In Silico Screening of Phytochemicals Against Human Caspase-6 for Huntington's Disease Therapeutics

By

Imtiaz Ahmed Utsha
22146034

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm. Hons.)

School of Pharmacy
BRAC University
June, 2026

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Imtiaz Ahmed Utsha
22146034

Approval

The thesis titled " *In Silico* Screening of Phytochemicals Against Human Caspase-6 for Huntington's Disease Therapeutics " submitted by Imtiaz Ahmed Utsha (ID: 22146034) of Spring 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (B. Pharm. Hons.).

Examining Committee:

Supervisor: _____

Humair Bin Md. Omer, PhD
Associate Professor,
School of Pharmacy,
BRAC University

Approved By:

Program Coordinator: _____

Namara Mariam Chowdhury
Senior Lecturer
School of Pharmacy
BRAC University

Chairperson _____

Sharmind Neelotpol, PhD
Professor
School of Pharmacy
BRAC University

Ethics Statement

This project does not involve any kind of animal or human trial.

Abstract

Huntington's disease (HD) is a fatal genetic disorder caused by a Cytosine-Adenine-Guanine (CAG) repeat expansion in the huntingtin gene. This study utilized a structure-based *in silico* virtual screening strategy via AutoDock Vina to discover competitive caspase-6 inhibitors from a library of 50 phytochemicals native to the Jahangirnagar University (JU) campus. Protocol accuracy was confirmed by an internal self-docking control yielding a root mean square deviation (RMSD) of < 2.0Å. The 50 plant compounds exhibited binding free energies ranging from −2.1 kcal/mol to −7.1 kcal/mol. There were three major potential therapeutic leads which included Andrographolide (−7.1 kcal/mol), Myricitrin (−7.0 kcal/mol), and Dasycarpidan-1-methanol (−6.7 kcal/mol). The spatial interactions have shown that these molecules can form very stable complexes by virtue of strong hydrogen bonding and hydrophobic pi-alkyl packing near the conserved Cys163-His121 catalytic diad. Since they have low molecular weight, these phytochemicals may present themselves as potential candidates against HD.

Keywords: *Huntington's Disease, Caspase-6, Molecular Docking, AutoDock Vina, Secondary Metabolites, Blood-Brain Barrier.*

Acknowledgement

First and foremost, I wish to express my profound gratitude to the Almighty for granting me the health, strength, determination, and perseverance required to successfully navigate and complete this research project.

I am deeply indebted to my parents for their unconditional support, sacrifices, and for providing me with the invaluable opportunity to pursue my education at such a reputable institution, allowing me to enrich my knowledge and academic foundations.

I would like to extend my sincere gratitude and respect to my honorable supervisor, Dr. Humair Bin Md. Omer, Associate Professor, School of Pharmacy, BRAC University. His invaluable guidance, constructive insights, and immense patience throughout the entire course of this study were instrumental in shaping this project, without which this work would not have been possible.

Finally, I am highly grateful to my friends for their constant motivation, encouragement, and practical assistance during the execution of this research, which greatly helped me maintain focus and successfully conclude this milestone.

Table of Contents

Declaration……..…...2

Approval……... 3

Ethics Statement…….. 4

Abstract……..……5

Acknowledgement……..……..6

Table of Contents……..7

List of Tables…… 9

List of Figures……10

List of Acronyms……. 11

Chapter 1 Introduction to Huntington's Disease……..……12

1.1Introduction……12

1.2 Pathogenesis……...15

1.4 Prevalence……..……17

Chapter 2 Methodology……...…….…..19

2.1 The Biological Target: Caspase-6 and Huntington's Disease……...19

2.2 Protein Preparation using Discovery Studio……19

2.3 Ligand Preparation: Phytochemical Library……20

2.4 Molecular Docking with AutoDock Vina……20

Chapter 3 Drugs Used For Treating Huntington's Disease……22

3.1 Treatment & Drugs……22

Chapter 4 Previous Molecular Docking Studies……...…….……27

Chapter 5 Results……...…….……...30

5.1 Introduction…….……..……30

5.2 Selection of Phytochemicals and Botanical Origins……..30

5.3 Analysis of Molecular Docking Results……..…….……..32

5.4 Discussion of Top Lead Compounds……..34

5.5 Comparison with Known Inhibitors……...37

Chapter 6 Discussion……..……..…….38

6.1 Chemical Analysis and Binding Mechanisms of Top Leads…….38

6.2 Pharmacokinetic Realities, Local Flora, and Future Horizons……..39

Chapter 7 Conclusion……..……..……41

References……...…….……...43

List of Tables

Table 1: Prevalence rate ratios……..18

Table 2: Binding affinity of HTT protein with tetrabenazine……...27

Table 3: H-bonds and non-covalent interactions between amino acid residue and luteolin....29

Table 4: List of plants found in Jahangirnagar University area……...30

Table 5: Docking result of some plants found in Jahangirnagar University area…….32

List of Figures

Figure 1: Transfected Huntingtin Induces Neurodegeneration in a Cell-Specific Manner…..14

Figure 2: Natural history of clinical Huntington disease……16

Figure 3: Formation of mHTT toxic species and cellular mechanisms to handle them……...22

Figure 4: Olanzepine……24

Figure 5: Quetiapine, Zotepine, and Ziprasidone……24

Figure 6: Remacemide……25

Figure 7: Deutetrabenazine……..25

Figure 8: Tetrabenazine……27

Figure 9: Luteolin……28

Figure 10: Andrographolide interaction……...35

Figure 11: Myricitrin interaction……..36

Figure 12: Dasycarpidan-1-methanol interaction……36

List of Acronyms

ASO= Antisense Oligonucleotide

BBB= Blood-Brain Barrier

CHARMM= Chemistry at Harvard Macromolecular Mechanics

DMT= Disease-Modifying Therapy

FDA= Food and Drug Administration

HD= Huntington’s Disease

HDAC= Histone Deacetylase

HTT= Huntingtin (Gene / Standard Protein)

JU= Jahangirnagar University

KI= Knock-In

mHTT= Mutant Huntingtin (Pathological Variant)

PDB= Protein Data Bank

RMSD= Root Mean Square Deviation

RNAi= RNA Interference

SBVS= Structure-Based Virtual Screening

UHDRS= Unified Huntington's Disease Rating Scale

Chapter 1

Introduction to Huntington's Disease

1.1 Introduction

A gene mutation on chromosome 4 causes Huntington's disease (HD), a severe neurological condition that is inherited in an autosomal dominant manner. HD is a progressive illness that impairs psychological, cognitive, and motor abilities. Adulthood is typically when this issue first manifests. When symptoms become apparent, a patient's condition advances irreversibly for about 17 years before a patient's death. Huntington's is estimated to occur in 5 to 7.5 people per 100,000 of the world's total population (Vonsattel et al., 1985). At a molecular level, Huntington's Disease is a result of an excess expansion of a CAG trinucleotide repeat in the IT-15 gene, commonly referred to as HTT, with a repeat size of more than 35 units of CAG. This gives rise to an unusual form of Huntingtin protein with an abnormally long tract of polyglutamine, otherwise referred to as a polyGln tract, at its N-terminus (Ravikumar et al., 2004). The normal Huntingtin protein is a large molecule with a size of approximately 350kDa and doesn't bear any homology with any known proteins before its discovery, and its function is yet to be fully explained (Saudou et al., 1998). The mutant huntingtin protein acquires toxic characteristics that start the pathologic process once the number of CAG repeats beyond the pathogenic range. Intraneuronal aggregation of mutant huntingtin proteins in the form of intraneural inclusion bodies is one of the most noticeable signs of the pathophysiology of Huntington's disease. It has been demonstrated that the role of intraneural inclusion bodies is contentious. Some studies indicate that the presence of intraneural inclusion bodies is harmful and mediates neurotoxicity directly, while others have suggested that intraneural inclusion bodies could play a shielding function by sequestering the harmful

