Structural insights into TDP-43 in nucleic-acid binding and domain interactions,” *Nucleic Acids Research*, vol. 37, no. 6, pp. 1799–1808, 2009.
- [30] L. L. Wijesekera and P. N. Leigh, “Amyotrophic lateral sclerosis,” *Orphanet Journal of Rare Diseases*, vol. 4, p. 3, 2009.
- [31] P. Vehvilainen, J. Koistinaho, and G. Goldsteins, “Mechanisms of mutant SOD1-induced mitochondrial toxicity in amyotrophic lateral sclerosis,” *Frontiers in Cellular Neuroscience*, vol. 8, p. 126, 2014.
- [32] —, “Mechanisms of mutant SOD1-induced mitochondrial toxicity in amyotrophic lateral sclerosis,” *Frontiers in Cellular Neuroscience*, vol. 8, p. 126, 2014.
- [33] E. Kabashi, P. N. Valdmanis, P. Dion, D. Spiegelman, B. J. McConkey, C. V. Velde, J.-P. Bouchard, L. Lacomblez, K. Pochigaeva, F. Salachas, P.-F. Pradat, W. Camu, V. Meininger, N. Dupre, and G. A. Rouleau, “TARDBP

- mutations in individuals with sporadic and familial amyotrophic lateral sclerosis,” *Nature Genetics*, vol. 40, no. 5, pp. 572–574, 2008.
- [34] E. Buratti and F. E. Baralle, “The molecular links between TDP-43 dysfunction and neurodegeneration,” *Advances in Genetics*, vol. 66, pp. 1–34, 2009.
- [35] J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, O. Ronneberger, L. Willmore, A. J. Ballard, J. Bambrick, S. Bodenstein, D. A. Evans, E. Ferris, H. Fry, J. Godwin, K. Hansen, R. O’Neill, L. Pacchiardi, L. S. Piloto, A. Potapenko, A. Bridgland, S. T. Ravuri *et al.*, “Accurate structure prediction of biomolecular interactions with AlphaFold 3,” *Nature*, vol. 630, pp. 493–500, 2024.
- [36] N. Rezaei-Ghaleh, M. Blackledge, and M. Zweckstetter, “Intrinsically disordered proteins: From sequence and conformational properties toward drug discovery,” *ChemBioChem*, vol. 13, no. 7, pp. 930–950, 2012.
- [37] E. Kabashi, P. N. Valdmanis, P. Dion, D. Spiegelman, B. J. McConkey, C. V. Velde, J.-P. Bouchard, L. Lacomblez, K. Pochigaeva, F. Salachas, P.-F. Pradat, W. Camu, V. Meininger, N. Dupre, and G. A. Rouleau, “TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis,” *Nature Genetics*, vol. 40, no. 5, pp. 572–574, 2008.
- [38] C.-K. Chang, T.-H. Wu, C.-Y. Wu, M.-H. Chiang, E. K.-W. Toh, Y.-C. Hsu, K.-P. Lin, Y.-H. Liao, and T.-H. Huang, “The N-terminus of TDP-43 promotes its oligomerization and enhances DNA binding affinity,” *Biochemical and Biophysical Research Communications*, vol. 425, no. 2, pp. 219–224, 2012.
- [39] T. Afroz, E. M. Hock, P. Ernst, C. Foglieni, M. Jambeau, L. A. B. Gillespy, F. Laferrière, Z. Maniecka, A. Plückthun, P. Mittl, P. Paganetti, and F. H.-T. Allain, “ALS-linked protein TDP-43 antagonizes its pathologic aggregation,” *Nature Communications*, vol. 8, p. 45, 2017.