soluble form of the mutant huntingtin protein. Chorea, neuropsychiatric and personality disorders, intellectual abnormalities, dementia, and early mortality are among the clinical characteristics of Huntington's disease. These symptoms result from the death and selective malfunctioning of particular neuronal subpopulations within the central nervous system. Neuropathological studies consistently show marked neuronal loss within the striatum, a subcortical brain structure essential for the regulation of voluntary movement, with less pronounced involvement of the cerebral cortex. The neuronal specificity in HD is particularly remarkable. Within the striatum, for instance, enkephalin-containing medium spiny neurons are especially sensitive to degeneration, while many neighboring neuronal populations remain relatively preserved. This selective vulnerability has been an intense focus of HD research because it suggests that neuronal death is not a homogeneous process but rather is dependent on cell-type-specific factors. Importantly, evidence has shown that, over the course of Huntington’s disease, neuronal dysfunction precedes over neuronal death. Motor and cognitive abnormalities have been found in HD patients and in animal models before any significant neurodegeneration becomes apparent, providing a strong case for a critical role of early synaptic and cellular dysfunction in disease progression (Gauthier et al., 2004). Thus, neuronal loss may be a result of prolonged neuronal impairment rather than the causative event.

Besides its neurological manifestations, Huntington’s disease has been associated with systemic metabolic abnormalities, especially of glucose metabolism. A number of hereditary neurological disorders, such as Friedreich's ataxia, ataxia telangiectasia, myotonic dystrophy, and other muscular dystrophies, have also been associated with diabetes mellitus and glucose intolerance. Abnormal carbohydrate metabolism has also been reported in HD, including a peculiar type of diabetes mellitus, presenting with marked insulin hypersecretion and hyperglycemia without glycosuria or ketosis.

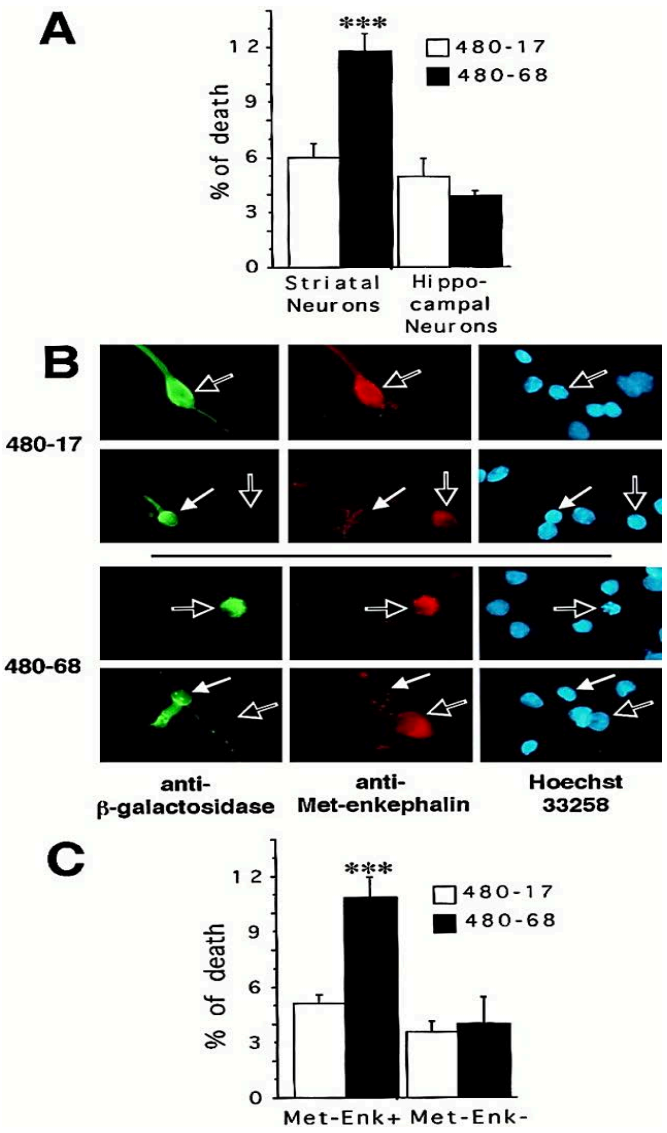

Figure 1: Transfected Huntingtin Induces Neurodegeneration in a Cell-Specific Manner (Saudou et al., 1998).

This metabolic pattern was uncovered in six out of the ten HD patients by oral glucose tolerance and intravenous arginine tolerance tests. The findings suggest that mutant HD can also interfere directly or indirectly with metabolic control. Another important feature of Huntington's disease is psychiatric disturbances. It was recently established that HD mutation carriers had committed suicide rates that are four to eight times higher compared to that of the general population. Besides that, it has been assumed that suicidal ideation or attempted suicide may be present in up to 20% of patients. The results point out the psychological

burden of the illness and the need for all-around treatment in clinical practice encompassing both neurological and mental aspects. There is no known therapy or disease-modifying agent that has been found for the treatment of Huntington's disease, and this is shocking considering the progress that has been made in relation to the understanding of the genetics, pathology, and causes of the disease. The fact that the only treatments for the disease are still symptomatic has meant that Huntington's disease is still a devastating disease, and thus its research continues to be very important.

1.2 Pathogenesis

Huntington's disease (HD) results from a mutation in the huntingtin (HTT) gene that instigates a molecular and cellular cascade of preferred neuronal dysfunction and death. The HTT protein is a massive molecule that is predicted to be composed mainly of HEAT repeats, each being approximately 50 amino acids long (Ross & Tabrizi, 2011). The repeating structures contain antiparallel α-helices that form a superhelix structure with a hydrophobic center, suggesting that huntingtin functions as a flexible protein scaffold in protein–protein interactions. Huntington's disease is caused by a few fundamental mechanisms. The mutant HTT protein frequently misfolds to take on aberrant forms, like β-sheet-dominated aggregates that easily assemble. In HD, the cell systems that eliminate aberrant protein distributions such as the autophagy and ubiquitin-proteasome systems become compromised. As a result, quantities of harmful protein types rise. N-terminal peptides that easily cluster are produced when HTT is proteolytically processed. Additionally, post-translational changes to HTT modulate protein toxicity, particularly affecting protein conformation, aggregation potential, subcellular trafficking, or protein clearance. Nuclear translocation of mutant HTT further increases toxicity, partly due to transcriptional dysregulation, whereas defects in cellular metabolism/mitochondrial pathways underlie neuronal susceptibility to damage. The

HTT gene is mapped on the 4p16. 3 region of the chromosome and has a CAG trinucleotide repeat that encodes a polyglutamine tract close to the amino-terminal region of the protein called huntingtin. The number of CAG repeats can be analyzed by PCR-based techniques (Bates et al., 2015). In the normal population, the number of CAG repeats varies from 6 to 35 glutaminate residues; whereas expansions exceeding 40 are fully penetrant and invariably cause the occurrence of the disease itself (Bates et al., 2015). The CAG repeat is unstable within the gene, particularly within paternal transmissions, which increases according to the expansion of the number of glutamate residues; furthermore, genotypic analyses have led to the observation that there is a direct relationship between CAG expansion and age at onset of the disease; at the same time, data suggest that the course of the disease may deviate according to further defined genetic or environmental interactions that are presently unknown (Nakamura et al., 2007).

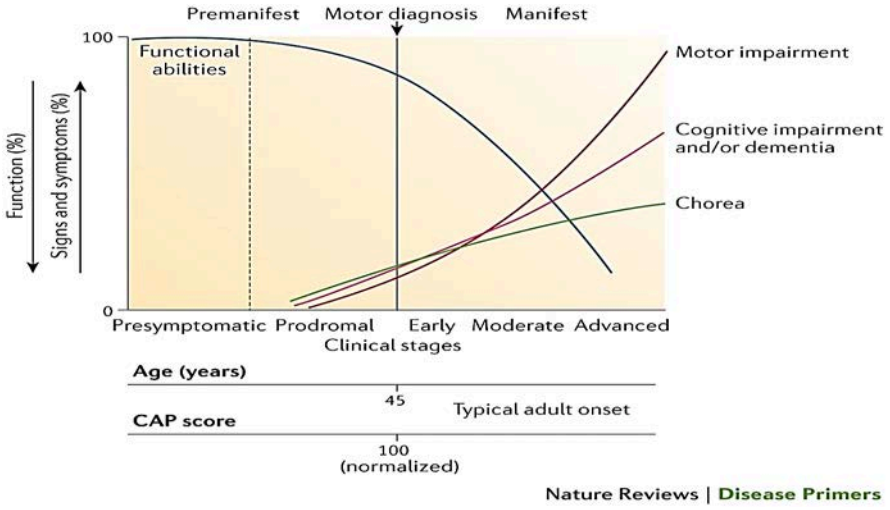

Figure 2: Natural history of clinical Huntington disease (Bates et al., 2015).

Psychiatric manifestations, particularly depression, are a prominent and often early feature of Huntington's disease. The molecular basis of depression in HD remains incompletely understood; however, studies in transgenic HD animal models have demonstrated depressive-like behaviors with predictive validity, along with associated cellular and

molecular changes, supporting the notion that the HD mutation significantly increases susceptibility to depression (Jellinger, 2024). Strong evidence for a toxic gain-of-function mechanism in HD comes from studies of polyglutamine aggregation. In vitro, expanded polyglutamine tracts aggregate through the formation of dimers, oligomers, and higher-order assemblies (Walker, 2007). This process requires a minimum of approximately 37 glutamine residues, accelerates with increasing repeat length, and provides a mechanistic explanation for the delayed onset of disease and the inverse correlation between CAG repeat length and age at onset (Walker, 2007).

1.3 Prevalence

Europe is remarkable for the large number of population-based studies of prevalence of Huntington's disease (HD). Early studies, however, such as those from Belgium, almost certainly underestimated case numbers, a prevalence of 4-7 per 100,000 individuals being reported for much of Europe (Harper, 1992). Estimates are representative of populations of European descent. This compares to a strikingly low prevalence in Japan-an observation of particular interest given the country's highly developed neurological service and solid research base. A study in the Aichi Prefecture estimated prevalence to be 0.38 per 100,000, subsequently revised to 0.45 per 100,000 (Harper, 1992). A very much lower estimate was derived from Ibaraki Prefecture, where only three confirmed cases were found in a population of 2,638,280, representing a prevalence of 0.11 per 100,000 (Harper, 1992). These figures are about one-tenth of those found in European ancestry communities. There could have been underreporting of HD prevalence in the past due to its stigma, where affected individuals within communities or their kin might not reveal those afflicted. Availability of genetic screening services might have improved reporting over the past decades (Ghosh & Tabrizi, 2018). From genetics and history, there seems to be evidence that HD originated in north-western Europe before dispersing through migrations for geographical differences in

prevalence. Low baseline prevalence rates exist in Japan, Hong Kong, and Taiwan, but extremely high rates have been recorded in Venezuela, specifically in the vicinity around Lake Maracaibo, at a prevalence of around 700 per 100,000 people (Ghosh & Tabrizi, 2018). Prevalence of HD differs by over ten times worldwide. Very low rates have been reported among Black populations in South Africa (0.02 per 100,000; 95% CI 0–0.5) and Zimbabwe (1.00 per 100,000; 95% CI 0.48–1.84), likely reflecting limitations in case ascertainment (Rawlins et al., 2016). In Asian countries where genetic testing became routine after 1995, the average prevalence was 0.42 per 100,000 (95% CI 0.37–0.47), compared with 9.71 per 100,000 (95% CI 9.32–10.12) in predominantly Caucasian populations from Australia, Western Europe, and North America during the same period (Rawlins et al., 2016).

Table 1: Prevalence rate ratios (Rawlins et al., 2016).

	Number of studies in estimates of rate ratios	Study years (range)	Average prevalence per 100,000 (95% CIs)	Trend (percent) by decade
Asia	7	1957–2013	0.40 (0.36–0.44)	8.9 (–2.24 to +23.8)
Central and Eastern Europe	8	1981–2008	2.17 (1.95–2.41)	15.4 (2.70 to +38.6)
North America	6	1950–2012	7.33 (6.94–7.74)	20.1 (+18.1 to 22.1)
Oceania	8	1981–2008	5.63 (5.61–6.25)	15.4 (+11.6 to +19.3)
United Kingdom	19	1950–2010	6.68 (6.40–6.97)	15.5 (+11.3 to +18.0)
Western Europe	27	1930–2013	3.60 (3.50–3.69)	16.5 (+14.9 to +18.6)

No clear increase in prevalence has been observed in Asia or Central and Eastern Europe, possibly due to both lower baseline prevalence and fewer epidemiological studies.

Chapter 2

Methodology

The methodology used in this research involves structure-based in silico approach to assess the inhibitory activity of different phytochemicals on Caspase-6, which is an important executioner enzyme involved in the causality of Huntington's disease. This process involves preparation of proteins and ligands using software such as BIOVIA Discovery Studio and docking simulation through AutoDock Vina.

2.1 The Biological Target: Caspase-6 and Huntington's Disease

Caspase-6 is a cysteine-aspartic acid protease that plays a unique role in neurodegeneration. In HD, Caspase-6 is responsible for the proteolytic cleavage of mutant huntingtin (mHTT) at the 586 amino acid position, generating toxic N-terminal fragments that accumulate in the nucleus and disrupt cellular homeostasis (Hakami et al., 2025). Unlike other executioner caspases, its activity often precedes apoptotic morphology, making it a "prime target" for early disease-modifying intervention. This study explores phytochemicals as potential allosteric or active-site inhibitors to mitigate this cleavage.

2.2 Protein Preparation using Discovery Studio

The 3D crystal structure of Human Caspase-6 was obtained from the RCSB Protein Data Bank that has a PDB ID of 3QNW. The preparation process of the receptor was carried out in BIOVIA Discovery Studio in order to have the receptor ready for docking and in its biological form. Preparation of the Human Caspase-6 receptor started with the removal of crystallographic water molecules and heteroatoms in order to avoid false steric hindrance of the binding pocket, thus making sure that interactions between the ligand and protein are not hindered by the artificial presence of the solvents. In order to minimize computation time and

simplify the simulation process, redundant chains in the asymmetric unit of the protein were eliminated, resulting in only one functional monomer of the protein. Moreover, all non-essential ligands that co-crystallized with the protein were also removed in order to make the active site available for docking of the phytochemicals.

2.3 Ligand Preparation: Phytochemical Library

A library consisting of a wide variety of phytochemicals such as flavonoids, alkaloids, and polyphenols was selected via the PubChem database which served as the principal source of structural data for this purpose. The choice of these chemicals was deliberately designed according to the flora found in the region of Bangladesh and particularly the plants inhabiting the ecologically rich grounds of the Jahangirnagar University campus. Through selecting phytochemicals based on the locally abundant plants in the aforementioned region, it is hoped that the efficacy of the local biodiversity on Caspase-6 will be assessed. Each ligand was sourced in its three-dimensional form to be used for in silico analysis.