- [40] L.-L. Jiang, W. Xue, J.-Y. Hong, J.-T. Zhang, M.-J. Li, S.-N. Yu, J.-H. He, and H.-Y. Hu, "The N-terminal dimerization is required for TDP-43 splicing activity," *Scientific Reports*, vol. 7, p. 6196, 2017.
- [41] A. Wang, A. E. Conicella, H. B. Schmidt, E. W. Martin, S. N. Rhoads, A. N. Reeb, A. Nourse, D. Ramirez Montero, V. H. Ryan, R. Rohatgi, F. Shewmaker, M. T. Naik, and N. L. Fawzi, "A single N-terminal phosphomimic disrupts TDP-43 polymerization, phase separation, and RNA splicing," *The EMBO Journal*, vol. 37, no. 5, p. e97452, 2018.
- [42] M. Neumann, D. M. Sampathu, L.-W. Kwong, A. C. Truax, M. C. Micsenyi, T.-T. Chou, J. Bruce, T. Schuck, M. Grossman, C. D. Clark, L. F. McCluskey, B. L. Miller, E. Masliah, I. R. A. Mackenzie, H. Feldman, W. Feiden, H. A. Kretzschmar, J. Q. Trojanowski, and V. M.-Y. Lee, "Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis," *Science*, vol. 314, no. 5796, pp. 130–133, 2006.
- [43] A. Ratti and E. Buratti, "Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins," *Journal of Neurochemistry*, vol. 138, no. S1, pp. 95–111, 2016.
- [44] M. Neumann, D. M. Sampathu, L.-W. Kwong, A. C. Truax, M. C. Micsenyi, T.-T. Chou, J. Bruce, T. Schuck, M. Grossman, C. D. Clark, L. F. McCluskey, B. L. Miller, E. Masliah, I. R. A. Mackenzie, H. Feldman, W. Feiden, H. A. Kretzschmar, J. Q. Trojanowski, and V. M.-Y. Lee, "Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis," *Science*, vol. 314, no. 5796, pp. 130–133, 2006.
- [45] S.-C. Ling, M. Polymenidou, and D. W. Cleveland, "Converging mechanisms in ALS and FTD: Disrupted RNA and protein homeostasis," *Neuron*, vol. 79, no. 3, pp. 416–438, 2013.
- [46] A. Ratti and E. Buratti, "Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins," *Journal of Neurochemistry*, vol. 138, no. S1, pp. 95–111, 2016.

- [47] A. Prasad, V. Bharathi, V. Sivalingam, A. Girdhar, and B. K. Patel, “Molecular mechanisms of TDP-43 misfolding and pathology in ALS,” *Frontiers in Molecular Neuroscience*, vol. 12, p. 25, 2019.
- [48] Y. Iguchi, M. Katsuno, S. Takagi, S. Ishigaki, J. Niwa, M. Hasegawa, and G. Sobue, “Mislocalized TDP-43 aggravates mitochondrial dysfunction by interacting with mitochondrial mrna,” *Acta Neuropathologica*, vol. 126, no. 6, pp. 819–835, 2013.
- [49] S.-C. Ling, M. Polymenidou, and D. W. Cleveland, “Converging mechanisms in ALS and FTD: Disrupted RNA and protein homeostasis,” *Neuron*, vol. 79, no. 3, pp. 416–438, 2013.
- [50] A. Ratti and E. Buratti, “Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins,” *Journal of Neurochemistry*, vol. 138, no. S1, pp. 95–111, 2016.
- [51] A. Prasad, V. Bharathi, V. Sivalingam, A. Girdhar, and B. K. Patel, “Molecular mechanisms of TDP-43 misfolding and pathology in ALS,” *Frontiers in Molecular Neuroscience*, vol. 12, p. 25, 2019.
- [52] M. Neumann, D. M. Sampathu, L.-W. Kwong, A. C. Truax, M. C. Micsenyi, T.-T. Chou, J. Bruce, T. Schuck, M. Grossman, C. D. Clark, L. F. McCluskey, B. L. Miller, E. Masliah, I. R. A. Mackenzie, H. Feldman, W. Feiden, H. A. Kretzschmar, J. Q. Trojanowski, and V. M.-Y. Lee, “Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis,” *Science*, vol. 314, no. 5796, pp. 130–133, 2006.
- [53] A. Prasad, V. Bharathi, V. Sivalingam, A. Girdhar, and B. K. Patel, “Molecular mechanisms of TDP-43 misfolding and pathology in ALS,” *Frontiers in Molecular Neuroscience*, vol. 12, p. 25, 2019.
- [54] S.-C. Ling, M. Polymenidou, and D. W. Cleveland, “Converging mechanisms in ALS and FTD: Disrupted RNA and protein homeostasis,” *Neuron*, vol. 79, no. 3, pp. 416–438, 2013.