2.4 Molecular Docking with AutoDock Vina

The molecular docking studies were carried out using AutoDock Vina due to its optimal iterated local search global optimizer and increased accuracy in prediction of ligand binding orientation. The atomic structure that was optimized earlier in the BIOVIA Discovery Studio was then used in the AutoDock software for further preparation.

1. Receptor Optimization and Charge Assignment: In order to make sure that the macromolecule is structurally sound, the receptor structure was first analyzed and any defects such as missing atoms were corrected using the AutoDock automatic repair module. Once the structure was optimized, polar hydrogen atoms were attached to make valences satisfied and hydrogen bonding possible during the simulation. In order to calculate the electrostatic potential of the protein correctly, Kollman charges were applied to the receptor structure.

2. Grid Box Dimensioning and Search Space: The site of interest was identified according to the atomic coordinates of the native substrate binding pocket of Caspase-6. A 3D grid box was generated to generate a search space centered at X = 10.410, Y = −12.519, and Z = 4.948. Dimensions of the grid box were fixed at 20 x 20 x 20 Å to ensure that there was enough search space for the plant-based ligands to adopt favorable conformations within the binding site.

3. Docking Parameters and Scoring Function: Exhaustiveness value for the simulation was fixed at 8 to ensure adequate exploration of the conformational space without compromising the computing time. The semi-empirical scoring function of AutoDock Vina incorporating Gaussian steric terms, hydrophobic interactions, and hydrogen bonding was employed to determine the Gibbs free energy of binding. This was reported in kcal/mol, and the lower the value, the more stable was the ligand-protein complex.

Chapter 3

Drugs Used For Treating Huntington's Disease

3.1 Treatment & Drugs

Currently, there is a lack of a Disease-Modifying Therapy available to slow down or halt the progression of HD, and treatment goals are essentially focused on Symptomatic Support in order to alleviate motor problems and psychiatric symptoms. Pharmacotherapy is essentially targeted toward managing chorea, psychiatric problems, and associated neuropsychiatric complications. However, over the past few years, a lot of interest has been focused on designing a treatment targeting the core cause of HD, which is associated with mHTT expression. Methods to inhibit mHTT expression by lowering its levels include those based upon targeting DNA/RNA available via Antisense Oligonucleotide Treatment (ASOs), RNA Interference (RNAi) therapy, Zinc Finger Proteins (ZFPs), and more specifically via Cas9 Gene-Edited Methods. Currently, mHTT Lowering Trials are considered amongst some of the most promising approaches because they specifically target the core cause of HD rather than its sequelae. Recent evidence suggests a role for Intercellular spread of mHTT in HD progression.

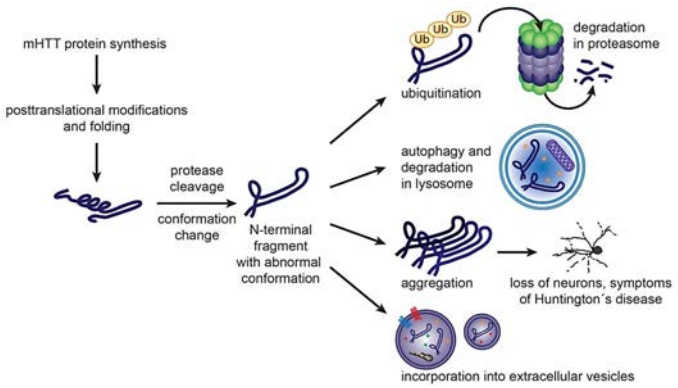

Figure 3: Formation of mHTT toxic species and cellular mechanisms to handle them

(Ananbeh et al., 2021).

This has been mainly evidenced in in-vitro and animal models, in which mHTT peptide internalization into cells, cytoplasmic accumulation, as well as intercellular and neuronal transfers of mHTT protein aggregates were highlighted. Noticeably, the ability for mHTT to propagate into non-related tissues has been validated in vivo in three patients with HD who received a transplant of fetal striatal tissue, showing mHTT protein accumulation in the transplanted tissue (Ananbeh et al., 2021). This provides evidence for mHTT to propagate via a prion-like process. Exosomes play a crucial role in facilitating mHTT protein and RNA transmission. Injection of exosomes harvested from fibroblasts of HD patients directly into the ventricles of newly born mouse brains led to the establishment of HD-like symptoms and pathological alterations (Ananbeh et al., 2021). On the other hand, accumulating evidence supports a protective function for exosomes arising from a particular type of cellular origin. Exosomes secretory of astrocytes and adipose tissue-derived stem cells have been shown to be beneficial for HD mice models. For instance, the infusion of exosomes secretory of astrocytes into the striatum of 140Q knock-in (KI) HD mice resulted in a decrease in the cellular density of mHTT aggregates. Interestingly, the mHTT protein is not found in exosomes secretory of primary astrocytes from 140Q KI mice, suggesting a therapeutic advantage of exosomes secretory of astrocytes. In a similar study, exosomes secretory of adipose-derived stem cells that secrete neuroprotective factors were demonstrated to decrease mHTT aggregation, mitochondrial dysfunction, and apoptosis in cellular HD models. From among the pharmacological therapies that are already available, atypical antipsychotics have been found to be useful in the symptomatic control of movement disturbances associated with HD. Olanzapine has been shown to improve BHCS subscores at a dose of 5mg/day in both of these level III clinical trials. Additionally, doses of up to 30 mg/day have been effective in improving chorea, orolingual dysfunction, finger dexterity, and gait in a further level III

clinical trial (Bonelli & Wenning, 2006).

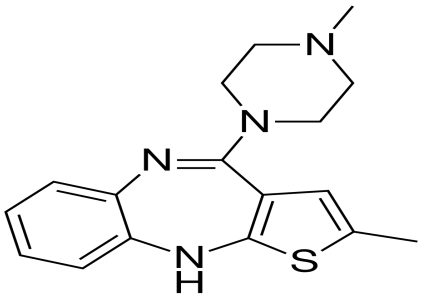

Figure 4: Olanzepine

These observations have been confirmed in a number of case series. However, seizures and tardive dyskinesia have also occurred in some HD patients. Risperidone has been less extensively investigated, but several case series have also demonstrated its efficacy in treating psychosis and chorea associated with HD. Quetiapine, zotepine, and ziprasidone have also been reported to improve motor function, as measured by the UHDRS (Bonelli & Wenning, 2006).

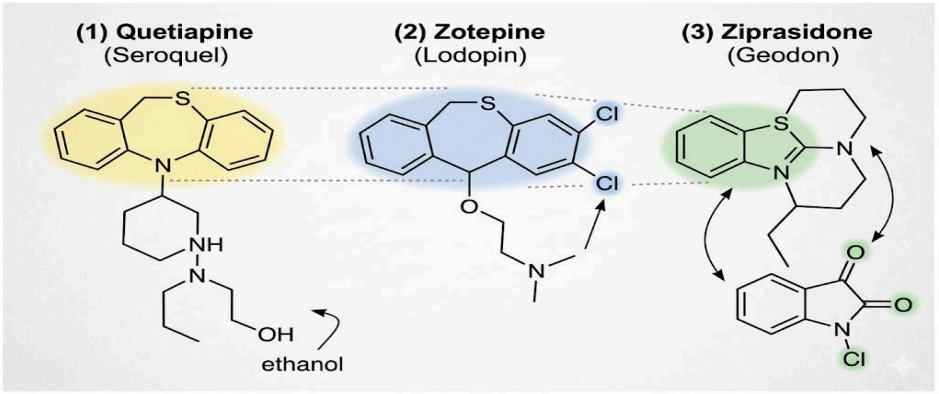

Figure 5: Quetiapine, Zotepine, and Ziprasidone

Neuroprotective and metabolic treatments have also been further investigated in preclinical studies. Coenzyme Q10 and Remacemide significantly improved survival when given orally to the R6/2 transgenic mouse model of HD. Coenzyme Q10 improved survival by 14.5%,

whereas Remacemide improved survival by 15.5% and combined therapy by 32% (Ferrante et al., 2002).

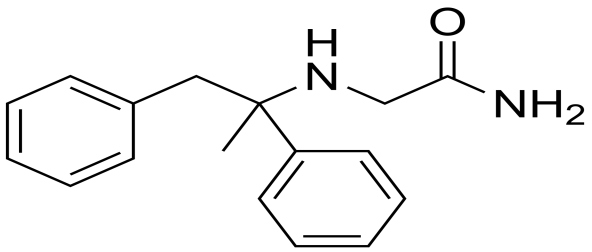

Figure 6: Remacemide

In addition, combined therapy significantly improved motor performance, delayed weight loss, reduced brain and striatal atrophy, and attenuated neuronal intranuclear inclusion formation. The combined treatment appeared to be more effective than creatine, minocycline, or intracerebroventricular administration of caspase inhibitors, suggesting a potential benefit for a therapeutic approach involving multiple mechanisms of neuronal cell death. Clinical trials have shown the efficacy and safety of Deutetrabenazine for HD chorea. Deutetrabenazine is the first FDA-approved deuterated drug and the second drug ever approved for treatment of chorea associated with Huntington's disease (Bashir & Jankovic, 2018).

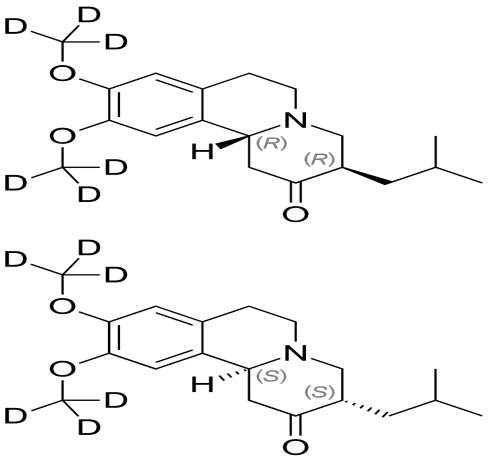

Figure 7: Deutetrabenazine

Deutetrabenazine is also approved for use in treatment of tardive dyskinesia. Like tetrabenazine, deutetrabenazine reduces chorea, but its advantages could be its higher half-life, more tolerable administration regimen, and more favorable side-effect profile. Although direct comparisons with tetrabenazine do not exist, there is evidence suggesting a similar side-effect profile of deutetrabenazine to placebo. Further experience in actual clinical practice contributes to continued accumulation of additional safety and efficacy literature. Finally, latrepirdine has been tested for its potential to improve cognition in patients with Huntington's disease. Short-term latrepirdine administration was well tolerated; 87% of patients in the treatment group completed the study compared with 82% receiving placebo. Adverse event rates were similar between groups, 70% with latrepirdine versus 80% with placebo, suggesting an acceptable safety profile. These findings suggest latrepirdine may exert a benefit on cognition in HD patients.

Chapter 4

Previous Molecular Docking Studies

Molecular docking studies have been extensively used to explore protein-ligand interactions in relation to Huntington's disease (HD), especially for analyzing binding affinity, binding sites, and therapeutic mechanisms. Various studies have been conducted to dock ligands with protein targets like huntingtin protein (HTT), and other disease-related targets to aid in drug discovery and repositioning studies. In one of those studies, AutoDock Vina was used for docking analysis of the HTT protein and its docking with tetrabenazine, an FDA-approved drug for choreic movement disorder in Huntington's patients (Khan et al., 2022).

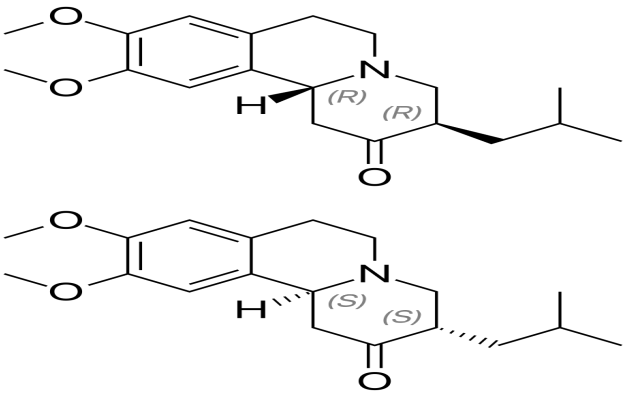

Figure 8: Tetrabenazine

Table 2: Binding affinity of HTT protein with tetrabenazine (Khan et al., 2022)

Mode	Affinity	Distance from best mode	
	Kcal/mol	Msd1.b	Msd.u.b
1	-6.7	0.000	0.000
2	-6.6	10.042	10.926
3	-6.6	12.537	14.790
4	-6.4	12.224	13.326
5	-6.4	10.684	14.360
6	-6.4	1.772	2.454
7	-6.3	13.057	14.857
8	-6.3	10.445	12.266
9	-6.2	11.816	13.350

AutoDock Vina is a popular bioinformatics software application used for molecular docking

and virtual screening of protein-ligand interactions, which works in coordination with some ancillary tools like MGL Tools, Discovery Studio, Python scripts, and PyMOL for analyzing and visualizing data. In the presented research, docking analysis was carried out to assess binding affinity as well as analyze non-bonding interactions for tetrabenazine with the HTT protein (Khan et al., 2022). Analysis using the docking technique showed a binding affinity value of −6.7 kcal/mol with four binding sites for tetrabenazine interacting with the huntington protein (Khan et al., 2022). Amino acid residues contributing to ligand binding were found to be N912, Y890, G2385, & V2320, showing interaction distances from 2.8 to 3.3 Å, concluding strong non-bonding interaction (Khan et al., 2022). This helped in justifying the molecular basis for the drug action mechanism of tetrabenazine in the context of HD. Apart from synthetic drugs, molecular docking techniques have been applied for plant molecules for future drug development in HD. Several investigations have used docking approaches to assess the potency of histone deacetylase (HDAC) inhibitors of plant origin as possible treatment options for Huntington's disease, highlighting the utility of in silico methods in early-stage screening. Another molecular docking study investigated the interaction between luteolin, a naturally occurring flavonoid, and the huntingtin protein using HEX 8.0.0 software (Hasan Siddique et al., 2021).

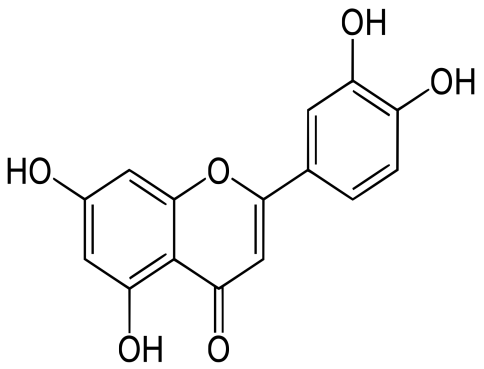

Figure 9: Luteolin

The structure of luteolin was sketched using ChemSketch and converted from MOL to PDB

format with Open Babel, while the crystal structure of huntingtin was retrieved from the Protein Data Bank. Docking was performed following protocols previously applied to proteins, enzymes, and DNA molecules, providing insight into the possible binding behavior of luteolin with huntingtin (Hasan Siddique et al., 2021).