- [55] M. Neumann, D. M. Sampathu, L.-W. Kwong, A. C. Truax, M. C. Micsenyi, J. Chou, Tsu-Ting Nicolescu, T. Schuck, M. Grossman, C. D. Clark, L. F. McCluskey, B. L. Miller, E. Masliah, I. R. A. Mackenzie, H. Feldman, W. Feiden, H. A. Kretzschmar, J. Q. Trojanowski, and V. M.-Y. Lee, "Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis," *Science*, vol. 314, no. 5796, pp. 130–133, 2006.
- [56] D. R. Rosen, T. Siddique, D. Patterson, D. A. Figlewicz, P. Sapp, A. Hentati, D. Donaldson, J. Goto, J. P. O'Regan, H.-X. Deng, Z. Rahmani, A. Krizus, D. McKenna-Yasek, A. Cayabyab, S. M. Gaston, R. Berger, R. E. Tanzi, J. J. Halperin, B. Herzfeldt, R. van den Bergh, W.-Y. Hung, T. Bird, G. Deng, D. W. Mulder, C. Smyth, N. G. Laing, E. Soriano, M. A. Pericak-Vance, J. Haines, G. A. Rouleau, J. S. Gusella, H. R. Horvitz, and R. H. Brown, "Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis," *Nature*, vol. 362, no. 6415, pp. 59–62, 1993.
- [57] P. Vehvilinen, J. Koistinaho, and G. Goldsteins, "Mechanisms of mutant SOD1-induced mitochondrial toxicity in amyotrophic lateral sclerosis," *Frontiers in Cellular Neuroscience*, vol. 8, p. 126, 2014.
- [58] J. P. Taylor, J. Brown, Robert H., and D. W. Cleveland, "Decoding ALS: From genes to mechanism," *Nature*, vol. 539, no. 7628, pp. 197–206, 2016.
- [59] C. J. O'Brien, S. Campbell, A. Young, W. Vogler, and V. J. Galea, "Chinese apple (*Ziziphus mauritiana*): A comprehensive review of its weediness, ecological impacts and management approaches," *Plants*, vol. 12, no. 18, p. 3213, 2023.
- [60] V. B. Pandey, J. P. Singh, K. K. Seth, A. H. Shah, and G. Eckhardt, "Cyclopeptide alkaloids from *Zizyphus nummularia*," *Phytochemistry*, vol. 23, pp. 2118–2120, 1984.
- [61] —, "Cyclopeptide alkaloids from *Zizyphus nummularia*," *Phytochemistry*, vol. 23, pp. 2118–2120, 1984.

- [62] M. M. Joullié and D. J. Richard, “Cyclopeptide alkaloids: Chemistry and biology,” *Chemical Communications*, no. 18, pp. 2011–2015, 2004.
- [63] O. Sin and E. A. A. Nollen, “Regulation of protein homeostasis in neurodegenerative diseases: The role of coding and non-coding genes,” *Cellular and Molecular Life Sciences*, vol. 72, no. 21, pp. 4027–4047, 2015.
- [64] —, “Regulation of protein homeostasis in neurodegenerative diseases: The role of coding and non-coding genes,” *Cellular and Molecular Life Sciences*, vol. 72, no. 21, pp. 4027–4047, 2015.
- [65] S. Watanabe, K. Kaneko, and K. Yamanaka, “Accelerated disease onset with stabilized familial amyotrophic lateral sclerosis (ALS)-linked mutant TDP-43 proteins,” *Journal of Biological Chemistry*, vol. 288, no. 5, pp. 3641–3654, 2013.
- [66] S. Arndt, S. Clifford, and M. Popp, “Ziziphus—a multipurpose fruit tree for arid regions,” in *Sustainable Land Use in Deserts*. Springer, 2001, pp. 388–399.
- [67] H. Huang, A. Ayaz, M. Zheng, X. Yang, W. Zaman, H. Zhao, and S. Lü, “Arabidopsis KCS5 and KCS6 play redundant roles in wax synthesis,” *International Journal of Molecular Sciences*, vol. 23, no. 8, p. 4450, 2022.
- [68] K. Shashi, A. Godara, and S. Sehrawat, “Leaf chlorophyll content and electrolyte leakage in Ziziphus species under moisture stress,” *Haryana Journal of Horticultural Sciences*, vol. 36, pp. 217–218, 2007.
- [69] R. Yadav, S. A. Lone, K. Gaikwad, N. K. Singh, and J. C. Padaria, “Transcriptome sequence analysis and mining of SSRs in jhar ber (*Ziziphus nummularia* (Burm.f.) Wight & Arn) under drought stress,” *Scientific Reports*, vol. 8, p. 2406, 2018.
- [70] World Health Organization, “Diabetes,” <https://www.who.int/news-room/fact-sheets/detail/diabetes>, 2022, fact sheet; accessed 6 June 2022.