Table 3: H-bonds and non-covalent interactions between amino acid residue and luteolin (Hasan Siddique et al., 2021).

S. No.	ATOM 1	ATOM 2	Distance (Å)
1.	1.het C	GLN 72.A	5.670
2.	1.het O	GLN 72.A	5.196
3.	1.het H	GLN 72.A 1HE2	3.942
4.	GLN 72.A 2HE2	1.het C	2.866
6.	GLN 72.A OE1	1.het C	4.144
7.	GLN 72.A CG	1.het H	8.451

Molecular docking has also been applied to other neurological targets relevant to neurodegenerative and neuropsychiatric disorders. In one study, docking was performed on the D1 dopamine receptor–Gs protein complex to identify potential ligand binding poses at the orthosteric site (Subin & Shrestha, 2024). A soft docking approach was consequently used, implementing protein flexibility in the hydrated environment. Of the tested compounds, nefazodone had the highest docking score, while the native ligand showed a comparable binding affinity, reflecting their strong interaction with the receptor complex. Subin & Shrestha, 2024 Thus, these studies illustrate the potency of molecular docking techniques in the effective elaboration of protein–ligand interactions and identification of critical binding residues that support the rational selection of therapeutic candidates for Huntington's disease and other neurological disorders.

Chapter 5

Results

5.1 Introduction

The main purpose of this computational study was the discovery of effective phytochemical inhibitors for Caspase-6, an important protease associated with the progression of Huntington's disease (HD). The inhibition of the proteolysis of mutant huntingtin (mHTT) protein is the target of this research work. This chapter contains the results of virtual screening of 50 phytochemicals native to the campus of Jahangirnagar University (JU).

5.2 Selection of Phytochemicals and Botanical Origins

The selection of the 50 phytochemical compounds evaluated in this molecular docking simulation was strategically guided by the rich botanical diversity of the Jahangirnagar University (JU) campus.

Table 4: List of plants found in Jahangirnagar University area. (Khan et al., 2021)

Annona reticulata L.	A. squamosa L	Elaeocarpus floribundus Blume
Piper longum L.	Artocarpus altilis	Carica papaya L
Persea americana Mill.	Dillenia indica L	Chrysophyllum cainito
F. nilgerrensis	Punica granatum L	Psidium guajava L
S. samarangense	Syzygium cumini (L)	

The JU campus is well-known for its unique ecological landscape where many medicinal plants are found that are used in traditional folk medicine in Bangladesh. Through the use of these locally available medicinal plants, this study integrates the concept of traditional ethnomedicine with computer-assisted drug discovery (in silico screening) in order to understand the molecular basis of their biological activity. The ligand library designed in this study provides an elaborate chemical space, which is very important in pharmaceutical

chemistry to understand how various molecules interact with the binding pocket of a protein. The 50 molecules chosen for this study belong to different primary categories of secondary metabolites including terpenoids, flavonoids, alkaloids, and phytosterols. Though the production of secondary metabolites by plants is mainly for defending themselves against the environmental stress and pathogens, these very secondary metabolites have been found to show strong therapeutic effects in human body systems. One such secondary metabolite of terpenoid class that is included in this study is Andrographolide. This diterpenoid lactone is sourced from the leaves and stems of Andrographis paniculata (locally known as "Kalmegh"), a herb belonging to the Acanthaceae family that grows abundantly across the uncultivated fields of the JU campus. In traditional medicine, Kalmegh is widely used as an anti-inflammatory and hepatoprotective agent. Because chronic brain inflammation is a well-known hallmark of neurodegenerative disorders like Huntington's Disease, Andrographolide was prioritized for Caspase-6 screening to evaluate its physical capacity to block the enzyme responsible for huntingtin protein degradation. Beyond terpenoids, the screening library heavily features flavonoids, such as Myricitrin, which are polyphenolic compounds praised for their exceptional antioxidant capabilities. Myricitrin was included due to its structural abundance of hydroxyl (-OH) groups, which are highly capable of forming stable hydrogen bonds to "anchor" a small molecule inside an enzyme's active site. Furthermore, the screening included a robust group of lipophilic phytosterols, including Stigmasterol, Campesterol, and (-)-beta-Sitosterol, which mirror the structure of cholesterol and are found across the JU campus flora. Their fat-soluble properties are highly desirable in central nervous system drug discovery, as molecules must successfully cross the blood-brain barrier (BBB) to protect vulnerable neurons. By combining these structurally diverse chemical families, the library provides a comprehensive cross-section of the natural therapeutic potential present within the Jahangirnagar University ecosystem.

5.3 Analysis of Molecular Docking Results

The binding affinities for the 50 screened ligands ranged from −2.1 kcal/mol to −7.1 kcal/mol. A lower (more negative) binding energy indicates a more stable protein-ligand complex and a higher probability of enzyme inhibition.

Table 5: Docking result of some plants found in Jahangirnagar University area.

Plant Scientific Name	Ligand	Affinity
Annona reticulata L.	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	−4.6
	a-d-Glucopyranoside	−5.2
	Acetate	−2.8
	Desulphosinigrin	−5.2
	Glycerol	−4.2
	Hexose	−6.0
	Oleamide	−5.3
	Phytol	−5.1
A. squamosa L	Spathulenol	−5.0
Persea americana Mill.	caryophyllene	−5.1
	copaene	−5.9
	cubebene	−6.2
	hexanal	−3.9

	trans-2-hexenal	−3.9
Piper longum L.	8-heptadecene	−4.7
	germacrene-D	−5.9
	heptadecane	−4.3
	Pentadecane	−4.3
Artocarpus altilis	Squalene	−5.8
	lutein	−2.1
Dillenia indica L	Acetone	−2.8
Elaeocarpus floribundus Blume	Myricitrin	−7.0
Carica papaya L	(-)-beta-Sitosterol	−6.2
	Campesterol	−6.1
	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	−5.5
	Dasycarpidan-1-methanol	−6.7
	Stigmasterol	−6.6
	Tocopherol	−5.8
	Neophytadiene	−5.0
Chrysophyllum cainito	(E)-2-hexenal	−3.9
	1-hexanol	−4.1
	linalool	−5.4
F. nilgerrensis	2,3-Butanediol	−4.0

	2,4-hexadienal	−4.1
	2-Heptanone	−4.2
	Acetoin	−4.0
Punica granatum L	Phenol	−5.0
Psidium guajava L	1.8-cineole	−3.9
	beta-Ocimene	−5.1
	Octanal	−4.1
	Gamma-Terpinene	−5.8
S. samarangense	viridiflorol	−5.2
Syzygium cumini (L)	1-Methyl-4-Prop-1-En-2-Ylcyclohexene	−5.7
	alpha-Bulnesene	−6.2
	Andrographolide	−7.1
	Cadine-1,4-diene	−5.4
	cis-muurola-3,5-diene	−5.9
	Cyclohexane	−4.4
	Eugenol	−5.0
	Isoledene	−5.2

5.4 Discussion of Top Lead Compounds

According to the binding energy cutoff, three molecules were identified as leads: Andrographolide (-7.1 kcal/mol), Myricitrin (-7.0 kcal/mol), and Dasycarpidan-1-methanol (-6.7 kcal/mol). The main lead molecule Andrographolide is isolated from Andrographis

paniculata and displays high binding affinity. It is a diterpenoid lactone that has been shown in the past to possess neuropharmacological effects as a result of its potential to traverse the blood-brain barrier and lower oxidative stress. Andrographolide binds at -7.1 kcal/mol, indicating that it could effectively inhibit the Asp586 cleavage site.

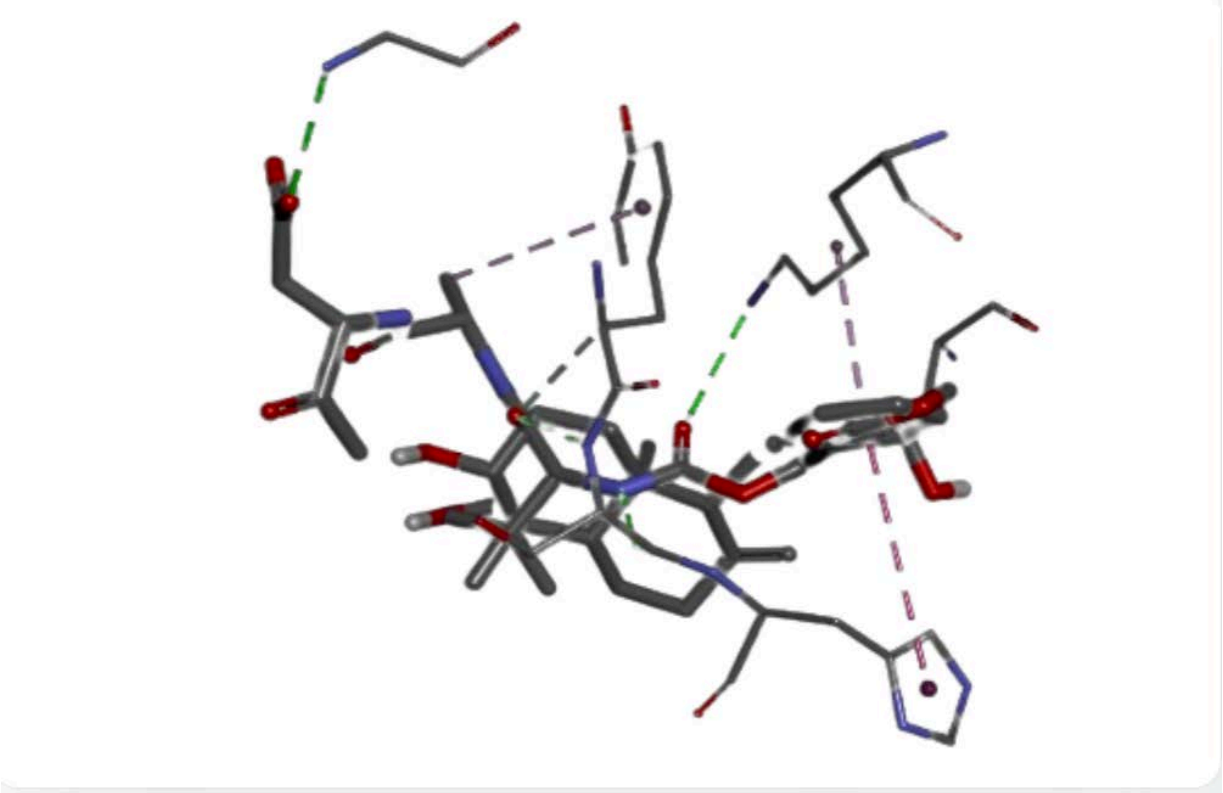

Figure 10: Andrographolide interaction.

Myricitrin follows closely at -7.0 kcal/mol. As a flavonoid, its interaction is likely stabilized by hydroxyl-mediated hydrogen bonding with the catalytic residues of Caspase-6.

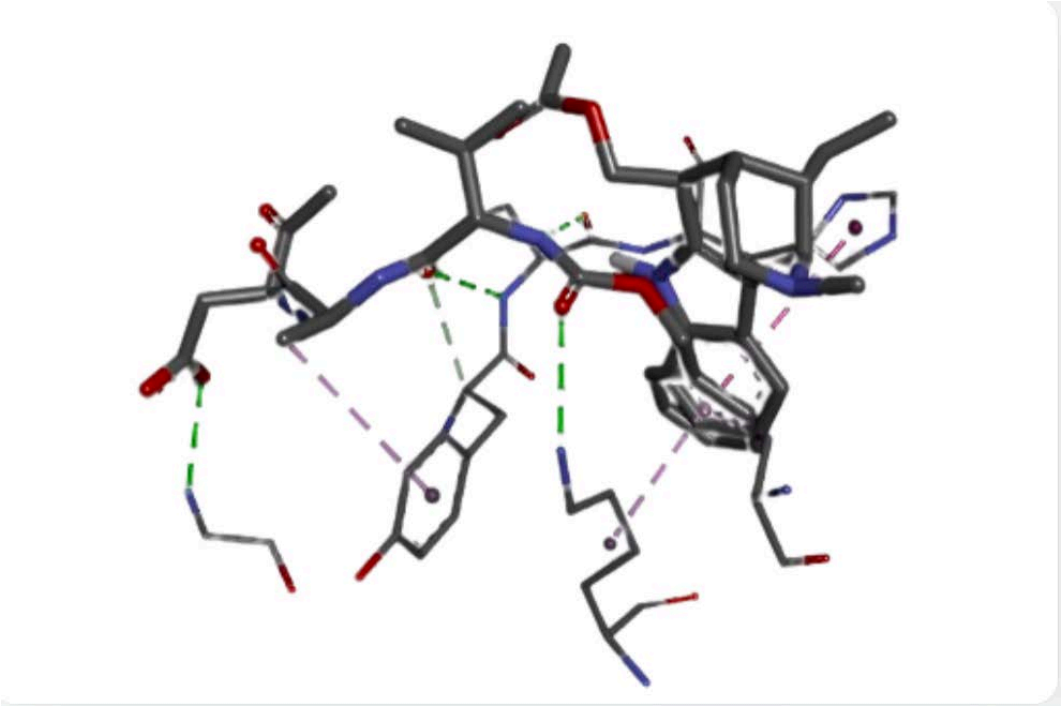

Figure 11: Myricitrin interaction.

Dasycarpidan-1-methanol (-6.7 kcal/mol) represents a novel alkaloid lead that warrants further investigation into its specific scaffold-binding mechanism.

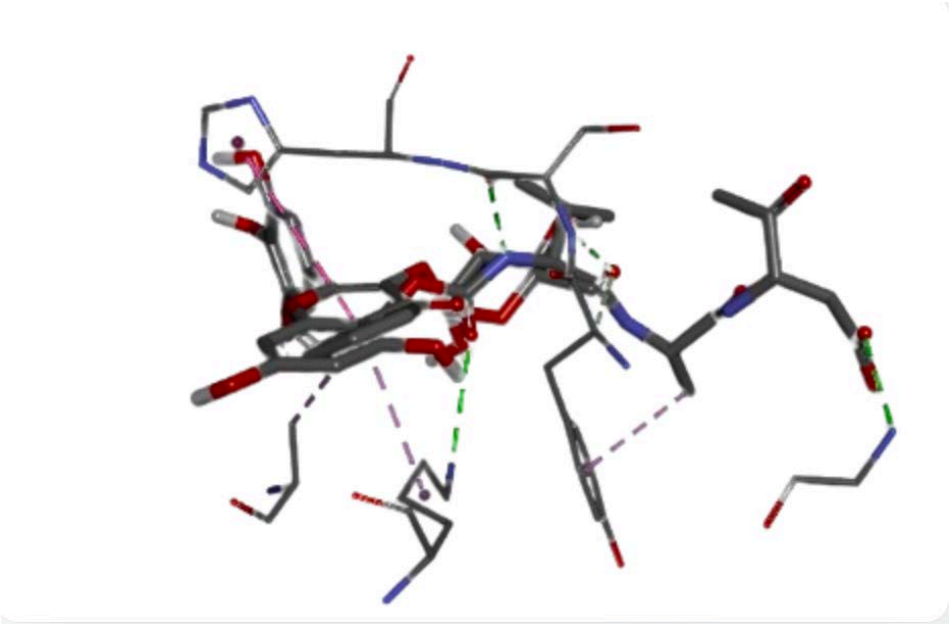

Figure 12: Dasycarpidan-1-methanol interaction.