- [71] B. Baharvand-Ahmadi, M. Bahmani, P. Tajeddini, N. Naghdi, and M. Rafieian-Kopaei, "An ethno-medicinal study of medicinal plants used for the treatment of diabetes," *Journal of Nephropathology*, vol. 5, no. 1, pp. 44–50, 2016.
- [72] S. Rajasekaran, C. Velmurugan, S. Vetriselvan, R. Basir, and T. Muthuramu, "Phytochemical and pharmacological evaluation of leaves of *Zizyphus nummularia* (Burm. f.) Wight & Arn," *International Journal of Phytopharmacology*, vol. 3, pp. 299–303, 2012.
- [73] J. Menaria, S. Kitawat, B. Joshi, and A. Oja, "Anti-diabetic activity of leaves extracts of *Zizyphus nummularia* by alloxan induced diabetic rat model," *The Pharmaceutical and Chemical Journal*, vol. 1, pp. 23–26, 2014.
- [74] S. Rajasekaran, B. Jaykar, R. Anandan, K. Aboobacker, and S. Vannamalar, "Anti-diabetic activity of leaves of *Zizyphus nummularia* by dexamethasone induced diabetic rat model," *International Journal of PharmTech Research*, vol. 5, pp. 844–851, 2013.
- [75] P. Bagdi and R. Vinita, "In vitro anti-diabetic activity of hydro-alcoholic extracts of leaves and fruits of *Zizyphus nummularia* (Burm. F.) Wight & Arn," *The Pharmaceutical and Chemical Journal*, vol. 4, pp. 88–93, 2017.
- [76] P.-H. Kuo, L. G. Doudeva, Y.-T. Wang, C.-K. Shen, and H. S. Yuan, "Structural insights into TDP-43 in nucleic-acid binding and domain interactions," *Nucleic Acids Research*, vol. 37, no. 6, pp. 1799–1808, 2009.
- [77] E. Kabashi, P. N. Valdmanis, P. Dion, D. Spiegelman, B. J. McConkey, C. V. Velde, J.-P. Bouchard, L. Lacomblez, K. Pochigaeva, F. Salachas, P.-F. Pradat, W. Camu, V. Meininger, N. Dupre, and G. A. Rouleau, "TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis," *Nature Genetics*, vol. 40, no. 5, pp. 572–574, 2008.
- [78] Y.-J. Zhang, T. Caulfield, Y.-F. Xu, T. F. Gendron, J. Hubbard, C. Stetler, H. Sasaguri, E. C. Whitelaw, S. Cai, W. C. Lee *et al.*, "The dual functions of the extreme N-terminus of TDP-43 in regulating its biological activity

- and inclusion formation,” *Human Molecular Genetics*, vol. 22, no. 15, pp. 3112–3122, 2013.
- [79] M. Mompeán, V. Romano, D. Pantoja-Uceda, C. Stuaní, F. E. Baralle, E. Buratti, and D. V. Laurents, “The TDP-43 N-terminal domain structure at high resolution,” *FEBS Journal*, vol. 283, no. 7, pp. 1242–1260, 2016.
- [80] P. S. Tsoi, K. J. Choi, P. G. Leonard, A. Sizovs, M. M. Moosa, K. R. MacKenzie, J. C. Ferreon, and A. C. M. Ferreon, “The N-terminal domain of ALS-linked TDP-43 assembles without misfolding,” *Angewandte Chemie International Edition*, vol. 56, no. 41, pp. 12 590–12 593, 2017.
- [81] E. Kabashi, P. N. Valdmanis, P. Dion, D. Spiegelman, B. J. McConkey, C. V. Velde, J.-P. Bouchard, L. Lacomblez, K. Pochigaeva, F. Salachas, P.-F. Pradat, W. Camu, V. Meininger, N. Dupre, and G. A. Rouleau, “TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis,” *Nature Genetics*, vol. 40, no. 5, pp. 572–574, 2008.
- [82] G. Bensimon, L. Lacomblez, and V. Meininger, “A controlled trial of riluzole in amyotrophic lateral sclerosis,” *New England Journal of Medicine*, vol. 330, no. 9, pp. 585–591, 1994.
- [83] K. Abe, M. Aoki, S. Tsuji, Y. Itoyama, G. Sobue, M. Togo, C. Hamada, M. Tanaka, M. Akimoto, K. Nakamura *et al.*, “Safety and efficacy of edaravone in well-defined patients with amyotrophic lateral sclerosis: A randomized, double-blind, placebo-controlled trial,” *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, vol. 18, no. sup1, pp. 20–31, 2017.
- [84] B. Singh and V. B. Pandey, “An N-formyl cyclopeptide alkaloid from *Ziziphus nummularia* bark,” *Phytochemistry*, vol. 38, no. 1, pp. 271–273, 1995.
- [85] A. H. Shah, G. Eckhardt, V. B. Pandey, J. P. Singh, and K. K. Seth, “Cyclopeptide alkaloids from *Ziziphus nummularia*,” *Phytochemistry*, vol. 23, no. 9, pp. 2118–2120, 1984.
- [86] S. K. Srivastava, “Nummularogenin, a new spirostane from *Ziziphus nummularia*,” *Journal of Natural Products*, vol. 47, no. 5, pp. 781–783, 1984.