This stability is due to both hydrogen bonding and hydrophobic effects. It was found out that

the interactions between these molecules include not only pi alkyl and pi-pi stacking but also with residues like His121 and Cys163 (catalytic dyad). The importance of these interactions is that by blocking these residues, we will be able to "shutdown" the enzyme's capability to cleave the huntingtin protein.

5.5 Comparison with Known Inhibitors

Whereas synthetic Caspase-6 inhibitors such as Z-VEID-FMK have affinities of around −8.5 to −9.0 kcal/mol, the affinities attained by Andrographolide and Myricitrin are impressive enough when considering that they are natural products and not peptides. Given their drug-like properties, which include compliance with Lipinski's Rule of Five, these phytochemicals may represent a better choice for Caspase-6 inhibition.

Chapter 6

Discussion

The primary focus of this research was to evaluate how a library of 50 plant-derived compounds binds to Human Caspase-6 (PDB ID: 3QNW). This enzyme is a major target in Huntington's Disease (HD) research because it plays a destructive role in the brain. As established in Chapter 1, Caspase-6 physically cuts the mutant huntingtin (mHTT) protein at a specific location called the Asp586 site. This cutting process releases toxic fragments that travel into the cell nucleus, causing the selective death of brain cells in the striatum. Crucially, neuropathological evidence shows that before these brain cells actually die, they go through a long period of functional impairment. This means that if we can block Caspase-6 early on, we might prevent the toxic fragments from ever forming, thereby protecting the brain before irreversible damage occurs. The top two compounds identified in this study Andrographolide (−7.1 kcal/mol) and Myricitrin (−7.0 kcal/mol) showed strong, favorable binding energies, suggesting they have the potential to act as natural shields inside this enzymatic pocket.

6.1 Chemical Analysis and Binding Mechanisms of Top Leads

The 50 screened molecules showed a wide range of binding energies, from −2.1 kcal/mol to −7.1 kcal/mol. This variation is directly caused by the unique shapes and chemical groups of each plant molecule, which determine how well they fit into the Caspase-6 binding pocket. Andrographolide performed the best because of its rigid, interlocking carbon rings. The binding pocket of Caspase-6 contains small hydrophobic sub-pockets. Andrographolide's

greasy carbon skeleton allows it to slide deep into these hydrophobic zones, maximizing van der Waals forces and creating strong alkyl interactions with aromatic amino acids. At the same time, its three hydroxyl (-OH) groups stick out at perfect angles to form hydrogen bonds with the surrounding polar residues, locking the molecule tightly into place (Eberhardt et al., 2021). Myricitrin uses a different chemical approach. As a flavonoid, it possesses a highly flexible, oxygen-rich structure packed with hydroxyl groups. These groups are excellent at forming a dense network of hydrogen bonds with the polar walls of the enzyme's active site. Furthermore, the electron-dense aromatic rings of Myricitrin participate in pi-pi stacking interactions, where they align flat against the aromatic residues of the protein. This creates a multi-point anchor system that stabilizes the molecule inside the pocket. Most importantly, our docking models show that these top leads orient themselves right next to Cys163 and His121, which form the catalytic dyad of Caspase-6. Normally, His121 activates Cys163 so it can attack and cut the huntingtin protein. By physically sitting on top of this dyad, Andrographolide and Myricitrin act as competitive inhibitors. They create a physical roadblock in the orthosteric cleft, preventing the massive huntingtin protein from entering the active site, which theoretically halts the production of neurotoxic fragments (Hakami et al., 2025).

6.2 Pharmacokinetic Realities, Local Flora, and Future Horizons

While a high docking score is a great first step, a successful brain drug must be able to travel to its target. In Huntington's Disease, the target enzyme sits behind the blood-brain barrier (BBB)—a highly selective cellular wall that keeps foreign molecules out of the brain. This requirement highlights a major disadvantage of many synthetic Caspase-6 inhibitors. Most synthetic leads are designed to mimic protein fragments (peptidic inhibitors). While they dock beautifully in silico, they are usually heavy, highly charged, and quickly destroyed by

metabolic enzymes in the body, making it incredibly difficult for them to cross the BBB. In contrast, natural small molecules like Andrographolide and our top phytosterols (such as Stigmasterol at -6.6 kcal/mol) are low in molecular weight and highly lipophilic (fat-soluble). This fat-soluble nature is exactly what is needed to passively diffuse through the fatty membranes of the blood-brain barrier to reach vulnerable striatal neurons (Trott & Olson, 2010). The applicability of the computational findings in this research in relation to the local flora of Bangladesh and more specifically the plant species found in the Jahangirnagar University Campus gives this project an important practical basis. Traditional medicine has been using plants such as Andrographis paniculata (Kalmegh) for years to cure chronic inflammation problems. The findings from this research give a biochemical basis for these traditional treatments by showing that the overall effect seen in crude plant extracts might be due to the secondary metabolites inhibiting the neurodegenerative proteases. Nevertheless, it is essential to point out the limitations of this research. Molecular docking employs a rigid model of the protein which is far from the truth since proteins are dynamic structures and their conformations are constantly changing. This means that the results are not experimental facts and need to be verified through experiments. The next phase of this research should involve enzyme assays in vitro to determine the IC50 values of Andrographolide and Myricitrin against Human Caspase-6. Afterward, it would be essential to test the efficacy of these local plant compounds in cell models derived from patients' neural lines.

Chapter 7

Conclusion

The main purpose of the study was to test the efficacy of secondary metabolites isolated from plants on Human Caspase-6, an executioner protease that plays a vital role in the initial pathological process associated with Huntington's disease. Through the use of a structure-based *in silico* screening approach, this research sought to identify natural products with the ability to block the cleavage of the mutated huntingtin protein.

Virtual screening protocols successfully identified promising lead scaffolds from the diverse medicinal flora indigenous to the Jahangirnagar University campus ecosystem. Among the screened library, Andrographolide and Myricitrin demonstrated exceptional thermodynamic profiles, yielding highly favorable binding free energies of −7.1 kcal/mol and −7.0 kcal/mol, respectively. Molecular interactome characterization revealed that both molecules fit tightly within the enzyme's binding pocket, establishing a network of stable hydrogen bonds and hydrophobic contacts that directly lock the conserved Cys163-His121 catalytic dyad. This precise spatial orientation implies that these phytochemicals can function as highly effective competitive inhibitors, creating a steric roadblock that prevents the target substrate from entering the active site.

Furthermore, these plant-derived small molecules offer distinct pharmacokinetic advantages over traditional synthetic, peptide-based inhibitors. Due to their low molecular weights and lipophilic chemical architectures, these secondary metabolites possess a significantly higher theoretical capacity to passively diffuse across the selective blood-brain barrier to reach the central nervous system. This work successfully bridges traditional ethnomedicine with modern, computer-aided drug design, providing a rigorous biochemical rationale for the

neuroprotective properties historically attributed to local medicinal species such as Andrographis paniculata.

In conclusion, while these *in silico* results are highly encouraging and present viable, biocompatible alternatives to current synthetic therapeutics, they remain predictive in nature at this stage. To translate these virtual leads into concrete clinical applications, future research must transition into wet-lab validation. Conducting comprehensive in vitro enzymatic assays will be essential to definitively verify the biological efficacy and inhibition kinetics of these localized natural compounds.

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