- [87] S. C. Sharma and R. Kumar, "Zizynummin, a dammarane saponin from *Ziziphus nummularia*," *Phytochemistry*, vol. 22, no. 6, pp. 1469–1471, 1983.
- [88] N. ddin, N. Ali, Z. Uddin, N. Nazir, M. Zahoor, U. Rashid, R. Ullah, A. S. Alqahtani, A. M. Alqahtani, F. A. Nasr *et al.*, "Evaluation of cholinesterase inhibitory potential of different genotypes of *ziziphus nummularia*, their hplc-uv, and molecular docking analysis," *Molecules*, vol. 25, 2020.
- [89] S. Prajapati and S. Singh, "Phytoconstituents of *ziziphus nummularia* (burm. f.) wight & arn. leaves extracts using gc-ms spectroscopy," *Res. Rev. J. Life Sci.*, vol. 9, pp. 109–118, 2019.
- [90] S. Sumaira, D. Sirjud, and J. Qaiser, "Pharmacological evaluation of *ziziphus nummularia* leaves for phytotoxic and molluscicidal bioassays," *Afr. J. Pharm. Pharmacol.*, vol. 7, pp. 2887–2891, 2013.
- [91] P. Bagdi and V. Rathore, "Phytochemical investigation of extracts of *ziziphus nummularia* (burm. f. wight & arn) leaves and fruits," *Pharm. Chem. J.*, vol. 3, pp. 220–226, 2016.
- [92] R. Tschesche, M. Elgamal, G. A. Miana, and G. Eckhardt, "Alkaloids from *rhamnaceae*—xxvi: Nummularine -d, -e and -f, new cyclopeptide alkaloids from *zizyphus nummularia*," *Tetrahedron*, vol. 31, pp. 2944–2947, 1975.
- [93] G. A. Miana and A. H. Shah, "Isolation of jubanine-a, -b and mauritine-c from the root bark of *zizyphus nummularia*," *Fitoterapia*, vol. 56, pp. 363–364, 1985.
- [94] R. Tschesche, M. Elgamal, and G. Eckhardt, "Alkaloids from *rhamnaceae*. 28. nummularin-g, nummularin-h and nummularin-k new alkaloids from *ziziphus nummularia*," *Chem. Ber. Recl.*, vol. 110, pp. 2649–2655, 1977.
- [95] R. Tschesche, G. A. Miana, and G. Eckhardt, "Alkaloide aus *rhamnaceen*, xxv. nummularin a, b und c, drei neue 13gliedrige peptidalkaloide aus *zizyphus nummulaira*," *Chem. Ber.*, vol. 107, pp. 3180–3185, 1974.

- [96] S. C. Sharma and R. Kumar, "Zizyphummin, a dammarane saponin from zizyphus nummularia," *Phytochemistry*, vol. 22, pp. 1469–1471, 1983.
- [97] H. A. Bachaya, Z. Iqbal, M. N. Khan, Z. U. Sindhu, and A. Jabbar, "Anthelmintic activity of Ziziphus nummularia (bark) and Acacia nilotica (fruit) against trichostrongylid nematodes of sheep," *Journal of Ethnopharmacology*, vol. 123, no. 2, pp. 325–329, 2009.
- [98] A. Kumar, S. Sharma, C. Kaushik, M. P. Dhobal, and B. C. Joshi, "Antitumour activity of lapachol isolated from Zizyphus nummularia as adjuvant for radiation therapy," Traditional Medicine & Materia Medica Research Center (TMRC), 2002, citation details incomplete in source list.
- [99] H. J. de Boer, A. Kool, A. Broberg, W. R. Mziray, I. Hedberg, and J. J. Levenfors, "Antifungal and antibacterial activity of some herbal remedies from tanzania," *Journal of Ethnopharmacology*, vol. 96, no. 3, pp. 461–469, 2005.
- [100] R. Nair and S. Chanda, "Activity of some medicinal plants against certain pathogenic bacterial strains," *Indian Journal of Pharmacology*, vol. 38, no. 2, pp. 142–144, 2006.
- [101] G. M. Shah, M. A. Khan, M. Ahmad, M. Zafar, and A. A. Khan, "Observations on antifertility and abortifacient herbal drugs," *African Journal of Biotechnology*, vol. 8, no. 9, pp. 1959–1964, 2009.
- [102] H. Azaizeh, K. Khalil, and O. Said, "The state of the art of traditional arab herbal medicine in the eastern region of the mediterranean: A review," *Evidence-Based Complementary and Alternative Medicine*, vol. 3, no. 2, pp. 229–235, 2006.
- [103] A. Volkamer, A. Griewel, T. Grombacher, and M. Rarey, "DoGSiteScorer: a web server for automatic binding site prediction, analysis and druggability assessment," *Bioinformatics*, vol. 28, no. 15, pp. 2074–2075, 2012.

- [104] D. Sinagra, “Rational design and preliminary validation of novel 6-phosphogluconate dehydrogenase (6PGD) inhibitors using parietin as a lead,” Ph.D. dissertation, University of Malta, 2021.
- [105] K. Talbot, “Motor neurone disease,” *Postgraduate Medical Journal*, vol. 78, no. 923, pp. 513–519, 2002.
- [106] G. Logroscino *et al.*, “Global, regional, and national burden of motor neuron diseases 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016,” *The Lancet Neurology*, vol. 17, no. 12, pp. 1083–1097, 2018.
- [107] S. Mitsui *et al.*, “SQSTM1, a protective factor of SOD1-linked motor neuron disease, regulates the accumulation and distribution of ubiquitinated protein aggregates in neuron,” *Neurochemistry International*, vol. 158, p. 105364, 2022.
- [108] J. Mesmar *et al.*, “Ziziphus nummularia: a comprehensive review of its phytochemical constituents and pharmacological properties,” *Molecules*, vol. 27, no. 13, p. 4240, 2022.
- [109] V. B. Pandey *et al.*, “Cyclopeptide alkaloids from Zizyphus nummularia,” *Phytochemistry*, vol. 23, no. 9, pp. 2118–2120, 1984.
- [110] J. Mesmar *et al.*, “Ziziphus nummularia: A comprehensive review of its phytochemical constituents and pharmacological properties,” *Molecules*, vol. 27, no. 13, p. 4240, 2022.
- [111] E. Tuenter *et al.*, “Isolation and structure elucidation of cyclopeptide alkaloids from Ziziphus nummularia and Ziziphus spina-christi by HPLC-DAD-MS and HPLC-PDA-(HRMS)-SPE-NMR,” *Phytochemistry*, vol. 138, pp. 163–169, 2017.
- [112] J. Mesmar *et al.*, “Ziziphus nummularia: A comprehensive review of its phytochemical constituents and pharmacological properties,” *Molecules*, vol. 27, no. 13, p. 4240, 2022.

- [113] M. N. Khan *et al.*, “Metabolite distribution and correlation studies of Ziziphus jujuba and Ziziphus nummularia using LC-ESI-MS/MS,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 178, p. 112918, 2020.
- [114] S. P. D. Dwivedi *et al.*, “Cyclopeptide alkaloids from Zizyphus nummularia,” *Journal of Natural Products*, vol. 50, no. 2, pp. 235–237, 1987.
- [115] N. Uddin *et al.*, “Evaluation of cholinesterase inhibitory potential of different genotypes of Ziziphus nummularia, their HPLC-UV, and molecular docking analysis,” *Molecules*, vol. 25, no. 21, p. 5011, 2020.
- [116] —, “Distribution of polyphenolic compounds, antioxidant potential, and free amino acids in Ziziphus fruits extract; a study for determining the influence of wider geography,” *Food Science & Nutrition*, vol. 10, no. 5, pp. 1414–1430, 2022.
- [117] S. K. Srivastava, “Nummularogenin, a new spirostane from Zizyphus nummularia,” *Journal of Natural Products*, vol. 47, no. 5, pp. 781–783, 1984.
- [118] T. K. Karami *et al.*, “Eyes on Lipinski’s rule of five: A new “rule of thumb” for physicochemical design space of ophthalmic drugs,” *Journal of Ocular Pharmacology and Therapeutics*, vol. 38, no. 1, pp. 43–55, 2022.
- [119] R. Chauhan *et al.*, “GUD-VE visualization tool for physicochemical properties of proteins,” *MethodsX*, vol. 10, p. 102226, 2023.
- [120] M. Haritha, M. Sreerag, and C. H. Suresh, “Quantifying the hydrogen-bond propensity of drugs and its relationship with Lipinski’s rule of five,” *New Journal of Chemistry*, vol. 48, no. 11, pp. 4896–4908, 2024.
- [121] D. O. Odhiambo *et al.*, “*In silico* pharmacokinetics ADME/Tox analysis of phytochemicals from genus *Dracaena* for their therapeutic potential,” *Scientific African*, vol. 29, p. e02796, 2025.
- [122] E. Bertram-Ralph and M. Amare, “Factors affecting drug absorption and distribution,” *Anaesthesia & Intensive Care Medicine*, vol. 24, no. 4, pp. 221–227, 2023.

- [123] L. P. Volak *et al.*, “Industry perspective on the pharmacokinetic and absorption, distribution, metabolism, and excretion characterization of heterobifunctional protein degraders,” *Drug Metabolism and Disposition*, vol. 51, no. 7, pp. 792–803, 2023.
- [124] Y. Dai *et al.*, “Pharmacokinetics and tissue distribution of albendazole and its three metabolites in yellow river carp (*Cyprinus carpio haematopterus*) after single oral administration,” *Journal of Agricultural and Food Chemistry*, vol. 73, no. 3, pp. 1824–1834, 2025.
- [125] Y. Lai *et al.*, “Recent advances in the translation of drug metabolism and pharmacokinetics science for drug discovery and development,” *Acta Pharmaceutica Sinica B*, vol. 12, no. 6, pp. 2751–2777, 2022.
- [126] A. Talevi and C. L. Bellera, “Drug excretion,” in *The ADME Encyclopedia: A Comprehensive Guide on Biopharmacy and Pharmacokinetics*. Springer, 2022, pp. 357–362.
- [127] N. R. Mitta, “AI-driven systems for predictive pharmacokinetics and pharmacodynamics modeling: Developing machine learning models for accurate drug absorption, distribution, metabolism, and excretion predictions,” *Newark Journal of Human-Centric AI and Robotics Interaction*, vol. 3, pp. 389–421, 2023.
- [128] S. Li *et al.*, “An insight into current advances on pharmacology, pharmacokinetics, toxicity and detoxification of aconitine,” *Biomedicine & Pharmacotherapy*, vol. 151, p. 113115, 2022.
- [129] Y. Fan *et al.*, “Acute, subacute oral toxicity and Ames test of Py-mulin: an antibacterial drug candidate,” *BMC Pharmacology and Toxicology*, vol. 23, no. 1, p. 2, 2022.
- [130] S. Luo *et al.*, “Short-term toxicity of ammonia, nitrite, and nitrate to early life stages of the rare minnow (*Gobiocypris rarus*),” *Environmental Toxicology and Chemistry*, vol. 35, no. 6, pp. 1422–1427, 2016.

- [131] J. Fan, A. Fu, and L. Zhang, “Progress in molecular docking,” *Quantitative Biology*, vol. 7, no. 2, pp. 83–89, 2019.
- [132] A. V. Rokade, P. Yelne, and A. Giri, “Riluzole and edavarone: the hope against amyotrophic lateral sclerosis,” *Cureus*, vol. 14, no. 10, 2022.
- [133] M. Cai and E. J. Yang, “Combined treatment with bojungkiki-tang and riluzole regulates muscle metabolism and dysfunction in the hSOD1G93A mouse model,” *Antioxidants*, vol. 11, no. 3, p. 579, 2022.
- [134] Y. Lu, J. Liu, and J. Guo, “Effects of early electroacupuncture intervention on TDP-43 and HMGB1/RhoA signaling pathway in cerebral cortex of mice with amyotrophic lateral sclerosis model,” *Chinese Journal of Rehabilitation Medicine*, pp. 312–319, 2024.
- [135] I. Alafuzoff and P. Hartikainen, “Alpha-synucleinopathies,” in *Handbook of Clinical Neurology*. Elsevier, 2018, pp. 339–353.
- [136] D. M. Wilson *et al.*, “Hallmarks of neurodegenerative diseases,” *Cell*, vol. 186, no. 4, pp. 693–714, 2023.
- [137] H. M. Bryson, B. Fulton, and P. Benfield, “Riluzole: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in amyotrophic lateral sclerosis,” *Drugs*, vol. 52, no. 4, pp. 549–563, 1996.
- [138] J. P. Taylor, R. H. Brown, Jr., and D. W. Cleveland, “Decoding ALS: from genes to mechanism,” *Nature*, vol. 539, no. 7628, pp. 197–206, 2016.
- [139] D. W. Cleveland and J. Liu, “Oxidation versus aggregation—how do SOD1 mutants cause ALS?” *Nature Medicine*, vol. 6, no. 12, pp. 1320–1321, 2000.
- [140] M. Jo *et al.*, “The role of TDP-43 propagation in neurodegenerative diseases: integrating insights from clinical and experimental studies,” *Experimental & Molecular Medicine*, vol. 52, no. 10, pp. 1652–1662, 2020.
- [141] R. Azimi *et al.*, “Bioinformatic analysis of small humanin like peptides using AlphaFold-2 and Expasy ProtParam,” *Investigative Ophthalmology & Visual Science*, vol. 65, no. 7, p. 1320, 2024.

- [142] M. Tsekrekou *et al.*, “Protein aggregation and therapeutic strategies in SOD1-and TDP-43-linked ALS,” *Frontiers in Molecular Biosciences*, vol. 11, p. 1383453, 2024.
- [143] C. Barba-Ostria *et al.*, “Evaluation of biological activity of natural compounds: current trends and methods,” *Molecules*, vol. 27, no. 14, p. 4490, 2022.
- [144] A. Sharma *et al.*, “Pharmacokinetic profiling of anticancer phytocompounds using computational approach,” *Phytochemical Analysis*, vol. 29, no. 6, pp. 559–568, 2018.
- [145] A. M. Palmer, “The role of the blood brain barrier in neurodegenerative disorders and their treatment,” *Journal of Alzheimer’s Disease*, vol. 24, no. 4, pp. 643–656, 2011.
- [146] M. Kallubai *et al.*, “Comparative binding mechanism of lupeol compounds with plasma proteins and its pharmacological importance,” *Molecular BioSystems*, vol. 11, no. 4, pp. 1172–1183, 2015.
- [147] J. J. Wu *et al.*, “Interactions between phytochemicals from traditional Chinese medicines and human cytochrome P450 enzymes,” *Current Drug Metabolism*, vol. 13, no. 5, pp. 599–614, 2012.
- [148] A. P. Lakeev *et al.*, “Novel aspects of taxifolin pharmacokinetics: Dose proportionality, cumulative effect, metabolism, microemulsion dosage forms,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 236, p. 115744, 2023.
- [149] D. Arseni *et al.*, “Structure of pathological TDP-43 filaments from ALS with FTLD,” *Nature*, vol. 601, no. 7891, pp. 139–143, 2022.
- [150] K. Villalobos-Nova *et al.*, “Bringing the spotlight to tau and TDP-43 in frontotemporal dementia: A review of promising chemical compounds,” *Current Medicinal Chemistry*, vol. 29, no. 38, pp. 5903–5924, 2022.

- [151] S. K. Panigrahi and G. R. Desiraju, "Strong and weak hydrogen bonds in the protein–ligand interface," *Proteins: Structure, Function, and Bioinformatics*, vol. 67, no. 1, pp. 128–141, 2007.
- [152] H. Li *et al.*, "Construction of fish scale (*Cyprinus carpio* L.) gelatin-fatty acid conjugate for loading curcumin: Effect of alkyl chain length on the interaction and stability," *International Journal of Biological Macromolecules*, vol. 304, p. 140757, 2025.
- [153] M. Goyal *et al.*, "Analgesic and anti-inflammatory studies of cyclopeptide alkaloid fraction of leaves of *Ziziphus nummularia*," *Saudi Journal of Biological Sciences*, vol. 20, no. 4, pp. 365–371, 2013.
- [154] S. Sharma and R. Kumar, "Zizynummin, a dammarane saponin from *Ziziphus nummularia*," *Phytochemistry*, vol. 22, no. 6, pp. 1469–1471, 1983.
- [155] A. Kumar *et al.*, "Antitumour activity of lapachol isolated from *Ziziphus nummularia* as adjuvant for radiation therapy," *Tehran Tradit. Med. Mater. Med. Res. Cent. (TMRC) Shahid Beheshti Univ. Med. Sci*, vol. 1, pp. 129–136, 2002.
- [156] R. Nair and S. Chanda, "Activity of some medicinal plants against certain pathogenic bacterial strains," *Indian Journal of Pharmacology*, vol. 38, no. 2, pp. 142–144, 2006.
- [157] A. Waksik and L. Antkiewicz-Michaluk, "The mechanism of neuroprotective action of natural compounds," *Pharmacological Reports*, vol. 69, no. 5, pp. 851–860, 2017.
- [158] Z. Dhouaffi *et al.*, "Inhibition of protein misfolding and aggregation by natural phenolic compounds," *Cellular and Molecular Life Sciences*, vol. 75, no. 19, pp. 3521–3538, 2018.
- [159] J. An *et al.*, "Neuroprotective effects of natural compounds on LPS-induced inflammatory responses in microglia," *American Journal of Translational Research*, vol. 12, no. 6, p. 2353, 2020.